

## Article

# Elevated Ozone Reduces the Quality of Tea Leaves but May Improve the Resistance of Tea Plants

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**Abstract:** Tropospheric ozone (O<sub>3</sub>) pollution can affect plant nutritional quality and secondary metabolites by altering plant biochemistry and physiology, which may lead to unpredictable effects on crop quality and resistance to pests and diseases. Here, we investigated the effects of O<sub>3</sub> (ambient air, Am; ambient air +80 ppb of O<sub>3</sub>, EO<sub>3</sub>) on the quality compounds and chemical defenses of a widely cultivated tea variety in China (*Camellia sinensis* cv. 'Baiye 1 Hao') using open-top chamber (OTC). We found that elevated O<sub>3</sub> increased the ratio of total polyphenols to free amino acids while decreasing the value of the catechin quality index, indicating a reduction in leaf quality for green tea. Specifically, elevated O<sub>3</sub> reduced concentrations of amino acids and caffeine but shows no impact on the concentrations of total polyphenols in tea leaves. Within individual catechins, elevated O<sub>3</sub> increased the concentrations of ester catechins but not non-ester catechins, resulting in a slight increase in total catechins. Moreover, elevated O<sub>3</sub> increased the emission of biogenic volatile organic compounds involved in plant defense against herbivores and parasites, including green leaf volatiles, aromatics, and terpenes. Additionally, concentrations of main chemical defenses, represented as condensed tannins and lignin, in tea leaves also increased in response to elevated O<sub>3</sub>. In conclusion, our results suggest that elevated ground-level O<sub>3</sub> may reduce the quality of tea leaves but could potentially enhance the resistance of tea plants to biotic stresses.

**Keywords:** ozone pollution; tea quality; catechins; BVOCs; chemical defenses



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## 1. Introduction

Tropospheric ozone (O<sub>3</sub>), a secondary photochemical pollutant with an oxidant nature, is considered the most widespread air pollutant threatening agricultural production [1,2]. Due to increasing emissions of O<sub>3</sub> precursors in industrialization, China has become a hotspot of O<sub>3</sub> pollution, with an increasing rate of 1–3 ppb year<sup>-1</sup> [3–5]. Ozone pollution over China has resulted in an estimated 8.6–32.8% reduction in three major staple crops (rice, maize, wheat), equivalent to a loss of 63 billion U.S. dollars per year [6]. While the deleterious impacts of elevated O<sub>3</sub> on crop yield have been well documented [6–8], our understanding of O<sub>3</sub> impacts on crop quality lags far behind. In addition, current studies about crop quality focused exclusively on the grain quality of rice, maize, wheat, and soybeans [1]. The influence of elevated O<sub>3</sub> on other crops, especially for lucrative cash crops, is poorly understood.

Tea (*Camellia sinensis* L.) is a well-known cash crop native to China that has been widely planted in tropical and subtropical areas [9]. Due to its significant economic benefits,

tea has been recognized as one of the most important cash crops that plays a significant role in rural development and poverty reduction around the world. China is the world's biggest tea producer, with 3.39 Mha of tea plantation and around 3 Mt of dry semifinished tea production per year [10], accounting for about 70% of the global tea planting area and more than 40% of global tea production [9]. Primary and secondary metabolites, such as amino acids, catechins, and biogenic volatile organic compounds (BVOCs), account for the flavor and defense capacity of tea leaves and thus determine the quality and economic value of tea production [11]. These metabolites are susceptible to environmental disturbances (such as elevated CO<sub>2</sub>, strong light, and drought), causing fluctuations in chemical concentrations of up to 50% in both positive and negative directions [12]. Despite most of the tea plantations in China being located in areas where O<sub>3</sub> concentrations exceed the threshold for plant injury (i.e., 40 ppb) [13], very little is known about how elevated O<sub>3</sub> affects the major chemical compounds in tea [14].

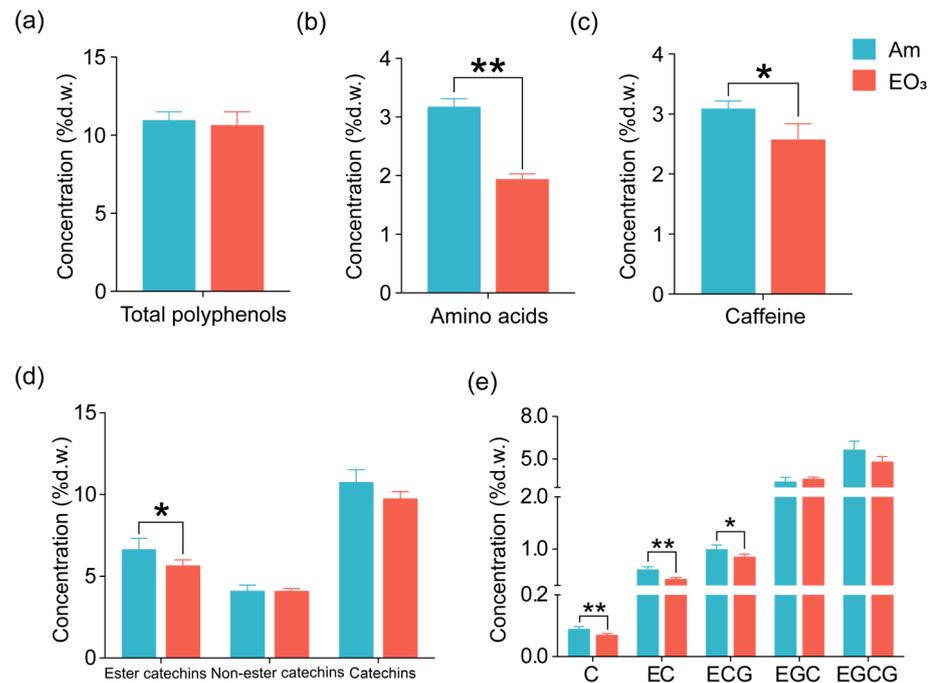
Elevated tropospheric O<sub>3</sub> may alter phytochemical profiles via several mechanisms. On one hand, reactive oxygen species (ROS) generated by O<sub>3</sub> can disrupt photosynthesis and inhibit the production of primary and secondary metabolites [15]. On the other hand, O<sub>3</sub> and ROS can trigger defensive responses and increase the production of antioxidant compounds [16,17]. In general, elevated O<sub>3</sub> leads to reduced amino acid concentrations due to increased consumption for antioxidant synthesis and repair processes upon O<sub>3</sub> damage [18]. The responses of phenolics to elevated O<sub>3</sub> are complex: lignin typically accumulates in plants grown under elevated O<sub>3</sub> to provide a physical barrier to O<sub>3</sub> [19], while concentrations of other phenolics (e.g., catechins, condensed tannins) generally increase at a relatively low dose but may decline under a higher dose of O<sub>3</sub> exposure [20,21]. The effects of O<sub>3</sub> on BVOCs vary among types of volatiles, with total BVOCs increased but isoprene generally reduced in response to elevated O<sub>3</sub> [22–24]. Ozone impacts on alkaloids are poorly understood; however, the key precursors to alkaloids (polyamines) are reported to be associated with O<sub>3</sub> tolerance in plants [17].

This study investigated the effects of elevated O<sub>3</sub> on the phytochemicals that contribute to tea quality and plant resistance in tea leaves. The main quality compounds, including total polyphenols, amino acids, caffeine, and catechins, as well as chemicals with defense functions such as BVOCs, condensed tannins, and lignin, were determined. Leaf quality for green tea was estimated using tea quality indexes. We hypothesized that (1) elevated O<sub>3</sub> would decrease the concentration of amino acids in tea leaves; (2) elevated O<sub>3</sub> would enhance the synthesis of secondary metabolites in tea leaves; and (3) impacts of O<sub>3</sub> on chemical compounds might influence tea quality.

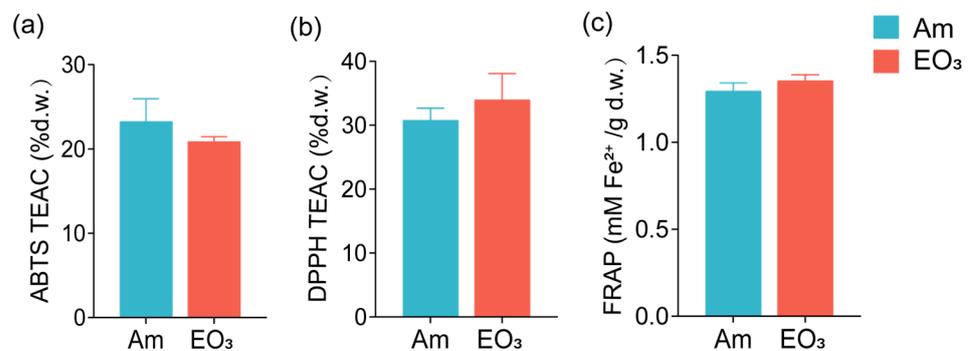
## 2. Results

### 2.1. Effect of Elevated O<sub>3</sub> on Main Quality Compounds

Elevated O<sub>3</sub> exhibited varied influences on the concentrations of key compounds (total polyphenols, amino acids, caffeine, and catechins) that contribute to tea quality. Specifically, elevated O<sub>3</sub> led to a decrease in amino acids and caffeine concentrations in tea leaves by 39% and 17%, respectively, while showing no significant impact on the concentrations of total polyphenols and total catechins (Figure 1). Impacts of elevated O<sub>3</sub> on catechins varied depending on their structure. In general, O<sub>3</sub> decreased the total concentration of ester catechins (ECG, epicatechin gallate; EGCG, epigallocatechin gallate) by 15% while having minimal effects on the total concentration of non-ester catechins (C, catechin; EC, epicatechin; EGC, epigallocatechin) in tea leaves (Figure 1e). Among the detected individual catechin components, we observed significant decreases in concentrations for C (by 22%), EC (by 31%), and ECG (by 15%), with a non-significant decreasing trend for EGCG concentrations in response to elevated O<sub>3</sub> in tea leaves. Similar to the response observed in total polyphenols, both trolox equivalent antioxidant capacity (TEAC) and ferric reducing antioxidant power (FRAP) also exhibited no significant changes in response to elevated O<sub>3</sub> (Figure 2).



**Figure 1.** Effect of elevated O<sub>3</sub> on main quality compounds in tea leaves. (a) Total polyphenols; (b) amino acids; (c) caffeine; (d) ester catechins ( $\Sigma$  ECG, EGCG), non-ester catechins ( $\Sigma$  C, EC, EGC) and total catechins; (e) individual catechin compounds. Each bar represents the mean value  $\pm$  SD ( $n = 4$  trees). % d.w. = percentage of foliar dry weight. Stars indicate significant differences between O<sub>3</sub> treatments (\*,  $0.01 < p < 0.05$ ; \*\*,  $p < 0.01$ ). Am, ambient air; EO<sub>3</sub>, ambient air +80 ppb of O<sub>3</sub>; C, catechin; EC, epicatechin; ECG, epicatechin gallate; EGC, epigallocatechin; EGCG, epigallocatechin gallate.



**Figure 2.** Effect of elevated O<sub>3</sub> on the antioxidant capacity of tea leaves. (a) Trolox equivalent antioxidant capacity (TEAC) in 2,20-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assay; (b) TEAC in 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay; (c) ferric reducing antioxidant power (FRAP). Each bar represents the mean value  $\pm$  SD ( $n = 4$  trees). % d.w. = percentage of foliar dry weight. Am, ambient air; EO<sub>3</sub>, ambient air +80 ppb of O<sub>3</sub>.

Additionally, we calculated commonly used tea quality indices based on the results of the main quality compounds in tea leaves. Our results showed that high concentrations of O<sub>3</sub> decreased the ratio of total polyphenols to amino acids (TP/AA) and catechin quality index (CQI) (Table 1), suggesting decreased leaf quality for green tea.

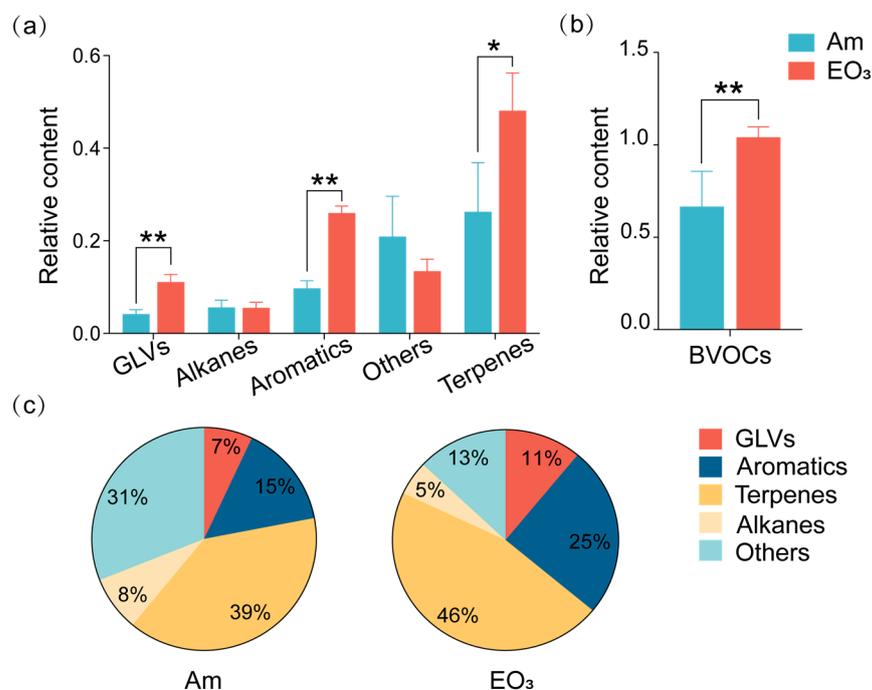
**Table 1.** Effect of elevated O<sub>3</sub> on tea quality indexes.

Tea Quality Index	Am	EO <sub>3</sub>
TP/AA	3.45 ± 0.10 <sup>b</sup>	5.50 ± 0.45 <sup>a</sup>
CQI	1.96 ± 0.23 <sup>a</sup>	1.57 ± 0.07 <sup>b</sup>

Values are depicted as the mean ± SD (*n* = 4 trees). Different lower-case letters denote significant differences between O<sub>3</sub> treatments (*p* < 0.05). Am, ambient air; EO<sub>3</sub>, ambient air +80 ppb of O<sub>3</sub>; TP/AA, the ratio of total polyphenols to amino acids; CQI, catechin quality index.

## 2.2. Effect of Elevated O<sub>3</sub> on Chemicals with Defensive Functions

Elevated O<sub>3</sub> increased the emission of total BVOCs in tea leaves (Figure 3). Our study identified 18 volatiles, classified into five categories: green leaf volatiles (GLVs), aromatics, terpenes, alkanes, and other volatiles (Table 2). Among the detected monomeric BVOCs, O<sub>3</sub> increased the relative abundances of toluene, (E)-2-hexenal, 6-epi-shyobunol, neophytadiene, and tridecane, while decreasing the relative abundance of 2-ethyl-1-hexanol. Overall, the content of total BVOCs was enhanced by the elevated O<sub>3</sub>, with the relative abundances of GLVs, aromatics, and terpenes increased by 166%, 167%, and 83%, respectively, when compared with controlled plants.



**Figure 3.** Effect of elevated O<sub>3</sub> on major BVOC classes in tea leaves. (a) Relative content of five major BVOC categories; (b) relative content of total BVOCs; (c) proportional representation of five major BVOC categories in total measured BVOC pool in tea leaves. Each bar represents the mean value ± SD (*n* = 4 trees). Stars above the bars indicate significant differences between O<sub>3</sub> treatments (\*, 0.01 < *p* < 0.05; \*\*, *p* < 0.01). Am, ambient air; EO<sub>3</sub>, ambient air +80 ppb of O<sub>3</sub>; GLVs, green leaf volatiles.

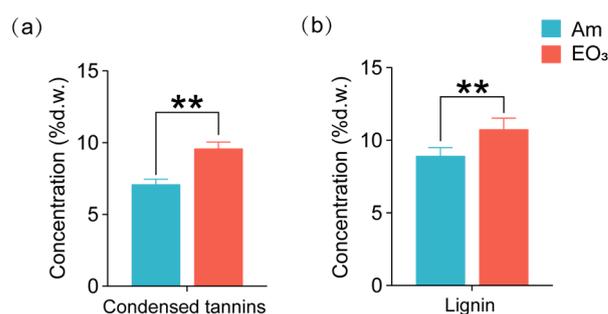
In our study, GLVs, aromatics, and terpenes collectively accounted for over 80% of total BVOCs in tea plants, and their proportions increased significantly in response to elevated O<sub>3</sub>. When compared with controlled tea plants, the proportion of terpenes increased from 39% to 46%, GLVs increased from 7% to 11%, and aromatics increased from 15% to 25% in tea plants exposed to elevated O<sub>3</sub>. Conversely, decreased proportions were observed for alkanes (from 8% to 5%) and others (from 31% to 13%) in tea plants grown under elevated O<sub>3</sub> conditions, primarily due to the O<sub>3</sub>-induced increase of relative abundances of GLVs, aromatics, and terpenes.

**Table 2.** Effect of elevated O<sub>3</sub> on major volatile organic compounds in tea leaves.

Volatile Class	Individual Compounds	Retention Time (min)	Am	EO <sub>3</sub>
Green leaf volatiles	E-2-hexenal	6.632	0.025 ± 0.010 <sup>b</sup>	0.106 ± 0.014 <sup>a</sup>
	2-Ethyl-1-hexanol	11.901	0.016 ± 0.008 <sup>a</sup>	0.005 ± 0.001 <sup>b</sup>
Aromatics	Toluene	5.303	0.050 ± 0.007 <sup>b</sup>	0.228 ± 0.008 <sup>a</sup>
	Benzyl alcohol	12.156	0.005 ± 0.003 <sup>a</sup>	0.003 ± 0.001 <sup>a</sup>
Terpenes	2,4-Di-tert-butylphenol	25.103	0.042 ± 0.006 <sup>a</sup>	0.029 ± 0.009 <sup>a</sup>
	Trans-Furanic linalool oxid	13.591	0.005 ± 0.003 <sup>a</sup>	0.005 ± 0.001 <sup>a</sup>
	Geraniol	18.901	0.003 ± 0.001 <sup>a</sup>	0.003 ± 0.001 <sup>a</sup>
Alkanes	Neophytadiene	32.261	0.172 ± 0.064 <sup>b</sup>	0.362 ± 0.059 <sup>a</sup>
	Phytol	37.536	0.078 ± 0.024 <sup>a</sup>	0.091 ± 0.020 <sup>a</sup>
	6-epi-shyobunol	28.565	0.005 ± 0.002 <sup>b</sup>	0.021 ± 0.004 <sup>a</sup>
	Undecane	11.312	0.002 ± 0.001 <sup>a</sup>	0.003 ± 0.001 <sup>a</sup>
	Dodecane	12.635	0.010 ± 0.005 <sup>b</sup>	0.015 ± 0.003 <sup>a</sup>
	Tridecane	17.246	0.009 ± 0.001 <sup>a</sup>	0.007 ± 0.001 <sup>a</sup>
Others	Pentadecane	19.088	0.032 ± 0.009 <sup>a</sup>	0.027 ± 0.007 <sup>a</sup>
	1-Methoxyadamantane	16.628	0.003 ± 0.001 <sup>a</sup>	0.004 ± 0.001 <sup>a</sup>
	2,4-Dimethyl-1-heptanol	13.976	0.007 ± 0.002 <sup>a</sup>	0.006 ± 0.001 <sup>a</sup>
	Hexyl octyl ether	14.151	0.002 ± 0.001 <sup>a</sup>	0.001 ± 0.000 <sup>a</sup>
	Cyclohexanol	7.442	0.201 ± 0.076 <sup>a</sup>	0.128 ± 0.021 <sup>a</sup>

Values are depicted as the mean ± SD ( $n = 4$  trees). Different lower-case letters denote significant differences between O<sub>3</sub> treatments ( $p < 0.05$ ). Am, ambient air; EO<sub>3</sub>, ambient air +80 ppb of O<sub>3</sub>.

In addition, elevated O<sub>3</sub> led to a 35% increase in the concentrations of condensed tannins and a 21% increase in lignin (Figure 4).



**Figure 4.** Effect of elevated O<sub>3</sub> on major defensive metabolites in tea leaves. (a) Condensed tannins; (b) lignin. Each bar represents the mean value ± SD ( $n = 4$  trees). % d.w. = percentage of foliar dry weight. Stars indicate significant differences between O<sub>3</sub> treatments (\*\*,  $p < 0.01$ ). Am, ambient air; EO<sub>3</sub>, ambient air +80 ppb of O<sub>3</sub>.

### 3. Discussion

#### 3.1. O<sub>3</sub> Impacts on Main Quality Compounds

Amino acids, caffeine, and polyphenols are the three major taste components in tea, contributing to umami, bitterness, and astringency, respectively [11]. Consistent with our first hypothesis, elevated O<sub>3</sub> decreased the concentration of amino acids in tea leaves. This finding aligns with previous studies on poplars, which also observed decreased amino acid concentrations following O<sub>3</sub> exposure. This response is likely attributed to the increased demand of amino acids in the detoxification and repair processes following O<sub>3</sub> injury [18,25].

Contrary to our second hypothesis, elevated O<sub>3</sub> shows varied impacts on secondary compounds. Ozone fumigation resulted in reduced caffeine concentrations in tea leaves, partially supporting previous studies in tea plants that observed decreased caffeine synthesis under stressful conditions [26]. Interestingly, elevated O<sub>3</sub> showed minimal effects on the concentrations of total polyphenols, aligning with the findings of Da Rosa Santos et al. [16] in *Tibouchina pulchra* (Cham.), where no significant response of total polyphenols to increased O<sub>3</sub> concentration was observed. Nevertheless, our results diverge from other

studies reporting either increased or decreased foliar total polyphenols in response to elevated  $O_3$  [27,28]. The inconsistent response of polyphenols across different studies might be attributed to the concept of  $O_3$ -induced hormesis in plants. This theory suggests that the production of antioxidative compounds may increase at low-to-moderate  $O_3$  exposures but decline when the defense response is overwhelmed under higher  $O_3$  exposure [21,29]. In the present study, antioxidant capacity in tea leaves mirrored the response of phenolics and showed no response to elevated  $O_3$ , which potentially supports the concept of hormesis. Additionally, other factors, including plant species, genotypes, plant development status, and the duration of  $O_3$  fumigation, may also contribute to the variable responses of total polyphenols to elevated  $O_3$  treatments [20].

It is worth noting that, while the total polyphenols and overall catechins concentrations exhibited a minimal response to elevated  $O_3$ , concentrations of C, EC, and ECG reduced significantly under elevated  $O_3$  treatment. According to the results of the TEAC assay, the scavenging capacity of the five main catechin compounds in tea follows this order: EGCG > EGC > ECG > EC > C [30]. In this context,  $O_3$ -induced decline in the concentrations of C, EC, and ECG may suggest that tea plants tend to prioritize the synthesis of compounds with relatively higher radical scavenging capacity, such as EGC and EGCG, under  $O_3$  stresses. Moreover, the concentrations of ester catechins—serving as the primary astringent compounds in tea—decrease with increasing  $O_3$  concentration, which may lead to changes of tea quality.

Quality indices, specifically represented by TP/AA and CQI, provide a quick assessment of the taste and overall quality of tea based on changes of flavoring compounds. Our results showed that both the TP/AA ratio and CQI value decreased in response of elevated  $O_3$ . The TP/AA ratio is widely acknowledged as a reliable marker of tea quality, influencing the taste by making tea either brisk and mellow with a lower TP/AA ratio or more astringent with a higher ratio [31]. Meanwhile, the value of CQI has a relatively limited scope of application compared to the TP/AA ratio, primarily due to the varied catechin accumulation characteristics among species during shoot elongation [32]. In our present study, however, the use of tea cutting seedlings from the same cultivar ensured no variation in catechin accumulation characteristics among treatment plants. Consequently, the CQI value becomes a valuable tool to discern the impact of  $O_3$  fumigation on tea quality, with an increased CQI value indicating decreased tea quality upon  $O_3$  stresses.

Collectively, our results suggest that elevated  $O_3$  may decrease the quality of tea leaves, as predicted in our third hypothesis. Specifically, the taste profile of green tea may be altered by elevated  $O_3$ , potentially leading to a decrease in umami, bitterness, and astringency. Given the potential interactions among taste compounds in modifying taste intensity [33], future studies incorporating sensory analysis can be conducted to clearly identify the impacts of elevated  $O_3$  on the taste of tea.

### 3.2. $O_3$ Impacts on Chemicals with Defensive Functions

Biogenic volatile organic compounds generally function as communication media between plants and insects [34]. In the present study,  $O_3$  was found to increase the release of BVOCs categories that have herbivore defense and/or predator attractive functions, represented as elevated loads of GLVs, terpenes, and aromatic compounds. Our results are in agreement with studies on broccoli and hybrid aspens, which reported higher amounts of GLV and terpenes when exposed to elevated  $O_3$  [35,36]. However, our findings contrast with one study on *Brassica napus* that observed a decreased emission of terpenes under elevated  $O_3$  conditions [37]. It has been well documented that GLVs serve as non-specific signals of on-going damage that can be utilized by natural predators [38,39], while terpenes function as highly specific defense signals that synthesized through specialized biosynthetic processes [40]. Therefore, the  $O_3$ -induced increase in emissions of GLVs and terpenes may suggest an enhanced defense capacity of tea plants against insect herbivores.

It is noteworthy that elevated  $O_3$  significantly increased the relative abundance of (*E*)-2-hexenal, a GLV component acting as an infochemical for insects, in tea leaves. (*E*)-

2-hexenal has been reported to attract both insect pests and natural predators within tea plantations [41,42]. Using both Y-tube olfactometer and field trapping tests, Mu et al. [43] demonstrated the role of (*E*)-2-hexenal in attracting tea shoot volatiles to tea green leafhoppers (*Empoasca vitis* Gothe). In the electroantennogram responses and the wind tunnel bioassays, Han and Chen [44] found that several natural predators, including *Chrysopa sinica* and *Coccinella septempunctata*, exploit (*E*)-2-hexenal as a long-range cue to locate and approach tea aphids (*Aphidius* sp.). Therefore, the O<sub>3</sub>-induced enhanced emission of (*E*)-2-hexenal in our study indicates that elevated O<sub>3</sub> may exert an unpredictable influence on the relationship between tea plants and insect pests.

In line with findings in other plant species [19,20,45], our study observed increased concentrations of condensed tannins and lignin in tea plants exposed to elevated O<sub>3</sub> compared to those grown in ambient air. These results supported the notions that plants defend against O<sub>3</sub> stresses by accumulating antioxidative components and reinforcing structural barrier to O<sub>3</sub> [19]. However, some studies have reported unchanged or even reduced condensed tannins in response to elevated O<sub>3</sub> [36,45,46]. This variation has been attributed to the inhibition of carbon-based defensive compounds synthesis due to heavily suppressed plant photosynthetic carbon fixation under elevated O<sub>3</sub> [47]. As two, well-studied defense chemicals in plants, condensed tannins and lignin are believed to reduce herbivory by increasing toxicity or decreasing palatability of host plants to insect herbivores [14,45,46]. They are also responsible for plant protection against pathogens [48]. An ozone-induced increase in condensed tannins and lignin concentrations has been reported to impact the performance of herbivores [45,49].

## 4. Materials and Methods

### 4.1. Experimental Site and Plant Materials

The study was conducted within open-top chambers (OTCs; 3 m in height, 4 m in diameter, octagonal shape, glass-covered) at the Tea Research Institute, Chinese Academy of Agricultural Sciences located in Hangzhou, China (30°10' N, 120°5' E, 19 m a.s.l.), from early spring to early summer of 2023. The experimental site features a subtropical monsoon climate with a mean annual air temperature of approximately 17.0 °C and a mean annual precipitation of exceeding 1500 mm. The widely cultivated tea variety 'BaiYe 1 Hao' was used for analysis. Tea cutting seedlings were planted in a sterilized germination mix (vermiculite: perlite: nutritive soil = 1:1:3) and allowed to grow for two years before being transplanted into 5-gallon pots in October 2022. On February 28th, tea plants were transferred to OTCs for a 7-day adaptation period. Subsequently, 16 tea plants with similar growth conditions were selected and randomly distributed into four OTCs for different O<sub>3</sub> treatments, with a total of four plants per OTC. Throughout the entire experimental period, tea plants were supplied with water as needed and maintained through standardized horticultural practices. At the time of the experiment, the tea plants were three years old.

### 4.2. Experimental Design

The experiment set up two O<sub>3</sub> concentration levels (Am, ambient air; EO<sub>3</sub>, ambient air + 80 ppb O<sub>3</sub>) with two replicated OTCs per O<sub>3</sub> treatment. Ozone was generated from pure oxygen using an O<sub>3</sub> generator (CFG-20, Sankang Co., Jinan, China), and a mixture of O<sub>3</sub> and ambient air was ventilated into each OTC through Teflon tubes. Continuous monitoring of O<sub>3</sub> concentration in the OTCs was achieved using a UV photometric O<sub>3</sub> detector (Model 49i, Thermo Fisher Scientific, Franklin, MA, USA). The flow rate of O<sub>3</sub> was controlled by an automatic controller (SC200, SevenStar Flow Co., Kunshan, China) to achieve the target concentration. Ozone fumigation was implemented for 8 h per day (from 9:00 to 17:00) over a duration of 96 days (from 6 March to 10 June 2023), with temporary suspension on days of precipitation. Throughout the fumigation period, the mean daily O<sub>3</sub> concentrations within the OTCs under Am and EO<sub>3</sub> treatment were 22.72 ± 1.28 ppb and 91.92 ± 1.43 ppb, respectively. Correspondingly, the AOT40 values (i.e., the accumulated O<sub>3</sub> exposure over a threshold of 40 ppb in daily hours) for the Am and EO<sub>3</sub> treatments

during the entire experimental period were  $1.57 \pm 0.58$  ppm h and  $56.36 \pm 1.60$  ppm h, respectively.

#### 4.3. Sample Collection

Following O<sub>3</sub> fumigation, two plants were randomly chosen from each OTC for sampling. The shoot tips (one bud and two leaves) were collected from tea plants, promptly immersed into liquid nitrogen, and transported to the laboratory within 2 h. Each sample was divided into two halves for distinct analyses. The first half was vacuum dried, pulverized by ball milling, and then stored at  $-20$  °C for analyses of non-volatile chemical compounds. The other half was stored at  $-80$  °C to facilitate the analyses of biogenic BVOCs.

#### 4.4. Chemical Analyses

Concentrations of total polyphenols were analyzed using a Folin–Ciocalteu assay with a slight modification [21]. In brief, 20–30 mg of leaf powder was extracted with 1.5 mL methanol, with 30 min of sonication. Properly diluted supernatant was then mixed with 10-fold diluted Folin–Ciocalteu reagent and 10% sodium carbonate water solution. The mixture was incubated in the dark for 30 min, and the absorbance at 760 nm was measured using a spectrophotometer (SpectraMax M2, Molecular Devices, Sunnyvale, CA, USA). The concentration of each sample was computed relative to standardization with gallic acid.

Free amino acids were quantified using the ninhydrin method modified from the Standardization Administration of China, GB/T 8314-2013 [50]. Leaf powder (20–30 mg) was extracted with boiled distilled water (1 mL) for 5 min. A subsample of the supernatant (100 µL) was mixed with 50 µL of 2% ninhydrin solution (augmented with 0.08% stannous oxide) and 50 µL of phosphate buffer (pH 8.0). After being heated in a boiling water bath for 5 min to facilitate the reaction, absorbance of the mixture was determined at 570 nm, and amino acid concentrations were calculated against theanine authentic standards.

Caffeine and the major individual catechins (C, EC, EGC, ECG, EGCG) were extracted into methanol and quantified using a high-performance liquid chromatography (HPLC; Alliance e2695, Waters, Milford, MA, USA) with a photodiode array detector (ACQUITY 2998, Waters, Milford, MA, USA) as described by Li et al. [30]. The retention time and chromatogram of each analyte are detailed in Table S1 and Figure S1. Concentrations of caffeine and individual catechins were quantified based on their peak areas with calibration against authentic standards. Total catechins were determined by summing the concentrations of the five individual catechins. Ester catechins were determined by summing the concentrations of ECG and EGCG. Non-ester catechins were determined by summing the concentrations of C, EC, and EGC.

Condensed tannins were determined using the acid–butanol method with modifications [51]. In brief, condensed tannins were extracted from 20–30 mg of leaf powder into 1.5 mL 70% acetone solution (include 10 mM ascorbic acid) with 30 min of sonication. A subsample of the supernatant (100 µL) was mixed with 400 µL of 70% acetone solution, 2 mL N-butanol/hydrochloric acid (95:5) solution, and 100 µL acidified 2% ferric ammonium sulfate solution (dissolved with 2N hydrochloric acid), followed by a 50 min boiling water bath. Condensed tannins were quantified by measuring the absorbance at 550 nm with standardization against proanthocyanins. Lignin concentrations were determined by the thioglycolic acid method following the steps described in Suzuki et al. [52], with alkali lignin as standards.

Quantification of BVOCs was performed using a gas chromatograph-mass spectrometer (GC-MS; 8890/5977B GC-MS system, Agilent Technologies, Santa Clara, CA, USA). The organic solvent was extracted with ethyl acetate (1:30,000) containing n-octanol as internal standard. Samples (2 µL) were injected into an HP-5MS chromatographic column (30 m × 0.25 mm, 1909IS-433UI, Agilent Technologies, Santa Clara, CA, USA). The injection temperature was 250 °C, and a temperature gradient of 5 °C/min from 40 °C (hold 3 min) to 250 °C was employed. The mass spectrometer (MS/MS) was operated in the electronic

ionization (EI) mode with 70 eV of electron energy. All MS data were collected from 40 to 400  $m/z$  [53]. The relative content of BVOCs was calculated by dividing the total ion current peak areas by the peak area of the internal standard. The sample chromatograms are shown in Figure S2.

#### 4.5. Antioxidant Activity

Trolox equivalent antioxidant capacity (TEAC) of tea samples was determined using DPPH radical and ABTS radical cation assays following the steps described in Li et al. [30]. The ferric reducing antioxidant power (FRAP) was measured by incubating a mixture of 100  $\mu$ L leaf extract and 900  $\mu$ L FRAP reagent (contained 8.3 mM 2,4,6-tripyridyl-s-triazine and 16.7 mM  $\text{FeCl}_3$ ) at 37 °C for 10 min, followed by measuring the absorbance at 593 nm.

#### 4.6. Assessment of Tea Quality Indexes

The ratio of total polyphenols to amino acids (TP/AA) reflects the balance between mellow tastes and astringency. Typically, the TP/AA ratio is negatively correlated with the quality of green tea, where a lower TP/AA ratio leads to a more brisk and mellow, but less bitter in taste [54]. Another quality index, catechin quality index (CQI), is calculated as the ratio of (EGCG + ECG) to EGC and is negatively correlated with the tenderness and overall quality of green tea [55].

#### 4.7. Statistical Analyses

SPSS 26.0 (SPSS Inc., Apache Software Foundation, Chicago, IL, USA) was used for statistical analysis of the calculated data under different treatments. To assess the effects of elevated  $\text{O}_3$  on the concentrations of main chemical compounds, tea quality indexes, relative abundances of BVOCs, and defense chemical compounds, the data were subjected to a one-way analysis of variance (one-way ANOVA). The Shapiro–Wilk normality test was applied to verify normality and homogeneity of variance. Differences were considered significant if  $p < 0.05$ . All quantitative data were expressed as mean  $\pm$  SD ( $n = 4$  trees).

## 5. Conclusions

Our study revealed that elevated  $\text{O}_3$  altered the concentrations of both quality and defense compounds in tea leaves. Impacts of elevated  $\text{O}_3$  on the quality of tea leaves is primarily attributed to decreased amino acid and caffeine concentrations and an altered catechins composition. Ozone was found to have decreased TP/AA and CQI, signifying a decrease in the quality of green tea. Furthermore, elevated  $\text{O}_3$  increased the release of BVOCs compounds with herbivore defense and/or predator-attractive functions in tea, as evidenced by the increased relative abundance of GLVs, aromatics, and terpenes. Elevated  $\text{O}_3$  also enhanced the accumulation of condensed tannins and lignin, indicating an improved resistance of tea plants to insect pests. In summary, our findings suggest that ozone concentrations of around 90 ppb reduce the quality of tea but may enhance the ability of tea plants to resist pests and diseases. Future studies should prioritize investigating the dose–response relationships between elevated tropospheric  $\text{O}_3$  and tea quality to accurately estimate the impacts of  $\text{O}_3$  pollution in tea production. Additionally, developing methods to mitigate the adverse effects of  $\text{O}_3$  pollution requires a deeper understanding of the mechanisms underlying  $\text{O}_3$  impacts on tea quality compounds.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/plants13081108/s1>, Table S1: the retention time of caffeine and individual catechins; Figure S1. HPLC chromatograms of tea leaves under (a) ambient air and (b) elevated  $\text{O}_3$  treatment; Figure S2. The total ion chromatogram of volatile components in tea leaves under (a) ambient air and (b) elevated  $\text{O}_3$  treatment.

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supervision, D.M.; writing—original draft, N.W., Y.W. (Yuxi Wang) and Z.L.; writing—review and editing, G.L. and J.F. All authors have read and agreed to the published version of the manuscript.

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## References

1. Wang, T.; Xue, L.; Feng, Z.; Dai, J.; Zhang, Y.; Tan, Y. Ground-level ozone pollution in China: A synthesis of recent findings on influencing factors and impacts. *Environ. Res. Lett.* **2022**, *17*, 063003. [CrossRef]
2. Yan, D.; Zhang, Z.; Jin, Z.; Li, M.; Sheridan, S.C.; Wang, T. Ozone variability driven by the synoptic patterns over China during 2014–2022 and its implications for crop yield and economy. *Atmos. Pollut. Res.* **2023**, *14*, 101843. [CrossRef]
3. Li, K.; Jacob, D.J.; Liao, H.; Shen, L.; Zhang, Q.; Bates, K.H. Anthropogenic drivers of 2013–2017 trends in summer surface ozone in China. *Proc. Natl. Acad. Sci. USA* **2019**, *116*, 422–427. [CrossRef]
4. Lu, X.; Hong, J.; Zhang, L.; Cooper, O.R.; Schultz, M.G.; Xu, X.; Wang, T.; Gao, M.; Zhao, Y.; Zhang, Y. Severe surface ozone pollution in China: A global perspective. *Environ. Sci. Technol. Lett.* **2018**, *5*, 487–494. [CrossRef]
5. Chen, B.; Wang, Y.; Huang, J.; Zhao, L.; Chen, R.; Song, Z.; Hu, J. Estimation of near-surface ozone concentration and analysis of main weather situation in China based on machine learning model and Himawari-8 TOAR data. *Sci. Total Environ.* **2023**, *864*, 160928. [CrossRef]
6. Feng, Z.; Xu, Y.; Kobayashi, K.; Dai, L.; Zhang, T.; Agathokleous, E.; Calatayud, V.; Paoletti, E.; Mukherjee, A.; Agrawal, M.; et al. Ozone pollution threatens the production of major staple crops in East Asia. *Nat. Food* **2022**, *3*, 47–56. [CrossRef]
7. Mills, G.; Sharps, K.; Simpson, D.; Pleijel, H.; Broberg, M.; Uddling, J.; Jaramillo, F.; Davies, W.J.; Dentener, F.; Van den Berg, M.; et al. Ozone pollution will compromise efforts to increase global wheat production. *Glob. Chang. Biol.* **2018**, *24*, 3560–3574. [CrossRef]
8. Li, S.; Leakey, A.D.B.; Moller, C.A.; Montes, C.M.; Sacks, E.J.; Lee, D.; Ainsworth, E.A. Similar photosynthetic but different yield responses of C3 and C4 crops to elevated O<sub>3</sub>. *Proc. Natl. Acad. Sci. USA* **2023**, *120*, e2313591120. [CrossRef]
9. FAO; CAAS. *Carbon Neutral Tea Production in China—Three Pilot Case Studies*; FAO: Rome, Italy, 2021.
10. National Bureau of Statistics. Available online: <https://data.stats.gov.cn/easyquery.htm?cn=C01> (accessed on 20 December 2023).
11. Zhang, L.; Cao, Q.-Q.; Granato, D.; Xu, Y.-Q.; Ho, C.-T. Association between chemistry and taste of tea: A review. *Trends Food Sci. Technol.* **2020**, *101*, 139–149. [CrossRef]
12. Ahmed, S.; Unachukwu, U.; Stepp, J.R.; Peters, C.M.; Long, C.; Kennelly, E. Pu-erh tea tasting in Yunnan, China: Correlation of drinkers' perceptions to phytochemistry. *J. Ethnopharmacol.* **2010**, *132*, 176–185. [CrossRef]
13. Yang, G.; Liu, Y.; Li, X. Spatiotemporal distribution of ground-level ozone in China at a city level. *Sci. Rep.* **2020**, *10*, 7229. [CrossRef]
14. Cotrozzi, L.; Conti, B.; Lorenzini, G.; Pellegrini, E.; Nali, C. In the tripartite combination ozone-poplar-*Chrysomela populi*, the pollutant alters the plant-insect interaction via primary metabolites of foliage. *Environ. Res.* **2021**, *201*, 111581. [CrossRef]
15. Feng, Z.; Agathokleous, E.; Yue, X.; Oksanen, E.; Paoletti, E.; Sase, H.; Gandin, A.; Koike, T.; Calatayud, V.; Yuan, X.; et al. Emerging challenges of ozone impacts on asian plants: Actions are needed to protect ecosystem health. *Ecosyst. Health Sustain.* **2021**, *7*, 1911602. [CrossRef]
16. da Rosa Santos, A.C.; Furlan, C.M. Levels of phenolic compounds in *Tibouchina pulchra* after fumigation with ozone. *Atmos. Pollut. Res.* **2013**, *4*, 250–256. [CrossRef]
17. Singh, A.A.; Ghosh, A.; Agrawal, M.; Agrawal, S.B. Secondary metabolites responses of plants exposed to ozone: An update. *Environ. Sci. Pollut. Res.* **2023**, *30*, 88281–88312. [CrossRef]
18. Shang, B.; Feng, Z.; Peng, J.; Xu, Y.; Paoletti, E. Effects of elevated ozone and nitrogen addition on leaf nitrogen metabolism in poplar. *J. Plant Ecol.* **2021**, *14*, 555–568. [CrossRef]
19. Li, Z.; Yang, J.; Shang, B.; Xu, Y.; Couture, J.J.; Yuan, X.; Kobayashi, K.; Feng, Z. Water stress rather than N addition mitigates impacts of elevated O<sub>3</sub> on foliar chemical profiles in poplar saplings. *Sci. Total Environ.* **2020**, *707*, 135935. [CrossRef]
20. Couture, J.J.; Holeski, L.M.; Lindroth, R.L. Long-term exposure to elevated CO<sub>2</sub> and O<sub>3</sub> alters aspen foliar chemistry across developmental stages. *Plant Cell Environ.* **2014**, *37*, 758–765. [CrossRef]
21. Li, Z.; Yang, J.; Shang, B.; Agathokleous, E.; Rubert-Nason, K.F.; Xu, Y.; Feng, Z. Nonlinear responses of foliar phenylpropanoids to increasing O<sub>3</sub> exposure: Ecological implications in a Populus model system. *Sci. Total Environ.* **2021**, *767*, 144358. [CrossRef]
22. Cui, H.; Su, J.; Wei, J.; Hu, Y.; Ge, F. Elevated O<sub>3</sub> enhances the attraction of whitefly-infested tomato plants to *Encarsia formosa*. *Sci. Rep.* **2014**, *4*, 5350. [CrossRef]

23. Yuan, X.; Calatayud, V.; Gao, F.; Fares, S.; Paoletti, E.; Tian, Y.; Feng, Z. Interaction of drought and ozone exposure on isoprene emission from extensively cultivated poplar. *Plant Cell Environ.* **2016**, *39*, 2276–2287. [[CrossRef](#)]
24. Feng, Z.; Yuan, X.; Fares, S.; Loreto, F.; Li, P.; Hoshika, Y.; Paoletti, E. Isoprene is more affected by climate drivers than monoterpenes: A meta-analytic review on plant isoprenoid emissions. *Plant Cell Environ.* **2019**, *42*, 1939–1949. [[CrossRef](#)]
25. Dumont, J.; Keski-Saari, S.; Keinänen, M.; Cohen, D.; Ningre, N.; Kontunen-Soppela, S.; Baldet, P.; Gibon, Y.; Dizengremel, P.; Vaultier, M.-N. Ozone affects ascorbate and glutathione biosynthesis as well as amino acid contents in three *Euramerican poplar* genotypes. *Tree Physiol.* **2014**, *34*, 253–266. [[CrossRef](#)]
26. Munivenkatappa, N.; Sarikonda, S.; Rajagopal, R.; Balakrishnan, R.; Nagarathana, C.K. Variations in quality constituents of green tea leaves in response to drought stress under south Indian condition. *Sci. Hortic.* **2018**, *233*, 359–369. [[CrossRef](#)]
27. Saviranta, N.M.; Julkunen-Tiitto, R.; Oksanen, E.; Karjalainen, R.O. Leaf phenolic compounds in red clover (*Trifolium pratense* L.) induced by exposure to moderately elevated ozone. *Environ. Pollut.* **2010**, *158*, 440–446. [[CrossRef](#)]
28. He, X.; Huang, W.; Chen, W.; Dong, T.; Liu, C.; Chen, Z.; Xu, S.; Ruan, Y. Changes of main secondary metabolites in leaves of *Ginkgo biloba* in response to ozone fumigation. *J. Environ. Sci.* **2009**, *21*, 199–203. [[CrossRef](#)]
29. Agathokleous, E.; Calabrese, E.J. Hormesis can enhance agricultural sustainability in a changing world. *Glob. Food Secur.* **2019**, *20*, 150–155. [[CrossRef](#)]
30. Li, Z.; Wu, Y.; Zhang, L.; Hasan, M.K.; Zhang, L.; Yan, P.; Fu, J.; Han, W.; Li, X. A chemical explanation for variations in antioxidant capacity across *Camellia sinensis* L. cultivars. *Forests* **2023**, *14*, 249. [[CrossRef](#)]
31. Tounekti, T.; Joubert, E.; Hernández, I.; Munné-Bosch, S. Improving the polyphenol content of tea. *Crit. Rev. Plant Sci.* **2013**, *32*, 192–215. [[CrossRef](#)]
32. JIANG, C.-K.; NI, D.-J.; YAO, M.-Z. Metabolic and transcriptome analysis reveals metabolite variation and flavonoid regulatory networks in fresh shoots of tea (*Camellia sinensis*) over three seasons. *Front. Agric. Sci. Eng.* **2021**, *8*, 215–230.
33. Xu, Y.-Q.; Zhang, Y.-N.; Chen, J.-X.; Wang, F.; Du, Q.-Z.; Yin, J.-F. Quantitative analyses of the bitterness and astringency of catechins from green tea. *Food Chem.* **2018**, *258*, 16–24. [[CrossRef](#)]
34. Masui, N.; Agathokleous, E.; Tani, A.; Matsuura, H.; Koike, T. Plant-insect communication in urban forests: Similarities of plant volatile compositions among tree species (host vs. non-host trees) for alder leaf beetle *Agelastica coerulea*. *Environ. Res.* **2022**, *204*, 111996. [[CrossRef](#)]
35. Li, T.; Blande, J.D. Associational susceptibility in broccoli: Mediated by plant volatiles, impeded by ozone. *Glob. Chang. Biol.* **2015**, *21*, 1993–2004. [[CrossRef](#)]
36. Sugai, T.; Okamoto, S.; Agathokleous, E.; Masui, N.; Satoh, F.; Koike, T. Leaf defense capacity of Japanese elm (*Ulmus davidiana* var. *japonica*) seedlings subjected to a nitrogen loading and insect herbivore dynamics in a free air ozone-enriched environment. *Environ. Sci. Pollut. Res.* **2020**, *27*, 3350–3360.
37. Acton, W.J.F.; Jud, W.; Ghirardo, A.; Wohlfahrt, G.; Hewitt, C.; Taylor, J.E.; Hansel, A. The effect of ozone fumigation on the biogenic volatile organic compounds (BVOCs) emitted from *Brassica napus* above-and below-ground. *PLoS ONE* **2018**, *13*, e0208825. [[CrossRef](#)]
38. Shiojiri, K.; Kishimoto, K.; Ozawa, R.; Kugimiya, S.; Urashimo, S.; Arimura, G.; Horiuchi, J.; Nishioka, T.; Matsui, K.; Takabayashi, J. Changing green leaf volatile biosynthesis in plants: An approach for improving plant resistance against both herbivores and pathogens. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 16672–16676. [[CrossRef](#)]
39. Matsui, K.; Kurishita, S.; Hisamitsu, A.; Kajiwara, T. A lipid-hydrolysing activity involved in hexenal formation. *Biochem. Soc. Trans.* **2000**, *28*, 857–860. [[CrossRef](#)]
40. Jin, J.; Zhao, M.; Jing, T.; Zhang, M.; Lu, M.; Yu, G.; Wang, J.; Guo, D.; Pan, Y.; Hoffmann, T.D. Volatile compound-mediated plant–plant interactions under stress with the tea plant as a model. *Hortic. Res.* **2023**, *10*, uhad143. [[CrossRef](#)]
41. Allmann, S.; Baldwin, I.T. Insects betray themselves in nature to predators by rapid isomerization of green leaf volatiles. *Science* **2010**, *329*, 1075–1078. [[CrossRef](#)]
42. Scala, A.; Allmann, S.; Mirabella, R.; Haring, M.A.; Schuurink, R.C. Green leaf volatiles: A plant’s multifunctional weapon against herbivores and pathogens. *Int. J. Mol. Sci.* **2013**, *14*, 17781–17811. [[CrossRef](#)]
43. Mu, D.; Cui, L.; Ge, J.; Wang, M.X.; Liu, L.F.; Yu, X.P.; Zhang, Q.H.; Han, B.Y. Behavioral responses for evaluating the attractiveness of specific tea shoot volatiles to the tea green leafhopper, *Empoasca vitis*. *Insect Sci.* **2012**, *19*, 229–238. [[CrossRef](#)]
44. Han, B.Y.; Chen, Z.M. Composition of the volatiles from intact and mechanically pierced Tea Aphid—Tea shoot complexes and their attraction to natural enemies of the Tea Aphid. *J. Agric. Food Chem.* **2002**, *50*, 2571–2575. [[CrossRef](#)]
45. Lindroth, R.L. Impacts of elevated atmospheric CO<sub>2</sub> and O<sub>3</sub> on forests: Phytochemistry, trophic interactions, and ecosystem dynamics. *J. Chem. Ecol.* **2010**, *36*, 2–21. [[CrossRef](#)]
46. Sakikawa, T.; Shi, C.; Nakamura, M.; Watanabe, M.; Oikawa, M.; Satoh, F.; Koike, T. Leaf phenology and insect grazing of Japanese white birch saplings grown under free-air ozone exposure. *J. Agric. Meteorol.* **2016**, *72*, 80–84. [[CrossRef](#)]
47. Duque, L.; Poelman, E.H.; Steffan-Dewenter, I. Plant-mediated effects of ozone on herbivores depend on exposure duration and temperature. *Sci. Rep.* **2019**, *9*, 19891. [[CrossRef](#)]
48. Miranda, M.; Ralph, S.G.; Mellway, R.; White, R.; Heath, M.C.; Bohlmann, J.; Constabel, C.P. The transcriptional response of hybrid poplar (*Populus trichocarpa* × *P. deltoids*) to infection by *Melampsora medusae* leaf rust involves induction of flavonoid pathway genes leading to the accumulation of proanthocyanidins. *Mol. Plant Microbe Interact.* **2007**, *20*, 816–831. [[CrossRef](#)]

49. Couture, J.J.; Lindroth, R.L. Atmospheric change alters performance of an invasive forest insect. *Glob. Chang. Biol.* **2012**, *18*, 3543–3557. [[CrossRef](#)]
50. GB/T 8314-2013; Tea-Determination of Free Amino Acids Concentration. Standardization Administration of China: Beijing, China, 2013.
51. Porter, L.J.; Hrstich, L.N.; Chan, B.G. The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. *Phytochemistry* **1985**, *25*, 223–230. [[CrossRef](#)]
52. Suzuki, S.; Suzuki, Y.; Yamamoto, N.; Hattori, T.; Sakamoto, M.; Umezawa, T. High-throughput determination of thioglycolic acid lignin from rice. *Plant Biotechnol.* **2009**, *26*, 337–340. [[CrossRef](#)]
53. Liu, G.; Yang, M.; Yang, X.; Ma, X.; Fu, J. Five TPSs are responsible for volatile terpenoid biosynthesis in *Albizia julibrissin*. *J. Plant Physiol.* **2021**, *258–259*, 153358. [[CrossRef](#)]
54. Li, X.; Ahammed, G.J.; Li, Z.-X.; Zhang, L.; Wei, J.-P.; Shen, C.; Yan, P.; Zhang, L.-P.; Han, W.-Y. Brassinosteroids improve quality of summer tea (*Camellia sinensis* L.) by balancing biosynthesis of polyphenols and amino acids. *Front. Plant Sci.* **2016**, *7*, 1304. [[CrossRef](#)]
55. Yuan, Y.; Cheng, C. The relation between the components of tea catechins and the quality of green tea. *Acta Hortic. Sin.* **1964**, *3*, 287–300.

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