



HAL
open science

The Effects of Ozone on Herbivore-Induced Volatile Emissions of Cultivated and Wild Brassica Rapa

Agnès Brosset, Amélie Saunier, Adedayo Mofikoya, Minna Kivimäenpää,
James Blande

► **To cite this version:**

Agnès Brosset, Amélie Saunier, Adedayo Mofikoya, Minna Kivimäenpää, James Blande. The Effects of Ozone on Herbivore-Induced Volatile Emissions of Cultivated and Wild Brassica Rapa. *Atmosphere*, 2020, 11 (11), pp.1213. 10.3390/atmos11111213 . hal-04440690

HAL Id: hal-04440690

<https://hal.science/hal-04440690v1>

Submitted on 11 Jan 2025

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.




L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

Article

The Effects of Ozone on Herbivore-Induced Volatile Emissions of Cultivated and Wild *Brassica Rapa*

Agnès Brosset ^{1,*}, Amélie Saunier ¹, Adedayo O. Mofikoya ², Minna Kivimäenpää ¹ and James D. Blande ¹

¹ Department of Environmental and Biological Sciences, University of Eastern Finland, FIN-70211 Kuopio, Finland; amelie.saunier@uef.fi (A.S.); minna.kivimaenpaa@uef.fi (M.K.); james.blande@uef.fi (J.D.B.)

² School of Agriculture, Policy and Development, University of Reading, Reading RG6 6AR, UK; adedayo.mofikoya@uef.fi

* Correspondence: agnes.brosset@uef.fi

Received: 6 October 2020; Accepted: 6 November 2020; Published: 10 November 2020



Abstract: Since preindustrial times, concentrations of tropospheric ozone, a phytotoxic pollutant, have risen in the Northern Hemisphere. Selective breeding has intentionally modified crop plant traits to improve yield but may have altered plant defenses against abiotic and biotic stresses. This study aims to determine if cultivated and wild plants respond differently to herbivory under elevated ozone. We studied the volatile emissions of four cultivated *Brassica rapa* ssp. *oleifera* varieties and one wild population after exposure to ozone or *Plutella xylostella* larval feeding either individually or together. Ozone modulated the volatiles emitted in response to herbivory by all plant varieties to different extents. We did not observe a clear difference in the effects of ozone on wild and cultivated plants, but cultivated plants had higher volatile emission rates in response to herbivory and ozone had either no effect or increased the herbivore-induced response. Larvae tended to feed more on elevated ozone-treated plants; however, we could not link the increase of feeding to the change in volatile emissions. Our study complements recent studies reporting that selective breeding might not have weakened chemical defenses to biotic and abiotic stresses of cultivated plants.

Keywords: tropospheric ozone; volatile organic compound (VOC); herbivory; multiple stresses; selective breeding; plant induced-defense; *Plutella xylostella*

1. Introduction

Since preindustrial times, concentrations of greenhouse gases and toxic pollutants have increased in the troposphere (the zone reaching from the Earth's surface to 10–12 km in altitude) [1,2]. Tropospheric ozone is one of the most important atmospheric pollutants and is formed from reactions involving nitrogen oxides and volatile organic compounds in the presence of sunlight [1–3]. Unlike stratospheric ozone, which protects life on the planet, tropospheric ozone can be harmful to living organisms and seriously affect human health [4]. Current guidelines established by the World Health Organization recommend not exceeding a mean threshold of 40 ppb during an eight-hour period. Tropospheric ozone is a phytotoxic air pollutant that can cause serious damage to plants depending on the concentration and duration of exposure [5]. In 1996, Europe implemented an AOT40 index (tropospheric ozone concentration accumulated over a threshold of 40 ppb) for the protection of vegetation [6]. Exceedance of a 3000 ppbh threshold for the three-month growing season (from May to July) may lead to chronic or acute exposure that can result in declines in photosynthesis and growth [7], induction of reactive oxygen species (ROS) and cell death in sensitive plants [8,9]. Studies have reported that an increase in ground-level ozone concentrations can have negative effects on plant production [10,11], leading to global yield losses estimated to be from 2.2 to 5.5% for maize,

3.9 to 15% for wheat and 8.5 to 14% for soybeans in 2000 [12], making ozone a crucial threat to global food security [11].

Today, food production depends on modern varieties of plants that are the result of extensive cultivation and artificial selection for traits suitable for human consumption and growth in agronomic conditions [13,14]. Over the selection process, humans have selected varieties for increased yield and other desirable properties, bringing considerable genotypic and phenotypic changes to cultivated plants [15], such as larger seeds [16], which have led to cultivated plants being ecologically different from their wild relatives [14,15]. The focus on yield and changes in plant chemistry related to palatability may have reduced plants' ability to cope with biotic and abiotic stresses [17–19] by directly reducing the level of secondary metabolites in plant leaves [20,21] due to their undesirable effects on food quality [22]. For example, domestication intentionally reduced glucosinolate content in the *Brassicaceae* [22,23] because they are harmful and distasteful to humans or livestock [22]. Several studies also showed that domestication reduced herbivore-induced plant volatiles (HIPVs) in potatoes *Solanum tuberosum* L. [24], maize *Zea mays* L. [25,26], cranberries *Vaccinium macrocarpon* Aiton [27] and Brussels sprout *Brassica oleracea* L. var. *gemmifera* (DC.) Zenker [28]. However, plants benefit from the emission of HIPVs and a diverse array of constitutive VOCs for protection against biotic stresses such as herbivores [29]. It has been frequently documented that cultivated plants are more susceptible to herbivory than their wild relatives [15,28]. No-choice tests have shown that *Pieris Brassicae* L. and *Pieris rapae* L. perform better on cultivated cabbages (*Brassica oleracea* L.) [28,30] and *Drosophila suzukii* M. feed more on cultivated blueberries (*Vaccinium corymbosum* L.) than wild relatives [31]. However, recent studies have shown that cultivated plants generally emit more HIPVs [32–34]. It was predicted that a trade-off between growth and defense occurred, through which selective breeding focused on yield and productivity resulted in a reduction in resources allocated to defense against biotic and abiotic stresses [21]. However, as induced defenses are considered less expensive to plants than constitutive defenses, by growth promotion, domesticated varieties could be improved by a shift from constitutive to induced defenses [32]. Induced plant volatiles also play a major role in plant defenses against abiotic stresses such as tropospheric ozone [35]. For instance, to limit ozone damage, plants have been shown to increase emissions of VOCs with protective and antioxidant functions such as terpenes [36]. This has been observed in several plants including *Nicotiana tabacum* L. [37], *Pinus sylvestris* L. and *Populus nigra* L. [38]. By modifying HIPV emissions, domestication may have affected the ability of plants to respond to increases in tropospheric ozone.

The effects of combined ozone and herbivore-feeding stresses have earlier been addressed in *Brassica napus* ssp. *oleifera* (Moench) Metzg, where a decrease in HIPVs emitted in response to *Plutella xylostella* L. feeding was observed in ozone-polluted conditions [39]. By contrast, exposure to elevated ozone increased HIPV emissions in *N. tabacum* [40], *B. nigra* [41] and *R. raphanistrum* [42]. More recently, a study on diverse wild and cultivated plants of the *Brassicaceae* family indicated that elevated ozone affected monoterpene and sesquiterpene emissions in response to *P. xylostella* feeding in wild plants but not in cultivated varieties [42]. The results suggested that elevated ozone affects volatiles emitted in response to herbivore feeding by cultivated plants to a lesser extent than in wild plants.

Brassica species are good examples of cultivated plants. *Brassica rapa* L. is the most widely distributed and domesticated of the agricultural *Brassica* species [43]. The long history of selection has created a wide range of morphotypes such as oilseed (ssp. *oleifera*), leafy vegetables (ssp. *chinensis*), root vegetables (ssp. *rapa*) and fodder crops (ssp. *rapifera*), all drastically different from the wild *B. rapa* [44]. A number of studies have shown that elevated ozone could affect volatile emissions of *Brassica* plants and affect responses to the specialist herbivore *P. xylostella* [45–47]. Controlled-environment studies suggest that ozone degrades herbivore-induced terpenes emitted by *Brassica oleracea* L. in response to *P. xylostella* feeding [45]. Elevated ozone enhanced the total emission rate of volatiles emitted by *B. nigra* in response to *P. xylostella* feeding, which was shown to affect resulting tritrophic interactions [41]. In addition, clear changes in the abundance of volatiles emitted

by *Brassica* plants induced by elevated ozone were shown to negatively affect the performance and feeding preferences of the specialist *P. brassicae* L. with larvae favoring plants exposed to elevated ozone [48]. However, a field experiment suggested that elevated ozone does not affect *P. xylostella* feeding behavior [46].

This study aims to determine if cultivated plants differ from their wild relatives in their volatile responses to ozone alone and in interaction with herbivory. We examined volatile emission rates of five *B. rapa* varieties, four cultivated varieties and one wild variety in response to feeding by larvae of the diamondback moth, *P. xylostella*, under ambient and elevated ozone. We also measured the feeding of *P. xylostella* larvae to estimate if any changes in volatile emissions could be related to changes in the feeding behaviors of larvae under elevated ozone. We hypothesized that plants would respond differently to each stress scenario tested and that cultivated plants would respond differently to wild plants. We also hypothesized that the change in volatile emissions in response to herbivory under elevated ozone could be linked to a change in the feeding behavior of *P. xylostella* larvae.

2. Materials and Methods

2.1. Insect and Plant Growth Conditions

Plutella xylostella L. was reared on broccoli (*B. oleracea* var. *italica*) with an artificial light–dark cycle of 16 h day/8 h night at 22 ± 0.5 °C. Wild *Brassica rapa* L. plants used for the experiments came from a wild population obtained from the Netherlands (supplied by E. Poelman, Wageningen University, Wageningen, the Netherlands) and the four cultivated spring turnip rape varieties were *B. rapa* ssp. *oleifera* Lam. var. Cordelia (2010), Valo (2010), Petita (2001) and Legato (2001) (Boreal Plant Breeding Ltd, Jokioinen, Finland). Seeds were sown in individual 0.8 L plastic pots containing a mixture of peat, soil and sand (3:1:1). Plants were grown in controlled-environment chambers (Weiss Bio 1300, Umwelttechnik GmbH, Germany) with an artificial light–dark cycle of 16 h day (light intensity of $800 \mu\text{mol m}^{-2} \text{s}^{-1}$)/8 h night. Day and night temperatures were 21 °C and 16 °C with a relative humidity of 60.0% and 80.0%, respectively. Plants were watered daily and fertilized twice per week with a 0.1% solution containing nitrogen, phosphorus and potassium (19:4:20, Kekkilä Oyj, Finland).

2.2. Treatments

Four-week-old plants were divided into four groups (Figure 1) with N = 5 plants per treatment for each variety:

- Ab: ambient ozone (15 ppb);
- O₃: elevated ozone (80 ppb);
- HB: 48 h herbivore feeding;
- O₃_HB: 48 h herbivore feeding under elevated ozone.

The elevated ozone concentration was set to 80 ppb between 07:00 and 20:00 and 30 ppb between 20:00 and 07:00 to approximate the real daily ozone oscillation. For the ambient treatment, ozone levels were not controlled and corresponded with a relatively low level in the laboratory, which was 15 ppb over the course of 24 h each day. Five days after the start of the plant exposure to ambient or elevated ozone, 20 third-instar larvae were added to each individual plant for 48 h, after which VOCs were collected. We used third-instar larvae because it is known to be a good stage for inducing plant defenses [49] and larvae would not pupate in the duration of the experiment. Twenty larvae were used to avoid causing excessive damage to the plants. Each plant variety was grown separately and was tested in a different chamber with the same controlled conditions as described above. This method was selected to avoid surface deposition and release of VOCs from one variety to another with a different VOC profile.

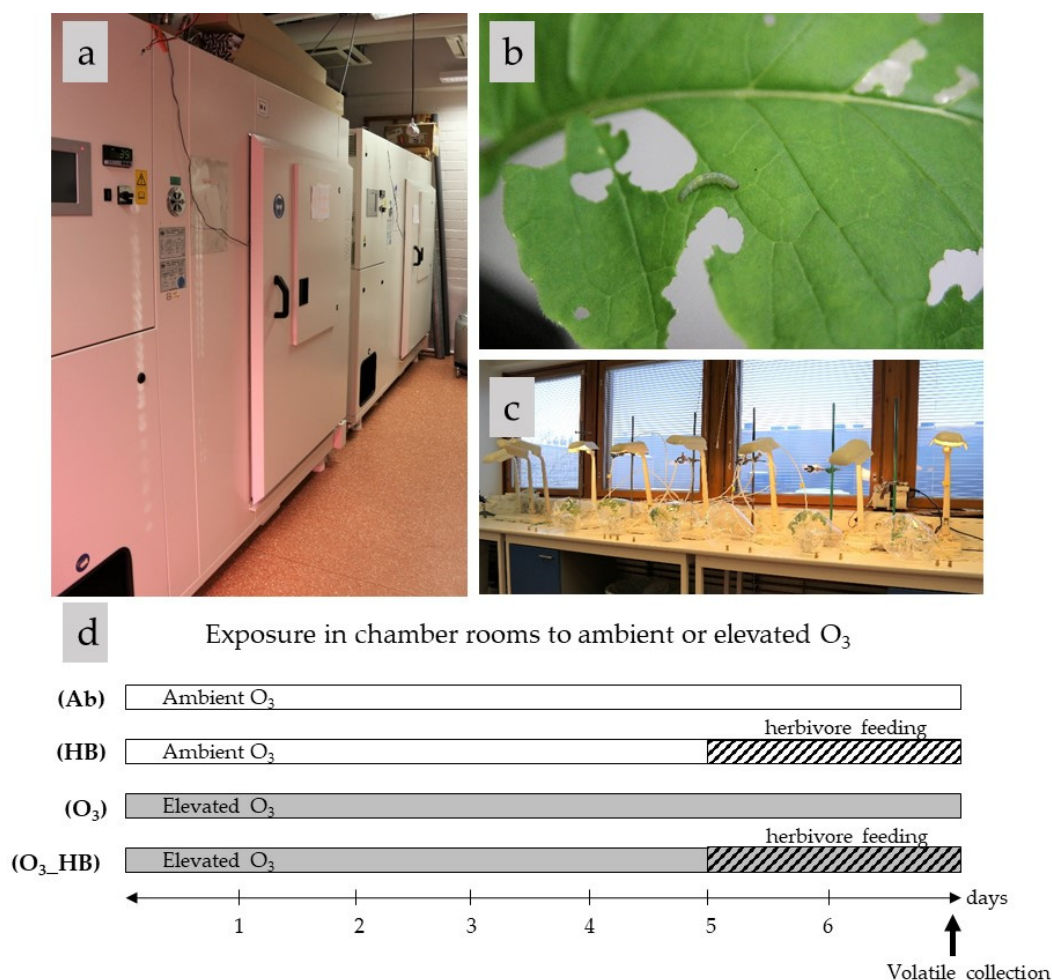


Figure 1. The experimental design consisted of plants exposed for seven days to ambient or elevated ozone in plant growth chambers (a). On the fifth day, all plants in the 48 h herbivore feeding (HB) and 48 h herbivore feeding under elevated ozone (O_3 _HB) chambers were infested with 20 third-instar *Plutella xylostella* larvae for 48 h (b). After 48 h of feeding, 7 days from the initiation of ozone exposure, volatile emissions from plants were sampled for 1 h by dynamic headspace sampling (c). Ozone was elevated to 80 ppb at 7:00 and reduced to 30 ppb at 20:00 to follow a realistic daily ozone oscillation for elevated ozone treatments (O_3 and O_3 _HB) (d). The ozone ranged around 15 ppb all day long in the ambient ozone treatments (Ab) and HB).

2.3. Volatile Organic Compounds (VOCs) Collection and Analysis

The collection of VOCs was conducted using dynamic headspace sampling. Six plants were simultaneously sampled in laboratory conditions at room temperature with lamps set to illuminate each sample at a level of approximately $250 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Figure 1d). Plants were enclosed in precleaned (1 h at 120°C) plastic bags (polyethylene terephthalate; dimensions: 35×43 cm; Look[®] Isopussi Eskimo oy, Helsinki, Finland) to avoid contamination with any compounds released from the plastic during sampling. Pressurized and filtered (through active charcoal) inlet air was introduced into bags at a flow rate of 300 mL min^{-1} . Bags were flushed with a clean air flow for 20 min. After flushing, VOCs were trapped by pulling the headspace in the bags through stainless steel tubes filled with 200 mg Tenax TA 60/80 adsorbent (Markes International Ltd, Llantrisant, UK) for 1 h. Tenax TA-filled tubes were connected via clean silicone tubing to a vacuum pump (KNE, Freiburg, Germany), which pulled air through the tubes at a flow rate of 220 mL min^{-1} . Inlet and outlet airflows were calibrated with a flowmeter (mini-Buck Calibrator, Buck, New York, NY, USA). Blanks (collected from empty bags) were also sampled with the same method at the same time in order to identify potential contaminants.

The collected VOCs were thermally desorbed with an automated thermal desorption unit (Perkin Elmer ATD400 Automatic Thermal Desorption System, Wellesley, MA, USA) at 250 °C for 10 min and cryofocused at −30 °C. Compounds were then analyzed by gas chromatography–mass spectrometry (Hewlett Packard GC type 6890, Wilmington, NC, USA; MSD 5973, Agilent Technology, Santa Clara, CA, USA) with a split mode of 1:20 and an HP-5 MS capillary column (0.25 µm × 60 m × 0.25 µm, Agilent Technology, Santa Clara, CA, USA). The carrier gas was helium. The oven temperature started with a rise to 40 °C for 2 min, which was then increased by 5 °C min^{−1} to 210 °C and then by 20 °C min^{−1} to 250 °C under a constant flow of 1.2 mL min^{−1}. The temperature was then held at 250 °C for 5 min. VOC identification was made by comparison with a series of 30 analytical standards (Sigma-Aldrich, Munich, Germany) and by comparison of their mass spectra to those in the Wiley 275 mass spectral library. Compound quantification was based on using total ion chromatograms (TIC) and according to the responses of analytical standards. Emission rates (ER) were calculated following the formula $ER = X \cdot A_i / D_w \cdot t \cdot A_o$, where ER is expressed as ng g_{DW}^{−1} h^{−1}, X is the compound quantity (ng), A_i and A_o are the inlet and outlet air flows (mL min^{−1}), respectively, t is the sampling time of 1 h and D_w is the dry weight of the plant sampled (g). After sampling, plants were dried in paper bags in an oven at 60 °C for 3 days.

2.4. Feeding Assessment

After the volatile collection, leaves of plants exposed to the HB and O₃_HB treatments were cut and placed on a sheet of 5 mm squared paper. The leaves were digitally photographed and the leaf area consumed by larvae (Figure 1b) was calculated using the Image J software (<https://imagej.nih.gov/ij/>).

2.5. Statistical Analyses

Analyses were performed with the SPSS 25 software (IBM Corp. Armonk, NY, USA). First, the emission rates of all compounds were grouped and summed as total VOC emissions. The normality of the data and their homogeneity were checked with Shapiro–Wilk and Levene’s tests, respectively. Data were log-transformed and analyzed with a two-way ANOVA to assess the main and interactive effects of ozone and herbivory on the total VOC emissions for each species. One interactive effect was found and further studied by calculating *p*-values for simple main effects (SME, i.e., post hoc tests for interactions) with Bonferroni corrections. Emission rates of individual volatile compounds were analyzed with generalized linear models (GLMs) to test the main and interactive effects of ozone and herbivory. All compounds for which an herbivory effect or an interactive effect was found were reported as HIPVs. HIPVs were summed for the treatments HB and O₃ + HB and differences in emissions between the HB and O₃ + HB treatments were analyzed for each variety with a *t*-test. Finally, we assessed the ozone and variety effects on the area consumed by *P. xylostella* larvae with a two-way ANOVA followed by Tukey’s post hoc test to determine if differences in *P. xylostella* larvae-feeding existed between plant varieties.

3. Results

3.1. The Main Effect of Herbivore Feeding on Volatile Emission Rates

Herbivore feeding had a significant effect on volatile emission rates of all cultivated varieties (Figure 2, Table 1). Analysis by two-way ANOVA on total volatile emission rates showed significant main effects of herbivory for the varieties Cordelia, Legato, Petita and Valo (Table 1). In addition, analysis by GLM showed that all cultivated plants emitted significant amounts of HIPVs (Table 2). For example, herbivore feeding increased or induced several green leaf volatiles (GLVs) and (*E*)-4,8-dimethylnona-1,3,7-triene ((*E*)-DMNT) in all cultivated plants. Herbivore feeding in Cordelia, Legato and Valo significantly induced emissions of methyl salicylate (MeSA) (Table 2). The sesquiterpene (*E, E*)- α -farnesene (Table 2) was significantly emitted in response to herbivory in three of the four cultivated plant varieties tested; Cordelia, Legato and Petita. Similarly, to cultivated plants, GLM analysis showed that the wild variety

responded to herbivore feeding by emitting several HIPVs (Figure 2 and Table 2). Herbivore feeding increased or induced the emissions of sesquiterpenes and GLVs such as α -humulene, (*E*)-caryophyllene and (*Z*)-3-hexenyl acetate. Herbivore feeding also induced emissions of (*E*)-DMNT and MeSA emissions as well as emissions of 2-methyl-5-hexenenitrile. Moreover, wild plants emitted a wider range of N- and S-containing compounds with five different compounds found while cultivated plant varieties emitted one to three N- and S-containing compounds (Appendix A). We also did not find N- and S-containing compounds to be significantly increased or induced for the cultivated plant varieties.

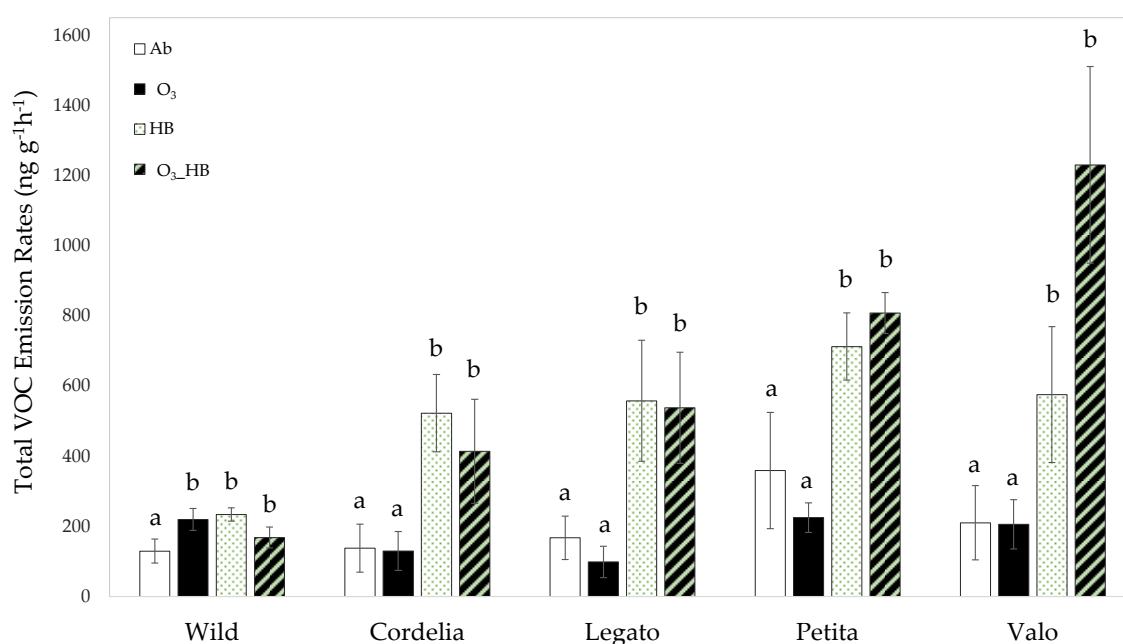


Figure 2. Mean (\pm SE) emission rates ($\text{ng g}^{-1} \text{h}^{-1}$) of total volatiles emitted by each species under the four treatments. Ab: ambient ozone (15–20 ppb); O₃: 80 ppb ozone; HB: ambient ozone and 48 h of *P. xylostella* larvae feeding; O₃_HB: 80 ppb ozone and 48 h of herbivore feeding. A two-way ANOVA on total volatiles emitted was performed separately for each species and results are presented in Table 1. One significant interaction was found and further studied by calculating *p*-values for simple main effects (SME, i.e., post hoc tests for interactions) with Bonferroni corrections. Letters a and b indicate differences between treatments.

Table 1. Results of the two-way ANOVA performed on the total emission rates for each variety. The significant interaction for the wild *B. rapa* variety was further studied by calculating *p*-values for simple main effects (SME, i.e., post hoc tests for interactions) with Bonferroni corrections. Differences between treatments observed after studying the interaction are indicated in Figure 2 by the letters a and b. ns = *p* > 0.1.

Variety	O ₃	HB	HB*O ₃
Wild	ns	ns	0.012
Cordelia	ns	0.002	ns
Legato	ns	0.001	ns
Petita	ns	0.001	ns
Valo	0.094	0.003	ns

3.2. The Main Effect of Ozone on Volatile Emission Rates

Analysis by two-way ANOVA on total volatile emission rates did not show a significant main effect of ozone for any of the varieties. Further analysis by GLM showed that ozone induced the emission of β -Pinene in the wild *B. rapa*. GLM analysis also showed that ozone affected some cultivated plant varieties at the compound level. Ozone-induced emission of benzyl alcohol in the Legato cultivar

(Table 2) and tended to increase emission rates of hexanal for Valo. Petita also emitted MeSA but only under elevated ozone conditions (Appendix A).

Table 2. Volatile compounds selected as herbivore-induced volatiles and ozone-induced volatiles. All compounds for which an herbivory effect was found with generalized linear models (GLMs) were reported as herbivore-induced plant volatiles (HIPVs). All compounds with the main ozone effect with GLMs were reported as ozone-induced volatiles.

Variety	Herbivore-Induced Plant Volatiles	Ozone-Induced Volatiles	Interactive Effect
Wild	(E)-DMNT (E)-Caryophyllene α -Humulene (Z)-3-Hexenyl acetate Methyl salicylate 2-methyl-5-hexenenitrile	β -Pinene	(Z)-3-Hexenyl acetate
Cordelia	α -Pinene (E)-DMNT (E,E)- α -Farnesene (E)-2-Hexenal (Z)-3-Hexenol (Z)-3-Hexenyl acetate Hexyl acetate Methyl salicylate		Methyl salicylate
Legato	(E)-DMNT (E)-Caryophyllene Unknown RI 1459.2 (E, E)- α -Farnesene (Z)-3-Hexenyl acetate Methyl salicylate	Benzyl alcohol	
Petita	(E)-DMNT Unknown RI 1461.5 Unknown RI 1438.6 Unknown RI 1426.9 (E, E)- α -Farnesene (E)-2-Hexenal		(E, E)- α -Farnesene
Valo	Myrcene (E)-DMNT Hexanal Methyl salicylate	Hexanal	Methyl salicylate

3.3. The Interactive Effect of Ozone and Herbivore Feeding on Volatile Emissions

Analysis by two-way ANOVA on total volatile emission rates did not show a significant effect on any of the cultivated plant varieties. However, a further analysis focusing only on HIPVs, showed that ozone tripled the HIPVs emitted by the variety Valo (Figure 3). Valo only emitted MeSA in response to herbivory under elevated ozone (Table 2 and Appendix A). In Cordelia, ozone reduced MeSA emissions in response to herbivory. Analysis by GLMs also showed that ozone halved (E, E)- α -farnesene emission rates in response to herbivore feeding in Petita (Appendix A). For the wild variety, analysis by two-way ANOVA on total volatile emission rates showed an interactive effect between ozone and herbivory. Wild plants increased their volatile emission rates in response to elevated ozone in the absence of herbivory, but no increase was found when plants were exposed to ozone and herbivory simultaneously. Further GLM analysis showed that ozone decreased the (Z)-3-hexenyl acetate emitted in response to herbivore feeding for the wild variety.

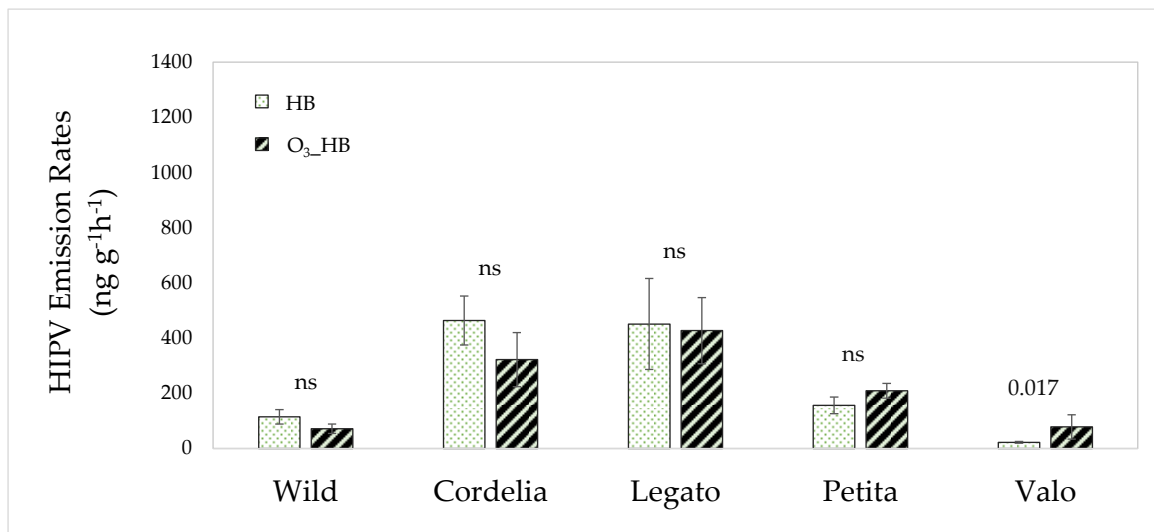


Figure 3. Total HIPV (volatile emitted in significantly greater amounts in response to herbivore feeding) emission rates ($\text{ng g}^{-1} \text{h}^{-1}$) of each species under ambient ozone (15–20 ppb) (green bars) and 80 ppb ozone (hatched bars). ns = $p > 0.1$. HB: ambient ozone and 48 h of *P. xylostella* larvae feeding; O₃_HB: 80 ppb ozone and 48 h of herbivore feeding.

3.4. The Effects of Ozone Exposure on Herbivore Feeding

The *P. xylostella* larvae fed on all varieties but to different extents. Larvae fed the most on wild plants and Legato, with consumption around 50% higher than on Cordelia, Petita and Valo (Figure 4). Plants under elevated ozone tended to have greater areas of the leaf removed by larvae than plants under ambient ozone irrespective of the variety.

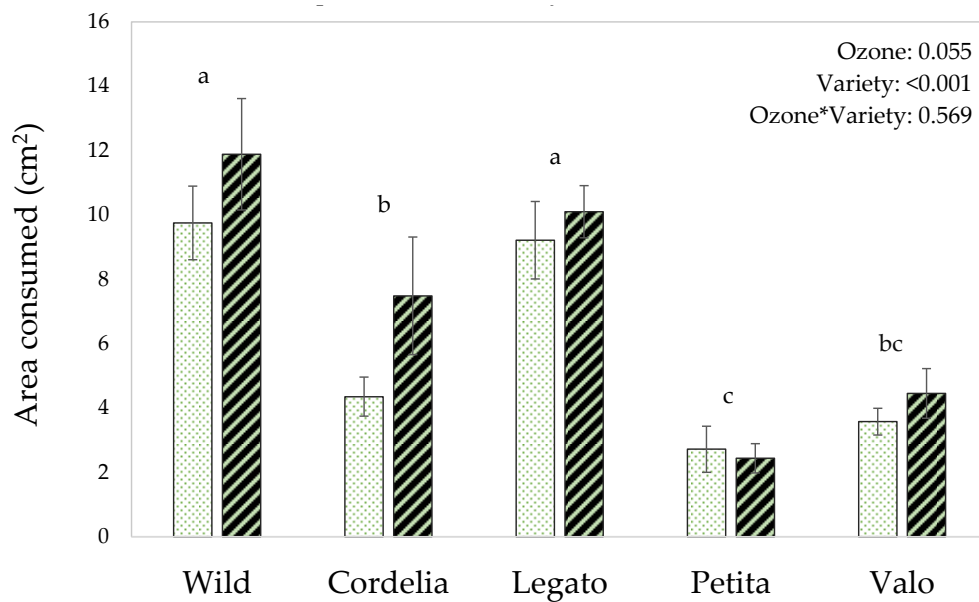


Figure 4. Area consumed by *Plutella xylostella* larvae on each plant variety after 48 h of herbivory under ambient (15–20 ppb) ozone (green) and 80 ppb ozone (hatched). Different letters denote the significant differences ($p < 0.05$, Tukey test) between varieties.

4. Discussion

4.1. Differences in Volatile Responses to Herbivore Feeding in Cultivated and Wild Plants

All plants responded to herbivore feeding by increasing their total volatile emission rates and emitted several HIPVs such as (*E*)-DMNT, MeSA, several sesquiterpenes and GLVs. Irrespective of whether the plant was a cultivated or wild variety, (*E*)-DMNT and MeSA were found in all the volatile blends. MeSA, as well as (*E*)-DMNT, are involved in plant defense against herbivorous insects and participate in particular to the attraction of predators of herbivorous insects [50,51]. Thus, the results supported that both cultivated and wild plants were able to respond to herbivore feeding by emitting key HIPVs. However, plants responded to herbivore feeding to varying degrees. Cultivated plant varieties generally responded by emitting higher levels of VOCs than the wild variety. It could be possible that cultivated varieties have stronger inducible defenses than the wild variety, which could have a higher constitutive defense [21]. This hypothesis is supported by work on tomatoes and maize showing that cultivated plant varieties increased their VOC emissions in response to herbivory to a greater extent than the wild varieties studied [33,34]. Cultivated tomatoes also had fewer trichomes than wild tomatoes and were preferred for oviposition by *Helicoverpa zea* Boddie [34], suggesting that cultivated plants have a stronger induced-defense but weaker constitutive defense than their wild relative. However, our data did not allow us to investigate if cultivated plants had a lower constitutive defense, other than their basal volatile emissions. Further work is needed to determine if cultivated and wild plants differ in their constitutive defense against herbivory.

Cultivated plant varieties also tended to have a lower diversity of N- and S-containing compounds (Appendix A). However, N- and S-containing compounds are derived from the breakdown of glucosinolates and constitute an important part of *Brassica* plant defense against herbivores [22,52]. Due to their adverse effects on animal health and their poor palatability, breeding processes have reduced glucosinolate concentrations in *Brassicaceous* plant leaves [22,23], supporting our results. Such observations have also been reported in *B. oleracea* [53]. On the other hand, N- and S-containing compounds are associated with a higher preference of *P. xylostella* for oviposition and greater feeding by larvae [53–55]. In our study *P. xylostella* larvae tended to feed most on the wild *B. rapa*, supporting the idea that N- and S-containing compounds could stimulate feeding.

4.2. Differences in Volatile Responses to Ozone in Cultivated and Wild Plants

When considered in the analysis as the main effect, elevated ozone did not affect the total VOC emission rates of plants. Only individual compounds were affected by elevated ozone. Among them, benzyl alcohol was induced by elevated ozone in Legato and ozone tended to increase emission rates of hexanal by Valo. Elevated ozone also induced β -pinene emissions in wild plants. Elevated ozone is well known to increase the emissions of volatiles with an antioxidant function that protects plants against oxidative damage [35,36]. Terpenes constitute a particular group of VOCs with antioxidant activity and can be induced by ozone [56], which could explain why we found an increase of VOC emissions including induction of β -pinene under ozone stress. Elevated ozone also induced higher volatile emissions in wild plants, but this was not observed in cultivated plants. Few studies have investigated the effects of ozone on wild plants compared to their cultivated relatives. Most of the studies have focused on the effects of elevated ozone on agricultural or horticultural crops [57]. However, several studies suggested that wild plants could be as sensitive or more sensitive to elevated ozone than the most sensitive crops [58]. In 2015, a study reported that of over 473 wild plant species, 80% were sensitive to elevated ozone with visible injuries to leaves and ozone-induced changes in photosynthesis, stomatal conductance and growth [59]. The weaker effect of ozone on cultivated plants could be due to a coincidental effect of their selection for other traits involved in plant volatile defense. A study assessing the genetic relatedness of ozone sensitivity of newly developed varieties and wild wheat showed that selective breeding indirectly improved the genotype of modern wheat crops for greater tolerance to elevated ozone (100 ppb) than wild wheat varieties [60]. The fact that

recent varieties were selected for optimum seed production under current environmental conditions may have improved their tolerance to ozone.

4.3. Differences in Volatile Responses to Interactive Ozone and Herbivory Exposure in Cultivated and Wild Plants

At the scale of the total VOC emission rates, no changes were found when cultivated plants were exposed to herbivory under elevated ozone compared with a similar scenario under ambient conditions. However, elevated ozone interacted with herbivore feeding in wild plants. In the absence of herbivory, elevated ozone increased the total VOC emission rates while it did not affect the response to herbivory. Elevated ozone also decreased (Z)-3-hexenyl acetate in wild plants suggesting an antagonistic effect between the two stresses. It is well known that ozone reduces the carbon assimilation in plants [61,62] and could reduce the production of volatile compounds emitted against herbivory as we found for the wild variety.

With regard to the effect of ozone on induced responses, we found that the variety Valo, increased the total emission of HIPVs by threefold and tended to increase the total VOC emitted in response to herbivory, suggesting a synergistic effect of the two stresses. It has been reported several times that ozone could increase HIPV emissions due to the formation of reactive oxygen species (ROS) increasing the production of antioxidant VOCs to counteract ozone stress [63,64]. An increase of HIPVs by elevated ozone has been found in *B. nigra* [42] and *R. raphanistrum* [43].

For Valo, ozone also induced MeSA emissions in response to herbivory. Increasing MeSA under elevated ozone has been reported for volatile leaf emissions of tobacco plants [41] and emissions of lima bean in response to feeding by *Tetranychus urticae* C. L. Koch [65]. MeSA is a VOC derived from the salicylic acid (SA) pathway [66]. As stated above, ozone is a phytotoxic pollutant that triggers the formation of ROS in plant cells [9]. The ROS in plant cells induced by ozone exposure can activate the salicylic acid pathway [67,68] and cause defensive responses in plants, resembling those occurring in response to pathogen infections [9]. Hence, elevated ozone might disturb biosynthesis of some volatile compounds by influencing the SA molecular pathways, supporting the change in MeSA concentrations in response to herbivore feeding under elevated ozone. As for the variety Valo, MeSA emissions were also affected by elevated ozone in Cordelia and Petita varieties, where ozone reduced their MeSA emissions, supporting that ozone might influence MeSA biosynthesis.

MeSA is an important HIPV, well known to be induced by mechanical damage and herbivore feeding [66–69]. It is a common HIPV emitted by several Brassicaceous plants after herbivore-damage [40,45] and is involved in indirect defense by attracting parasitoids [70]. The change in MeSA emissions in response to herbivory may affect tritrophic interactions. For instance, *B. nigra* plants subjected to herbivory at 120 ppb ozone increased their volatile emissions, attracting fewer *Cotesia glomerata* L. parasitoids compared to plants subjected to herbivory alone [42]. MeSA and GLVs also play roles in plant-to-plant interactions by inducing or priming neighboring plant defenses [71,72] or adjacent leaves of the same plant [73]. Thus, a change in these emissions in response to elevated ozone could affect interactions between neighboring plants.

4.4. Effect of Elevated Ozone on the Feeding Behavior of *P. xylostella* Related to the Change in VOC Emissions

Plutella xylostella larvae tend to feed more on elevated ozone-treated plants. Several studies have reported an increase in the amount of leaf material consumed by insects on plants previously exposed to elevated ozone. Jones and Coleman [74] showed that exposure of cotton-wood to elevated ozone led to an increase in the consumption of foliage by the leaf beetle *Plagioderma versicolora* Laicharting. Another study reported that growth and consumption by caterpillars of the monarch butterfly *Danaus plexippus* L. were greater on *Asclepias syriaca* L. and *Asclepias curassavica* L. leaves that had been exposed to elevated ozone [75]. More recently, Khaling et al. [48] observed that *P. brassicae* preferred to feed on *B. nigra* plants exposed to 120 ppb ozone than on plants exposed to clean air but performed better on plants exposed to ambient air. Metabolic analyses suggested that the change in larvae

preference and performance was associated with a change in glucosinolate concentrations in leaves under elevated ozone [48]. Field experiments also reported an increase of area consumed by *P. xylostella* on cauliflower plants exposed to elevated ozone and ammonium sulfate, which has been associated with an increase in nitrogen content in leaves [46]. In general, when herbivorous insects feed on plants with lower nitrogen concentration, they increase consumption to compensate for the lower nitrogen acquisition [46]. Moreover, elevated ozone generally tends to increase the volatile emissions emitted by plants and especially the terpenes [36–38]. The increase of terpenes could decrease leaf palatability, which could also affect insect feeding behavior. In our study, we could not link the increase of feeding to the change in volatile emissions.

Greater feeding under elevated ozone could be explained by an increase in concentrations of nonvolatile compounds in exposed leaves, such as sugar content. Leaves richer in sugars could have been subject to increased consumption. This hypothesis is supported by a study showing that elevated ozone increases the carbohydrate content in both ozone sensitive and resistant lines of *B. rapa* and that the increase of carbohydrate was correlated with increased leaf area consumption by insects [76]. The greater induction of volatiles by herbivore feeding on cultivated plants may be the result of a greater leaf area consumed by larvae. However, *P. xylostella* larvae tended to feed the most on the wild *B. rapa*, suggesting that the emissions of volatiles in response to herbivore feeding were not correlated with the quantity of damage. It is possible that other traits associated with the domestication of varieties led to greater volatile emissions in cultivated plants [11]. Loss of defensive structures involved in constitutive defense, as mentioned earlier, may result in greater quantities of volatiles emitted in response to herbivore feeding [32–34].

Most studies investigating the effects of elevated ozone on insects have focused on effects mediated by plants. To determine the direct effect of elevated ozone on insects, future research should separate insects from plants.

5. Conclusions

The results indicated that ozone modulated volatile emissions in all varieties studied to differing extents. MeSA was an HIPV often affected by elevated ozone, suggesting that elevated ozone might disturb biosynthesis of some volatile compounds by influencing the SA pathway. Our results also showed that cultivated plants had greater induction of volatiles emitted in response to herbivory and ozone had either no effect or increased the volatiles emitted in responses to herbivory for the cultivated plant varieties. Thus, our study complements recent studies reporting that domestication might not have weakened the chemical defenses of cultivated plants. The study also shows differences within varieties of the same species in their responses to stresses and supports that studies comparing wild and cultivated species should take into account this variation by studying different varieties. Further studies should explore whether the increase of pollutants and greenhouse gases differentially affect wild and cultivated plants. In the context of a polluted atmosphere, as we have with global change [77], it is crucial to understand the susceptibility of cultivated and wild plants to herbivory. This knowledge should be taken into consideration for future selective breeding. It would be better to favor varieties with the same defense level (e.g., Legato, Petita) or a greater defense level (e.g., Valo) against pests under elevated ozone.

Author Contributions: Conceptualization and methodology, J.D.B.; formal analysis, A.B. and M.K.; investigation, A.B. and A.O.M.; data curation, A.B.; writing—original draft preparation, A.B.; writing—review and editing, J.D.B., A.S., A.O.M. and M.K.; supervision, J.D.B.; funding acquisition, J.D.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Academy of Finland, Project Number: 309425.

Acknowledgments: We thank Timo Oksanen and Jaana Rissanen for their technical support in the laboratory, Erik Poelman and Boreal Plant Breeding Ltd, Jokioinen, Finland, for donating the seeds. We also thank Helena Ruhanen for her help with the insect rearing.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Appendix A

Table A1. Mean (\pm SE) emission rates ($\text{ng g}^{-1} \text{h}^{-1}$) of volatile compounds emitted by each *B. rapa* varieties under the four treatments tested: ambient ozone (Ab) (15 ppb); elevated ozone (O_3) (80 ppb), 48 h of *Plutella xylostella* larvae feeding (HB); and 48 h *P. xylostella* larvae feeding under elevated ozone (O_3 + HB) (80 ppb). RI indicated the retention index on the GC-MS (column HP5-MS) calculated for unknown compounds through the injection of alkanes C8–C20. The main effects of ozone (O_3), herbivory (HB) and their interaction (O_3 *HB) were tested with generalized linear models. ns = $p > 0.1$.

Compounds	Ab	O_3	HB	O_3 _HB	HB	O_3	HB* O_3
<i>B. rapa</i> (wild variety)							
Monoterpenes							
α -Pinene	2.1 \pm 0.4	3.2 \pm 1.1	3.7 \pm 1.4	2.9 \pm 1.6	ns	ns	ns
β -Pinene	0.0 \pm 0.0	0.3 \pm 0.2	0.0 \pm 0.0	0.0 \pm 0.0	ns	0.050	ns
Myrcene	0.7 \pm 0.5	2.8 \pm 2.3	3.7 \pm 0.6	4.0 \pm 2.2	ns	ns	ns
3-carene	1.2 \pm 0.6	1.3 \pm 0.9	4.0 \pm 0.9	3.1 \pm 1.1	ns	ns	ns
Limonene	4.3 \pm 0.7	6.0 \pm 1.6	5.1 \pm 1.9	4.4 \pm 1.4	ns	ns	ns
1.8 cineole	0.0 \pm 0.0	0.1 \pm 0.1	0.0 \pm 0.0	0.1 \pm 0.1	ns	ns	ns
Homoterpenes							
(<i>E</i>)-DMNT ¹	0.0 \pm 0.0	0.0 \pm 0.0	3.0 \pm 3.0	8.8 \pm 6.3	0.070	ns	ns
Sesquiterpenoids							
Unknown RI 1339.8	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.2 \pm 0.1	ns	ns	ns
(<i>E</i>)-Caryophyllene	0.7 \pm 0.5	0.0 \pm 0.0	8.3 \pm 1.5	8.2 \pm 5.4	0.002	ns	ns
α -Humulene	0.0 \pm 0.0	0.0 \pm 0.0	0.9 \pm 0.4	0.6 \pm 0.4	0.007	ns	ns
(<i>E, E</i>)- α -Farnesene	0.6 \pm 0.6	0.0 \pm 0.0	1.2 \pm 0.4	3.8 \pm 3.0	ns	ns	ns
GLVs + MeSA ²							
(<i>Z</i>)-3-Hexenal	9.6 \pm 7.6	33.2 \pm 21.6	30.1 \pm 4.4	19.9 \pm 6.7	ns	ns	ns
Hexanal	6.0 \pm 0.3	13.1 \pm 5.8	10.4 \pm 2.6	8.6 \pm 1.4	ns	ns	ns
(<i>E</i>)-2-Hexenal	6.7 \pm 4.6	10.9 \pm 6.1	8.1 \pm 2.4	10.7 \pm 3.9	ns	ns	ns
(<i>Z</i>)-3-Hexenol	9.9 \pm 5.2	29.0 \pm 6.8	20.2 \pm 2.4	18.4 \pm 7.4	ns	ns	ns
(<i>Z</i>)-3-Hexenyl acetate	69.0 \pm 25.0	114.7 \pm 12.8	101.7 \pm 28.7	47.6 \pm 9.3	ns	ns	0.022
Hexyl acetate	0.0 \pm 0.0	0.0 \pm 0.0	4.0 \pm 4.0	0.0 \pm 0.0	ns	ns	ns
MeSA ²	0.0 \pm 0.0	0.0 \pm 0.0	0.4 \pm 0.4	3.0 \pm 1.9	0.070	ns	ns
N-/or S-containing compounds							
Dimethyl disulfide	15.9 \pm 7.1	1.9 \pm 1.0	22.6 \pm 21.6	13.9 \pm 8.9	ns	ns	ns
Methyl isothiocyanate	0.3 \pm 0.3	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	ns	ns	ns
2-methyl-5-hexenenitrile	0.0 \pm 0.0	0.0 \pm 0.0	0.3 \pm 0.3	3.5 \pm 1.6	0.073	ns	ns
t-butyl isothiocyanate	0.1 \pm 0.1	0.4 \pm 0.4	2.8 \pm 1.4	2.7 \pm 2.6	ns	ns	ns
3-butenyl isothiocyanate	2.2 \pm 0.9	2.4 \pm 0.8	3.1 \pm 0.8	3.5 \pm 2.5	ns	ns	ns
<i>Cordelia</i>							
Monoterpenes							
α -Pinene	0.0 \pm 0.0	0.5 \pm 0.2	0.3 \pm 0.1	0.2 \pm 0.1	ns	ns	ns
Myrcene	0.0 \pm 0.0	0.9 \pm 0.5	1.4 \pm 0.5	2.5 \pm 1.1	ns	ns	ns
Limonene	7.9 \pm 1.7	10.2 \pm 3.6	11.2 \pm 5.2	5.5 \pm 3.8	ns	ns	ns
Linalool	15.7 \pm 15.7	0.0 \pm 0.0	14.3 \pm 13.9	1.0 \pm 1.0	ns	ns	ns
Homoterpenes							
(<i>E</i>)-DMNT ¹	0.5 \pm 0.5	0.0 \pm 0.0	8.5 \pm 5.1	3.7 \pm 2.5	0.1	ns	ns
Sesquiterpenoids							
Unknown RI 1423.4	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.2 \pm 0.2	ns	ns	ns
Unknown RI 1442.1	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.3 \pm 0.3	ns	ns	ns
(<i>E, E</i>)- α -Farnesene	0.6 \pm 0.6	0.8 \pm 0.5	8.8 \pm 2.6	3.9 \pm 2.0	0.095	ns	ns
GLVs + MeSA ²							
(<i>Z</i>)-3-Hexenal	0.0 \pm 0.0	0.0 \pm 0.0	25.1 \pm 25.1	74.2 \pm 57.0	ns	ns	ns
Hexanal	1.7 \pm 0.8	4.2 \pm 2.4	4.7 \pm 2.0	3.9 \pm 1.0	ns	ns	ns
(<i>E</i>)-2-Hexenal	0.0 \pm 0.0	0.0 \pm 0.0	6.4 \pm 4.6	14.4 \pm 10.2	0.029	ns	ns
(<i>Z</i>)-3-Hexenol	4.2 \pm 1.9	6.5 \pm 3.1	36.1 \pm 7.2	70.1 \pm 44.7	0.001	ns	ns

Table A1. Cont.

Compounds	Ab	O ₃	HB	O ₃ _HB	HB	O ₃	HB*O ₃
(Z)-3-Hexenyl acetate	101.8 ± 49.2	103.6 ± 49.5	395.6 ± 80.5	226.8 ± 57.5	0.005	ns	ns
Hexyl acetate	0.7 ± 0.6	0.0 ± 0.0	4.7 ± 1.2	2.3 ± 0.9	0.049	ns	ns
MeSA ²	0.0 ± 0.0	0.0 ± 0.0	3.5 ± 1.6	0.2 ± 0.2	0.054	ns	0.092
N-/or S-containing compounds							
Dimethyl disulfide	3.2 ± 1.6	2.9 ± 1.4	1.8 ± 0.7	4.0 ± 0.8	ns	ns	ns
2-methyl-5-hexenenitrile	0.4 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	ns	ns	ns
t-butyl isothiocyanate	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.1	ns	ns	ns
Legato	Ab	O ₃	HB	O ₃ _HB	HB	O ₃	HB*O ₃
Monoterpenes + Benzyl alcohol							
α-Pinene	1.1 ± 0.7	0.6 ± 0.5	0.8 ± 0.4	0.4 ± 0.4	ns	ns	ns
β-Pinene	0.3 ± 0.2	0.2 ± 0.2	0.5 ± 0.4	0.1 ± 0.1	ns	ns	ns
Myrcene	6.8 ± 6.8	2.2 ± 1.5	18.4 ± 12.6	6.3 ± 2.8	ns	ns	ns
3-carene	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.4 ± 0.4	ns	ns	ns
Limonene	1.2 ± 0.2	1.0 ± 0.6	3.4 ± 1.9	1.5 ± 0.8	ns	ns	ns
Benzyl alcohol	0.0 ± 0.0	1.3 ± 0.8	0.1 ± 0.1	1.3 ± 0.4	ns	0.036	ns
Linalool	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	5.4 ± 5.4	ns	ns	ns
Homoterpenes							
(E)-DMNT ¹	0.0 ± 0.0	0.0 ± 0.0	158.8 ± 69.4	104.2 ± 36.3	<0.001	ns	ns
Sesquiterpenoids							
(E)-Caryophyllene	1.7 ± 1.0	0.0 ± 0.0	12.0 ± 8.4	3.5 ± 0.4	0.05	ns	ns
Unknown RI 1459.2	0.0 ± 0.0	0.0 ± 0.0	0.7 ± 0.4	0.8 ± 0.5	0.02	ns	ns
α-Humulene	1.9 ± 1.9	0.0 ± 0.0	1.0 ± 0.6	0.3 ± 0.3	ns	ns	ns
(E, E)-α-Farnesene	4.4 ± 2.3	6.4 ± 3.8	68.3 ± 30.0	103.7 ± 39.9	0.015	ns	ns
GLVs + MeSA ²							
Hexanal	1.5 ± 1.1	2.6 ± 1.8	7.4 ± 5.0	4.1 ± 1.5	ns	ns	ns
(E)-2-Hexenal	5.0 ± 3.2	1.9 ± 1.9	14.7 ± 2.3	18.8 ± 3.1	ns	ns	ns
(Z)-3-Hexenol	23.6 ± 11.3	8.4 ± 4.2	42.1 ± 4.2	46.3 ± 12.6	ns	ns	ns
(Z)-3-Hexenyl acetate	116.0 ± 47.2	65.1 ± 30.5	192.8 ± 69.5	206.2 ± 49.1	0.057	ns	ns
Hexyl acetate	2.2 ± 2.2	8.1 ± 5.1	9.4 ± 4.7	20.0 ± 17.1	ns	ns	ns
MeSA ²	0.0 ± 0.0	0.0 ± 0.0	18.6 ± 10.3	8.3 ± 3.8	0.022	ns	ns
N-/or S-containing compounds							
Dimethyl disulfide	1.6 ± 1.1	0.0 ± 0.0	8.4 ± 6.6	4.7 ± 2.5	ns	ns	ns
t-butyl isothiocyanate	0.0 ± 0.0	0.3 ± 0.3	0.5 ± 0.5	1.8 ± 0.8	ns	ns	ns
Petita	Ab	O ₃	HB	O ₃ _HB	HB	O ₃	HB*O ₃
Monoterpenes							
α-Pinene	9.4 ± 3.2	7.7 ± 1.4	9.1 ± 2.0	7.7 ± 1.2	ns	ns	ns
β-Pinene	1.6 ± 0.8	0.7 ± 0.4	0.5 ± 0.3	1.0 ± 0.4	ns	ns	ns
3-carene	5.1 ± 2.1	2.8 ± 1.2	4.1 ± 1.5	3.0 ± 1.6	ns	ns	ns
Benzyl alcohol	4.7 ± 2.9	4.1 ± 2.8	2.1 ± 1.2	2.5 ± 1.4	ns	ns	ns
Limonene	16.9 ± 10.6	12.0 ± 8.4	14.5 ± 5.9	7.0 ± 2.0	ns	ns	ns
Linalool	0.0 ± 0.0	0.0 ± 0.0	5.0 ± 5.0	11.6 ± 11.5	ns	ns	ns
Homoterpenes							
(E)-DMNT ¹	0.0 ± 0.0	0.0 ± 0.0	74.6 ± 22.9	67.0 ± 23.8	0.001	ns	ns
Sesquiterpenoids							
Unknown RI 1461.5	0.0 ± 0.0	0.0 ± 0.0	2.3 ± 1.4	4.3 ± 1.4	0.033	ns	ns
Unknown RI 1438.6	0.0 ± 0.0	0.0 ± 0.0	5.7 ± 2.6	6.7 ± 2.2	0.015	ns	ns
Unknown RI 1426.9	0.0 ± 0.0	0.0 ± 0.0	2.8 ± 1.6	6.6 ± 0.6	0.007	ns	ns
(E, E)-α-Farnesene	0.0 ± 0.0	0.0 ± 0.0	105.1 ± 31.9	44.3 ± 16.7	<0.001	ns	0.054
GLVs + MeSA ²							
Hexanal	34.2 ± 12.8	29.2 ± 11.2	43.7 ± 13.9	43.5 ± 9.3	ns	ns	ns
(E)-2-Hexenal	0.0 ± 0.0	8.8 ± 8.8	70.8 ± 15.4	124.5 ± 24.6	0.071	ns	ns
(Z)-3-Hexenol	73.4 ± 65.6	30.5 ± 4.0	86.4 ± 12.4	124.2 ± 32.3	ns	ns	ns
(Z)-3-Hexenyl acetate	211.7 ± 118.6	124.3 ± 38.3	198.2 ± 17.7	316.4 ± 66.5	ns	ns	ns
Hexyl acetate	1.9 ± 1.9	0.0 ± 0.0	4.5 ± 2.8	9.6 ± 5.0	ns	ns	ns
MeSA ²	0.0 ± 0.0	0.0 ± 0.0	73.0 ± 51.5	20.8 ± 20.8	ns	ns	ns

Table A1. Cont.

Compounds	Ab	O ₃	HB	O ₃ _HB	HB	O ₃	HB*O ₃
N-/or S-containing compounds							
Dimethyl disulfide	0.0 ± 0.0	6.5 ± 0.6	7.8 ± 3.6	7.8 ± 3.6	ns	ns	ns
Valo							
Monoterpenes							
α-Pinene	0.0 ± 0.0	0.0 ± 0.0	3.2 ± 2.0	1.0 ± 1.0	ns	ns	ns
β-Pinene	0.0 ± 0.0	0.0 ± 0.0	0.7 ± 0.5	0.2 ± 0.2	ns	ns	ns
Myrcene	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.3	0.5 ± 0.4	0.042	ns	ns
3-carene	0.5 ± 0.2	0.1 ± 0.05	0.8 ± 0.8	0.7 ± 0.7	ns	ns	ns
Limonene	2.2 ± 0.3	2.7 ± 2.1	8.6 ± 2.7	3.7 ± 1.2	ns	ns	ns
Linalool	0.0 ± 0.0	11.2 ± 9.5	4.6 ± 4.6	29.6 ± 17.3	ns	ns	ns
Homoterpenes							
(E)-DMNT ¹	0.0 ± 0.0	0.0 ± 0.0	4.6 ± 2.9	20.6 ± 9.2	0.096	ns	ns
Sesquiterpenoids							
Unknown RI 1438.6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	ns	ns	ns
Unknown RI 1506.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.3 ± 1.2	ns	ns	ns
(E, E)-α-Farnesene	0.6 ± 0.6	0.3 ± 0.3	7.9 ± 2.3	19.2 ± 7.1	ns	ns	ns
GLVs + MeSA²							
(Z)-3-Hexenal	13.1 ± 13.1	0.0 ± 0.0	20.3 ± 12.9	55.70 ± 34.50	ns	ns	ns
Hexanal	6.8 ± 2.7	11.06 ± 3.36	17.0 ± 2.0	36.57 ± 18.33	0.005	0.061	ns
(E)-2-Hexenal	7.5 ± 7.5	0.0 ± 0.0	45.6 ± 21.8	111.02 ± 37.78	ns	ns	ns
(Z)-3-Hexenol	69.8 ± 62.0	33.20 ± 16.0	92.5 ± 29.8	190.26 ± 52.01	ns	ns	ns
(Z)-3-Hexenyl acetate	187.6 ± 145.2	147.1 ± 54.2	359.2 ± 128.5	719.8 ± 177.9	ns	ns	ns
Hexyl acetate	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	6.7 ± 4.7	ns	ns	ns
MeSA ²	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	20.1 ± 8.2	0.017	ns	0.017
N-/or S-containing compounds							
Dimethyl disulfide	4.3 ± 2.9	0.0 ± 0.0	3.5 ± 1.0	3.50 ± 1.24	ns	ns	ns
2-methyl-5-hexenenitrile	0.0 ± 0.0	0.0 ± 0.0	6.3 ± 6.3	9.5 ± 9.5	ns	ns	ns

¹ (E)-DMNT: (E)-4,8-dimethylnona-1,3,7-triene. ² MeSA: methyl salicylate.

References

1. Kirtman, B.; Power, S.B. Near-term climate change: Projections and predictability. In *Climate Change 2013 Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*; Cambridge University Press: Cambridge, UK; New York, NY, USA, 2013; pp. 953–1028.
2. Mills, G.; Pleijel, H.; Malley, C.S.; Sinha, B.; Cooper, O.R.; Schultz, M.G. Tropospheric ozone assessment report: Present-day ozone distribution and trends relevant to vegetation. *Elem. Sci. Anthr.* **2018**, *6*, 2–46. [CrossRef]
3. Sitch, S.; Cox, P.M.; Collins, W.J.C.; Huntingford, C. Indirect radiative forcing of climate change through ozone effects on the land-carbon sink. *Nature* **2007**, *448*, 791–794. [CrossRef]
4. Lehtomäki, H.; Geels, C.; Brandt, J.; Rao, S.; Yaramenka, K.; Åström, S.; Skou Andersen, M.; Frohn, L.M.; Im, U.; Hänninen, O. Deaths attributable to air pollution in Nordic countries: Disparities in the estimates. *Atmosphere* **2020**, *11*, 467. [CrossRef]
5. Iriti, M.; Faora, F. Oxidative stress, the paradigm of ozone toxicity in plants and animals. *Water Air Soil Pollut.* **2008**, *187*, 285–301. [CrossRef]
6. Viaene, P.; Deutsch, F.; Mensink, C.; Vandermeiren, K.; Vancraeynest, L.; Vanpoucke, C.; Fierens, F. Assessment of damage to vegetation in Belgium based on an ozone flux model approach. *Air Pollut. Model. Appl.* **2016**, *24*, 147–153.
7. Leisner, C.P.; Ainsworth, E.A. Quantifying the effects of ozone on plant reproductive growth and development. *Glob. Chang. Biol.* **2012**, *18*, 606–616. [CrossRef]
8. Kangasjärvi, J.; Talvinen, J.; Utriainen, M.; Karjalainen, R. Plant defence systems induced by ozone. *Plant Cell Environ.* **1994**, *17*, 783–794. [CrossRef]

9. Kangasjärvi, J.; Jaspers, P.; Kollist, H. Signalling and cell death in ozone-exposed plants. *Plant Cell Environ.* **2015**, *28*, 1021–1036. [[CrossRef](#)]
10. Pleijel, H.; Norberg, P.A.; Selldén, G.; Skärby, L. Tropospheric ozone decreases biomass production in radish plants (*Raphanus sativus*) grown in rural south-west Sweden. *Environ. Pollut.* **1999**, *106*, 143–147. [[CrossRef](#)]
11. Wilkinson, S.; Mills, G.; Illidge, R.; Davies, W.J. How is ozone pollution reducing our food supply? *J. Exp. Bot.* **2012**, *63*, 527–536. [[CrossRef](#)]
12. Avnery, S.; Mauzerall, D.L.; Liu, J.; Horowitz, L.W. Global crop yield reductions due to surface ozone exposure: 1. Year 2000 crop production losses and economic damage. *Atmos. Environ.* **2011**, *45*, 2284–2296. [[CrossRef](#)]
13. Evans, L.T. The History of Agricultural Production. *Tree* **1993**, *8*, 461.
14. Chen, Y.H.; Langelotto, G.A.; Barrion, A.T. Cultivation of domesticated rice alters arthropod biodiversity and community composition. *Annu. Rev. Entomol.* **2013**, *106*, 100–110. [[CrossRef](#)]
15. Chen, Y.H.; Gols, R.; Benrey, B. Crop domestication and its impact on naturally selected trophic interactions. *Annu. Rev. Entomol.* **2015**, *60*, 35–58. [[CrossRef](#)]
16. Eckardt, N.A. Evolution of domesticated bread wheat. *Plant Biol.* **2010**, *22*, 993. [[CrossRef](#)]
17. Whitehead, S.R.; Turcotte, M.M.; Poveda, K. Domestication impacts on plant-herbivore interactions: A meta-analysis. *Phil. Trans. R. Soc.* **2017**, *372*, 20160034. [[CrossRef](#)]
18. Turcotte, M.M.; Turley, N.E.; Johnson, M.T.J. The impact of domestication on resistance to two generalist herbivores across 29 independent domestication events. *New Phytol.* **2014**, *204*, 671–681. [[CrossRef](#)]
19. Chaudhary, B. Plant domestication and resistance to herbivory. *Int. J. Plant Genom.* **2013**, *2013*, 11–14. [[CrossRef](#)]
20. Chacón-Fuentes, M.; Parra, L.; Lizama, M.; Seguel, I.; Urzúa, A.; Quiroz, A. Plant flavonoid content modified by domestication. *Environ. Entomol.* **2017**, *46*, 1080–1089. [[CrossRef](#)] [[PubMed](#)]
21. Moreira, X.; Abdala-Roberts, L.; Gols, R.; Francisco, M. Plant domestication decreases both constitutive and induced chemical defences by direct selection against defensive traits. *Sci. Rep.* **2018**, *8*, 12678. [[CrossRef](#)]
22. Mithen, R.F.; Lewis, B.G.; Heaney, R.K.; Fenwick, G.R. Glucosinolates of wild and cultivated *Brassica* species. *Phytochemistry* **1987**, *26*, 1969–1973.
23. Moyes, C.L.; Collin, H.A.; Britton, G.; Raybould, A.F. Glucosinolates and differential herbivory in wild population of *Brassica oleracea*. *J. Chem. Ecol.* **2000**, *26*, 2625–2641. [[CrossRef](#)]
24. Altesor, P.; Garcia, A.; Font, E.; Rodríguez-Haralambides, A.; Vilaró, F.; Oesterheld, M.; Soler, R.; González, G. Glycoalkaloids of wild and cultivated solanum: Effects on specialist and generalist insect herbivores. *J. Chem. Ecol.* **2014**, *40*, 599–608. [[CrossRef](#)]
25. Maag, D.; Erb, M.; Bernal, J.S.; Wolfender, J.; Turlings, T.C.J. Maize domestication and anti-herbivore defences: Leaf-specific dynamics during early ontogeny of maize and its wild ancestors. *PLoS ONE* **2015**, *8*, 1–21. [[CrossRef](#)]
26. De Lange, E.S.; Balmer, D.; Mauch-mani, B.; Ted, C.J. Insect and pathogen attack and resistance in maize and its wild ancestors; the teosintes. *New Phytol.* **2014**, *204*, 329–341. [[CrossRef](#)]
27. Rodríguez-saona, C.; Vorsa, N.; Singh, A.P.; Johnson-Cicalese, J.; Szendrei, Z.; Mescher, M.C.; Forst, C.J. Tracing the history of plant traits under domestication in cranberries: Potential consequences on anti-herbivore defences. *J. Exp. Botany* **2011**, *62*, 2633–2644. [[CrossRef](#)]
28. Gols, R.; Bukovinszky, T.; Van Dam, N.M.; Dicke, M.; Bullock, J.M.; Harvey, J.A. Performance of generalist and specialist herbivores and their endoparasitoids differs on cultivated and wild *Brassica* populations. *J. Chem. Ecol.* **2008**, *34*, 132–143. [[CrossRef](#)]
29. Kessler, A.; Baldwin, I.T. Defensive Function of herbivore-induced plant volatile emission in nature. *Science* **2001**, *291*, 2141. [[CrossRef](#)]
30. Benrey, B.; Callejas, A.; Rios, L.; Oyama, K.; Denno, R.F. The effects of domestication of brassica and phaseolus on the interaction between phytophagous insects and parasitoids. *Biol. Cont.* **1998**, *11*, 130–140. [[CrossRef](#)]
31. Rodríguez-Saona, C.; Cloonan, K.R.; Sanchez-Pedraza, F.; Zhou, Y.; Giusti, M.M.; Benrey, B. Differential susceptibility of wild and cultivated blueberries to an invasive frugivorous pest. *J. Chem. Ecol.* **2019**, *45*, 286–297. [[CrossRef](#)] [[PubMed](#)]
32. Rowen, E.; Kaplan, I. Eco-evolutionary factors drive induced plant volatiles: A metaanalysis. *New Phytol.* **2016**, *210*, 284–294. [[CrossRef](#)] [[PubMed](#)]
33. Gaillard, M.D.P.; Glauser, G.; Robert, C.A.M.; Turlings, T.C.J. Fine-tuning the ‘plant domestication-reduced defense’ hypothesis: Specialist vs generalist herbivores. *New Phytol.* **2018**, *217*, 355–366. [[CrossRef](#)] [[PubMed](#)]

34. Paudel, S.; Lin, P.A.; Foolad, M.R.; Ali, J.G.; Rajotte, E.G.; Felton, G.W. Induced plant defenses against herbivory in cultivated and wild tomato. *J. Chem. Ecol.* **2019**, *45*, 693–707. [[CrossRef](#)] [[PubMed](#)]
35. Loreto, F.; Schnitzler, J.P. Abiotic stresses and induced BVOCs. *Trends Plant Sci.* **2010**, *15*, 154–166. [[CrossRef](#)]
36. Vickers, C.E.; Gershenzon, J.; Lerdau, M.T.; Loreto, F. A unified mechanism of action for volatile isoprenoids in plant abiotic stress. *Nat. Chem. Biol.* **2009**, *5*, 283–291. [[CrossRef](#)]
37. Heiden, A.; Hoffmann, T.; Kahl, D.; Flockow, D.; Wildt, J. Emission of volatile organic compounds from ozone-exposed plants. *Ecol. Appl.* **1999**, *9*, 1160–1167. [[CrossRef](#)]
38. Fares, S.; Loreto, F.; Kleist, E.; Wildt, J. Stomatal uptake and stomatal deposition of ozone in isoprene and monoterpene emitting plants. *Plant Biol.* **2008**, *10*, 44–54. [[CrossRef](#)]
39. Himanen, S.J.; Nerg, A.M.; Nissinen, A.; Pinto, D.M.; Stewart Jr, C.N.; Poppy, G.M.; Holopainen, J.K. Effects of elevated carbon dioxide and ozone on volatile terpenoid emissions and multitrophic communication of transgenic insecticidal oilseed rape (*Brassica napus*). *New Phytol.* **2008**, *181*, 174–186. [[CrossRef](#)]
40. Beauchamp, J.; Wisthaler, A.; Hansel, A.; Kleist, E.; Miebach, M. Ozone induced emissions of biogenic VOC from tobacco: Relationships between ozone uptake and emission of LOX products Volatile organic compound (VOC) emissions from tobacco. *Cell* **2005**, *28*, 1334–1343.
41. Khaling, E.; Li, T.; Holopainen, J.K.; Blande, J.D. Elevated ozone modulates herbivore-induced volatile emissions of *Brassica nigra* and alters a tritrophic interaction. *J. Chem. Ecol.* **2016**, *42*, 368–381. [[CrossRef](#)]
42. Brosset, A.; Saunier, A.; Kivimäenpää, M.; Blande, J.D. Does ozone exposure modify herbivore induced plant volatile emissions? *Environ. Sci. Pollut. Res.* **2020**, *27*, 30448–30459. [[CrossRef](#)] [[PubMed](#)]
43. Guo, Y.; Chen, S.; Li, Z.; Cowling, W.A. Center of origin and centers of diversity in an ancient crop, *Brassica rapa* (turnip rape). *J. Hered.* **2014**, *105*, 555–565. [[CrossRef](#)] [[PubMed](#)]
44. Gómez-Campo, C.; Prakash, S. Origin and domestication. *Biol. Brassica Coenospecies* **1999**, *4*, 33–57.
45. Pinto, D.M.; Blande, J.D.; Nykänen, R. Ozone degrades common herbivore-induced plant volatiles: Does this affect herbivore prey location by predators and parasitoids? *J. Chem. Ecol.* **2007**, *1*, 683–694. [[CrossRef](#)]
46. Agathokleous, E.; Yu, W.L.; Ntatsi, G.; Konno, K.; Costas, J.S.; Kitaoa, M.; Koike, T. Effects of ozone and ammonium sulfate on cauliflower: Emphasis on the interaction between plants and insect herbivores. *Sci. Total Environ.* **2019**, *659*, 995–1007. [[CrossRef](#)]
47. Niinemets, Ü.; Kännaste, A.; Copolovici, L. Quantitative patterns between plant volatile emissions induced by biotic stresses and the degree of damage. *Front. Plant Sci.* **2013**, *4*, 1–15. [[CrossRef](#)]
48. Khaling, E.; Papazian, S.; Poelman, E.H.; Holopainen, J.K.; Albrechtsen, B.R.; Blande, J.D. Ozone affects growth and development of *Pieris brassicae* on the wild host plant *Brassica nigra*. *Environ. Pollut.* **2015**, *199*, 119–129. [[CrossRef](#)]
49. Van Dam, N.M.; Hermenau, U.; Baldwin, I. Instar-specific sensitivity of specialist *Manduca sexta* larvae to induced defences in their host plant *Nicotina attenuate*. *Ecol. Entomol.* **2001**, *26*, 578–586. [[CrossRef](#)]
50. Do Boer, J.D.; Dicke, M. The Role of Methyl salicylate in prey searching behavior of the predatory mite *Phytoseiulus persimilis*. *J. Chem. Ecol.* **2004**, *30*, 255–271. [[CrossRef](#)]
51. Zhu, J.; Park, K. Methyl salicylate, a soybean aphid-induced plant volatile attractive to the predator *Coccinella septempunctata*. *J. Chem. Ecol.* **2005**, *31*, 1733–1746. [[CrossRef](#)]
52. Hopkins, R.J.; van Dam, N.M.; van Loon, J.J.A. Role of glucosinolates in insect-plant relationships and multitrophic interactions. *Annu. Rev. Entomol.* **2009**, *54*, 57–83. [[CrossRef](#)]
53. Gols, R.; Bullock, M.; Dicked, M.; Bukovinszky, T.; Harvey, J.A. Smelling the wood from the trees: Non-linear parasitoid responses to volatile attractants produced by wild and cultivated cabbage. *J. Chem. Ecol.* **2011**, *37*, 795–807. [[CrossRef](#)]
54. Marazzi, C.; Patrian, B.; Städler, E. Secondary metabolites of the leaf surface affected by sulphur fertilisation and perceived by the diamondback moth. *Chemoecology*. **2004**, *14*, 81–86. [[CrossRef](#)]
55. Badenes-Perez, F.R.; Reichelt, M.; Heckel, D.G. Can sulfur fertilisation improve the effectiveness of trap crops for diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae)? *Pest. Manag. Sci.* **2010**, *66*, 832–838. [[CrossRef](#)]
56. Loreto, F.; Mannozi, L.; Maris, C.; Nascetti, P.; Ferranti, F.; Pasqualini, S. Ozone quenching properties of isoprene and its antioxidant role in leaves. *Plant Physiol.* **2001**, *126*, 993–1000. [[CrossRef](#)]
57. Black, V.J.; Black, C.R.; Roberts, J.A.; Stewart, C.A. Impact of ozone on the reproductive development of plants. *New Phytol.* **2000**, *147*, 421–447. [[CrossRef](#)]

58. Ashmore, M.R.; Davison, A.W. Towards a critical level of ozone for natural vegetation. In *Critical Levels for Ozone in Europe: Testing and Finalising the Concepts*; UNECE Workshop Report; University of Kuopio: Kuopio, Finland, 1996; pp. 58–71.
59. Agathokleous, E.; Saitanis, C.J.; Koike, T. Tropospheric O₃, the nightmare of wild plants: A review study. *J. Agric. Meteorol.* **2015**, *71*, 142–152. [[CrossRef](#)]
60. Biswas, D.K.; Li, Y.G.; Chen, Y.H.; Sun, J.Z.; Jiang, G.M. Assessing the genetic relatedness of higher ozone sensitivity of modern wheat to its wild and cultivated progenitors relatives. *J. Exp. Bot.* **2008**, *59*, 951–963. [[CrossRef](#)]
61. Morgan, P.B.; Ainsworth, E.A.; Long, S.P. How does elevated ozone impact soybean? A meta-analysis of photosynthesis, growth and yield. *Plant Cell Environ.* **2003**, *26*, 1317–1328. [[CrossRef](#)]
62. Fiscus, E.L.; Booker, F.L.; Burkey, K.O. Crop responses to ozone: Uptake, modes of action, carbon assimilation and partitioning. *Plant Cell Environ.* **2005**, *28*, 997–1011. [[CrossRef](#)]
63. Baéso Moura, B.; Alves, E.S.; Marabesi, M.A.; Ribeiro de Souza, S.; Schaub, M.; Vollenweider, P. Ozone affects leaf physiology and causes injury to foliage of native tree species from the tropical Atlantic Forest of southern Brazil. *Sci. Total Environ.* **2018**, *610–611*, 912–925. [[CrossRef](#)] [[PubMed](#)]
64. Vainonen, J.P.; Kangasjärvi, J. Plant signalling in acute ozone exposure. *Plant Cell Environ.* **2015**, *38*, 240–252. [[CrossRef](#)] [[PubMed](#)]
65. Vuorinen, T.; Nerg, A.M.; Holopainen, J.K. Ozone exposure triggers the emission of herbivore-induced plant volatiles, but does not disturb tritrophic signalling. *Environ. Pollut.* **2004**, *131*, 305–311. [[CrossRef](#)] [[PubMed](#)]
66. Dudareva, N.; Klemplen, A.; Muhlemann, J.K.; Kaplan, I. Biosynthesis, function and metabolic engineering of plant volatile organic compounds. *New Phytol.* **2013**, *198*, 16–32. [[CrossRef](#)]
67. Caarls, L.; Pieterse, C.M.J.; Van Wees, S.C.M. How salicylic acid takes transcriptional control over jasmonic acid signaling. *Front. Plant Sci.* **2015**, *6*, 1–11. [[CrossRef](#)]
68. Mur, L.A.J.; Kenton, P.; Lloyd, A.J.; Ougham, H.; Prats, E.; Gogerddan, P. The hypersensitive response, the centenary is upon us but how much do we know? *J. Exp. Bot.* **2008**, *59*, 501–520. [[CrossRef](#)]
69. Mithöfer, A.; Wanner, G.; Boland, W. Effects of feeding *Spodoptera littoralis* on lima bean leaves. ii. continuous mechanical wounding resembling insect feeding is sufficient to elicit herbivory-related volatile emission. *Plant Physiol.* **2005**, *137*, 1160–1168. [[CrossRef](#)]
70. Vuorinen, T.; Nerg, A.; Ibrahim, M.A.; Reddy, G.V.P.; Holopainen, J.K. Emission of *Plutella xylostella*-induced compounds from cabbages grown at elevated CO₂ and orientation behavior of the natural enemies. *Plant Physiol.* **2004**, *135*, 1984–1992. [[CrossRef](#)]
71. Zebelo, S.A.; Matsui, K.; Ozawa, R.; Maffei, M.E. Plasma membrane potential depolarization and cytosolic calcium flux are early events involved in tomato (*Solanum lycopersicon*) plant-to-plant communication. *Plant Sci.* **2012**, *196*, 93–100. [[CrossRef](#)]
72. Sugimoto, K.; Matsui, K.; Akakabe, Y.; Muramoto, S.; Ozawa, R.; Uefune, M. Intake and transformation to a glycoside of (Z)-3-hexenol from infested neighbors reveals a mode of plant odor reception and defense. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 7144–7149. [[CrossRef](#)]
73. Heil, M.; Carlos, J.; Bueno, S. Within-plant signaling by volatiles leads to induction and priming of an indirect plant defense in nature. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 5467–5472. [[CrossRef](#)] [[PubMed](#)]
74. Jones, C.G.; Coleman, J.S. Plant stress and insect behavior: Cottonwood ozone and the feeding and oviposition preference of a Beetle. *Oecologia* **1988**, *76*, 51–56. [[CrossRef](#)] [[PubMed](#)]
75. Bolsinger, M.; Lier, M.E.; Hughes, P.R. Influence of ozone air pollution on plant- herbivore interactions. Part 2: Effects of ozone on feeding preference, growth and consumption rates of monarch butterflies (*Danaus plexippus*). *Environ. Pollut.* **1992**, *77*, 31–37. [[CrossRef](#)]
76. Jøndrup, P.M.; Barnes, J.D.; Port, G.R. The effect of ozone fumigation and different *Brassica rapa* lines on the feeding behaviour of *Pieris brassicae* larvae. *Entomol. Exp. Appl.* **2002**, *104*, 143–151. [[CrossRef](#)]
77. Vingarzan, R. A review of surface ozone background levels and trends. *Atmos. Environ.* **2004**, *38*, 3431–3442. [[CrossRef](#)]

Publisher’s Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).