

# Behavioural response of wheat bulb fly (*Delia coarctata*, Diptera: Anthomyiidae) larvae to the primary plant metabolite carbon dioxide

C.D. Rogers<sup>1,2\*</sup>, K.A. Evans<sup>2</sup>, J. Parker<sup>2</sup> and V.A. Pappa<sup>3</sup>

<sup>1</sup>Institute of Evolutionary Biology, University of Edinburgh Ashworth Laboratories, Edinburgh EH9 3JT, UK; <sup>2</sup>SRUC, West Mains Road, Edinburgh EH9 3JG, UK; <sup>3</sup>Züricher Hochschule für Angewandte Wissenschaften, Institut Umwelt und Natürliche Ressourcen, Grüental 8820, Wädenswil, Switzerland

## Abstract

Wheat bulb fly (WBF) larvae use chemotaxis to orientate towards host-plant root exudates. This study aimed to investigate the role of the primary plant metabolite carbon dioxide (CO<sub>2</sub>) in host-plant location by WBF. Arena based behavioural experiments were used to identify whether CO<sub>2</sub> induced chemotaxis (directional movement in response to a chemical stimulus) or kinesis (non-directional movement in response to a stimulus) from WBF larvae. No chemotactic response was observed when larvae were presented to a point source of CO<sub>2</sub>. However, elevated levels of CO<sub>2</sub> induced kinesis, with both track length and tortuosity (number of twists and turns in the movement path) increasing at elevated CO<sub>2</sub> levels of 1000–2000 ppm, demonstrating increased searching behaviour. Soil emission of CO<sub>2</sub> was quantified to compare soil levels with those identified as eliciting behavioural effects on the larvae. Samples removed from soil gave a mean CO<sub>2</sub> concentration of 557 (±46) ppm, which is lower than the lowest concentration of CO<sub>2</sub> found to induce a behavioural response and higher than the lowest CO<sub>2</sub> concentration tested, which was found not to alter behaviour. It is proposed that increased CO<sub>2</sub> concentrations in the soil act as a behavioural trigger, inducing intensive searching of an area by WBF larvae. This increases the likelihood of finding more host-specific identifiers, such as secondary metabolites when near a potential host-plant.

**Keywords:** chemical ecology, CO<sub>2</sub>, host location, pest, phytophagous, semiochemical

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## Introduction

Primary and secondary metabolites exuded by plants are utilized by soil-dwelling phytophagous insect larvae to locate

and identify host-plant species (Johnson & Gregory, 2006; Johnson & Nielsen, 2012). Secondary plant metabolite attractants generally include species, genus and family-specific chemicals that may allow both monophagous and oligophagous larvae to identify their specific host-plants (Johnson & Gregory, 2006). Primary plant metabolites are metabolites directly involved in essential plant processes and are thus ubiquitous to all plants (Taiz & Zeiger, 2010). Primary plant metabolites such as carbon dioxide (CO<sub>2</sub>) and carbohydrates have been shown to have an important role in

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\*Author for correspondence  
Fax: +44 (0)131 535 4144  
Phone: +44 (0)7725649393  
E-mail: craigrogers22@hotmail.com

host-plant location in a number of insect species (Thorpe *et al.*, 1947; Strnad *et al.*, 1986; Honda & Ishikawa, 1987; Galbreath, 1988; Hopkins *et al.*, 1993; Huang & Mack, 2001; Johnson *et al.*, 2006), including a number important to agriculture, such as *Diabrotica* spp. (corn rootworm, Strnad *et al.*, 1986; Jewett & Bjostad, 1996), *Agriotes* spp. (wireworm, Klingler, 1957), *Delia radicum* (cabbage root fly, Jones & Coaker, 1978) and *Psilia rosae* (carrot root fly, Jones & Coaker, 1977).

Despite being found to induce behavioural changes in a number of soil-dwelling phytophagous insects, CO<sub>2</sub> gradients as a means for host-plant location is difficult to conceive, due to: (1) The universal production of CO<sub>2</sub> by plants prevents distinction from host and non-host species. (2) There are a number of potential non-host point sources of CO<sub>2</sub> in the soil such as microbial interactions (Hanson *et al.*, 2000). (3) Interaction between atmospheric and soil CO<sub>2</sub> concentrations disrupt CO<sub>2</sub> gradients from plants (Gollany *et al.*, 1993; Sheppard & Lloyd, 2002).

In some systems, CO<sub>2</sub> has been proposed as being attractive to host-plant seeking insects, with larvae orientating towards a source (Jones & Coaker, 1977; Jewett & Bjostad, 1996). However, for species such as *Sitona lepidus* (clover weevil) no attraction has been demonstrated, but behavioural changes are observed when larvae are exposed to concentrations of CO<sub>2</sub> comparable with that found around the root system of its host-plant (Johnson *et al.*, 2006). It has been suggested that CO<sub>2</sub> does not always play a direct role in identifying suitable hosts in the subterranean environment, but plays a role in initiating searching behaviour (Johnson *et al.*, 2006; Hiltbold & Turlings, 2012; Johnson & Nielsen, 2012; Turlings *et al.*, 2012). Understanding the mechanisms underpinning host-plant location in pest species can be key to the development of novel pest control strategies.

*Delia coarctata* (Fallén, Diptera: Anthomyiidae, wheat bulb fly (WBF)) oviposits in bare and exposed soil such as that colonized by its suspected natural host couch grass (*Elytrigia repens*) (Marriott & Evans, 2003). In agricultural environments, WBF display a preference for fallow ground or fields containing potato, sugar beet and open canopy vegetables (Petherbridge, 1921; Morris, 1925). Often, crop rotations result in wheat (*Triticum aestivum* L.) being sown after these crops, allowing WBF larvae to infest vulnerable wheat seedlings when the eggs hatch in January and February (Way, 1959). Since the eggs are not laid in association with a host-plant (Petherbridge, 1921), hatching larvae are required to seek out host-plants quickly before energy reserves run out.

Wheat bulb larvae have been shown to display positive taxis towards exudates of host-plants (Marriott & Evans, 2003; Rogers & Evans, 2013). However, no research has looked at the role of primary plant metabolites such as CO<sub>2</sub> in host-plant location by WBF larvae. The studies on WBF larval behavioural responses to exudates and chemical constituents have previously concentrated on analysing the final resting position of the larvae or observing movement to a source (Stokes, 1956; Scott, 1974; Greenway *et al.*, 1976; Marriott & Evans, 2003; Rogers & Evans, 2013), and these methodologies simply allow the description of attraction or repellence. However, valuable information contained in the movement path of the larvae is not recorded.

Analysis of the tracks left by insects exposed to semi-chemicals can provide evidence of the role these chemicals play in the insects' ecology (Nams, 2005). The analysis of the fractal dimension of track shape is commonly used to interpret tracks left by animals (Doerr & Doerr, 2004). The fractal

dimension of a track shape allows the inference of the behaviour of the animal based on its movements (Nams, 1996). The lower the fractal dimension value attributed to a track in a homogeneous environment the more direct the animal is moving from one point to another, suggesting movement between resource patches (Wiens *et al.*, 1995). However, a higher fractal dimension associated with a more tortuous track can indicate searching behaviour within a resource, with the area being covered more intensively.

Understanding the chemical ecology of WBF allows the potential to develop new control measures that can lessen the current dependence on the use of organophosphate insecticides in control of this species. The aim of this study was to observe the behavioural response of WBF larvae in relation to CO<sub>2</sub>. Bioassays were conducted to identify whether larvae orientated towards a point source of CO<sub>2</sub> (chemotaxis) (Adler, 1966; Eisenbach, 2004) or alter their searching behaviour (turning pattern, indicated by a change in fractal dimension) in the presence of differing concentrations of CO<sub>2</sub> without moving towards the source (kinesis) (Gillott, 2005). Finally, the concentration of soil CO<sub>2</sub> in relation to host-plant seedlings was determined to relate the bioassay response to CO<sub>2</sub> to the concentration typically encountered by WBF larvae in soil.

## Materials and methods

### Biological material

Field collected WBF eggs were surface sterilized with 0.3% NaOCl before being rinsed in sterile distilled water (Bellows & Fisher, 1999; Marriott, 2001). The eggs were stored between sections of nylon, positioned on top of moist vermiculite in a sealed 90 mm Petri dish. Eggs were stored at 5°C for at least 2 months; methyl paraben was added to the eggs to control fungal infections as required. Eggs were stored at 10°C to initiate hatching after the two month diapause had elapsed, and larvae were used within 24 h of hatching, with individual larvae used once only.

Wheat seeds (cv. Aristos) were surface-sterilized in 3% NaOCl solution for 2 min, before being thoroughly rinsed in sterile distilled water. The seeds were then germinated in 90 mm Petri dishes containing 0.3% technical grade agar under sterile conditions. The seeds were germinated in the dark at 13°C for 7 days (until coleoptiles were at least 80 mm in length), before being used in the experiment.

### CO<sub>2</sub> as an attractant

Larval tracks were observed in circular arenas consisting of a 90 mm diameter Petri dish with an inlet and an outlet valve at opposite sides of the arena (fig. 1). The base of the arena was lined with a 5-mm thick film of agar (Oxoid, Technical grade 0.3%), while an opening on the lid allowed access for placement of test larvae, without disrupting the atmosphere of the arena. A cap coated with petroleum jelly was used to seal the opening during the experiment.

A constant stream of CO<sub>2</sub> (>99%) or CO<sub>2</sub>-free air (BOC, UK) (absorbed using sodium hydroxide (Tepe & Dodge, 1943)) was pumped into the arena at 8 ml h<sup>-1</sup> (the most attractive rate found by Jones & Coaker, 1977; Johnson *et al.*, 2006), while air was drawn out of the arena directly opposite the inlet at the same rate. The CO<sub>2</sub> gradient was allowed to build for 1 h before a larva was introduced into the centre of

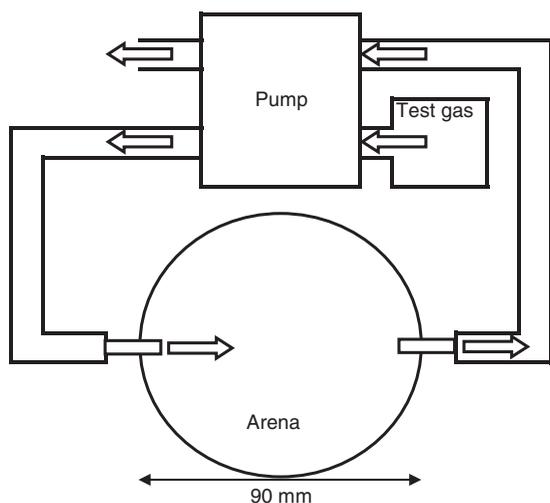


Fig. 1. Diagram of area bioassay used to record the behavioural response of WBF larvae to different concentrations of CO<sub>2</sub>: 0, 380, 1000, 1500, 2000 and 2500 ppm. Direction of system airflow indicated by: ⇨.

the arena, presenting it towards the airflow. Arenas were stored in the dark at 18 ± 2°C for 20 min after which the larval track visible on the agar film was traced on to the base of the arena, and the number of larvae that moved to within 20 mm of the inlet were recorded. The number of individuals tested for each treatment was 20, with each larva being used once only.

#### CO<sub>2</sub> as a behavioural modifier

Larvae were released in the centre of Petri dish arenas (the same as described in the previous experiment), which were then sealed and saturated with specific concentrations of either CO<sub>2</sub>: 380 (ambient), 1000, 1500, 2000 and 2500 ppm, or a control consisting of CO<sub>2</sub>-free air. CO<sub>2</sub> concentrations were verified using a CO<sub>2</sub> meter (Gascard II, Edinburgh Instruments Ltd.). Larval start position was recorded and arenas were stored in the dark at 18 ± 2°C for 20 min, after which the resulting larval track visible on the agar film was traced on to the base of the arena. Tracks were digitized and *x*-*y* co-ordinates for the tracks were captured. The number of individuals tested for each treatment was 20, each larva being used once only.

#### Behavioural statistical analysis

Larval attraction to CO<sub>2</sub> was assessed by analysing the movement of larvae to within 20 mm of the point source using Fisher's exact *P* test (Johnson *et al.*, 2006). Larval behavioural response to constant CO<sub>2</sub> atmospheres were assessed by analysing larval tracks using Fractal (v. 5.18.0) software (Nams, 1996). Fractal dimension is commonly used to interpret tracks left by animals (Doerr & Doerr, 2004). A fractal is a geometric shape that has 'self similarity', meaning when divided into sections, each section looks like a smaller sized version of the original shape. The fractal dimension is a statistical quantity that indicates how completely the fractal fills space as the scale is reduced (Mandelbrot, 1967;

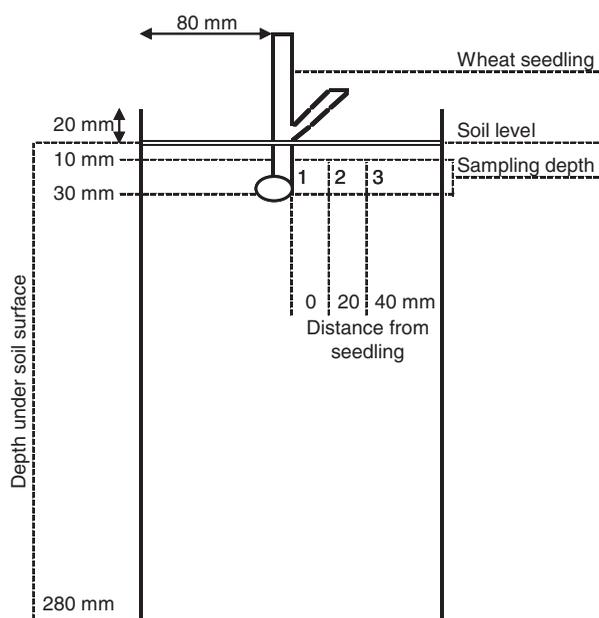


Fig. 2. Diagram of the setup and sampling positions for CO<sub>2</sub> analysis of wheat seedlings grown within microcosms.

Milne, 1991). When analysing an animal track its fractal dimension is a measure of track tortuosity (how tortuous the track is i.e., how much it twists and turns) (Milne, 1997). The fractal dimension of a track lies between the values 1.0 and 2.0, with a value of 1.0 representing a track that is a straight line and 2.0 being a tortuous track that completely covers a plane (Nams, 1996).

Tracks that are produced in heterogeneous environments will show direct lines from resource to resource with tortuous sections within each resource patch, and not display self-similarity (Wiens *et al.*, 1995). The assumption that the environment in the arena was homogeneous was tested by examination of the correlation between track length (number of fractal dividers) and the fractal divider size. If the relationship between the track length and the divider size conforms to a power-law relationship, then it is seen to be scale-invariant, displaying self-symmetry (Mandelbrot, 1983), therefore laid by larvae experiencing a homogeneous environment (Johnson *et al.*, 2006).

Fractal dimension (FD, a dimensionless quantity) and length (mm) were calculated for each track using Fractal (v. 5.18.0) software (Nams, 1996) and transformed by log<sub>10</sub> and square root respectively to normalize the data (tested by an Anderson-Darling test), as a normal distribution is a requirement for analysis by one-way analysis of variance (ANOVA) (Fowler *et al.*, 1998). Fractal dimension values and track length were analysed with ANOVA, with *post-hoc* comparisons by Tukey's test (Johnson *et al.*, 2006), and finally 95% confidence intervals (CI) were calculated. All statistics were conducted using Minitab statistical software version 15 (Minitab Inc.). The number of individuals tested for each treatment was 20.

#### CO<sub>2</sub> concentration in soil, sample collection

Germinated wheat seedlings (cv. Aristos) were planted at the depth of 30 mm in top soil (packed to a bulk density of

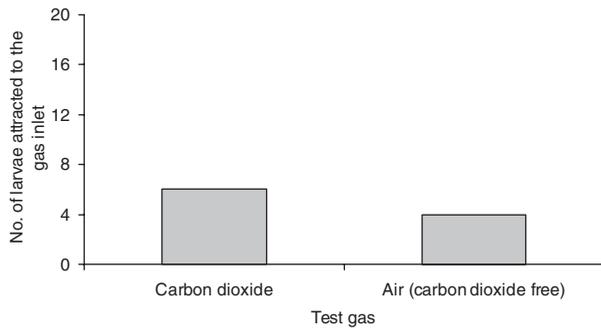


Fig. 3. Number of WBF larvae that moved to within 20 mm of the gas inlet in arenas, introducing either CO<sub>2</sub> or CO<sub>2</sub>-free air (the control), at a rate of 8 ml h<sup>-1</sup>, *N* = 20.

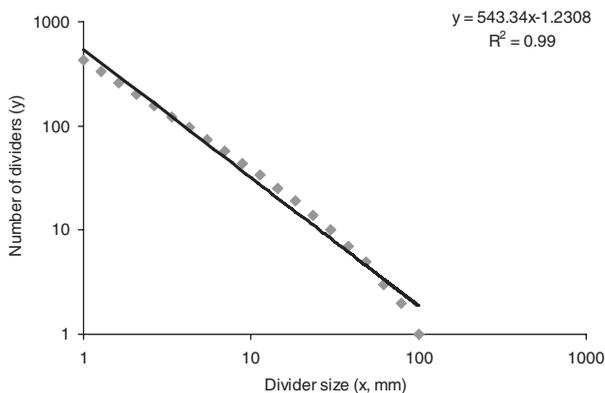


Fig. 4. Power-law relationship between number of dividers (track length) and divider size (mm).  $R^2$  = coefficient of determination.

1.2 g cm<sup>-3</sup>) in the centre of cylindrical containers measuring 160 mm in diameter and 130 cm in height (fig. 2). The seedlings were grown in these microcosms, housed in Snijdes Scientific (Microclima 1000E) growth cabinets under the following conditions: a constant light and dark period temperature of 12:7°C, respectively; a photoperiod of 12:12 (light:dark); light intensity of 12,000 Lux; relative humidity of ≥50%. The wheat seedlings were grown for 11 days, individual microcosms were weighed at regular intervals and watered gravimetrically to maintain soil moisture at 45%. After 11 days microcosms were sealed using parafilm and left to stand for 1 h. Gas samples, 11 ml in volume, were taken from sampling ports using a sterile syringe to analyse the CO<sub>2</sub> concentration. A sampling port was situated at either: 0, 20 or 40 mm distance from the seedling at a depth of between 10 and 30 mm (fig. 2). A single distance was sampled from each microcosm, with each distance replicated eight times.

#### Analysis through gas chromatography

CO<sub>2</sub> samples were analysed using a Hewlett Packard Series II 5890 gas chromatograph (GC), utilizing a thermal conductivity detector (TCD). Column temperature was held at 45°C throughout the analysis (total run time approximately 3 min, per sample). The injector temperature was 35°C, with an injection volume of 1 ml. The carrier gas was hydrogen (BOC,

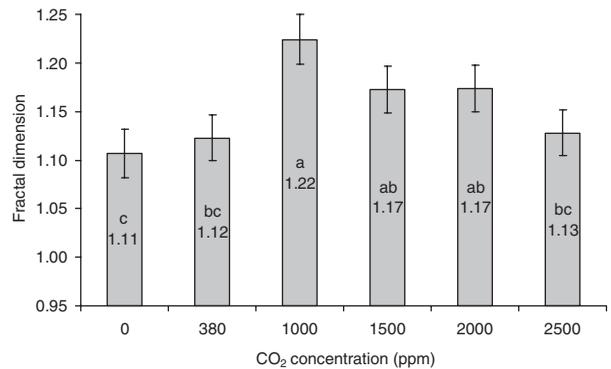


Fig. 5. Mean fractal dimensions (back-transformed) ( $\pm$ 95% CI) of tracks laid by WBF larvae exposed to different concentrations of CO<sub>2</sub>: 0, 380 (atmospheric), 1000, 1500, 2000, 2500 ppm. The greater the fractal dimension value, the more tortuous the path taken, *N* = 20. Statistical differences between groups are indicated by differing lower case letters (one-way ANOVA *post-hoc* comparisons by Tukey's test).

UK), the calibration standards (390; 1093; 5262; 10,100 ppm CO<sub>2</sub> were supplied by BOC, UK) were run in triplicate. The mean of the two most similar results were taken and plotted to produce a linear standard curve so as to estimate the unknown samples.

## Results

### CO<sub>2</sub> as an attractant

Analysis of arena bioassays offering a constant stream of CO<sub>2</sub> or CO<sub>2</sub>-free air at 8 ml h<sup>-1</sup> showed no significant difference in larval numbers entering a 20 mm radius of the inlet (fig. 3) when analysed using Fisher's exact test ( $P = 0.716$ ).

### CO<sub>2</sub> as a behavioural modifier

When assessing the larval behavioural response to constant CO<sub>2</sub> concentrations through analysis of track fractal dimensions, the assumption was made that the habitat (arena) encountered by the larvae was homogeneous in nature. If this was the case then the relationship between the 'number of dividers' (track length) and 'divider size' would be scale invariant (scaling divider size by a constant causes a proportionate scaling of the track length). This was demonstrated by plotting track length and the divider size, proportionate scaling was observed as track length and the divider size conformed to a power-law relationship ( $R^2 = 0.99$ ) (fig. 4). This scale-invariant relationship meant that larvae did not encounter a heterogeneous environment in the arenas, i.e., the atmosphere in arena was homogeneous.

Fractal dimension and distance of track data were normally distributed when transformed by log<sub>10</sub> and square root, respectively. ANOVA conducted on the fractal dimension data showed that there were significant differences within the group of CO<sub>2</sub> concentrations:  $F_{5, 114} = 8.38$ ;  $P < 0.001$ . Tukey's *post-hoc* test provided information on which groups were significantly different from each other (fig. 5). Larval tracks produced in the elevated CO<sub>2</sub> concentration level of 1000 ppm (fig. 6a) were shown to be significantly more tortuous than those produced under 0 (fig. 6b) or 380 ppm

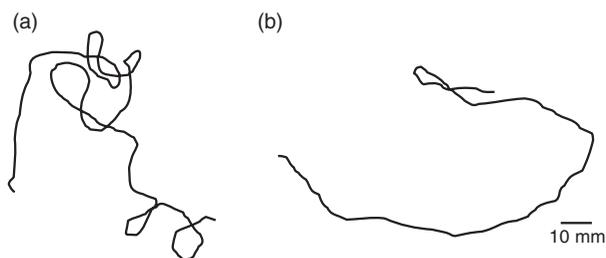


Fig. 6. Track of WBF larva made in an arena with a homogeneous atmosphere containing (a) 1000 ppm CO<sub>2</sub>. The track displays higher tortuosity (higher fractal dimension, FD=1.3), indicating the animal was travelling within a resource; (b) 0 ppm CO<sub>2</sub>. The track displays low tortuosity (low fractal dimension, FD=1.1), indicating the animal was travelling between resources.

CO<sub>2</sub> concentrations ( $P < 0.001$  and  $< 0.001$ , respectively), and also more so than the highest concentration tested, 2500 ppm ( $P < 0.001$ ). Although not as pronounced, the higher concentrations of CO<sub>2</sub>; 1500 ppm ( $P = 0.024$ ) and 2000 ppm ( $P = 0.021$ ), were significantly more tortuous than those produced under a 0 ppm CO<sub>2</sub> environment.

ANOVA of track distance data collected under different constant CO<sub>2</sub> concentrations also showed significant differences between treatments;  $F_{5,114} = 5.70$ ,  $P < 0.001$ . The *post-hoc* Tukey's test highlighted significant differences between track lengths (fig. 7); tracks formed under the 1000 ppm CO<sub>2</sub> environment were significantly longer than those formed under the 0 ( $P = 0.018$ ), 380 ( $P = 0.042$ ) and 2500 ppm ( $P \leq 0.001$ ) environments. The length of tracks produced under a CO<sub>2</sub> concentration of 1500 ppm were significantly longer than those produced under 0 ppm conditions ( $P = 0.021$ ), whereas tracks formed under 1500 and 2000 ppm conditions were significantly longer than those created under 2500 ppm CO<sub>2</sub> ( $P = 0.004$  and  $0.04$ , respectively).

#### Seedling derived CO<sub>2</sub> concentration in soil

Soil CO<sub>2</sub> gradients were assed using GC analysis of gas samples removed at set distances from wheat seedlings, with the hypothesis that CO<sub>2</sub> concentration in the soil would increase with proximity to the seedling. The data collected were not normally distributed; therefore a non-parametric Kruskal–Wallis test was used to compare the median CO<sub>2</sub> concentrations from the three distance categories sampled (0, 20 and 40 mm) (fig. 8). A trend was noted for a decrease in the concentration of CO<sub>2</sub> as the sampling distance increased. However, analysis through the Kruskal–Wallis test reported no significant change in the CO<sub>2</sub> concentrations in relation to distance from the seedling;  $H = 2.94$ , 2 d.f.,  $P = 0.229$ .

#### Discussion

It was observed that WBF larvae produced more tortuous and longer tracks when exposed to CO<sub>2</sub> concentrations of 1000 ppm than when exposed to concentrations of 0 and 380 ppm (atmospheric). A trend was observed for tracks to be longer and more tortuous at CO<sub>2</sub> levels above 380 ppm, but these were not always significant at the 1500 and 2000 ppm levels, up until 2500 ppm where track tortuosity and length returned to a similar level to that found under 0 and 380 ppm conditions. When presented with a point source of CO<sub>2</sub> WBF

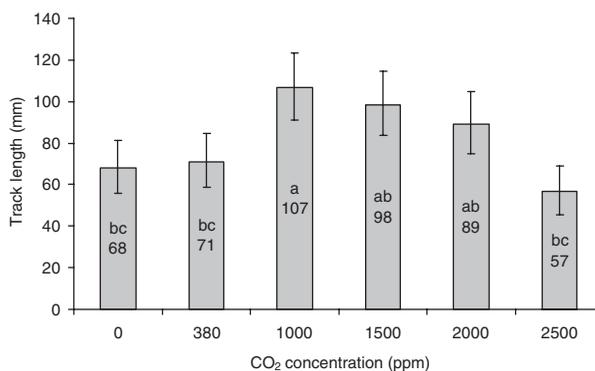


Fig. 7. Mean length (back-transformed) (mm  $\pm$  95% CI) of tracks laid by WBF larvae exposed to different constant concentrations of CO<sub>2</sub>: 0, 380 (atmospheric), 1000, 1500, 2000, 2500 ppm,  $N = 20$ . Statistical differences between groups are indicated by differing lower case letters (one-way ANOVA, *post-hoc* comparisons by Tukey's test).

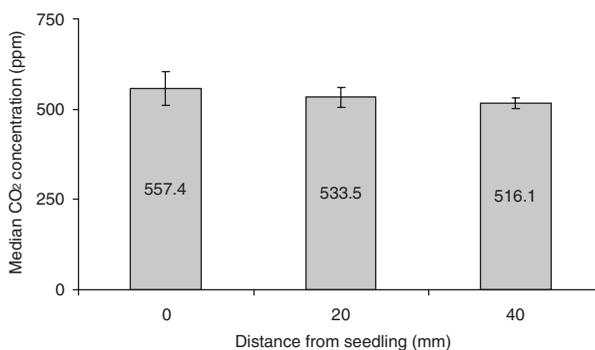


Fig. 8. Median concentration of CO<sub>2</sub> (ppm  $\pm$  95% CI) detected from sampling ports situated at: 0, 20 and 40 mm distances from wheat seedlings,  $N = 8$ .

larvae did not orientate within 20 mm of the source significantly more frequently than when presented with air containing 0 ppm CO<sub>2</sub>, indicating that larvae are not attracted to a point source of CO<sub>2</sub>.

These results suggest that WBF, like many other phytophagous larvae, utilize CO<sub>2</sub> to aid in the location of host-plants. As a primary metabolite, CO<sub>2</sub> is produced by all plants (Taiz & Zeiger, 2010) and is the most abundant gas released from plant roots (Payne & Gregory, 1988). For many less specific polyphagous species locating the nearest host-plant could be a relatively simple undertaking through following CO<sub>2</sub> emissions alone. However, monophagous and oligophagous larvae need to be more selective when it comes to locating food sources. Oligophagous larvae such as WBF would be less likely to locate a host-plant by merely following CO<sub>2</sub> gradients in the soil, due to there being more CO<sub>2</sub> sources in the soil than their host-plant alone; therefore it is unsurprising that attraction to this compound was not observed in this study. Johnson *et al.* (2006) found that the monophagous weevil *S. lepidus* also did not show a directional response to CO<sub>2</sub>, but did observe increased movement tortuosity similar to that which was observed in WBF during this

study. This increase in searching behaviour by *S. lepidus* was noted at a concentration of 1000 ppm CO<sub>2</sub>, the same concentration as was found to induce the greatest tortuosity and track length in WBF larvae. It was also demonstrated that 1000 ppm was similar to the CO<sub>2</sub> concentration found surrounding the roots of the clover host-plant (Johnson *et al.*, 2006). When sampling CO<sub>2</sub> levels in soil around wheat seedlings in this study, it was noted that there was a trend for higher concentrations of CO<sub>2</sub> close to the seedling, which reduced with distance. However, statistical analysis did not show this as significant. The relatively low level of observed CO<sub>2</sub> at close proximity to the plant, compared with the Johnson *et al.* study, could be put down to the lack of a mesh screen in the microcosms, as these limit the spread of the roots, and were used in that and other similar studies (Johnson *et al.*, 2006). By limiting the spread of the root system, such screens keep the roots closer to the sampling sites and potentially result in a higher and more consistent soil concentration of CO<sub>2</sub>. The level of CO<sub>2</sub> found near the seedling was less than the lowest concentration found to stimulate the larvae (1000 ppm) and was greater than the highest concentration that did not stimulate the larvae (380 ppm).

Host-plant locations in *Diabrotica* spp. have been extensively studied, and both hydroxamic acids (Bjostad & Hibbard, 1992) and CO<sub>2</sub> (Jewett & Bjostad, 1996) have been identified as attractive, with CO<sub>2</sub> being the primary chemical attractant involved in host-plant location (Bernklau & Bjostad, 1998). The hypothesis of CO<sub>2</sub> being the primary attractant utilized by host-finding larvae is plausible for *Diabrotica* spp. as adult females oviposit in fields of corn, the larval host-plant (Levine & Oloumi-Sadeghi, 1991). The larvae are more likely to hatch in close proximity to their host-plant, therefore simply orientating along CO<sub>2</sub> gradients is more likely to locate them a host-plant. This scenario appears to be too simplistic a method of host-plant location in WBF, given the complex ecology associated with oviposition in this species, resulting from the lack of association between oviposition sites and larval host-plants (Petherbridge, 1921).

These results suggest that WBF larvae do not use CO<sub>2</sub> gradients to locate host-plants. However, CO<sub>2</sub> does induce a behavioural response from the larvae. As CO<sub>2</sub> gradients in soil are unstable due to microbial interactions, pore structures and interaction with the atmosphere (Gollany *et al.*, 1993; Hanson *et al.*, 2000; Sheppard & Lloyd, 2002), combined with the ubiquitous nature of CO<sub>2</sub> emission by plants (Taiz & Zeiger, 2010), it makes host-plant location by this method unlikely for an oligophagous larvae such as WBF. WBF larvae are stimulated by increased CO<sub>2</sub> levels in the soil; however, instead of being induced to orientate with respect to the gradient, they are induced to perform searching behaviour, such as an increase in turning as indicated by the increased tortuosity of the track or increased track length. Once induced to search, the larvae would then rely more on family or genus-specific chemicals to locate and identify their host-plant such as the hydroxamic acids DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one) and MBOA (6-methoxybenzoxazolone), which have been found to be attractive (Rogers, 2011; Rogers & Evans, 2013).

CO<sub>2</sub> has been identified as a target compound for controlling a number of insect species deleterious to human health, mainly to control parasitic dipterans (DeFoliart & Morris, 1967) including Tabanidae (horse flies, Hayakawa, 1988), *Culicoides impunctatus* (Highland midge, Bhasin *et al.*, 2001) and *Triatoma infestans* (kissing bug, Barrozo & Lazzari,

2004). CO<sub>2</sub> baited traps have been proposed to lure and kill mosquitoes (Williams *et al.*, 2006) and *Culicoides* midges (Nelson, 1965), while the targeting of CO<sub>2</sub> receptors with chemical inhibitors and activators to disrupt host-finding behaviour of mosquitoes has also been proposed (Turner *et al.*, 2011). Attracting larvae or disrupting searching behaviour underground through the creation of CO<sub>2</sub> lures and false CO<sub>2</sub> gradients has been discussed (Bernklau *et al.*, 2004), although it is both impractical and counter to the current move to curb agricultural output of greenhouse gasses such as CO<sub>2</sub>. However, the use of chemicals that over stimulate or block the CO<sub>2</sub> receptors of larvae could be developed. Work with lepidopteran pests has shown that exposing adult males to high levels of sex pheromone can render the moth incapable of locating female conspecifics (Nansen *et al.*, 2007); this can be due to over excitation of the sensory receptors causing sensory fatigue or by competing with natural pheromone plumes (Cardé & Minks, 1995). The principles behind this 'mating disruption' method of control could also be adapted for use to control host-finding phytophagous larvae, by using the chemical inhibitors and activators of CO<sub>2</sub> receptors reported by Turner *et al.* (2011) or similar. Through this method, larval host finding could be disrupted, inducing larvae to waste valuable resources by increasing their activity unnecessarily or by preventing them locating host-plants by blocking their perception of CO<sub>2</sub> gradients. Larval host finding in WBF is a critical period given the lack of associated host-plant when adults select oviposition sites. Larvae must locate a host-plant rapidly to avoid predation and to avoid death through starvation. If CO<sub>2</sub> gradients could be disrupted through the addition of compounds that sequester CO<sub>2</sub> or if the perception of these gradients were removed through disruption of larval CO<sub>2</sub> receptors, then there would be a greater chance of larval mortality during their vulnerable host finding stage. The study of insect chemical ecology has been investigated for a number of subterranean phytophagous larvae. However, unlike above ground herbivorous insect pests, little has been done to utilize these results within pest management systems.

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