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Wheat bulb fly (*Delia coarctata*, Fallén, Diptera: Anthomyiidae) larval response to hydroxamic acid constituents of host-plant root exudates

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Abstract

Wheat bulb fly (WBF, *Delia coarctata*, Fallén, Diptera: Anthomyiidae) is a pest of commercial importance in wheat, barley and rye, with attacked crops failing to produce full potential yields. Females do not oviposit in association with a host-plant; therefore, prompt location of a suitable host is critical to the survival of the newly hatched larvae. The objective of this study was to conduct choice test bioassays to assess the attraction of WBF larvae to specific chemical constituents of WBF host-plant root exudates, the hydroxamic acids DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one) and MBOA (6-methoxy-benzoxazolin-2-one). The larval response to four concentrations of each test compound was assessed in arena bioassays. Analysis using a Rayleigh test of uniformity of the final resting positions of larvae in response to these chemicals indicated attraction. These results go some way to explaining the mechanisms by which WBF larvae locate host plants, giving the potential to develop semiochemical based control strategies.

Keywords: chemical ecology, semiochemical, behaviour, *Avena sativa*, attraction, pest, cereal

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Introduction

Wheat bulb fly (WBF, *Delia coarctata*, Fallén, Diptera: Anthomyiidae) is a pest of commercial importance in wheat, barley and rye, with attacked crops failing to produce full potential yields (Young & Ellis, 1996). Adult WBF emerge in June and oviposit from July to September, in advance of crop sowing in October and November. The preferred ovipostion site of WBF is bare soil (Petherbridge, 1921) and open canopy crops (Young & Ellis, 1996). This is thought to be because, in natural situations, bare soil is often colonized by couch grass (*Elytrigia repens*), the proposed natural host plant of WBF (Marriott & Evans, 2003), which is an aggressive colonizer of disturbed ground.

The eggs overwinter in the soil, hatching through January and February in the UK. Unlike other anthomyiid flies, such as cabbage root fly, *Delia radicum* (Baur *et al*., 1996) and onion fly, *Delia antiqua* (Judd & Whitfield, 1997), eggs are not laid in close association with a host plant; therefore, WBF larvae must move through the soil to locate host plant seedlings before invading and feeding within the stem of the plant. The damage caused by the larvae in wheat results in the yellowing and eventual death of the central stem – commonly referred to as a 'deadheart'. The seedling may be killed outright if the plant has not developed sufficiently prior to invasion, with the larvae moving to new stems once the originally invaded stem is depleted (Gough, 1947).

Effective control of WBF is difficult; precise timing is required for some insecticidal applications, and treatments are

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often not cost effective. Three methods of control are used against WBF in the UK. Prior to egg laying, the pyrethroid seed treatment tefluthrin can be used on the seed to provide early protection (Frost *et al.*, 1994), although later hatching larvae may not be controlled. Eggs hatching in January and February can be targeted with an organophosphate insecticide soil spray (chlorpyrifos). Finally, larvae can be targeted systemically using dimethoate, an organophosphate foliar spray, once larvae have infested the stem (Young, 1992). The limited use of chemical controls gives no margin for error with application timing, and the risk of further restrictions on insecticide use make the development of new control methodologies desirable.

Wheat bulb fly larvae have been shown to respond behaviourally to host plant seedlings, extracts and root exudates (Stokes, 1956; Scott, 1974; Greenway *et al.*, 1976; Marriott & Evans, 2003). These studies suggest that WBF larvae utilize plant-specific chemicals or plant-specific ratios of ubiquitous chemicals to locate host plants. Marriott (2001) proposed that the hydroxamic acid, 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), may play a role in host plant location by WBF larvae, due to higher levels being identified in the favoured host-plants, wheat and couch grass, than in other host plants, and be absent from non-host Poaceae such as oat (*Avena sativa*) (Hamilton, 1964; Tang *et al.*, 1975), which are thought to have a repellent effect on WBF (Scott *et al.*, 1971; Scott & Greenway, 1973).

Hydroxamic acids are distributed throughout wheat plants (Wu *et al.*, 2000) and in root exudates (Pérez & Ormeño-Núñez, 1991), where they have been shown to act as plant defence agents against insects (including the corn borers, *Ostrinia nubilalis* (Klun *et al.*, 1967) and *SeSamia nonagrioides* (Gutiérrez & Castañera, 1986) and the aphids *Metopolophium dirhodum* (Argandoña *et al.*, 1980), *Schizaphis graminum* (Argandoña *et al.*, 1981), *Sitobion avenae* (Bohidar *et al.*, 1986) and *Rhopalosiphum maidis* (Long *et al.*, 1977), as well as bacteria and fungi (Niemeyer, 1988; Wilkes *et al.*, 1999). DIMBOA is the major hydroxamic acid found in wheat (Zuniga & Massardo, 1991) and couch grass (Friebe *et al.*, 1995); although, when in solution, it decomposes to the less toxic 6-methoxy-benzoxazolin-2-one (MBOA) (Pratt *et al.*, 1995) with the release of formic acid (Bravo & Niemeyer, 1985).

Understanding the mechanisms involved in the infestation of host plant crops by WBF is necessary for the development of sustainable management of this pest. If attractant chemicals from host plant root exudates can be identified, there is the potential to develop semiochemical control systems for WBF. Employing such a system could reduce dependence on organophosphates for control of the WBF by replacing the current chemical control methods, or by including them in an integrated pest management programme. The aim of this study was to identify chemicals that induce a chemotactic response from WBF larvae. Choice test arena assays were used to assess attraction of WBF larvae to hydroxamic acid (DIMBOA and MBOA) chemical constituents of root exudates from wheat and couch grass, both host plants of the WBF.

Materials and methods

Experimental insects

Wheat bulb fly eggs were harvested from potato fields in East Lothian (NT 515 835 GB Grid), the Borders (NT 669 343 GB Grid) and Fife (NO 305 145 GB Grid) regions of the UK.

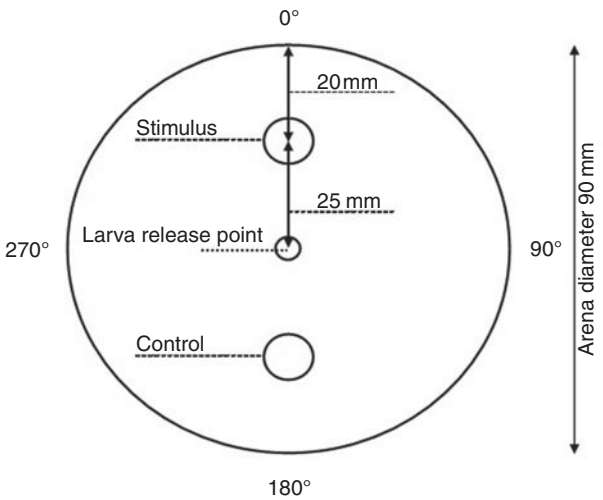

Fig 1. Diagram of the arena used to test attractiveness of stimuli to WBF larvae.

The eggs were sterilized in 0.3% NaOCl solution for two minutes and rinsed in sterile distilled water (Bellows & Fisher, 1999; Marriott, 2001). After being sterilized, the eggs were kept in 90 mm diameter Petri dishes containing moist vermiculite on black nylon mesh and stored at 5°C. Eggs were monitored and moistened regularly, and methyl paraben was applied when necessary to control fungal infections (Kishaba *et al.*, 1968; Hedin *et al.*, 1974). After two months, eggs were removed as needed to a Petri dish containing moistened black filter paper and kept at approximately 10°C to encourage egg hatch. Larvae were used in experiments within 24 h of hatching and individual larvae were used only once.

Chemicals

All chemicals were sourced from Sigma-Aldrich (MBOA (purity 97%), Oxoid technical agar), except DIMBOA (purity >95%), which was supplied by Prof. Brigitte Kopp, University of Vienna.

Arena

Arenas consisted of 90-mm diameter pieces of black filter paper (Whatman 551) placed in the centre of 140-mm diameter glass Petri dishes and moistened with 1 ml of distilled water. The stimulus (an exudate agar plug or a test chemical agar plug) was positioned at one side of the filter paper, and the control (a blank agar plug) was situated at the opposite side of the arena and used only once, before being replaced. Each was positioned 25 mm from the centre point and both were equidistant from the edge of the filter paper (fig. 1).

Seedling assay

Seedlings for bioassays were prepared as follows, based on the methodology of Marriott & Evans (2003). Wheat seeds (cv. Aristos) were surface-sterilized in 3% NaOCl solution for two minutes before being thoroughly rinsed in sterile distilled water (Abdul-Baki, 1974). The seeds were then germinated in 90-mm Petri dishes containing Whatman 181 filter paper moistened with sterile distilled water. The seeds were

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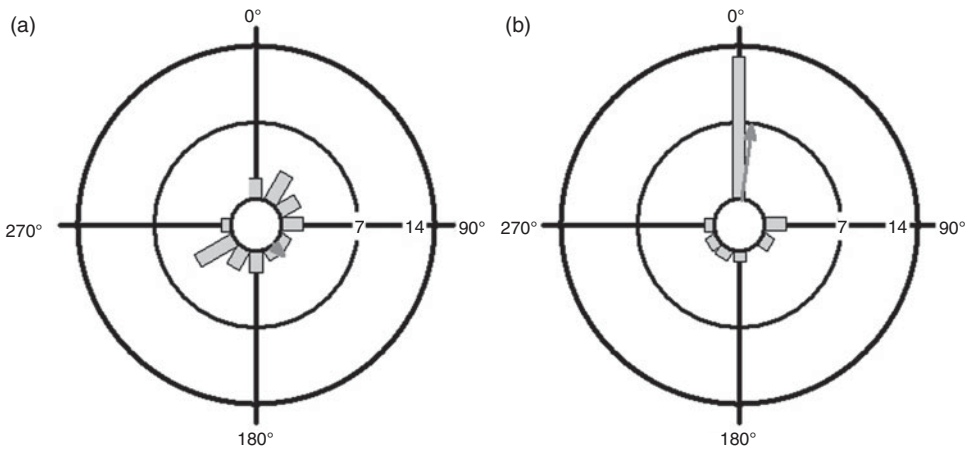

Fig 2. Directional response of larvae to (a) agar, (b) wheat exudates (0°), $N = 20$. The direction of the grey arrow indicates the direction of the mean angle, while the length indicates the mean vector strength. The arena was divided into segments of 30° (the x-axis 0, 90, 180 and 270° around the perimeter of the circle shows the direction of the segment). The grey bars radiating out from the centre indicate the number of larvae located in each of these segment of the arena, with the segment at 0° indicating the position of the stimulus plug and the segment at 180° indicating the control plug. The right side of the y-axis contains the scale for the number of larvae located in each 30° segment (grey bars).

Table 1. Rayleigh test of uniformity against unimodal direction 0° for resting positions of larvae in response to; no stimulus control (agar) and wheat seedling exudates in agar.

	Control treatments	
	Agar	Wheat
Sample number	20	20
Mean direction (°)	137.1	6.6
Mean vector length (R)	0.10	0.49
Rayleigh test of uniformity	$P = 0.683$	$P \leq 0.001$

germinated in the dark at 13°C for four days (until coleoptiles were 20 mm in length). Following germination, six seedlings were transplanted into sterilized glass vials containing 25 ml of 0.3% technical grade agar. The vials were sealed with Parafilm™ to prevent contamination and evaporation of the agar. The seedlings were placed in a controlled-environment incubator maintained at a constant temperature of 20°C with a 16:8 L:D cycle for seven days before use in experiments. A 1 g plug of the exudate agar was used as the stimulus in the bioassays. Seedlings were removed from the agar prior to the extraction of the plugs, which were taken away from where roots had been present to reduce contamination of root material in the plug. Control plugs of technical agar (0.3%), prepared in the same way without the addition of wheat seedlings, were used as controls.

Semiochemical assay

Agar plugs consisting of sterile technical grade agar 0.3% w/w in water were prepared and left to cool to approximately 30°C. Test chemicals were added to the agar, mixed and then poured into 90-mm diameter Petri dishes to the depth of 10 mm and allowed to set. One gram plugs of these mixtures were used as attractant point sources in the bioassays. Agar plugs were used as controls, and these were prepared in the same way but excluding the test chemical.

Experimental procedure

Individual larvae were placed onto the centre of the filter paper. The Petri dish lid was replaced and the arena was kept in the dark at 18°C ± 3°C for 20 min. After that time, the final resting position of the larva was recorded. In all assays, larvae that failed to move from the centre of the filter paper were excluded from the analysis.

Statistical analysis

Final resting positions of larvae were analysed using circular statistics (Batschelet, 1981; Fisher, 1995). The mean angle of orientation and its resultant mean vector strength (R) were calculated for each treatment. The mean angle ranges between 0° and 360°, with 0° indicating orientation directly towards the test stimulus and 180° indicating orientation directly toward the control. The mean vector strength ranges from 0–1.0, the more dispersed around the circle the samples are, the more mean vector strength tends toward zero. The more concentrated they are, the more the mean vector strength tends towards one. The mean angle and R were estimated and tested for significance using the Rayleigh test of uniformity against the direction 0° (0° toward the attractant), $P > 0.1$ is equal to no deviation from circular uniformity while $P \leq 0.1$ is equal to significant deviation towards the attractant. The sample size for each treatment was 20 larvae. Mean angle and mean vector strength is indicated on the circular graphs by a grey arrow, the direction of which indicates the mean angle, while the length indicates the mean strength. The arena was divided into 12 segments of 30° (the perimeter of the circular graph is the x-axis with the numbers 0, 90, 180 and 270° around it providing the scale to show the direction of the segments). The grey bars radiating out from the centre indicate the number of larvae located in each of these segments of the arena, with the segment at 0° indicating the position of the stimulus plug and the segment at 180° indicating the control plug. The right side of the y-axis contains the scale for the number of larvae located in each 30° segment (grey bars).

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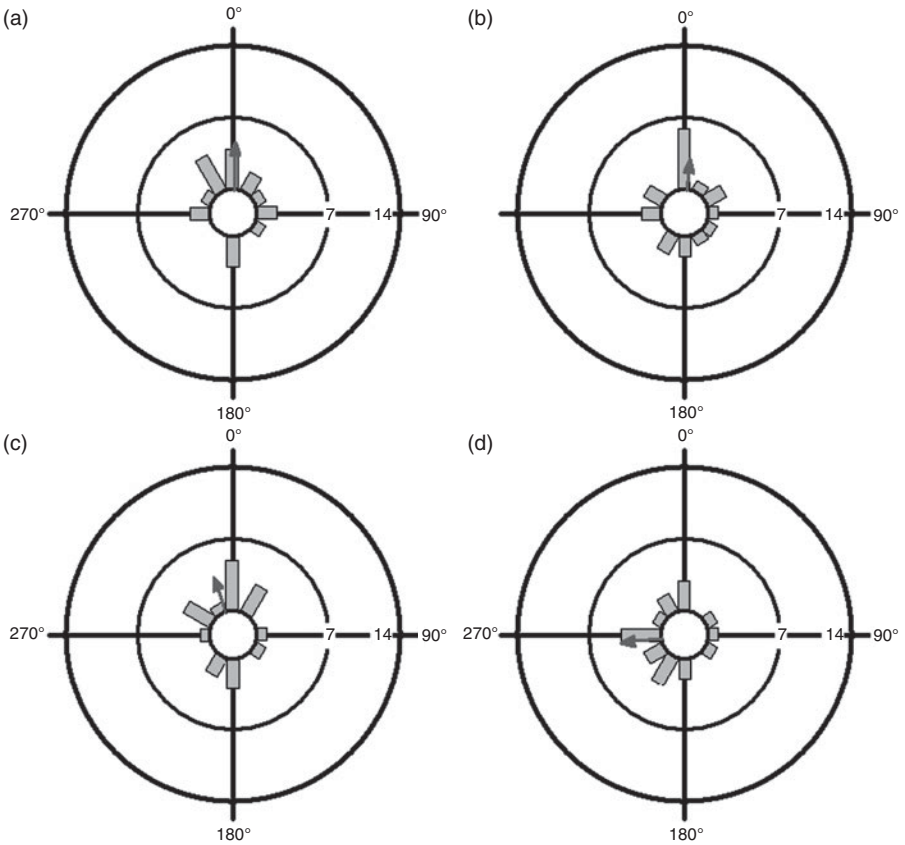

Fig 3. Directional response of larvae to DIMBOA at (a) 0.009 mg l^{-1}, (b) 0.9 mg l^{-1}, (c) 9 mg l^{-1} and (d) 90 mg l^{-1}, test stimulus at 0° and $N = 20$ in each case. The arena was divided into segments of 30° (the x-axis 0, 90, 180 and 270° around the perimeter of the circle shows the direction of the segment). The grey bars radiating out from the centre indicate the number of larvae located in each of these segment of the arena, with the segment at 0° indicating the position of the stimulus plug and the segment at 180° indicating the control plug. The right side of the y-axis contains the scale for the number of larvae located in each 30° segment (grey bars).

Statistics were done using GenStat Release 11.1 (VSN International Ltd, Oxford, UK) and graphs were made using Oriana4 (Kovach Computing Services, Pentraeth, UK).

Results

Neonate larvae resting positions 20 min after being released on filter paper containing no stimulus (two blank agar plugs) were found to be uniformly distributed (table 1, fig. 2a). No statistically significant mean direction or vector length can be inferred for a random distribution such as this. However, when one blank agar plug was replaced with agar containing the exudates of wheat seedlings, the larvae displayed a significant directional response towards the wheat exudate stimulus (table 1, fig. 2b).

Larvae displayed positive taxis in the direction of agar plugs containing the hydroxamic acid DIMBOA (fig. 3, table 2). Larvae in response to DIMBOA at 0.009 mg l^{-1} orientated with a statistically significant mean direction towards the source of DIMBOA. While DIMBOA concentrations of 0.9 mg l^{-1} and 9 mg l^{-1} also stimulated positive orientation from the larvae but at a reduced level. Larval response to a concentration of DIMBOA at 90 mg l^{-1} gave no statistically significant Rayleigh test of uniformity; therefore, no significant mean direction and vector length could be inferred.

Table 2. Rayleigh test of uniformity against unimodal direction 0° for resting positions of larvae in response to DIMBOA at four concentrations.

	DIMBOA			
	0.009 mg l^{-1}	0.9 mg l^{-1}	9 mg l^{-1}	90 mg l^{-1}
Sample number	20	20	20	20
Mean direction (°)	1.4	5.6	340.1	262.6
Mean vector length (R)	0.33	0.21	0.26	0.29
Rayleigh test of uniformity	$P = 0.018$	$P = 0.094$	$P = 0.061$	$P = 0.592$

Concentrations of MBOA of 0.009 and 0.9 mg l^{-1} (fig. 4, table 3) induced no statistically significant mean direction or vector length from WBF larvae. However, the MBOA concentrations of 9 mg l^{-1} and 90 mg l^{-1} elicited a significant Rayleigh test of uniformity, with 90 mg l^{-1} inducing a highly significant larval response.

Discussion

The observed difference in attraction of the two hydroxamic acids could be explained by the decomposition of

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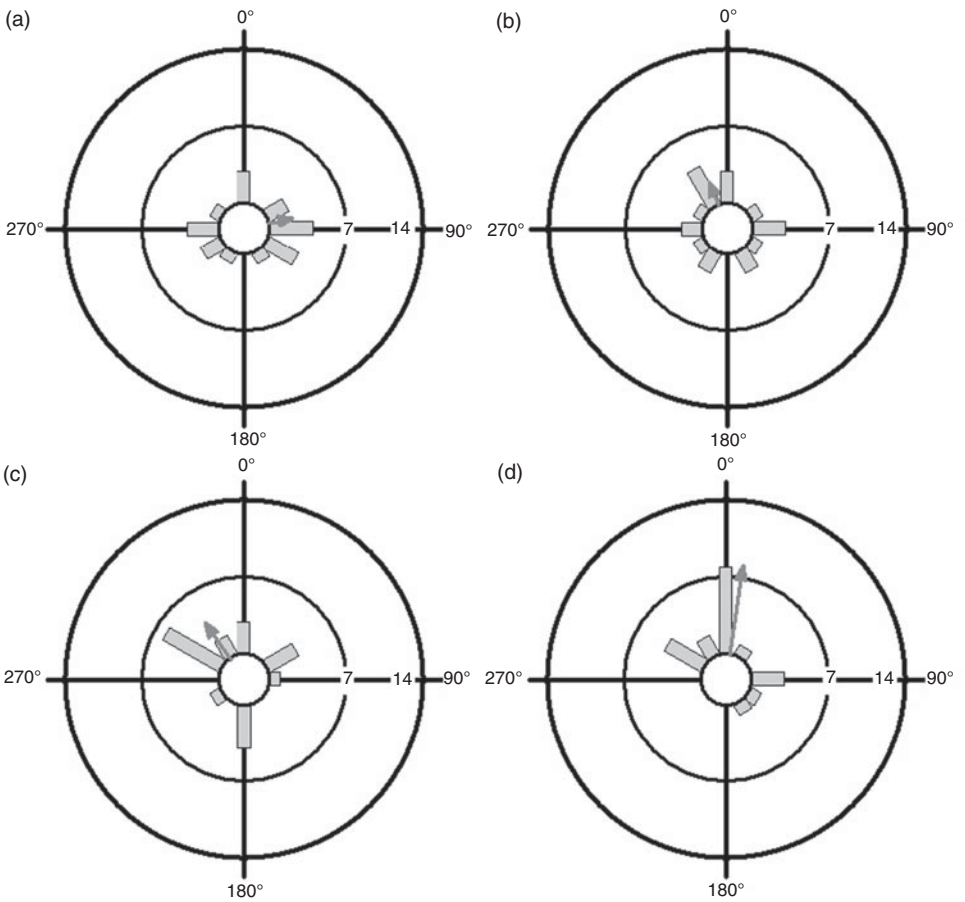

Fig 4. Directional response of larvae to MBOA at (a) 0.009 mg l^{-1}, (b) 0.9 mg l^{-1}, (c) 9 mg l^{-1} and (d) 90 mg l^{-1}, test stimulus at 0° and $N = 20$ in each case. The arena was divided into segments of 30° (the x-axis 0, 90, 180 and 270° around the perimeter of the circle shows the direction of the segment). The grey bars radiating out from the centre indicate the number of larvae located in each of these segment of the arena, with the segment at 0° indicating the position of the stimulus plug and the segment at 180° indicating the control plug. The right side of the y-axis contains the scale for the number of larvae located in each 30° segment (grey bars).

DIMBOA to MBOA. The composition of root exudates change over time through differing decomposition rates, and spatially as solubility effects transportation in the soil, creating a concentration gradient of chemicals around the plant root system. In wheat, DIMBOA concentrations in root exudates start from approximately 0.001–0.01 mg l^{-1} when present (Pérez & Ormeño-Núñez, 1991; Wu *et al*., 2001), a similar level to that found to be attractive in this study (table 2). DIMBOA exuded by wheat roots has been shown to exhibit a half-life of approximately 30 h before decomposing to MBOA (Macías *et al*., 2004), although decomposition is strongly affected by pH. Therefore, this rate can be greatly reduced (Woodward *et al*., 1978). It is, therefore, expected that DIMBOA is only found at low concentrations in close proximity of the exuding plant, making it unsuitable for host-plant location by WBF larvae over longer distances and is, therefore, more likely to act as a close contact cue, arrestant or phagostimulant. MBOA is a more stable compound with a half-life of up to six days (Macías *et al*., 2004). Consequently, MBOA would build up in the soil, potentially having a greater concentration than that of DIMBOA, and be distributed over a greater distance surrounding the plant due to its higher solubility. It is possible that the WBF larvae use the more stable MBOA as an attractant in the soil over longer distances. Attraction to MBOA looked to be dose dependent, with Rayleigh scores becoming stronger with increasing dose. This dose dependent response can be seen in other phytophagous soil dwelling larvae such cabbage root fly, which are suspected of using concentration gradients to orientate to the root systems of their host plants (Koštál, 1992).

Table 3. Rayleigh test of uniformity against unimodal direction 0° for resting positions of larvae in response to MBOA at four concentrations.

	MBOA			
	0.009 mg l^{-1}	0.9 mg l^{-1}	9 mg l^{-1}	90 mg l^{-1}
Sample number	20	20	20	20
Mean direction (°)	77.6	338.3	324.6	8.6
Mean vector length (R)	0.15	0.17	0.29	0.58
Rayleigh test of uniformity *P*	$P = 0.417$	$P = 0.163$	$P = 0.07$	$P \leq 0.001$

Hydroxamic acids are exuded by young seedlings, with concentrations within the plant gradually declining with

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plant growth (Belz & Hurle, 2005). The concentration of DIMBOA and MBOA in soil adjacent to host plants could allow WBF larvae to differentiate between susceptible young seedlings and potentially harder to penetrate older host plants.

DIMBOA plays a key role as a plant defence agent against insects, as well as bacteria and fungi (Niemeyer, 1988; Wilkes *et al*., 1999). These results show that WBF would need to have overcome the deleterious effects of the compound before utilising it as an attractant. MBOA has a reduced toxicity to organisms in comparison to that of DIMBOA (Pratt *et al*., 1995) and that could allow WBF larvae to be attracted to the higher concentration observed in this study. It is not uncommon for specialist insect herbivores to overcome plant defence chemicals and utilize these chemicals (such as allyl isothiocyante being used by *Delia radicum* (Koštál, 1992), *Delia floralis* (Rygg & Sömme, 1972) and *Delia antiqua* (Ross & Anderson, 1992) to locate host plants). Previous studies have indicated the use of hydroxamic acids as attractants to insects such as the larvae of *Spodoptera frugiperda* (Lepidoptera: Noctuidae), which are attracted to DIMBOA (Rostás, 2007), and the Hessian fly *Mayetiola destructor* (Diptera: Cecidomyiidae), which uses MBOA as an oviposition cue (Morris *et al*., 2000). The hydroxamic acid, 2,4-dihydroxy-1,4-benzoxazin-3-one (DIBOA), which is also found in cereals, has also been seen to induce a behavioural response in soil-dwelling pests, having been implicated in the initiation of hatching of nematodes from cysts (Mitchinson, 2009). Previously, MBOA was attributed as an attractant to western corn rootworm, *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae), in conjunction with CO_2 (Bjostad & Hibbard, 1992) but was later retracted in favour of CO_2 as the lone volatile compound responsible for attraction in this species (Bernklau & Bjostad, 1998).

Wheat bulb fly are an oligophagous herbivore species, feeding on a restricted family of plants. Unlike polyphagous species that tend to use ubiquitous host plant semiochemicals due to the less specific target plant, oligophagous herbivores are thought to be more likely to utilize a combination of ubiquitous cues and family specific semiochemicals to locate suitable host plants (Jones & Coaker, 1978). The larvae of related soil herbivores, such as *D. radicum*, *D. floralis* (turnip fly) and *D. antiqua*, exploit a number of compounds when identifying their host plants, as well as family specific compounds such as isothiocyanates (*D. radicum* and *D. floralis*) (Rygg & Sömme, 1972; Finch & Skinner, 1982; Ross & Anderson, 1992), and sulphur metabolites (*D. antiqua*) (Matsumoto & Thorsteinson, 1968; Soni & Finch, 1979; Ross & Anderson, 1992). This appears to be the case with WBF, with family specific host plant cues (the hydroxamic acids) playing a role in host plant location and/or recognition. Hydroxamic acids are restricted to the Poaceae family, being found in higher quantities in WBF-preferred host plants while being absent altogether in *Avena sativa* (Hamilton, 1964), a Poaceae rejected by WBF larvae (Scott *et al*., 1971; Scott & Greenway, 1973). In this study, it was noted that the attraction of the test chemicals were not as strong as the seedling exudate. Different fractions of wheat extracts have been found to have varying levels of attraction to WBF (Greenway *et al*., 1976), suggesting that a number of compounds are responsible for host plant location. It is highly probable, therefore, that further compounds, including more ubiquitous exudates, may play a role in host-plant location in WBF. Phenolic compounds have been implicated (Greenway *et al*., 1976) and it is possible that primary metabolites, such as carbohydrates and CO_2, may also play a role in locating host plants.

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References

Abdul-Baki, A.A. (1974) Pitfalls in using sodium hypochlorite as a seed disinfectant in ^{14}C incorporation studies. *Plant Physiology* **53**, 768–771.

Argandoña, V.H., Luza, J.G., Niemeyer, H.M. & Corcuera, L.J. (1980) Role of hydroxamic acids in the resistance of cereals to aphids. *Phytochemistry* **19**, 1665–1668.

Argandoña, V.H., Niemeyer, H.M. & Corcuera, L.J. (1981) Effect of content and distribution of hydroxamic acids in wheat on infestation by the aphid *Schizaphis gramineum*. *Phytochemistry* **20**, 673–676.

Batschelet, E. (1981) *Circular statistics in biology*. Academic Press, London.

Baur, R., Birch, A.N.E., Hopkins, R.J., Griffiths, D.W., Simmonds, M.S.J. & Stadler, E. (1996) Oviposition and chemosensory stimulation of the root flies *Delia radicum* and *D. floralis* in response to plants and leaf surface extracts from resistant and susceptible Brassica genotypes. *Entomologia Experimentalis et Applicata* **78**, 61–75.

Bellows, T.S. & Fisher, T.W. (1999) *Handbook of Biological Control: Principles and Applications*. San Diego, CA, USA, Academic Press.

Belz, R.G. & Hurle, K. (2005) Differential exudation of two benzoxazinoids one of the determining factors for feeding allelopathy of Triticeae species. *Journal of Agricultural and Food Chemistry* **53**, 250–261.

Bernklau, E.J. & Bjostad, L.B. (1998) Re-investigation of host location by western corn rootworm larvae (Coleoptera: Chrysomelidae): CO_2 is the only volatile attractant. *Journal of Economic Entomology* **91**, 1331–1340.

Bjostad, L.B. & Hibbard, B.E. (1992) 6-Methoxy-2-benzoxazolinone: A semiochemical for host location by western corn rootworm larvae. *Journal of Chemical Ecology* **18**, 931–944.

Bohidar, K., Wratten, S.D. & Niemeyer, H.M. (1986) Effects of hydroxamic acids on the resistance of wheat to the aphid *Sitobion avenae*. *Annals of Applied Biology* **109**, 193–198.

Bravo, H.R. & Niemeyer, H.M. (1985) Decomposition in aprotic solvents of 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one, a hydroxamic acid from cereals. *Tetrahedron* **41**, 4983–4986.

Finch, S. & Skinner, G. (1982) Trapping female cabbage root flies (*Delia radicum* (L.)) (Diptera: Anthomyiidae) with allylisothiocyanate-baited traps. *Bulletin of Entomological Research* **72**, 165–173.

Fisher, N.I. (1995) *Statistical Analysis of Circular Data*. Cambridge, UK, Cambridge University Press.

Friebe, A., Schulz, M., Kuck, P. & Schnabl, H. (1995) Phytotoxins from shoot extracts and root exudates of *Agropyron repens* seedlings. *Phytochemistry* **38**, 1157–1159.

https://doi.org/10.1017/S0007485312000661 Published online by Cambridge University Press

Frost, M.J., Elsworth, S.G. & Moran, A. (1994) Tefluthrin: A cereal seed treatment for the control of wheat bulb fly (*Delia coarctata*). *Brighton Crop Protection Conference: Pests and Disease* **1–3**, 217–222.

Gough, H.C. (1947) Studies on wheat bulb fly, *Leptohylemyia coarctata* Fall. II. numbers in relation to crop damage. *Bulletin of Entomological Research* **37**, 439–454.

Greenway, A.R., Scott, G.C., Calam, D.H. & Smith, M.C. (1976) Chemistry of components in wheat and oats that influence behaviour of wheat bulb fly larvae. *Journal of Insect Physiology* **22**, 445–451.

Gutiérrez, C. & Castañera, P. (1986) Efecto de los tejidos de maíz con alto y bajo contenido en DIMBOA sobre la biología del taladro Sesamia nonagrioides Lef. (Lepidoptera: Noctuidae). *Investigación Agraria. Producción y Protección Vegetal* **1**, 109–119.

Hamilton, R.H. (1964) Metabolism of herbicides, tolerance of several grass species to 2-chloro-s-triazine herbicides in relation to degradation and content of benzoxazinone derivatives. *Journal of Agricultural and Food Chemistry* **12**, 14–17.

Hedin, P.A., Thompson, A.C., Gueldner, R.C. & Henson, R.D. (1974) Analysis of the antimicrobial agents, potassium sorbate and methyl-*p*-hydroxybenzoate, in boll weevil diets. *Journal of Economic Entomology* **67**, 147–149.

Jones, O.T. & Coaker, T.H. (1978) A basis for host plant finding in phytophagous larvae. *Entomologia Experimentalis et Applicata* **24**, 272–284.

Judd, O.J.R. & Whitfield, G.H. (1997) Visual and olfactory behaviour of *Delia antiqua* (Diptera: Anthomyiidae) in relation to time of day and ovarian development. *European Journal of Entomology* **94**, 199–209.

Kishaba, A.N., Henneberry, T.J., Pangaldan, R. & Tsao, P.H. (1968) Effects of mold inhibitors in larval diet on the biology of the cabbage looper. *Journal of Economic Entomology* **61**, 1189–1194.

Klun, J.A., Tipton, C.L. & Brindley, T.A. (1967) 2,4-Dihydroxy-7-methoxy-1,4 benzoxazin-3-one (DIMBOA), an active agent in the resistance of maize to the European corn borer. *Journal of Economic Entomology* **60**, 1529–1533.

Koštál, V. (1992) Orientation behavior of newly hatched larvae of the cabbage maggot, *Delia radicum* (L.) (Diptera: Anthomyiidae), to volatile plant metabolites. *Journal of Insect Behavior* **5**, 61–70.

Long, B.J., Dunn, G.M., Bowman, J.S. & Routley, D.G. (1977) Relationship of hydroxamic acid content in corn and resistance to corn leaf aphid. *Crop Science* **17**, 55–58.

Macías, F.A., Oliveros-Bastidas, A., Marín, D., Castellano, D., Simonet, A.M. & Molinillo, J.M.G. (2004) Degradation studies on benzoxazinoids. Soil degradation dynamics of 2,4-Dihydroxy-7-methoxy-(2H)-1,4-benzoxazin-3(4H)-one (DIMBOA) and its degradation products, phytotoxic allelochemicals from Gramineae. *Journal of Agricultural and Food Chemistry* **52**, 6402–6413.

Marriott, C. (2001) Host plant location, selection and preference by wheat bulb fly Delia coarctata Fall. (Diptera: Anthomyiidae). PhD thesis, University of Edinburgh, Edinburgh, Scotland, UK.

Marriott, C. & Evans, K.A. (2003) Host plant choice and location by larvae of the wheat bulb fly (*Delia coarctata*). *Entomologia Experimentalis et Applicata* **106**, 1–6.

Matsumoto, Y. & Thorsteinson, A.J. (1968) Olfactory response of larvae of the onion maggot, *Hylemya antiqua* Meigen (Diptera: Anthomyiidae) to organic sulfur compounds mercaptan. *Applied Entomology and Zoology* **3**, 107–111.

Mitchinson, S. (2009) New cyst nematode threats to cereals in the UK: PhD. Summary Report No. 13. Home Grown Cereals Authority: Project No. RD-2005–3190.

Morris, B.D., Foster, S.P. & Harris, M.O. (2000) Identification of 1-octacosanol and 6-methoxy-2-benzoxizolinone from wheat as ovipositional stimulants for the Hessian fly, *Mayetiola destructor*. *Journal of Chemical Ecology* **26**, 859–873.

Niemeyer, H.M. (1988) Hydroxamic acids (4-hydroxy-1,4-benzoxazin-3-one), defence chemicals in the Gramineae. *Phytochemistry* **27**, 3349–3358.

Pérez, F.J. & Ormeño-Núñez, J. (1991) Difference in hydroxamic acid content in roots and root exudates of wheat (*Triticum aestivum* L.) and rye (*Secale cereale* L.): Possible role in allelopathy. *Journal of Chemical Ecology* **17**, 1037–1043.

Petherbridge, F.R. (1921) Observations on the life history of the wheat-bulb fly *Leptohylemyia coarctata*, Fall. *Journal of Agricultural Science* **11**, 99–105.

Pratt, K., Kumar, P. & Chilton, W.S. (1995) Cyclic hydroxamic acids in dicotyledonous plants. *Biochemical Systematics and Ecology* **23**, 781–785.

Ross, K.T.A. & Anderson, M. (1992) Larval responses of three vegetable root fly pests of the genus *Delia* (Diptera, Anthomyiidae) to plant volatiles. *Bulletin of Entomological Research* **82**, 393–398.

Rostás, M. (2007) The effects of 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one on two species of *Spodoptera* and the growth of *Setosphaeria turcica* in vitro. *Journal of Pest Science* **80**, 35–41.

Rygg, T. & Sömme, L. (1972) Oviposition and larval development of *Hylemya floralis* (Fallén) (Diptera, Anthomyiidae) on varieties of swedes and turnips. *Norsk Entomologisk Tidsskrift* **19**, 81–90.

Scott, G.C. (1974) The response of wheat bulb fly larvae to cereal exudates and extracts. *Annals of Applied Biology* **77**, 107–111.

Scott, G.C. & Greenway, A.R. (1973) Effects of cereal extracts on attack on wheat by wheat bulb fly, *Leptohylemyia coarctata*. *Entomologia Experimentalis et Applicata* **16**, 554–556.

Scott, G.C., Griffiths, D.C. & Greenway, A.R. (1971) The nature and mode of action of extracts of wheat and oats that influence the behaviour of wheat bulb fly larvae. Report: Rothamsted Experimental Station, 167–168.

Soni, S.K. & Finch, S. (1979) Laboratory evaluation of sulphur bearing chemicals as attractants for larvae of the onion fly, *Delia antiqua* (Meigen) (Diptera: Anthomyiidae). *Bulletin of Entomological Research* **69**, 291–298.

Stokes, B.M. (1956) A chemotactic response in wheat bulb fly larvae. *Nature* **178**, 801.

Tang, C.S., Chang, S.H., Hoo, D. & Yanagihara, K.H. (1975) Gas chromatographic determination of 2(3)-benzoxazolinones from cereal plants. *Phytochemistry* **14**, 2077–2079

Wilkes, M.A., Marshall, D.R. & Copeland, L. (1999) Hydroxamic acids in cereal roots inhibit the growth of take-all. *Soil Biology and Biochemistry* **31**, 1831–1836.

Woodward, M.D., Corcuera, L.J., Helgeson, J.P. & Upper, C.D. (1978) Decomposition of 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one in aqueous solutions. *Plant Physiology* **61**, 796–802.

Wu, H.W., Haig, T., Pratley, J., Lemerle, D. & An, M. (2000) Distribution and exudation of allelochemicals in wheat *Triticum aestivum*. *Journal of Chemical Ecology* **26**, 2141–2154.

https://doi.org/10.1017/S0007485312000661 Published online by Cambridge University Press

Wu, H.W., Haig, T., Pratley, J., Lemerle, D. & An, M. (2001) Allelochemicals in wheat (*Triticum aestivum* L.): Production and exudation of 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one. *Journal of Chemical Ecology* **27**, 1691–1700.

Young, J.E.B. (1992) Control of wheat bulb fly in winter wheat: I. Chemical methods. II. Varietal susceptibility. Home Grown Cereals Authority. Project Report No. 67.

Young, J.E.B. & Ellis, S.A. (1996) Impact of changes in arable agriculture on the biology and control of wheat bulb fly. Home Grown Cereals Authority: Research Review No. 33.

Zuniga, G.E. & Massardo, F. (1991) Hydroxamic acid content in undifferentiated and differentiated tissue of wheat. *Phytochemistry* **30**, 3281–3283.

https://doi.org/10.1017/S0007485312000661 Published online by Cambridge University Press