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ELEVATED ATMOSPHERIC CO_2 IMPAIRS APHID ESCAPE RESPONSES TO PREDATORS AND CONSPECIFIC ALARM SIGNALS

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ABSTRACT – Research into the impacts of atmospheric change on predator-prey interactions

has mainly focused on density dependent responses and trophic linkages. As yet, the chemical

ecology underpinning predator-prey interactions has received little attention in environmental

change research. Group living animals have evolved behavioural mechanisms to escape

predation, including chemical alarm signalling. Chemical alarm signalling between conspecific

prey could be susceptible to environmental change if the physiology and behaviour of these

organisms are affected by changes in dietary quality resulting from environmental change.

Using *Rubus idaeus* plants, we show that elevated concentrations of atmospheric CO_2 (eCO_2)

severely impaired escape responses of the aphid *Amphorophora idaei* to predation by ladybird

larvae (*Harmonia axyridis*). Escape responses to ladybirds was reduced by >50% after aphids

had been reared on plants grown under eCO_2. This behavioural response was rapidly induced,

occurring within 24h of being transferred to plants grown at eCO_2 and, once induced, persisted

even after aphids were transferred to plants grown at ambient CO_2. Escape responses were

impaired due to reduced sensitivity to aphid alarm pheromone, (E)-β-farnesene, via an

undefined plant-mediated mechanism. Aphid abundance often increases under eCO_2, however,

reduced efficacy of conspecific signalling may increase aphid vulnerability to predation,

highlighting the need to study the chemical ecology of predator-prey interactions under

environmental change.

INTRODUCTION

Animals that live in groups have evolved numerous behavioural mechanisms for escaping

predation, ranging from aggregation for protection (e.g. the 'selfish herd' hypothesis (Hamilton,

1971)) to more 'altruistic' alarm signalling to conspecifics (Zuberbuehler, 2009). The latter has

evolved in many different invertebrate and vertebrate taxa, employing a variety of acoustic,

visual and chemical cues to warn conspecifics of a predation risk (Ruxton et al., 2004). Alarm

signalling between individuals via pheromones is particularly prevalent amongst insects (Blum,

1969).

Predicting how ecosystems will respond to climate change requires greater understanding of

the impacts on community processes, like herbivore prey-predator interactions (Jamieson et al.,

2012; Facey et al., 2014). Atmospheric carbon dioxide (CO_2) has reached 400ppm and is

predicted to increase to 421-936 ppm by 2100 (IPCC, 2013). This may have profound

consequences for predator-prey interactions (Robinson et al., 2012; Facey et al., 2014).

Experimental studies have hitherto focussed on density dependent population responses and

trophic interactions (e.g. Chen et al., 2005; Hentley et al., 2014). Elevated atmospheric CO_2

(eCO_2) could, however, also alter behavioural aspects of predator-prey interactions, such as

how prey respond to conspecific chemical signals (e.g. pheromones) to avoid predators. Such

communication could be indirectly altered by eCO_2 affecting the physiology of herbivorous prey

via changes to their plant resources (Zavala et al., 2013).

Aphids emit the alarm pheromone (E)-β-farnesene (EβF) to alert conspecifics of imminent

attack (Bowers et al., 1972). This pheromone facilitates a variety of evasive tactics used by

aphids, including cessation of feeding, walking from the signal source or dropping from the

plant (Pickett et al., 1992; Vandermoten et al., 2012). Reduced evasion responses of aphids

when physically disturbed by the experimenter have been shown under eCO_2 (e.g. squeezing

with forceps, Awmack et al., 1997 or prodding the thorax, Mondor et al., 2004). While these

studies did not explicitly link this to EβF, a subsequent study that subjected wheat aphids

54   (*Sitobium avenae*) to periodic release of *E*βF showed population declines at ambient

55   atmospheric CO_2 (aCO_2), but no change at eCO_2 (Sun et al., 2010). They suggested that this was

56   due to aphids becoming insensitive to *E*βF under eCO_2 (Sun et al., 2010). To date, however, the

57   behavioural response of aphids to both *E*βF and predators when feeding on intact plants under

58   eCO_2 has not been investigated; previous studies have used excised leaves (Awmack et al., 1997;

59   Mondor et al., 2004) or focused solely on the response to *E*βF (Sun et al., 2010).

60   We used the large raspberry aphid (*Amphorophora idaei*) feeding on red raspberry (*Rubus*

61   *idaeus*) as a model system. Densities of *A. idaei* increase in response to eCO_2 on some *R. idaeus*

62   genoptypes (Martin and Johnson, 2011), but predation at eCO_2 can negate this increase (Hentley

63   et al., 2014) potentially due to increased susceptibility of aphid prey. We therefore hypothesised

64   that aphids have diminished escape responses to predator attack under eCO_2, compared to

65   aCO_2, which will be underpinned by reduced sensitivity to *E*βF.

66

67   **MATERIALS AND METHODS**

68   *Insects, plants and environmental chamber conditions*

69   The large raspberry aphid (*Amphorophora idaei*) population was initiated from field aphids and

70   then maintained in the laboratory for multiple generations. Cultures were maintained at 18 ±

71   1°C with a 16 h photoperiod. The predatory harlequin ladybird (*Harmonia axyridis*) cultures

72   were first collected from lime trees (*Tilia* spp.) in Oxfordshire, UK. The population was then

73   maintained in the same environment as the aphid cultures (full details of collection and

74   maintenance given in Hentley et al., 2014). Prior to the experiment, insect cultures were reared

75   for at least four generations at aCO_2 (390 ± 50 μmol/mol) and eCO_2 (650 ± 50 μmol/mol)

76   conditions in four environmentally controlled chambers (two per CO_2 regime, all at 20 ± 4°C,

77   50-70% relative humidity and 16h photoperiod). These chambers (full details in Hentley et al.,

78   2014) were also used for growing plants. Forty *Rubus idaeus* (cv. Glen Clova) were grown from

79   rootstock; at approximately 6 weeks old (1 cm height) plants were transferred to 3L pots, and

80   randomly assigned to the four chambers. To minimize chamber effects, plants were moved

81   between corresponding treatment chambers once a week for five weeks prior to assays (*sensu*

82   Bezemer et al., 1998; Johnson and McNicol, 2010).

83   *Behavioural assays*

84   Behavioural assays used intact plants exposed to the different CO_2 treatments for five weeks.

85   Fully-crossed combinations (Fig. 1) of aphids and plants maintained under the two CO_2 regimes

86   were tested in response to stimuli from the presence of (i) ladybird (*Harmonia axyridis*)

87   predators, (ii) 200 ng of *E*βF in 5µl hexane solvent and (iii) control of 5µl hexane (both

88   analytical standard, Sigma-Aldrich, UK). For each assay, a leaf was randomly selected and 50

89   mixed-age, apterous aphids were confined to the underside of the leaf with a 20mm diameter

90   clip-cage for 24h. The cage was then removed and the number of aphids feeding counted, aphids

91   not feeding were removed prior to the onset of the assay. Assays proceeded as follows: (i) a

92   single fourth instar ladybird larva, starved for 24h, was introduced onto the leaf c. 2 cm from the

93   aphids and observed for 5 mins. The ladybird was replaced if it did not attack aphids after 5

94   mins. For the assays with (ii) *E*βF and (iii) the control hexane only, 5µl of the solution was

95   placed on the underside of the leaf surface, c. 2 cm from the aphid colony and, again, observed

96   for 5 mins for escape responses. For each assay, the proportion of aphids that stopped feeding

97   and showed predator avoidance behaviour (e.g. walking away, dropping) in response to stimuli

98   (i-iii) was quantified. Each assay was repeated 10 times.

99   *Statistical analysis*

100   The proportion of aphids responding to stimuli was modelled with generalised linear mixed

101   effect models fitting a binomial error distribution (GLIMMIX, SAS Institute). The random effect

102   was environmental chamber nested within date of bioassay. Mean temperature was fitted as a

103   fixed effect to account for spatial (between chambers) and temporal (between days) variation

104   during the experiment (Table 1-f). A minimum adequate model was obtained with forward

105   stepwise selection of fixed effects. F-ratio and p-values presented are adjusted for other

106   significant fitted terms (SAS type III), two-way interactions are only reported where $p < 0.05$.

107   Degrees of freedom were estimated with Sattherthwaites' approximation.

108

109   **RESULTS**

110   Compared to aphids and plants under aCO_2 (Fig. 2-I), aphid escape responses to ladybirds were

111   significantly reduced when they had been reared under eCO_2 (Fig. 2-II & IV, Table 1-b & c), even

112   when transferred to plants grown under aCO_2 (Fig. 2-II). Moreover, aphids reared under aCO_2,

113   but subsequently transferred to plants grown under eCO_2 for <24h, showed the same reduction

114   in escape responses (Fig. 2-III, Table 1-d) as seen in aphids reared under eCO_2 (Fig. 2-II & IV).

115   Over twice as many aphids initiated escape responses when they fed under aCO_2 conditions

116   (Fig. 2a-I). Aphids exposed to their alarm pheromone $E\beta F$, exhibited a reduction in escape

117   behaviour, similar to when being attacked by the predator (Fig. 3a, Table 1-a & e). Aphids

118   showed no behavioural response to the control solvent hexane (Fig. 3b).

119

120   **DISCUSSION**

121   Aphid escape responses to predator presence or exposure to the aphid alarm pheromone $E\beta F$

122   were rapidly (within 24h) and significantly (>50%) impaired when feeding on a plant reared in

123   eCO_2, which persisted individual aphids reared in eCO_2 fed on plants grown at aCO_2.

124   Aphid behaviour often differs when feeding on experimentally excised leaves compared with

125   intact plants, because phloem hydraulics and chemistry are altered by excision (Van Emden and

126   Bashford, 1976; Douglas, 1993). This study, using whole plants, an insect predator, and a

127   controlled dose of alarm pheromone $E\beta F$ - rather than a mechanical disturbance of aphids to

128   stimulate its release (as performed by Awmack et al., 1997; Mondor et al., 2004), - in a

129   reciprocal experimental design permitted us to conduct a more realistic test of eCO_2 impacts on

130   this tri-trophic interaction.

131   Impairment of aphid escape responses by eCO_2 is likely to have been mediated via changes in

132   the plant quality which inturn impacted aphids, this is because the impairment always occurred

133   when aphids had been, or were, feeding on plants grown at eCO_2. At least two possible

134   mechanisms may underpin reduced escape responses. Firstly, aphids may 'hold their ground'

135   rather than escape if feeding on a good quality host. In particular, *Amphorophora idaei*

136   performance is known to be enhanced by eCO_2 induced changes in plant suitability (Martin and

137   Johnson, 2011) and these species is known to continue feeding, even under threat, if the host

138   plant is of good quality (Mitchell et al., 2010). Alternatively, if eCO_2 reduces host plant quality,

139   then aphids can engage in more intense and sustained feeding activity (Sun and Ge, 2011; Guo et

140   al., 2013a; Guo et al., 2013b), akin to the compensatory feeding responses of chewing insect

141   herbivores (e.g. Docherty et al., 1996; Johnson et al., 2014a). Ingesting more phloem will

142   inevitably be energetically costly and may require deeper penetration of plant tissues, and

143   possibly the manipulation of the plants metabolism (Guo et al., 2013b). This investment may

144   result in aphids being either physiologically less able, or behaviourally less inclined, to abandon

145   a host plant. Either way, it is feasible that enhancement or deterioration in the nutritional

146   quality of plants grown under eCO_2 is enough to make aphids to continue feeding, even under

147   risk of predation.

148   Multi-trophic interactions must be accounted for to accurately predict the net effect of eCO_2 on

149   plants (Harrington et al., 1999; Robinson et al., 2012; Facey et al., 2014). Crops may become

150   more susceptible to insect pests, including aphids (Martin and Johnson, 2011; Johnson et al.,

151   2014b), in an eCO_2 environment. Top-down control of aphids by natural enemies is a major

152   factor in regulation of aphid populations (Dixon, 2000). Here we demonstrate that atmospheric

153   change modified the behavioural response of a herbivore when a conspecific is being attacked

154   by a natural enemy, which could increase the net impact of the predator. The lack of behavioural

155   response from conspecifics will increase prey availability for the natural enemy, but also limit

156   beneficial, non-consumptive effects, such as the herbivore dropping from the plant. Such

157 modified predator-prey interactions clearly have implications for crop security in a changing

158 world.

159 Together with previous evidence using other aphid species (Awmack et al., 1997; Mondor et al.,

160 2004; Sun et al., 2010), it seems that the interference effect of eCO_2 on aphid escape responses

161 is a general, and possibly widespread, reaction. However, further work is needed to establish

162 the mechanistic basis of how atmospheric change mediates the chemical ecology of predator-

163 prey interactions. Moreover, whether aphid populations will adapt to such atmospheric changes

164 over time to re-establish responsiveness to predator-related conspecific alarm signals remains

165 an unanswered question.

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Figure Legends

Figure 1 Schematic of behavioural assays using reciprocal treatments of aphids and plants

maintained under aCO_2 and eCO_2

Figure 2 Mean (± S.E.) percentage of aphids reared at aCO_2 (grey bars) or eCO_2 (white bars)

showing escape responses to ladybird larva (*Harmonia axyridis*). Roman numerals refer to

treatment combinations (see Fig. 1)

Figure 3 Mean (± S.E.) percentage of aphids reared at aCO_2 (grey bars) or eCO_2 (white bars)

showing escape responses to a) *E*βF mixed with hexane solvent, and b) hexane alone. Roman

numerals refer to treatment combinations (see Fig. 1).

280   Table 1. Final GLMM results summary for aphid escape response to predator/ $E\beta F$ stimulus and

281   CO_2 regimes.

Response variable	Explanatory variables	Estimate	$F_{(ndf,ddf)}$	p
% aphids per plant showing escape behaviours	a) Stimulus *Hexane/Ladybird/ EβF*	*2.17 / 3.54 / 2.68*	$6.46_{(2,6)}$	0.0031
	b) Plant growing environment *aCO₂/eCO₂*	*2.74/2.68*	$15.28_{(1,6)}$	0.0089
	c) Aphid rearing environment *aCO₂/eCO₂*	*3.29/2.68*	$21.43_{(1,145)}$	<0.0001
Random effect estimate = 0.07 ± 0.07	d) Plant growing * aphid rearing environment *aCO₂ plant * aCO₂ aphid / eCO₂ plant * aCO₂ aphid* *aCO₂ plant * eCO₂ aphid / eCO₂ plant * eCO₂ aphid*	*4.04 / 2.68* *2.68 / 2.68*	$38.40_{(1,38)}$	<0.0001
	e) Aphid rearing environment * stimulus aCO₂ * Hexane / aCO₂ * Ladybird / aCO₂ * *E*βF eCO₂ * Hexane / eCO₂ * Ladybird / eCO₂ * *E*βF	*1.30 / 2.16 / 2.68* *2.68 / 2.68 / 2.68*	$6.02_{(2,145)}$	0.0031
	f) Mean temperature	-0.275	$5.41_{(1,5)}$	0.0544

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