The Effects of Calcium Chloride and Ammonium Sulfate on Onion Bulb Quality at Harvest and During Storage

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Abstract. Sandy soils in the onion (*Allium cepa* L.) growing region of southeastern Georgia are generally low in calcium (Ca). Bulbs grown in these soils are often soft and susceptible to postharvest diseases. Preliminary greenhouse studies have indicated that supplemental calcium chloride (CaCl₂) can improve bulb firmness. The effects of supplemental CaCl₂ on the quality of field-grown onions were therefore investigated. Other preliminary studies indicated that CaCl₂ may inhibit sulfur (S) uptake in onion and decrease bulb pungency. Thus, ammonium sulfate (NH₄)₂SO₄ and CaCl₂ levels were varied to determine if CaCl₂ could improve flavor at different levels of nitrogen (N) and S fertility. Onions, cv Georgia Boy, were grown with 0, 250, and 500 kg·ha⁻¹ (NH₄)₂SO₄ and 0, 115, and 230 kg·ha⁻¹ CaCl₂ in a factorial combination in 2005 and 2006. Total bulb yield increased with increasing (NH₄)₂SO₄, but was unaffected by CaCl₂. The percentage of diseased bulbs increased during storage in both years, and was affected by (NH₄)₂SO₄ fertility in 2006. Bulb scale firmness increased with supplemental CaCl₂ fertility and decreased significantly during storage in both years. Fertility treatments had little effect on bulb pectin composition, although total pectin concentrations fell during storage in 2005 and 2006. In addition, bulb pungency decreased with additional CaCl₂ in 2006. However, CaCl₂ had a limited effect on flavor precursor concentrations. There were no interactions between fertility treatments, but there were CaCl₂ and (NH₄)₂SO₄ by storage duration interactions affecting firmness and disease incidence, respectively. With the exception of yield, differences among years in the parameters measured were generally small.

Onions (*Allium cepa* L.) are grown worldwide for their flavor attributes. In some regions, niche markets have developed for producing sweet, mild-flavored bulbs. Onions grown in southeastern Georgia are sold under the trademark of Vidalia onions and account for nearly \$100 million in farm-gate income annually (Maw, 2006). Valued for their low pungency, these bulbs are typically soft and store poorly (Kopsell and Randle, 1997). Although genetic composition is important in determining bulb qualities such as flavor, firmness, and disease susceptibility, several studies have indicated that the environment also can affect these characteristics (Kopsell and Randle, 1997; Randle, 2000; Uzo and Currah, 1990; Yoo et al., 2006). Empirical observations have indicated that Vidalia onions grown on sandy, low-calcium (Ca) soils are softer and more susceptible to disease than on high-Ca soils. Furthermore, greenhouse studies (Randle, 2005) have suggested that Ca status

can affect bulb firmness and postharvest quality.

Calcium has many roles in the plant. A high proportion of Ca in plant cells is found in the cell wall/middle lamella region (Marschner, 1995). Here it is bound to carboxyl groups of polygalacturonic acids (pectin), where it links adjacent galacturonic acid chains through ionic bonds (Carpita and Gilbeaut, 1993). Because of its role in cell wall architecture, Ca is considered important in determining cell wall strength and firmness in fruits and vegetables (DeEll et al., 2001; Sams and Conway, 1984; Van Buren, 1979). Numerous studies report improvements in fruit and vegetable firmness when supplemental Ca is supplied during growth (DeEll et al., 2001; Manganaris et al., 2006; Toivonen and Bowen, 1999). In addition to improvements in firmness, Ca applications can enhance disease resistance in some crops (Conway et al., 1991; Volpin and Elad, 1991). Because mild-flavored, sweet onions are often soft and highly susceptible to disease during storage, they may be suitable candidates for supplemental Ca fertility. Furthermore, Somers (1973) reported that onion cell walls had a high affinity for Ca ions. Earlier studies on the effects of Ca fertility on

onion have focused on nutrient balancing or yield (Boyhan et al., 2002; Coolong et al., 2004; Fenn and Feagley 1999). Hence, little is known about the effects of Ca on bulb quality attributes.

In addition to improving bulb firmness, CaCl₂ applications have been correlated to decreases in bulb pungency in greenhouse studies (Randle, 2005). Previous studies indicate that enhanced N and S fertility can lead to increases in pungency (Coolong and Randle, 2003; Randle and Lancaster 2002). The decrease in bulb pungency with additional CaCl₂ may be the result of several mechanisms. Chloride may compete with nitrate or sulfate for uptake by the plant, therefore reducing pungency (Barbier-Brygoo et al., 2000). Alternatively, the application of Ca may lead to the formation of calcium sulfate, which is much less soluble than other sulfate-containing minerals (Doner and Lynn, 1989). This could lead to a removal of available S, thus reducing onion pungency. Therefore, supplemental CaCl₂ may have several benefits to sweet onion growers. In addition to improving firmness and postharvest quality, CaCl₂ applications may decrease N or S availability to the plant, thus reducing pungency. However, if N or S availability is too low, other parameters such as yield could be negatively influenced.

We had several objectives: to determine the suitability of CaCl₂ as an amendment to improve onion bulb firmness and postharvest quality in field-grown bulbs; to test the possible inhibitory effects of CaCl₂ on bulb flavor, and N and S uptake, and to understand possible mechanisms by which CaCl₂ affected bulb firmness, total pectin and pectin fractions were measured. In addition, bulb S-flavor compounds were measured to determine if CaCl₂ applications would affect flavor in field-grown onions.

Materials and Methods

Plant material. All plants used in this study were grown at the Vidalia Onion and Vegetable Research Center, Lyons, GA (≈32°N). Soils at the farm are an Irvington loamy sand with a pH 6.2. Seeds for cv Georgia Boy were sown into seed beds at a rate of 200 seeds per linear meter on 19 Sept. 2004 and 19 Sept. 2005. Seedlings were grown for 8 weeks following the guidelines of The University Georgia Cooperative Extension Service (Boyhan et al., 2001). On 23 Nov. 2004 and 1 Dec. 2005, seedlings were pulled and foliage cut to a length of ≈15 cm. Transplants were set into raised beds spaced 1.8 m on center, with in-row spacing of 14 cm and 30.5 cm between rows with four rows per bed. Each experimental plot was 9.15 m in length, resulting in a population of 260 plants per treatment plot. There were 1.8-m spaces between adjacent plots within a row. The study was arranged as a 3 × 3 factorial randomized complete block design with three treatment levels each of (NH₄)₂SO₄ and CaCl₂. Each treatment was replicated four times, for a total of 36

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experimental plots. Border plots planted with cv Georgia Boy surrounded the study on all sides and received no supplemental CaCl_2 or $(\text{NH}_4)_2\text{SO}_4$. With the exception of fertility treatments, onions were grown according to Cooperative Extension Guidelines (Boyhan et al., 2001). Soil samples for both growing seasons were obtained from each test plot before planting and after harvest and soil nutrient concentrations determined (University of Georgia Soils Testing Laboratory). Concentrations of soil N ranged from 610 to 722 $\text{kg}\cdot\text{ha}^{-1}$ N, and soil S levels ranged from 158 to 230 $\text{kg}\cdot\text{ha}^{-1}$. Soil Ca levels ranged from 1003 to 1122 $\text{kg}\cdot\text{ha}^{-1}$ Ca. There were no significant differences between years or among plots tested. Soil N concentrations were slightly higher before planting than after harvest in 2006 ($P = 0.11$), but generally nutrient levels at harvest were not significantly different from before planting. Plants were overhead irrigated with well water to provide at least 2.54 cm of water (rainfall and irrigation) weekly. Irrigation water was determined to contain 3.6 $\text{mg}\cdot\text{L}^{-1}$ of sulfate, 28.3 $\text{mg}\cdot\text{L}^{-1}$ of Ca, and negligible levels of nitrate (University of Georgia Soil, Plant, and Water Laboratory).

Raised beds received 455 $\text{kg}\cdot\text{ha}^{-1}$ 5N–10P₂O₅–15K₂O with 9% S 3 weeks before transplant. At 6 and 8 weeks post-transplant, all plants received KNO_3 and KH_2PO_4 at a rates of 145 and 75 $\text{kg}\cdot\text{ha}^{-1}$, respectively. At 12 weeks post-transplant, all plants received CaNO_3 (15.5N–0–0) at a rate of 225 $\text{kg}\cdot\text{ha}^{-1}$. Calcium fertility treatments consisted of three levels of CaCl_2 (0, 28.75, and 57.5 $\text{kg}\cdot\text{ha}^{-1}$ CaCl_2) applied as a liquid band at the base of the plants at 8, 12, 16, and 20 weeks after transplant to ensure an even distribution throughout the growing season. This resulted in a total season application of 0, 115, and 230 $\text{kg}\cdot\text{ha}^{-1}$ CaCl_2 . The $(\text{NH}_4)_2\text{SO}_4$ treatments consisted of two applications of 0, 125, and 250 $\text{kg}\cdot\text{ha}^{-1}$ $(\text{NH}_4)_2\text{SO}_4$ applied in granular bands at the base of the plants at 6 and 10 weeks post-transplant, resulting in a total application of 0, 250 and 500 $\text{kg}\cdot\text{ha}^{-1}$ $(\text{NH}_4)_2\text{SO}_4$ for the growing season. These fertility treatments were chosen based on low, medium, and high levels of S fertility and levels of CaCl_2 typically used by growers in the region.

Plants were undercut and hand harvested on 11 May 2005 and 10 May 2006. Entire plots (260 plants) were harvested. Bulbs were cured with forced air at 36 °C for 48 h. Average bulb weights were obtained by dividing the cured yield of each plot by 260. Yields were then estimated using an average plant population of 197,600 bulbs per hectare, which is typical for Georgia. Bulbs were sorted into 20-bulb nylon mesh bags for immediate analysis or refrigerated storage. Bulbs were stored for 10 and 20 weeks at 1.5 °C and 70% relative humidity. Upon removal from storage, bulbs were weighed, cut longitudinally, and disease symptoms were visually assessed (Schwartz and Mohan, 1995). Subsequent analyses were performed on the combined tissue from each 20-bulb replication.

Mineral analysis. A 5-mm longitudinal slice was taken from each bulb, weighed, and oven dried for 7 d at 70 °C. After dry weights were determined, plant tissue was ground to a fine powder using a coffee grinder. Total bulb S was determined by combining 0.2 g of dry tissue with 0.1 g of vanadium pentoxide accelerant and was analyzed using a Leco 232 S analyzer (Leco Corp., St. Joseph, MI; Coolong et al., 2004). Bulb N concentrations were determined with ≈ 0.25 g of dried bulb tissue using a Leco CNS 2000 (Leco Corp.). Calcium concentrations were determined using the wet acid digestion method and atomic absorption spectrometry (AAAnalyst 300; Perkin-Elmer, Norwalk, CT; Mills and Jones, 1996).

Firmness. Bulb scale firmness was measured by cutting a 2 × 4-cm section from the first fully fleshy scale (usually the second or third scale from the outside of the bulb) at the equatorial region of each bulb. Firmness was measured as the force in Newtons (N) required to penetrate the scale using a 1-mm diameter probe coupled to a fruit penetrometer mounted to a motorized press operated at a speed of 1.5 $\text{mm}\cdot\text{s}^{-1}$ (model 327; McCormick Fruit Tech, Yakima, WA). Firmness for each 2 × 4-cm slice was measured three times and averaged.

Alcohol-insoluble residue (AIR), pectin fractioning, and total pectin determination. The AIR was prepared from onion tissue according to a modification of the method of Huber and Lee (1986). Longitudinal slices, 5 to 10 mm thick, were cut from bulbs and homogenized in a blender for 60 s with four volumes (w/v) of 95% ethanol. Two more volumes of 95% ethanol were added and the homogenate was boiled for 20 min with slow stirring. The homogenate was cooled in an ice-water bath for 30 min. The residue was filtered under vacuum through glass fiber filters (0.7 μm , APFF; Millipore, Billerica, MA). Based on the initial sample weight, the residue was sequentially washed with six volumes of 95% ethanol, with four volumes of 100% ethanol, and with four volumes of acetone. The residue was dried overnight in a fume hood. The dried AIR was weighed and ground to a fine powder using a coffee grinder and was stored at –20 °C until analysis.

The pectin in the AIR was fractionated into water-, chelator-, acid-, and alkali-soluble pectins according to a modification of the method of DeVries et al., (1981). About 30 mg of onion AIR was extracted at 60 °C for 90 min in 40 mL of 0.05 M sodium acetate buffer, pH 5.2, to obtain water-soluble pectin (WSP). The WSP was obtained by centrifuging the extract at 30,000 g_a for 15 min and filtering the supernatant through one layer of Miracloth (CalBiochem, EMD Biosciences, San Diego, CA). The remaining pellet was suspended in 40 mL of 0.05 M sodium oxalate, 0.05 M ammonium oxalate, and 0.05 M sodium acetate, pH 5.2, and incubated for 90 min at 60 °C to obtain chelator-soluble pectin (CSP). The remaining pellet was again resuspended in 40 mL of HCl, pH 2.5, and incubated for 90 min at 60

°C. The extract was centrifuged and filtered to obtain the acid-soluble pectin (ASP). The remaining pellet was resuspended in 40 mL of 0.05 M NaOH and incubated for 90 min at 60 °C, centrifuged, and filtered as previously to obtain the alkaline-soluble pectin (AKSP). The pectin (uronic acid) content of each fraction was determined with the *m*-hydroxydiphenol method (Blumenkrantz and Asboe-Hansen, 1973).

Total pectin was determined with the method of Ahmed and Labavitch (1977). About 5 mg of AIR was weighed into a 50-mL beaker and placed in an ice-water bath. Five milliliters of concentrated cold sulfuric acid was slowly added to the AIR followed by 5 mL of cold deionized water with slow stirring. Then cold deionized water was added to bring the solution to a volume of 25 mL in a volumetric flask. An aliquot of the solution was analyzed for galacturonic acid content (Blumenkrantz and Asboe-Hansen, 1973). Galacturonic acid content of samples was estimated from a linear regression using galacturonic acid as a standard at concentrations of 0 to 20 μg .

Pungency and lachrymatory factor. Pungency was determined by crushing bulbs and analyzing the juice for pyruvic acid (Randle and Bussard, 1993a). The concentration of propanethial S-oxide (lachrymatory factor; LF) was determined according to a minor modification of Schmidt et al. (1996). In brief, 2.0 mL of juice was added to 2.0 mL of chilled methylene chloride containing 0.4% *m*-xylene (internal standard). The mix was inverted several times and centrifuged at 1,000 g_a for 5 min. The methylene chloride fraction was analyzed using gas chromatographic (GC) analysis. The LF was analyzed on a GC-17A gas chromatograph with a flame ionization detector (Shimadzu Corp., Kyoto, Japan). A 5-m × 0.53-mm fused silica 2.65- μm film thickness capillary column was used (DB-1; J&W Scientific, Agilent Technologies, Santa Clara, CA). Injector and detector temperatures were 210 °C and 250 °C, respectively. The initial column temperature was 60 °C, held for 20 s, then increased at 15 °C·min^{–1} to 100 °C and held for 30 s. Helium was used as the carrier gas and column flow rates were 8.2 mL·min^{–1}. A 1- μL sample injection was made with a 10:1 split ratio. Identity of the LF was confirmed using GC-mass spectrometry (MS; The University of Georgia Chemical and Biological Sciences Mass Spectrometry Facility).

Flavor precursors. The S-alk(en)yl cysteine sulfoxides (ASCOS) were determined according to a modification of the GC method of Kubec et al. (1999). First, 2–5-mm-thick longitudinal slices were obtained from bulbs and extracted (10:1, w/v) in 80% methanol at –20 °C for 5 d. To each 10-mL extract, 0.5 mg of ethyl cysteine sulfoxide (ECOS), synthesized according to Lancaster and Kelly (1983), was added and dried using forced air (Evap-o-Rac; Cole Parmer, Vernon Hills, IL). The dry samples were redissolved in 1 mL of high-performance liquid chromatography (HPLC) water and passed through a

column (1.5 × 12 cm) containing 1 × 5 cm of cation exchange resin (Dowex 50W-X8, 200–400 mesh; Bio-Rad, Richmond, CA) pre-treated with 20 mL of 3% HCl. Interfering substances were removed with 10 mL of 3% HCl followed by 15 mL of HPLC water. The ACSOs were eluted from the column with 15 mL of 2 M ammonium hydroxide. The eluate was evaporated using forced air. The dry residue was dissolved in 1 mL of 32:60:8 ethanol:water:pyridine, 0.4 mL of which was derivatized with 0.1 mL of ethyl chloroformate. After 1 h, derivatized ACSOs were reduced to alk(en)yl cysteines by the addition of 0.2 mL of sodium iodide solution (0.5 g·mL⁻¹) and 50 μ L of acetyl chloride. The derivatized alkenyl cysteines were extracted with 0.3 mL of methylene chloride and were analyzed by GC.

Samples were analyzed on a GC-17A GC with flame ionization detector (Shimadzu Corp.). A 30-m × 0.32-mm fused silica 0.25- μ m film thickness capillary column was used (HP-5, J&W Scientific, Agilent Technologies). Injector and detector temperatures were 180 °C and 250 °C, respectively. The temperature program was as follows: initial temperature was 120 °C held for 0 s, increased at 2 °C·min⁻¹ to 160 °C held for 30 s, then to 280 °C at a rate of 10 °C·min⁻¹ and held for 5 min. Helium was used as the carrier gas with a column flow rate of 8.2 mL·min⁻¹. A 1- μ L injection was made with a split ratio of 10:1. Response factors for the derivatized alk(en)yl cysteines were determined using standards. The response factor for *S*-1-propenyl-L-cysteine sulfoxide [isochlorogenic acid (PECSO)], which is not commercially available and is very difficult to synthesize, was estimated to be the same as that of *S*-2-propenyl-cysteine sulfoxide (alliin), as has been done previously (Kubec et al., 1999). Propyl cysteine was prepared according to the method of Lancaster and Kelly (1983), and methyl cysteine, ethyl cysteine (Sigma, St. Louis, MO), and allyl cysteine (TCI America, Portland, OR) were purchased. The identities of individual derivatized alk(en)yl cysteines and cycloalliin were confirmed using GC-MS. Because PECSO will easily cyclize when subjected to alkaline conditions such as those incurred during cation exchange chromatography, it was necessary to include cycloalliin in our measurements of PECSO (Virtanen and Matikkala, 1959). Kubec et al. (1999) found almost complete conversion PECSO to cycloalliin using a similar method of sample preparation. Therefore, the areas of both isomers of cycloalliin were added to the area found for PECSO to get an estimate of PECSO concentration in the samples.

An Agilent 6870N GC coupled to an Agilent 5973 MS (Agilent Technologies, Palo Alto, CA) was used for GC-MS analysis of derivatized alkenyl cysteines and cycloalliin. A 30-m × 0.25-mm capillary column with a 0.25- μ m film thickness was used for compound separation (HP-5MS; Agilent Technologies). The temperature program was as mentioned previously for GC analysis.

One microliter of sample was injected into a split injector (10:1) ratio with He carrier gas and an injector temperature of 180 °C. Column flow was 1.0 mL·min⁻¹. The MS ion source was held at 230 °C and mass spectra were obtained over the range of 50 to 550 mass units.

Statistical analysis. All data were subjected to the GLM procedure testing the significance of main effects and interactions SAS statistical software (version 9.1.3; SAS Institute, Cary, NC). Mean separations were performed using a Waller-Duncan test of mean separation when appropriate. Percentage data were subjected to the arcsin transformation before analysis. Interactions among treatments or sampling time were uncommon and generally did not affect the interpretation of the results. Therefore, main effects are primarily described in the proceeding sections, although interactions are discussed when significant.

Results and Discussion

Yield and mineral nutrients. In 2005 and 2006, (NH₄)₂SO₄ applications increased bulb yield, whereas CaCl₂ treatments had no effect (Table 1). No treatment interactions were observed. The yield increase resulting from (NH₄)₂SO₄ could be expected as N and S fertility have been shown to increase yields in onion (Coolong and Randle, 2003; Hussaini et al., 2000). The addition of 250 and 500 kg·ha⁻¹ (NH₄)₂SO₄ resulted in similar yield increases, indicating that the application of 250 kg·ha⁻¹ (NH₄)₂SO₄ was sufficient to increase yields. In addition, yields were significantly greater in 2006 than in 2005. In 2005, environmental conditions were conducive for onions to flower (bolt) during maturation. During 2005, up to 20% of the bulbs in a given plot bolted, although no treatment differences in bolting were observed. In 2006, however, only a very small number of plants (<0.1%) formed inflorescences. Because bulbs with an inflorescence stalk were not harvested, their prevalence in the 2005 grow-

ing season may be responsible for the lower yields observed that year.

To confirm the efficacy of the fertility treatments, bulb N, S, and Ca concentrations were measured at harvest. In both years, bulb N concentrations increased with (NH₄)₂SO₄ fertility, indicating that the additional supply of N affected the plants (Table 2). Bulb S increased with the addition of (NH₄)₂SO₄ in 2006 but not in 2005. The increase in bulb N in 2005 indicates that the (NH₄)₂SO₄ treatment affected the mineral concentration in bulbs, even though bulb S remained unchanged. Supplemental CaCl₂ increased bulb Ca concentrations in 2005 and 2006 (Table 2). Additional CaCl₂ also lead to a significant decrease in bulb S in 2006. This confirms earlier results observed in greenhouse studies (Randle, 2005). No interactions were observed between (NH₄)₂SO₄ and CaCl₂ for N, S, or Ca concentrations in the bulb. Although further study may be necessary, the results from 2006 suggest that CaCl₂ could potentially be used to reduce S uptake by onions. The mechanism for CaCl₂ to decrease bulb levels of S is not clear. However, it is plausible that chloride and sulfate compete for availability and uptake by anion channels (Barbier-Brygoo et al., 2000). It is also possible that additional Ca could combine with available sulfate to form gypsum, therefore reducing S availability to the plant. Bulb concentrations of N, S, and Ca were lower in 2006 than in 2005. The higher yields in 2006 may have resulted in diluting the pool of available nutrients in the root zone, leading to lower bulb concentrations of N, S, and Ca (Zink, 1966).

Disease status. One of our primary objectives in this study was to determine if CaCl₂ affected disease incidence during storage. Onion neck rot caused by *Botrytis* spp. was the most prevalent disease observed, although some bulbs displayed symptoms of sour skin and center rot caused by *Burkholderia cepacia* and *Pantoea ananatis*, respectively.

Calcium chloride did not affect disease incidence. In 2005, (NH₄)₂SO₄ applications had no effect on disease incidence, but in 2006, interacted with storage duration to affect the number of bulbs displaying disease symptoms at 10 and 20 weeks of storage (Table 3). In 2006, the application of 250 kg·ha⁻¹ (NH₄)₂SO₄ lead to a decrease in disease incidence in bulbs during storage. Bulbs receiving 500 kg·ha⁻¹ (NH₄)₂SO₄ had a decrease in visible disease symptoms when compared with the 0 kg·ha⁻¹ (NH₄)₂SO₄ at 20 weeks of storage, but had a greater incidence of disease symptoms than the 250 kg·ha⁻¹ (NH₄)₂SO₄ treatment. The reason for the decrease in storage disease with 250 kg·ha⁻¹ (NH₄)₂SO₄ compared with the 0 kg·ha⁻¹ (NH₄)₂SO₄ treatment could be that bulbs grown with no additional (NH₄)₂SO₄ in 2006 may have been slightly deficient in S (1416 mg·kg⁻¹ dry weight; Table 2). However, the application of 500 kg·ha⁻¹ (NH₄)₂SO₄, although providing sufficient S, may have provided excessive N, which can lead to an increase in storage rot (Batal et al.,

Table 1. Means for the main effects of ammonium sulfate [(NH₄)₂SO₄] and calcium chloride (CaCl₂) applications for cured yield of cv Georgia Boy onion in 2005 and 2006. No treatment interactions were observed.

	2005	2006
	Yield (kg·ha ⁻¹)	
(NH ₄) ₂ SO ₄ (kg·ha ⁻¹)		
0	56,170 b ^c	95,843 b
250	61,081 a	105,589 a
500	61,389 a	107,697 a
LSD	2,923	3,755
CaCl ₂ (kg·ha ⁻¹)		
0	60,388 a	103,065 a
115	58,540 a	102,048 a
230	59,429 a	104,016 a
LSD	3,706	5,149

^aAny means within the same treatment and year not followed by the same letter are significantly different at *P* < 0.05.

Table 2. Main effects and means separation for ammonium sulfate [(NH₄)₂SO₄] and calcium chloride (CaCl₂) applications for total bulb nitrogen (N), sulfur (S), and calcium (Ca) concentrations in field-grown cv Georgia Boy onion in 2005 and 2006. No treatment interactions were observed.

	2005			2006		
	N	S	Ca	N	S	Ca
	----- (mg·kg ⁻¹ dry wt) -----			----- (mg·kg ⁻¹ dry wt) -----		
(NH ₄) ₂ SO ₄ (kg·ha ⁻¹)						
0	9,076 b ^z	2,923 a	1,747 a	8,079 b	1,416 c	1,304 a
250	10,048 b	2,682 a	1,677 a	8,785 b	2,363 b	1,332 a
500	11,908 a	2,869 a	1,742 a	11,159 a	2,973 a	1,272 a
LSD	1,262	622	241	875	206	151
CaCl ₂ (kg·ha ⁻¹)						
0	10,318 a	2,829 a	1,624 b	9,469 a	2,421 a	1,214 b
115	10,512 a	2,676 a	1,640 b	9,310 a	2,198 ab	1,306 ab
230	10,139 a	2,975 a	1,890 a	9,243 a	2,057 b	1,376 a
LSD	1,724	609	191	1,243	247	132

^zAny means within the same treatment and year not followed by the same letter are significantly different at *P* < 0.05.

Table 3. Means for ammonium sulfate [(NH₄)₂SO₄] and storage duration for the percentage of diseased cv Georgia Boy onion bulbs displaying visual symptoms of neck rot, center rot, or sour skin caused by *Botrytis* spp., *Pantoea ananatis*, and *Burkholderia cepacia*, respectively, at harvest, and 10 and 20 weeks of storage.

	2005	2006
	% of diseased bulbs	% of diseased bulbs
Harvest		
(NH ₄) ₂ SO ₄ (kg·ha ⁻¹)		
0	0 b ^z	1.7 f
250	4.2 b	3.3 f
500	1.9 b	3.8 f
10 weeks (NH ₄) ₂ SO ₄		
0	8.9 b	22.5 bc
250	7.3 b	7.1 ef
500	6.7 b	12.5 de
20 weeks (NH ₄) ₂ SO ₄		
0	29.6 a	40.4 a
125	26.7 a	17.1 cd
250	28.5 a	29.6 b
LSD	8.8	9.3

^zAny means within the same column not followed by the same letter are significantly different at *P* < 0.05.

1994). This may be why the application of 500 kg·ha⁻¹ (NH₄)₂SO₄ had less disease incidence than the 0 kg·ha⁻¹ (NH₄)₂SO₄, but more than the 250 kg·ha⁻¹ (NH₄)₂SO₄ at 20 weeks of storage in 2006.

Disease incidence in bulbs increased during storage for both years (Table 3). At harvest, about 3% to 4% of bulbs displayed visible disease symptoms; however, this increased to 17% to 40% of bulbs after 20 weeks of storage. Increases in disease incidence in onion during storage are typical (Williams-Woodward, 2001).

Bulb scale firmness. In 2005 and 2006, supplemental CaCl₂ interacted with storage duration to affect onion bulb firmness (Table 4a). Additional CaCl₂ increased scale firmness by 5% and 8% in 2005 and 2006, respectively, at harvest. However, after 10 weeks of storage, there were no differences in firmness among the CaCl₂ treatments. This indicates that at the levels used in this experiment, additional CaCl₂ improved firmness at harvest, but the increase in firmness was not enough to compensate for the soft-

Table 4A. Mean onion scale firmness for different levels of calcium chloride (CaCl₂) fertility and storage duration from harvest for cv Georgia Boy onion bulbs in 2005 and 2006.

	2005	2006
	Firmness (N)	
Harvest		
CaCl ₂ (kg·ha ⁻¹)		
0	3.36 b ^z	3.13 b
115	3.46 ab	3.30 a
230	3.54 a	3.38 a
10 weeks CaCl ₂		
0	3.09 cd	2.81 cd
115	3.07 cd	2.78 cd
230	3.17 c	2.85 c
20 weeks CaCl ₂		
0	3.00 d	2.72 d
115	2.99 d	2.72 d
230	3.00 d	2.76 cd
LSD	0.09	0.08

^zAny means within the same column not followed by the same letter are significantly different at *P* < 0.05.

Table 4B. Main effect means for onion scale firmness for cv 'Georgia Boy' onion bulbs grown with different levels of ammonium sulfate fertility [(NH₄)₂SO₄] in 2005 and 2006.

	2005	2006
	Firmness (N)	
(NH ₄) ₂ SO ₄ (kg·ha ⁻¹)		
0	3.19 a ^z	2.98 a
250	3.22 a	2.96 a
500	3.15 a	2.87 b
LSD	0.07	0.05

^zAny means within the same column not followed by the same letter are significantly different at *P* < 0.05.

ening that occurs during storage. The application of greater levels of CaCl₂ may be necessary to realize gains in firmness during storage. The application of 500 kg·ha⁻¹ (NH₄)₂SO₄ lead to a decrease in bulb firmness in 2006. Mean bulb firmness decreased from 2.97 N in the 0 and 250 kg·ha⁻¹ (NH₄)₂SO₄ treatment to 2.88 N in the 500 kg·ha⁻¹ (NH₄)₂SO₄ treatment (Table 4b). There was no interaction between storage duration and (NH₄)₂SO₄, as the differences in firmness remained throughout storage. A decrease in firmness with additional (NH₄)₂SO₄ may be expected as additional N has been reported to decrease the firmness of hydroponically grown bulbs at harvest (Randle, 2000).

Although storage duration interacted with CaCl₂ to affect firmness, it is worth discussing the degree of softening that occurred during storage. After 10 weeks of storage, bulb firmness decreased by 8% and 14% in 2005 and 2006, respectively. Declines in firmness are often associated with a decrease in cell turgor that accompanies water loss in fruits and vegetables (Gomez-Galindo et al., 2004). Water loss is generally considered to be responsible for much of the weight loss of bulbs in storage (Komochi, 1990). However, bulb weight loss from harvest in 2005 and 2006 was not correlated (*P* = 0.22, *r* = 0.1) to changes in bulb scale firmness during storage. This suggests that water loss may not be the primary mechanism for softening of onion scales during storage. Recent findings in our laboratory suggest that softening in onion bulbs may be related to changes in the middle lamella region resulting in cell slippage (T. Coolong, unpublished data).

Pectin. Supplemental Ca fertility is thought to improve the firmness of fruits and vegetables by increasing the Ca available to interact with carboxyl groups in adjacent polygalacturonic acid (pectin) chains strengthening the primary cell wall (Micheli, 2001).

Therefore, we chose to measure total pectin and individual pectin fractions to determine if supplemental CaCl₂ or (NH₄)₂SO₄ affected the composition of structural carbohydrates in onion. Total pectin concentrations were unaffected by CaCl₂ or (NH₄)₂SO₄ treatments in 2005 or 2006. Total pectin concentrations decreased during storage in 2005 and 2006 (Table 5). The decrease in uronic acid concentrations can be explained by changes in the amount of extractable AIR. In both years, total uronic acid concentrations expressed per unit of AIR did not change during storage. However, in 2005 and 2006, the amount of AIR extracted per unit of fresh tissue decreased during storage. These differences in extractable AIR resulted in changes in total pectin concentrations when expressed in milligrams per gram of dry weight. Significant decreases in extractable AIR have been observed in apple fruit during development and postharvest ripening (Fisher and Amado, 1994). The decrease in AIR in apple was attributed to changes in starch content during storage (Fisher and Amado, 1994). However, onions contain very little starch (Darbyshire and Steer, 1990), suggesting that the decrease in extractable AIR is the result of the metabolism of other alcohol-insoluble compounds in the bulb. There were no significant interactions between treatments or storage duration and treatments for total pectin or pectin fractions in 2005 or 2006.

The WSP fraction is characterized by highly esterified, low-branched polymers that may loosely interact with surrounding components of the cell wall (Heredia et al., 1995). The concentration of WSP was unaffected by CaCl₂ or (NH₄)₂SO₄ applications in 2005 and 2006. However, WSP levels were affected by storage duration. The WSP

increased during storage in 2005, but fell during storage in 2006 (Table 5). The small increase in WSP during storage in 2005 could be expected as increases in WSP have been correlated to softening in many fruits and vegetables (Brummell, 2006). In 2006, WSP decreased slightly between 10 and 20 weeks of storage. This change mirrored a small increase in AKSP, suggesting changes in the solubility of the pectin during storage. Cantor et al. (1992) also found changes in the solubility of pectin during cold storage of peach.

The CSP generally represents low to medium esterified pectin chains that are stabilized by forming ionic bonds with Ca ions, and as such, are solubilized by chelating agents (Heredia et al., 1995). It was thought that, in the presence of an active pectin methyl esterase system, supplemental CaCl_2 would increase the concentration of CSP. However, in this study, CaCl_2 did not affect the amount of CSP in the bulbs. Additional $(\text{NH}_4)_2\text{SO}_4$ applications lead to a small increase in CSP concentrations in 2005 and 2006 (Table 5). O'Donoghue et al. (2004) reported that additional S fertility did not affect onion CSP. This suggests that the increase in CSP observed in bulbs grown with additional $(\text{NH}_4)_2\text{SO}_4$ may be because of N or the combined effects of N and S together. In 2005, there was an increase in CSP during storage, although in 2006, CSP concentrations simply fluctuated during storage (Table 5). This indicates that pectin methyl esterase (PME) may be active in bulbs during storage, as WSP pectin chains must be first de-esterified before forming CSP. Garcia et al. (2002) reported PME activity in fresh onion tissue, but did not investigate PME activity during storage. Because PME activity is related to softening in some fruits and vegetables, in future studies it may be appropriate to investigate the role of PME in the softening of stored onions.

The ASP consists of covalently bound pectins that are solubilized by weak acids (Heredia et al., 1995). The ASP made up the smallest portion of pectin fractions measured and was unaffected by fertilizer treatments or storage duration in either year (Table 5), although the concentration of ASP was higher in 2005 than in 2006. The AKSP, which primarily consists of highly branched pectin polysaccharides in onion (Ng et al., 1998), was unaffected by $(\text{NH}_4)_2\text{SO}_4$ and CaCl_2 in 2005 and 2006. However, the AKSP was affected by storage duration in both years. In 2005, the AKSP decreased during storage, suggesting an increase in solubility of long-chain pectins during storage. In 2006, AKSP concentrations declined at 10 weeks of storage and then increased to harvest levels after 20 weeks, mirroring changes in CSP that occurred during storage in 2006. These results indicate that the increases in firmness observed with supplemental CaCl_2 fertility may be too small to be realized through changes in pectin concentration. Although the composition of onion cell wall pectin has previously been investigated (Ng et al.,

Table 5. The main effect means for different levels of ammonium sulfate fertility $[(\text{NH}_4)_2\text{SO}_4]$ and storage duration for uronic acid [total pectin, (TP)], water soluble pectin (WSP), chelator soluble pectin (CSP), acid soluble pectin (ACSP), and alkaline soluble pectin (AKSP) in $\text{mg}\cdot\text{g}^{-1}$ dry weight (DW) of field-grown cv Georgia Boy onions in 2005 and 2006. No treatment interactions were present.

	2005					2006				
	TP	WSP	CSP	ACSP	AKSP	TP	WSP	CSP	ACSP	AKSP
	mg·g ⁻¹ DW					mg·g ⁻¹ DW				
$(\text{NH}_4)_2\text{SO}_4$ (kg·ha ⁻¹)										
0	48.0 a ^z	11.5 a	13.5 b	4.1 a	19.5 a	43.9 a	12.4 a	7.1 b	1.3 a	18.8 a
250	48.6 a	10.9 a	13.3 b	3.9 a	19.1 a	43.8 a	12.3 a	8.1 a	1.5 a	19.6 a
500	50.4 a	11.7 a	15.1 a	4.5 a	19.6 a	46.3 a	12.1 a	8.2 a	1.4 a	19.7 a
LSD	3.4	1.4	1.19	0.73	1.9	5.3	1.9	0.94	0.34	2.0
Storage duration										
Harvest	55.5 a	10.2 b	9.6 c	4.5 a	21.6 a	48.1 a	12.6 ab	8.7 a	1.1 a	19.4 ab
10 weeks	46.9 b	11.1 b	11.9 b	3.9 a	19.0 b	43.7 ab	12.9 a	6.4 b	1.4 a	18.1 b
20 weeks	44.4 b	12.8 a	20.5 a	4.1 a	17.6 b	42.3 b	11.3 b	8.3 a	1.7 a	20.7 a
LSD	2.6	1.1	1.0	0.7	1.4	4.7	1.5	1.8	0.3	1.6

^zAny means within the same treatment and year not followed by the same letter are significantly different at $P < 0.05$.

1998), to our knowledge, this is the first attempt to quantify changes in pectin composition in onion during storage. The results obtained here suggest that pectin metabolism in onion is a dynamic process that may affect the quality of stored bulbs.

Pungency, LF, and flavor precursors. Although sugars contribute to onion flavor, S-containing compounds dominate the flavor profile of fresh bulbs (Block, 1992). Additional S fertility has been shown to increase flavor intensity in numerous greenhouse studies (Coolong and Randle, 2003; Randle et al., 1995; Randle and Bussard, 1993b). Recently, S and N fertilizer applications have been shown to affect flavor potential in field-grown onions (McCallum et al., 2005). Calcium chloride has also been reported to decrease bulb S concentrations and reduce flavor potential in greenhouse-grown onions, thus improving palatability of bulbs for fresh consumption (Randle, 2005). To determine the effects of CaCl_2 on onion flavor potential, LF concentrations were measured.

Pyruvic acid is a byproduct of the enzymatic hydrolysis ACSOs (flavor precursors) by the enzyme alliinase (EC 4.4.1.4) when

bulb tissue is disrupted (Block, 1992). Pyruvic acid is relatively stable and easy to measure and therefore has been used to estimate the overall flavor intensity or pungency of bulbs (Schwimmer and Weston, 1961; Wall and Corgan, 1992). Total pyruvic acid (TPY) increased with $(\text{NH}_4)_2\text{SO}_4$ application in 2005 and 2006 (Table 6). Additionally, in 2006, TPY levels decreased when additional CaCl_2 was supplied. Although not observed in 2005, these results indicate that additional CaCl_2 could lead to a decrease in bulb pungency. Accordingly, additional CaCl_2 also reduced bulb S levels in 2006 (Table 2). Bulb TPY concentrations increased during storage in 2005 and 2006. Increases in pungency during storage have been observed previously as additional ACSOs are believed to be synthesized during storage, leading to an increase in pungency (Kopsell and Randle, 1997; Kopsell et al., 1999). No treatment or storage duration interactions were observed with bulb TPY.

The LF [(Z,E) propanethial S-oxide] is a direct product of the hydrolysis of PECSO and can dominate the onion flavor profile when present in high levels. As the name

Table 6. The main effect means for ammonium sulfate $[(\text{NH}_4)_2\text{SO}_4]$, calcium chloride (CaCl_2), and storage duration for total pyruvic acid (TPY), and lachrymatory factor (LF) in $\mu\text{mol}\cdot\text{mL}^{-1}$ juice, and S-methyl cysteine sulfoxide (MCSO), S-propyl cysteine sulfoxide (PCSO), and S-1-propenyl-cysteine sulfoxide (PECSO) in $\mu\text{mol}\cdot\text{g}^{-1}$ dry weight (DW) for cv Georgia Boy onions in 2005 and 2006.

	2005					2006				
	TPY	LF	PECSO	PCSO	MCSO	TPY	LF	PECSO	PCSO	MCSO
	$(\mu\text{mol}\cdot\text{mL}^{-1}$ juice)		$(\mu\text{mol}\cdot\text{mg}^{-1}$ DW)			$(\mu\text{mol}\cdot\text{mL}^{-1}$ juice)		$(\mu\text{mol}\cdot\text{mg}^{-1}$ DW)		
$(\text{NH}_4)_2\text{SO}_4$ (kg·ha ⁻¹)										
0	4.25 b ^z	5.85 b	5.15 c	1.92 c	2.14 c	4.05 b	3.58 b	4.08 b	1.28 c	1.97 a
250	5.00 a	6.86 a	6.46 b	2.46 b	4.12 b	5.05 a	4.34 a	6.15 a	1.78 b	4.89 b
500	5.04 a	7.08 a	7.71 a	2.88 a	5.11 a	5.25 a	4.23 a	6.82 a	2.34 a	6.71 a
LSD	0.21	0.56	0.94	0.37	0.65	0.24	0.50	0.94	0.26	0.47
CaCl_2 (kg·ha ⁻¹)										
0	4.75 a	6.47 a	6.25 a	2.34 a	3.68 a	5.03 a	4.06 a	5.92 a	1.97 a	4.72 a
115	4.82 a	6.59 a	6.60 a	2.37 a	3.77 a	4.64 b	4.03 a	5.58 a	1.82 ab	4.34 a
230	4.68 a	6.66 a	6.37 a	2.52 a	3.82 a	4.68 b	4.07 a	5.56 a	1.62 b	4.51 a
LSD	0.30	0.78	1.29	0.51	0.94	0.27	0.68	1.33	0.30	0.64
Storage duration										
Harvest	4.08 c	6.46 b	7.03 a	2.28 b	2.54 b	4.43 b	4.48 a	8.77 a	1.79 b	2.93 b
10 weeks	4.76 b	5.63 c	6.35 a	1.77 c	4.17 a	4.62 b	3.84 b	3.61 c	1.48 c	5.07 a
20 weeks	5.41 a	7.66 a	5.21 b	3.21 a	4.57 a	5.31 a	3.84 b	4.67 b	2.13 a	5.57 a
LSD	0.21	0.54	1.05	0.36	0.66	0.24	0.52	0.91	0.27	0.47

^zAny means within the same treatment and year not followed by the same letter are significantly different at $P < 0.05$.

implies, it is responsible for the tearing sensation associated with chopping onions. Although unaffected by CaCl_2 , LF concentrations increased with $(\text{NH}_4)_2\text{SO}_4$ (Table 6). Although McCallum et al. (2005) reported that N and S fertility affected LF concentrations in a field-grown pungent cv Kojak, to our knowledge, this is the first time that N and S supply have been shown to affect LF concentrations in a field-grown mild onion cultivar. Onion LF concentrations were significantly affected during storage in both years (Table 6). However, while LF concentrations generally increased after 20 weeks of storage in 2005, in 2006, LF concentrations decreased during storage. The LF is the product of the hydrolysis of the flavor precursor, PECISO. However, during storage, changes in the LF did not always correspond to changes in PECISO. In 2005, concentrations of PECISO decreased in storage, whereas LF levels increased. In 2006, PECISO concentrations decreased by 47% after 20 weeks in storage, but LF concentrations only decreased by 14%. The reason for the poor correlation between LF and PECISO concentrations in storage could be because of differences in alliinase activity in the onion macerate at different storage durations in 2005 and 2006 (Uddin and MacTavish, 2003). In 2005 and 2006, PECISO concentrations were unaffected by CaCl_2 , but increased with additional $(\text{NH}_4)_2\text{SO}_4$, as was the case with the LF (Table 6). No treatment or storage interactions were observed with LF or PECISO concentrations.

Two additional ACSOs, methyl cysteine sulfoxide (MCSO) and propyl cysteine sulfoxide (PCSO) were measured. Found in the majority of *Allium* and some *Brassica* spp., MCSO, when hydrolyzed by alliinase, imparts a cabbage-like flavor to onion (Lancaster and Boland, 1990; Randle et al., 1994). In 2005 and 2006, bulb MCSO concentrations were unaffected by CaCl_2 , but increased when grown under additional $(\text{NH}_4)_2\text{SO}_4$ (Table 6). Of the three ACSOs measured, MCSO had the greatest response to applications of $(\text{NH}_4)_2\text{SO}_4$. McCallum et al. (2005) reported similar increases in MCSO concentrations in field-grown bulbs subjected to an additional 200 kg S ha^{-1} . Bulb MCSO concentrations increased in storage in 2005 and 2006, indicating an active synthesis of MCSO during storage. Propyl cysteine sulfoxide is typically found in the lowest concentrations among the three flavor precursors routinely detected in onion (Randle and Lancaster, 2002). Volatiles generated from the hydrolysis of PCSO lend a chive-like flavor to bulbs (Randle et al., 1994). As expected, PCSO was present in the lowest concentration of the three ACSOs measured (Table 6). Onion PCSO concentrations increased with additional $(\text{NH}_4)_2\text{SO}_4$ in 2005 and 2006. Interestingly, PCSO concentrations decreased with additional CaCl_2 in 2006. It is likely that this decrease in PCSO is responsible for the decrease in TPY observed with supplemental CaCl_2 in 2006. Bulb PCSO concentrations also increased

during storage in 2005 and 2006. No treatment or storage interactions were evident when measuring bulb MCSO and PCSO concentrations.

As expected, supplemental $(\text{NH}_4)_2\text{SO}_4$ increased bulb flavor potential. To our knowledge, this is only the second time that supplemental N and S have been reported to affect flavor precursors and the LF in field-grown onions (McCallum et al., 2005). Additionally, CaCl_2 applications did lead to a small decrease in TPY and PCSO concentrations in 2006. Although the effects were small, they support earlier findings (Randle 2005). The lack of interactions between CaCl_2 and $(\text{NH}_4)_2\text{SO}_4$ suggest that CaCl_2 could be used over a wide range of N and S levels. However, higher levels of CaCl_2 may be necessary to receive substantial benefits in reducing onion pungency.

Conclusions

The results of this experiment indicate that supplemental CaCl_2 could be used to improve onion bulb firmness at harvest on low Ca soils. However, at the levels used in this study, CaCl_2 did not decrease the degree of bulb softening during storage. Larger applications of CaCl_2 may be necessary to realize improvements in firmness during storage. Although pectin fractions were unaffected by CaCl_2 and were only minimally affected by $(\text{NH}_4)_2\text{SO}_4$, the results obtained here indicate that there are significant changes in the pectin composition of onion bulbs during storage. These changes may provide insight into the mechanisms involved in onion bulb softening and degradation during storage. The effects of CaCl_2 and $(\text{NH}_4)_2\text{SO}_4$ on flavor were also noteworthy. Although applications of CaCl_2 did not affect flavor potential in 2005, they did lead to a decrease in pungency and PCSO in 2006, regardless of the level of $(\text{NH}_4)_2\text{SO}_4$ applied. Further research with higher levels of CaCl_2 may result in further reductions in onion pungency. If successful, this may be a tool that growers could use to produce firmer and milder bulbs in regions low in soil Ca.

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