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Temporal Accumulation of Geosmin, Oxalic Acid, and Total Dissolved Solids in Table Beet

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Abstract. **Consumers perceive flavor as a critically important attribute of vegetable crops. Gas chromatography-mass spectrometry (GC-MS), spectrophotometry, and refractometry of tissue samples collected during multiple years from table beet (*Beta vulgaris*) at various stages of maturity were performed to characterize the endogenous production of geosmin, oxalic acid, and total dissolved solids within the root. The geosmin concentration was primarily influenced by the cultivar and peaked early during the growing season, with root concentrations at 6 weeks after planting that were 312% higher, on average, than those found in harvest stage roots at 15 weeks after planting. The highest average concentration of geosmin in harvest stage roots was detected in tissue from the cultivar Bull's Blood (16.08 $\mu g \cdot kg^{-1}$). The oxalic acid concentration showed a strong cultivar influence and statistically significant variability across the growing season. Hybrid beet cultivar Boro had the lowest soluble oxalic acid concentration (95.73 $mg \cdot 100\ g^{-1}$ fresh tissue) at all locations and during all years. The oxalic acid concentration peaked 12 weeks after planting, and it was lower at the postharvest sampling date 18 weeks after planting. Total dissolved solids (TDS) concentrations were strongly influenced by year and growing environment and displayed crossover interactions for environment × week. TDS measurements had a moderate negative correlation with root mass. 'Chioggia Guardsmark' consistently had the highest TDS during all years and at all locations at 12.01 °Brix. The TDS varied significantly according to time, and diurnal sampling revealed fluctuations as large as 4 °Brix over the course of a 12-hour period. The TDS concentrations increased throughout the growing season, although the rate at which they increased changed according to plant age. The results from this study suggest that interactions between cultivar, time, and environment are important determinants of oxalic acid and TDS concentrations, but they have less influence on geosmin. This information may influence the methods that plant breeders use to collect phenotypic data of important flavor compounds in beets.**

Table beet (*Beta vulgaris* L. Arcang) possesses a unique suite of compounds that impact its culinary potential, including earthy flavors and high levels of sucrose. A third factor influencing edibility is oxalic acid. As consumers continue to demand produce with new and superior flavors, aromas, and culinary quality, plant breeders must develop methods to improve flavor and eating quality traits without sacrificing shelf life, aesthetics, or agronomic traits (Folta and Klee 2016). In recent years, in addition to its traditional use as a cooked, canned, or pickled product, table beet has been promoted as a vegetable crop suitable for eating fresh (Bach et al. 2015; Hanson 2020; Hanson et al. 2022; Prasad et al. 2018).

Other crop species with a high market value in the fresh eating produce sector, such as blueberries (*Vaccinium* sp.), have experienced large-scale investment in molecular-assisted breeding for flavor (Klee 2010). Using gas chromatography, researchers quantified a diverse set of volatile organic compounds suspected to have an impact on consumer liking (Farneti et al. 2017), characterized how important flavor compounds change with the species, cultivar, growing environment, and stage of ripeness (Beaulieu et al. 2014), and performed a successful genome-wide association study followed by the identification of the candidate gene or genes responsible for important fluctuations of volatile organic compounds in blueberry (Ferrão et al. 2020). In recent decades, substantial efforts have been expended to understand flavor profiles of root and bulb crops, such as carrot and onion, and to develop genomic databases and tools that can be used by plant breeders (Khosa et al. 2016; Rolling et al. 2022). Such an approach may prove to be an effective pipeline for improving the culinary quality of table beet; however, a critical first step is developing reliable and robust methods for measuring compounds that influence flavor and eating quality.

Regarding species such as blueberries or other climacteric fruits, breeders and growers are often most concerned with the flavor of ripe marketable produce. However, many vegetable crops lack such a distinction in maturity. Baby beet greens are harvested as early as 28 to 30 d after planting, whereas fresh market "bunch" beets (roots and leaves) are typically harvested between 45 and 60 d after planting. Beets intended for processing are often left in the field for 90 to 120 d, and their size is controlled by the high planting density or by mowing the tops off of actively growing plants (Goldman and Navazio 2002). Thus, if flavor compounds are found to change significantly with regard to plant maturity, then it is possible that cultivars may vary in their flavor profiles depending on their end uses and how they are grown. Furthermore, plant breeders who wish to evaluate the flavor and eating quality of table beets should evaluate these traits earlier in the season to allow an examination of larger populations and increase selection efficiency. Understanding the relationship of flavor compounds with the developmental stage would enable breeders to determine the earliest time when a phenotype predictive of the trait at the harvest stage root could be collected. For example, early-season detection of culinary quality before the harvest stage would allow for greater efficiency during the harvest and postharvest period, when breeding programs require the largest amount of resource deployment. The following experiment presents a framework for evaluating the trajectory of three important flavor and eating quality traits of table beet over the course of the growing season; this topic has not been well-researched.

A large component of the earthy flavor of beet is the volatile terpene, geosmin (Murray et al. 1975). Geosmin is also produced by *Streptomyces* spp. bacteria living in the soil or in stagnant water, as well as other microbial species. Humans are quite sensitive to small changes in geosmin concentrations; as a result, geosmin is considered to be a contaminant in processed food and beverages (Buttery and Garibaldi 1976; Liato and Aïder 2017; Zhang et al. 2016), as well as in drinking water (Lvova et al. 2020; Park et al. 2021). Beets high in geosmin may be off-putting or even unpalatable.

Although the biosynthetic pathway of geosmin in *Streptomyces* bacteria has been characterized (Jiang et al. 2006), this pathway has not been characterized in plants. A search using the Position-Specific Iterative Basic Local Alignment Search Tool (PSI-BLAST) database procured only two potential matches for a geosmin synthase protein analog in table beet cultivars Pacemaker III, Touchstone Gold, Bull's Blood, and the inbred line W364B (Maher and Goldman 2018); however, neither match has been confirmed to synthesize geosmin. One of these proteins is a terpene synthase, and the other is an isoprenoid synthase; both are located on chromosome 8 (Maher and Goldman 2017).

Previous studies have shown that geosmin production in table beets is cultivar-specific (Freidig and Goldman 2011), and that geosmin is being produced endogenously within the beet (Maher and Goldman 2018). Geosmin has been shown to be a heritable trait

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that can be selected both for and against (Maher and Goldman 2017), although concentrations may be affected by genotype × year and year × location interactions (Hanson and Goldman 2019). A mapping study performed by Hanson (2020) identified an association between the geosmin concentration and a quantitative trait locus (QTL) on chromosome 8. After consumer taste testing, Hanson (2020) concluded that geosmin may not be the only compound responsible for the earthy flavor of beets, and that the geosmin concentration was not the primary determinant of hedonic liking by participants in the study.

Oxalic acid is a molecule found in a wide variety of plants. Many food crops in the Amaranthaceae family, including beets, are classified as high-oxalate foods. High concentrations of oxalic acid can cause a burning sensation when beets are eaten raw (Freidig and Goldman 2011), and diets rich in oxalic acid may contribute to the formation of kidney stones (Noonan and Savage 1999). Additionally, oxalic acid may possess antinutritive qualities because it binds to calcium and other minerals and reduces the bioavailability of these nutrients (Wanasundera and Ravindran 1992). Okutani and Sugiyama (1994) observed that the concentration of oxalic acid in spinach (*Spinacia oleracea*) leaves typically increases as the plant ages, and they posited that oxalate synthesis may be a byproduct of metabolism, could serve as a buffer system for cation–anion regulation, or could serve a role in disease and pest resistance (Fasset 1973). Oxalic acid is often cited as an important pathogenicity factor for fungal pathogens (Bennett and Hindal 1989; Cessna et al. 2000; Dutton et al. 1996; Kirkland et al. 2005; Magro et al. 1984); as such, it has been shown to activate systemic-acquired resistance (SAR) to the fungal pathogen *Rhizoctonia solani* in rice plants (Jayaraj et al. 2010). The production of oxalic acid by plants has also been shown to have a role in resistance to insect (Nakata 2015) and animal (Prasad and Shivay 2017) herbivory. A biosynthetic pathway of oxalic acid in bacteria such as *Burkholderia glumae* has been described (Nakata and He 2010); however, a complete synthesis pathway for oxalic acid in plants has not been published.

Sucrose is one of the main compounds associated with the sweetness of fruits and vegetables; however, consumer perception of sweetness can be impacted by other compounds present in plant tissue. Table beet contains substantial amounts of sucrose, and these amounts are often quantified using total dissolved solids (TDS), which are primarily composed of sucrose (Wolyn and Gabelman 1990). TDS, as measured using a refractometer, were chosen as a proxy for sucrose concentration because of the ease of data collection and use as a measure of table beet quality (Feller and Fink 2004). Modern sugar beets are believed to have been domesticated from sugar-accumulating transgressive segregants from a cross between Swiss chard and fodder beet (Dohm et al. 2014). Although researchers attempted to resynthesize sugar beet by crossing Swiss chard and fodder beet and selecting for sugar beet shape and composition, they found that recurrent selection for sugar content did not appear to increase the sugar content of progeny (Fischer 1989). After three cycles of half sib selection for the TDS concentration, Wolyn and Gabelman (1990) found nonsignificant changes in TDS and observed a low realized heritability ($h^2 = 0.25$) for TDS. Tobi et al. (2021) calculated an even lower estimate of narrow sense heritability ($h^2 = 0.025$) for TDS in sugar beet.

Hanson and Goldman (2019) found that complex genotype × environment interactions influenced the variance present in TDS measurements of beet root tissue. The sucrose concentration in sugar beet was found to increase throughout the growing season and slightly decrease after harvest (Kenter and Hoffmann 2006). Several studies have reported that the sucrose concentration in table beet increases during the first half of the growing season before reaching a saturation point; thereafter, the sucrose concentration does not increase (Fasahat et al. 2018; Kenter and Hoffmann 2006). To date, no such study involving table beet has been performed.

The table beet is generally considered to be descended from a relatively narrow genetic base. Modern cultivars consist of open pollinated varieties and hybrid cultivars produced by crossing inbred parents, with one of which possessing cytoplasmic male sterility. The complexity of identifying and maintaining male sterility in inbred parents is one of the impediments to smaller breeding programs seeking to gain market penetrance with new hybrid beet varieties boasting improved flavor and easting quality. The processing sector is dominated by relatively few varieties, whereas more diversity is seen in operations growing for the fresh market (Goldman and Navazio 2002). Recently, a newfound public interest in consumer quality traits spurred the release of table beet cultivars such as Badger Flame, which was bred for its low geosmin and oxalic acid contents, sweet flavor, interesting shape, and unique colors, as well as five new cultivars that were developed as part of a participatory plant breeding program using farmer and consumer taste test data to guide selections (Hanson 2020).

Understanding the mechanisms and trends by which compounds that affect flavor and eating quality change with regard to time can assist plant breeders with the development and optimization of methods of selecting for or against these compounds by allowing them to more efficiently allocate resources, personnel, and equipment, and by enabling them to make selections earlier or with greater confidence. Although the effects of early and late planting dates have been examined in the table beet oxalic acid concentration (Freidig and Goldman 2011) and TDS (Gaertner and Goldman 2005), an analysis of geosmin, oxalic acid, and TDS in tissue collected at multiple time points over the season has never been performed. Diurnal measurements of the geosmin concentration and TDS in table beet have not been reported, and the findings of these experiments may influence the degree to which researchers rely on refractometer data as a proxy for the sucrose concentration in table beet. Our objective was to assess key determinants of table beet flavor during the growing season across several table beet cultivars.

Methods

Field design. Four cultivars of beets were planted in a randomized complete block design with three blocks flanked by border rows of beet cultivar Bull's Blood. Open pollinated cultivars Bulls Blood, Chioggia Guardsmark, and Touchstone Gold and hybrid cultivar Boro were selected for their contrasting phenotypes and relative geosmin content (Fig. 1) (Freidig and Goldman 2014; Hanson and Goldman 2019; Lu et al. 2003). 'Bull's Blood' had the highest previously reported geosmin concentration in field-grown beet root (20.82 $\mu g \cdot kg^{-1}$ geosmin), with 'Touchstone Gold' having the lowest geosmin concentration (4.84 $\mu g \cdot kg^{-1}$) and 'Chioggia Guardsmark' displaying an intermediate geosmin concentration (14.1 $\mu g \cdot kg^{-1}$), as measured in field-grown beets (Freidig and Goldman 2014). Boro is a popular hybrid beet cultivar that has not been included in previous studies that quantified geosmin concentrations. 'Bull's Blood', 'Chioggia Guardsmark', and 'Touchstone Gold' were previously found to have similar levels of soluble oxalate in root tissue (Freidig and Goldman 2011). Previously, 'Chioggia Guardsmark' exhibited higher TDS (9.4 °Brix) than 'Bull's Blood' and 'Touchstone Gold', which both had a mean TDS concentration of 8.3 °Brix (Hanson et al. 2022).

Each plot consisted of two 3.7-m paired rows of a single cultivar with between-row spacing of 46 cm. Two rows were necessary to provide enough plant material for repeated random sampling throughout the season without significantly changing the plant density. Rows were overseeded using a hand-propelled Planet Jr. planter with a cone seeder attachment; then, they were thinned to a density of 20 seedlings per meter at 3 weeks after planting. Fields were treated with S-metolachlor-based pre-emergent herbicides Nortron SC and Dual II Magnum (Syngenta, Basel, Switzerland) immediately after planting.

Fig. 1. Four cultivars of table beet selected for use during this experiment (left to right): Touchstone Gold, Chioggia Guardsmark, Boro F_1 Hybrid, and Bull's Blood.

This experiment was conducted at the Hancock Agricultural Experiment Station (Hancock, WI, USA) and the Arlington Agricultural Research Station (Arlington, WI, USA) over the course of the 2021 and 2022 growing seasons for a total of four year–location trials.

In 2021, the Hancock and Arlington fields were planted on 17 May and 25 May, respectively. In 2022, Hancock was planted on 17 May; Arlington was planted on 24 May. As necessitated by the sandy soils of the Hancock Research Station, we sowed a barley nurse crop at the time of planting and irrigated consistently throughout the growing season. Hancock received 31 cm of precipitation during both the 2021 season and 2022 season. The barley nurse crop was ended 2 weeks after planting, and applications of urea and ammonium nitrate were performed weekly over the growing season. The plot at the Arlington Research Station was planted in silt loam soil and did not receive any irrigation or fertilization; it received rain totals of ∼26 cm in 2021 and 41 cm in 2022 (Weather Underground, Ann Arbor, MI, USA).

Sample collection was performed tri-weekly at each location for a total of six sample collection dates (3, 6, 9, 12, 15, and 18 weeks after planting) per location per year. At the time of sampling, six plants were randomly chosen from each row; then, 1-cm-diameter cores containing both epidermal and core root tissues were taken and subsequently placed in a labeled polyethylene bag for transport and storage. Beet cores were kept frozen at −20 °C until processing. Samples collected 3 weeks after planting consisted of only stems, two cotyledons, and unswollen root tissue; as such, they were excluded from analysis of root metabolites. Samples obtained at 6 weeks after planting showed signs of swelling in the hypocotyl tissue; therefore, they were used in the experiment. At 15 weeks after planting, all roots in the row were harvested, packed in cedar shavings, and stored in a walk-in cooler at 7 °C (Walnut Street Greenhouse, Madison, WI, USA) for 3 weeks to simulate postharvest storage and handling conditions. Then, they were randomly selected for the week 18 sample collection.

Size sampling. The relationship between root size and flavor compound concentration was examined by harvesting 63 roots of the cultivar Bull's Blood from the Arlington Research Farm on 28 Aug, 2021. The masses of individual roots were recorded before 1-cm cores containing both epidermal and core root tissues were obtained, placed in labeled polyethylene bags, and stored at −20 °C until sample preparation and laboratory analysis.

Diurnal sampling. To assess the changes in geosmin and TDS over the course of the day, samples from the cultivar Bull's Blood were collected from three experimental blocks at the Arlington Research Farm at 2-h intervals over a 24-h period, resulting in a total of 36 block × time samples. At each sampling time, three plants were randomly chosen from each block, the mass of each root was recorded, and 1-cm-diameter cores containing both epidermal and core root tissues were obtained from each root and subsequently placed in a labeled polyethylene bag for transport and storage. Beet cores were kept frozen at −20 °C until processing. This experiment was performed on 28 Aug 2021, and again on 6 Sep 2022.

Sample preparation. To prepare beets for chemical analyses, frozen cores were placed in an industrial Waring (McConnellsburg, PA, USA) blender and ground for 2 min at 13,000 rpm. To account for the variations in geosmin contents of epidermal tissue and core root tissue, which would skew the results in a manner correlated with the surface area-to-volume ratio of the beet, samples were standardized to 5% epidermal tissue by weight. A portion of ground tissue was used to collect measurements of TDS by pressing 10 μL of liquid through a Kimwipe (Kimberly-Clark, Irving, TX, USA) onto an ABBE-3L refractometer (Thermo Fisher Scientific, Waltham, MA, USA). Six refractometer measurements were performed for each sample; then, 10 g of homogenized tissue was stored for later analysis of the oxalic acid concentration. The remaining tissue was mixed in a 1:1 ratio with MilliQ water to create a uniform beet slurry, which was later placed into a 25-mL test tube filled to the top of the tube to minimize headspace, sealed with parafilm, and placed in the −20 °C freezer again.

Geosmin. Geosmin was quantified using headspace solid-phase microextraction gas chromatography mass spectrometry (GC-MS), as described by Freidig and Goldman (2014), and each sample was run in triplicate. After thawing the beet slurry, 5 g of the sample was placed in a headspace vial containing 1 g of NaCl and sealed with a PTFE septum cap. An internal standard of −(−)menthone was added to each sample, as well as to water standards with geosmin concentrations of 5, 10, and 21.6 $\mu g \cdot kg^{-1}$ geosmin. Analytical-grade geosmin and menthone were purchased from Sigma Aldrich (St. Louis, MO, USA). Vials were placed in a Shimadzu Model QP2010SE GC-MS with an AOC-5000 Shimadzu Autoinjector.

The Maher (2017) adaptation of the Lu et al. (2003) GC-MS protocol was used, which required 3 min of 500-rpm agitation at 60 °C before extraction of the volatilized headspace components by a polydimethylsiloxane/divinylbenzene fiber (Supelco, Bellefonte, PA, USA) over the course of a 10-min period under the same conditions. The fiber was thermally desorbed at 250 °C and subsequently injected using the split injection mode with a split ratio of 10 and a split/splitless liner (Restek, Bellefonte, PA, USA). A 30-m SH-Rxi-5Sil column with a 0.25-mm inner diameter and degrees of freedom of 0.25 mm (Shimadzu, Kyoto, Japan) was used. Temperatures were maintained at 40 °C for 1 min before being increased at a rate of $35\ ^\circ C \cdot min^{-1}$ to 300 °C; at that point, it was maintained for 2 min. A 10-min incubation period at 250 °C was used to condition the fiber between samples.

Relative recovery was calculated using samples of the beet cultivar Bull's Blood spiked with 5, 10, 15, and 21.6 $\mu g \cdot kg^{-1}$ root tissue of geosmin and 2.82 $\mu g \cdot kg^{-1}$ root tissue −(−)menthone using the equation described by Lu et al. (2003):

$$RR = \frac{\mu g\ \text{Geosmin}_{Total} - \mu g\ \text{Geosmin}_{Unmodified}}{\mu g\ \text{Geosmin}_{Spiked}} \times 100\%$$

Relative recovery rates of geosmin from the beet root slurry matrix wEre 42.44% in 2021 and 41.15% in 2022, similar to that reported previously (Hanson and Goldman 2019).

Oxalic acid. To measure the oxalic acid present in the samples, an adaptation of a protocol using an oxalic acid-catalyzed reaction of bromophenol blue and potassium dichromate was chosen (Xu and Zhang 2000). Oxalic acid measurements had two technical replicates for each sample.

Soluble oxalic acid was extracted by adding 0.250 g of ground beet tissue in 2.5 mL of MilliQ water and incubating at 60 °C for 30 min. After oxalate extraction, 1 mL of supernatant was removed and stored at 35 °C. Then, 100 μL of the supernatant was added to 4.493 mL of MilliQ water. Next, 340 μL of a reaction master mix containing 49.6 mM potassium dichromate and 558.4 mM sulfuric acid in MilliQ water was added, and the solution was mixed by pipetting. The addition of 1.5×10^{-5} M bromophenol blue followed by 5 s of vortexing began a 10-min incubation period in a 60 °C water bath. At 10 min, the reaction was quenched with 1 mL of 2 M sodium hydroxide, and 1 mL of the solution was immediately placed in a semimicro cuvette (Greiner Bio-One, Kremsmünster, Austria). The absorbance at 600 nm was measured using a Spectronic Genesys 5 ultraviolet/Visible Spectrophotometer (Spectronics Corporation, Melville, NY, USA), and the absorbance values were compared with those of a standard curve (Fig. 2). The linear analytical range of the assay was 0 to 200 $mg \cdot 100\ g^{-1}$ fresh tissue.

Statistical analysis. A mixed-model analysis of variance (ANOVA) was selected to analyze this experiment to account for repeated measures using a replicated randomized complete block design. Each year–location combination was treated as a separate environment because of changes in field location and orientation. Environment, weeks, and genotype were treated as fixed effects, with the effect of block nested within a year–site combination defined as random. An analysis was conducted using RStudio version 2022.07.2 (RStudio, Boston, MA, USA) and R version 4.2.2 (R Core Team, Vienna, Austria). R package "lme4" was used for mixed-effects modeling and the ANOVA (Bates et al. 2015), "emmeans" was used for drawing pairwise comparisons and generating linear contrasts, and "ggplot2" was chosen for data visualization and to produce graphics for this publication (Wilkinson 2011).

Results

An ANOVA of all data showed evidence of interactions among weeks, environment, and cultivar for most traits (Table 1). Significant interactions resulting in rank changes occurred primarily for samples during week 6.

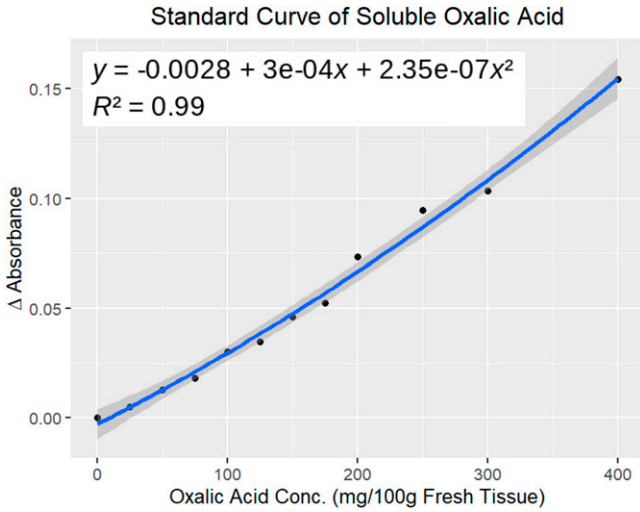

Fig. 2. Standard curve for the soluble oxalic acid in beet root tissue produced using the kinematic spectrophotometric assay. The curve is fit with a polynomial regression curve but has a linear analytical range of 0 to 200 mg·100 g^{-1} fresh tissue.

These samples exhibited large variations because of differences in the germination time, early vigor, and establishment of the cultivars. Thus, data from samples collected 6 weeks after planting were excluded from the analysis. This had the effect of limiting interaction effects, and the data from all subsequent weeks were explained primarily by main effects.

Geosmin. The ANOVA revealed that the geosmin concentration displayed a significant cultivar × week interaction. This interaction was not of a crossover nature, and rank was conserved. Significant main effects were found for cultivar and environment. The effect of environment may be explained by the fact that geosmin concentrations in 2021 were higher in Arlington than in Hancock, but they were lower in Arlington in 2022.

Cultivar Bull's Blood consistently had the highest root geosmin concentrations, with an experiment-wide average concentration of 16.08 μg·kg^{-1}, which was higher than the average concentration for 'Chioggia Guardsmark' (13.05 μg·kg^{-1}). Cultivars Boro and Touchstone Gold had the lowest concentrations of geosmin, averaging 8.50 μg·kg^{-1} and 7.64 μg·kg^{-1}, respectively.

By multiplying the concentration of geosmin by the average mass of the roots in the sample, we were able to approximate the total geosmin content of the beet root. The total geosmin content increased throughout the growing season, but it stopped increasing when roots were harvested and placed in postharvest storage (Fig. 2). However, the total accumulation was different across locations (Fig. 3).

Oxalic acid. Statistically significant environment × weeks and environment × cultivar interactions for root oxalic acid concentration occurred. These interactions were not of a crossover nature. Cultivar and weeks after planting had the strongest effect on the oxalic acid concentration ($P \leq 0.001$ for both). Oxalic acid was most strongly influenced by cultivar, but it also changed over the course of a growing season.

'Boro' consistently had the lowest soluble oxalic acid concentration of all location × year combinations, with an experiment-wide average concentration of 95.73 mg·100 g^{-1} in roots at 18 weeks after planting. 'Chioggia Guardsmark' displayed the highest oxalate concentration (253.17 mg·100 g^{-1}) at both locations during the 2022 season compared with 'Touchstone Gold' (209.41 mg·100 g^{-1}) and 'Bull's Blood' (162.35 mg·100 g^{-1}), but the three were not significantly different in 2021.

Total dissolved solids. An ANOVA of TDS concentrations showed three significant interaction terms, with the environment × week interaction having a crossover nature. When separate ANOVAs were performed for each environment, there were no significant interactions present in any of the environments. Hancock showed significant main effects of block and cultivar in both 2021 and 2022. There were no significant factors affecting TDS in Arlington 2021, and Arlington 2022 showed significant effects of cultivar and was the only environment to show a highly significant effect of weeks after planting on TDS.

The TDS level was highest in 'Chioggia Guardsmark' across all years and locations, with an experiment-wide average of 12.01 °Brix in roots harvested 15 weeks after planting, which was higher than that of 'Touchstone Gold' (10.80 °Brix), 'Bull's Blood' (10.66 °Brix), and 'Boro' (10.42 °Brix) (Fig. 4). In 2022, TDS peaked for all cultivars in both locations at 9 weeks after planting. This same trend was not observed in 2021. The TDS level was lowest in the first root tissue sampling at 6 weeks after planting; however, by 12 weeks after planting, it had stabilized to levels found in harvest-stage roots.

Relationship among traits. An ANOVA of the average root mass from beets harvested 15 weeks after planting at Arlington and Hancock in 2022 (Table 2) revealed a location × cultivar interaction; however, this interaction was not of a crossover nature.

The relationship between root size, geosmin concentration, and TDS was explored using data collected from 63 individuals roots of the cultivar Bull's Blood that were harvested on 28 Aug 2021. An analysis of the samples found that there was a weak-to-moderate negative correlation ($R^2 = -0.048$) between °Brix and root mass. The geosmin concentration had only a weak correlation ($R^2 = 0.012$) with root mass. An ANOVA showed that mass is not a significant factor affecting the root geosmin concentration ($P = 0.3765$) or TDS concentration ($P = 0.0827$), and that the geosmin concentration was not significantly correlated with the TDS concentration ($P = 0.433$) (Table 3).

Diurnal sampling. An ANOVA of the results obtained during the diurnal sampling experiment indicated that time (hours) had a significant effect ($P < 0.05$) on TDS readings of root tissue (Table 4, Fig. 5). Although a graphical analysis of the data revealed what appears to be a cyclic trend in the geosmin concentration, the ANOVA did not reveal a significant effect of hours on the root geosmin concentration. The fact that the same trend was not observed over multiple years suggests that this may be an artifact of sampling, and that the fluctuations in geosmin concentration can be attributed to experimental error. Therefore, we concluded that although the TDS level changes significantly

Table 1. Degrees of freedom (df) and F statistics from an analysis of variance (ANOVA) of the randomized complete block design with repeated measures for the effects of environment (E), cultivar (C), weeks after planting (W), and block (B) on the geosmin concentration, oxalic acid concentration, and total dissolved solids in four table beet cultivars grown at two sites over 2 years in Wisconsin and sampled at 9, 12, 15, and 18 weeks after planting. Geosmin concentration and oxalic concentration both underwent square-root transformation to correct for nonconstant variance.

	Geosmin			Oxalic acid			Total dissolved solids		
Source	df	F		df	F		df	F	
E	3	9.58	***	3	2.92		3	3.11	*
C	3	69.84	***	3	39.02	***	3	39.57	***
W	3	1.30		1	66.28	***	3	7.50	***
E × Block	8	1.92		8	1.60		8	4.62	**
E × C	9	1.04		9	2.72	*	9	2.75	*
E × W	9	1.97		3	11.27	***	9	3.43	**
C × W	9	2.99	**	3	0.53		9	1.81	
E × C × W	27	0.62		9	1.92		27	0.80	

*, **, *** Significant at $P \leq 0.05$, 0.01, and 0.001, respectively.

Table 2. Sources of variation, degrees of freedom (df), F statistics, and *P* values from an analysis of variance of the effects of location and cultivar on the average mass of beets roots harvested at 15 weeks after planting at two locations (Arlington, WI, USA and Hancock, WI, USA) in 2022.

Source	df	F	*P*	
Location	1	109.00	5.46e-08	***
Cultivar	3	4.27	0.02457	*
Location × cultivar	3	4.00	0.02980	*

*, **, *** Significant at $P \leq 0.05$, 0.01, and 0.001, respectively.

with respect to the time of sample collection, geosmin does not display a similar behavior.

Discussion

This experiment characterized the changes of important quality-related compounds over the course of the season and examined the influence of factors such as year, location, root mass, and sampling time on these compounds. Additionally, an accurate, low-cost assay for measuring the concentrations of soluble oxalic acid in table beet root tissue was developed, and findings regarding relatively large daily fluctuations in TDS were identified through diurnal sampling.

In concordance with previously published research (Hanson and Goldman 2019), the concentration of geosmin in mature table beet root tissue appeared to be cultivar-specific and strongly controlled by genetics. Geosmin concentrations previously reported for 'Bull's Blood' and 'Touchstone Gold' (Hanson and Goldman 2019) were similar to the concentrations found during this study. We found that geosmin synthesis occurred throughout the growing season, but that it was present in the highest concentration early in the season. Changes in the root geosmin concentration during the growing season as large as 75.2% were observed for the cultivar Chioggia Guardsmark.

The concentrations of soluble oxalic acid found in beet roots at 18 weeks after planting during this experiment were, on average, 11.5% higher than concentrations previously reported by Freidig and Goldman (2011); however, this may be attributed to the use of a different assay. Freidig and Goldman (2011) used an enzymatic colorimetry kit to measure the concentration of oxalic acid in beet roots and leaves. Part of this project involved developing an accurate, lower-cost assay for measuring oxalic acid in beetroots. Initial efforts were focused on adapting a protocol for the spectrophotometric determination of oxalic acid through the kinematic degradation of potassium permanganate, as described by Franco and Krinitz (1973) and Karamad et al. (2019); however, the absorbance spectrum of potassium permanganate overlapped with that of the betalin pigments found in some beets. To avoid interference by the varying levels of red pigment in beets, this experiment used a kinematic spectrophotometric protocol that exploited an oxalic acid-catalyzed reaction of bromophenol blue and potassium dichromate, which was then quenched by sodium hydroxide to shift the absorbance outside of the spectra of betalain pigments. Although the new kinetic spectrophotometric assay is significantly less expensive than the previously used methods for measuring oxalic acid in table beet, the kinetic nature of the reaction means that even small fluctuations in the amount of time that a sample is allowed to react before being placed into the refractometer can cause variation of the data. Therefore, great care must be taken to ensure consistent reaction times and conditions to minimize experimental error.

Table 3. Results of three separate analyses of variance that examined the effects of root mass on total dissolved solids (TDS) and root geosmin concentrations, and the correlation between the TDS and geosmin concentration. A linear model was fitted to data collected from 63 roots of the beet cultivar Bull's Blood harvested on 28 Aug 2021 at the Arlington Research Farm.

Source	df	t-value	*P*
Average mass of TDS	61	−1.76	0.0827
Average mass of geosmin	60	0.89	0.3765
TDS of geosmin	60	0.79	0.4330

*, **, *** Significant at $P \leq 0.05$, 0.01, and 0.001, respectively.

Table 4. Results of two separate analyses of variance examining the effects of the hour of sampling on the beet root geosmin concentration and total dissolved solids (TDS) concentration. Samples of the table beet cultivar Bull's Blood at 15 weeks after planting were collected from three experimental blocks at 2-h intervals for 24 h, and the experiment was repeated in 2021 and 2022. A mixed-effects model was fitted with block as a random effect to account for repeated measures.

Source	df	t-value	*P*	
Geosmin: hour	11	0.91	0.5366	
TDS: hour	11	2.15	0.0296	*

*, **, *** Significant at $P \leq 0.05$, 0.01, and 0.001, respectively.

The presence of rank-changing interactions among location × year × week suggests that the concentration of oxalic acid for a particular cultivar may vary across years and location, and that the accumulation of oxalic acid throughout the season and during postharvest storage occurs at a rate that is dependent on both year and location. Thus, it is difficult to accurately predict the nature of seasonal trends in the concentration of oxalic acid for a given cultivar across locations and years. Oxalic acid concentrations in harvest stage roots at 18 weeks after planting showed a noncrossover year × cultivar interaction and large effects of cultivar and year, from which we can infer that the oxalic acid concentration in harvest stage roots is controlled by genetics but influenced by year-to-year changes in growing conditions.

Future studies attempting to further characterize these interactions may benefit from the higher resolution offered by increasing the frequency of sampling. Despite this, the hybrid beet cultivar Boro had an oxalic acid concentration significantly lower than that of the other cultivars during the experiment across both years and locations, with Boro exhibiting oxalic acid concentrations 46.2% lower than the average of all other cultivars at 18 weeks after planting. Thus, it may be possible for breeders to select cultivars with lower and more stable levels of oxalic acid. At the time of writing, there have been no formal efforts to breed for reduced levels of oxalic acid in beets; however, breeders who seek to release cultivars with improved eating quality may be inadvertently selecting for low-oxalate beets because of the negative impact of oxalic acid on eating quality. Coordinated efforts to breed for lower oxalate levels in other crop species, such as spinach (Shi et al. 2016), rhubarb (Libert 1987), tomato (Chakraborty et al. 2013), winter wheat (Matros et al. 2017), and soybean (Francis et al. 2020), are in progress. Regarding rhubarb, a crop species in the same Order as beet, low (h^2 = 0.3–0.38) narrow-sense heritabilities have been estimated for oxalic acid concentration, whereas estimates of broad-sense heritability are moderate (H^2 = 0.58) for winter wheat (Matros et al. 2017) and high (H^2 = 0.97) for populations resulting from a hybridization event between longan and lychee (Wang et al. 2022). Research of other crop species has suggested that the type and quantity of exogenous nitrogen applied may influence the amount of oxalic acid found in plant tissues (Tian et al. 2008; Rahman et al. 2012; Liu et al. 2015). Therefore, nitrogen management and the timing of fertilizer application may be a useful tool for growers and an important consideration for plant breeders seeking to breed lower levels of oxalic acid in table beets.

Crossover cultivar × week, location × week, and year × week interactions led us to infer that cultivars do not perform consistently with regard to TDS across the growing season, and that both location and week impact TDS differentially in ways that are not well-characterized. These findings are corroborated elsewhere in the literature (Hanson and Goldman 2019). Our data suggest that TDS levels in beet may be not only cultivar-specific but also location-specific and tied to particular developmental trajectories in the plant.

TDS was the only trait examined during this study that showed a significant difference between blocks, which was indicative of an underlying field gradient. This provides further support that TDS is more influenced by environmental conditions than are geosmin or oxalic acid concentrations. Fluctuations in TDS in beets harvested from the same cultivar in the same row over a 24-h period were as large as 4.00 °Brix, which is a fluctuation representing 32.7% of the highest TDS concentration for that row. The average within-row diurnal variation for all blocks and years was 26.6%, with TDS being the lowest at dawn and highest before sunset. We noted that the cultivar used for this experiment is open-pollinated and, thus, may inherently possess plant-to-plant variability. However, the magnitude of the variability measured

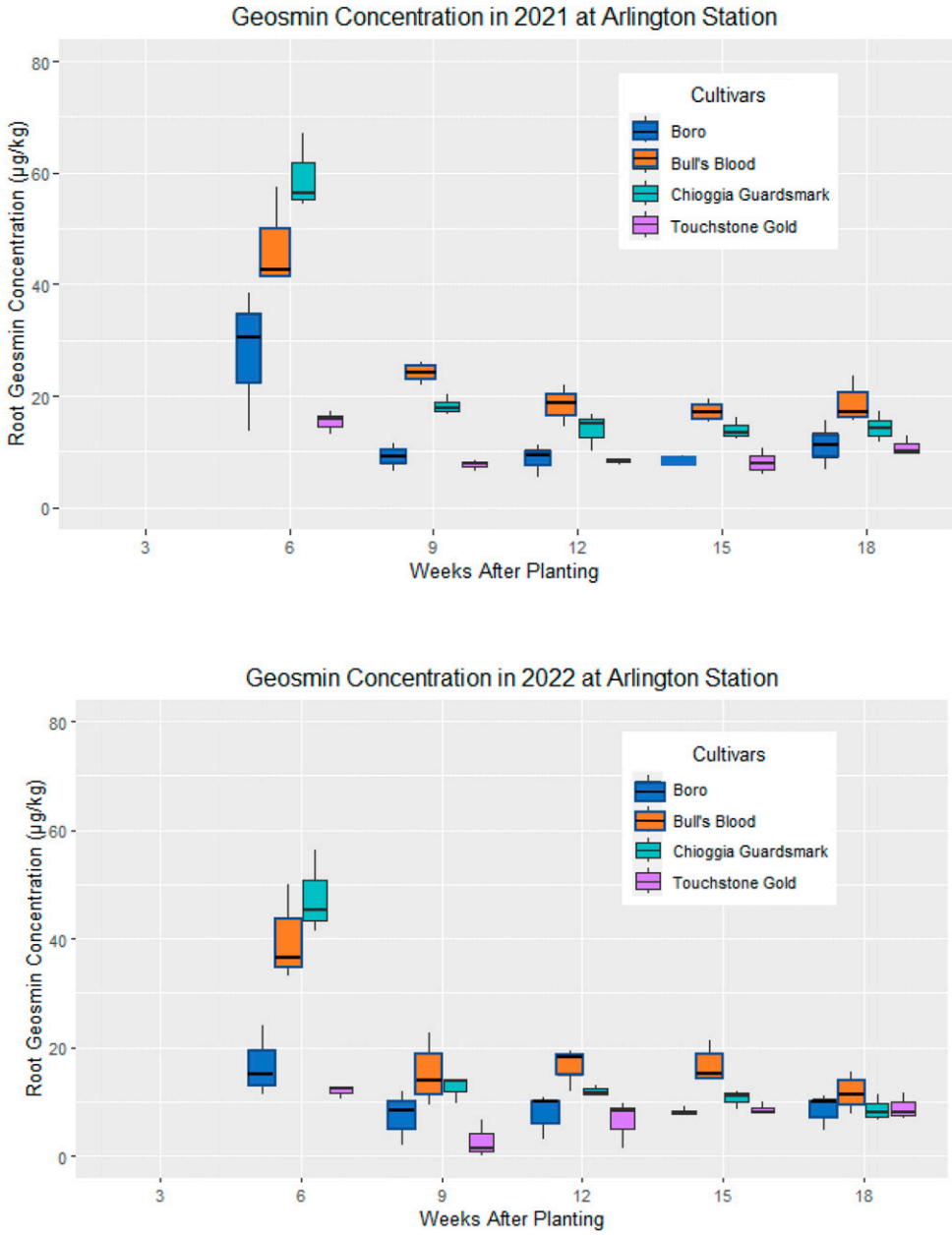

Fig. 3. Average concentration of geosmin found in root tissue samples collected from four cultivars of table beet every 3 weeks throughout the 2021 and 2022 growing seasons at the Arlington Research Farm.

suggests that other factors influenced the range of TDS measured. This is the first report of fluctuations in TDS concentrations in table beet over the course of a diurnal period; therefore, this may account for some of the unexplained variations in previous research involving TDS in table beet (Goldman et al. 1996). Because the daily changes in the measured values were larger than the published differences in TDS between cultivars (Hanson and Goldman 2019), breeders may find that if they wish to use TDS as an indicator of sucrose content, then they must take measures to ensure consistency in sample data, such as collecting data at the same time each day and implementing random checks of each experimental block.

A graphical analysis of the root geosmin concentration data from the diurnal sampling experiment indicated that the geosmin concentration may also have a diurnal trend; however, the diurnal change detected during this study was not deemed statistically significant. Although cacti show diurnal cycling in the amount of geosmin released by flowers, which is likely to attract pollinators that associate the earthy scent with water (Schlumpberger et al. 2004), there is no published data indicating a diurnal fluctuation in the geosmin content of any crop species.

Measurements of the geosmin concentration and TDS generally had higher variance in samples obtained at week 6 of the temporal experiment. This may be attributable to cultivar differences in the rate of germination, establishment, and early season vigor, which became less apparent at later sampling dates, and to experimental error as a result of the small amount of root tissue available for analysis. Additionally, notable changes in rank interactions were observed for week × year for geosmin only during week 6, and the exclusion of the week 6 data resulted in no crossover interactions for the geosmin concentration at any of the subsequent weeks of sampling.

Sampling was performed on a triweekly basis to facilitate consistency in sample collection across years and locations; however, the results may have differed if sampling was instead based on the growth stages of the beet plants. The cultivar Bull's Blood had the lowest root mass at 15 weeks after planting at both locations in 2022, whereas Chioggia Guardsmark, Touchstone Gold, and Borohad had similar masses at Arlington in 2021, but varied root masses at Hancock, with Touchstone Gold having the largest, followed by Boro and Chioggia.

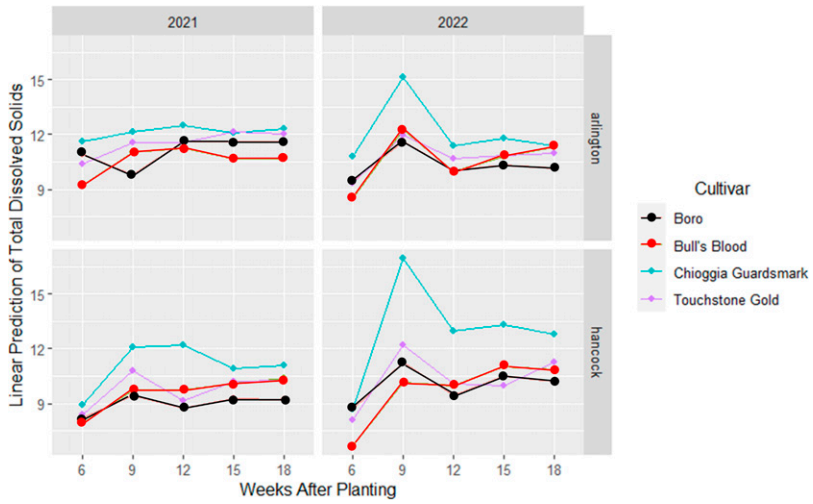

Fig. 4. Linear predictions of total dissolved solids (TDS) in root tissue samples collected from four table beet cultivars grown at two locations over 2 years in Wisconsin and sampled at 6, 9, 12, 15, and 18 weeks after planting.

One source of error in the temporal study included bulk sampling, which was necessary early during the season to provide enough tissue for the appropriate number of technical replicates (three for geosmin and oxalic acid; six for TDS). It is possible that the matrix interference of the beet root tissue changed over the course of the growing season after the shift in the ratio of vascular tissue to storage parenchyma as the bands of parenchyma swelled with the growing root. Adjustments to account for this could be made by calculating the relative recovery of geosmin and oxalic acid using spiked samples from each stage of tissue maturity and using this value to adjust the measured concentrations. Of the 63 roots sampled as part of the size study that examined the relationship between root size, geosmin concentration, and TDS concentration, inherent bias exists because enough tissue to complete the analysis for geosmin, oxalic acid, and TDS could not be collected from a single root smaller than 25 g; however, the majority of beets in the field at 15 weeks after planting were larger than 25 g.

In 2021, herbicide drift from a neighboring carrot field damaged the east side of the Hancock experiment. Damage primarily occurred to the border rows, but the death of a percentage of the beet plants led to the surviving plants growing larger roots in several experimental rows because of the reduced competition for resources. Results of the size study indicated that root mass may have a moderate negative correlation with TDS, as has been found by previous research (Gaertner and Goldman 2005); therefore, this damage introduced another source of variability not accounted for by our experimental design.

One potential application of the findings of this research could be a more informed approach to breeding for flavor and eating quality of table beet. Plant breeders may be able to make selections based on the geosmin concentration, oxalic acid concentration, and TDS as early as 12 weeks after planting, which could enable them to evaluate larger populations and make selections before harvest.

Prudent areas of future inquiry may include experiments examining the effects of biotic and abiotic stresses such as drought, heat, early frost, foliar disease, and herbivory on the concentration of oxalic acid. The heritability of oxalic acid concentration could be estimated and bidirectional recurrent selection could be performed to test the efficacy of breeding for reduced oxalate contents in table beet. Trials evaluating various fertilizer types and regimes may also prove useful for making informed recommendations to farmers seeking a culture-based approach to reduce oxalic acid levels in table beets. The diurnal nature of TDS may also be explored by comparing TDS measurements with the results of sucrose assays, performing diurnal sampling for longer periods, and observing diurnal patterns of plants under simulated long-day and short-day conditions.

This study is the first to examine the dynamic nature of geosmin, oxalic acid, and TDS production and their accumulation in table beet with regard to time. The results of this analysis showed that the geosmin concentration was primarily influenced by cultivar,

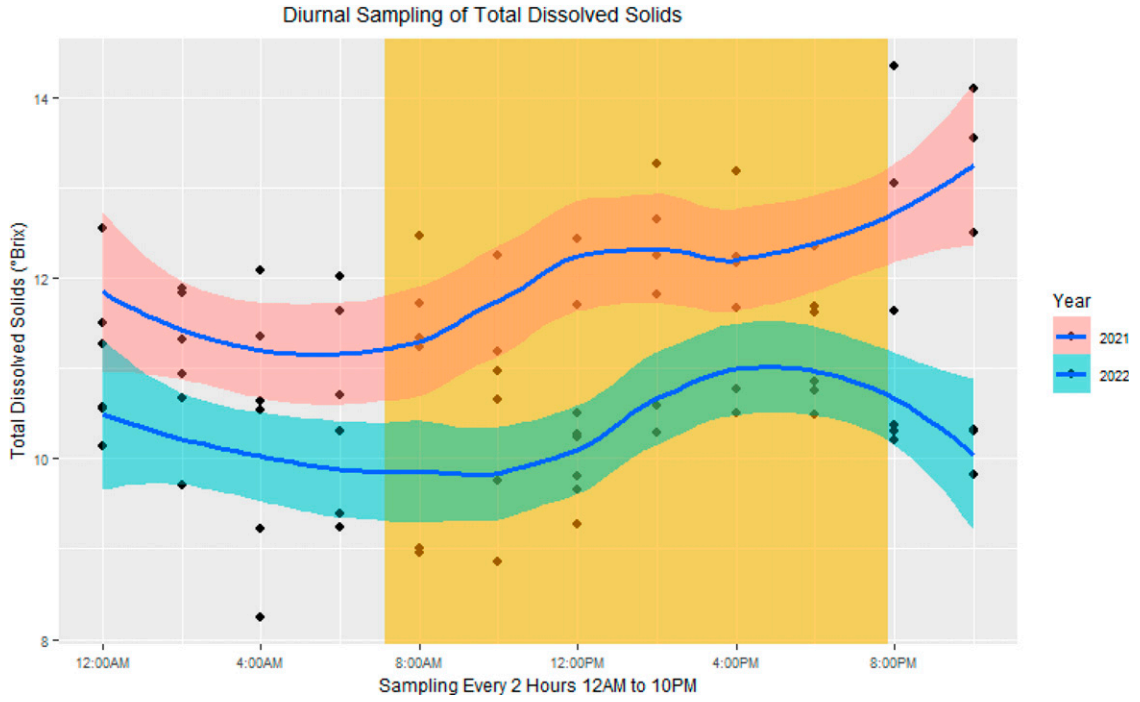

Fig. 5. Average total dissolved solids (TDS) concentration found in beet root tissue samples from the cultivar Bull's Blood that were randomly selected from three blocks at 2-h intervals. The experiment was performed on 28 Aug 2021 and 6 Sep 2022.

and that it changes significantly over the course of the growing season but generally stabilizes to concentrations found in harvest stage roots by 12 weeks after planting. The oxalic acid concentration was strongly influenced by cultivar and was higher during the midseason, although further sampling is necessary to fully characterize the nature of the interactions between location and years and their effects on the beet root oxalic acid concentration. The ranking of TDS concentrations was also cultivar-specific; however, the magnitude of the values was subject to the influence of complex year × week interactions. Over the course of 24 h, TDS concentrations in the cultivar Bull's Blood fluctuated by as much as 32.7%; however, geosmin concentrations did not change in a significant way with regard to time. This represents a previously unidentified source of variation that impacts the efficacy of TDS measurements as a proxy for the sucrose concentration in table beet. Breeders can use this information to decide when they can accurately select for certain traits and how they can design experiments and breeding nurseries to improve the ability to capture the portion of phenotypic variation controlled by heritable genetic factors.

References Cited

Bach V, Mikkelsen L, Kidmose U, Edelenbos M. 2015. Culinary preparation of beetroot (*Beta vulgaris* L.): The impact on sensory quality and appropriateness. J Sci Food Agr. 95(9):1852–1859. https://doi.org/10.1002/jsfa.6886.

Bates D, Mächler M, Bolker BM, Walker SC. 2015. Fitting linear mixed-effects models using lme4. J Stat Softw. 67. https://doi.org/10.18637/jss.v067.i01.

Beaulieu JC, Stein-Chisholm RE, Boykin DL. 2014. Qualitative analysis of volatiles in rabbiteye blueberry cultivars at various maturities using rapid solid-phase microextraction. J Am Soc Hortic Sci. 139(2):167–177. https://doi.org/10.21273/JASHS.139.2.167.

Bennett AR, Hindal DF. 1989. Mycelial growth and oxalate production by five strains of *Cryphonectria parasitica* in selected liquid culture media. Mycologia. 81(4):554–560.

Buttery RG, Garibaldi JA. 1976. Geosmin and methylisoborneol in garden soil aroma production by cultures of *Ceratocystis rnoniliformis*. J Agr Food Chem. 24(6):1246–1247.

Cessna SG, Sears VE, Dickman MB, Low PS. 2000. Oxalic acid, a pathogenicity factor for *Sclerotinia sclerotiorum*, suppresses the oxidative burst of the host plant. Plant Cell. 12:2191–2199.

Chakraborty N, Ghosh R, Ghosh S, Narula K, Tayal R, Datta A, Chakraborty S. 2013. Reduction of oxalate levels in tomato fruit and consequent metabolic remodeling following overexpression of a fungal oxalate decarboxylase. Plant Physiol. 162(1):364–378. https://doi.org/10.1104/pp.112.209197.

Dohm JC, Minoche AE, Holtgräwe D, Capella-Gutiérrez S, Zakrzewski F, Tafer H, Rupp O, Sörensen TR, Stracke R, Reinhardt R, Goesmann A, Kraft T, Schulz B, Stadler PF, Schmidt T, Gabaldón T, Lehrach H, Weisshaar B, Himmelbauer H. 2014. The genome of the recently domesticated crop plant sugar beet (*Beta vulgaris*). Nature. 505(7484). https://doi.org/10.1038/nature12817.

Dutton Mv, Evans CS, Evans CS. 1996. Oxalate production by fungi: Its role in pathogenicity and ecology in the soil environment. Can J Microbiol. 42:881–895.

Farneti B, Khomenko J, Grisenti M, Ajelli M, Betta E, Algarra AA, Cappellin L, Aprea E, Gasperi F, Biasioli F, Giongo L. 2017. Exploring blueberry aroma complexity by chromatographic and direct-injection spectrometric techniques. Front. Plant Sci. 8. https://doi.org/10.3389/fpls.2017.00617.

Fasahat P, Aghaeezadeh M, Jabbari L, Sadeghzadeh Hemayati S, Townson P. 2018. Sucrose accumulation in sugar beet: From fodder beet selection to genomic selection. Sugar Tech. 20(6):635–644. https://doi.org/10.1007/s12355-018-0617-z.

Fasset D. 1973. Oxalates, p 346–362. Toxicants occurring naturally in foods (2nd ed). National Academy of Sciences, Washington DC, USA.

Feller C, Fink M. 2004. Nitrate content, soluble solids content, and yield of table beet as affected by cultivar, sowing date and nitrogen supply. HortScience. 39(6):1255–1259. https://doi.org/10.21273/hortsci.39.6.1255.

Ferrão LFV, Johnson TS, Benevenuto J, Edger PP, Colquhoun TA, Munoz PR. 2020. Genome-wide association of volatiles reveals candidate loci for blueberry flavor. New Phytol. 226(6):1725–1737. https://doi.org/10.1111/nph.16459.

Fischer HE. 1989. Origin of the 'Weisse Schlesische Rübe' (white Silesian beet) and resynthesis of sugar beet. Euphytica. 41:75–80. https://doi.org/10.1007/BF00022414.

Folta KM, Klee HJ. 2016. Sensory sacrifices when we mass-produce mass produce. Hortic Res. 3. https://doi.org/10.1038/hortres.2016.32.

Francis A, Luper Tsenum J, Gbamwuam DE. 2020. Evaluation of soybean varieties for oxalate, phytate and polyunsaturated fatty acids content [MS Diss.], Makurdi, Nigeria.

Franco V, Krinitz B. 1973. Determination of oxalic acid in foods. J AOAC Int. 56(1):164–166. https://academic.oup.com/jaoac/article/56/1/164/5711724.

Freidig AK, Goldman IL. 2011. Variation in oxalic acid content among commercial table beet cultivars and related crops. J Am Soc Hortic Sci. 136(1):54–60. https://doi.org/10.21273/JASHS.136.1.54.

Freidig AK, Goldman IL. 2014. Geosmin (2β,6α-dimethylbicyclo[4.4.0]decan-1β-ol) production associated with *Beta vulgaris* ssp. vulgaris is cultivar specific. J Agr Food Chem. 62(9):2031–2036. https://doi.org/10.1021/jf4047336.

Gaertner VL, Goldman IL. 2005. Pigment distribution and total dissolved solids of selected cycles of table beet from a recurrent selection program for increased pigment. J Am Soc Hortic Sci. 130(3):424–433. https://doi.org/10.21273/JASHS.130.3.424.

Goldman IL, Navazio JP. 2002. History and breeding of table beet in the United States. Plant Breed Rev. 22:357–388.

Goldman IL, Eagen KA, Breitbach DN, Gabelman WH. 1996. Simultaneous selection is effective in increasing betalain pigment concentration but not total dissolved solids in red beet. J Am Soc Hortic Sci. 121(1):23–26. https://doi.org/10.21273/jashs.121.1.23.

Hanson SJ. 2020. Geosmin concentration in table beet: Genetic characterization and participatory cultivar development. PhD Dissertation, University of Wisconsin-Madison, Madison, WI, USA.

Hanson SJ, Dawson JC, Goldman IL. 2022. Participatory plant breeding reveals that geosmin concentration is not the central determinant of hedonic liking in table beet. Euphytica. 218(2). https://doi.org/10.1007/s10681-021-02959-w.

Hanson SJ, Goldman IL. 2019. Genotype is primarily responsible for variance in table beet geosmin concentration, but complex genotype environment interactions influence variance in total dissolved solids. J Am Soc Hortic Sci. 144(6):429–438. https://doi.org/10.21273/JASHS04758-19.

Jayaraj J, Bhuvaneswari R, Rabindran R, Muthukrishnan S, Velazhahan R. 2010. Oxalic acid-induced resistance to Rhizoctonia solani in rice is associated with induction of phenolics, peroxidase and pathogenesis-related proteins. J Plant Interact. 5(2):147–157. https://doi.org/10.1080/17429140903291012.

Jiang J, He X, Cane DE. 2006. Geosmin biosynthesis. *Streptomyces coelicolor* germacradienol/germacrene D synthase converts farnesyl diphosphate to geosmin. J Am Chem Soc. 128(25):8128–8129. https://doi.org/10.1021/ja062669x.

Karamad D, Khosravi-Darani K, Hosseini H, Tavasoli S. 2019. Analytical procedures and methods validation for oxalate content estimation. Biointerface Res Appl Chem. 9(5):4305–4310. https://doi.org/10.33263/BRIAC95.305310.

Kenter C, Hoffmann CM. 2006. Seasonal patterns of sucrose concentration in relation to other quality parameters of sugar beet (*Beta vulgaris* L.). J Sci Food Agr. 86(1):62–70. https://doi.org/10.1002/jsfa.2332.

Khosa JS, McCallum J, Dhatt AS, Macknight RC. 2016. Enhancing onion breeding using molecular tools. Plant Breed. 135:9–20. https://doi.org/10.1111/pbr.12330.

Kirkland BH, Eisa A, Keyhani NO. 2005. Oxalic acid as a fungal acaracidal virulence factor. J Med Entomol. 42:346–351. https://academic.oup.com/jme/article/42/3/346/849106.

Klee HJ. 2010. Improving the flavor of fresh fruits: Genomics, biochemistry, and biotechnology. New Phytol. 187(1):44–56. https://doi.org/10.1111/j.1469-8137.2010.03281.x.

Liato V, Aïder M. 2017. Geosmin as a source of the earthy-musty smell in fruits, vegetables and water: Origins, impact on foods and water, and review of the removing techniques. Chemosphere. 181:9–18. https://doi.org/10.1016/j.chemosphere.2017.04.039.

Libert B. 1987. Breeding a low-oxalate rhubarb (*Rheum* sp. L.). J Hortic Sci. 62(4):523–529. https://doi.org/10.1080/14620316.1987.11515816.

Liu X, Lu L, Chen Q, Ding W, Dai P, Hu Y, Yu Y, Jin C, Lin X. 2015. Ammonium reduces oxalate accumulation in different spinach (*Spinacia oleracea* L.) genotypes by inhibiting root uptake of nitrate. Food Chem. 186:312–318. https://doi.org/10.1016/j.foodchem.2014.06.122.

Lu G, Fellman JK, Edwards CG, Mattinson DS, Navazio J. 2003. Quantitative determination of geosmin in red beets (*Beta vulgaris* L.) using headspace solid-phase microextraction. J Agr Food Chem. 51(4):1021–1025. https://doi.org/10.1021/jf020806d.

Lvova L, Jahatspanian I, Mattoso LHC, Correa DS, Oleneva E, Legin A, Di Natale C, Paolesse R. 2020. Potentiometric E-tongue system for geosmin/isoborneol presence monitoring in drinkable water. Sensors (Switzerland). 20(3). https://doi.org/10.3390/s20030821.

Magro P, Marciano P, di Lenna P. 1984. Oxalic acid production and its role in pathogenesis of *Sclerotinia sclerotiorum*. FEMS Microbiol Lett. 24(1):9–12. https://doi.org/10.1111/j.1574-6968.1984.tb01234.x.

Maher L, Goldman IL. 2017. Bidirectional recurrent half-sib family selection for geosmin concentration

in table beet. Crop Sci. 57(5):2564–2574. https://doi.org/10.2135/cropsci2017.02.0111.

Maher L, Goldman IL. 2018. Endogenous production of geosmin in table beet. HortScience. 53(1):67–72. https://doi.org/10.21273/HORTSCI12488-17.

Matros A, Liu G, Hartmann A, Jiang Y, Zhao Y, Wang H, Ebmeyer E, Korzun V, Schachschneider R, Kazman E, Schacht J, Longin F, Reif JC, Mock HP. 2017. Genome-metabolite associations revealed low heritability, high genetic complexity, and causal relations for leaf metabolites in winter wheat (*Triticum aestivum*). J Expt Bot. 68(3):415–428. https://doi.org/10.1093/jxb/erw441.

Murray KE, Bannister PA, Buttery RG. 1975. Geosmin: An important colatile constituent of beetroot. Chem Ind. 15:973–974.

Nakata PA. 2015. An assessment of engineered calcium oxalate crystal formation on plant growth and development as a step toward evaluating its use to enhance plant defense. PLoS One. 10. https://doi.org/10.1371/journal.pone.0141982.

Nakata PA, He C. 2010. Oxalic acid biosynthesis is encoded by an operon in *Burkholderia glumae*. FEMS Microbiol Lett. 304(2):177–182. https://doi.org/10.1111/j.1574-6968.2010.01895.x.

Noonan S, Savage G. 1999. Oxalate content of foods and its effect on humans. Asia Pac J Clin Nutr. 8(1):64–74.

Okutani I, Sugiyama N. 1994. Relationship between oxalate concentration and leaf position in various spinach cultivars. HortScience. 29(9):1019–1021. https://doi.org/10.21273/HORTSCI.29.9.1019.

Park SJ, Seo SE, Kim KH, Lee SH, Kim J, Ha S, Song HS, Lee SH, Kwon OS. 2021. Real-time monitoring of geosmin based on an aptamer-conjugated graphene field-effect transistor. Biosens Bioelectron. 174. https://doi.org/10.1016/j.bios.2020.112804.

Prasad BVG, Chakravorty S, Rao PG, Deb P. 2018. Effect of post-harvest treatments and packaging on spinach beet (*Beta vulgaris* var *bengalensis* Hort.) under ambient conditions. Int J Curr Microbiol Appl Sci. 7(11):711–720. https://doi.org/10.20546/ijcmas.2018.711.085.

Prasad R, Shivay YS. 2017. Oxalic acid/oxalates in plants: From self-defense to phytoremediation. Curr Sci. 112(8):1665–1667.

Rahman MM, Ishii Y, Niimi M, Kawamura O. 2012. Effect of application of nitrogen on oxalate accumulation and mineral uptake by napiergrass (*Pennisetum purpureum*). Grassl Sci. 56:141–144.

Rolling WR, Senalik D, Iorizzo M, Ellison S, Van Deynze A, Simon PW. 2022. CarrotOmics: A genetics and comparative genomics database for carrot (Daucus carota). Database (Oxford).baac079. https://doi.org/10.1093/database/baac079.

Schlumpberger BO, Jux A, Kunert M, Boland W, Wittmann D. 2004. Musty-earthy scent in cactus flowers: Characteristics of floral scent production in dehydrogeosmin-producing cacti. Int J Plant Sci. 165(6):1007–1015. https://doi.org/10.1086/423878.

Shi A, Mou B, Correll JC. 2016. Association analysis for oxalate concentration in spinach. Euphytica. 212(1):17–28. https://doi.org/10.1007/s10681-016-1740-0.

Tian H, Jiang L, Liu E, Zhang J, Liu F, Peng X. 2008. Dependence of nitrate-induced oxalate accumulation on nitrate reduction in rice leaves. Physiol Plant. 133(2):180–189. https://doi.org/10.1111/j.1399-3054.2008.01052.x.

Tobi G, Bahloul YE, Oumouss S, Rahmouni I, Birouk A, Benlhabib O. 2021. Productivity, heritability and stability analysis of a moroccan sugar beet germplasm. Agron Res (Tartu). 19(2):612–628. https://doi.org/10.15159/AR.21.022.

Wanasundera JPD, Ravindran G. 1992. Effects of cooking on the nutrient and antinutrient contents of yam tubers (*Dioscorea alata* and *Dioscorea esculenta*). Food Chem. 45(4):247–250. https://doi.org/10.1016/0308-8146(92)90155-U.

Wang J, Liu L, Dong J, Zhu L, Wang Y, Hu G, Shi S. 2022. Inheritance of fruit weight, size and organic acid content in a distant hybrid population of longan 'Huanongzao' and Lychee 'Ziniangxi'. Horticulturae. 8(11):999. https://doi.org/10.3390/horticulturae8110999.

Wilkinson L. 2011. ggplot2: Elegant graphics for data analysis. Biometrics. 67(2):678–679. https://doi.org/10.1111/j.1541-0420.2011.01616.x.

Wolyn DJ, Gabelman WH. 1990. Selection for betalain pigment concentrations and total dissolved solids in red table beets. J Am Soc Hortic Sci. 115(1):165–169. https://doi.org/10.21273/jashs.115.1.165.

Xu XQ, Zhang ZQ. 2000. Kinetic spectrophotometric determination of oxalic acid based on the catalytic oxidation of bromophenol blue by dichromate. Mikrochim Acta. 135:169–172.

Zhang T, Xue Y, Li Z, Wang Y, Yang W, Xue C. 2016. Effects of ozone on the removal of geosmin and the physicochemical properties of fish meat from bighead carp (*Hypophthalmichthys nobilis*). Innov Food Sci Emerg Technol. 34:16–23. https://doi.org/10.1016/j.ifset.2016.01.001.