EFFECTS OF AIR POLLUTION ON THE SEARCHING BEHAVIOUR OF AN INSECT PARASITOID

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Abstract. To assess the impact of air pollutants on the population dynamics of herbivores, the effects of pollutants on their natural enemies including predators, parasites, and pathogens must be evaluated in addition to direct effects and indirect effects mediated via the host plant. Insect parasitoids are an important group of such natural enemies providing many examples of partial or complete biological control of pest species. This study examined the effects of air pollutants (ozone (O_3), sulphur dioxide (SO_2), and nitrogen dioxide (NO_2)) on the searching behaviour of insect parasitoids.

A series of experiments comprising short-term, closed chamber fumigations of O_3, SO_2, and NO_2 (100 nl l^{-1}) of the braconid parasitoid (*Asobara tabida*) and aggregated distributions of its host larvae (*Drosophila subobscura*) was set up. Analysis of chamber results showed that the proportion of hosts parasitised and the searching efficiency of the parasitoids were both significantly reduced with O_3 fumigation, but not with NO_2 or SO_2 fumigations. O_3 fumigation reduced percentage parasitism by approximately 10%.

Parasitoids were able to avoid patches with no hosts, both in filtered air controls and when exposed to pollutants. However in the O_3 and NO_2 treatments they appeared less able to discriminate between different host densities, suggesting that pollutants may interfere with the olfactory responses of the parasitoids.

These results indicate the potential for air pollutants, particularly O_3, to negatively influence the searching behaviour of parasitoids, and hence reduce the efficiency of natural enemy control of many pest species.

Key words: insects, parasitoid, ozone, nitrogen dioxide, sulphur dioxide, searching behaviour, natural enemy control, Asobara tabida, Drosophila subobscura.

1. Introduction

The performance of herbivorous insects has been consistently shown to increase when their host plants are exposed to sulphur dioxide (SO_2) or nitrogen dioxide (NO_2), with responses such as increased growth rates, increased adult size, increased fecundity and reduced developmental time being demonstrated (Hughes et al., 1982; Houlden et al., 1990; Brown et al, 1993). The plant mediated responses of herbivores to ozone (O_3) are more complex, being dependent on the pattern of exposure and other environmental factors, but are also often beneficial to the insect (Brown, 1995). However, to assess the impact of pollutants on the population dynamics of herbivores it is important to include the effects of pollutants on their natural enemies including predators, parasites and pathogens. Insect parasitoids are an important group of such natural enemies providing many examples of partial or complete biological control of pest species (Debach and Rosen, 1991). This study examines the effects of ozone (O_3), sulphur dioxide (SO_2) and nitrogen dioxide (NO_2) on the searching behaviour of insect parasitoids.

Many parasitoids have been shown to use olfactory cues or kairomones from their hosts or host plants to aid host location (Vet and Dicke, 1992). Pollutants could reduce the searching efficiency of parasitoids by direct effects on the functioning of olfactory receptors or the integration of receptor responses, or by induced changes in physiology or behaviour. Pollutants could also cause indirect effects, either by chemical reaction with the kairomones, or by altering their quantitative or qualitative production as a result of

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induced changes in plant secondary chemistry.

Evidence of pollutant effects on parasitoids has been provided by a number of field studies showing reduced % parasitism along pollution gradients towards industrial sources (Templin, 1962; Wentzel and Ohnesorge, 1961, Heliövaara et al, 1982) or in urban environments (Ruszcyk, 1986). However such observations of reduced rates of parasitism are confounded by increased host performance and so do not necessarily infer any detrimental effects on the parasitoids. There are also studies which show unchanged parasitism rates in polluted industrial areas (Villemant, 1981; Heliövaara and Väisänen, 1986) and roadsides (Braun and Flückiger, 1984) despite elevated populations of hosts. There is very little experimental evidence with controlled fumigations of individual pollutants, and there are no observations relating to O_3 pollution and its possible effects on parasitoids. However it has been suggested that O_3 may modify the production of plant volatiles important as insect feeding stimulants (Bolsinger et al., 1992), so similarly the production of volatiles associated with parasitoid host location could also be affected.

The experimental system chosen for this study was the braconid parasitoid (*Asobara tabida* Nees) and its fruit fly larval host (*Drosophila subobscura* Collin). Drosophilid parasitoids are known to respond to substrate and host odours to aid host location (van Alphen and Vet, 1986) with the final location and orientation to individual potential hosts being by vibrotaxis (orientation by movement detection) (van Alphen and Drijver, 1982). *Drosophila* larvae can be reared on an apple and yeast based substrate allowing standardisation of larval age, manipulation of larval density, and the creation of uniform substrate units or patches. *Asobara* is a solitary parasitoid in which only a single adult can emerge from each host; therefore superparasitism (i.e. the subsequent parasitisation of a previously parasitised host) is in most cases disadvantageous.

2. Materials and methods

A series of 3 experiments was conducted, comprising short-term fumigations of *Asobara* females and *Drosophila* larvae with O_3, NO_2, and SO_2 (100nl l^{-1}). Aggregated distributions of hosts were used since they were more representative of natural situations and required the parasitoids to respond to host density in order to utilise the hosts efficiently.

Fumigations were conducted in a closed-chamber system (6 chambers) sited within a controlled temperature room (20°C) providing a constant temperature for the experiments. Air flow through each chamber (70x50x50 cm) was restricted to 1 air change per minute in order to minimise disruption of parasitoid behaviour. Chambers were supplied with filtered air drawn from the C.T.room, 3 chambers having pollutant added to give a concentration of 100 nl l^{-1} (i.e. 3 chambers x FA, 3 x pollutant). Pairs of chambers were lit by a metal halide lamp (Philips HP1-T, 400w)

Preparation of insects:

Asobara and *Drosophila* were cultured at 18°C, 70% R.H., with fluorescent lighting giving a 16 hour light/ 8 hour dark cycle, on an apple, yeast and agar based medium.

Asobara females used in the experiments were 7-11 days old, and were "experienced" by exposure to hosts and substrate for a period of 4 hours (11:00-15:00) on the day prior to the experiment. During the subsequent hour, females visiting the substrate were caught and placed in gauze-topped 3"x1" glass tubes with damp cotton wool until the start of the experiment. It should be noted that this procedure does not guarantee previous egg laying experience.

Drosophila larvae, which averaged 34 hours old (second instar) at the start of the experiment, were obtained by allowing adult flies to lay eggs for a period of 7 hours (10:00-17:00) 3 days prior to the experiment. 24 hours before the experiment larvae averaging 10 hours old were transferred to experimental patches to allow accumulation of host kairomones.

A 'patch' consisted of a 5cm diameter petri dish filled with agar to a depth of 5mm, into which a central well (1cm diameter) was cut and filled with apple medium topped with a layer of active yeast.

Experimental protocol:

In each chamber 200 standardised host larvae were distributed between 10 patches (2x0, 2x10, 2x20, 2x30, 2x40) giving a total of 1200 larvae for each experiment. The patches were arranged in 2 rows of 5 at right angles to the air flow and 30cm upwind of the point of parasitoid release.

Experimental patches were pre-exposed to the pollution treatments for 1 hour (10:00-11:00) and then 20 "experienced" *Asobara* females were released and allowed to search for and parasitise hosts for a period of 5 hours (11:00-16:00). During this period, the number of parasitoids on each patch was noted every 15 minutes. At the end of the experiment the patches were removed, covered and stored in a refrigerator (5°C). After a period of 3 days to allow the parasitoid eggs to swell, the host larvae were dissected to determine the rates of parasitism and superparasitism.

The measurement of patch time allowed the calculation of searching efficiency (a measure of the proportion of hosts attacked per unit of search time). Chamber searching efficiency (s) was calculated by the equation:-

$$s = \frac{1}{Pt} \ln\left(\frac{Nt}{Nt-Na}\right)$$

where Pt = total patch time for all parasitoids (hrs), Nt = total number of hosts, Na = number of hosts parasitised.

Results

ANOVA analysis of chamber results (Table I) showed that the proportion of hosts parasitised (arcsine transformed) was significantly reduced by O_3 fumigation (P=0.030, d.f.1,4) but not by NO_2 or SO_2 fumigations (P=0.582, d.f.1,4 and P=0.813, d.f.1,4 respectively). The searching efficiency of the parasitoids was also significantly reduced in

O_3 (P=0.018, d.f.1,4) but not by NO_2 or SO_2 fumigations (P=0.918, d.f.1,4 and P=0.738, d.f.1,4). Since there were no significant differences in total chamber patch times between FA (filtered air) and pollutant treatments for any of the experiments, the above differences in the proportion parasitised were not due to differences in searching intensity. There were no significant differences in the degree of superparasitism between FA and pollutant treatments. There was, however considerable variability in measured parameters between experiments. These differences must be attributed to the fact that experiments could not be carried out simultaneously and hence there was potential for changes in the fitness of cultured insects, (and changes in environmental conditions which could not be controlled (e.g. air pressure)).

TABLE I

Treatment means for chamber estimates of proportion parasitised, searching efficiency, proportion superparasitised and total patch time, for O_3, NO_2, and SO_2 experiments with aggregated distributions of hosts.

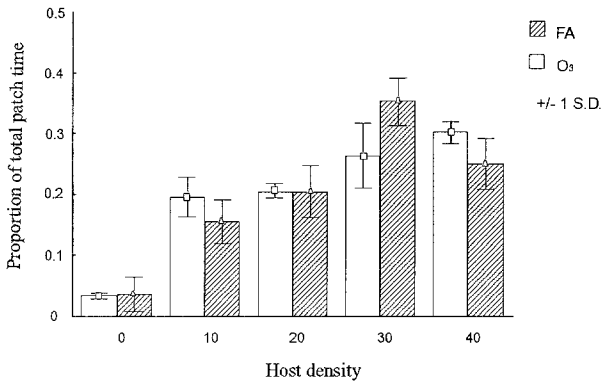
		O_3	NO_2	SO_2
Proportion parasitised	Pollutant	0.697*	0.504	0.824
	FA	0.789	0.461	0.800
Searching efficiency	Pollutant	0.0265 *	0.0335	0.0424
	FA	0.0415	0.03	0.0468
Proportion superparasitised	Pollutant	0.230	0.138	0.174
	FA	0.242	0.031	0.137
Total Patch time (hrs)	Pollutant	46.08	23.92	42.75
	FA	38.25	19.00	36.92

* P<0.05 (Statistical analyses were computed on arcsine transformed proportions)
FA = filtered air

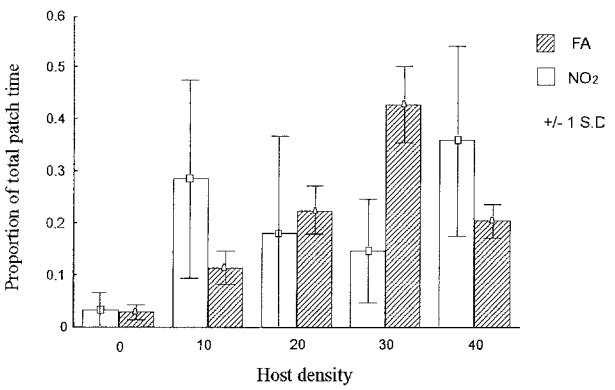
To establish whether the above differences in searching efficiency were associated with changes in the distribution of search time between patches of differing host density, the proportions of total chamber patch time were plotted against host density (Figure 1). In all experimental treatments, the parasitoids spent little time on patches with no hosts. Where hosts were present, the patch times in the FA treatments of the O_3 and NO_2 experiments showed a domed relationship, indicating a preference for a host density of 30. This may result from a trade off between the greater attractiveness of patches with high host densities and the need to identify individual hosts by vibrotaxis prior to oviposition. However, this domed pattern was less apparent for the FA treatment in the SO_2 experiment. In the O_3 treatment, there was a trend for the proportions of patch times associated with the range of host densities (other than 0) to be more similar. This observation may indicate a reduced ability of the parasitoid to discriminate between host

Fig. 1. Proportion of total chamber patch time spent on patches of different host density for a) O_3 experiment b) NO_2 experiment c) SO_2 experiment.

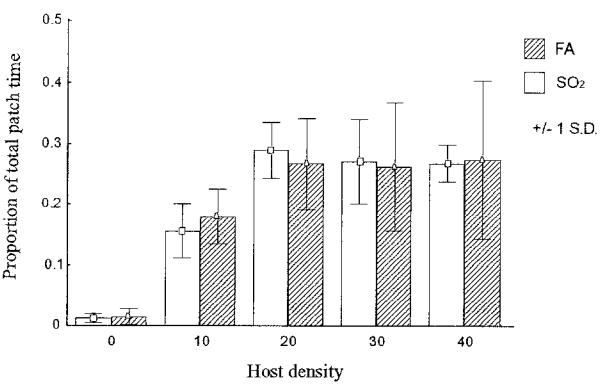
a)



b)



c)



densities in the presence of O_3 ; although this interpretation cannot be formerly supported by statistical analysis of the data presented in figure 1, it is consistent with the results of some recent olfactometry experiments (Gate et al, in prep). In the NO_2 treatment the increase in the variability of patch times associated with different host densities may also suggest a loss of discrimination

Discussion

These results suggest that O_3 can have a significant negative effect on the searching efficiency of an insect parasitoid. In these experiments, the behaviour of the parasitoid is determined by the summation of responses to olfactory cues, patch experience including oviposition, and possibly other stimuli, e.g. visual. The mechanism for the reduction in searching efficiency by O_3 is unknown, but may prove to be a direct or indirect effect on the olfactory responses of the parasitoids, resulting in a reduced ability to discriminate host density and hence assess the availability of potential hosts.

These results indicate the potential for important reductions in the levels of parasitism in the field by exposure to realistic levels of O_3. The associated effects on the dynamics of host-parasitoid interactions could have significant implications for natural enemy control of pest species, particularly given the current and predicted importance of O_3 in rural areas.

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