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Elevated Carbon Dioxide Alters Hydrocarbon Emissions and Flavor in Onion

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ABSTRACT

Bulb onion (*Allium cepa*), non-bulbing Japanese bunching onion (*A. fistulosum*), common chives (*A. schoenoprasum*) and garlic chives (*A. tuberosum*) have markedly different harvest indices. With the onset of bulbing, leaf production ceases, photosynthates are reallocated to the bulb, lowering production of new shoots and crop canopy. Successive harvests from the same planting allow for a cumulative harvest index. In testing the influence of growing plants under different CO_2 conditions, a set of volatile methyl-ketones have been identified from onion that are emitted at higher levels when plants are grown at elevated CO_2 compared to controls grown at ambient CO_2 levels. Sensory panel taste testing has indicated differences in flavor for some cultivars when comparisons were made between plants grown at ambient and elevated CO_2 conditions. In future studies we will examine if thiosulfinates generated from the enzymatic conversion of alk(en)yl cysteine sulphoxides contribute to flavor differences detected between ambient and elevated CO_2 grown plants.

INTRODUCTION

Onion is a bulb crop with unusual leaf morphology, unique essential oils, secondary metabolites, and phytochemicals not found in other crop plants[1]. The target of vegetable Allium production is a high yield of superior quality produce. Bulbing is affected mainly by temperature and length of the dark period; however, precise conditions for physiological (bulb) maturity are cultivar dependent and are determined empirically. Onion development can be divided into three phases: a juvenile stage in which roots and leaves are produced, an adult vegetative stage in which the stem lengthens and widens, and a mature stage in which the bulb swells and scales begin to dry. Bulbing, non-bulbing and perennial Allium species with varying growth habits were evaluated to assess suitable conditions for optimum growth throughout these different developmental stages.

Onion is a rich source of low molecular weight secondary metabolites. In addition to being high in antioxidant flavonoids that are associated with a reduction in blood lipids, cholesterol and platelet activity[2], onion release a variety of low-molecular-weight hydrocarbons and sulfur derivatives. Odd-chain ketones are emitted from onion in undamaged leaf tissue and may confer insect resistance against agricultural pests[3]. Onion leaves also contain high levels of the sulfur-substituted amino acid S-methyl-L-cysteine sulfoxide. Onion pungency develops when the enzyme alliinase interacts with the sulfur-substituted amino acids S-propenyl-L-cysteine sulfoxide, and *S*-alk(en)yl-L-cysteine sulfoxides (ACSO) after cutting or rushing the onion tissue[4]. Alliinase converts the ACSOs to highly unstable methyl, propyl, and propenyl sulfenic acids. This reaction produces volatile sulfur compounds, pyruvic acid and ammonia. By growing plants in glass-enclosed flow-through chambers, volatile hydrocarbons from undamaged plants can be collected under different environmental conditions (Fig. 1).

Figure 1: Individual Growth Chambers (interior dimension 45 cm tall x 7 cm diameter) with onion seedlings: port with syringe for exchanging the nutrient media [**A**], port with absorbent filter for volatile organic collections [**B**], port with sensor for temperature/moisture measurements [**C**], nutrient media pack wrapped in aluminum foil [**D**], and flow meters for measuring the air pulled from the chamber [**E**].

At the time of harvest, sulfur VOCs generated from leaf tissue with mechanical damage can also be measured.

Plants grown in space develop differently than that of their land based counterparts[5]. Space flight conditions may initiate changes in root growth, whole plant development, soluble sugar and starch content, and the emissions of VOCs including ethylene[6]. Although many of these modifications have been attributed to micro-gravitational forces[7], other space flight conditions such as elevated carbon dioxide or VOCs imposed by growth in a closed system may influence the growth, physiology and development of plants. In this study growth parameters for a select group of onion cultivars under ambient CO_2 conditions were established. Volatile emission's for one of the cultivars was measured with undamaged plants under ambient and elevated CO_2 conditions. To examine whether there were perceivable differences in flavor between ambient and elevated CO_2 growth conditions a sensory panel was asked to discern between ambient- and elevated CO_2-grown plants. Studies continue to determine if sensory quality varies as a result of elevated CO_2.

PLANT GROWTH CONDITIONS AND FLAVOR ANALYSIS

OPTIMIZING HARVEST INDICES

Biomass production of *A. cepa* 'Cal 296', 'Deep Purple', and 'Purplette'; *A. fistulosum* 'Choesty' and 'Kinka'; *A. schoenoprasum* 'Fine Leaf', 'Purly', and 'Staro'; *A. tuberosum* 'Garlic' was investigated. Plants were grown in an Environmental Growth Chamber (EGC) (Shagria Falls, OH) under baseline conditions of 16 h /8 h day/night photoperiod, light of 600 mol m^{-2} s^{-1}, $24/20^{0}$ C (day/night on a 16/8 h thermoperiod), ambient CO_2 concentration and 75/100% relative humidity (day/night). Onion seeds were sown into Oasis solid support media (Hummert International Earth City, MO). Half strength Hydro-Sol (Scotts, Marysville, OH) nutrient solution adjusted and maintained to pH 6.8 was circulated below the solid support media. By covering the Oasis with aluminum foil, bacterial growth was minimized.

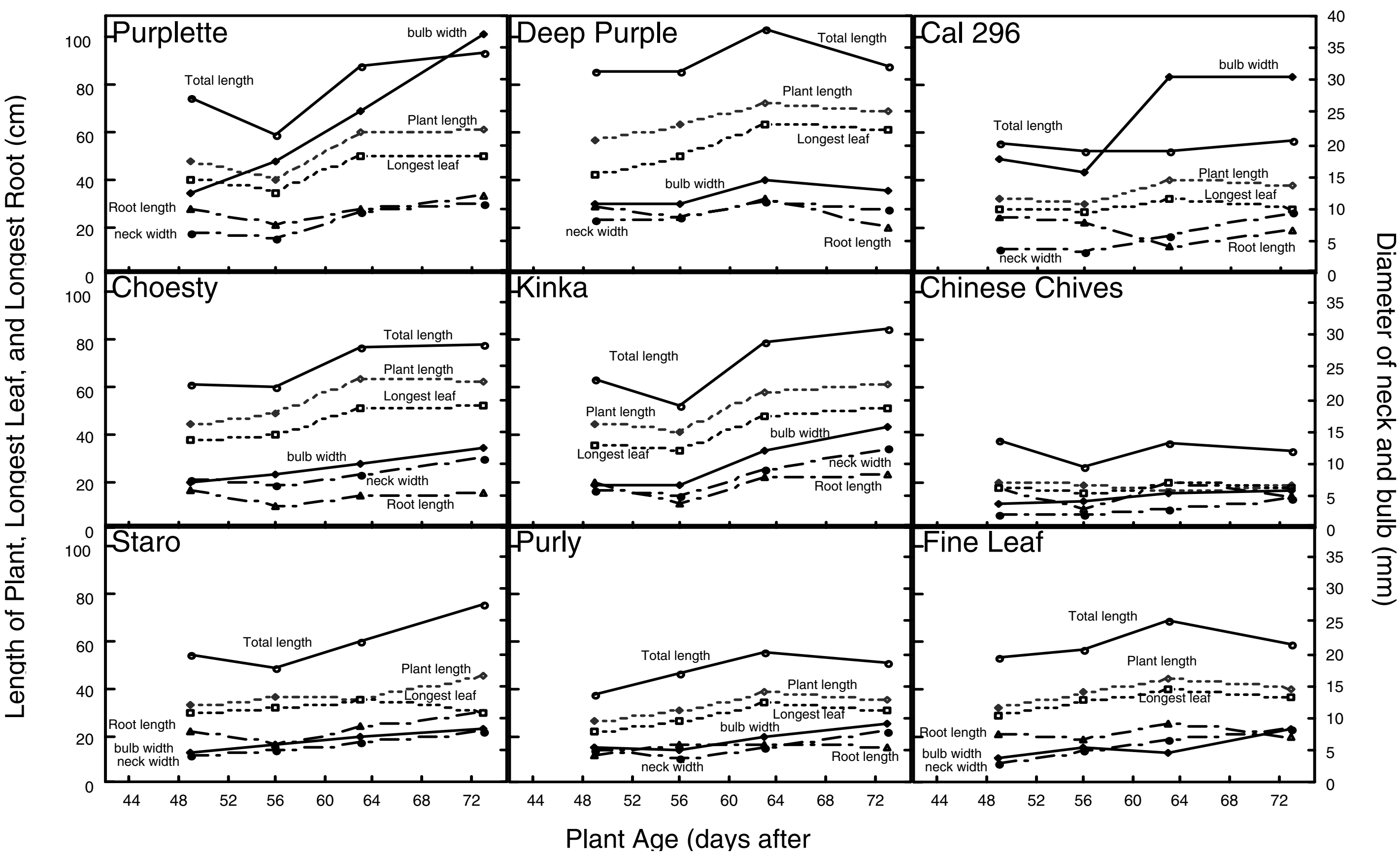


Figure 2. Total plant length, length of leaves and roots, and diameter of the neck and bulb of nine cultivars of four species of *Allium* grown in a hydroponic unit. Top panel includes three *A. cepa* cultivars (Purplette, Deep Purple, and Cal 296), middle panel includes two *A. fistulosum* cultivars (Choesty and Kinka) as well as *A. tuberosum* (Chinese Chives), and the bottom panel shows three *A. schoenoprasum* cultivars (Staro Purly and Fine Leaf). Plant age (days after sowing) is indicated on the x-axis. Data are presented as means (n = 3).

Phenology of cultivars of *A. cepa* (bulb onion), *A. fistulosum* (bunching onion), *A. schoenoprasum* (common chives), and *A. tuberosum* (Garlic chives) were evaluated according to bulb diameter and fresh weight, pseudostem length and color, leaf length, and root growth. Bulb diameter of all *A. cepas'* was significantly larger than *A. fistulosum, A. schoenoprasum* or *A. tuberosum* with no difference in bulb diameter among the latter three. *A. cepa* cultivars produced significantly greater biomass (total weight) and leaf length than cultivars of the other species, with no difference among cultivars of the other species. Purplette and Cal 296 produced a defined bulb. Deep Purple formed a slight bulb, not unlike a bunching onion under these conditions. 'Cal 296' matured earliest with slightly less biomass. Deep Purple, Choesty, and Kinka are all Japanese bunching onion types which produce a slight bulb and chives do not form a bulb (Fig 2). Each cultivar is phenologically unique and provides different edible biomass. Total flavonols were not significantly different among the three cultivars.

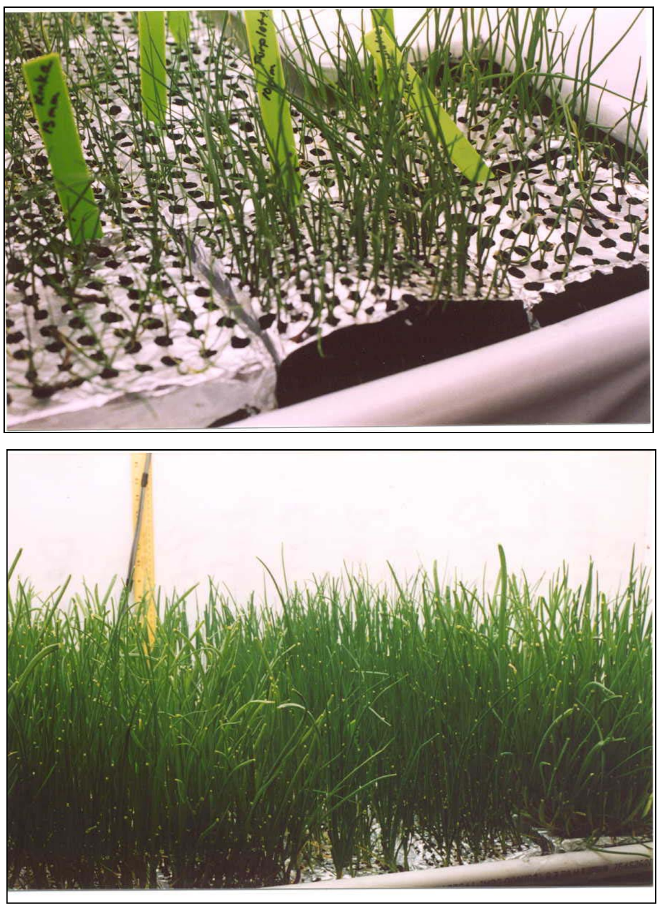


Figure 3 Plants at 25 days after planting at 15-mm spacing (4,445 plants m^{-2}) [top] and re-growth of *Alliums* following 1st cutting [bottom].

In a follow-up study biomass of these same *Allium* cultivars were grown hydroponically in an EGC at 10-mm plant spacing (10,000 plants m^{-2}) and 15-mm plant spacing (4,445 plants m^{-2}) with multiple harvests. We were interested in investigating whether harvest indices could be increased with multiple harvests without increasing the growing area utilized. Plants were grown at two plant densities and edible biomass removed such that repeated harvests could be made. Seeds were sown and grown in an EGC under above defined baseline conditions. Harvests of shoot edible biomass were taken by cutting the plant shoots above the pseudostem at 5 cm above the Oasis. The first cutting occurred 32 days after planting (dap) with successive harvests at 7-d intervals, for a total of seven harvests (Fig 3). For both spacings, the greatest amount of biomass was harvested at the first cutting with lesser amounts of biomass produced with successive harvests (Fig 4). Data taken at each harvest were fresh shoot weight per block (1 block is 12.5 cm x 12 cm), number of plants per block, and length of longest leaf. The number of plants at harvest varied among the blocks, therefore harvest shoot weights are reported as weight per individual, calculated by dividing the total block shoot weight by the number of established plants.

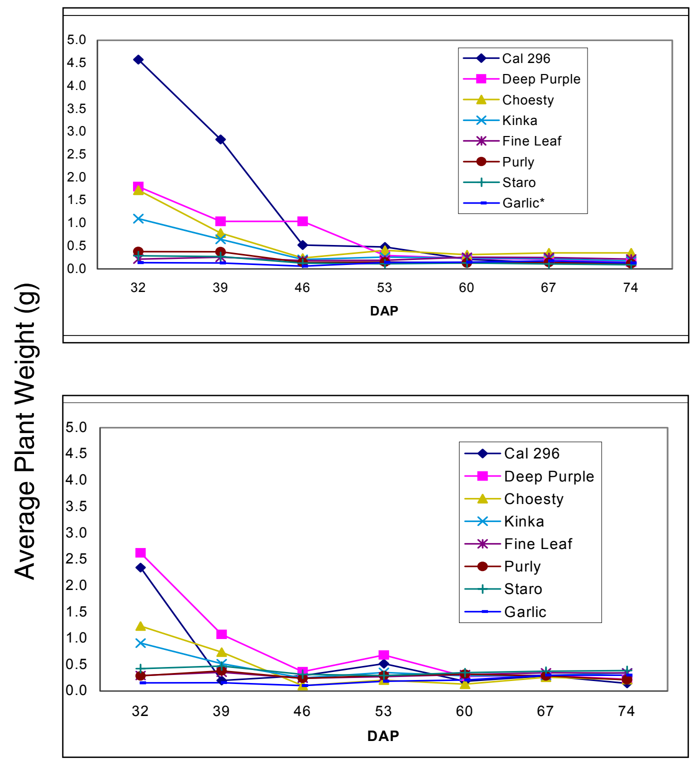
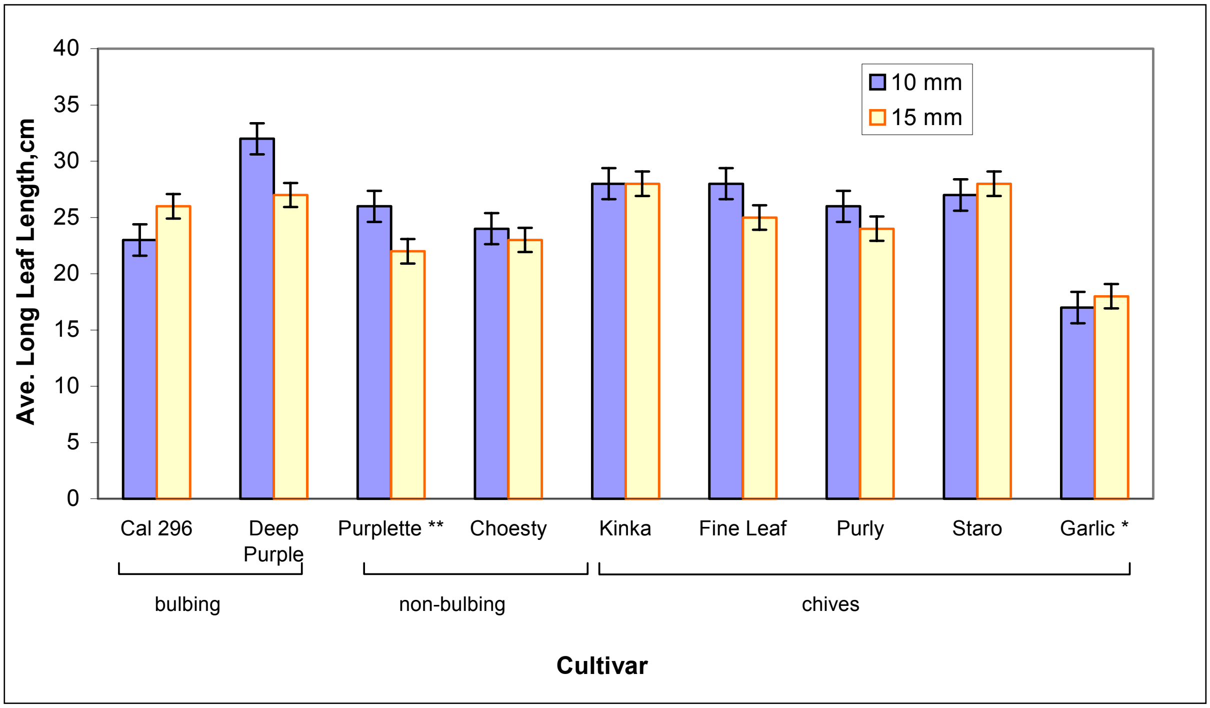


Figure 4 Biomass production (grams) of *Alliums* grown hydroponically in Oasis blocks at 10-mm (10,000 plants m^{-2}) [top] and 15-mm (4,445 plants m^{-2}) [bottom] spacings; x-axis shows the age of plants (Days After Planting).

Biomass production across all cultivars was greatest in the first two harvests regardless of planting density. The regrowth rate diminished with each successive cutting. Cultivars producing the most biomass were *A. cepa* 'Cal 296', 'Deep Purple', 'Purplette'; and *A. fistulosum* 'Kinka' for both spacings. Greater biomass was produced by the leaves in the 15-mm spacing even though leaves were shorter. Morphology of biomass produced at the different densities was dissimilar. Leaves of plants grown at 10-mm spacing (10,000 plants m^{-2}) were longer, whereas shoots of plants grown at 15-mm spacing (4,445 plants m^{-2}) weighed more (Fig 5). This is consistent with Ellison (1988)[8] and Egli (1987)[9] who found increased height:dry weight ratios at narrow spacings. Likewise, Ballare *et al*. (1994)[10] reported elongation and weight decreases with increasing population density. Plans are underway to compare plants grown at 5-mm spacing (40,000 plants m^{-2}) with those at 10-mm spacing (10,000 plants m^{-2}) and 15-mm spacing (4,445 plants m^{-2}). Cumulative biomass varied with density. The greatest biomass was produced by 'Cal 296' at the higher plant density followed by 'Purplette' at the lower plant population. With the exception of 'Cal 296' and 'Choesty', plants at the wider spacing produced the greatest amount of biomass. Except for 'Cal 296', these observations were consistent with increased height to weight ratio resulting from denser plantings reported elsewhere[11].



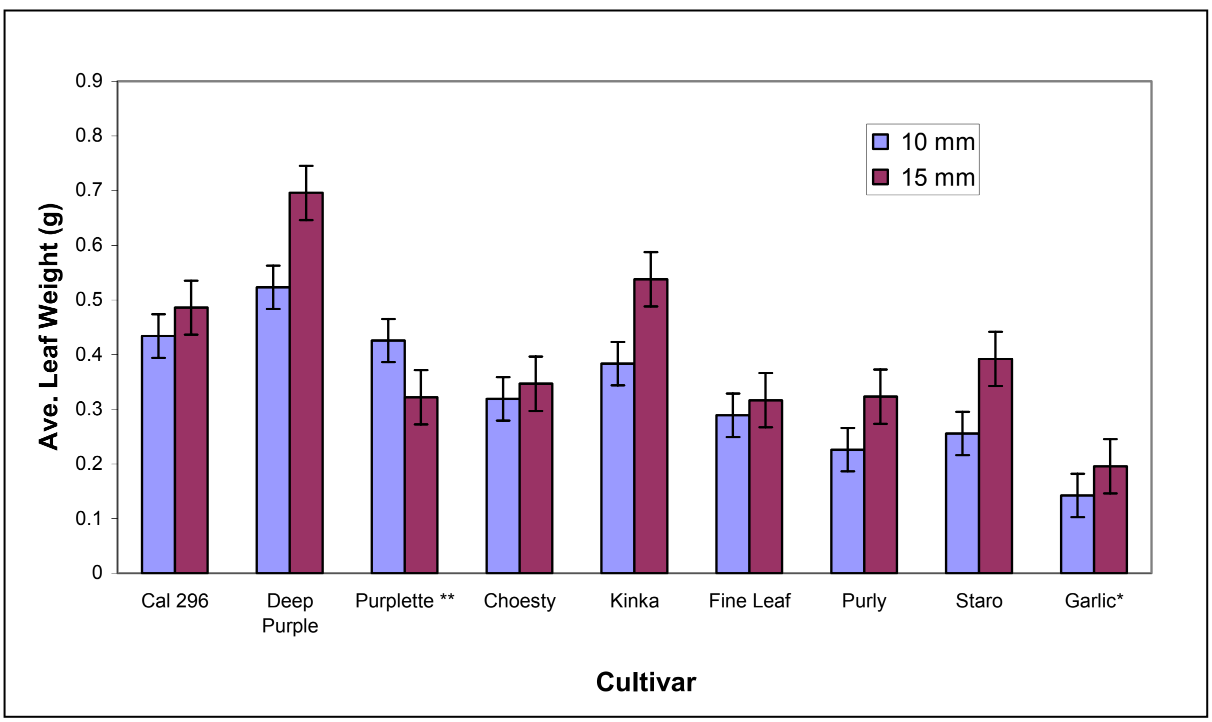


Figure 5 Average length of longest leaf (mm) [left top] and average leaf weight (g) of nine *Allium* cultivars grown at 10-mm (10,000 plants m^{-2}; 156 plants / block and 15-mm spacing (4,445 plants m^{-2}; 72 plants / block) hydroponically [left bottom] both 74 days after planting; error bar represents ± 1 standard error.

WHOLE PLANT VOLATILE ANALYSIS

The chemical identification of 2-tridecanone and 2-undecanone emitted from undamaged onion plants was based on comparisons of gas chromatography retention time and mass spectral (MS) fragmentation patterns of plant collected samples (Fig. 6) with those of authentic synthetic standards.

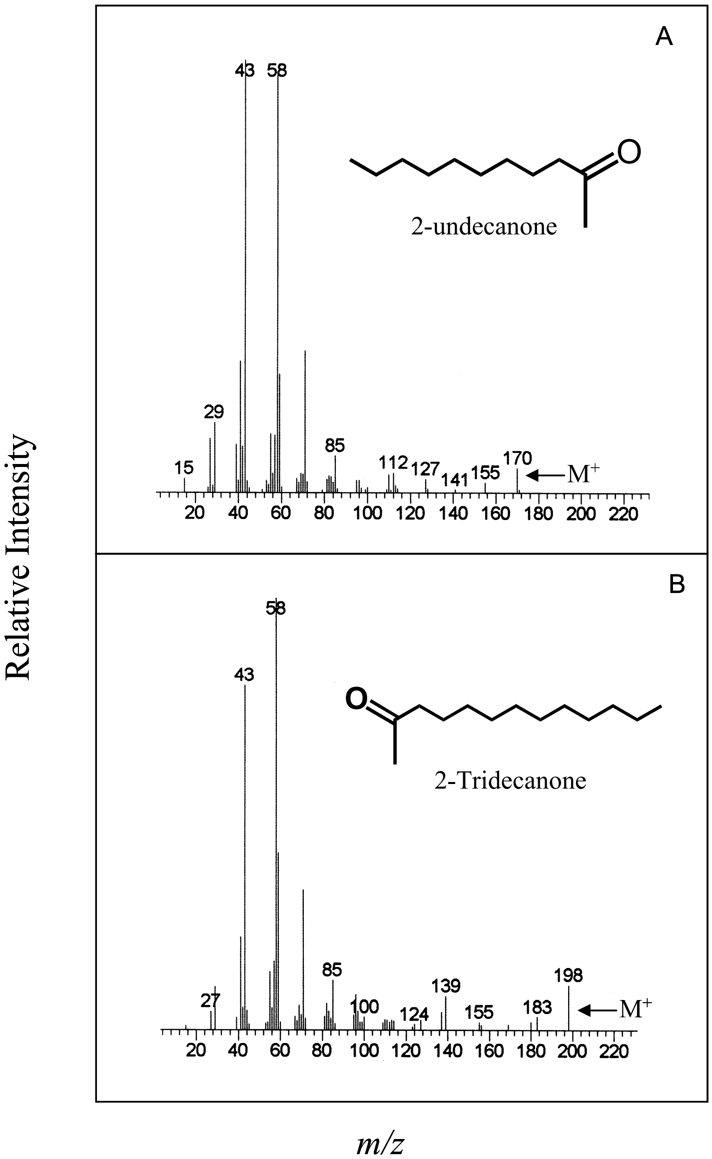


Figure 6. Electron ionization mass spectra of 2-undecanone [A] and 2-tridecanone [B] with *m/z* indicating the mass to charge ratio. The molecular ion is marked as M^+ and numbers above individual bars indicate the molecular weight of the fragment.

Volatiles were analyzed by capillary gas chromatography (GC) (Hewlett-Packard 6890) on a 15-m x 0.25-mm (i.d.) crosslinked-methylsiloxane column with a 0.1-µm-film thickness. The injector temperature was 230 °C and a detector temperature of 250 °C. The column was held at 40 °C for 0.5 min, increased 12 °C min^{-1} to 180°C, and then increased 40 °C min^{-1} to 220 °C and held for 2 min. Helium was used as a carrier gas at a linear flow velocity of 35 cm sec^{-1}. Both of these compounds are released from onion as significantly higher levels when plants are grown at elevated (1000 ppm) carbon dioxide levels compared to ambient (400 pm) conditions. Average daily VOC emissions with elevated CO_2 for 2-undecanone and 2-tridecanone was 17-fold (88 ng g^{-1} fresh weight) and 38-fold (610 ng g^{-1} fresh weight) greater, respectively, on a fresh weight basis on day 30 compared to plants exposed to ambient CO_2.

VOLATILE SULFUR METABOLITES

Based on a method of Mondy et al[12] we have been able to extract and positively identify thiosulfinates produced from onion in a rapid and reproducible manner. The GC analysis time is 40 minutes per analysis and the extraction incubation time for alliinase generation of pyruvate and thiosulfinates is 80 minutes. High percent recovery of thiosulfinates was obtained using a short column at higher temperatures and high carrier gas flow rates. Under the present extraction and analysis procedures, enzyme generated thiosulfinates (methanesulfinothioic acid *S*-1-propyl ester, 1-propanesulfinothioic acid S-1-propyl ester, and the zwiebelane isomer) as well as proposed rearrangement products including dipropyl disulfide and 1-propenyl propyl disulfide are observed. Mass spectral data for some of the sulfur volatile compounds are shown in Fig 7. Nonyl acetate was used as an internal standard since its retention time with GC analysis did not coincide with other peaks found in onion extracts, it yields a sharp, well-defined peak and its volatility is similar to the VOCs present in onion.

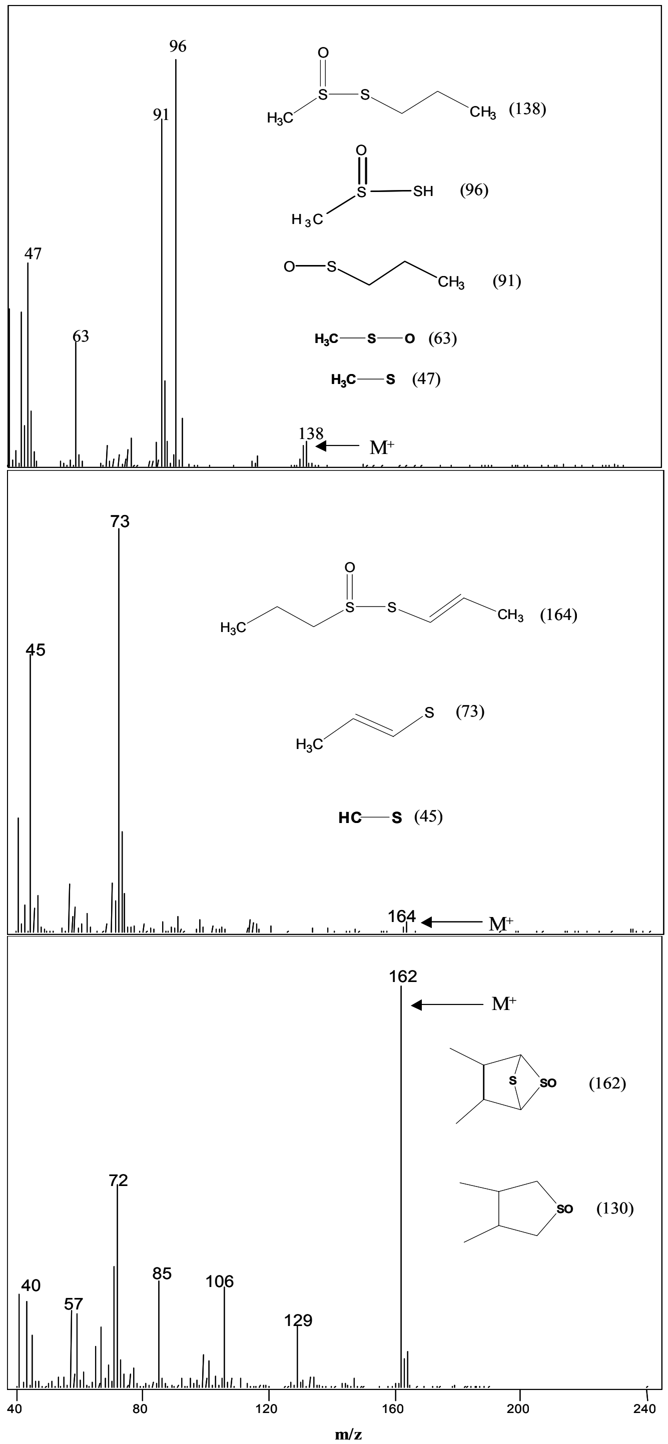


Figure 7 Electron ionization mass spectra of methanesulfinothioic acid S-1-propyl ester [A], 1-propanesulfinothioic acid S-1-propyl ester [B], and zwiebelane isomer [C] collected from onion plant tissue crushed and extracted with CH_2Cl_2.

SENSORY STUDIES

Whole green onions of each cultivar were harvested at 83 dap. The roots were removed at the basal plate and any undesirable (wilted, yellowing, dehydrated) plant material was removed. After the plants were rinsed with tap water, sanitized in a 50-ppm sodium hypochlorite solution and drained, they were chopped into about 0.5-cm portions and mixed with iceberg lettuce in a 1:1 ratio providing about a 5-g "salad" sample. Samples were placed in plastic portion control cups with lids and labeled with 3-digit random numbers. Four consumer panels with 16 to 18 members per panel performed triangle tests, in which elevated CO_2- and ambient-grown onions from each cultivar were presented to panelists. In each triangle test two like samples and one odd sample were presented, and panelists were asked to select the odd sample. In between each triangle test panelists cleansed their palettes with distilled water and unsalted soda crackers. Chi-square analysis at the P<0.05 level was used to determine if the onions grown in the two different CO_2 concentrations tasted or appeared different. Because variable amounts of plant materials were available, the number of respondents ranged from 9 to 18.

Table 1. Chi-square analysis of sensory panel triangle tests of six onions cultivars harvested at 83 dap grown in EGCs under ambient (400 ppm) or elevated CO_2 (1200 ppm).

Cultivar	Number of respondents	Number of correct judgments	alpha at $P \leq 0.05$
'Choesty'	16	10	9
'Kinka'	15	3	9
'Staro'	9	5	6
'Purplette'	15	3	9
'Cal 296'	18	10	10
'Deep Purple'	16	8	9

Results indicate that the sensory quality of some cultivars may be affected by CO_2 levels. 'Choesty' and 'Cal 296' were identified as two cultivars whose sensory attributes were altered by CO_2 levels. Interestingly, 'Cal 296' was not identified in an earlier sensory study (data not shown) as a cultivar affected by CO_2 level. Plants in the two studies were harvested at different dap, thus it is possible that CO_2 level and age interact to affect sensory attributes in some cultivars. Further research will attempt to identify which sensory attributes (pungency, onion flavor intensity, onion flavor aroma, off flavor intensity) seem to be affected by growth at elevated CO_2, to investigate the underlying causes for the modified attributes, and to determine whether changes in quality attributes are detrimental or desirable to the overall acceptance of the onions. Because sulfur components play a key role in onion pungency and flavor we are particularly interested in studying how the production of sulfur products is affected by elevated CO_2 growth conditions. Environmental[13] and genetic factors[14], such as temperature, water availability or stress, stage of development, and genetic background, have already been shown to affect sulfur composition in onion.

ACKNOWLEDGMENTS

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DEFINITIONS, ACRONYMS, ABBREVIATIONS

ACSO S-alk(en)yl-L-cysteine sulfoxides; dap days after planting; EGC Environmental Growth Chamber; ppm parts per million; VOC Volatile Organic Compound.

REFERENCES

[1] Brewster JL (1994) Onion and other vegetable alliums. In “Crop Production Science in Horticulture” Vol. 3 CAB International Wallingford, Oxon UK.

[2] Kendler BS (1987) Garlic (*Allium sativum*) and onion (*Allium cepa*): a review of their relationship to cardiovascular disease. Prev. Med. 16: 670-685.

[3] Jasoni R, Kane C, Green C, Peffley E, Tissue D, Thompson L, Payton P, and Paré PW (2004) Altered leaf and root emissions from onion (*Allium cepa* L.) grown under elevated CO_2 conditions. Environmental and Experimental Botany 51: 273-280.

[4] Shen C, Xiao H, Parkin KL (2002) *In Vitro* stability and chemical reactivity of thiosulfinates. J. Agric. Food Chem. 50: 2644-2651.

[5] Kiss JZ, Edelmann RE, Wood PC (1999) Gravitropism of hypocotyls of wild-type and starch–deficient mutants of Arabidopsis seedlings in spaceflight studies. Planta 209: 96-103.

[6] Kiss Z, Brinckmann Brillouet C (2000) Development and growth of several strains of Arabidopsis seedlings in microgravity. Intl J. Plant Sci. 161: 55-62

[7] Krikorian AD, Levine HG (1991) Development and growth in space. Pages 491-555 in FC Steward, ed. Plant Physiology: a treatise. Vol. 10 Academic Press, Orlando, FL.

[8] Ellison, A. M. (1987) Density-dependent dynamics of *Salicornia europaea* monocultures. Ecology 68: 737-741

[9] Egli DB (1988) Plant density and soybean yield. Crop Science 28: 977-981

[10] Ballare CL., Scopel AL, Jordan ET, Vierstra RD (1994) Signaling among neighboring plants and the development of size inequalities in plant populations. Proc Natl Acad Sci USA 91: 10094-10098

[11] Harper, JL (1977) Population Biology of Plants Academic, London

[12] Mondy N, Duplat D, Christides JP, Arnault I, Auger J (2002) Aroma analysis of fresh and preserved onion and leek by dual solid-phase microextraction-liquid extraction and gas chromatography-mass spectrometry. J Chrom. A 963: 89-93.

[13] Lancaster JE, Farrant J, Shaw ML (2001) Sulfur nutrition affects cellular sulfur, dry weight distribution, and bulb quality in onion. J Am Soc Hort Sci 126: 164-168

[14] McCallum JA, Pither-Joyce M, Shaw M (2002) Sulfur deprivation and genotype affect gene expression and metabolism of onion roots. J. Am Soc Hort Sci 127: 583-589