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Elevated CO_2 changes the interactions between nematode and tomato genotypes differing in the JA pathway

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ABSTRACT

Interactions between the root-knot nematode *Meloidogyne incognita* and three isogenic tomato (*Lycopersicon esculentum*) genotypes were examined when plants were grown under ambient (370 ppm) and elevated (750 ppm) CO_2. We tested the hypothesis that, defence-recessive genotypes tend to allocate 'extra' carbon (relative to nitrogen) to growth under elevated CO_2, whereas defence-dominated genotypes allocate extra carbon to defence, and thereby increases the defence against nematodes. For all three genotypes, elevated CO_2 increased height, biomass, and root and leaf total non-structural carbohydrates (TNC):N ratio, and decreased amino acids and proteins in leaves. The activity of anti-oxidant enzymes (superoxide dismutase and catalase) was enhanced by nematode infection in defence-recessive genotypes. Furthermore, elevated CO_2 and nematode infection did not qualitatively change the volatile organic compounds (VOC) emitted from plants. Elevated CO_2 increased the VOC emission rate only for defence-dominated genotypes that were not infected with nematodes. Elevated CO_2 increased the number of nematode-induced galls on defence-dominated genotypes but not on wild-types or defence-recessive genotypes roots. Our results suggest that CO_2 enrichment may not only increase plant C : N ratio but can disrupt the allocation of plant resources between growth and defence in some genetically modified plants and thereby reduce their resistance to nematodes.

Key-words: elevated CO_2; jasmonic acid; *Meloidogyne incognita*; tomato mutants; volatile organic compounds.

INTRODUCTION

Atmospheric CO_2 levels are increasing rapidly and is expected to double in the next century (IPCC 2007). The global increase of atmospheric CO_2 directly affects the physiology of plants and generally accelerates the photosynthetic rate and increases plant growth, yield and carbon- : nitrogen (C : N) ratio (Agrell, McDonald & Lindroth 2000; Jablonski, Wang & Curtis 2002). Elevated CO_2 has caused plants to re-allocate carbon and nitrogen resources among tissues, and to alter the synthesis of nutrition and secondary metabolites in plant tissue and chemical components in root exudates (Hartley *et al.* 2000; Allard *et al.* 2006; Phillips, Fox & Six 2006). These changes may cascade to influence the interactions between nematodes and their host plants (Yeates & Newton 2009).

The response of plants to elevated CO_2 varies among species (Bezemer & Jones 1998) and genotypes (Goverde *et al.* 1999; Lindroth, Roth & Nordheim 2001), but few studies can explain why plant genotypes that have very high similarities in their genetic background still responded differently to elevated CO_2. Li *et al.* (2008) identified and compared transcriptional changes of two ecotypes of *Arabidopsis thaliana* in response to elevated CO_2, which indicated that the imbalance in the C : N plant metabolism is one main driver for changes in gene expression and metabolism, causing the genotype-specific response to elevated CO_2. Thus, plant genotypes may not only have different carbon assimilation but also have different partitioning patterns between growth and defence in responding to elevated CO_2 (Cseke *et al.* 2009).

Most studies addressing how plant–herbivore interactions are affected by elevated CO_2 have focused on aboveground herbivory (Bezemer & Jones 1998; Chen *et al.* 2005b; Wu, Chen & Ge 2006). Few studies, however, have considered how CO_2 affects plant resistance against root-feeding nematodes. Yeates & Newton (2009) found that elevated CO_2 increased population abundance of root-feeding nematodes, microbial-feeding nematodes and predacious nematodes in soil, suggesting that elevated CO_2 might modify the interaction between nematodes and their host plants. Furthermore, as predicted from the Carbon Nutrient Balance (CNB) hypothesis (Hamilton *et al.* 2001), elevated CO_2 results in accumulation of excess carbon in plant tissues, which is probably allocated to more carbon-based secondary metabolites such as terpenes, sesquiterpenes and phenolics. On the other hand, elevated CO_2 increases the plant photosynthetic capacity and root growth, as well as changes the nutrient level of plant tissue. Thus, the performance of root-feeding nematodes would logically be changed under elevated CO_2.

The root-knot nematode, *Meloidogyne incognita*, is a soil-dwelling, microscopic nematode that parasitizes roots and feeds exclusively on the cytoplasm of living plant cells. (Davis, Hussey & Baum 2004). Plant reaction to *M.*

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incognita includes the presence of galls on infested plants, which may also have secondary infections by other organisms (Jasmer, Goverse & Smant 2003). This nematode and related species cause substantial losses to many crops, including tomatoes (*Lycopersicon esculentum*), throughout the world (Barker & Koenning 1998). Recent studies suggest that the activation of the jasmonic acid (JA) pathway is an important component of plant resistance to nematodes (Bhattarai *et al.* 2008). Cooper, Jia & Goggin (2005) demonstrated that artificial induction of JA-pathway defences reduced reproduction of the root-knot nematode on tomato plants. Moreover, the activation of the JA-pathway is considered central for defence against a broad spectrum of herbivores, including leaf chewers and cell-content feeders (i.e. aphids, nematodes, mites and thrips) (Li *et al.* 2002; Thaler *et al.* 2002; Cooper & Goggin 2005). The signalling compounds associated with this pathway trigger the expression of defensive proteins, up-regulation of secondary metabolites and induction of plant volatile organic compounds (VOC) (Howe & Ryan 1999; Howe & Jander 2007). Thus, the JA-pathway is known to be very important in herbivore–plant interactions, but apparently, not much is known about the effects of elevated CO_2 on JA-mediated interactions, especially below-ground interactions.

The current study examined how three isogenic tomato genotypes that differ in the JA pathway responded to elevated CO_2, both alone and combined with *M. incognita* infection. We test the hypothesis that depending on plant genotype, elevated CO_2 results in accumulation of excess carbon in plant tissues and thereby alters plant carbon-based defences against nematodes. Owing to a very different partitioning patterns among these three tomato genotypes, defence-dominated genotypes may tend to allocate 'extra' carbon (relative to nitrogen) to defence under elevated CO_2, whereas defence-recessive genotypes tend to allocate it to growth. Thus, the specific objectives in this study were: (1) to determine whether elevated CO_2 changes the growth, nutrition, the activities of anti-oxidant enzymes and carbon-based secondary metabolites of the isogenic tomato genotypes; (2) to determine whether elevated CO_2 influence the response of tomato genotypes to nematode infection in terms of these variables; and (3) to determine whether elevated CO_2 alters the resistance of genotypes differing in the JA pathway against *M. incognita*.

MATERIALS AND METHODS

Open-top chambers

The experiment was carried out in eight octagonal, open-top chambers (OTCs) (1.6 m wide, 4.2 m diameter and 2.4 m high) at the Observation Station on Global Change Biology of the Institute of Zoology, CAS in Xiaotangshan County, Beijing, China (40°11′N, 116°24′E). The current ambient level of CO_2 (375 ppm) and double the current ambient level (750 ppm, the predicted level in about 100 years) (IPCC 2007) were applied continuously in the OTCs. Four blocks were used for CO_2 treatment. Each block was split into paired OTCs, one with elevated and one with ambient CO_2.

During the 2 months of the experiment (9 August to 9 October 2007), 750 ppm CO_2 concentrations were monitored and controlled by an infrared CO_2 transmitter (Ventostat 8102, Telaire Company, Goleta, CA, USA) and were maintained throughout the experiment. CO_2 concentrations were measured hourly; the measured CO_2 concentrations (mean ± SD per day) were 383 ± 26 ppm in ambient CO_2 chambers versus 769 ± 23 ppm in elevated CO_2 chambers). Details of the automatic control system for CO_2 levels and OTCs were provided in Chen, Ge & Su (2005a) and Chen *et al.* (2005b). The tops of the OTCs were covered with nylon netting to exclude insects. Air temperature was measured three times a day throughout the experiment and did not differ significantly between the two sets of OTCs (24.8 ± 3.40 °C in ambient CO_2 chambers versus 25.5 ± 4.55 °C in elevated CO_2 chambers).

Host plants and nematodes

Wild-type (Wt) tomato plants (*L. esculentum* cv. Castlemart), the jasmonate-deficient *spr2* mutants (*spr2*), and the *35S:Prosystemin* transgenic tomato plants (*35S*) were kindly provided by Professor C. Li of the Institute of Genetics and Developmental Biology, the Chinese Academy of Sciences. The JA-biosynthesis mutant, *suppressor of prosystemin-mediated responses2* (*spr2*), reduces chloroplast ω3 fatty acid desaturase, which impairs the synthesis of JA (Li *et al.* 2003). In contrast, *35S:prosystemin* (*35S*) transgenic plants over-express prosystemin (Howe & Ryan 1999), which constitutively activates system defence in unwounded plants and results in stronger and quicker induced resistance (Li *et al.* 2002). Tomato (*L. esculentum*) cv. Castlemart was the wild type (Wt) parent for the *spr2* mutant and the *35S* transgenic plant. After growing in sterilized soil for 2 weeks, tomato seedlings were individually transplanted into small plastic pots (15 cm diameter and 13 cm height) containing sterilized loamy field soil and placed in OTCs on 9 August 2007. Each OTC contained 90 plants (30 of each tomato genotype × three genotypes).

The root-knot nematode, *M. incognita*, was cultured in Wt plants grown under ambient CO_2. To prepare nematode inoculum, nematode eggs were extracted from infected tomato roots by blending them in water containing 1.0% bleach ($CaCl_2{\cdot}Ca(OCl)_2{\cdot}2H_2O$) with an electric blender. Eggs and root debris were collected on a 25-μm-pore sieve. The second-stage juveniles (J2) were hatched from the eggs (Hussey & Barker 1973) and used as inoculum (see next paragraph).

On the same day (9 August) that seedlings were transplanted into pots and placed in the OTCs, 15 plants of each genotype in each OTC were randomly selected and inoculated with freshly hatched *M. incognita* J2 and any associated microorganisms, and another 15 plants of each tomato genotype in each OTC were treated with sterilized water as

the control. Thus, the experiment had two levels of CO_2, three tomato genotypes, and two levels of nematodes. All the nematode-treated pots received ≈ 1000 J2 in 5 mL of water applied with a pipette over the surface of the soil around the primary roots.

Plants were maintained in the OTCs for 2 months. Pot placement was re-randomized within each OTC once every week. No chemical fertilizers and insecticides were used. Water was added to each pot once every 2 days.

Assessment of disease symptoms caused by the nematode

When J2 of *M. incognita* infect roots, they induce galls, and galls were quantified to estimate root infection in the present study. On 9 October (2 months after inoculation), roots of nematode-inoculated plant from three randomly selected plants of each of three tomato genotypes per OTC (= 9 plants per OTC and 72 plants in total) were carefully removed from soil and washed. A stereomicroscope was used to determine the numbers of galls produced on the entire root system of each plant.

Collection and quantification of plant volatiles

On 9 October, volatiles were collected from one randomly selected plant from each tomato genotype and nematode treatment per OTC (= 6 plants per OTC and 48 plants in total). The method used to collect headspace volatiles was similar to that described by Turlings *et al.* (1998) and Wei, Zhu & Kang (2006). The shoots and leaves of each plant, except for the stem extending 4–5 cm from the soil surface, were sealed in a plastic bag (40 cm wide and 46 cm long). Purified air was pumped (Beijing Institute of Labour Instruments, Beijing, China) into the bag through a freshly activated charcoal trap (Beijing Chemical Company, Beijing, China) and then withdrawn through a glass cartridge (3.0 mm internal diameter and 12.6 cm long) packed with 100 mg of the adsorbent Porapak Q (80–100 mesh, Supelco, Bellefonte, PA, USA); the flow rate was 0.25 L/min. Volatile compounds were rinsed from the Porapak Q with 600 μL *n*-hexane (HPLC grade, Sigma-Aldrich, St Louis, MO, USA) containing internal standards (200 ng ethyl heptanoate) for quantification. The aeration extracts were stored at –20 °C until chemical analyses. After headspace volatiles were collected, the fresh weights of the plant leaves were immediately measured.

Volatiles were quantified and identified using a gas chromatography-mass spectrometry (GC-MS) system (Hewlett Packard 6890N GC model coupled with 5973 MSD) equipped with a HP-5MS column (30 m long, 0.25-mm-inner diameter, and 0.25-μm-film thickness; Agilent Technologies, Palo Alto, CA, USA). The initial oven temperature was kept at 50 °C for 1 min, and then increased to 250 °C at a rate of 5 °C/min. Volatile compounds were identified by comparing their retention times and spectra with those of compounds in the NIST02 library (Scientific Instrument Services, Inc., Ringoes, NJ, USA) and those of pure standards.

Assessment of plant traits and foliar chemical components

Four plants from each tomato genotype and nematode treatment per OTC (= 24 plants per OTC and 192 plants in total) were randomly selected on 8 October After plant height (from base to terminal) was measured, the leaves and roots from each plant were collected and stored at –20 °C until subjected to chemical analysis, except that a sample of fresh leaves (600 mg) from each plant was removed and used to measure enzyme activity, as described later in this subsection. The soil from each pot was air-dried in a ventilation room and cleaned of roots and organic debris before being prepared for chemical analysis. Four additional plants per genotype and nematode treatment per OTC (192 plants in total) were removed from the soil and dried at 80 °C for 72 h to measure the biomass per plant.

Total non-structural carbohydrates (TNCs), mainly starch and sugar, in leaves and roots were assayed by acid hydrolysis following the method of Tissue & Wright (1995). The organic carbon in soil was measured following the Mebius method with minor modification (Nelson & Sommers 1982). Soil samples (0.5 g) were digested with 5 mL of 1 mol/L $K_2Cr_2O_7$ and 10 mL of concentrated H_2SO_4 (≈ 98%) at 150 °C for 30 min, followed by titration of the digests with standardized $FeSO_4$. Nitrogen content in leaves, roots, and soil were assayed using Kjeltec nitrogen analysis (Foss automated Kjeltec™ instruments, Model 2100).

Fresh tomato leaves (600 mg from each of 192 plants; see the first paragraph of this subsection) were homogenized for 1.5 min at 4 °C in 1:10 (fresh weight/buffer volume ratio) 100 mM phosphate buffer, pH 7.4, containing 100 mM KCl and 1 mM EDTA. Homogenates were centrifuged at 10 000 *g* for 10 min, and the supernatants were subjected to chemical component analysis. Protein concentration was determined by the Bradford (1976) assay. Total amino acids (TAA) were analysed with a reagent kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu Province, China) (Wu *et al.* 2007). The activities of the anti-oxidant enzymes, superoxide dismutase (SOD) and catalase (CAT), were also measured with a reagent kit (Nanjing Jiancheng Bioengineering Institute) (Wu *et al.* 2007). As indicated by kit protocol, SOD activity was assayed spectrophotometrically at 550 nm by use of the xanthine and xanthine oxidase system. One unit (*U*) of SOD activity was defined as the amount of SOD required for 50% inhibition of the xanthine and xanthine oxidase system reaction per minute and per milligram of total protein in the homogenate. CAT activity was based on the decomposition rate of H_2O_2 by the enzyme, which can be measured as absorbance decrease per minute at 405 nm. Enzyme activity values were also expressed in CAT units, where one unit is the amount of enzyme needed to hydrolyse 1 μmol H_2O_2 per minute and per milligram of total proteins present in the homogenate.

Statistical analyses

A split–split plot design was used to analyse the univariate responses of the measured variables (i.e. plant height, biomass, VOC, foliar chemical components, C : N ratio) (ANOVA, SAS Institute, 1996). In the following ANOVA model, CO_2 and block (a pair of ambient and elevated OTCs) were the main effects, tomato genotype was the subplot effect, and nematode level was the sub-subplot effect:

$$X_{ijklm} = \mu + C_i + B(C)_{j(i)} + G_k + CG_{ik} + GB(C)_{kj(i)} + N_l + CN_{il} + NB(C)_{lj(i)} + GNB(C)_{klj(i)} + e_{m(ijkl)}$$

where C is the CO_2 treatment ($i = 2$), B is the block ($j = 4$), G is the tomato genotypes ($k = 3$), and N is the nematode treatment ($l = 2$). X_{ijklm} represents the error because of the smaller scale differences between samples and variability within blocks (ANOVA, SAS institute, 1996). Effects were considered significant if $P < 0.05$. The effect of block and the interactive effects of block and other factors were not significant ($P > 0.45$), and the effect of block and its interaction with other factors are not presented in order to facilitate data presentation in tables and in text. Least significant difference (LSD) tests were used to separate the levels within the same variable. To quantify the nematode reproduction on different tomato genotypes under two CO_2 levels, split-plot was also applied, with CO_2 and block as the main effects and tomato genotype as the subplot effect.

Proportional data were transformed using the arcsine square root to satisfy assumptions of normality. Data from plant height were square root-ln(X) transformed, and VOC and numbers of galls per gram of root were ln(X + 10) transformed if necessary.

RESULTS

Plant height, biomass, and C : N ratio

CO_2 level, genotype and their interaction had significant effects on plant height (Table A1). All factors, with the exception of the interaction between CO_2 level and genotype, as well as the interaction among CO_2 level, genotype and nematode, significantly affected plant biomass. Furthermore, all factors and their interactions had significant effects on the foliar TNC : N ratio, and with exception of CO_2 level, nematode, and interactions among the three factors, all factors and their interactions were significant for root TNC : N ratio. In contrast, only genotype and the interaction between genotype and nematode affected the soil C : N ratio (Table A1).

Elevated CO_2 increased the height, biomass and the foliar TNC : N ratio of all the genotypes (Fig. 1). Regardless of CO_2 level, uninfected *35S* had highest foliar TNC : N ratio among genotypes. Nematode infection reduced the biomass of *spr2* plant under ambient CO_2 and the biomass of all the genotypes under elevated CO_2, but was not significant for plant height under both CO_2 levels (Fig. 1).

SOD, CAT, amino acids and protein

CO_2 level, genotype, nematode infection and their interactions (except for $CO_2 \times$ nematode) significantly affected the activity of SOD in tomato leaves (Table A1). Genotype, nematode infection, and their interaction had significant effects on CAT activity in tomato leaves. Furthermore, all factors and their interactions significantly influenced total amino acid and protein contents of leaves (Table A1).

Regardless of CO_2 level, uninfected *35S* had highest enzyme activities of SOD and CAT among the three genotypes (Fig. 2). Furthermore, elevated CO_2 decreased the foliar total amino acid ($F_{1,36} = 325.6$, $P < 0.001$) and protein ($F_{1,36} = 27.4$, $P < 0.001$) contents of uninfected tomato genotypes. Moreover, among the uninfected three tomato genotypes, *spr2* leaves had the highest amino acid and protein contents under both ambient and elevated CO_2 (Fig. 2).

Elevated CO_2 decreased amino acid content of all the genotypes infected by nematodes. In the leaves of jasmonate-deficient *spr2* mutants grown under ambient CO_2, SOD activity was higher in nematode-infected than in uninfected plants ($F_{3,12} = 15.0$, $P < 0.001$) (Fig. 2a), which was opposite the trend found with elevated CO_2. In contrast, nematode infection was associated with higher SOD activity under elevated CO_2 rather than ambient CO_2 in the leaves of Wt plants ($F_{3,12} = 32.6$, $P < 0.001$) and *35S* transgenic plants ($F_{3,12} = 13.1$, $P < 0.001$). Under both CO_2 levels, CAT activity was higher in the leaves of *spr2* and *35S* plants infected with nematodes than in those not infected with nematodes (Fig. 2b). Furthermore, among the three tomato genotypes, *35S* plants had the highest SOD and CAT activities regardless of treatment.

Nematode infection caused higher amino acid content in *spr2* and *35S* leaves, and lower amino acid content in Wt leaves, under both ambient and elevated CO_2 (Fig. 2c) For the three tomato genotypes, foliar protein content decreased in response to nematode infection under ambient CO_2 but increased in response to nematode infection under elevated CO_2 (Fig. 2d).

Volatile emission rate

CO_2 level, tomato genotype and nematode infection had significant effects on the total amount of VOC. Interactive effects between tomato genotype and nematode and among CO_2 level, tomato genotype, and nematode were also significant for total amount of plant VOC (Table A2).

In the absence of nematodes, elevated CO_2 increased the total amount of VOC released by only *35S* plants ($F_{3,12} = 11.9$, $P = 0.001$). The jasmonate-deficient *spr2* plants released less VOC than *35S* plants under both ambient and elevated CO_2 (Fig. 3). Elevated CO_2 reduced emission of β-myrcene in uninfected *spr2* plants and increased emission of β-phellandrene in uninfected *35S* plants (Table A3). *spr2* plants emitted less of each volatile terpene than *35S* plants under elevated CO_2.

VOC were increased in response to nematode infection under ambient CO_2 treatments in all three tomato

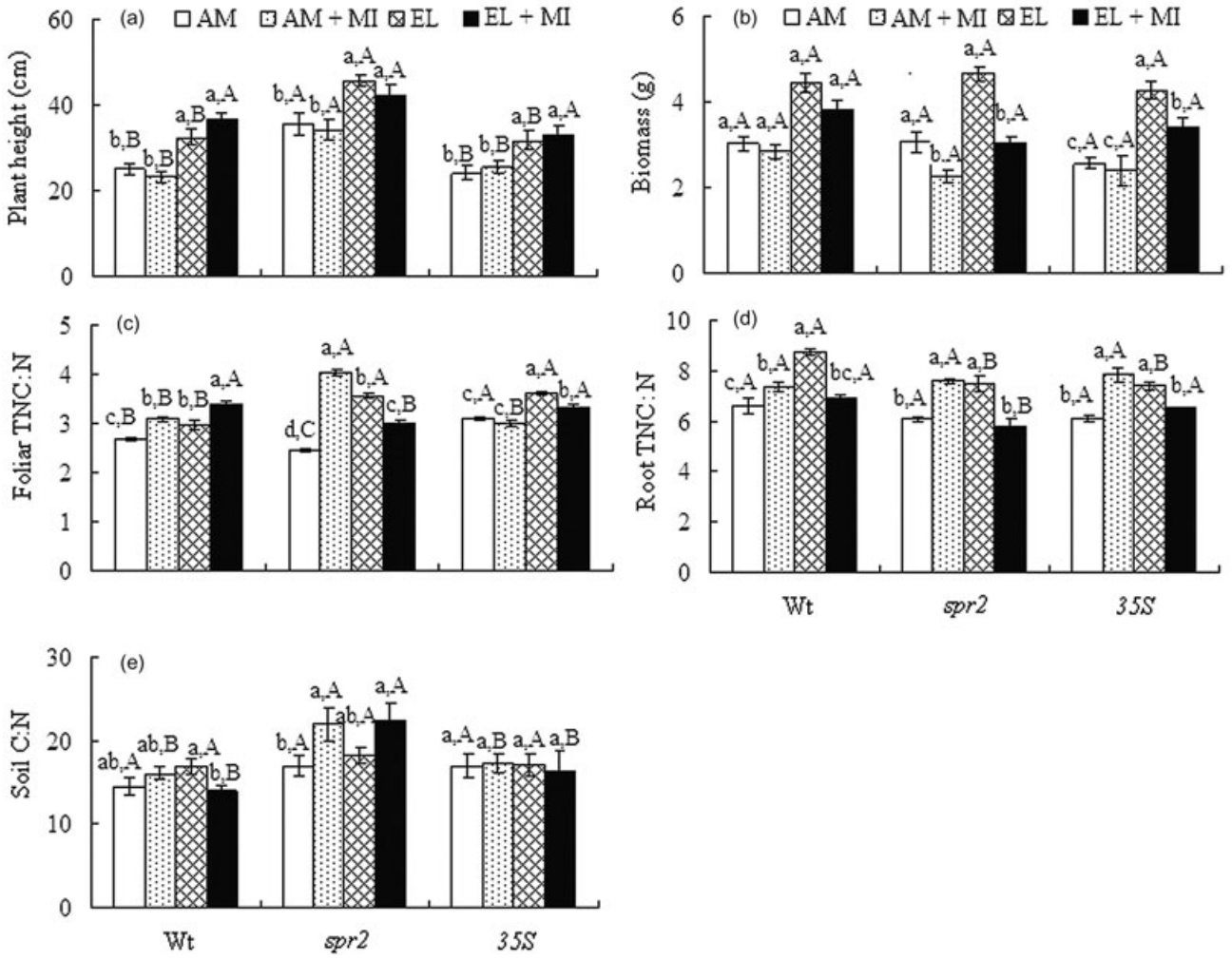

Figure 1. Growth traits and C : N ratio of tomato genotypes grown under ambient (AM) and elevated CO_2 (EL) without and with *M. incognita* (MI). Each value represents the average (±SE) of four replicates. Different lowercase letters indicate significant differences among combinations of nematode and CO_2 level within the same tomato genotype (LSD test: d.f. = 3.12; $P < 0.05$). Different uppercase letters indicate significant differences among tomato genotypes within the same CO_2 and nematode treatment (LSD test: d.f. = 2.9; $P < 0.05$). TNC : N ratio in foliage and root represents the total non-structural carbohydrates: total nitrogen ratio.

genotypes, while VOC were not changed in *35S* plants grown under elevated CO_2 and with nematode infection (Fig. 3). With the exception of tomato genotypes grown under elevated CO_2 and with nematode infection ($F_{2,9} = 4.12$, $P = 0.122$), the jasmonate-deficient *spr2* plants released less VOC than *35S* plants in all treatments (Fig. 3). Under ambient CO_2, nematode infection increased emission of β-myrcene in *spr2* and *35S* plants and camphene in *35S* plants. Furthermore, under elevated CO_2, nematode infection increased emission of α-phellandrene and β-phellandrene in Wt plants, and camphene and β-phellandrene in *spr2* plants (Table A3).

Galls resulting from nematode infection

Tomato genotype affected the number of nematode-induced galls per gram of dry root ($F_{2,48} = 23.38$, $P < 0.001$). Elevated CO_2 and the interaction with tomato genotype, however, were not significant for the number of galls on plant root (Fig. 4). Furthermore, the number of galls on *35S* root was greater under elevated CO_2 than under ambient CO_2 ($F_{1,22} = 6.23$, $P = 0.021$). Regardless of CO_2 level, there were fewer galls on *35S* plants than on Wt or *spr2* plants. Under elevated CO_2, galls were more abundant on *spr2* roots than on the roots of the other two genotypes ($F_{2,33} = 16.1$, $P < 0.001$). Whether data were analysed as numbers of galls per gram of root (Fig. 4) or as numbers of galls per root system, the patterns and statistical analysis were the same (data not shown).

DISCUSSION

Under our experimental conditions, elevated CO_2 increases the C : N ratio in plant tissue (both leaf and root) and increases plant height and biomass in all treatment. It is suggested that regardless of nematode infection, three

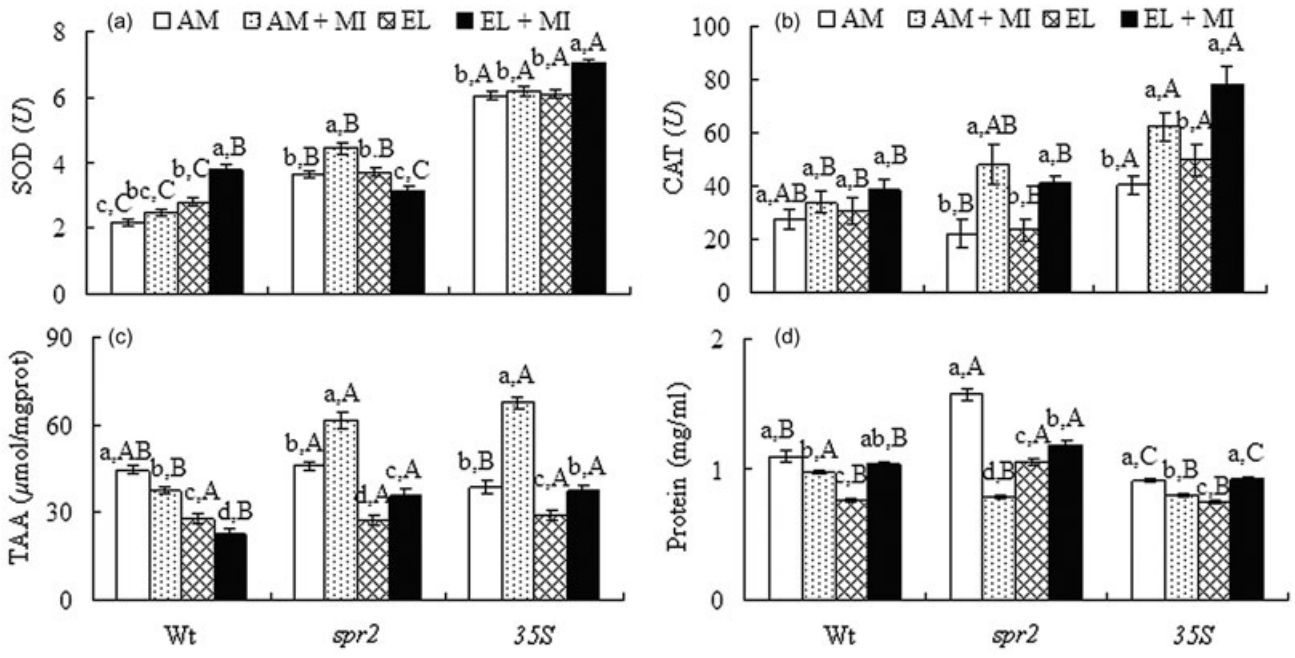

Figure 2. Foliar chemical components of tomato genotypes grown under ambient (AM) and elevated CO_2 (EL) without and with *M. incognita* (MI). Each value represents the average (±SE) of four replicates. Different lowercase letters indicate significant differences among combinations of nematode and CO_2 level within the same tomato genotype (LSD test: d.f. = 3.12; $P < 0.05$). Different uppercase letters indicate significant differences among tomato genotypes within the same CO_2 and nematode treatment (LSD test: d.f. = 2.9; $P < 0.05$).

genotypes of tomato allocated more carbon to growth under elevated CO_2. Furthermore, the defence-dominated genotypes reduced their defence (in terms of gall numbers) against nematodes under elevated CO_2, whereas two of other genotypes were not changed. But our plants were vigorous and better able to support nematodes as shown in plant sized; the plants were not impacted by a stress in addition to *Meloidogyne* and it is when two stresses occur that 'nematode damage' is likely to be shown. Our results debate the hypothesis that defence-dominated genotypes tend to increase the defence against nematodes under elevated CO_2. To the best of our knowledge, our report is the first to consider how isogenic genotypes respond to nematode infection under elevated CO_2. This study demonstrates that elevated CO_2 not only increases the plant C : N ratio but also alters partitioning patterns of plant resources

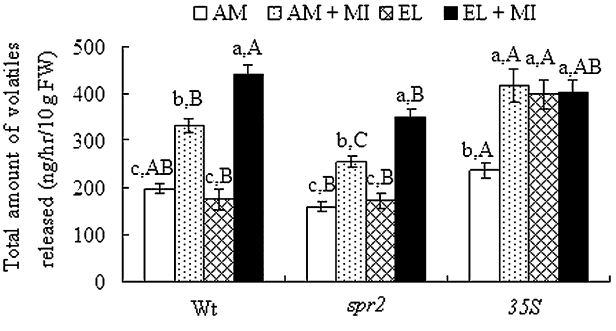

Figure 3. Emission rate of total volatile organic compounds (VOC) from tomato genotypes grown under ambient (AM) and elevated CO_2 (EL) without and with *M. incognita* (MI). Each value represents the average (±SE) of four replicates. Different lowercase letters indicate significant differences among combinations of nematode and CO_2 level within the same tomato genotype (LSD test: d.f. = 3.12; $P < 0.05$); Different uppercase letters indicate significantly different among tomato genotypes within the same CO_2 and nematode treatment (LSD test: d.f. = 2.9; $P < 0.05$). Emission rate represents ng of compound released by 10g (fresh weight) of leaves per hour.

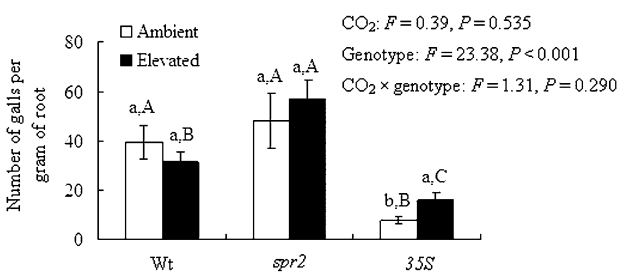

Figure 4. Number of galls per gram of dry root infected by *M. incognita* on tomato genotypes grown under ambient (370 ppm) and elevated CO_2 (750 ppm). Each value represents the average (±SE) of 12 replicates. *P*- and *F*-value of ANOVA are shown. Different lowercase letters indicate significant differences between CO_2 levels within the same tomato genotype (LSD test: d.f. = 1.6, $P < 0.05$); different uppercase letters indicate significant differences among tomato genotypes within CO_2 levels (LSD test: d.f. = 3.12, $P < 0.05$).

between growth and defence, and elevated CO_2 may reduce the resistance or increase the tolerance of plants to the parasitic nematode *M. incognita* when that resistance is based on the JA pathway.

JA-induced defence plays an important role in protecting plant roots from pests and pathogens (Bhattarai *et al.* 2008), and the systemic defence signals associated with the JA pathway are rapidly transported between above-ground and below-ground plant parts (Ryan 2000; Van Dam *et al.* 2003). For example, root application of methyl jasmonate induced nematode resistance in spinach (Soriano *et al.* 2004), and foliar application of JA also induced systemic defence and suppressed the reproduction of the nematode *Melodoigyne javanica* on tomatoes (Cooper *et al.* 2005). In this study, *35S* plants had the most resistance to *M. incognita*, probably because *35S* transgenic plants over-express prosystemin, which can constitutively activate the JA pathway in unwounded plants and result in stronger and quicker induced resistance; among the three genotypes, *35S* leaves have been found to contain the highest JA levels (≈ 1.2 nmol/g in fresh weight) (Bergey, Howe & Ryan 1996). We note that the jasmonate-deficient mutant *spr2* was not significantly more susceptible than the Wt under ambient CO_2. It follows that, although enhanced systemic defence in *35S* can increase the resistance to nematodes, the JA-pathway is not the only defence mechanism involved in plant resistance to nematodes.

To date, researchers have proposed several mechanisms of plant resistance to nematode infection, including the production of reactive oxygen species (ROS) in the hypersensitive reaction (Melillo *et al.* 2006). Thus, the activities of anti-oxidant enzymes could play an important role in resistance of the three tomato genotypes to nematode infection. Our data show that, regardless of CO_2, the regulation pattern of anti-oxidant enzymes SOD and CAT vary among tomato genotypes response to nematode infection. Although we do not know the mechanisms by which nematodes activate the anti-oxidant enzymes among isogenic tomatoes differing in JA pathway, the variety-specific regulation pattern of anti-oxidant enzymes may be involved in the process that elevated CO_2 changes plant resistance against nematode infection.

Elevated CO_2 is expected to increase the emission of VOC from plants because excess carbon is likely to be allocated to volatile secondary metabolites and/or to the larger and heavier leaves (Constable *et al.* 1999; Vuorinena *et al.* 2004). However, changes in VOC emissions in response to elevated CO_2 are highly variety-specific or genotype-specific (Loreto *et al.* 2001; Staudt *et al.* 2001). In our study, elevated CO_2 increased VOC emissions only for *35S* plants, in which elevated CO_2 enhanced the synthesis of terpenes and sesquiterpenes. Furthermore, several studies have measured VOC emission from tomatoes under current ambient CO_2 levels. Sánchez-Hernández, López & Délano-Frier (2006) reported that *spr2* plants produced lower levels of VOC than Wt and *35S* plants in response to herbivory by tobacco hornworm (Lepidoptera) and mechanical damage. However, minor quantitative and qualitative differences in volatile emissions were detected between intact *def-1* mutants (*defenseless-1*, deficient in JA accumulation) and Wt tomatoes, and no induced emission of volatiles was detected in spider mite-infested *def-1* tomatoes (Thaler *et al.* 2002; Ament *et al.* 2004). In our study, nematode infection increased VOC emissions from the three tomato genotypes under ambient CO_2 levels, whereas nematode infection only increased the VOC emissions of Wt and *spr2* plants under elevated CO_2 levels.

Although small pots could reduce or eliminate plant responses to elevated CO_2, some research indicates that CO_2-induced growth enhancement is not necessarily reduced in small pots (McConnaughay, Berntson & Bazzaz 1993). Kerstiens & Hawes (1994) even concluded that there is no evidence that inadequate pot size had a negative impact on the response of plant to elevated CO_2. In this study, elevated CO_2 enhanced plant growth, and the larger plants presumably absorbed more nitrogen from soil. However, soil C : N ratio did not differ among the three tomato genotypes or between ambient and elevated CO_2. Thus, elevated CO_2 provided excess carbon while nitrogen was not changed, so that the larger plants contained less nitrogen per unit of tissue, which was in accordance with the CNB hypothesis (Hamilton *et al.* 2001).

Genetic tradeoffs between growth and defence is manifested in variety-specific responses to (a)biotic environments (Herms & Mattson 1992). In our study, a higher level of basal defence was maintained in *35S* plants, while higher nutrients (proteins and amino acids) were found in *spr2* plants, which indicated *spr2* plants allocate more carbon and nitrogen resources to nutrient synthesis. Artificial genetic modification of *35S* plants apparently increased the physiological cost of defence. Although the biomass of *spr2* plants was 20% greater than that of *35S* plants under ambient CO_2, the difference was not statistically significant. Because of different metabolic/physiological processes in three tomato genotypes which may limit the response of plant genotypes to elevated CO_2, the partitioning patterns of these genetically modified plants may be changed under elevated CO_2 (Cseke *et al.* 2009). We suggest that elevated CO_2 increases the growth of defence-dominative genotypes whereas decreases their defence against nematodes. In contract, elevated CO_2 only enhances the growth of defence-recessive genotypes and wild-types, and is not significant for their defence against nematodes. Thus, elevated CO_2 not only increased the carbon resource and C : N ratio in plant tissue, but it also disrupted the allocation of carbon resources between growth and defence in different tomato genotypes.

Overall, in studying how the interactions of isogenic tomatoes and nematode are affected by elevated CO_2, we detected genotype-specific responses in VOC emission, nutrition content, and anti-oxidant defence. These results argue against our previous hypothesis and suggest that elevated CO_2 reduces the defence of defence-dominated genotypes against nematodes but has no effect on two of other genotypes. Plant defence pathways interact synergistically to the challenge of infection, and cross-talk between different defences pathways has been demonstrated

(Bostock 1999). In this respect, further work on the effects of elevated CO_2 on resistance to infection should consider multiple signalling pathways.

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APPENDIX

Table A1. *P*-values from ANOVAs for the effect of CO2 level, tomato genotype, nematode infection on growth traits, C : N ratio, and foliar chemical components of three tomato genotypes

	Main effects and interactions						
Dependent variable	CO_2[a]	Genotype[b]	Nematode[c]	CO_2 × Genotype	CO_2 × Nematode	Genotype × Nematode	CO_2 × Genotype × Nematode
Height	<0.001***	<0.001***	0.978	0.047*	0.896	0.548	0.764
Biomass	<0.001***	0.038*	<0.001***	0.679	0.012*	0.008**	0.756
Foliar TNC : N[d]	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***
Root TNC : N[d]	0.073	<0.001***	0.576	0.002**	<0.001***	0.006**	0.508
Soil C : N	0.802	<0.001***	0.137	0.838	0.19	0.022*	0.551
SOD[e]	0.001**	<0.001***	<0.001***	<0.001***	0.907	0.03*	<0.001***
CAT[f]	0.126	<0.001***	<0.001***	0.098	0.984	0.035*	0.564
TAA[g]	<0.001***	<0.001***	<0.001***	0.045*	<0.001***	<0.001***	0.001***
Protein content	<0.001***	<0.001***	<0.001***	0.003**	<0.001***	<0.001***	<0.001***

*<0.05, **<0.01, ***<0.001.
[a]Ambient CO_2 versus elevated CO_2.
[b]Three genotypes of tomato (*spr2*, Wt, and *35S:prosys*).
[c]Inoculated or not inoculated with the root-knot nematode *M. incognita*.
[d]TNC : N ratio in foliage and root represents the total non-structural carbohydrates: total nitrogen ratio.
[e]superoxide dismutase.
[f]catalase.
[g]total amino acids.

Table A2. *P*-values from ANOVAs for the effect of CO2 level, tomato genotype, and nematode infection on plant volatiles

Volatiles	CO_2[a]	Genotype[b]	Nematode[c]	CO_2 × Genotype	CO_2 × Nematode	Genotype × Nematode	CO_2 × Genotype × Nematode
α-pinene	0.101	0.025*	0.004**	0.595	0.809	0.424	0.704
p-cymene	0.194	0.013*	0.011*	0.499	0.718	0.769	0.375
carveol	0.557	0.165	0.004**	0.319	0.909	0.886	0.642
β-pinene	0.974	0.348	0.102	0.912	0.827	0.328	0.346
β-myrcene	0.856	0.416	0.068	0.084	0.557	0.863	0.434
camphene	0.081	0.01*	<0.001***	0.949	0.838	0.115	0.072
α-phellandrene	0.218	0.114	0.008**	0.248	0.356	0.002**	0.049*
β-phellandrene	0.017*	<0.001***	<0.001***	0.569	0.922	0.149	0.006**
Total release	<0.001***	<0.001***	<0.001***	0.354	0.639	0.001**	<0.001***

*<0.05, **<0.01, ***<0.001.
[a]Ambient CO_2 versus elevated CO_2.
[b]Three genotypes of tomato (s*pr2*, Wt, and *35S:prosys*).
[c]Inoculated or not inoculated with the root-knot nematode *M. incognita*.

Table A3. Emission rate[a] of volatile organic compounds (VOC) from tomato genotypes grown under ambient (370 ppm) and elevated CO2 (750 ppm) without and with *M. incognita*

		370 ppm		750 ppm	
Genotype	Volatiles	– *M. incognita*	+ *M. incognita*	– *M. incognita*	+ *M. incognita*
Wt	α-pinene	14.0 ± 3.90a,A	26.9 ± 5.76a,A	14.4 ± 4.14a,B	35.9 ± 11.8a,A
	p-cymene	7.45 ± 2.15a,AB	12.7 ± 1.68a,A	7.78 ± 1.6a,B	21.9 ± 9.76a,A
	carveol	0.92 ± 0.18a,A	1.57 ± 0.17a,A	1.04 ± 0.16a,AB	1.81 ± 0.63a,A
	β-pinene	3.14 ± 0.38a,A	2.77 ± 0.51a,A	2.98 ± 0.69a,AB	3.08 ± 0.79a,A
	β-myrcene	2.90 ± 0.85a,A	3.09 ± 0.86a,A	2.85 ± 0.80a,A	4.40 ± 1.24a,A
	camphene	45.3 ± 9.99a,A	88.0 ± 6.89a,A	41.2 ± 17.6a,B	113.7 ± 21.0a,A
	α-phellandrene	4.18 ± 1.17b,A	6.90 ± 1.21ab,A	2.83 ± 0.66b,B	16.0 ± 3.24a,A
	β-phellandrene	103.6 ± 21.2b,A	179.6 ± 16.6ab,AB	86.4 ± 11.0b,B	223.3 ± 23.6a,A
spr2	α-pinene	13.6 ± 3.65a,A	25.7 ± 10.3a,A	14.6 ± 3.52a,B	30.8 ± 6.87a,A
	p-cymene	5.94 ± 0.65a,B	17.2 ± 7.72a,A	8.62 ± 2.71a,B	12.7 ± 1.67a,A
	carveol	1.24 ± 0.10a,A	1.51 ± 0.29a,A	0.76 ± 0.060a,B	1.45 ± 0.27a,A
	β-pinene	2.67 ± 0.15a,A	3.07 ± 0.32a,A	1.98 ± 0.17a,B	3.29 ± 0.73a,A
	β-myrcene	4.16 ± 0.86a,A	4.00 ± 0.49a,A	1.51 ± 0.21b,B	3.06 ± 0.76ab,A
	camphene	38.3 ± 6.51b,A	52.0 ± 11.0b,A	41.5 ± 6.50b,B	82.9 ± 8.69a,A
	α-phellandrene	4.17 ± 0.64ab,A	6.61 ± 1.60a,A	3.39 ± 0.35b,B	5.24 ± 0.63ab,B
	β-phellandrene	65.5 ± 4.20c,A	122.7 ± 21.6b,B	86.4 ± 11.2 bc,B	192.0 ± 13.5a,A
35S	α-pinene	23.5 ± 3.93a,A	32.0 ± 7.92a,A	39.3 ± 6.83a,A	40.9 ± 7.69a,A
	p-cymene	12.9 ± 2.18a,A	21.7 ± 7.34a,A	23.9 ± 5.28a,A	24.9 ± 3.91a,A
	carveol	1.05 ± 0.10b,A	1.85 ± 0.49ab,A	1.68 ± 0.29ab,A	2.06 ± 0.16a,A
	β-pinene	0.98 ± 0.38a,B	3.33 ± 0.45a,A	2.14 ± 1.40a,A	2.56 ± 0.22a,A
	β-myrcene	2.53 ± 0.30a,A	4.50 ± 1.50a,A	4.04 ± 0.88a,A	4.76 ± 0.67a,A
	camphene	55.9 ± 9.78b,A	99.2 ± 19.8a,A	93.7 ± 14.0ab,A	89.2 ± 7.38ab,A
	α-phellandrene	6.05 ± 1.54a,A	7.53 ± 4.32a,A	9.29 ± 1.51a,A	7.40 ± 0.65a,B
	β-phellandrene	117.1 ± 19.2b,A	225.0 ± 22.4a,A	206.6 ± 35.4a,A	198.2 ± 15.7a,A

[a]Emission rate = ng of compound released by 10 g (fresh weight) of leaves per hour.
Each value represents the average (±SE) of 4 replicates. Different lowercase letters within a row indicate significant differences (LSD test: d.f. = 3.12; $P < 0.05$); Different uppercase letters indicate significantly different among tomato genotypes within the same CO_2 and nematode treatment (LSD test: d.f. = 2.9; $P < 0.05$).