Concurrent anthropogenic air pollutants enhance recruitment of a specialist parasitoid

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Running title: Air pollutants differentially modify parasitoid recruitment

Abstract

Air pollutants, such as nitrogen oxides, emitted in diesel exhaust, and ozone, disrupt interactions between plants, the insect herbivore pests that feed upon them, and natural enemies of those herbivores (e.g., parasitoids). Using eight field-based rings that emit regulated quantities of diesel exhaust and ozone, we investigated how both pollutants, individually and in combination, altered the attraction and parasitism rate of a specialist parasitoid (*Diaeretiella rapae*) on aphid-infested and un-infested *Brassica napus* plants. Individual effects of ozone decreased *D. rapae* abundance and emergence by 37% and 55%, respectively, compared with ambient (control) conditions. When ozone and diesel exhaust were emitted concomitantly, *D. rapae* abundance and emergence increased by 79% and 181%, respectively, relative to control conditions. This attraction response occurred regardless of whether plants were infested with aphids and was associated with an increase in concentration of aliphatic glucosinolates, especially gluconapin (3-butenyl glucosinolate), within *B. napus* leaves. Plant defensive responses and their ability to attract natural aphid enemies may be beneficially impacted by pollution exposure. These results demonstrate the importance of incorporating multiple air pollutants when considering the effects of air pollution on plant-insect interactions.

Keywords: Air pollution; aphid population; diesel exhaust; glucosinolates; ozone; parasitoid recruitment

Introduction

Insects utilise a variety of stimuli when interacting with their environment but rely heavily upon olfactory stimuli and particularly volatile organic compounds (VOCs) to perceive and interact with other organisms. As such, VOCs are used during critical stages of the life cycles of many insects, such as for locating hosts, food or mates [1]. Common atmospheric pollutants, such as nitrogen oxides (NO and NO_2, collectively NO_x) that are released from diesel vehicle exhausts, and ozone (O_3), are capable of chemically altering many of the VOCs that insects use for communication [2]. Disruption of these VOC cues may have wide ranging impacts on the important ecosystem services that insects provide (e.g., pest-regulation services or pollination), which are critical for the functioning of terrestrial ecosystems.

Parasitic wasps (or parasitoids) are a provider of critical pest-regulation services, both in natural ecosystems and in many horticultural and arable cropping systems [3]. Their larvae live as parasites on or in other host insects (which can be crop pests), feeding upon them until the host dies [4]. Parasitoids of insect herbivores locate their herbivore hosts using VOC cues released by: i) the herbivores and, ii) the herbivore's host plants, which are produced and released in response to herbivore feeding [5]. Air pollution has been shown, in laboratory assays, to negatively affect the ability of parasitoids to locate their insect hosts, because the pollutants: i) react with and chemically change the VOCs released by plants and insects in the air, and ii) cause physiological changes to the plants, altering the VOCs that the plants release [6, 7]. While these laboratory studies have been critical for identifying the mechanisms by which air pollution affects herbivore-parasitoid interactions, little is known about the ecological impacts of air pollution on these odour-mediated interactions, and whether the efficiency of pest-regulation (i.e., parasitoid recruitment) under field conditions would be affected by their disruption. Recent reviews [8, 9] have called for studies to bridge the gap in our knowledge on how VOCs influence the process of host location by parasitoids at larger spatial scales. In addition, there have also been calls for more work on the combined effects of different air pollutants [10]; emissions of NO_x occur alongside elevations in O_3 [11 and references therein], yet no studies to date have considered their combined effects on multi-trophic interactions.

The effects of air pollution on VOC-mediated interactions is of increasing concern because global tropospheric background O_3 concentrations are rising [12]. Concentrations of O_3 are typically higher in rural areas than urban areas because in urban areas there is more NO_x pollution from vehicles and industry, and O_3 and NO_x readily react with one another, reducing O_3 concentrations [13]. NO_x

emissions remain a serious problem, with areas throughout the UK continually exceeding limits imposed by the EU Ambient Air Quality Directive [14]. Transportation accounts for the majority of NO_x emissions in the UK (e.g., 47% in 2017; [15]) and many of the latest 'Euro 6' diesel cars that have been approved for sale continue to exceed air pollution limits (RDE test data; [16]) so diesel exhaust pollution is likely to remain a problem for decades to come [17]. Furthermore, as urbanisation and traffic congestion increases, those critical ecosystem services delivered by insects that rely upon odour-mediated interactions, such as pest-regulation and pollination, may be at increased risk [13, 14, 18]. Long-term, this risk may diminish as diesel exhaust emission sources reduce, at which point urban environments and polluted rural areas (e.g. those next to major roads) will have to contend with relatively higher levels of O_3 (because less O_3 is quenched by NO_x [19, 20]) and its effect on the natural ecosystem services that we rely on [10].

Using a unique set of Free-Air Diesel and Ozone Exposure (FADOE) rings over two years, this study aimed to determine how changes in diesel exhaust and O_3 pollution, individually and in combination, shape populations of insect herbivore pests (cabbage aphids, *Brevicoryne brassicae* Linnaeus.) and their parasitoids (specifically, *Diaeretiella rapae* MacIntosh). We used oilseed rape (or OSR; *Brassica napus* L.) as our model plant, which is commonly attacked by *B. brassicae* and is the most economically important brassica species in Europe [21]. The native European *B. brassicae*, now distributed throughout the world, are specialist feeders on Brassicaceous crops (e.g., OSR, cabbage, broccoli, cauliflower etc.). The parasitoid *D. rapae* commonly targets *B. brassicae* and has been shown to be attracted to aphid- and plant-released glucosinolate hydrolysis products that act as indicators of host presence [22-24], making these insects ideal model species for investigating the mechanisms underpinning the effects of diesel exhaust and O_3 on tri-trophic interactions. We identified the total number of parasitoids attracted to aphid-infested and non-infested plants under each pollution scenario (diesel exhaust, O_3, diesel exhaust plus O_3, and control) and recorded the abundance and oviposition success of the parasitoid, *D. rapae*. As such, we hypothesised that air pollution would result in reduced abundances of all species of parasitoid, including *D. rapae*,, by reacting with and depleting the VOCs that parasitoids use to locate their aphid hosts, and as a result decreased parasitism rates of their aphid hosts. Phloem-feeding aphids tend to respond positively, in terms of growth/reproductive rate, to pollution-mediated changes in plant quality due to stress-related increases in nitrogen-containing compounds and/or decreases in plant defensive compounds [25]; consequently, reductions in parasitism may act to further increase their pest status [18, 26]. We therefore hypothesised that pollution-mediated increases in aphid abundance (resulting in increases of aphid-emitted VOCs) would counteract the negative effects of air pollution on

parasitoid abundance. We also examined whether the concentrations of specific glucosinolates in leaf tissue changed as a result of exposure to pollution and, if so, whether they were correlated with any changes in parasitoid abundance. Understanding how air pollution could modify pest–parasitoid interactions in the field at temporal and spatial scales that are relevant to the insects could provide greater insight into how current or future levels of air pollution may mediate and influence insect pest outbreaks.

Materials and methods

Insect cultures and plant material

Four *Brevicoryne brassicae* aphid cultures were established from a single parthenogenetic adult female collected from an OSR field at Sonning Farm (latitude 51.480330, longitude -0.899504). Cultures were maintained at 20 °C on propagated OSR (cv. Tamarin, sourced from Senova, Cambridge, UK) for at least six generations (c. 8 weeks) prior to the experiment. For the experiment, OSR plants (cv. Tamarin) were grown from seed in 100 mL round cell trays in glasshouse rooms receiving natural light. After four weeks, plants were transplanted to 18 cm diameter pots containing c. 2.7 kg of vegetable topsoil (Quality Garden Supplies Ltd., Staffordshire, UK) and white mesh (organza) nets (55 x 75 cm) were placed over the plants and attached tightly around the rim of all pots to prevent any insect damage under field conditions. Bamboo sticks were placed inside the nets to prevent contact between the leaves and the mesh.

Field conditions and experimental procedures

In 2018, eight FADOE octagonal rings (8 m in diameter) were constructed at the University of Reading's Sonning farm within a field of winter wheat (*Triticum aestivum* cv. Skyfall), which maximised weed control (i.e. prevented weeds from growing, which themselves could have emitted different VOCs that may have altered the odour landscape inconsistently across the field). The centre of each ring was positioned 46 m from the centre of the field (latitude 51.482853, longitude -0.897749) in an octagonal formation, such that each ring was separated by a distance of at least 30 m. Full details of the FADOE configuration and layout are reported in [11]. Two rings were assigned to each of four treatments: i) diesel exhaust (D), ii) O_3, iii) diesel exhaust and O_3 combined (D+O_3), and iv) ambient air control. Concentrations of nitric oxide (NO), nitrogen dioxide (NO_2), nitrogen oxides (NO_x = NO + NO_2) and O_3 were monitored continuously and automatically maintained at field-realistic levels. The target concentrations were 120 ppb NO_x (based on average concentrations adjacent to major UK roadways and urban areas; [27]) and 90 ppb O_3 (based on peak concentrations

recorded in rural European sites in 1990-2012; [28]) but average concentrations achieved within the rings were significantly lower than these, as described in the results. Diesel and O_3 generators were turned on for up to 17 hours of the day (between 4.30 am and 9.30 pm), during which oviposition rates of *D. rapae* parasitoids are highest (females oviposit over 96% of their total eggs during the photophase; [29]). In 2019, the FADOE rings were moved to an adjacent field of wheat (latitude 51.482374, longitude -0.895855) and rotated within the field to account for the effects of pseudo-replication. A total of three experimental runs (described below) were undertaken, one in September-October 2018 and two in September-October 2019. Natural environmental conditions, including air temperature, wind speed and wind direction were monitored continuously throughout the experiment.

Netted OSR plants (16 plants in the first and third experimental runs and 28 plants in the second experimental run) were placed in each of the eight FADOE rings in four random groups (four plants per group in the first and third experimental runs and seven plants per group in the second experimental run; Fig. 1a). When plants were five weeks old (i.e. one week after plants were transplanted into pots within the rings), each plant in two groups (aphid treatments A10 and OPEN) was inoculated with 10 teneral adult *B. brassicae* aphids. A further group was inoculated with 50 aphids (A50) and the final group remained insect-free (CON). The aphids were left to establish for one week on the netted plants before placing a sticky trap (22 cm x 10 cm) on a stake in the centre of each group (outside of the nets), in order to capture naturally-occurring parasitoids. After a further week, the first set of sticky traps were stored at −20°C and replaced in all but the OPEN group. The nets from the OPEN group were removed so that the aphids were exposed (Fig. 1a). After a further week, the plants in the OPEN group were re-netted and a sticky trap was positioned inside the net of each plant to catch emerging parasitoids. The second set of sticky traps were collected from the three other treatments (A10, A50 and CON) on the same day and stored at −20°C until required. Aphids were removed from each plant within these three groups using a pooter and stored in 60 mL pots at −20°C before being freeze-dried for 72 h and weighed. Plants were oven-dried at 70°C and weighed. The sticky traps from inside each of the nets of the OPEN plants were collected 10 days later and stored at -20°C. *Diaretiella rapae*, as well as all other parasitoids, were identified and counted from all sticky traps, from which *D. rapae* was easily identified by its distribution and wing structure [30, 31]. Therefore, parasitism rate (i.e. parasitoid emergence of *D. rapae*) was recorded from the OPEN treatment only, and parasitoid abundance (i.e. total parasitoid abundance and the abundance of *D. rapae*) was recorded from the three other treatments (A10, A50 and CON). The timeline of experimental events is visualised in Fig. 1b.

Chemical analysis

For the second experimental run, three additional plants were added to each treatment within each ring for purposes of chemical analyses. Therefore, all seven plants in the A10, A50 and OPEN treatments were inoculated with aphids (described above). Upon harvest, three plants were selected from each of the A50 and CON treatments (Fig. 1a), from which four intact leaves were removed, freeze-dried and ground for glucosinolate analysis using liquid chromatography-mass spectrometry (LC-MS), following the protocol set out by [32]. Seven glucosinolates were detected using this method. Based on their side-chain structure and amino acid precursors [33], these glucosinolates were classified as aliphatic (glucoalyssin, progoitrin, glucobrassicanapin, gluconapin) and indolic (glucobrassicin, neoglucobrassicin, 4-methoxyglucobrassicin).

Solid phase micro-extraction gas chromatography mass spectrometry (SPME-GC-MS) was used to determine VOC relative abundances in fresh OSR leaf samples collected from three plants in each of the A50 and CON (i.e. no aphids) treatments within each ring. Sample preparation, headspace extraction from macerated leaves, chromatography and mass spectrometry conditions were as presented by [34]. VOCs were identified or tentatively identified by comparison of each mass spectrum with authentic compounds, or the NIST mass spectral database (NIST/EPA/NIH Mass Spectral database, 2014). A spectral quality value >80 was used alongside linear retention index (LRI) to support the identification of compounds where no authentic standards were available. All peak areas were normalised. LRI was calculated for each VOC using the retention times of a homologous series of C_6-C_{25} *n*-alkanes and by comparing the LRI with those of authentic compounds analysed under similar conditions.

Statistical analysis

Air pollution and aphid treatment effects on plant mass, final aphid population mass, parasitoid abundance and emergence, and glucosinolate concentrations were analysed using mixed models in the *lme4* statistical package [35] within the R statistical interface v4.1.1. The fixed effects included air pollution treatment (control, O_3, D and D+O_3) and aphid treatment (No aphids, A10 and A50) as well as the two-way interaction between these terms. The random terms included year, run and ring location to account for seasonal and spatial differences that could confound any treatment effects and their inclusion was confirmed by model reductions using AIC and QQ plots. Negative binomial models were used for dependent variables with count data (numbers of *D. rapae* and other parasitoids and numbers of *D. rapae* that emerged from parasitised aphids) based on model

deviance and critical chi-squared values. Where appropriate, response variables were transformed before analysis (Table S1) to standardise residuals, which were confirmed with AIC and QQ plots. The model df, total number of observations not accounting for replication (N_{obs}) and group N (i.e. N_{group}; Year/Run/Ring = 24) are included in all reported model statistics. Parasitoids captured in sticky traps within the OPEN group prior to net removal were not included in these analyses. Pairwise comparisons of means for treatment effects were made with Tukey's post hoc tests utilising the *glht* function in R's 'multcomp' package [36] Pearson's correlation tests using the R base function *cor.test* were used to determine whether indolic, aliphatic and total glucosinolate concentrations were correlated with average *D. rapae* abundances for each ring. Normalized VOC abundances were analysed using XLSTAT (Addinsoft, Paris, France) protected Analysis of Variance (ANOVA) with post hoc Tukey's Honestly Significant Difference (HSD) pairwise comparison ($P < 0.05$). As such, if the ANOVA model is not significant, post hoc pairwise comparisons are 'protected' from overinterpretation (e.g. through the generation of Type I statistical errors) by not being calculated, and simply stated as being non-significant. This approach has been used previously in relation to glucosinolate and VOC data in Brassicaceae plants [37].

Results

Air pollutant concentrations

Concentrations of NO_x and O_3 recorded during the experimental period of September 2018 and 2019 are reported and analysed in [38]. In short, individual pollution rings averaged low to moderate concentrations (as defined by DEFRA Air Quality Index; [39]) of 48.63 (± 1.39 SE) ppb NO_x and 38.85 (± 1.30 SE) ppb O_3 in the individual D and O_3 treatment rings, respectively, compared with 7.35 ± 0.18 ppb NO_x and 21.74 ± 0.28 ppb O_3 in the ambient (control) rings. In the combined (D+O_3) pollution treatment, NO_x concentrations (34.14 ± 1.14 ppb) were significantly lower than those in D, associated with the interaction between atmospheric NO_x and O_3 and the conversion of nitrogen dioxide to nitric oxide (see [11, 38] for details). Moreover, the concentrations of O_3 in the D+O_3 treatment (20.38 ± 0.27 ppb) were 48% lower than the O_3 treatment, decreasing to levels equivalent to those in the control treatment.

Aphid population and plant mass under air pollution and aphid treatments

Aphid population mass was not significantly affected by air pollution but was significantly higher in the A50 treatment compared with the A10 treatment (Fig. 2). Air pollution and aphid treatments, individually and in interaction, had no significant effects on plant mass (Table S1).

Parasitoid responses to air pollution and aphid treatments

Diaeretiella rapae parasitoid abundance was significantly affected by air pollution ($\chi^2_{3,10}$ = 20.00, P < 0.001, N_{obs} = 72, N_{group} = 24), whereby *D. rapae* decreased under O_3 but increased under D+O_3 compared with ambient (control) conditions (Fig. 3). The abundance of other parasitoids, in contrast, significantly decreased under all three pollution treatments relative to the control treatment ($\chi^2_{3,10}$ = 8.35, P = 0.039, N_{obs} = 72, N_{group} = 24). Air pollution had a significant effect on the percentage of all parasitoids that were *D. rapae* ($\chi^2_{3,10}$ = 25.68, P < 0.001, N_{obs} = 72, N_{group} = 24), which increased significantly under both diesel treatments (D and D+O_3) but not under O_3 (Fig. 3).

The abundance of *D. rapae* significantly increased when aphids were present (i.e., plants that were initially infested with 10 and 50 aphids) compared with plants that were not inoculated with aphids ($\chi^2_{2,10}$ = 16.60, P < 0.001, N_{obs} = 72, N_{group} = 24). The abundance of other parasitoids increased when plants were infested with 50 aphids but not when plants were infested with 10 aphids. There were no interactive effects of air pollution and aphid treatment on parasitoids. Full statistical results are shown in Table S1.

The number of *D. rapae* that successfully emerged from parasitised aphids (OPEN plants) was significantly affected by air pollution ($\chi^2_{3,8}$ = 25.39, P < 0.001, N_{obs} = 120, N_{group} = 24), whereby *D. rapae* emergence decreased under O_3 and increased under D+O_3 (Fig. 4).

Plant glucosinolate concentrations

Total glucosinolate concentrations in leaves of OSR increased in the combined D+O_3 treatment ($\chi^2_{3,8}$ = 11.89, P = 0.008, N_{obs} = 133, N_{group} = 24), which was driven by increases in aliphatic glucosinolates ($\chi^2_{3,8}$ = 14.57, P = 0.002, N_{obs} = 133, N_{group} = 24), especially gluconapin (3-butenyl-glucosinolate: Fig. 5). Concentrations of indolic glucosinolates did not vary significantly between pollutants or aphid treatments. Full statistical results are shown in Table S1. Concentrations of aliphatic glucosinolates were positively correlated with the abundance of *D. rapae* that were attracted to aphid-infested plants, which was driven by the positive association between gluconapin and *D. rapae* abundance (Fig. 6).

Plant volatile organic compound relative abundances

Pollution treatment and aphid infestation had a significant effect on 21 of the 44 VOC compounds identified (Table S2) and there was a two-way interactive effect of air pollution and aphid treatment

on six VOC compounds (Table S3). In general, VOCs from plants that were infested with aphids and subjected to both O_3 and diesel exhaust combined were significantly higher than other aphid and/or pollution treatment combinations. For example, normalized peak areas of four methyl esters (methyl hexanoate, 2-hexenoic acid, methyl octanoate and (*Z*)-3-hexenyl isobutyrate), one alcohol (2-hexen-1-ol), two aldehydes ((*E*)-tiglaldehyde and (*E,E*)-2,4-heptadienal), dimethyl disulfide and hexanoic acid were significantly higher in aphid-infested D+O_3-fumigated plants compared with un-infested D+O_3-fumigated plants. Furthermore, normalized peak areas of three methyl esters (methyl hexanoate, 2-hexenoic acid and methyl octanoate), two ketones (3-pentanone and β-ionone), two aldehydes ((*E*)-tiglaldehyde and (*E,E*)-2,4-heptadienal) and dimethyl disulfide were significantly higher in aphid-infested D+O_3-fumigated plants compared with aphid-infested plants under ambient (control) conditions (Table S2).

Discussion

Exposure to diesel exhaust and O_3 pollutants had no clear effect on the population mass of aphids that were not exposed to natural enemies, yet these pollutants, in isolation, had opposing effects on the parasitism rate of *D. rapae* and their attraction to aphid-infested plants. In particular, we demonstrated negative effects of O_3 on parasitoid recruitment, mirroring effects which have been reported by others [18, 40, 41] and that are generally considered to be a result of the degradation of behaviourally important plant-released VOCs [42]. However, it is also possible that air pollution could physiologically alter VOC perception and directly impair insect health or motility [43-46]. In the current study, plants exposed to diesel exhaust, alone and in combination with O_3, were generally more attractive to *D. rapae*, and those aphids on plants exposed to both pollutants experienced higher rates of parasitism. This change in attraction, which was especially pronounced under the combined pollution treatment, occurred regardless of whether the plants were aphid-infested or not, indicating that changes in plant-released VOCs, as opposed to insect-emitted VOCs (i.e., those released from aphids directly), are more likely be responsible for the increased attraction of *D. rapae*. It is also possible that compounds within the pollution mix attracted *D. rapae* directly and, as such, further studies examining the direct impacts of concurrent air pollutants on the behaviour of parasitoids will be essential for mechanistically determining how parasitoids will respond to changes in the atmosphere as we shift away from fossil fuel dependence.

The combined pollution treatment significantly increased plant glucosinolate concentrations, especially of the aliphatic glucosinolate gluconapin (3-butenyl-glucosinolate). These changes in leaf tissue concentrations suggest that the plants modified their glucosinolate production as a stress-

induced response to their exposure to both pollutants simultaneously [47, 48]. Increases in concentrations of gluconapin were positively correlated with increases in abundance of *D. rapae*. The hydrolysis product of gluconapin, 3-butenyl isothiocyanate, has been shown to act as an attractant for *D. rapae* in previous studies [23, 24]. We did not identify any glucosinolate hydrolysis products in the headspace of macerated OSR leaves, although it is possible that parasitoids may be attracted to hydrolysis products (i.e., isothiocyanates) that were below the detection threshold of instrumentation. OSR has typically been bred to contain low concentrations of glucosinolates and isothiocyanates [49], making them challenging to detect; however, further studies using non-destructive headspace sampling from whole living tissue (e.g., [5, 50]) could more effectively mimic the VOC emissions that the parasitoids would be exposed to and provide the identities of additional VOCs that may contribute to parasitoid recruitment [49]. The increase in some VOCs within the leaf tissue of OSR when aphids were present and when plants were fumigated with both pollutants provides further evidence to suggest a stress-induced systemic impact on the secondary metabolism of OSR.

Air pollution-mediated changes in the proportion of *D. rapae* in the parasitoid assemblage has the potential to impact the structure of insect communities associated with OSR and other brassica species via changes in pest regulation [51]. Parasitoids other than *D. rapae* differed in their response to air pollution and tended to decrease in all pollution treatments. These contrasting responses of *D. rapae*, a specialist parasitoid of *Brassica*-feeding aphids [23, 52], when compared with responses of other parasitoids to air pollution suggests that differences could be species-specific or a function of their diet (i.e., whether they are specialist or generalist feeders that target few or multiple host Orders, respectively). As specialist parasitoids (i.e. those with preferred prey limited to one or a few related host species [53, 54]), *D. rapae* may be more likely to respond to air pollution-mediated changes in VOCs because they tend to respond to a restricted set of stimuli, specific to their aphid hosts and the plants they feed upon [41, 52, 55]. Generalist parasitoids that target multiple hosts that may feed on many different plants, in contrast, may be less likely to rely on specific stimuli [38, 56]) but instead utilise a range of different VOCs that may be more prone to being degraded by oxidising air pollutants. It is also possible that specific VOCs are induced by host herbivory, therefore enhancing the signal that conveys host presence for *D. rapae* specifically.

Regardless of the specific mechanism used, parasitoids may be able to adapt to forage in polluted atmospheres by learning to associate altered VOC emission profiles with their target host [57]. As such, studies comparing parasitoids originating from polluted and unpolluted environments could

quantify their ability to adapt, which is also likely to differ according to their diet specialisation. Comparing generalist and specialist parasitoids originating from urban environments with those originating from more rural environments would be a useful next step. In general, mechanistically identifying how groups of generalist and specialist parasitoid species will respond to air pollution, using a combination of controlled laboratory studies and longer-term population studies, could contribute to the targeted formation and/or release of specific compounds that effectively attract natural enemies and reduce herbivore populations. This would be especially valuable for protected (i.e. crops grown under glass or plastic) and high-value crops that more often rely on biological control for herbivore pest management [58, 59]. From a wider ecological perspective, declines in the abundance of parasitoids other than *D. rapae* within polluted environments is likely to enhance the pest status of aphids and other plant pests in general, with negative consequences for food security.

Conclusions

Both diesel exhaust and O_3 pollution, individually and in combination, had deleterious effects on the abundance of parasitoid species other than *D. rapae*. In contrast, we demonstrated significant increases in *D. rapae* parasitoid recruitment under the combined effects of diesel exhaust and O_3 pollution, which contrasted with our hypothesis that both pollutants would deplete the VOCs that these parasitoids use to find their aphid hosts. This attraction response by *D. rapae* to diesel exhaust- and O_3-polluted environments is likely associated with an increase in the aliphatic glucosinolate, gluconapin, in OSR leaves, which is the precursor of an isothiocyanate which is attractive to *D. rapae*. These results stress the importance of studies incorporating the effects of multiple pollutants occurring in tandem in the natural environment. Concentrations of NO_x and O_3 emitted from the FADOE rings were lower than those considered safe under current air quality standards, emphasising how only moderate levels of air pollution can have significant impacts on plant-parasitoid dynamics. Shifting to sustainable energy generation and electrifying the fleet of diesel vehicles within the next two decades will again significantly alter the levels of atmospheric pollutants at times of peak daily activity of important ecosystem service providers, including those providing important pest-regulation services. A mechanistic understanding of how these service providers will respond to air pollution is, therefore, a vital, but hitherto neglected, component required to aid understanding and prediction of pest outbreaks.

Data accessibility

Data deposited in the EIDC Digital Repository (doi to follow).

Authors' contributions

JMWR conceived the experimental design, carried out data collection, carried out data analyses and drafted the manuscript. LMB participated in data collection. NJM contributed to the maintenance of the FADOE facility. LB and JJ performed chemical analysis and LB critically revised the manuscript. JDB participated in the design of the study and critically revised the manuscript. RDG participated in the design of the study and participated in drafting the manuscript. All authors gave final approval for publication and agree to be held accountable for the work performed therein.

Competing interests

We declare we have no competing interests.

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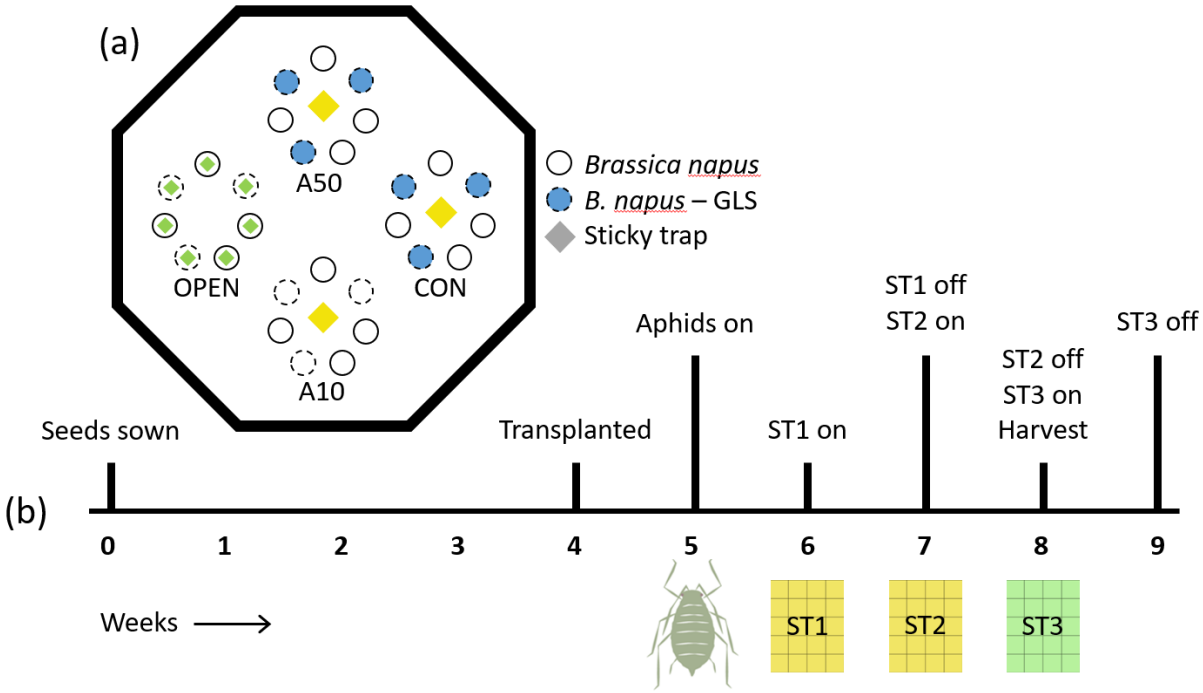
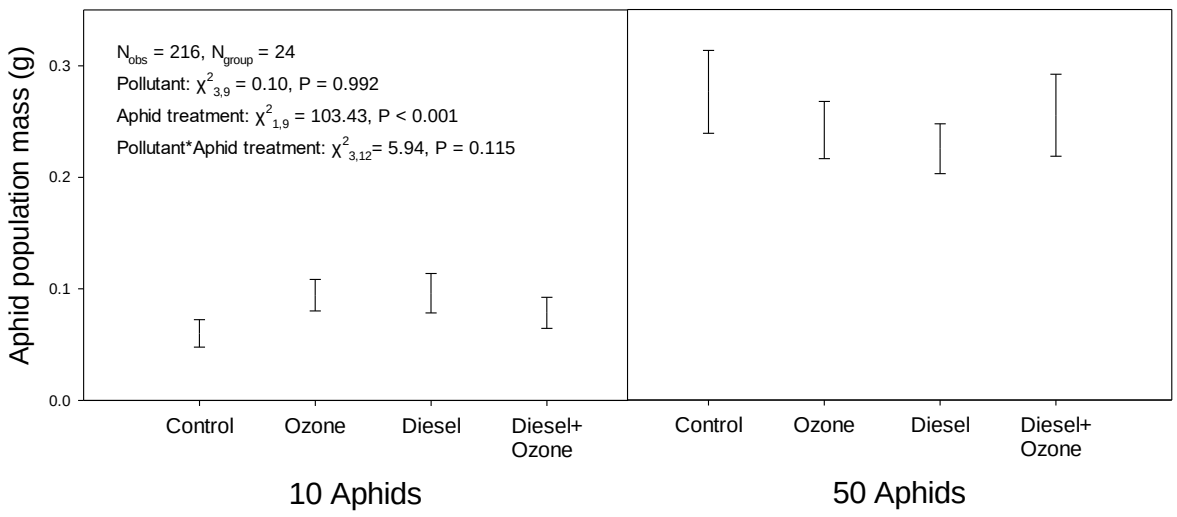


Fig. 1. Experimental layout and timeline of events. Plants and aphid treatments within the eight individual rings are shown for the second experimental run (a), with "*B. napus* – GLS" referring to OSR plants that were selected for glucosinolate analysis. The first and third experimental runs included four plants for each aphid treatment. Therefore, circles with dotted lines indicate plants that were included in the second experimental run only. The timeline of events (b) was the same for all three experimental runs. "ST" refers to sticky trap. Parasitoid numbers on ST1 and ST2 (yellow diamonds) were pooled, and ST3 (green diamonds) was used to trap emerging parasitoids from OPEN plants only.



574

575 **Fig. 2.** Aphid (*Brevicoryne brassicae*) final population mass under air pollution (control, ozone, diesel
576 exhaust or diesel exhaust and ozone) and aphid treatments (either 10 aphids or 50 aphids added as a
577 parent population at the start of the experiment to *Brassica napus* plants) three weeks after aphid
578 inoculation. Values are means ± SE. Statistical effects of treatments, and their interaction, on aphid
579 population mass shown. N_{obs} = total number of observations. N_{group} = group number associated with
580 the random effects of Year/Run/Ring.

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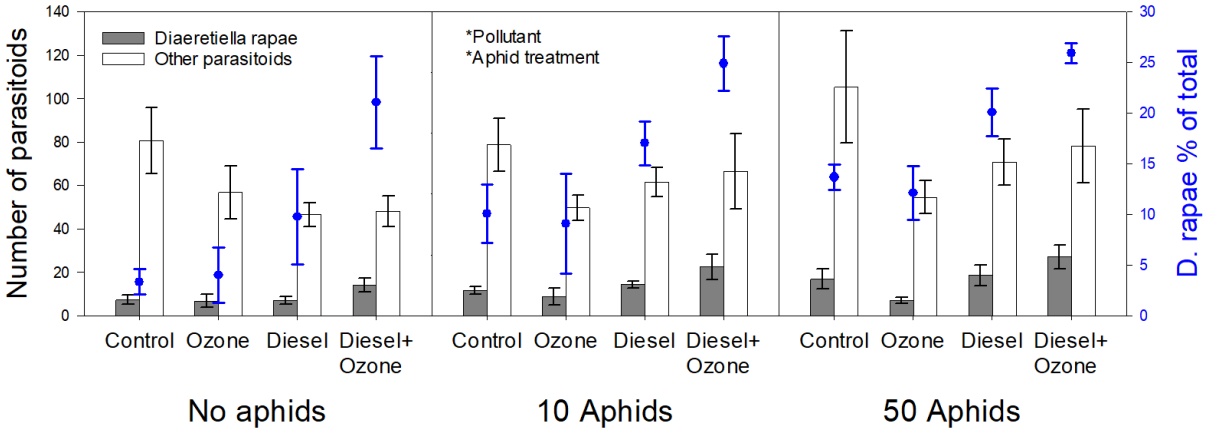
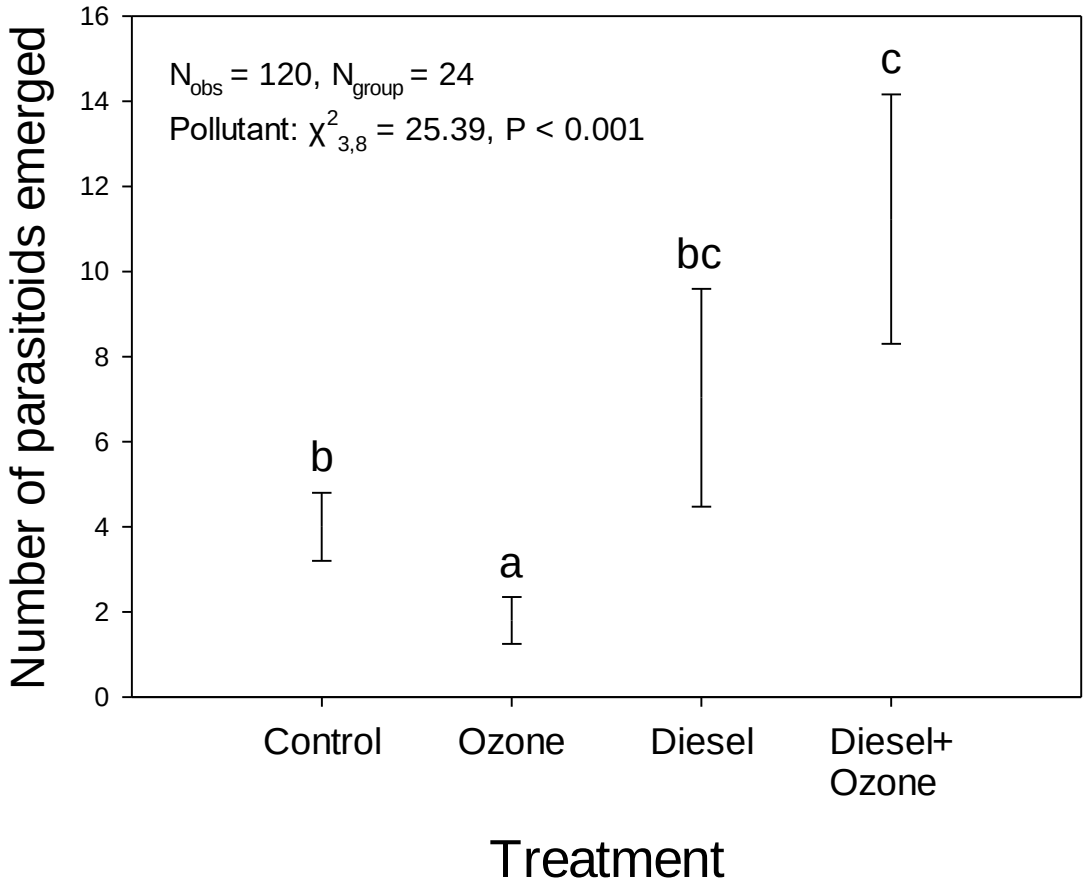


Fig. 3. Parasitoid abundance (left axis) and the percentage of total parasitoids that were *Diaeretiella rapae* (right axis, blue) under air pollution (control, ozone, diesel exhaust or diesel exhaust and ozone) and aphid treatments (i.e., plots with no *Brevicoryne brassicae* aphids, and either 10 aphids or 50 aphids added as a parent population to *Brassica napus* plants) after two weeks. Values are means ± SE. * indicates significant effects ($P > 0.05$) for all three dependent variables. Pairwise comparisons for the individual effects of air pollution and aphid treatments are shown in Figure S1.



591

592 **Fig. 4.** The effects of air pollution treatment (control, ozone, diesel exhaust or diesel exhaust and
593 ozone) on the number of *Diaeretiella rapae* parasitoids that emerged from parasitised aphids on
594 exposed (OPEN) *Brassica napus* plants. Values are means ± SE. Statistical effects of air pollution on
595 parasitoid emergence shown. Bars with the same letters were not significantly different ($P < 0.05$).
596 N_{obs} = total number of observations. N_{group} = group number associated with the random effects of
597 Year/Run/Ring.

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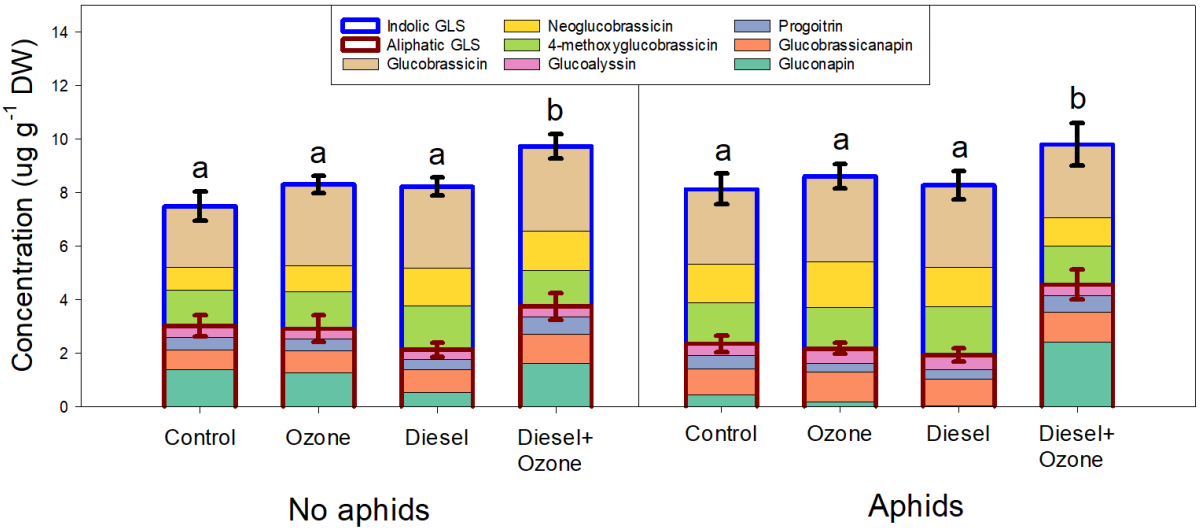
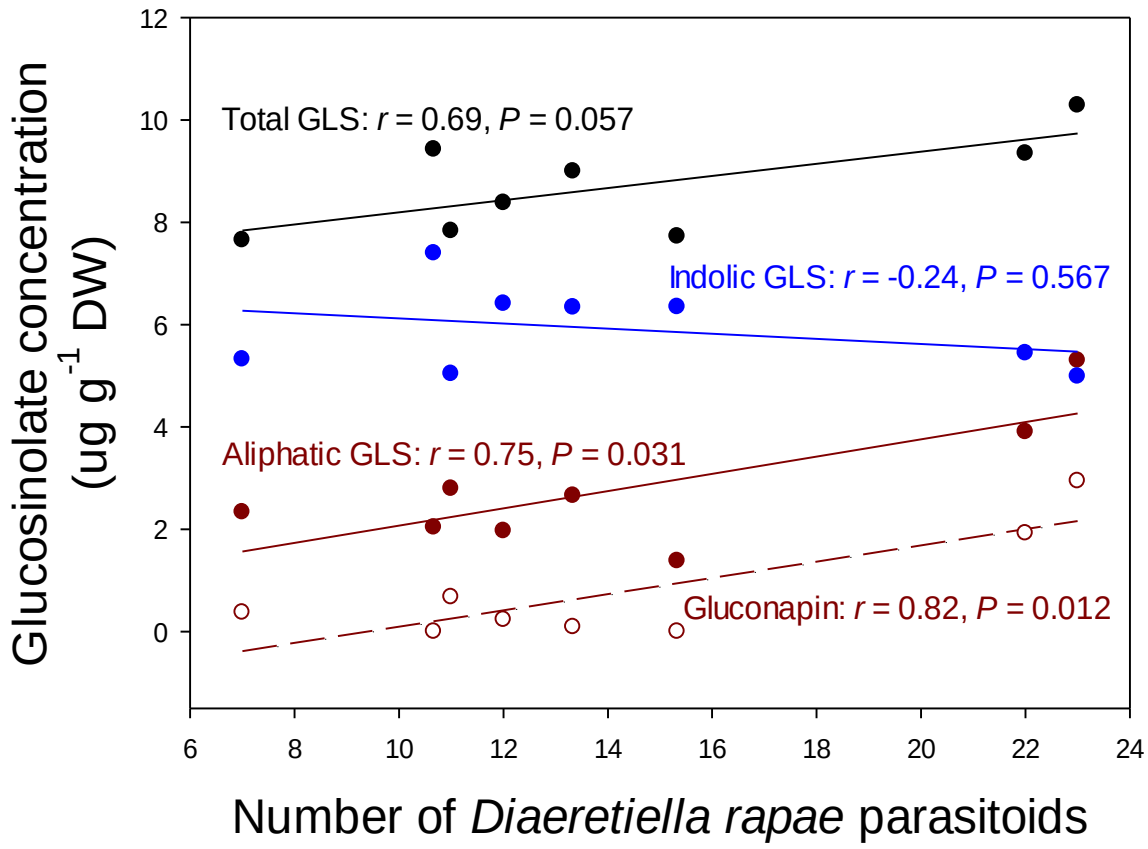


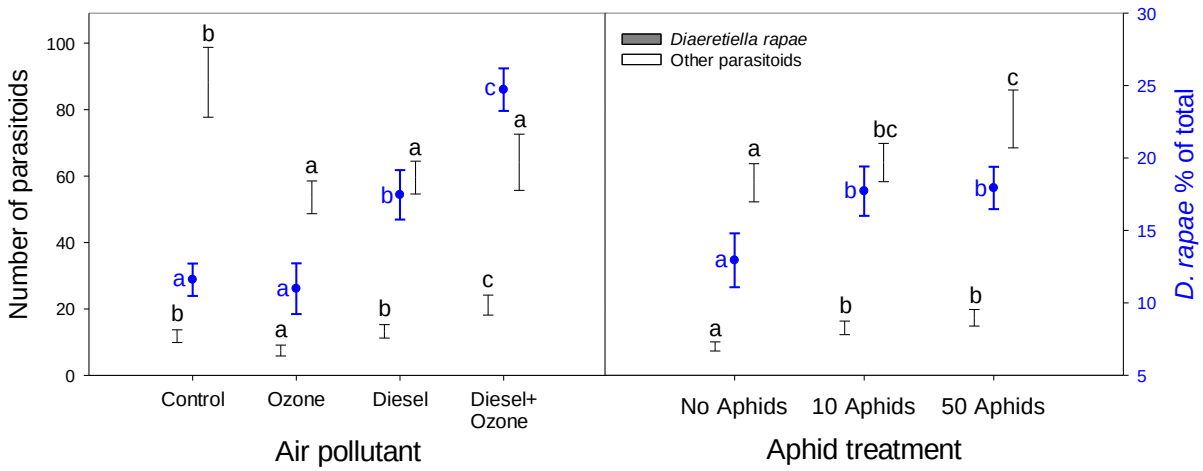
Fig. 5. Indolic (highlighted blue) and aliphatic (highlighted red) glucosinolate concentrations under air pollution treatments (control, ozone, diesel exhaust or diesel exhaust and ozone) and aphid treatments (control *Brassica napus* plants with no aphids and A50 plants with aphids). Black letters indicate significant differences between air pollution treatments for total glucosinolate concentrations; bars with the same letters were not significantly different ($P < 0.05$).



606

607 **Fig. 6.** Correlations between the mean number of *D. rapae* parasitoids counted on sticky traps within
608 each ring and mean glucosinolate concentrations (GLS) per ring. N = 8. Correlation test statistics
609 shown. Significant correlations between individual aliphatic GLS and parasitoid abundance (i.e.,
610 Gluconapin) are displayed. Other individual GLS were not significantly correlated with parasitoid
611 abundance.

612



613

614 **Figure S1.** Individual effects of air pollution (left panel) and aphid treatment (right panel) on
615 parasitoid abundance (left axis) and the percentage of total parasitoids that were *Diaeretiella rapae*
616 (right axis, blue). Values are means ± SE. Bars with the same letters for each dependent variable (i.e.
617 *Diaeretiella rapae* abundance, the abundance of other parasitoids and *D. rapae* % of total) within
618 each panel were not significantly different ($P < 0.05$).

619

Supplementary materials

Table S1. Effects of air pollution and aphid treatments on plant and insect measurements. [a]indicates variables that were square-root transformed. 'Diesel' refers to diesel exhaust pollution. Statistically significant effects ($P < 0.05$) are highlighted in bold. Means ± SE are shown for individual treatments. Models for plant mass and insect characteristics include random effects of Year, Run and Ring location (N = 24). Models for glucosinolates include random effects of Ring location and plant replicate (N = 8). N_{obs} refers to total observed N in statistical models. Treatment df followed by the number of model parameters is provided in subscript.

Response variable	Air pollution (Pollutant)				Aphid treatment (Aphid)			Statistical analyses
	Control	Ozone	Diesel	Diesel+ Ozone	None	A10	A50	
PLANT CHARACTERISTICS								
Plant mass (g) N_{obs} = 312	4.89 ± 0.19	4.60 ± 0.19	4.32 ± 0.23	4.62 ± 0.20	4.72 ± 0.17	4.76 ± 0.16	4.30 ± 0.20	Pollutant: $\chi^2_{3,10}$ = 1.48, P = 0.686 Aphid: $\chi^2_{2,10}$ = 4.79, P = 0.091 Pollutant × Aphid: $\chi^2_{6,16}$ = 7.93, P = 0.244
Indolic glucosinolates (µg g^{-1} DW) N_{obs} = 133	5.10 ± 0.31	5.87 ± 0.34	6.20 ± 0.28	5.60 ± 0.43	5.47 ± 0.25	-	5.91 ± 0.24	Pollutant: $\chi^2_{3,8}$ = 5.40, P = 0.145 Aphid: $\chi^2_{1,8}$ = 2.42, P = 0.120 Pollutant × Aphid: $\chi^2_{3,11}$ = 5.79, P = 0.122
Aliphatic glucosinolates (µg g^{-1} DW) N_{obs} = 133	2.70 ± 0.25	2.57 ± 0.29	2.04 ± 0.18	4.16 ± 0.38	2.95 ± 0.22	-	2.81 ± 0.23	Pollutant: $\chi^2_{3,8}$ = 14.57, **P = 0.002** Aphid: $\chi^2_{1,8}$ = 0.37, P = 0.542 Pollutant × Aphid: $\chi^2_{3,11}$ = 2.66, P = 0.447
Total glucosinolates (µg g^{-1} DW) N_{obs} = 133	7.80 ± 0.40	8.44 ± 0.27	8.24 ± 0.29	9.76 ± 0.45	8.42 ± 0.23	-	8.72 ± 0.31	Pollutant: $\chi^2_{3,8}$ = 11.89, **P = 0.008** Aphid: $\chi^2_{1,8}$ = 0.54, P = 0.463 Pollutant × Aphid: $\chi^2_{3,11}$ = 0.45, P = 0.931
INSECT CHARACTERISTICS								
Aphid population mass (g)[a] N_{obs} = 216	0.16 ± 0.02	0.16 ± 0.02	0.15 ± 0.02	0.16 ± 0.02	-	0.08 ± 0.01	0.25 ± 0.02	Pollutant: $\chi^2_{3,9}$ = 0.10, P = 0.992 Aphid: $\chi^2_{1,9}$ = 103.43, **P < 0.001** Pollutant × Aphid: $\chi^2_{3,12}$ = 5.94, P = 0.115
Diaeretiella rapae abundance N_{obs} = 72	11.83 ± 1.91	7.50 ± 1.63	13.28 ± 2.02	21.17 ± 3.01	8.71 ± 1.38	14.29 ± 2.04	17.33 ± 2.49	Pollutant: $\chi^2_{3,10}$ = 20.00, **P < 0.001** Aphid: $\chi^2_{2,10}$ = 16.60, **P < 0.001** Pollutant × Aphid: $\chi^2_{6,16}$ = 5.16, P = 0.524
Abundance of other parasitoids N_{obs} = 72	88.22 ± 10.51	53.61 ± 4.92	59.56 ± 4.94	64.17 ± 8.45	57.96 ± 5.74	64.04 ± 5.77	77.17 ± 8.71	Pollutant: $\chi^2_{3,10}$ = 8.35, **P = 0.039** Aphid: $\chi^2_{2,10}$ = 9.31, **P = 0.010** Pollutant × Aphid: $\chi^2_{6,16}$ = 5.81, P = 0.445
D. rapae % of total parasitoids[a] N_{obs} = 72	11.61 ± 1.12	10.99 ± 1.75	17.46 ± 1.71	24.73 ± 1.47	12.94 ± 1.86	17.72 ± 1.71	17.93 ± 1.46	Pollutant: $\chi^2_{3,10}$ = 25.68, **P < 0.001** Aphid: $\chi^2_{2,10}$ = 16.18, **P < 0.001** Pollutant × Aphid: $\chi^2_{6,16}$ = 1.82, P = 0.935

Table S2. Relative abundances of volatile organic compounds present in Solid Phase Microextraction headspace samples of *Brassica napus* leaves, from plants exposed to ambient air (Control), diesel exhaust (Diesel) and ozone pollution (Ozone) with and without aphids present (Aphids and No aphids, respectively). N = 3 per group. Identified and tentatively identified compounds are presented with PubChem Compound ID (CID) numbers and Linear Retention Indices (LRI).

Compound identification	**CID number**	**LRI** [$]	**ID***	**Normalized peak areas**																***P*-value**
				Control + No aphids		**Control + Aphids**		**Ozone + No aphids**		**Ozone + Aphids**		**Diesel + No aphids**		**Diesel + Aphids**		**Diesel + Ozone + No aphids**		**Diesel + Ozone + Aphids**		
Methyl esters																				
Methyl acetate	5971	<650	A	2680465	ns	3618619	ns	2488018	ns	3250038	ns	2574370	ns	2464986	ns	1465211	ns	3280177	ns	0.469
Methyl propionate	11124	<650	B	6807725	ns	2880745	ns	3792547	ns	9855903	ns	6928412	ns	2356247	ns	10664845	ns	6161987	ns	0.270
Methyl hexanoate	7824	925	A	4651735	a	45931758	ab	1031120	a	168531302	ab	3201879	a	120384488	ab	2778975	a	291137635	b	0.012
Methyl (*Z*)-3-hexenoate	5362819	932	B	5505523	ns	3881537	ns	3499786	ns	8120145	ns	5161060	ns	4451698	ns	5359849	ns	9875749	ns	0.124
2-hexenoic acid methyl ester	61310	967	B	2992757	ab	1345694	a	816817	a	1166615	a	2337748	a	980430	a	1818024	a	6672197	b	0.001
Methyl octanoate	8091	1124	B	879752	a	617730	a	258554	a	1419375	a	660633	a	1067536	a	1437922	a	5579506	b	0.000
(*E*)-3-hexenyl isobutyrate	5352539	1144	A	0	ns	0	ns	0	ns	0	ns	0	ns	0	ns	207631	ns	293393	ns	0.094
Methyl nonanoate	15606	1224	B	1095996	ns	214333	ns	714351	ns	1067931	ns	652062	ns	961201	ns	481155	ns	0	ns	0.503
Methyl decanoate	8050	1324	B	806253	a	223430	a	177845	a	687557	a	240224	a	391658	a	615085	a	1038469	a	0.027
(*Z*)-3-hexenyl hexanoate	5352543	1382	A	0	a	385096	ab	0	a	0	a	0	a	359490	ab	0	a	1350403	b	0.011
Methyl dodecanoate	8139	1524	B	806278	a	2473008	a	281385	a	11415225	a	557081	a	10813071	a	722498	a	8868901	a	0.008
Methyl tetradecanoate	31284	>1500	B	472840	a	7428844	ab	280134	a	50215128	b	609502	a	21461074	ab	858763	a	19861324	ab	0.007
Alcohols																				
Propanol	1031	<650	B	1455039	ns	1040798	ns	926693	ns	992870	ns	1468841	ns	922762	ns	661157	ns	1753979	ns	0.639
1-pentanol	6276	768	A	722875	ns	0	ns	518438	ns	0	ns	971131	ns	169945	ns	258840	ns	1366039	ns	0.094
(*E*)-2-penten-1-ol	5364919	769	A	3553966	ns	659865	ns	1046175	ns	645411	ns	1563366	ns	766700	ns	1451780	ns	1646934	ns	0.429
(*Z*)-2-penten-1-ol	5364919	771	A	4222093	ns	2580591	ns	4057377	ns	1108192	ns	3170362	ns	2700248	ns	5147437	ns	9424105	ns	0.209
(*E*)-2-hexen-1-ol	5318042	853	B	0	ns	0	ns	0	ns	1519454	ns	0	ns	1464711	ns	0	ns	0	ns	0.069
(*Z*)-3-hexen-1-ol	5281167	859	B	282424102	ns	198931122	ns	208071497	ns	197718831	ns	274509395	ns	164771891	ns	228653504	ns	399423283	ns	0.239
2-hexen-1-ol	5318042	867	A	18130245	a	10626701	a	17917639	a	3951654	a	17781634	a	4746952	a	24396497	ab	67228684	b	0.002
1-hexanol	8103	869	A	43600425	ns	13075272	ns	12908507	ns	14435390	ns	21868001	ns	10899992	ns	17090560	ns	38317179	ns	0.201
1-octen-3-ol	18827	980	B	4782584	ns	6547814	ns	4843795	ns	11103838	ns	5444648	ns	4463238	ns	2068140	ns	2748408	ns	0.072

Ketones																				
3-methyl-2-butanone	11251	674	B	973495	ns	1873301	ns	703707	ns	2720059	ns	1324203	ns	1979601	ns	1375508	ns	976932	ns	0.388
3-pentanone	7288	695	B	7225975	ab	2996176	a	3325887	a	3035797	a	5658284	ab	3463528	ab	6011825	ab	10381135	b	0.009
2-heptanone	8051	891	B	0	ns	710655	ns	0	ns	1392410	ns	0	ns	1302596	ns	0	ns	510025	ns	0.087
3-octanone	246728	987	B	3048703	ns	1740145	ns	1761972	ns	3425317	ns	2662196	ns	1941437	ns	2502971	ns	3158367	ns	0.610
2-nonanone	13187	1093	B	139461	a	1256735	a	0	a	944475	a	0	a	0	a	230382	a	0	a	0.009
Aldehydes																				
(*E*)-tiglaldehyde	5321950	755	B	2100028	ab	0	a	601920	a	0	a	1245495	ab	205703	a	1624857	ab	3951388	b	0.004
(*Z*)-3-hexenal	643941	799	A	0	ns	566877	ns	602488	ns	0	ns	468309	ns	0	ns	0	ns	0	ns	0.079
(*E*)-4-oxohex-2-enal	6365145	959	B	0	a	0	a	0	a	0	a	0	a	0	a	366724	a	1759040	a	0.040
(*E,E*)-2,4-heptadienal	5283321	1014	B	289838	a	0	a	340743	a	0	a	516128	a	0	a	472887	a	2372520	b	0.003
Alkenes																				
3-methyl-1,2-butadiene	11714	759	B	2093392	ab	1183270	ab	1133180	ab	953428	a	1635177	ab	990837	a	1839283	ab	3050479	b	0.022
3-ethyl-1,5-octadiene	5353002	948	B	2216815	ns	647329	ns	831741	ns	224053	ns	750351	ns	356473	ns	3890492	ns	5541592	ns	0.108
(*E*)-2-tetradecene	5352912	1292	B	833643	ns	809982	ns	428129	ns	929175	ns	971692	ns	780927	ns	685227	ns	1049354	ns	0.591
(*E*)-β-farnesene	5281517	1462	A	0	a	0	a	0	a	2316851	a	0	a	0	a	0	a	1048928	a	0.028
Alkanes																				
1,1-dimethylcyclopropane	74202	847	B	676049	ns	0	ns	0	ns	0	ns	0	ns	0	ns	403397	ns	1743730	ns	0.097
2-ethyl-3-vinyloxirane	534767	854	B	40921089	ns	6310846	ns	17041630	ns	1672222	ns	15187877	ns	2006088	ns	22372794	ns	93476322	ns	0.064
Cyclo-alcohols																				
1-cyclohexene-1-methanol	317542	1021	B	1294308	ab	791125	ab	202423	a	3236687	ab	274425	a	1527304	ab	533178	ab	3745310	b	0.005
2-methylenecyclopentanol	550922	1061	B	1073340	a	350773	a	0	a	0	a	309680	a	649465	a	85189	a	1563017	a	0.044
Other																				
Methyl thiocyanate	11168	714	B	0	ns	0	ns	1180570	ns	0	ns	1586797	ns	0	ns	0	ns	0	ns	0.078
Dimethyl disulfide	1068	746	B	0	a	229887	a	0	a	521310	ab	0	a	0	a	0	a	1197123	b	0.001
Hexanoic acid	8892	978	B	1561109	a	6883846	ab	3322245	ab	9288844	ab	1358160	a	2510199	a	825824	a	17423583	b	0.009
(*Z*)-3-hexenyl acetate	5363388	1006	B	5657761	ns	7006784	ns	8665609	ns	1819434	ns	13229012	ns	2391923	ns	5214277	ns	14803118	ns	0.228
1-chlorooctane	8142	1065	B	421837	ns	750760	ns	387697	ns	0	ns	258777	ns	0	ns	219243	ns	901789	ns	0.265
β-ionone	638014	1502	B	2683596	ab	498871	a	703767	ab	0	a	883199	ab	365862	a	1791658	ab	3354479	b	0.003

Unknown																			
<unknown 1>	-	664	-	2873258	ns	3129185	ns	3685174	ns	965650	ns	4301699	ns	1097426	ns	4044021	ns	5975188	ns	0.666
<unknown 4>	-	1234	-	2343247	ns	907894	ns	836757	ns	223509	ns	1299205	ns	375311	ns	1614294	ns	1902493	ns	0.090
<unknown 6>	-	1327	-	254217	ns	0	ns	0	ns	0	ns	309367	ns	0	ns	255865	ns	304227	ns	0.324
<unknown 7>	-	1513	-	0	a	299943	ab	0	a	4900756	c	0	a	2991504	bc	0	a	320200	ab	<0.0001

$ = LRI on a HP-5MS. * = A, compound mass spectrum and LRI agrees with authentic compound; B, mass spectrum agrees with reference spectrum in the NIST/EPA/NIH database and LRI agree with literature sources, tentatively identified. ns = not significant according to protected ANOVA with Tukey's Honestly Significant Difference pairwise comparison test; different letters within rows signify significant differences at the $P < 0.05$ threshold.

Table S3. ANOVA with *post hoc* Tukey HSD test levels of significance for the two-way interaction between aphid treatment (Aphid) and air pollution (Pollutant). Statistically significant effects ($P < 0.05$) are highlighted in bold.

Compound	*P*-value		
	Aphid	**Pollutant**	**Aphid x Pollutant**
Methyl esters			
Methyl acetate	**0.016**	0.912	0.451
Methyl propionate	0.517	0.401	0.191
Methyl hexanoate	**0.041**	0.217	0.245
Methyl (*Z*)-3-hexenoate	0.151	0.270	0.126
2-hexanoic acid methyl ester	0.273	**0.023**	**0.004**
Methyl octanoate	0.145	0.138	0.143
(*E*)-3-hexenyl isobutyrate	0.445	0.962	0.727
Methyl nonanoate	0.064	0.185	0.058
Methyl decanoate	0.987	0.243	0.309
(*Z*)-3-hexenyl-hexanoate	0.100	0.100	0.100
Methyl dodecanoate	0.116	0.822	0.640
Methyl tetradecanoate	0.778	0.998	0.456
Alcohols			
Propanol	0.682	0.838	0.443
1-pentanol	0.814	0.753	0.302
(*E*)-2-penten-1-ol	0.286	0.587	0.617
(*Z*)-2-penten-1-ol	0.768	**0.021**	**0.026**
(*E*)-2-hexen-1-ol	0.698	0.707	0.855
(*Z*)-3-hexen-1-ol	0.855	0.311	0.126
2-hexen-1-ol	0.494	**0.008**	**0.025**
1-hexanol	0.283	0.687	0.096
1-octen-3-ol	0.272	0.494	0.328
Ketones			
3-methyl-2-butanone	**0.000**	0.096	0.270
3-pentanone	0.577	**0.011**	**0.035**
2-heptanone	0.778	0.594	0.708
3-octanone	0.389	0.900	0.199
2-nonanone	0.124	**0.033**	0.120
Aldehydes			
(*E*)-tiglaldehyde	0.690	0.250	0.180
(*Z*)-3-hexenal	**0.005**	**0.001**	**0.004**
(*E*)-4-oxohex-2-enal	0.349	0.954	0.823
(*E,E*)-2,4-heptadienal	0.235	0.408	0.155
Alkenes			
3-methyl-1,2-butadiene	0.882	**0.041**	0.115
3-ethyl-1,5-octadiene	0.693	0.082	0.972
(*E*)-2-tetradecene	0.862	0.699	0.261
(*E*)-β-farnesene	0.696	0.696	0.696

Alkanes			
1,1-dimethylcyclopropane	0.157	0.980	0.580
2-ethyl-3-vinyloxirane	0.695	0.209	0.137
Cyclo-alcohols			
1-cyclohexene-1-methanol	**0.043**	0.668	0.300
2-methylenecyclopentanol	0.936	0.788	0.265
Other compounds			
Methyl thiocyanate	0.464	0.171	0.149
Dimethyl disulfide	0.134	0.392	**0.038**
Hexanoic acid	0.481	0.260	0.377
(*Z*)-3-hexenyl acetate	0.363	0.461	0.113
1-chlorooctane	0.219	0.313	0.489
β-ionone	0.845	0.112	0.270
Unknown compounds			
<unknown 1>	0.646	**0.006**	**0.001**
<unknown 4>	0.120	0.057	0.230
<unknown 6>	0.476	0.844	0.727
<unknown 7>	0.128	0.509	0.128