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Sequentially Reducing Sulfate Fertility During Onion Growth and Development Affects Bulb Flavor at Harvest

William M. Randle, David E. Kopsell, and Dean A. Kopsell
Department of Horticulture, 1111 Plant Sciences Building, University of Georgia, Athens, GA 30602-7273

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Abstract. **A major decision in producing onions with mild flavor on low sulfur soils is determining when to stop applying SO_4^{-2} to the crop. Sulfate (SO_4^{-2}) is necessary for good early growth, but high levels of available SO_4^{-2} late in the season increase bulb pungency. The objective of this research was to determine how sequentially reducing the availability of SO_4^{-2} during onion growth and development would affect flavor intensity and quality of Granex-type onions. Starting 77 days before harvest, SO_4^{-2} concentrations were lowered from 1 mM to 0.05 mM on different blocks of onions in a greenhouse experiment at bi-weekly intervals. Total leaf and bulb S were measured at harvest to monitor S accumulation as SO_4^{-2} fertility was sequentially reduced. Bulbs were harvested and analyzed for flavor precursors and their biosynthetic intermediates, gross flavor intensity as measured by enzymatically developed pyruvic acid (EPY), and soluble solids content. As SO_4^{-2} fertility reductions were delayed during the experiment, total leaf and bulb S increased linearly. In addition, bulb EPY concentrations increased linearly as SO_4^{-2} reduction was delayed, indicating increases in overall flavor intensity. While the total concentration of flavor precursors did not significantly change in response to lowering SO_4^{-2} fertility during the experiment, the concentrations of MCSO to 1-PRENCSO did. MCSO concentration decreased and then increased in a quadratic manner. MCSO produces fresh onion and cabbage like flavors. 1-PRENCSO, on the other hand, increased linearly as the high SO_4^{-2} fertility level was extended through bulb maturation. Increasing concentrations of 1-PRENCSO causes onions to have significantly more heat and mouth burn when eaten. Reducing available SO_4^{-2} 49 days prior to harvest coincided with a reduction in EPY and a change in the flavor biosynthetic pathway that appeared to be associated with the metabolic changes occurring with the onset of bulbing. Chemical names used: enzymatically developed pyruvic acid (EPY); methyl cysteine sulfoxide (MCSO); 1-propenyl cysteine sulfoxide (1-PRENCSO).**

Onion (*Allium cepa* L.) flavor is dominated by a special class of sulfur (S) precursor compounds, collectively known as *S*-alk(en)yl cysteine sulfoxides (ACSO) (Block, 1992). Upon maceration of the tissues, alliinase decomposes the ACSOs to form the lachrymatory factor (LF) and thiosulfinates that are responsible for the flavor attributes of cut onions (Randle, 1997). The thiosulfinates are unstable and randomly rearrange and dissociate over time forming other compounds, thereby affecting a time sensitive change in cut onion flavor. The three precursors that give onions their characteristic flavors and aromas are 1-PRENCSO, MCSO, and propyl cysteine sulfoxide (PCSO) (Block, 1992).

Up until the 1990s, onion flavor was reported to be dominated by the accumulation of 1-PRENCSO, which upon decomposition, gives rise to the LF and heat and mouth burn attributes (Block, 1992). MCSO and PCSO were reported to accumulate in lesser concentrations and upon decomposition, gave rise to fresh onion, sulfureous, and cabbage-like attributes. In the 1990s, research identified several mineral elements that affected the composition and concentration of the individual ACSOs. First, SO_4^{-2} fertility levels dramatically affected the concentration and composition of the ACSOs (Randle et al., 1995). With high SO_4^{-2} fertility, 1-PRENCSO accumulated in highest concentration. However, as SO_4^{-2} fertility incrementally decreased to a level that produced S deficiency symptoms in the plants, MCSO increased in concentration relative to 1-PRENCSO, and became the dominant precursor at the lower SO_4^{-2} fertility levels. Second, when onions were grown under high sodium selenate (Na_2SeO_4) fertility levels, MCSO became the dominant precursor (Kopsell and Randle, 1999). This response mimicked the low SO_4^{-2} fertility response and hinted to the competitive nature of S and Se in plant metabolism. And third, when onions were grown with luxuriant levels of nitrogen, MCSO accumulated in highest concentration of the three ACSOs (Randle, 2000). These experiments demonstrated that fertility impacted onion flavor intensity and quality. It is the accumulation pattern of the three individual ACSOs that give rise to differences in onion flavor intensity and quality (Randle, 1997).

The goal for quality onion production is to gradually deplete nutrients, especially N, from soils during advanced bulbing (Brewster, 1990). In doing so, bulbs mature properly and are able to better withstand postharvest handling and storage. For over-wintered mild onions produced on sandy loam soils, applications of S-containing fertilizers are discouraged after early spring (Vavrina and Granberry, 1988). High amounts of S applied before planting caused S to be available at high levels late in the season, and onions intended to be mild were pungent (Smittle, 1984). Mild onions can be produced by depleting S from the soils before high levels of ACSOs are synthesized in the leaves and translocated to the swelling bulbs (Lancaster and Boland, 1990; Randle et al., 1993). Liberal applications of S in the early stages of onion growth and development, however, are required to support good root and foliar growth that positively influence bulb yields. Restricting SO_4^{-2} availability to the plant early in growth and development reduced bulb fresh weight (Randle et al., 1995). Scheduling the reduction of S from the growing environment is a key management decision by mild onion producers. The object of this research was to determine how sequentially reducing the availability of SO_4^{-2} during onion growth and development would affect flavor intensity and quality in Granex-type onions.

Materials and Methods

Seeds of 'Sweet Vidalia' (Granex-type, Rio Colorado Seed, Yuma, Ariz.) were planted 3 Oct. 1997 in flats containing Fafard No. 3 (Fafard Co., Anderson, S.C.) artificial medium and greenhouse grown under 28 °C day/16 °C night temperatures until the plants had five true leaves. During this time, plants were fertilized weekly with 400 mL of Peters 20N–20P–20K soluble fertilizer (Scotts Sierra, Maryville, Ohio) at a rate of 200 mg/L (5% S). On 15 Dec. 1997, seedlings were transplanted into 30.5 × 25.4 × 8.9-cm flats containing 50% Fafard 6 M media and 50% washed river sand. Nine seedlings were planted per flat at 7.6 cm spacing on center. Plants in each flat were fertilized weekly with 1.8 L of a half-strength modified Hoagland's solution (Hoagland and Arnon, 1950) containing 1 mM SO_4^{-2} until SO_4^{-2} reduction treatments began. Plants were supplemented with deionized water as needed.

The experimental design was a randomized complete block having four blocks and six SO_4^{-2} reduction treatments per block. There were six flats per block. Starting 26 Jan. 1998, the SO_4^{-2} concentration in the nutrient solution applied to one flat in each block was reduced to 0.05 mM and maintained at that level until the experiment was terminated. Every two weeks thereafter, another flat in each block received the reduced SO_4^{-2} fertility. A 0.05 mM SO_4^{-2} concentration was that of the carrier ions of some micronutrients in the Hoagland's solution. The experiment was terminated 13 April 1998 when 50% of the onions' foliage in the experiment had lodged, indicating matura-

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tion. Six sequential SO_4^{-2} reduction treatments resulted at 77, 63, 49, 35, 21, and 7 d before harvest, respectively. Developmental stages associated with the sequential reduction dates were nonbulbing plants at 77 d and 63 d, early bulbing at 49 d, active bulbing at 35 d and 21 d, and bulb maturation at 7 d before harvest. Plants were considered to be bulbing when the leaf bases were two times the diameter of the sheath area.

At harvest, a 1-cm cross section of leaves were taken 10 cm above the bulb for each treatment combination for total S analysis. The roots and foliage were then removed from the bulbs. The bulbs were dried at ambient greenhouse temperatures for 7 d. The eight most uniform bulbs in each flat were selected for flavor analyses. All analyses were done on the combined tissues of each eight-bulb treatment/block combination. Five- to 10-mm-thick wedges were cut longitudinally from the bulbs. One wedge group was used to determine enzymatically developed pyruvic acid (EPY) and soluble solids content (SSC). EPY and SSC measure gross flavor intensity and sugars, respectively. A second wedge group was used to measure the ACSOs and precursor intermediates, and a third wedge group was used for mineral analysis.

Soluble solids content and gross flavor intensity. Wedges from each eight-bulb group were juiced in a pneumatic press. Several drops of the juice were applied to a hand-held refractometer (Kernco, Tokyo) to measure soluble solids content (SSC). Soluble solids content correlated well with water-soluble carbohydrate content in onions (Mann and Hoyle, 1945). Gross flavor intensity for each treatment combination was determined using the pyruvic acid method of Randle and Bussard (1993). EPY (total pyruvic acid–background pyruvic acid) in the juice was diluted 40-fold, reacted with 2,4-dinitrophenyl hydrazine, incubated for 10 min in a 37 °C water bath, developed with 0.6 N sodium hydroxide and measured at 420 nm on a Spectronic 21D spectrophotometer (Milton Roy, Rochester, N.Y.). Pyruvic acid content was calculated against a sodium pyruvate standard curve.

Flavor precursors and precursor intermediates. The ACSOs and their intermediates were extracted twice using a 12 methanol : 3 water solution, and once with an 80% ethanol solution that was modified for bulb tissue from Lancaster and Kelly (1983). *S*-Methyl glutathione (MeGTH; 0.5 $mg \cdot g^{-1}$ fresh weight), g-L-glutamyl-L glutamic acid (ggG; 0.2 $mg \cdot g^{-1}$ fresh weight), and (±)-*S*-1-butyl-L-cysteine sulfoxide (BCSO; 1.0 $mg \cdot g^{-1}$ fresh weight) were used as internal standards and added to 15 mL of the combined solutions (1 g fresh wt. equivalent). The solutions were dried using ambient air and re-dissolved in one mL of deionized/distilled water. A 0.5-mL aliquot was then subjected to ion exchange chromatography using a 10 × 40-mm column (Bio-Rad, Hercules, Calif.) with 3 mL Dowex 1 × 8 resin (200 to 400 mesh; Bio-Rad). Fractionation of the sample was carried out using acetic acid at 0.1, 0.2, 2, and 5 M concentrations. The fractions containing the ACSO and the intermediate compounds (0.1 and 2 M, respectively) were each collected and dried using ambient air. HPLC sample preparation and analysis were done according to Randle et al. (1995). Samples were derivatized using ethanol, triethylamine (TEA), and phenylisothiocyanate, dried and redissolved in 1 mL of acetonitrile and water before HPLC analysis.

A Waters (Milford, Mass.) 2690 HPLC separator module with a 996 photodiode array detector was used for analysis. A Sphri-5 RP-18 5 micron 250 × 4.6-mm column (Applied Biosystems, Foster City, Calif.) fitted with a 15 × 3.2-mm 7 micron guard column (RP-18 Newgard; Applied Biosystems) was used for separation. The column temperature was maintained at 30 °C. Eluted compounds were detected at 254 nm. Peak assignment was carried out by comparing retention times with authentic standards (supplied by J.E. Lancaster, Crop and Food Research, Christchurch, New Zealand).

Solvents were A) aqueous acetonitrile (60%) and B) 0.14 M sodium acetate with 0.05% TEA buffered to pH 6.35 using glacial acetic acid. Forty mL sample volumes were injected onto the column. A flow rate of 1.0 mL/min was used. The solvent gradient used was 15% A for 1.10 min, 15% to 45% A over 21.1 min, 45% to 100% A over 1 min, and a hold at 100% A for 14 min.

Sulfur and SO_4^{-2} analysis. Bulb and leaf tissues were dried at 60 °C in a forced-air oven (model 630; National Appliance Co., Portland, Ore.) for no less than 72 h. The dried tissue was ground through a 0.5-mm screen (Cyclotec, model 1093; Tector, Hoganas, Sweden). Total bulb and leaf S were determined using a Leco S determinator (model SC 232; St. Joseph, Mich.).

Sulfate concentrations were quantified using anion chromatography. One-half gram of ground bulb tissue was extracted in 100 mL HPLC grade water for 30 min with agitation. Ten mL of the solution were then centrifuged at 7000 rpm for 10 min (Sorvall, Norwalk, Conn.). The supernatant was filtered through a Millex-GV 0.22-μM filter unit (Millipore, Molsheim, France) and 40 μL were injected onto an IC-Pak Anion HR column equipped with a IC-Pak Anion guard pak (Waters, Milford, Mass.). Column temperature was maintained at 30 °C. A Waters (Milford, Mass.) 2690 HPLC separator module with a 432 conductivity detector was used for analysis. A flow rate of 1.0 $mL \cdot min^{-1}$ was used with an isocratic sodium borate/gluconate solvent. The solvent contained 16 g sodium gluconate, 18 g boric acid, 25 g sodium tertaborate and 250 mL glycerin in 1 L of water. Data were collected and recorded using a personal computer with Millenium chromatography manager software as described earlier. Quantification was performed using 10 $mg \cdot L^{-1}$ concentrations of the sodium sulfate salt (J.T. Baker, Phillipsburg, N.J.) as an external standard.

Statistical analysis. Data were subjected to analysis of variance and linear and polynomial regression procedures using StatMost software (DataMost, Salt Lake City, Utah). An arcsine transformation of percentage data was done prior to analysis.

Results and Discussion

Sulfur and SO_4^{-2} accumulation. Leaf and bulb S responded significantly to the sequential reduction of SO_4^{-2} fertility during plant growth and development ($P = 0.01$; F = 6.1 and 5.9, respectively). Leaf S increased linearly as SO_4^{-2} reduction was delayed during growth and development (Leaf S = 0.523 + 0.002 days, $R^2 = 0.93$, Fig. 1). Similarly, bulb S increased linearly as SO_4^{-2} reduction was delayed (Bulb S = 0.457 + 0.002 days, $R^2 = 0.90$, Fig. 1). Previously, bulb S was shown to change in response to increasing SO_4^{-2} fertility levels (Randle et al., 1999). As SO_4^{-2} fertility increased from 5 to 150 mg SO_4^{-2} per L of nutrient solution, total bulb S linearly increased in three onion cultivars. Therefore, the response of bulb S accumulation to prolonging the high SO_4^{-2} fertility regime as plants grew and developed in this study was similar to sequentially increasing the SO_4^{-2} fertility concentrations given throughout growth and development in the previous study.

Bulb SO_4^{-2} accumulation responded significantly to the sequential reduction of SO_4^{-2} fertility ($P = 0.007$, F = 5.03). Bulb SO_4^{-2} increased linearly as the reduction of SO_4^{-2} fertility was delayed to 7 d before harvest (Table 1). In a previous study, increasing SO_4^{-2} fertility concentrations corresponded to linear increases in bulb SO_4^{-2} for three onion cultivars (Randle et al., 1999). At low SO_4^{-2} fertility, onions were generally more efficient in metabolizing SO_4^{-2} to organic S when compared to the SO_4^{-2} metabolism of plants grown at high SO_4^{-2} fertility. As SO_4^{-2} fertility levels increased, bulb SO_4^{-2} began to accumulate, most likely in the vacuole, as an available S pool (Randle et al., 1999). No trend was found for bulb SO_4^{-2} as a percentage of total bulb S when SO_4^{-2} fertility was delayed (Table 1).

Soluble solids content and gross flavor intensity. Mature bulb SSC responded significantly to the sequential reduction of SO_4^{-2} during plant growth and development ($P = 0.003$; F = 6.03). There was a decrease in SSC between the SO_4^{-2} reduction 77 days before harvest to 21 days before harvest, followed by an increase in SSC for the last SO_4^{-2} reduction date (Fig. 2). The overall response of SSC was quadratic ($SSC = 7.37 - 0.0198\ d + 0.0003\ d^2$, $R^2 = 0.80$). The response of onion SSC to S fertility has been cultivar dependent (Randle, 1992a). When onions of broad genetic background were evaluated at high- and low-S fertility levels, 35 of the entries had higher SSC with the high-S treatment, 25 of the entries had higher SSC with the low-S treatment, and 15 entries had SSC that were unaffected by S fertility. However, mild-type cultivars, like 'Sweet Vidalia' in our study, generally had higher SSC with low-S fertility compared to plants grown at high-S fertility (Randle and Bussard, 1993). Similarly in our study, reducing SO_4^{-2} early in the bulbing process caused the onions to have higher SSC than those subjected to later SO_4^{-2} reductions, except

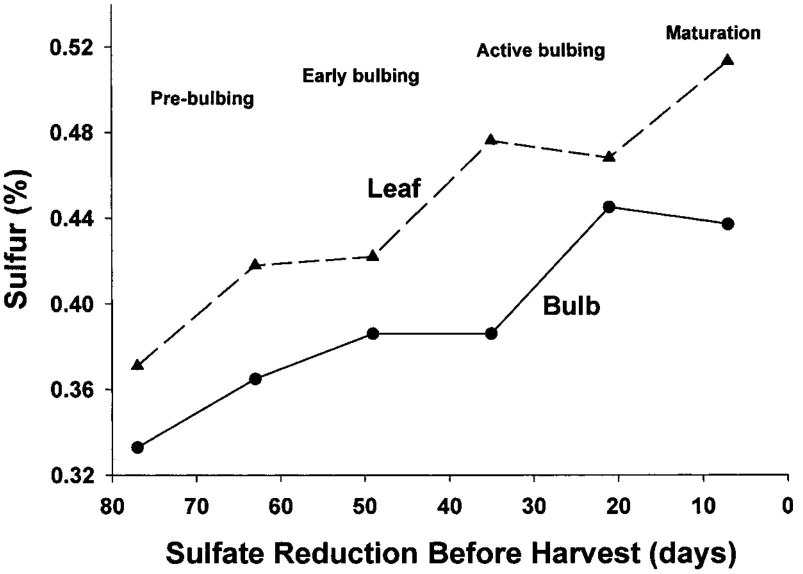

Fig. 1. Changes in total leaf and bulb S from 'Sweet Vidalia' onions when applied SO_4^{-2} concentrations from nutrient solutions were sequentially reduced from 1 mM to 0.5 mM at 14-d intervals beginning 77 d before harvest. Leaf and bulb S increased linearly as SO_4^{-2} reduction was delayed during growth and development (Leaf S = 0.523 – 0.002 d, R^2 = 0.93; Bulb S = 0.457 – 0.002 d, R^2 = 0.90).

Table 1. Bulb SO_4^{-2} (%) and SO_4^{-2} as a percentage of total bulb S in 'Sweet Vidalia' onions when SO_4^{-2} fertility was sequentially reduced at 14-d intervals beginning 77 d before harvest. Bulb SO_4^{-2} = 6.5 – 0.03 d; R^2 = 0.86.

SO_4^{-2} Reduction (d)	Bulb SO_4^{-2} (% dry wt)	S as SO_4^{-2} (%)
77	0.043	12.9
63	0.049	13.4
49	0.046	12.0
35	0.058	15.1
21	0.061	13.7
7	0.061	14.0

for the last date. As SSC increased in mild-type onions, greater amounts of sucrose, glucose and fructose accumulated, and these bulbs, therefore, would be perceived sweeter at low-S fertility (Randle and Bussard, 1993). Bulbs subjected to earlier SO_4^{-2} reductions should, therefore, have higher sugars and be perceived sweeter.

Gross flavor intensity, as measured by EPY, responded significantly to the sequential reduction of SO_4^{-2} during plant growth and development ($P = 0.01$; $F = 4.43$). The greater the EPY, the more intense overall onion flavor becomes (Wall and Corgan, 1992). As SO_4^{-2} reduction was delayed during growth and development, EPY increased linearly (EPY = 5.18 + 0.02 d, R^2 = 0.86, Fig. 2). Within the range of EPY concentrations measured in this experiment, a difference of one μmol is perceivable when bulbs are eaten (Randle, 1997). Available S is a primary environmental factor influencing flavor intensity in onions. Increasing SO_4^{-2} concentrations in nutrient solutions linearly increased EPY in three onion cultivars evaluated over five SO_4^{-2} concentrations (Randle et al., 1995). Although a significant positive linear trend was found for EPY in response to delayed SO_4^{-2} fertility reduction, a decrease in EPY was found when SO_4^{-2} was reduced 49 d before harvest (Fig. 2). Biochemical changes occur early in the bulbing process that prepare the plant for the translocation of metabolites to the leaf bases (Lercari, 1982). It is possible that these metabolic changes affect the synthesis or the composition of the ACSOs more than in previous or subsequent SO_4^{-2} reduction dates. Targeting this developmental stage for SO_4^{-2} fertility reduction may be advantageous in the production of low pungency onions.

Flavor precursors and precursor intermediates. The three ACSOs of onion and the biosynthetic intermediate γ-glutamyl propenyl cysteine sulfoxide (γGP) responded significantly to the sequential reduction of SO_4^{-2} during plant growth and development. 2-Carboxypropyl glutathione was unaffected by changes in SO_4^{-2} fertility during growth and development. 1-Propenyl cysteine sulfoxide (P = 0.001, F = 8.34) increased linearly as the reduction in SO_4^{-2} concentration was delayed during plant growth and development (1-PRENCSO = 0.735 – 0.004 day, R^2 = 0.95, Fig. 3). The lachrymatory factor is a primary product of the enzymatic decomposition of 1-PRENCSO which also produces organoleptic heat and mouth burn when eaten (Randle, 1997). Therefore, the longer high levels of SO_4^{-2} are made available to the plant during bulbing, the harsher the taste will be when the bulbs are eaten. γ-Glutamyl propenyl cysteine sulfoxide (P = 0.002, F = 6.78) increased in a cubic response to a delay in the reduction of SO_4^{-2} during growth and development (γGP = 1.054 – 0.025 d + 0.0005 d^2 – 4.13 day^3, R^2 = 0.96, Fig. 3). γ-Glutamyl propenyl cysteine sulfoxide is the penultimate peptide in the synthesis of 1-PRENCSO. γGP was generally found in higher concentrations than 1-PRENCSO. Recent evidence from our laboratory suggests that when onions are juiced, between 30 and 50% of the γGP is degraded to 1-PRENCSO in the macerate. Evidence suggests that some of the γGP is converted to 1-PRENCSO and is then hydrolyzed to form the lachrymatory factor (unpublished data). Increasing γGP concentrations in response to delayed SO_4^{-2} reduction could contribute to 1-PRENCSO concentrations and, therefore, affect the harshness of these onions. During bulb storage, γGP was also shown to be systematically converted to 1-PRENCSO in Granex-type onions (Kopsell et al., 1999), which lead to increases in overall flavor intensity during storage (Kopsell and Randle, 1997). Delaying SO_4^{-2} reduction should cause Granex-type onions to not only be harsh when eaten at harvest, but to also become even harsher during storage as γGP is converted to 1-PRENCSO.

In a quadratic response to delayed SO_4^{-2} reduction during growth and development, MCSO (P = 0.05, F = 2.89) decreased as plants went through pre-bulbing into active bulbing, and then increased as bulbs matured (MCSO = 1.19 – 0.016 day + 0.0002 day^2, R^2 = 0.88, Fig. 3). Methyl cysteine sulfoxide accumulated in highest concentration of the three ACSOs measured. Thiosulfinates from the enzymatic decomposition of MCSO produce fresh onion and cabbage-like flavors when the bulbs are eaten. Although PCSO was significant in response to the sequential reduction of SO_4^{-2} (P = 0.003, F = 5.93), the levels measured were very low (Fig. 3). The response followed a cubic trend (PCSO = 0.084 – 0.0018 d + 0.0006 d^2 – 0.0001 d^3, R^2 = 0.92). Thiosulfinates from the decomposition of PCSO give rise to raw, fresh onion-like flavors (Randle, 1997).

Because the decomposition products from 1-PRENCSO can dominate the organoleptic experience, 1-PRENCSO must be in low enough concentrations for sugars to be perceived over the heat and mouth burn. As the high SO_4^{-2} solution concentrations were provided closer to harvest, 1-PRENCSO accumulated in higher concentration while MCSO and SSC decreased in concentration. These onions would be harsher and perceived less sweet. Conversely, onions exposed to early SO_4^{-2} reductions should be perceived sweeter because of higher SSC and lower 1-PRENCSO concentrations. Typically, storage-type onions have higher SSC than mild sweet onions. However, storage onions are typically more pungent and the sugars are not perceived when eaten raw (Randle, 1992b, personal experience). As SO_4^{-2} reduction occurred earlier in growth and development, the response of the ACSOs were similar to what was found in a previous study when SO_4^{-2} concentrations were given at consistent levels through out the growing season (Randle, et al., 1995). In that study, luxuriant SO_4^{-2} fertility concentrations resulted in high 1-PRENCSO concentrations, and low PCSO and MCSO concentrations. Conversely, low to deficient SO_4^{-2} fertility levels resulted in lower 1-PRENCSO and higher PCSO and MCSO concentrations.

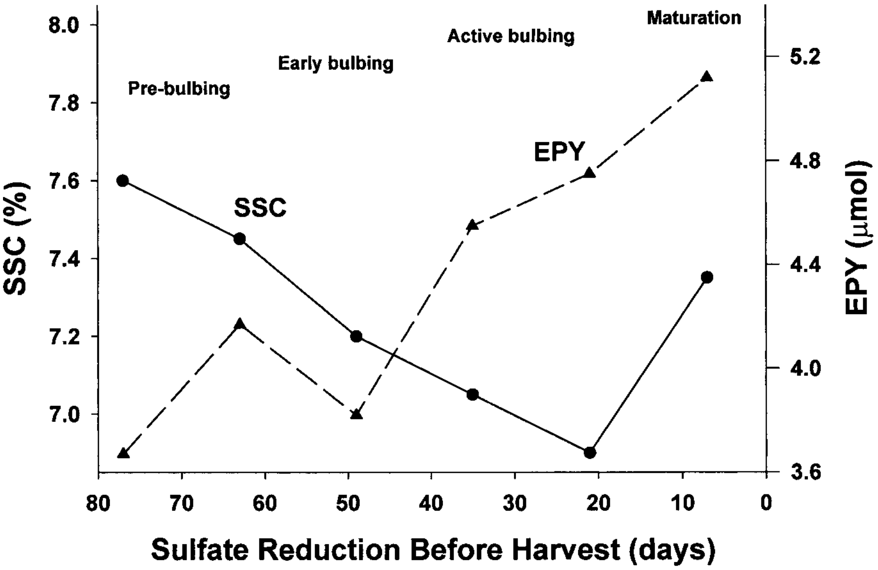

Fig. 2. Changes in soluble solids content (SSC) and enzymatically developed pyruvate (EPY) concentration from mature 'Sweet Vidalia' bulbs when applied SO_4^{-2} concentrations from nutrient solutions were sequentially reduced from 1 mM to 0.5 mM at 14-d intervals beginning 77 d before harvest. Soluble solids content decreased quadratically ($SSC = 7.37 - 0.0198\ d + 0.0003\ d^2, R^2 = 0.80$), and EPY increased linearly ($EPY = 5.18 + 0.02\ d, R^2 = 0.86$) in response to decreasing SO_4^{-2} concentrations.

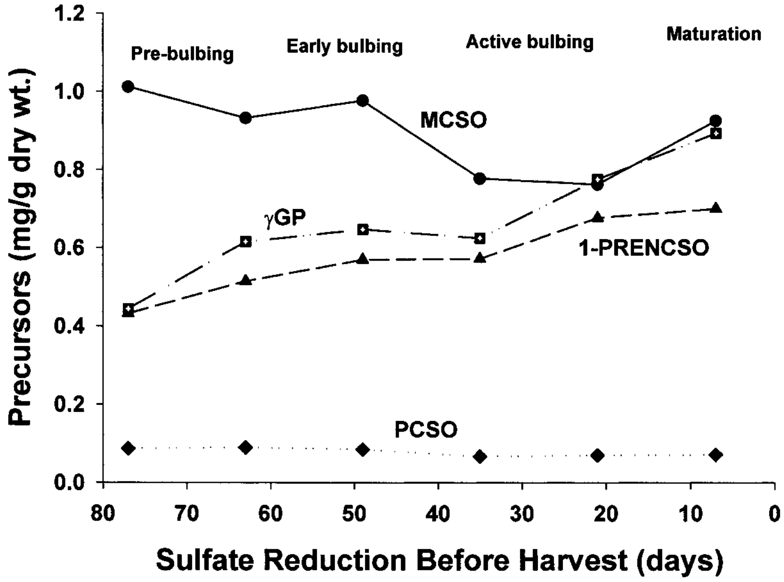

Fig. 3. Changes in methyl cysteine sulfoxide (MCSO), 1-propenyl cysteine sulfoxide (1-PRENCSO), propyl cysteine sulfoxide (PCSO), and γ–glutamyl cysteine sulfoxide (γGP) from mature 'Sweet Vidalia' bulbs when applied SO_4^{-2} concentrations from nutrient solutions were sequentially reduced from 1 mm to 0.5 mM at 14-d intervals beginning 77 d before harvest. The response of MCSO to decreasing SO_4^{-2} concentrations was quadratic ($MCSO = 1.19 - 0.016\ d + 0.0002\ d^2, R^2 = 0.88$), while 1-PRENCSO was linear ($1\text{-}PRENCSO = 0.735 - 0.004\ d, R^2 = 0.95$), PCSO was cubic ($PCSO = 0.084 - 0.0018\ d + 0.0006\ d^2 - 0.0001\ d^3, R^2 = 0.92$) and γGP was cubic ($\gamma GP = 1.054 - 0.025\ d + 0.0005\ d^2 - 4.13\ d^3, R^2 = 0.96$).

The significant linear increases in EPY, γGP, and 1-PRENCSO with delaying SO_4^{-2} reduction suggest that SO_4^{-2} should be reduced as early as possible for mild sweet onion production. However, restricting SO_4^{-2} too early adversely impacts bulb fresh weight (Randle et al., 1995). A potential target date for SO_4^{-2} reduction could be a time ≈7 weeks prior to harvest that coincides with the onset of bulbing. Changes that occurred in the flavor biosynthetic pathway during this time could be associated with the metabolic changes that occur in the early stages of bulbing, causing MCSO synthesis and accumulation to increase relative to 1-PRENCSO. It is possible that reducing SO_4^{-2} during early bulbing caused onions to be more responsive to low SO_4^{-2} concentrations, thereby triggering greater MCSO synthesis than at other developmental stages. Low SO_4^{-2} concentrations increased MCSO synthesis (Randle et al., 1995). Increasing MCSO relative to 1-PRENCSO can also decrease EPY measured (Randle, 2000). Early reductions of SO_4^{-2} would also prevent a decrease in soluble solids that was associated with later SO_4^{-2} reductions. Lower SSC would be associated with decreasing sugar content in the bulbs.

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