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EFFECT OF CROP DEVELOPMENT ON BIOGENIC EMISSIONS FROM PLANT POPULATIONS GROWN IN CLOSED PLANT GROWTH CHAMBERS

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Key Word Index—*Triticum aestivum*; Gramineae; wheat; *Lactuca sativa*; Compositae; lettuce; volatiles; furan; 2-methylfuran; carbon disulphide; dimethylsulphide; isoprene; α-pinene; 2-ethyl-1-hexanol; 1-butanol; nonanal; benzaldehyde; tetramethylurea; tetramethylthiourea.

Abstract—The Biomass Production Chamber at John F. Kennedy Space Center is a closed plant growth chamber facility that can be used to monitor the level of biogenic emissions from large populations of plants throughout their entire growth cycle. The head space atmosphere of a 26-day-old lettuce (*Lactuca sativa* cv. Waldmann's Green) stand was repeatedly sampled and emissions identified and quantified using GC-mass spectrometry. Concentrations of dimethyl sulphide, carbon disulphide, α-pinene, furan and 2-methylfuran were not significantly different throughout the day; whereas, isoprene showed significant differences in concentration between samples collected in light and dark periods. Volatile organic compounds from the atmosphere of wheat (*Triticum aestivum* cv. Yecora Rojo) were analysed and quantified from planting to maturity. Volatile plant-derived compounds included 1-butanol, 2-ethyl-1-hexanol, nonanal, benzaldehyde, tetramethylurea, tetramethylthiourea, 2-methylfuran and 3-methylfuran. Concentrations of volatiles were determined during seedling establishment, vegetative growth, anthesis, grain fill and senescence and found to vary depending on the developmental stage. Atmospheric concentrations of benzaldehyde and nonanal were highest during anthesis, 2-methylfuran and 3-methylfuran concentrations were greatest during grain fill, and the concentration of the tetramethylurea peaked during senescence.

INTRODUCTION

The Biomass Production Chamber (BPC) at John F. Kennedy Space Center, FL is a 113 m^3 closed chamber constructed to investigate the feasibility of growing food crops in enclosed environments for human life support [1]. When sealed, atmospheric volatiles from large populations of plants can be monitored throughout entire growth cycles. Determining whether volatiles reach levels toxic to plant growth in closed environments will be critical for assessing bioregenerative life support approaches for long duration space missions [1].

Previous work in monitoring plant volatiles has typically examined emissions from small populations of plants [2], a single point in the growth cycle [3], cut plants [4–7], macerated plants [4, 8, 9], vacuum steam distillates [10] or organic extractions [11, 12]. These techniques offer the advantage of eliminating background emissions (from chamber materials) and adsorption on to surfaces but are limited in that they do not reflect the overall emissions from, or exposure associated with, growing plant populations. The BPC can be considered a large sampling cuvette for identifying the volatile organic compounds (VOCs) from intact stands of plants with non-evasive sampling techniques.

In this report, we present data on the emissions from a 20 m^2 lettuce crop (192 plants) at full canopy coverage and a wheat crop (approximately 25 600 plants) throughout growth and development, including seedling establishment, vegetative growth, anthesis, grain fill and senescence.

RESULTS AND DISCUSSION

The BPC is constructed from a variety of materials, many of which also emit volatile organic compounds (VOCs) into the atmosphere. In order to separate the biogenic and chamber-related VOCs, atmospheric samples were collected from the BPC and analysed before plants were in the chamber. Compounds such as dichloromethane, 1,1,2-trichloro-1,2,2-trifluoroethane, methylbenzene, hexamethylcyclotrisiloxane and octamethylcyclotetrasiloxane are among those that were consistently defected in the BPC atmosphere when plants were not present. A detailed analysis of the non-biogenic VOC constituents in the BPC has been reported [13]. The biogenic compounds dealt with here were selected for discussion based on their absence in the BPC prior to planting, quality of the mass spectra and structural diversity.

Lettuce

Lettuce is well suited to hydroponic culture and has been extensively studied as a Controlled Ecological Life Support System (CELSS) crop in the BPC [14]. Waldmann's Green is a leaf lettuce which is typically harvested between 28 and 30 days after planting (DAP). At harvest, 95% of the dry mass is edible (leaves). Lettuce is considered horticulturally mature when in vegetative development and is harvested prior to either reproductive or physiological maturity. Because there were only vegetative structures present and the mass of the lettuce was greater late in the study, the atmosphere was intensely sampled on a single day late in the crop growth cycle when the lettuce was near full canopy. A series of atmospheric samples was collected on 26 DAP (four days prior to harvest), at which time the lettuce was in a stage of rapid vegetative growth and sufficient plant mass was present to give biogenic gas resolution. This permitted quantitative evaluation of the differences in volatile emission concentrations during the light and dark cycles. Previous studies have indicated that there can be significant differences in volatile emission between light and dark samples [2]. Additionally, intensive sampling during this strictly vegetative phase allows the precision of the analysis to be quantified without developmental differences in biogenic emissions being a factor. Two representative compounds of the sulphides (carbon disulphide and dimethylsulphide), the terpenes (isoprene and α-pinene) and the furans (furan and 2-methylfuran) were selected for quantification (Table 1).

Chamber concentrations of carbon disulphide and dimethylsulphide averaged 0.69 and 0.94 nmole mole^{-1} air and the standard errors as percents of the means were 2.8% and 8.2% respectively for the seven samples. Student's *t*-test results indicate that there was no significant difference between the atmospheric concentrations between the light and dark cycles. Dimethylsulphide and other sulphur derivatives have been reported as volatile components of *Brassica napus*, *B. campestris*, *B. juncea* [4], *B. oleracea* [12], *Scorzonera hispanica* [15] and *Azadirachta indica* [16].

The average concentrations of isoprene and α-pinene were 1.18 and 0.064 nmol mol^{-1} and the standard errors as percents of the means were 9.3% and 10.9%, respectively. Student's *t*-test results suggest that there was no significant difference in α-pinene concentration between the light and dark cycle, but there was a significant increase in the concentration of isoprene during the dark cycle. The average isoprene concentrations in samples collected during the light and dark cycles were 0.982 and 1.44 nmol mol^{-1}, respectively. Both isoprene and α-pinene are commonly reported in head-space analysis of vegetation. Isoprene and monoterpenes are characteristic VOCs in forest canopies [17], and α-pinene has been reported as a volatile in a variety of plant species [3, 4, 18].

Furan and 2-methylfuran were detected at average concentration levels of 2.47 and 0.103 nmol mole^{-1} air and the standard errors as percents of the means were 7.7% and 26%, respectively. Student's *t*-test results indicate that there was no significant difference between the atmospheric concentrations for the light and dark cycles. Furan or its derivatives have been reported in the head-space of *Brassica oleracea* [12], *Scorzonera hispanica* [15] and *Cicer arietinum* [19].

These results indicate that the concentrations of VOCs observed from lettuce are relatively insensitive to light/dark cycling. The exception was isoprene which increased 40% during the dark cycle. The air-handling systems provide three to four air cyclings per minute, suggesting that these values are realistic estimates of the atmospheric VOC concentration during the sampling period. It should be noted that absolute emission rates by the plants would be underestimated with this procedure since sampling losses associated with adsorption on to chamber surfaces are not accounted for.

Wheat

In addition to the screening methods used with the lettuce, head-space analyses [20] of BPC-grown wheat stems, leaves (photosynthetic and senescent), roots and heads (if present) were performed on days 24, 56, 65 and 82 (Table 2) in order to confirm that the VOCs to be dealt with here were of biogenic origin. The photosynthetic leaves were not analysed on day 82, because most were senescent at this point. The head-space analyses of the

Table 1. Atmospheric concentrations (nmole VOC mol^{-1} air) of volatile organic compounds from a 26-day-old lettuce stand during light and dark cycles

Compound	R_T min	Dark (Mean ± se)*	Light (Mean ± se)	T-test
Furan	5.85	2.18 ± 0.14	2.61 ± 0.26	NS†
Isoprene	6.18	1.43 ± 0.13	0.98 ± 0.04	‡
Dimethylsulphide	6.52	0.94 ± 0.07	0.94 ± 0.13	NS
Carbon disulphide	6.83	0.69 ± 0.06	0.68 ± 0.02	NS
2-Methylfuran	10.2	0.070 ± 0.011	0.130 ± 0.040	NS
α-Pinene	22.2	0.073 ± 0.016	0.057 ± 0.006	NS

*Dark cycle $n = 3$, light cycle $n = 4$.
†Non-significant.
‡Significant at $P = 0.01$.

Table 2. Volatile organic compounds detected in head-space analysis of BPC grown wheat stems, leaves and heads

Compound	Photo-synthetic leaves	Senescent leaves	Stems	Heads
1-Butanol	56, 65*	82		56, 65
2-Ethyl-1-hexanol		56, 82		65
Nonanal		56, 82	24, 82	
2-Methylfuran				56, 65, 82
Tetramethylurea	56		56, 65	
Tetramethylthiourea	56		56, 65	

*Day after planting that compounds were detected.

plant tissues resulted in the identification of many compounds that were not detected in the BPC atmosphere. This was probably due to the fact that the tissues were cut during sampling and the surface to volume ratio was much higher in the head-space analysis vessels. Although benzaldehyde and 3-methylfuran were not detected in any of the head-space analyses of the wheat tissue, these compounds were monitored and reported because they were detected in the BPC atmosphere only when a population of wheat plant was present.

Wheat is a crop with very well-described developmental stages [21] and for the purposes of this study, the following stages were defined: seedling establishment, vegetative growth, anthesis, grain fill and senescence. Seedling establishment (0–10 DAP) was characterized by seed imbibition and swelling, germination, coleoptile emergence and early shoot growth. Canopy photosynthetic rates were low and nutrient uptake was limited during seedling establishment. Vegetative growth (11–32 DAP) was characterized by rapid increases in plant height and leaf area (data not shown). The induction of reproductive structures occurred during this period, and both canopy photosynthetic rates and nutrient assimilation rates were high. Anthesis is when ovule fertilization occurs. In this study, the anthesis period (33–40 DAP) was defined as occurring in the canopy between the first appearance of flowering spikes (heading) in the crop and the first appearance of grain. It is assumed that this time range will account for developmental variability in anthesis. During the anthesis period canopy photosynthetic rates were high and nutrient uptake declined. Grain fill (41–60 DAP) was characterized by lack of vegetative growth, high chlorophyll content of grain heads and rapid increase in the dry matter of grain. During grain fill, canopy photosynthetic rates were high and nutrient uptake rates were low. Senescence (61–84 DAP) is defined here as 'ripening' of the grain and was characterized by a decline in vegetative dry mass, suggesting nutrient transport from vegetative to reproductive structures. During senescence, photosynthetic rates declined, nutrient uptake was low, chlorophyll degraded in the seed heads and seeds began to dry [22].

Atmospheric concentrations of the VOCs (nmol VOC per mol air $\pm$ s.e.) inside the BPC during each of the development periods are given in Table 3. The total amounts of each VOC (based on 5.6×10^4 l air per chamber) per unit dry mass of the whole plant and by different plant parts (based on an estimated 1.25×10^4 plants per chamber) are given in Fig. 1. Dry mass data of whole plant harvests (leaves, stem, roots and heads) over time were used to calculate values on a total plant mass basis for each of the compounds. Based on the results of the head-space analyses of the different plant tissues, dry mass data of the leaves and stems were used to calculate the tissue values for the aldehydes, alcohols and ureas; dry mass data of the heads were used to calculate the tissue values for the methylfurans.

The VOCs of biogenic origin were not detected in the BPC during seedling establishment. This is probably due to the high volume to biomass ratio during this period. 1-Butanol was at its highest atmospheric concentration in chamber 1 during anthesis, but the concentration in chamber 2 peaked during vegetative growth. Wheat heads appeared three days earlier in chamber 2, which indicates that the plant growth was slightly accelerated in chamber 2 and this may account for the concentation differences. The atmospheric concentration during vegetative growth, grain fill and senescence of 1-butanol in chamber 1 was about 20% of the 1-butanol concentration during anthesis. The amount of 1-butanol normalized to plant mass indicated that the production was highest during anthesis in chamber 1 and during vegetative growth in chamber 2.

2-Ethyl-1-hexanol concentrations were highest in the BPC atmospheres during the vegetative growth and anthesis periods. The atmospheric concentrations dropped about 60% after anthesis and remained at a basal level during grain fill and senescence. The amount of 2-ethyl-1-hexanol normalized for standing plant mass suggests that production was highest during vegetative growth. The mass-normalized production dropped sharply during anthesis and continued to decline during grain fill.

Nonanal was first detected in chamber 1 of the BPC during vegetative growth and in chamber 2 during anthesis. There was 22% less leaf mass on DAP 32 in chamber 2, which may have contributed to the concentration differences observed in the atmosphere. Nonanal was at

Table 3. Concentration (nmol mol^{-1} air) of volatiles detected in the wheat crop atmosphere

Period*	Retention time (min)	Chamber 1	Chamber 2
		1-Butanol	
VG	13.9	0.051 ± 0.017†	0.016 ± 0.023
A		0.34 ± 0.12	0.074 ± 0.034
GF		0.078 ± 0.010	0.011 ± 0.019
S		0.063 ± 0.006	0.076 ± 0.007
0		*2-Ethyl-1-hexanol*	
VG	30.3	0.24 ± 0.02	0.14 ± 0.03
A		0.16 ± 0.02	0.14 ± 0.04
GF		0.064 ± 0.008	0.044 ± 0.011
S		0.084 ± 0.011	0.067 ± 0.010
		Nonanal	
VG	33.1	0.014 ± 0.002	ND‡
A		0.025 ± 0.007	0.017 ± 0.007
GF		0.009 ± 0.002	0.009 ± 0.001
S		0.013 ± 0.003	0.010 ± 0.001
		Benzaldehyde	
VG	27.2	5.1 ± 1.4	3.5 ± 0.2
A		10. ± 2	6.7 ± 0.4
GF		4.1 ± 0.9	4.4 ± 1.1
S		5.0 ± 0.6	2.4 ± 0.3
		2-Methylfuran	
VG	8.3	ND	ND
A		0.12 ± 0.030	0.058 ± 0.020
GF		028 ± 0.067	0.16 ± 0.03
S		0.17 ± 0.021	0.10 ± 0.03
		3-Methylfuran	
VG	8.0	ND	ND
A		0.15 ± 0.02	0.052 ± 0.014
GF		0.22 ± 0.05	0.12 ± 0.04
S		0.10 ± 0.03	ND
		Tetramethylurea	
VG	29.5	2.7 ± 0.4	1.3 ± 0.7
A		1.6 ± 0.5	1.5 ± 0.1
GF		1.2 ± 0.1	0.58 ± 0.46
S		4.8 ± 0.9	2.8 ± 1.2
		Tetramethylthiourea	
VG	38.8	0.35 ± 0.05	ND
A		0.27	ND
GF		096 ± 0.48	0.56 ± 0.09
S		1.1 ± 0.3	0.66 ± 0.32

*Period: VG-vegetative growth, A-anthesis, GF-grain fill, S-senescence.

†s.e.

‡Not detected. Limits of detection (nmol mole^{-1} air): 1-butanol, 0.014; 2-ethyl-1-hexanol, 0.039; nonanal, 0.006; benzaldehyde, 0.010; 2-methylfuran, 0.10; tetramethylurea, 0.18; and tetramethylthiourea, 0.14.

highest atmospheric concentrations during the anthesis period in both chambers. The atmospheric concentrations dropped about 50% after anthesis and remained at this level until harvest. The amount of nonanal in the atmosphere normalized for standing plant mass suggests that the highest production occurred during vegetative growth and anthesis. After anthesis the mass-normalized production dropped by more than 60%. Nonanal has been previously reported as a volatile component of wheat stems and leaves [5, 8].

Benzaldehyde was first detected in the BPC during vegetative growth. The atmospheric concentration nearly doubled during anthesis then dropped to pre-anthesis concentrations during grain fill and senescence. The amount of benzaldehyde normalized for standing plant mass indicates that during grain fill and senescence production was about 25–35% of the pre-anthesis levels. This suggests that the mass-normalized production of benzaldehyde was greatest during periods of active vegetative growth and a basal level was maintained through

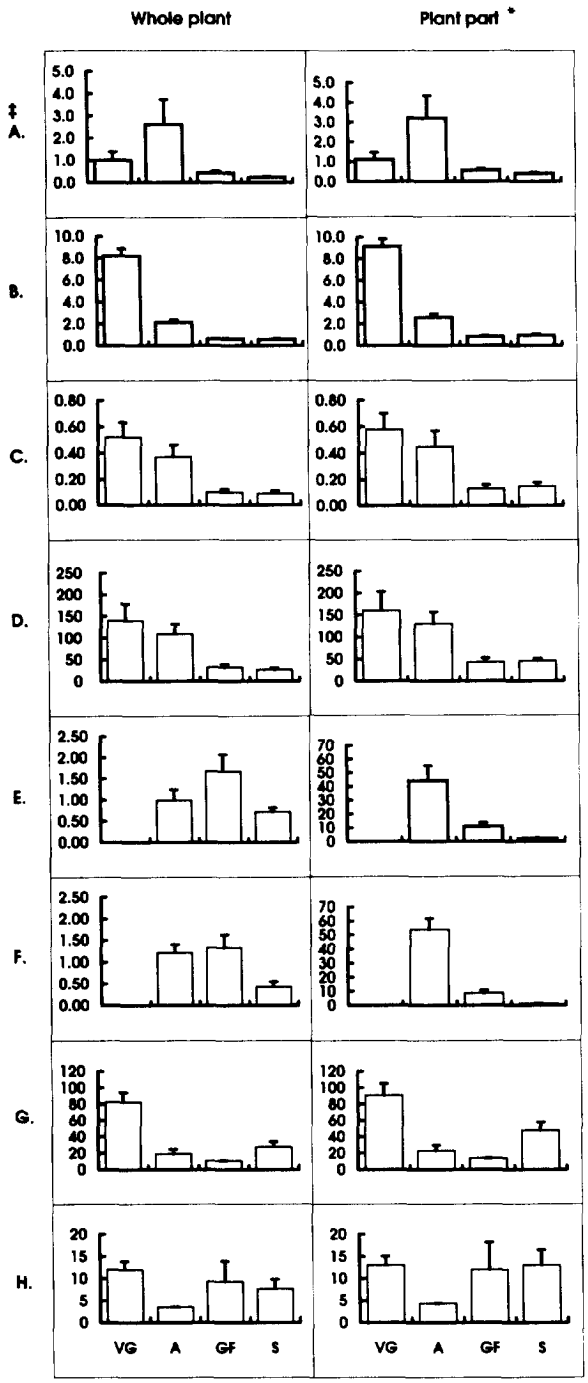

Fig. 1. Total atmospheric volatiles per dry mass ($\mu g\,kg^{-1}$) of the whole plants and specific plant tissue* during growth and development of wheat (chamber 1). *Dry mass of the heads was used to calculate the plant part value for 2- and 3-methylfuran; all others were calculated based on the dry mass of the leaves and stems. †Period: VG-vegetative growth, A-anthesis, GF-grain fill, S-senescence. ‡a = 1-butanol, b = 2-ethyl-1-hexanol, c = nonanal, d = benzaldehyde, e = 2-methylfuran, f = 3-methylfuran, g = tetramethylurea, h = tetramethylthiourea.

grain fill and senescence. Benzaldehyde has previously been reported as a volatile constituent of marcerated wheat leaves and stems [8].

No methylfurans were detected prior to head emergence. The methylfurans were first detected in the BPC during anthesis, with the total amount of the methylfurans per unit mass of the head tissue being highest in both chambers during this stage. Absolute concentrations of these compounds in the atmosphere increased following anthesis and were greatest during the grain fill period. On DAP 58 the mass of the grain and chaff was 5 and 50%, respectively, of their mass at harvest, suggesting that the methylfuran compounds were emitted from the growing chaff portion of the head. The concentration in the atmosphere and head mass-normalized production decreased during senescence. 3-Methylfuran was not detected during senescence in chamber 1, which may be related to greater photosynthetic rate decline (0.74 versus 0.34 $\mu mol\,m^{-2}\,s^{-1}$ per day for chambers 1 and 2, respectively) in the wheat in that chamber. Rembold *et al.* [18] have previously reported 2-methylfuran and other alkyl substituted furans in the headspace of chickpea seeds.

Urea compounds were detected in the BPC atmosphere during the vegetative growth period, with the exception of tetramethylthiourea in chamber 2, which was not detected until grain fill. The atmospheric concentration of tetramethylthiourea varied throughout the crop cycle in both chambers. Atmospheric concentrations of tetramethylurea were highest during senescence. However, the levels of these ureas normalized for standing plant mass were greatest during vegetative growth. The mass-normalized production of tetramethylurea decreased 20–35% during anthesis and grain fill but then increased three-fold during senescence. Mass-normalized concentrations of tetramethylthiourea in chamber 1 decreased during anthesis and then increased to 70% of pre-anthesis levels during grain fill and senescence. Urea compounds have not been previously reported as plant volatiles in the literature; however, few address plants during senescence. A review of urea metabolism in plants has been presented by Reinbothe and Mothes [23].

The results indicate that volatile emissions from wheat vary with the stages of plant development. The alcohol, aldehyde and urea compounds studied were detected in the BPC atmosphere during all the developmental periods, with the exception of seedling establishment. The aldehydes showed a significant atmospheric increase during anthesis, while the atmospheric concentrations of the ureas increased during senescence. The methylfurans were not detected until after the emergence of grain heads.

Because the crops were grown in soilless conditions using the nutrient film hydroponic technique, the biogenic VOCs could not have originated from soil-borne microorganisms. Further, since VOCs were analysed from a well-mixed atmosphere supporting a large plant population, sampling variation associated with short-term sampling from a limited canopy area is eliminated. The concentrations of several compounds are near the detection limit of the instrument because of the relatively

large head-space in the chamber (53 000 l). Since the original intent of the experiment was to determine the composition and concentration of biogenic VOCs which may have biological activity, no attempt was made to optimize sampling conditions to determine the absolute rate of VOC production. The VOC concentration, expressed as $\mu g\,kg^{-1}$ DM, provides a minimum production required by the plant population to have achieved the concentration in the chamber. Where possible, similar data are provided for the likely tissue of origin for a specific VOC in order to provide a baseline for VOC production from plant tissues.

These compounds may provide readily monitored, non-destructive developmental markers for assessing crop development and physiology in closed environments. For example, nonanal is a candidate compound for determining on-set of heading and elevated concentrations of urea derivatives may be indicators of senescence. No physiological damage or injury problems were apparent in either the lettuce or wheat, suggesting that the levels of VOCs measured in this study were not toxic to the plants.

EXPERIMENTAL

Plant growth chamber. The BPC is a two-chamber, atmospherically closed chamber that has a total volume of 113 m^3. Each chamber has two plant growth shelves that hold 16 0.25 m^2 hydroponic trays. A total of 20 m^2 of photosynthetic area is used as the growing area in order to correct for canopy edge effects. Lighting is provided by 96 400-W high-pressure sodium lamps, with air circulation and temp. control provided by two 30-kW blowers and two 53-kW chilling units. The air-handling units circulate the atmosphere at 200 $m^3\,min^{-1}$. This is equivalent to 3.7 air exchanges per min. The BPC atmospheric leakage, as determined by CO_2 decay curves without plants present [24], was approximately 10% of the chamber volume per day (0.4% volume hr^{-1}) with most of the leakage associated with areas of large pressure differences, for example before and after each of the 2 30-kW blowers for air circulation and episodes of diurnal temperature change [25]. This rate of leakage from a 113 m^3 BPC compares favourably with much smaller closed plant growth systems (2.4 m^3 or less), which have ranged in leakage from 4% volume day^{-1} [26] to 50–240% volume day^{-1} [27] and has allowed the system to be used for 'closed' gas exchange measurements of the 20 m^2 canopy [25].During the wheat study, $KMnO_4$ filters were placed in chamber 2 of the BPC to reduce the concentration of ethylene [28]. The $KMnO_4$ filters could reduce the concentration of selected hydrocarbons through either reaction or adsorption. The high rate of air mixing in the BPC should ensure that VOC concentrations reported are conservative estimates of canopy level exposures.

Plant growth. Lettuce (*Lactuca sativa* L. cv. Waldmann's Green) plants (192) were grown in chamber 1 of the BPC. Lettuce seeds were planted at day 0 and maintained under translucent plastic germination covers for 4 days. The essential nutrients were provided using a recirculating nutrient film culture technique (pH 5.8, electrical conductivity 0.12 $S\,m^{-1}$). Air and nutrient solution temps were maintained at 23° during the light and dark cycles. The lamps were cycled each day to provide 16 hr light and 8 hr dark (22:00 hrs to 06:00). Irradiance at the top of the plant canopy averaged 318 $\mu mol\,m^2\,sec^{-1}$ photosynthetic photon flux on the day of sampling. Relative humidity was controlled to 85% the first week and then maintained at 70%. CO_2 concn was maintained at 1200 $\mu mol\ CO_2\ mol^{-1}$ during the light cycle. Additional experimental details have been published previously [14].

Wheat (*Triticum aestivum* cv. Yecora Rojo) plants were grown from seed with a planting density of approximately 1250 plants per m^2 (1.25×10^4 plants per chamber). The essential nutrients were provided using a recirculating nutrient film culture (pH 5.8, electrical conductivity 0.12 $S\,m^{-1}$). Air temps were maintained at 24° in the light and 16° in the dark for the first 20 days, after which the temp. was lowered to 20° (light) and 16° (dark). The lamps were cycled each day to provide 20 hr light and 4 hr dark. Irradiance at the top of the plant canopy averaged 930 $\mu mol\,m^{-2}\,sec^{-1}$ photosynthetic photon flux throughout the experiment. Relative humidity was controlled to 75% and CO_2 conc to 1000 $\mu mol\ CO_2\ mol^{-1}$. Additional experimental details have been reported previously [29].

Crop development. Lettuce growth was monitored through cumulative CO_2 fixation. Full canopy coverage was obtained on the 25th DAP. Plants were harvested at 30 DAP and fresh and dry mass determined. Average head (shoot) fresh weight was 192 g per plant.

The wheat growth was monitored through cumulative CO_2 fixation and by harvesting two rows of wheat plants from two growing trays on 11-day intervals from each of the two growing shelves of each chamber of the BPC. The number of plants was recorded and tissues sepd into roots, stems, leaves chaff, and seed where appropriate. The tissues were dried at 70° in a forced-air oven for 72 hr, and dry mass determined.

Atmospheric sampling. Atmospheric samples were collected in 16 l passivated stainless steel canisters [30] from sampling ports on the low pressure side of the air-handling system. During the lettuce study, air samples were collected on day 26 of the planting. Seven samples were collected throughout the day at 08:15, 17:15, 19:15, 21:15, 22:35, 22:30 and 23:30 hrs. During the wheat study, air samples were collected approximately 2 hr after the lamps came on each morning. The numbers of samples taken per chamber during each period were as follows: seedling establishment, 4; vegetative growth, 10; anthesis, 4; grain fill, 8; and senescence, 13.

GC-MS. The air analyses were carried out by concentrating 10 l samples that were collected in clean, passivated 16 l stainless steel canisters on to a multibed graphitized carbon-adsorbent trap at the rate of 100 $ml\,min^{-1}$. The canister and pumping system were checked for cleanliness and background levels by filling the canisters with ultra pure N_2 and analysing the atmo-

sphere according to modified EPA method TO14. The adsorbent trap was interfaced through a cryofocusing unit to a GC-MS [30]. During the lettuce study the GC-MS was equipped with a 30 m × 0.25 mm DBVRX™ bonded phase (1.4 μm, film) column. The temp. was programmed from 0° to 220° at 6° min^{-1}. The carrier gas (He) flow rate was 1.8 ml min^{-1}. During the wheat study the GC was equipped with a 30 m × 0.596 mm DB™ 624 bonded phase (3 μm film) column and a jet separator. The temp. was programmed from 0° to 200° at 3° min^{-1}. The carrier gas (He) flow rate was 4 ml min^{-1}. The detector and injector temps were 250°. The ionization voltage of the MS was 70 eV.

Identification and quantification. Compound identification was confirmed by comparison of mass spectral data and retention time comparison to verified standards (with the exception of 3-methylfuran, furan and dimethylsulphate for which no standards were available for retention time comparison). Quantification was achieved with the external standard method using a 5-point calibration curve. Continuing calibration standards were analysed daily in order to confirm that the efficiency of the adsorbent trap and the stability of GC-MS were constant. A list of the 80 standards and their detection limits have been previously published [13]. The detection limit is defined as three times the signal/noise ratio. An estimation of the concentration of compounds for which standards were not available was achieved by comparison of the response of a standard with similar organic functional groups.

Head-space analysis. Head-space analysis was carried out by placing the specified plant part into a purge vessel that was on-line to the adsorbent trap–GC-MS system described for the atmospheric analysis [20]. Helium was passed through the sample vessel at 40 ml min^{-1} for 60 min for sampling. Samples were analysed at ambient temp.

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REFERENCES

1. Prince, R. P., Knott, W. M., Sager, J. C. and Hilding, S. E. (1987) *Soc. Auto. Eng. Tech.* Paper No. 87, 1437.
2. Loughrin, J. H., Hamilton-Kemp, T. R., Anderson, R. A. and Hildebrand, D. F. (1990) *Phytochemistry* **29**, 2473.
3. Lwande, W., McDowell, P. G., Amiani, H. and Amoke, P. (1989) *Phytochemistry* **28**, 421.
4. Tollesten, L. and Bergstrom, G. (1988) *Phytochemistry* **27**, 4013.
5. Buttery, R. G., Xu, C. and Ling, L. C. (1985) *J. Agric. Food Chem.* **33**, 115.
6. Hamilton-Kemp, T. R., Loughrin, J. H. and Anderson, R. A. (1990) *Phytochemistry* **29**, 2874.
7. Sutton, C. J., Keegans, S. J., Kirk, W. D. J. and Morgan, E. D. (1992) *Phytochemistry* **31**, 3427.
8. Hamilton-Kemp, T. R. and Anderson, R. A. (1984) *Phytochemistry* **23**, 1176.
9. Hatanaka, A. and Harada, T. (1973) *Phytochemistry* **12**, 2341.
10. Takeoka, G. R., Flath, R. A., Güntert, M. and Jennings, W. (1988) *J. Agric. Food. Chem.* **36**, 553.
11. Potter, T. L. and Fagerson, I. S. (1990) *J. Agric. Food Chem.* **38**, 2054.
12. MacLeod, G. and MacLeod, A. J. (1990) *Phytochemistry* **29**, 1183.
13. Batten, J. H., Peterson, B. V., Berdis, E. and Wheeler, R. M. (1993) *NASA Tech. Memo.* **109192**. Kennedy Space Center, FL.
14. Wheeler, R. M., Mackowiak, C. L., Sager, J. C., Knott, W. M. and Berry, W. L. (1994) *J. Amer. Soc. Hort. Sci.* **119**, 610.
15. MacLeod, G. and Ames, J. M. (1991) *Phytochemistry* **30**, 883.
16. Mubarak, A. M. and Kulatilleke, C. P. (1990) *Phytochemistry* **29**, 3351.
17. Hoffmann, T., Jacob, P., Linschied, M. and Klockow, D. (1993) *Intern. J. Environ. Anal. Chem.* **52**,
18. Ruberto, G., Biondi, D. and Piatelli, M. (1991) *Flav Fragr. J.* **6**, 121.
19. Rembold, H., Wallner, P., Nitz, S.,. Kollmannsberger, H. and Drawert, F. (1989) *J. Agric. Food Chem.* **37**, 659.
20. Berdis, E., Peterson, B. V., Yorio, N. C., Batten, J. H. and Wheeler, R. M. (1993) *NASA Tech. Memo.* **109199**. Kennedy Space Center, FL.
21. Simmons, S. R. (1987) in *Wheat and Wheat Improvement* (Hayne, E. G., ed.), 2nd edn., p. 77. CSSA, Madison.
22. Wheeler, R. M., Cory, K. A., Sager, J. C. and Knott, W. M. (1993) *Crop Sci.* **33**, 161.
23. Reinbothe, H. and Mothes, K. (1962) *Ann. Rev. Plant Physiol.* **13**, 129.
24. Acock, B. and Acock, M. C. (1989) *Agron. J.* **81**, 619.
25. Wheeler, R. M. (1992) *Hortscience* **27**, 777.
26. Schwartzkopf, S. H. and Stophan, P. E. (1981) *Amer. Soc. Mech. Eng.* Publ. 81-ENAs-37.
27. Acock, B., Charles-Edwards, D. A. and Hearn, A. R. (1977) *Ann. Bot.* **41**, 41.
28. Wheeler, R. W., Peterson, B. V., Mackowiak, C. L. and Stutte, G. W. (1993) *HortScience.* **28**, 466.
29. Stutte, G. W., Mackowiak, C. L., Markwell, G., Sager, J. C. and Wheeler, R. W. (1993) *NASA Tech. Memo.* **109191**. Kennedy Space Center, FL.
30. Stephens, J. and Myron, H. (1990) EPA Air and Waste Management International Symposium on Measurement of Toxic and Related Air Pollutants, U.S. EPA Region IV College Station, Athens, GA.