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ORIGINAL PAPER

Electrophysiological and behavioural responses of the pollen beetle, *Meligethes aeneus*, to volatiles from a non-host plant, lavender, *Lavandula angustifolia* (Lamiaceae)

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Abstract A semiochemical based push-pull strategy for control of oilseed rape pests is being developed at Rothamsted Research. This strategy uses insect and plant derived semiochemicals to manipulate pests and their natural enemies. An important element within this strategy is an understanding of the importance of non-host plant cues for pest insects and how such signals could be used to manipulate their behaviour. Previous studies using a range of non-host plants have shown that, for the pollen beetle *Meligethes aeneus* (Coleoptera: Nitidulidae), the essential oil of lavender, *Lavandula angustifolia* (Lamiaceae), was the most repellent. The aim of this study was to identify the active components in *L. angustifolia* oil, and to investigate the behaviour of *M. aeneus* to these chemicals, to establish the most effective use of repellent stimuli to disrupt colonisation of oilseed rape crops. Coupled gas chromatography-electroantennography (GC-EAG) and gas chromatography-mass spectrometry (GC-MS) resulted in the identification of seven active compounds which were tested for behavioural activity using a 4-way olfactometer. Repellent responses were observed with (±)-linalool and (±)-linalyl acetate. The use of these chemicals within a push-pull pest control strategy is discussed.

Keywords Coleoptera · Behaviour · Electroantennography · *Meligethes aeneus* · Nitidulidae · Non-host plant · Olfactometer · Pollen beetle · Repellent · Volatiles

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Introduction

The pollen beetle *Meligethes aeneus* Fab. (Coleoptera, Nitidulidae) is an important pest of oilseeds, particularly oilseed rape, *Brassica napus* L., across Europe (Winfield 1992) and has recently developed some resistance to conventional insecticides (Hansen 2003). Through their feeding and oviposition habits, both the adults and, to a lesser extent, the feeding larvae contribute to economic losses by the destruction of buds and flowers.

Phytophagous insects use plant volatiles to recognise and distinguish hosts from non-hosts (Bernays and Chapman 1994). *Meligethes aeneus* responds to general green leaf volatiles released by host and non-host plants (Ruther and Thiemann 1997), as well as to volatiles such as isothiocyanates (Smart and Blight 2000) that are characteristic of Brassicas, their ovipositional hosts. Allyl isothiocyanate, 3-butenyl isothiocyanate, 4-pentenyl isothiocyanate and phenylethyl isothiocyanate have been shown to be important host recognition chemicals (Free and Williams 1978; Smart et al. 1993, 1995; Blight and Smart 1999). Mixtures of these chemicals are attractive, but a complex mixture of general green leaf volatiles and host-specific chemicals was required for maximal attraction (Smart et al. 1995; Smart and Blight 2000).

Previous studies have shown that *M. aeneus* avoid some volatile plant chemicals (Smart and Blight 2000; Mauchline 2003; Mauchline et al. 2005). Of the chemicals extracted from brassicaceous plants and tested in the field using water traps, 15 were attractive to *M. aeneus*, five were inactive, but five reduced the number of beetles

caught (Smart and Blight 2000), indicating the importance of the blend of volatiles in eliciting different behaviours by *M. aeneus*. The repellents identified included 1-octen-3-ol, 1-hexanol, (*Z*)-3-hexen-1-ol, 1-pentanol and *cis*-jasmone. Many of these had variable behavioural effects depending upon the concentration used. Specific ratios of compounds can also be responsible for the repellency of non-host odours (Blight et al. 1995b; Al Abassi et al. 2000; Bruce et al. 2003).

There have been few published studies investigating olfactory detection of volatiles by *M. aeneus*, despite laboratory and field demonstrations of behavioural responses. The only study using coupled gas chromatography-electroantennography (GC-EAG) to investigate olfaction in *M. aeneus* showed that 26 compounds from an extract of oilseed rape elicited antennal responses in these insects, including several isothiocyanates and methyl salicylate (Blight et al. 1995a).

From a range of non-host plant essential oils, lavender (*Lavandula angustifolia*, Miller) (Lamiaceae) was demonstrated to be the most repellent to *M. aeneus* in a series of laboratory bioassays (Mauchline et al. 2005). In addition, field tests have shown it to be effective at reducing oilseed rape colonisation by *M. aeneus* (Mauchline, unpublished data) and, importantly, it does not interfere with the host location behaviour of two of their most important parasitoids (Cook et al. 2007b).

This paper reports on the identification and quantification of chemicals within *L. angustifolia* oil having a repellent effect on *M. aeneus*, with a view to their incorporation into a "push-pull" strategy; where the pests are manipulated using a combination of attractant and repellent semiochemicals (Pyke et al. 1987; Miller and Cowles 1990; Pickett et al. 1997; Agelopoulos et al. 1999; Cook et al. 2007a, c). Repellents that disrupt colonisation of the crop by *M. aeneus* could be released by non-host plant intercrops or formulated as a prophylactic spray within this strategy.

Materials and methods

Insects

Adult pollen beetles, *Meligethes aeneus*, were collected from flowering crops of oilseed rape, *Brassica napus*, on the farm at Rothamsted Research, Harpenden, Herts, UK. The beetles were maintained in ventilated plastic sandwich boxes (Stewart Plastics, Surrey, UK) and fed on flowering racemes of glasshouse grown oilseed rape. The insects were maintained in a controlled environment at 10°C, 16 h light:8 h dark, for a maximum of 4 days prior to use in experiments. The sex of the beetles was determined (Cook et al. 2002) and they were starved overnight prior to electrophysiological and behavioural assays.

Electroantennography (EAG)

EAG recordings from *M. aeneus* antennae were made using Ag–AgCl glass electrodes filled with saline solution (composition as in Maddrell 1969, but without glucose). Antennae were excised and suspended between the electrodes. Signals from the antenna were passed through a high impedance amplifier (UN-06, Syntech, The Netherlands) and analysed using a software package (Syntech, The Netherlands).

Stimulus delivery

For EAG studies, the stimulus delivery device has been described previously (Wadhams et al. 1982). *Lavandula angustifolia* essential oil in hexane (10 µl of oil in 1.5 ml redistilled hexane) was applied to strips of filter paper, with the solvent being allowed to evaporate before use, and delivered (2 s duration) through a Pasteur pipette into a purified air stream (1 l/min) flowing continuously over the antenna. Solutions were freshly prepared prior to each stimulation. An artificial 1 mV signal was applied via the amplifier to the EAG trace and antennal responses were calculated as a percentage of this. Antennae from 20 female and 8 male beetles were tested.

Gas chromatography (GC)

Volatiles in *L. angustifolia* cv. Mailette essential oil were separated on a Hewlett-Packard 5890A gas chromatograph (GC), equipped with a cool on-column injector, a flame ionization detector (FID) and two GC columns of differing polarity. A non-polar HP-1 bonded phase fused silica capillary column (50 m × 0.32 mm ID × 2.65 µm film thickness) and a polar DB-WAX column were used (30 m × 0.32 mm ID × 0.5 µm film thickness). The oven temperature was maintained at 40°C for 1 min, then 5°C/min to 150°C, held at 150°C for 0.1 min, then at 10°C/min to 250°C. The carrier gas was hydrogen. Quantities of electrophysiologically active components were calculated from the individual peak areas and response factors for the individual compounds. Response factors were calculated from injections of known amounts of each compound (100 ng) containing an internal standard (100 ng *n*-octadecane).

Gas chromatography-electroantennography (GC-EAG)

The coupled GC-electrophysiology system, in which the effluent from the GC column is simultaneously directed to

the antennal preparation and the GC detector, has been described previously (Wadhams 1990). Separation of *L. angustifolia* essential oil volatiles was achieved using an AI 93 GC equipped with a cold on-column injector, a non-polar HP-1 column (50 m × 0.32 mm ID × 2,065 µm film thickness) and an FID. The oven temperature was maintained at 40°C for 1 min, then programmed at 5°C/min to 100°C, and then at 10°C/min to 250°C. The carrier gas was hydrogen. Five coupled runs were conducted, and the outputs from the EAG amplifier and the FID were monitored simultaneously and analysed using a software package (Syntech, The Netherlands). Each antennal preparation was used for one GC coupled run, which lasted for 30 minutes, and showed responses at the end of the run.

Gas chromatography-mass spectrometry (GC-MS)

A capillary column (50 m × 0.32 mm ID HP-1), fitted in a Hewlett Packard 5890 GC with a cool on-column injector, was directly coupled to a mass spectrometer (VG Autospec, Fisons Instruments, Manchester, UK). Ionization was by electron impact (at 70 eV, 250°C). The oven temperature was maintained at 30°C for 5 min and then programmed at 5°/min to 250°C. Tentative identifications were confirmed by peak enhancement on GC using co-injections on non-polar and polar columns of lavender essential oil with authentic chemical samples (Pickett 1990).

Chemicals

The essential oil of lavender, *Lavandula angustifolia* cv. Mailette (Lamiaceae), Batch No. CB1007, was provided by Botanix Ltd. (Paddock Wood, Kent, UK). For electrophysiological studies, a solution was prepared in freshly distilled hexane (10 µl in 1.5 ml hexane). Chemicals identified from *L. angustifolia* oil, subsequently used in behavioural assays, were obtained from commercial sources. 3-Octanone and hexyl acetate were purchased from the Aldrich Chemical Company. Myrcene, (+)-terpinen-4-ol and (±)-linalyl acetate were obtained from Fluka Chemie AG. (−)-*β*-Caryophyllene was obtained from Koch-Light Laboratories and (±)-linalool was obtained from Avocado Research Chemicals. For peak enhancement by GC, solutions of chemicals (100 ng/µl) were prepared in freshly redistilled hexane. For behavioural studies, solutions of chemicals were prepared in acetone at the concentration found in a 1% v:v *L. angustifolia* oil solution previously shown to elicit avoidance behaviour (Mauchline et al. 2005).

Insect behaviour

A four-way olfactometer (11 cm diameter) (Pettersson 1970; Vet et al. 1983) was used to test the behavioural responses of female *M. aeneus* to *L. angustifolia* essential oil and the components identified by coupled GC-EAG and GC-MS. The experiments were conducted at 19°C and ~60–70% relative humidity. Illumination was provided by an overhead light source (132 Watt, high frequency, polarising white light; Clearvision Lighting Ltd.). Air was drawn through the olfactometer at 400 ml/min. For each experiment, a single female *M. aeneus* was introduced into the centre of the chamber. The total time spent by each beetle in each of the four arms was recorded for 5 min using 'The Observer' behavioural software (Noldus Information Technology, Wageningen, The Netherlands) and the null hypothesis of equal time spent in each arm was tested using Friedman's non-parametric ANOVA (Couty et al. 1999) using Genstat (VSN International Ltd., Hemel Hempstead, UK). Each experiment with a different stimulus was replicated 15 times. Stimuli were presented by applying the test solution (10 µl in acetone) to 1.8 cm diameter filter paper discs placed in three arms of the olfactometer, leaving one arm as the control (10 µl acetone alone).

Results

Electroantennography (EAG)

Electroantennogram recordings from the antennae of both sexes of *M. aeneus* showed strong electrophysiological responses to *L. angustifolia* oil. The mean EAG response from females ($n = 20$, ±SE) was 1.35 ± 0.22 mV, and from males ($n = 8$) was 1.25 ± 0.33 mV. GC analysis showed that the oil comprised a complex mixture of components (Fig. 1 shows the 47 peaks emitted over the ~30 min run).

Coupled GC-EAG and chemical identification

Coupled GC-EAG with female *M. aeneus*, followed by coupled GC-MS analysis and peak enhancement on GC, led to the identification of seven physiologically active compounds from the oil (Table 1); as an example, the antennal response to linalool is shown in Fig. 2. Consistent responses to fifteen compounds in the lavender oil were found over the five EAG preparations. However, seven of these compounds were present only in very low quantities, precluding identification. We therefore proceeded with chemical identification and behavioural assays for the major components. The identification of α-santalene (peak 7 in Fig. 1) remained tentative because an authentic sample was not available. The stereochemistry of linalool, terpinen-4-ol and linalyl acetate was not determined. Commercially available materials were used in further

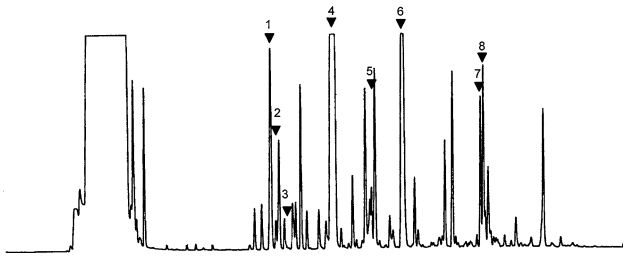

Fig. 1 Gas chromatogram of lavender (*Lavandula angustifolia* cv. Mailette) essential oil (HP-1 column). Numbered peaks showed electrophysiological activity for female pollen beetles, *Meligethes aeneus*

Table 1 Electrophysiologically active compounds for female pollen beetles, *Meligethes aeneus*, identified from lavender essential oil

Peak number	Compound	Concentration in 1% (v:v) lavender oil (mg/ml)[a]
1	3-octanone	0.215
2	Myrcene	0.056
3	Hexyl acetate	0.022
4	Linalool[b]	2.87
5	Terpinen-4-ol[b]	0.025
6	Linalyl acetate[b]	1.56
7	α-santalene[c]	–
8	β-caryophyllene	0.273

[a] Peaks quantified using *n*-octadecane as an internal standard

[b] Stereochemistry undefined

[c] Tentative identification only

experiments. Quantities of the identified compounds in a 1% v:v *L. angustifolia* oil solution were calculated using *n*-octadecane as an internal standard (Table 1).

Behavioural testing

Identified chemicals were tested in olfactometer assays with female *M. aeneus* at the level found in the 1% solution. Two of the chemicals, (±)-linalool and (±)-linalyl acetate, were shown to be significantly avoided by the beetle compared to the control (Table 2; $P = 0.028$ and $P = 0.002$, respectively). No statistically significant effects on *M. aeneus* were observed for any of the other compounds in this bioassay, although for 3-octanone ($P = 0.118$), the mean time spent in the control arm (129 s) was much higher than the mean time spent in the treatment arms (50 s).

Discussion

The aim of the current study was to investigate the possibility of using non-host plants as a source of new repellent

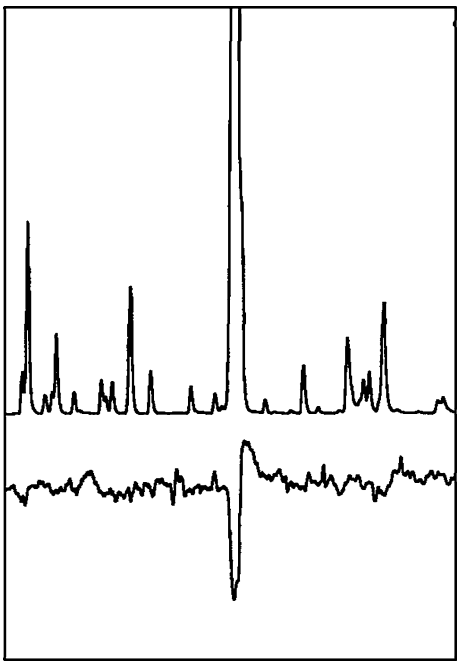

Fig. 2 Coupled GC-EAG of female *Meligethes aeneus* with volatiles from lavender oil. Upper trace: GC response; lower trace: EAG response to linalool (HP-1 column, linalool eluting at 7.86 min; response = approx 0.5 mV)

signals for *M. aeneus* in novel crop protection strategies. The essential oil of lavender, *L. angustifolia*, was selected as a model, because previous laboratory bioassays using a range of non-host plant essential oils had showed it to be one of the most repellent (Mauchline et al. 2005). Essential oils are usually extracted from plants by steam distillation and contain a mixture of volatile organic chemicals often different to the volatile profile of the plant. However, we chose to use plant essential oils as they can provide a standard non-host signal for chemical and behavioural testing.

Insect host location involves the detection of volatile chemical cues by olfactory receptors on the antenna (Bruce et al. 2005). Using coupled GC-EAG, complex blends of volatiles can be separated into individual components and the electrophysiologically active components located. Compounds showing EAG activity can then be identified and tested to determine whether their detection by the insect translates to behavioural activity. In EAG studies, male and female *M. aeneus* showed strong electrophysiological responses to the overall *L. angustifolia* odour, indicating that both sexes are capable of detecting non-host plant odours. Within *L. angustifolia* oil, a complex range of volatile components was shown to be present. From this, fifteen components were shown to elicit EAG responses from *M. aeneus*, of which seven were identified by coupled GC-MS and peak enhancement.

Table 2 Responses of female *Meligethes aeneus* in a 4-way olfactometer[a]

Compound	Control	Treatment (mean of 3 arms)	Friedman's statistic (adjusted for ties)	*P*-value
3-octanone	128.5 ± 29.2	49.6 ± 17.1	5.9	0.118
Myrcene	106.2 ± 23.4	59.6 ± 16.2	1.9	0.581
Hexyl acetate	71.4 ± 21.8	69.7 ± 17.0	1.6	0.655
(±)-linalool	161.2 ± 28.7	37.6 ± 12.6	9.1	0.028
(+)-terpinen-4-ol	63.7 ± 19.9	63.8 ± 20.3	2.4	0.488
(±)-linalyl acetate	120.3 ± 17.7	52.8 ± 14.6	15.1	0.002
(−)-*β*-caryophyllene	64.5 ± 10.6	72.4 ± 14.7	1.1	0.775
Lavender oil[b]	182.2 ± 28.2	29.6 ± 11.3	18.8	<0.001

[a] Responses measured as the mean time ± SE spent in control and treated arms ($n = 15$). The data were analysed using Friedman's non-parametric ANOVA

[b] Data from Mauchline et al. (2005) for 1% v:v lavender:acetone tested against 15 female *M. aeneus*

The ability of male and female *M. aeneus* to detect chemicals via olfactory receptors does not necessarily imply a role for these cues in the behavioural ecology of this insect. Behavioural bioassays are required to establish the behavioural effects, if any, of each chemical. Females were chosen for the behavioural bioassays as they are of most importance in pest control, and cause more damage to the oilseed rape plants through a combination of oviposition and feeding. The results of this study showed that, at the doses tested, the major components (±)-linalool and the related compound (±)-linalyl acetate were the two most repellent compounds of the seven tested. As the synthetic standards of linalool and linalyl acetate contain both possible isomers, the conclusion is that they must contain the stereoisomers found in the lavender oil. However, further work is needed to confirm the stereochemistry of the compounds in the oil and their behavioural activity.

Both linalool and linalyl acetate are floral volatiles found ubiquitously in the plant kingdom and different concentrations result in either attractancy or repellency for a variety of insects. There are many references to their importance in plant/insect interactions (e.g. Henning et al. 1992; Groot et al. 1999; Omura et al. 2000; Koschier and Sedy 2003). There is the further possibility that specific ratios of these two compounds are responsible for the repellent effect of *L. angustifolia* oil. In addition to confirming the activity of the correct stereoisomers as described above, it would be of interest to test the response of *M. aeneus* to varying ratios of these compounds, to determine whether there is a point at which maximum repellency is observed. Insects can determine ratios of volatile chemicals by comparing the stimulation of one type of receptor cell with that of another (Blight et al. 1995b; Bruce et al. 2005). For example, single cell recordings (SCR) (Wadhams 1990) identified 166 responding olfactory cells on the antenna of the cabbage seed weevil, *Ceutorhynchus assimilis* Paykull, most of which showed high specificity in their response profiles (Blight et al. 1995b). There were consistent pairings of specific cell types, for example, methyl salicylate with isothiocyanates, indicating that specific ratios of these compounds are likely to be important in the chemical ecology of this species. Electrophysiological studies have not yet demonstrated paired receptor cells on *M. aeneus* antennae (Blight et al. 1995a), although their existence seems likely.

Despite the lack of statistically significant avoidance patterns by *M. aeneus* to 3-octanone in the olfactometer, a clear behavioural pattern (turning away at the boundary of the odour stream) was observed. This is a general fungal derived volatile (Cardoza et al. 2002), so its detection by *M. aeneus* could provide a mechanism for avoidance of infected host plants. 3-Octanone also occurs in alfalfa floral volatiles and was repellent to honeybees when tested alone (Henning and Teuber 1992).

Previous investigations into the volatile chemicals detected by pollen beetles have identified repellent compounds. Volatiles produced by the host oilseed rape plant, *Brassica napus*, were tested individually and although most showed attractancy, there were some that had a repellent effect (Smart and Blight 2000). The repellents were mainly volatile fatty acid derivatives, including 1-octen-3-ol, 1-hexanol, (*Z*)-3-hexen-1-ol, 1-pentanol and *cis*-jasmone.

Botanical repellents are receiving increased interest (Isman 2006) and there are several semiochemical based push-pull systems for insect pest control that have been field tested, and for which the chemical basis underlying their efficacy has been established (Cook et al. 2007b). The work presented in this paper forms part of an ongoing research programme into the use of attractant and repellent compounds for use in push-pull crop protection strategies and has been followed up with field testing, which is showing encouraging results (Alice Mauchline, unpublished results). The information presented here identifies individual chemicals that are likely to be involved in the efficacy of lavender as a repellent and a future research path could be to maximise this effect by creating the optimal blend of the active compounds. This study presents a rational approach to investigating the chemical basis of repellent non-host plant volatiles, for assessing the

behavioural effects of such repellent volatiles on insects and in the development of push-pull crop protection strategies.

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References

Agelopoulos N, Birkett MA, Hick AJ, Hooper AM, Pickett JA, Pow EM, Smart LE, Smiley DWM, Wadhams LJ, Woodcock CM (1999) Exploiting semiochemicals in insect control. Pestic Sci 55:225–235

Al Abassi S, Birkett MA, Pettersson J, Pickett JA, Wadhams LJ, Woodcock CM (2000) Response of the seven-spot ladybird to an aphid alarm pheromone and an alarm pheromone inhibitor is mediated by paired olfactory cells. J Chem Ecol 26:1765–1771

Bernays EA, Chapman RF (1994) Host–plant selection by phytophagous insects. Chapman & Hall, New York

Blight MM, Smart LE (1999) Influence of visual cues and isothiocyanate lures on capture of the pollen beetle, *Meligethes aeneus* in field traps. J Chem Ecol 25:1501–1516

Blight MM, Pickett JA, Ryan J, Wadhams LJ, Woodcock CM (1995a) Recognition of oilseed rape volatiles by pollen beetles, *Meligethes* spp.: electrophysiological and chemical studies. In: Proceedings, GCIRC 9th international rapeseed congress 3, pp 1043–1045

Blight MM, Pickett JA, Wadhams LJ, Woodcock CM (1995b) Antennal perception of oilseed rape, *Brassica napus* (Brassicaceae), volatiles by the cabbage seed weevil *Ceutorhynchus assimilis* (Coleoptera: Curculionidae). J Chem Ecol 21:1649–1664

Bruce TJA, Martin JL, Pickett JA, Pye BJ, Smart LE, Wadhams LJ (2003) *cis*-Jasmone treatment induces resistance in wheat plants against the grain aphid, *Sitobion avenae* (Fabricius) (Homoptera: Aphididae) Pest Manage Sci 59:1031–1036

Bruce TJA, Wadhams LJ, Woodcock CM (2005). Insect host location: a volatile situation. Trends Plant Sci 10:269–274

Cardoza YJ, Alborn HT, Tumlinson JH (2002) In vivo volatile emissions from peanut plants induced by simultaneous fungal infection and insect damage. J Chem Ecol 28:161–174

Cook SM, Bartlet E, Murray DA, Williams IH (2002) The role of pollen odour in the attraction of pollen beetles to oilseed rape flowers. Entomol Exp Appl 104:43–50

Cook SM, Rasmussen HB, Birkett MA, Murray DA, Pye BJ, Watts NP, Williams IH (2007a) Behavioural and chemical ecology underlying the success of turnip rape (*Brassica rapa*) trap crops in protecting oilseed rape (*Brassica napus*) from the pollen beetle (*Meligethes aeneus*). Arthropod-Plant Interactions 1:57–67

Cook SM, Jönsson M, Skellern MP, Murray DA, Anderson P, Powell W (2007b) Responses of *Phradis* parasitoids to volatiles of lavender, *Lavandula angustifolia* – a possible repellent for their host, *Meligethes aeneus*. BioControl 52:591–598

Cook SM, Khan ZR, Pickett JA (2007c) The use of push-pull strategies in integrated pest management. Annu Rev Entomol 52:375–400

Couty A, Kaiser L, Huet D, Pham-Delegue MH (1999) The attractiveness of different odour sources from the fruit- host complex on *Leptopilina boulardi*, a larval parasitoid of frugivorous *Drosophila* spp. Physiol Entomol 24:76–82

Free JB, Williams IH (1978) The responses of the pollen beetle, *Meligethes aeneus*, and the seed weevil, *Ceutorhynchus assimilis*, to oilseed rape, *Brassica napus* and other plants. J Appl Ecol 15:761–774

Groot AT, Timmer R, Gort G, Lelyveld GP, Drijfhout FP, Van Beek TA, Visser JH (1999). Sex-related perception of insect and plant volatiles in *Lygocoris pabulinus*. J Chem Ecol 25:2357–2371

Hansen LM (2003) Insecticide-resistant pollen beetles (*Meligethes aeneus* F) found in Danish oilseed rape (*Brassica napus* L) fields. Pest Manage Sci 59:1057–1059

Henning JA, Teuber LR (1992) Combined gas-chromatography - electroantennogram characterization of alfalfa floral volatiles recognized by honey-bees (Hymenoptera, Apidae). J Econ Entomol 85:226–232

Henning JA, Peng YS, Montague MA, Teuber LR (1992) Honey-bee (Hymenoptera, Apidae) behavioral response to primary alfalfa (Rosales, Fabaceae) floral volatiles. J Econ Entomol 85:233–239

Isman MB (2006) Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annu Rev Entomol 51:45–66

Koschier EH, Sedy KA (2003) Labiate essential oils affecting host selection and acceptance of *Thrips tabaci* Lindeman. Crop Prot 22:929–934

Maddrell SHP (1969) Secretion by the Malpighian tubules of *Rhodnius*. The movement of ions and water. J Exp Biol 51:71–97

Mauchline AL (2003) Behavioural and chemical ecology of *Meligethes aeneus*: Effects of non-host plant volatiles. PhD Thesis, The Open University

Mauchline AL, Osborne JL, Martin AP, Poppy GM, Powell W (2005) The effects of essential oil volatiles on the behaviour of the pollen beetle *Meligethes aeneus*. Entomol Exp Appl 114: 181–188

Miller JR, Cowles RS (1990) Stimulo-deterrent diversion: a concept and its possible application to onion maggot control. J Chem Ecol 16:3197–3212

Omura H, Honda K, Hayashi N (2000) Floral scent of *Osmanthus fragrans* discourages foraging behaviour of cabbage butterfly, *Pieris rapae*. J Chem Ecol 26:655–666

Pettersson J (1970) An aphid sex attractant I. Biological studies. Entomol Scand 1:63–73

Pickett JA (1990) Gas chromatography-mass spectrometry in insect pheromone identification: three extreme cases. In: McCaffery AR, Wilson ID (eds) Chromatography and isolation of insect hormones and pheromones. Plenum Press, New York, pp 209–309

Pickett JA, Wadhams LJ, Woodcock CM (1997) Developing sustainable pest control from chemical ecology. Agric Ecosyst Environ 64:149–156

Pyke B, Rice M, Sabine G, Zalucki M (1987) The push-pull strategy – behavioural control of *Heliothis*. Australian Cotton Grower May–July

Ruther J, Thiemann K (1997) Response of the pollen beetle *Meligethes aeneus* to volatiles emitted by intact plants and conspecifics. Entomol Exp Appl 84:183–188

Smart LE, Blight MM (2000) Response of the pollen beetle, *Meligethes aeneus*, to traps baited with volatiles from oilseed rape, *Brassica napus*. J Chem Ecol 26:1051–1064

Smart LE, Blight MM, Hick AJ (1993) Development of a monitoring system for the cabbage seed weevil and the pollen beetle. IOBC/WPRS Bull 16:351–354

Smart LE, Blight MM, Ryan J (1995) Response of pollen beetles, *Meligethes* spp., to volatiles from *Brassica napus.* In: Proceedings 9th international rapeseed congress, pp 122–124

Vet LEM, van Lenteren JC, Heymans M, Meelis E (1983) An air-flow olfactometer for measuring olfactory responses of

hymenopterous parasitoids and other small insects. Physiol Entomol 8:97–106

Wadhams LJ (1990) The use of coupled gas chromatography: electrophysiological techniques in the identification of insect pheromones. In: McCaffery AR, Wilson ID (eds) Chromatography and isolation of insect hormones and pheromones. Plenum Press, New York, pp 289–298

Wadhams LJ, Angst ME, Blight MM (1982) Responses of the olfactory receptors of *Scolytus scolytus* (F.) (Coleoptera: Scolytidae) to the stereoisomers of 4-methyl-3-heptanol. J Chem Ecol 8:477–492

Winfield AL (1992) Management of oilseed rape pests in Europe. Agric Zool Rev 5:51–94