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

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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$$_2$ concentrations were lowered from 1.0 mM to 0.05 mM on different blocks of onions in a greenhouse experiment at bi-weekly intervals. Total leaf and bulb S were measured at harvest to monitor S accumulation as SO$_4$$_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 (ACSOs) (Block, 1992). Upon maceration of the tissues, alliinase decomposes the ACSOs to form the lachrymatory factor (LF) and thiosulfates that are responsible for the flavor attributes of cut onions (Randle, 1997). The thiosulfates 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, sulfuric, 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$_2$SeO$_3$) 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 (Smithie, 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, the 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 six treatments 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 per block was reduced the 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-
tion. Six sequential SO₄²⁻ 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 before harvest. Plants were considered to be bulbing when the leaf bases were two times the diameter of the sheath area.

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influencing flavor intensity in onions. Increases in low pungency onions could contribute to 1-PRENCSO concentrations, which lead to increases in overall onion flavor. The three ACSOs of onion and the biosynthetic intermediate γ-glutamyl propenyl cysteine sulfoxide (γGP) responded significantly to the sequential reduction of SO₄²⁻ during plant growth and development. A Carboxypropyl glutathione was unaffected by changes in SO₄²⁻ fertility during growth and development. γ-Glutamyl cysteine sulfoxide (γGP = 0.001, F = 8.34) increased linearly as the reduction in SO₄²⁻ concentration was delayed during plant growth and development (1-PRENSO = 0.735 – 0.004 day, R² = 0.95). The lachrymatory factor is a primary product of the enzymatic decomposition of 1-PRENSO which also produces organoleptic heat and mouth burn when eaten (Randle, 1997). Therefore, the longer high levels of SO₄²⁻ are made available to the plant during bulbing, the harsher the taste will become even harsher during storage as γGP is converted to 1-PRENSO. In a quadratic response to delayed SO₄²⁻ reduction during growth and development, γ-glutamyl propenyl cysteine sulfoxide (γGP) went through pre-bulbing into active bulbing, and then increased as bulbs matured (MCSO = 1.19 – 0.016 day + 0.0002 day², R² = 0.88, Fig. 3). Methyl cysteine sulfoxide accumulated to the highest concentration of the three ACSOs measured. Thiolsulfinates 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 SO₄²⁻ (R² = 0.003, F = 5.93), the levels measured were very low (Fig. 3). The response followed a cubic trend (PCS = 0.084 – 0.0018 d + 0.0006d² – 0.0001d³, R² = 0.92). Thiolsulfinates from the decomposition of PCSO give rise to raw, fresh onion-like flavors (Randle, 1997). Because the decomposition products from 1-PRENSO can be in low enough concentrations for sugars to be perceived over the heat and mouth burn. As the high SO₄²⁻ solution concentrations were provided closer to harvest, 1-PRENSO 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 SO₄²⁻ reductions should be perceived sweeter because of 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 SO₄²⁻ reduction occurred earlier in growth and development, the response of the ACSOs were similar to what was found in a previous study when SO₄²⁻ concentrations were given at consistent levels through out the growing season (Randle, et al., 1995). In that study, luxuriant SO₄²⁻ fertility concentrations resulted in high 1-PRENSO concentrations, and low PCSO and MCSO concentrations. Conversely, low to deficient SO₄²⁻ fertility levels resulted in lower 1-PRENSO and higher PCSO and MCSO concentrations.
be associated with the metabolic changes that biosynthetic pathway during this time could prior to harvest that coincides with the onset of SO\(_4^\text{2-}\) reductions suggest that SO\(_4^\text{2-}\) increases linearly (EPY = 5.18 + 0.02 \(d\), \(R^2 = 0.86\)) in response to decreasing SO\(_4^\text{2-}\) concentrations.

The significant linear increases in EPY, \(\gamma\)GP, and 1-PRENCSO with delaying SO\(_4^\text{2-}\) reduction suggest that SO\(_4^\text{2-}\) should be reduced as early as possible for mild sweet onion production. However, restricting SO\(_4^\text{2-}\) too early adversely impacts bulb fresh weight (Randle et al., 1995). A potential target date for SO\(_4^\text{2-}\) reduction could be a time \(-7\) weeks prior to harvest that coincides with the onset of bulbbing. 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 bulbbing, causing MCSO synthesis and accumulation to increase relative to 1-PRENCSO. It is possible that reducing SO\(_4^\text{2-}\) during early bulbng caused onions to be more responsive to low SO\(_4^\text{2-}\) concentrations, thereby triggering greater MCSO synthesis than at other developmental stages. Low SO\(_4^\text{2-}\) 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\(_4^\text{2-}\) would also prevent a decrease in soluble sulfurs that was associated with later SO\(_4^\text{2-}\) reductions. Lower SSC would be associated with decreasing sugar content in the bulbs.

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**Fig. 2.** Changes in soluble solids content (SSC) and enzymatically developed pyruvate (EPY) concentrations from mature ‘Sweet Vidalia’ bulbs when applied SO\(_4^\text{2-}\) concentrations 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 \(d^2\), \(R^2 = 0.86\)), and EPY increased linearly (EPY = 5.18 + 0.02 \(d\), \(R^2 = 0.86\)) in response to decreasing SO\(_4^\text{2-}\) concentrations.

**Fig. 3.** Changes in methyl cysteine sulfoxide (MCSO), 1-propenyl cysteine sulfoxide (1-PRENCSO), propyl cysteine sulfoxide (PCSO), and \(\gamma\)-glutamyl cysteine sulfoxide (\(\gamma\)GP) from mature ‘Sweet Vidalia’ bulbs when applied SO\(_4^\text{2-}\) 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\(_4^\text{2-}\) concentrations was quadratic (MCSO = 1.19 – 0.016 \(d\) + 0.0002 \(d^2\), \(R^2 = 0.88\)), while 1-PRENCSO was linear (1-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 \(\gamma\)GP was cubic (\(\gamma\)GP = 1.054 – 0.025 \(d\) + 0.0005 \(d^2\) – 4.13 \(d^3\), \(R^2 = 0.96\)).