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# Elevated O<sub>3</sub> enhances the attraction of whitefly-infested tomato plants to *Encarsia formosa*

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We experimentally examined the effects of elevated O<sub>3</sub> and whitefly herbivory on tomato volatiles, feeding and oviposition preferences of whiteflies and behavioural responses of *Encarsia formosa* to these emissions on two tomato genotypes, a wild-type (Wt) and a jasmonic acid (JA) defence-enhanced genotype (JA-OE, 35S). The O<sub>3</sub> level and whitefly herbivory significantly increased the total amount of volatile organic compounds (VOCs), monoterpenes, green leaf volatiles (GLVs), and aldehyde volatiles produced by tomato plants. The 35S plants released higher amount of total VOCs and monoterpene volatiles than Wt plants under O<sub>3</sub>+herbivory treatments. The feeding and oviposition bioassays showed that control plants were preferred by adult whiteflies whereas the 35S plants were not preferred by whiteflies. In the Y-tube tests, O<sub>3</sub>+herbivory treatment genotypes were preferred by adult *E. Formosa*. The 35S plants were preferred by adult *E. formosa* under O<sub>3</sub>, herbivory and O<sub>3</sub>+herbivory treatments. Our results demonstrated that elevated O<sub>3</sub> and whitefly herbivory significantly increased tomato volatiles, which attracted *E. formosa* and reduced whitefly feeding. The 35S plants had a higher resistance to *B. tabaci* than Wt plant. Such changes suggest that the direct and indirect defences of resistant genotypes, such as 35S, could strengthen as the atmospheric O<sub>3</sub> concentration increases.

Plants are a source of a large diversity of volatile organic compounds (VOCs), such as monoterpenes, sesquiterpenes and homoterpenes<sup>1</sup>. These VOCs can serve as semiochemicals that can be detected by other organisms to reveal the host presence<sup>2</sup>, to signify availability of food and mating sites<sup>3,4</sup> and also to aid in navigating to food sources<sup>5,6</sup>.

Insect feeding influences the nutrient condition<sup>7</sup>; secondary metabolite production, i.e., jasmonic acid (JA is a plant hormone involved in regulating secondary metabolite production among other things)<sup>8–10</sup>; and the type and amount of herbivore-induced plant volatiles (HIPVs) released by the plant<sup>11,12</sup>. HIPVs can function as plant defences by directly repelling herbivores from ovipositing and host-seeking<sup>13</sup> and by attracting herbivore enemies, such as parasitic wasps, predatory arthropods and possibly even insectivorous birds<sup>14–16</sup>. For example, the monoterpene volatiles of *Chrysanthemum morifolium* repel ovipositing females of the diamondback moth (*Plutella xylostella*), a lepidopteran that does not normally lay eggs on *C. morifolium*<sup>17</sup>. Volatiles, such as the aphid alarm pheromone from transgenic *Arabidopsis thaliana*, repelled the green peach aphid (*Myzus persicae*) from feeding, although wild-type *Arabidopsis* does not normally produce this pheromone<sup>18</sup>. Laboratory and field studies have shown that HIPVs attract parasitoids and/or predators to many plant species that reduce the risk of damage from herbivorous insects<sup>19–21</sup>. Predatory mites preferred the odour of Lima bean plants infested with two-spotted spider mites (*Tetranychus urticae*) to that of uninfested plants<sup>11</sup>. The pea leafminer, *Liriomyza huidobrensis*, caused an increased emission of volatiles in tomato plants and consequently increased the attraction of the parasitoid *Opius dissitus*<sup>22</sup>. Enhancement of these interactions between the HIPVs and the natural enemies of herbivores can aid the development and delivery of integrated pest management programs<sup>12,22</sup>.

Recently, the global atmospheric concentration of ozone (O<sub>3</sub>) has risen from less than 10 ppb (parts per billion) a century ago to 40 ppb today and is projected to continue to increase at an annual rate of 1–2%<sup>23,24</sup> to 68 ppb by the year 2050<sup>25</sup>. Elevated O<sub>3</sub> changes plant biochemistry, including the activation of many plant defence responses<sup>26–28</sup>. Elevated O<sub>3</sub> is also changing the atmospheric life times of phytochemical VOCs, including the HIPVs<sup>29</sup>. Due to these alterations, the behavioural and life history parameters of herbivorous insects are also influenced<sup>30,31</sup>. O<sub>3</sub> stress can increase VOC emissions from plants, including terpenoids and green leaf volatiles (GLVs)<sup>32,33</sup>. For


**Table 1 | ANOVA analyses on the effects of the O<sub>3</sub> level, whitefly herbivory and tomato genotypes on the volatile emission rate of tomato plants**

Factor	Measured Indices F(P)			
	Monoterpene volatile emissions	GLVs <sup>a</sup>	Aldehyde volatiles	VOCs <sup>b</sup>
O <sub>3</sub>	<0.001	<0.001	<0.001	<0.001
Herbivory	<0.001	<0.001	<0.001	<0.001
Genotype	<0.001	<0.001	<0.001	<0.001
O <sub>3</sub> × herbivory	<0.001	<0.001	0.41	<0.001
O <sub>3</sub> × genotype	<0.001	0.19	0.2	<0.001
Herbivory × genotype	<0.001	<0.001	<0.001	<0.001
O <sub>3</sub> × herbivory × genotype	<0.001	0.5	0.03	<0.001

<sup>a</sup>Green leaf volatiles.  
<sup>b</sup>Total amount of volatile organic compounds.

example, Blande *et al.* reported increased emissions of some monoterpenes ( $\alpha$ -pinene and/or  $\beta$ -pinene, (E)- $\beta$ -ocimenes) and higher total monoterpene emissions from hybrid aspens (*Populus tremula* × *tremuloides Michx.*) grown under moderately elevated O<sub>3</sub><sup>12</sup>. Furthermore, O<sub>3</sub> fumigation has been reported to promote the emission of herbivore-induced volatiles<sup>1</sup>. Differences in the quality and quantity of the volatile mixtures induced by a high O<sub>3</sub> concentration will affect tritrophic interactions among plants, herbivorous insects and natural enemies<sup>34</sup>. However, little is known regarding HIPVs induction by piercing-sucking insects and the effects of herbivores and their natural enemies under elevated levels of O<sub>3</sub>.

Tomato, *Solanum lycopersicum*, is an economically important vegetable worldwide and a commonly used model plant for biologists<sup>35,36</sup>. It is also an O<sub>3</sub>-sensitive species<sup>37</sup>. *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae)<sup>38</sup> is one of the most noxious insect pests on field and greenhouse crops worldwide<sup>39,40</sup>. *Encarsia formosa* is the main parasitoid of *B. tabaci*, and the use of *E. formosa* for whitefly management is now universally accepted as a biological control agent<sup>41</sup>. Previous research indicated that several JA-overexpression mutants exhibit greater resistance against insects than wild-type plants<sup>42–44</sup>. Wei *et al.* (2011) showed that there are ecological trade-offs between JA-dependent direct and indirect defences in genetically modified plants<sup>22</sup>. Our previous research indicated that the JA-overexpression tomato mutant 35S was resistant to *B. tabaci* under a high O<sub>3</sub> concentration and whitefly infestation and there was a reduction in the fitness of conspecific *B. tabaci* that fed on three previously infested tomato genotypes that differed in the JA pathway<sup>15,46</sup>. However, the mechanism(s) by which VOCs affect whitefly feeding and oviposition and the behavioural responses of *E. formosa* induced by whitefly infestation remain unclear under elevated O<sub>3</sub> conditions.

Here, we hypothesise that elevated O<sub>3</sub> levels and whitefly infestation will affect the type and amount of tomato volatiles produced, which will affect the feeding and oviposition preferences of whiteflies and their interaction with *E. formosa*. To test this hypothesis, the effects of elevated O<sub>3</sub> and whitefly infestation on two tomato genotypes, including the wild-type and a JA-defence-enhanced genotype (35S), in association with the phloem feeder *B. tabaci* Gennadius biotype B and a natural enemy, *E. Formosa*, were examined in open-top chambers in the field. Our specific objectives were to determine: (1) whether elevated O<sub>3</sub> levels alter the emission of whitefly-induced volatiles, (2) the effects of VOCs on the feeding and oviposition preferences of *B. tabaci* and (3) the effects of VOCs on the behavioural responses of *E. formosa*.

## Results

**Volatile emission rate.** O<sub>3</sub> level, whitefly herbivory, tomato genotypes and the interactions between the whitefly herbivory and tomato genotypes significantly affected the total amount of VOCs, monoterpene volatile emissions, GLVs and aldehyde production. The interactions between O<sub>3</sub> and whitefly herbivory significantly

affected the total amount of VOCs, monoterpenes and GLVs. The interactions between O<sub>3</sub> and tomato genotypes significantly affected the total amount of VOCs and monoterpene volatile emissions. Ozone level × tomato genotype × whitefly herbivory significantly affected the total amount of VOCs, monoterpene volatile emissions and aldehyde volatiles (Table 1).

The monoterpene volatile emissions included terpinolene, (+)- $\alpha$ -pinene, (+)-3-carene, D-limonene,  $\alpha$ -phellandrene, ocimene and  $\beta$ -phellandrene in the two tomato genotypes. The GLVs were Z-3-hexanol and E-2-hexanol. The aldehyde volatiles referred to nonanal and octanal (Fig. 1A, B, C, D).

Elevated O<sub>3</sub> levels increased the VOC emissions 4.85-fold in the Wt genotype ( $F_{3, 12} = 382.234, P = 0.000$ ) and 7.48-fold in the 35S genotype ( $F_{3, 12} = 195.509, P = 0.000$ ) (Table 1, Fig. 1A). Elevated O<sub>3</sub> levels enhanced the monoterpene emissions 5.12-fold in the Wt genotype ( $F_{3, 12} = 372.234, P = 0.000$ ) and 7.80-fold in the 35S genotype ( $F_{3, 12} = 198.668, P = 0.000$ ) (Table 1, Fig. 1B).

Whitefly herbivory increased the total amount of VOC emissions 5.12-fold in the Wt genotype ( $F_{3, 12} = 382.234, P = 0.000$ ) and 3.41-fold in the 35S genotype ( $F_{3, 12} = 195.509, P = 0.000$ ) (Table 1, Fig. 1A). Whitefly herbivory increased the monoterpene emissions 5.56-fold in the Wt genotype ( $F_{3, 12} = 372.234, P = 0.000$ ) and 3.38-fold in the 35S genotype ( $F_{3, 12} = 198.668, P = 0.000$ ) (Table 1, Fig. 1B).

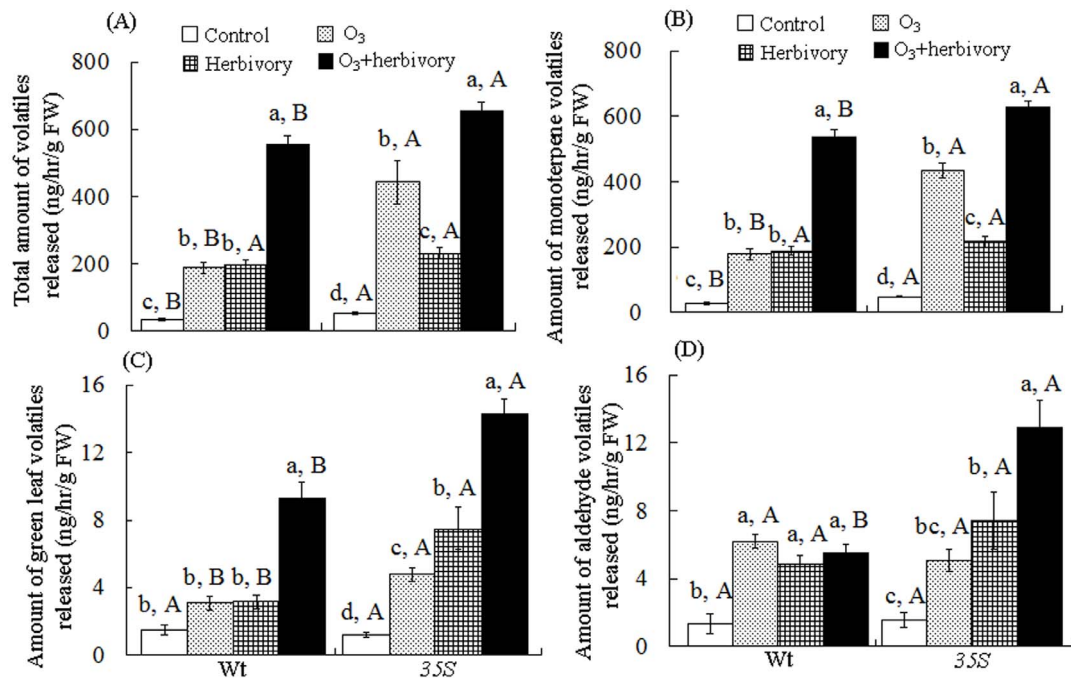
For the two plant genotypes, the total amount of VOCs is highest under O<sub>3</sub>+herbivory treatment. The sum of the peak areas show the total amount of VOCs from the 35S plants was  $656.75 \pm 22.85$ , whereas that from Wt plants was  $554.53 \pm 56.65$ . The 35S plants released higher levels of monoterpene volatiles than the Wt plants under control, O<sub>3</sub> and O<sub>3</sub>+herbivory treatments (Fig. 1A, B).

**Feeding and Oviposition Preferences of *B. tabaci*.** O<sub>3</sub> level, whitefly herbivory and tomato genotypes significantly affected the feeding and oviposition preferences of *B. tabaci*.

Adult whiteflies more often preferred the control plants for feeding (Wt:  $X^2 = 87.063, P < 0.001$ ; 35S:  $X^2 = 62.349, P < 0.001$ ) and oviposition (Wt:  $X^2 = 385.914, P < 0.001$ ; 35S:  $X^2 = 660.616, P < 0.001$ ) (Fig. 2A, B).

Adult whiteflies preferred Wt plants for feeding under control ( $X^2 = 11.645, P = 0.001$ ), O<sub>3</sub> ( $X^2 = 25.712, P = 0.000$ ) and herbivory ( $X^2 = 3.939, P = 0.047$ ) treatments. Adult whiteflies preferred Wt plants for oviposition under four treatments (control:  $X^2 = 177.888, P = 0.000$ ; O<sub>3</sub>:  $X^2 = 200.166, P = 0.000$ ; herbivory:  $X^2 = 50.797, P = 0.000$ ; O<sub>3</sub>+herbivory:  $X^2 = 5.69, P = 0.017$ ). Adult whiteflies did not prefer 35S plants for feeding and oviposition under the four treatments (Fig. 3A, B).

**The behavioural responses of *E. formosa* to the odors of two tomato genotypes in various treatments.** In a two-choice behavioural assay, O<sub>3</sub>+herbivory treatment plants were preferred



**Figure 1** | Emission rate<sup>1</sup> of volatile organic compounds (VOCs) from tomato genotypes grown under ambient and elevated O<sub>3</sub> with and without *Bemisia tabaci* of herbivore preconditioning after three weeks. Different lowercase letters within a row indicate significant differences among the four treatments in a specific tomato cultivar (LSD test:  $P < 0.05$ ); Different uppercase letters indicate significant differences among tomato genotypes within the same O<sub>3</sub> and whitefly treatment (LSD test:  $P < 0.05$ ).<sup>1</sup> Emission rate = ng of compound released per g (fresh weight) of leaves per hour. Each value represents the average ( $\pm$ SE) of 4 replicates.

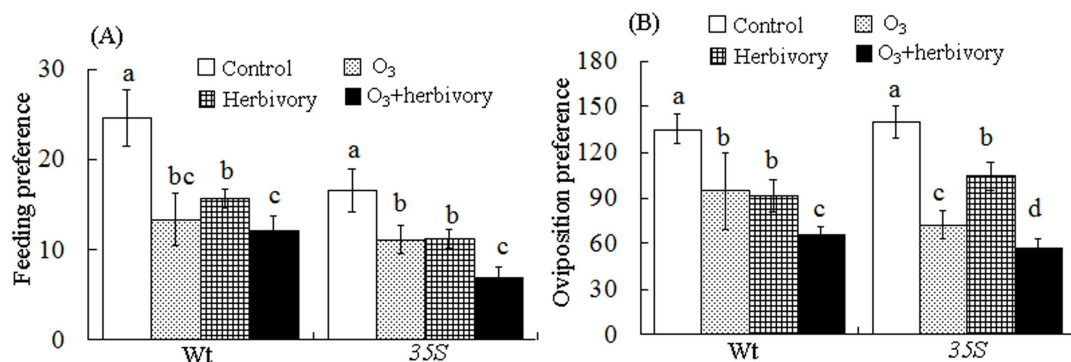
by adult *E. formosa* for the two tomato genotypes in the Y-tube tests (Fig. 4A). Control treatment plants were not preferred by adult *E. formosa* for the two tomato genotypes in the Y-tube tests (Fig. 4A).

To determine the preference of *E. formosa* for volatiles emitted by different tomato genotypes, behavioural responses were investigated in a dual-choice olfactometer. Behavioural assays in the Y-tube olfactometer showed that the 35S plants were preferred by adult *E. formosa* under O<sub>3</sub>, herbivory and O<sub>3</sub>+herbivory treatments (Fig. 4B). Adult parasitoid preferences for the two tomato genotypes were not significantly different under the control treatment (Fig. 4B).

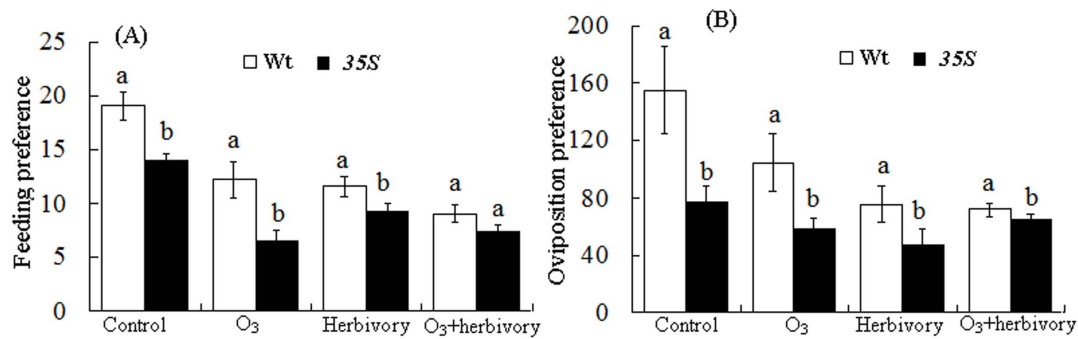
## Discussion

The herbivorous insect/plant interaction has proven to be a complex process that extends beyond compromising plant fitness to include directly inducing defensive responses by the plant to the herbivore and indirect effects involving VOC emissions by the plant that affect natural enemies<sup>9,47</sup>. Elevated O<sub>3</sub> levels can alter nutritional quality, secondary metabolites and the resistance of plants as well as result in

significantly increased VOC emissions<sup>45,48</sup>. Elevated O<sub>3</sub> concentrations (100 ppb up to 250 ppb) resulted in increased monoterpene emissions of 3-year-old *Quercus ilex* L. plants<sup>49</sup>. Either the acute ozone-exposure or spider mite-infestation could induce the volatile emission of the Lima bean, but under elevated ozone condition, spider mite-damaged plants could release significantly higher amount of volatile emission<sup>11</sup>. Our previous research showed that elevated O<sub>3</sub> levels altered the nutritional content and secondary metabolites in tomato plants<sup>45</sup>. In this study, we report that elevated O<sub>3</sub> concentrations and whitefly infestation significantly increased the total amount of VOCs, monoterpene emissions, GLVs and aldehyde volatile productions of the tomato plants. The monoterpene volatile emissions remain the dominant product among the VOCs. Moreover, the tomato VOC emissions in the O<sub>3</sub>+herbivory treatment are the highest among the four treatments. The results indicated that the O<sub>3</sub> factor and the whitefly infestation factor are related, suggesting that elevated O<sub>3</sub> levels significantly abet the emission of whitefly-induced volatiles.



**Figure 2** | Effects of elevated O<sub>3</sub> concentration and whitefly infestation on the feeding and oviposition preferences of whiteflies after three weeks on the two tomato genotypes. Each value represents the average ( $\pm$ SE) of 15 replicates. Different lowercase letters indicate significant differences between the four treatments for a specific tomato cultivar (X<sup>2</sup> test:  $P < 0.05$ ).



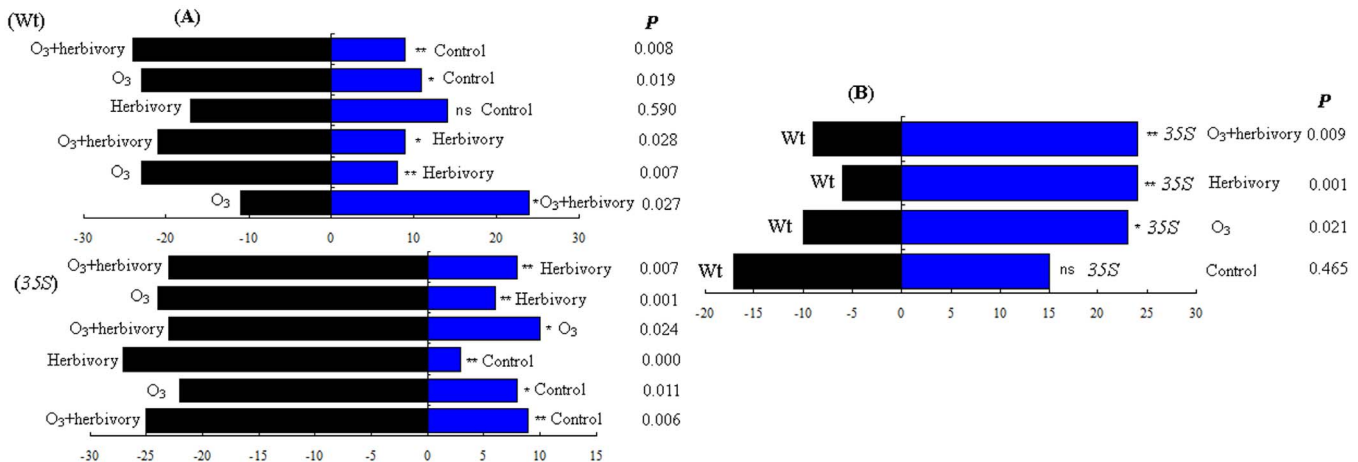
**Figure 3** | Effects of the tomato genotypes on the feeding and oviposition preferences of feeding whiteflies after three weeks under four treatments. Each value represents the average ( $\pm$ SE) of 15 replicates. Different lowercase letters indicate significant differences between the tomato cultivars under four treatments ( $X^2$  test:  $P < 0.05$ ).

VOC release following elevated O<sub>3</sub> levels; herbivore and mechanical damage can repel herbivores<sup>13,22,50</sup>. For example, volatiles from the infested transgenic *A. thaliana* repelled the green peach aphid (*Myzus persicae*) in comparison to volatiles from control transgenic plants<sup>18</sup>. Cannon (1990) found that O<sub>3</sub>-induced VOCs from red spruce needles repel spruce budworm larvae<sup>51</sup>. Some studies showed that monoterpenes are biogenic volatile organic compounds that play an important role in repelling herbivore oviposition and feeding<sup>17,48</sup>. Our results found that adult whiteflies preferred feeding and oviposition on control plants to three other O<sub>3</sub> and herbivory treatments, suggesting that the increasing VOCs (especially monoterpenes) following elevated O<sub>3</sub> levels and herbivore exposure will repel the whitefly.

VOC release following elevated O<sub>3</sub> levels, herbivore and mechanical damage can also attract herbivore enemies<sup>13,14,32</sup>. The predatory mite *Phytoseiulus persimilis* prefers volatiles from the Lima bean leaves of plants injured by spider mites to volatiles from uninfested Lima bean leaves<sup>52</sup>. The aphid *Myzus persicae* caused an increased emission of volatiles triggered by spider mites in pepper plants and consequently increased the attraction of predators<sup>53</sup>. Pinto *et al.* (2007) found that VOCs, such as terpenes and GLVs of *Brassica oleracea*, induced by elevated O<sub>3</sub> levels are crucial for the orientation of the wasps *Cotesia plutellae*<sup>48</sup>. Previous studies showed that monoterpenes and GLVs are important cues for natural enemies in host finding<sup>54–56</sup>. Our results showed that O<sub>3</sub>+herbivory treatment plants were preferred by adult *E. formosa* compared to control treatment plants in the two tomato genotypes in the Y-tube tests. The enrichment of VOCs in the O<sub>3</sub>+herbivory tomato plant treatment significantly attracted *E. formosa*.

JA-overexpression tomato genotype 35S has higher resistance to sucking insects than Wt plant and thus is considered one of the best plant genotype for insect resistance<sup>22,43</sup>. Cui *et al.* (2012a and 2012b) showed that the 35S has the highest resistance to *B. tabaci* under elevated O<sub>3</sub> and whitefly infestation<sup>45,46</sup>. We report here that the 35S tomato genotypes released higher VOC emissions (including the total amount of VOCs, monoterpene volatile emissions, GLVs and aldehyde volatiles) than the Wt tomato plants. The 35S plants were not preferred by adult whiteflies for feeding or oviposition under four treatments, while the 35S plants were preferred by adult *E. formosa* under O<sub>3</sub>, herbivory and O<sub>3</sub>+herbivory treatments. Moreover, the differences between the two tomato genotypes were accentuated under elevated O<sub>3</sub>. These results showed that JA-overexpression tomato genotype 35S has a significantly higher resistance to the whitefly under elevated O<sub>3</sub>.

To our knowledge, this is the first systematic study of the responses to elevated O<sub>3</sub> levels on tritrophic interactions among plants, herbivorous insects and natural enemies. Our results indicate that elevated O<sub>3</sub> and whitefly infestation significantly increased the VOC emissions, especially the monoterpene volatile emissions. The tomato VOC emissions in the O<sub>3</sub>+herbivory treatment were the highest among the four treatments. 35S tomato genotypes released higher VOC emissions, especially monoterpene volatile emissions, than did the Wt tomato plants. The 35S plants had a higher repellent effect on the whitefly and a higher attraction of *E. formosa*. Such changes suggest that the direct and indirect defence of resistant genotypes, such as 35S, would be further strengthened as the atmospheric concentration of O<sub>3</sub> continues to increase.



**Figure 4** | Effects of elevated O<sub>3</sub> levels and whitefly infestation after three weeks on the behavioural responses of *E. formosa* to the two host tomato genotypes (A). Effects of the tomato genotypes on the behavioural responses of *E. formosa* after three weeks under four treatments (B).  $X^2$  test was used for differences between the numbers of *E. Formosa* in each arm (\*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; ns, non significant).



## Methods

**Open-top chambers and O<sub>3</sub> Treatment.** Experiments were conducted using eight octagonal open-top chambers (OTCs), each 2.2 m in height and 2 m in diameter, at the Observation Station for Global Change Biology at the Institute of Zoology of the Chinese Academy of Science, in Xiaotangshan County, Beijing, China (40°11'N, 116°24'E). Four OTCs were used for each O<sub>3</sub> concentration treatment. In the elevated O<sub>3</sub> treatment, O<sub>3</sub> was generated from ambient air by an O<sub>3</sub> generator (3S-A15, Tonglin Technology, Beijing, China) and then transported to the entrances of the OTCs using a fan (HB-429, 4.1 m<sup>3</sup> min<sup>-1</sup>, Ruiyong Mechanical and Electrical Equipment Company). Mixed air (O<sub>3</sub> and ambient air) was ventilated into each OTC through columniform polyvinyl chloride pipes (inner diameter 11 cm, outer diameter 16 cm). O<sub>3</sub> concentrations were monitored at both the fan output (Shenzhen Yiyuntian Electronic CO. LTD) and within the OTCs (AQL-200, Aeroqual). From July 28<sup>th</sup> to August 21<sup>th</sup>, 2010, except for 2 rainy days, the OTCs were ventilated with air daily from 9:00 a.m. to 5:00 p.m. through a hemispherical stainless steel sprayer (diameter = 30 cm) situated 0.5 m above the canopy at a rate of approximately 4.1 m<sup>3</sup> min<sup>-1</sup>, resulting in approximately 0.59 air changes per minute in each OTC. Gas concentrations were measured once every hour in each chamber receiving O<sub>3</sub> treatment<sup>45</sup>.

The two O<sub>3</sub> concentration treatments employed were as follows: (1) current ambient atmospheric O<sub>3</sub> levels (average value from 9:00 a.m. to 5:00 p.m. on all air-treated days of 37.3 nmol mol<sup>-1</sup>) and (2) twice the current ambient O<sub>3</sub> levels (average value from 9:00 a.m. to 5:00 p.m. on all air-treated days of 72.2 nmol mol<sup>-1</sup>)<sup>45</sup>.

**Herbivory Treatment.** Individuals of *B. tabaci* biotype B were collected on 5 April 2010 from cabbage growing at the Beijing Academy of Agriculture and Forestry. The offspring of these whiteflies were reared on tomatoes. For the infestation treatments, three leaves of each intact plant were encased in a mesh gauze bag and infested with 90 male whiteflies (to avoid the production of offspring) that were replaced every three days from July 28<sup>th</sup> to August 21<sup>th</sup>, 2010.

**Host Plants.** Two tomato genotypes were selected for the present study: wild-type (Wt) tomato plants (*Solanum esculentum* cv. Castlemart) and 35S::*prosystemin* transgenic tomato plants (35S). Professor C. Li of the Institute of Genetics and Developmental Biology, Chinese Academy of Science provided these plants. *L. esculentum* cv. Castlemart was the Wt parent for the 35S transgenic plants. The 35S::*prosystemin* (35S) JA-biosynthesis mutant transgenic plants overexpress *prosystemin*, which constitutively activates the defence system in unwounded plants and results in a stronger and more rapidly induced resistance<sup>43</sup>. After being grown in sterilised soil for two weeks, the tomato seedlings were individually transplanted into small plastic pots (14 cm diameter, 12 cm height) containing sterilised loamy field soil. Plants that were approximately 40 days old with heights of 20–30 cm were moved to the OTCs on 27 July 2010.

**Feeding and Oviposition Preferences of *B. tabaci*.** One control plant and one herbivore-preconditioned plant of each tomato genotype were placed into the same cage (dimensions = 60 × 60 × 60 cm) on 7 August 2010 in the open cylinders. They were control Wt plant/preconditioned Wt plant and control 35S plant/preconditioned 35S plant, respectively. The experiment had 15 replicates. For the preconditioning treatment, a cohort of 90 male whiteflies was established and replaced every three days on the lateral 3 leaves to provide a continuous infestation for three weeks in the open cylinders. After three weeks, 50 pairs of adult whiteflies were put into each cage, and three days later, the feeding and oviposition preference of each whitefly was recorded.

The second experiment was prepared in the same manner as the above mentioned experiment. The differences were each of 15 cages containing two control plants (one Wt and one 35S plant). Each of the other 15 cages contained two preconditioned treatment plants (one preconditioned Wt and one preconditioned 35S plant).

**Adult parasitoid *Encarsia formosa* preferences for two tomato genotypes. Y-tube experiments** A Y-tube olfactometer was used to investigate the behavioural responses of *E. formosa* to the volatile blends from different treatments within a tomato cultivar and different tomato cultivars within a same treatment. Each parasitoid *E. formosa* was placed in the olfactometer and observed for up to 5 minutes. A 'no choice' outcome was recorded when the adults remained inactive during the testing period. A 'first choice' outcome was recorded when the adults moved > 5 cm onto either arm (visually assessed by a line marked on each arm). Each experiment involved at least 30 *E. formosa* that made a choice.

**Collection and quantification of plant volatiles.** Volatiles were collected from one randomly selected plant from each combination of O<sub>3</sub> concentration, tomato genotype and whitefly herbivory treatment in each chamber (8 individuals from each tomato genotype × four treatments). The headspace volatiles were collected according to Turlings *et al.*<sup>47</sup>. The shoots and leaves of each plant, except for the stem extending 4 to 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 Labor Instruments, China) into the bag through a freshly activated charcoal trap (Beijing Chemical Company) 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 0.2 L/min. Volatile compounds were rinsed from the Porapak Q with 1000 μl of n-pentane (HPLC grade, Sigma-Aldrich, USA) containing internal standards (200 ng of ethyl heptanoate) for quantification. The

aeration extracts were stored at -20°C until analysed. Immediately after headspace volatiles were collected, the fresh weights of the plant leaves were 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 (60 m long, 0.25 mm inner diameter, and 0.25 mm film thickness; Agilent Technologies, Palo Alto, CA, USA). The initial oven temperature was kept at 35°C for 1 min, which was 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 (terpinolene, (+)-3-carene, (+)- $\alpha$ -pinene, D-limonene,  $\alpha$ -phellandrene, ocimene,  $\beta$ -phellandrene, Z-3-hexanol, E-2-hexanol, nonanal and octanal were purchased from Sigma-Aldrich (St. Louis, MO) and Fluka (Buchs, Switzerland) and the purity of them exceeds 95%).

**Statistical Analyses.** To study the impacts of elevated O<sub>3</sub> on the volatile emission rate, we used a split-split plot design, with O<sub>3</sub> and block (a pair of ambient and elevated OTCs) as the main effects, whitefly herbivory as the subplot effect, and tomato genotypes as the sub-subplot effect according to the following model:

$$X_{ijklm} = \mu + O_i + B(O)_{j(i)} + H_k + OH_{ik} + HB(O)_{kj(i)} + T_l + OT_{il} + TB(O)_{jl(i)} + HTB(O)_{klj(i)} + e_{m(ijkl)}$$

where O is the O<sub>3</sub> treatment ( $i = 2$ ), B is the block ( $j = 4$ ), H is the herbivore treatment ( $k = 2$ ), and T is the tomato genotypes ( $l = 2$ ).  $X_{ijklm}$  represents the error because of the smaller scale differences between samples and variability within blocks (SPSS 13.0, USA). 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 to simplify the presentation. LSD's multiple range tests were used to separate means when ANOVAs were significant. X<sup>2</sup> tests were used to analyse adult whitefly feeding and oviposition preference. The chi-squared test was used to examine the significance of differences between the numbers of parasitoids choosing each olfactometer arm<sup>58,59</sup>.

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## Author contributions

C.H.Y. performed the experiments and wrote the main manuscript text. G.F. designed the experiments. S.J.W., W.J.N. and H.Y.J. helped interpret the data. All of the authors read and approved the final manuscript.

## Additional information

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