

Factors influencing the aggregative response of the blue willow beetle, *Phratora vulgatissima*

Lori Peacock, Mervyn Lewis & Sarah Herrick

IACR-Long Ashton Research Station, Department of Agricultural Science, University of Bristol, Long Ashton, Bristol, BS41 9AF, UK

Accepted: September 7, 2000

Key words: host-finding behaviour, green leaf volatiles, chemical ecology, semiochemicals, aggregation kairomones, short-rotation coppice

Abstract

Factors influencing the aggregative response of the blue willow beetle, *Phratora vulgatissima* (L.) (Coleoptera: Chrysomelidae), on potted plants of *Salix dasyclados* (Wimm) were investigated in the field. A significantly higher female beetle density was recorded on plants that had a combination of beetle-feeding damage and conspecific beetles present as compared with control plants and plants having only damage or beetles. Volatile chemicals emitted from undamaged *S. dasyclados* (Wimm) leaves were found to be benzaldehyde and the green leaf volatiles (GLVs) cis-3-hexenyl acetate and cis-3-hexenol. Upon beetle-feeding damage, there was a higher concentration of these compounds and an increase in the number of different volatile chemicals emitted. These results are discussed in relation to the behavioural ecology of the willow beetle.

Introduction

Many insect species aggregate, an important factor in explaining insect distribution (Bach & Carr, 1990). Aggregated distributions can be a result of multiple behaviours such as beetle oriented movement to attractions like pheromones or plant kairomones with a subsequent arrest and possible feeding due to the palatability of the plant. Examples of such attractions can be found in Giblin-Davis et al. (1996), Loughrin et al. (1996), Landolt (1997), Schütz et al. (1997), Cerda et al. (1998) and Fadamiro et al. (1998).

The chrysomelid willow beetles, *Phratora vulgatissima* (L.) and *P. vitellinae* (L.) have been shown to have an aggregated distribution on host plants and to be highly mobile (Peacock et al., 1999). Beetle density on individual trees in a willow plantation was positively correlated during the early season (indicating that trees with high beetle density at one time tended to have a high beetle density at the next assessment and vice versa) with indications of subsequent movement to different trees (as the correlation became negative). The willow beetle overwinters generally away from

the willow plantation in cracks, crevices, loose bark, etc. (Kendall et al., 1996). In early spring (April–May), they leave these sites and enter the plantation where they eat, mate and lay eggs. In 2 weeks the eggs hatch and the larvae feed on willows where they develop through three instars before pupating in the soil. Two weeks later, adults emerge and commence feeding until late summer/early autumn when they return to overwintering sites. A preliminary study revealed that *P. vulgatissima* males were significantly attracted to *Salix viminalis* Bowles Hybrid with no firm conclusions drawn for females due to the lack of available data (Ansell, 1996). This attraction was synergistic with the addition of a minimum of five females.

These willow beetles are the most serious and widespread insect pests of willows and poplars grown as short-rotation coppice (SRC). These crops are increasing in importance as a potential source of alternative energy as they produce no net CO₂ emissions. The UK is legally bound to reduce gases implicated in global warming by 12.5% by 2010 following the 1997 Kyoto Climate Change Conference (Armstrong, 1999). It is suggested that biomass crops could sub-

stantially contribute to the reduction of these gases, with a potential cultivated area in the EU of 6 million hectares (European Commission, 1997). Application of pesticides to coppice is difficult, environmentally undesirable and probably uneconomic (Armstrong, 1999) as it is a low-value crop. Therefore, current research places an emphasis on non-chemical pest control methods (Hunter et al., 1996).

The use of semiochemicals that influence insect behaviour is a promising method of non-chemical crop protection. The success of such a technique necessitates an understanding of insect/insect/plant interactions and insect chemical ecology (Pickett, 1998). Moreover, understanding what factors influence aggregation patterns can help explain herbivore/host-plant interactions.

The current study was undertaken to investigate whether certain factors can influence the aggregative response of *P. vulgatissima* on a favourable willow host, *Salix dasyclados* (Wimm). Specifically, we sought to determine whether the density of beetles on plants was affected by the presence of conspecifics and if the sex of the conspecifics affected this density. We also examined whether beetle-feeding damage, both alone and coupled with conspecifics, influenced the beetle density. A concurrent study was made of the volatile emissions from potted *S. dasyclados* plants and the effects of beetle-feeding damage on these volatile chemicals.

Materials and methods

Plants, insects. Cuttings (25 cm) of *S. dasyclados* (Wimm) willows were taken from cold storage in February 1999, planted individually into 10 cm diameter pots and maintained in a greenhouse for 3½ months, at which time they were approximately 60 cm high (same height as coppiced field plants). *Phratora vulgatissima* beetles used in the net bags were collected from willows at IACR-Long Ashton Research Station, sexed for experiment two, and held for a few days in cages at 15 °C and L16:D8.

Field assays. Three separate field experiments were performed between 15–27 May 1999, in a bare patch of ground 0.5 m adjacent to the willows of the National Willow Collection at Long Ashton Research Station, which had a moderate to heavy beetle population (average of approximately 10–30 beetles/plant). Pots with willows as described below were submerged

into the soil, the pot top being at ground level, placed 40 cm apart and watered throughout the experiment to prevent desiccation.

The initial two experiments tested for pheromones emitted by the beetles, as the insects were not allowed to feed on leaves, thus reducing any potential confounding effects of feeding-induced kairomones. In the first experiment, the response of beetles to conspecifics was examined by placing 15 randomly collected *P. vulgatissima* adults in a mesh net bag, which was loosely tied to a willow plant, while the control plant had an empty bag attached. This was replicated 8 times. As it has been shown that the sex ratio for this beetle under natural field conditions is very nearly 1:1 (Kendall et al., 1996), it was assumed that half the beetles captured were male and half female (these will be referred to as mixed-sex). The second experiment, tested for a sex-specific response both of, and to, these beetles by attaching net bags containing either 15 male or 15 female *P. vulgatissima* (ten replicates of each), with no plants having an empty bag. In both experiments, plants were paired by treatments (either beetles/no beetles or males/females). For both experiments, beetles on each plant after 24 h were counted and collected in a perspex tube (and sexed in experiment two), and repeated after a further 24 h.

The third experiment tested for a synergistic beetle response to conspecifics and beetle-feeding damage with willow plants under the following five treatments tested for attractiveness to blue willow beetle: (1) undamaged plant; (2) undamaged plant with a mesh net bag containing 15 mixed-sex beetles, which were not allowed to feed on leaves; (3) plant that had been placed in field for 48 h with approximately 25 beetles of mixed-sex and then, after feeding damage, had beetles removed; (4) plant as in number three, yet with a net bag containing 15 mixed-sex beetles that were not allowed to feed on leaves; (5) undamaged plant with net bag containing 15 mixed-sex beetles confined to a single leaf and able to feed. The experiment was arranged as 10 blocks of randomised treatments. The beetles on each plant after 24 h were collected and sexed and this repeated after a further 24 h. The fifth treatment was included to test whether current feeding damage elicited a different response from 48 h damage.

It is assumed that the density of beetles on plants after 24 h relates to the relative attractiveness of that plant. The willow, *S. dasyclados*, was the most preferred willow genotype by *P. vulgatissima* adults in a study of 105 different willows (Kendall & Wiltshire,

1996), so beetles are likely to remain to feed on this favoured willow. In a dispersal study in a glasshouse, beetles were generally found to remain on an individual tree of a favourable willow genotype for a few days before moving to another (L. Peacock, unpubl.).

As the data was in the form of counts it was analysed by fitting a Poisson Generalised Linear Model (GLM) with a log link function, using Genstat 5 [Release 4.1 Third Edition, ©Lawes (IACR-Rothamsted) 1997]. Chi-squared values and the corresponding degrees of freedom and P-values for treatment (adjusted for blocking factors) obtained from the analysis of deviance are presented in the text. For experiments one and two, treatment means and approximate standard errors are also given in the text; for experiment three these are presented in a figure. However, in a few cases the model showed significant evidence of lack of fit. This was adjusted for by performing an F-test in the analysis of deviance (which takes into account the actual residual deviance), rather than using a Chi-squared test, and the approximate standard errors of the means are based on the residual deviance, rather than on a dispersion parameter of one. In each case, F-values and their corresponding P-values from the analysis of deviance are presented in the text. For experiment three, a square root transformation was performed on the means in order to stabilise the standard errors so that the means could be compared using a *t*-test.

Volatile compound collection, analysis. The emissions of volatile compounds from *S. dasyclados* were collected using an Ametek MG-4 air sampling pump (Shaw City Limited) sucking at 2 ml min^{-1} through a Tenax-GC trap (Scientific Glass Engineering). A system blank was performed by placing the trap within an empty inverted glass beaker (155 mm \times 90 mm) held by a retort stand that had the opening loosely sealed with aluminium foil, and collecting the headspace for 90 min. Collection of the headspace from the willows was performed for the same length of time by placing the terminal 20 cm of a $3 \frac{1}{2}$ month-old willow plant (approx. 60 cm high) in the glass beaker held by a retort stand and the opening loosely sealed with aluminium foil. The trap was placed within the beaker, avoiding contact with the plant and glass sides, and air collected from the headspace. The trap was desorbed in a SGE Unijector fitted to a Carlo Erba Mega GC at 250 °C for 4 min into a 50 m \times 0.32 mm \times 0.3 μm Superox II column (Alltech) held at 5 °C. The GC temperature programme was 5 °C, heating to 50 °C

(at an increment of 20 °C min^{-1}), then heating to 210 °C (at an increment of 5 °C min^{-1}). This was then held for 30 min. Helium was used as the carrier gas and the column was connected to a Kratos MS80RFA mass spectrometer. Source temperature was 200 °C and interface temperature was 250 °C. A scan speed of 1 s/decade from 500–40 daltons and ionising voltage of 70 eV were used and the instrument operated in electron impact mode.

Once the volatiles had been collected, the plant was removed and had 10 *P. vulgatissima* adults confined in a net on to each of the first five fully expanded leaves for 24 h. All leaves had approximately 50% of the leaf area damaged, as assessed visually. This is a plausible damage level as a high population density of beetles is capable of total defoliation under field conditions (T. Hunter, pers. comm.). Beetles were removed and the volatiles from this newly damaged plant were measured as before. This procedure was repeated on three different plants.

Compound identities were confirmed by a library search against the Wiley/NBS Registry of Mass Spectral Data, that which contains 1.3×10^5 compounds, and the area under each peak was estimated (the programme presents this in units). To estimate the concentration of compounds detected (in ng), stock standard solutions of the two major volatiles detected (cis-3-hexen-1-ol and cis-3-hexenyl acetate) (both Sigma) were made up in ethyl acetate. Serial dilutions of these solutions were then used to prepare the calibration graphs to convert area to approximate concentration. Differences in the quantity of each compound between damaged and undamaged plants were compared using a *t*-test.

Results

Field assays. The majority of willow beetles were feeding or mating at the time of collection, with the occasional female ovipositing. The initial two experiments showed indications (albeit non-significant) of an attractant effect of beetles to conspecifics. In the first experiment, more beetles were attracted to plants with conspecifics (4.0 ± 1.00 [mean no. of beetles \pm SE], $n = 8$) than to plants with none (1.5 ± 0.61) ($F = 4.73$, $\text{df} = 1, 7$; $P = 0.066$). In experiment two, which tested for a sex-specific attractant effect, plants with confined females attracted more beetles (7.1 ± 1.35 , $n = 10$) than did plants with confined males (4.4 ± 1.10 , $n = 10$) although the differences were

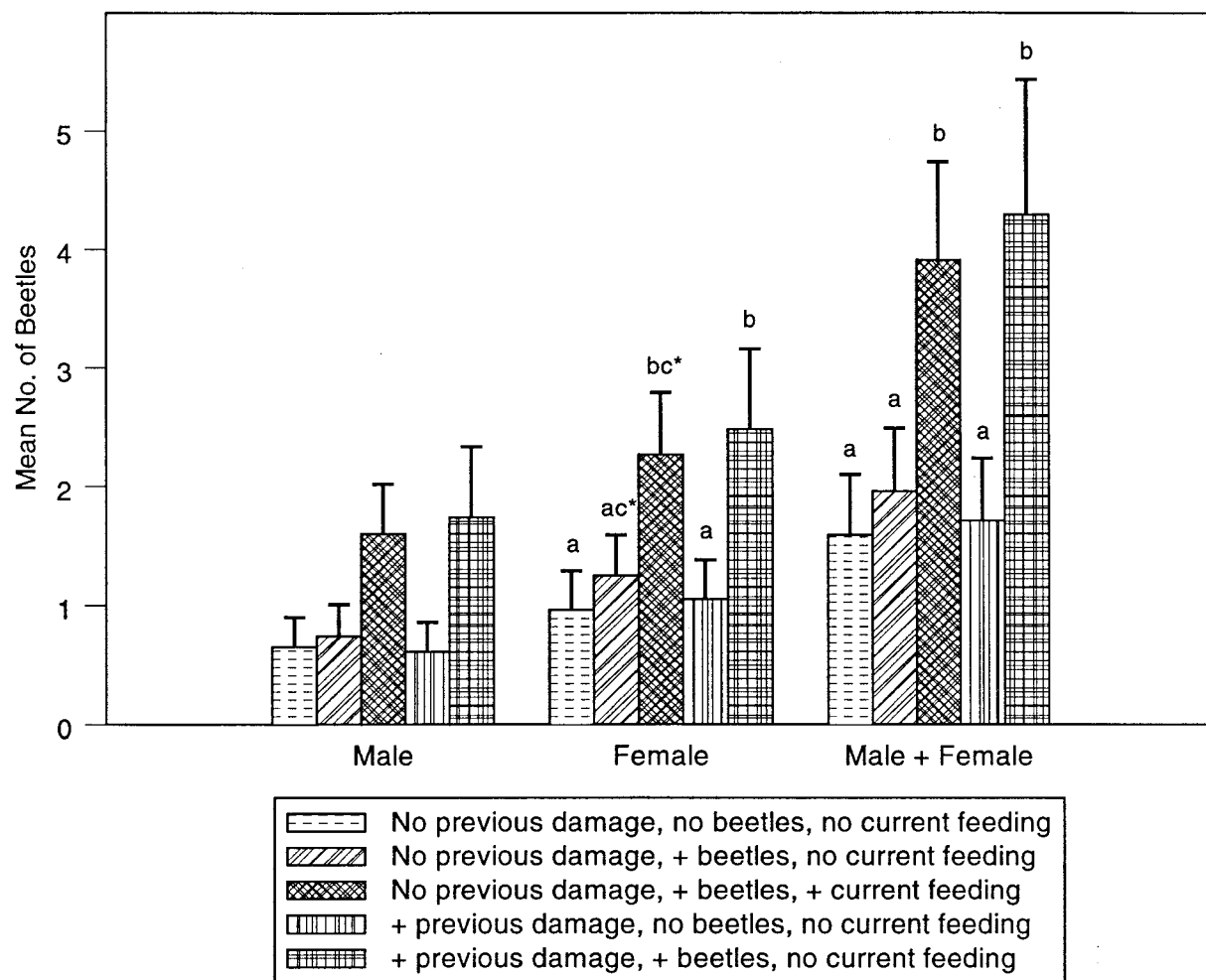


Figure 1. Mean number (and s.e.) of males, females and total *Phratora vulgatissima* collected on potted plants of *Salix dasyclados*. There were four treatments with yes/no previous feeding damage, yes/no confined non-feeding beetles and one treatment with no previous damage yet confined beetles allowed to feed. Bars with different letters above are significantly different ($P < 0.05$, $n = 10$; t -test), * ($P = 0.08$, $n = 10$; t -test).

not statistically significant ($F = 2.42$, $df = 1, 8$; $P = 0.16$). However, female beetles were possibly more responsive than males to conspecific females, as more females landed on plants with confined females than confined males (3.9 ± 0.63 female beetles on plants with females, 2.4 ± 0.52 female beetles on plants with males) ($\chi^2 = 3.3$, $df = 1$; $P = 0.069$). This is in contrast to the male beetles where the differences were not as great (3.2 ± 0.83 male beetles on plants with females, 2.0 ± 0.66 male beetles on plants with males) ($F = 1.38$, $df = 1, 8$; $P = 0.27$).

Results from the GLM analyses for experiment three showed significant treatment effects ($F = 3.00$, $df = 4, 31$; $P = 0.033$). Subsequent t -tests showed that significantly ($P < 0.05$, $n = 10$) more beetles landed

on plants that had the combination of confined beetles and current or previous willow beetle feeding damage than on plants with none or only damage or beetles present (Figure 1). There was no significant difference in beetle density between treatments with beetles together with previous or current feeding damage, so beetles did not need to be actively feeding to elicit an aggregative response. Female *P. vulgatissima* were more responsive than males to plants with different treatments of conspecifics and feeding damage ($\chi^2 = 9.67$, $df = 4$; $P = 0.046$ for females; $\chi^2 = 8.53$, $df = 4$; $P = 0.074$ for males). Subsequent t -tests again showed that significantly ($P < 0.05$, $n = 10$) more females landed on plants that had confined beetles and

Table 1. The mean retention time and quantity (ng) of volatile compounds emitted from the top 20 cm of stem of undamaged and 24-h beetle-feeding damaged *Salix dasyclados* willow plants ($n = 3$)

Compound	Retention time (min)	Quantity (ng)			
		Undamaged	Damaged	<i>t</i>	P*
3-Hexenal	12.1	n.d.	14.3		
2-Hexenal	14.1	n.d.	38.7		
<i>n</i> -hexyl acetate	15.4	n.d.	9.3		
cis-3-hexenyl acetate	16.8	0.7	34.3	−2.07	0.087
Trans-2-hexenyl acetate	17.2	n.d.	7.3		
<i>n</i> -hexanol	17.5	n.d.	15.3		
Cis-3-hexen-1-ol	18.5	1.3	15.7	−3.97	0.029
Trans-2-hexen-1-ol	19.2	n.d.	8.3		
Hexenyl pentanoate	21.1	n.d.	2.7		
Benzaldehyde	22.4	0.5	55.3	−8.66	0.007

n.d. = not detectable.

**t* and P values from *t*-test comparison of differences between undamaged and damaged plants.

feeding damage, with no differences between age of damage (Figure 1).

Volatile compound collection, analysis. The examination of volatile compounds showed a higher level and complexity of volatile emissions from the damaged, as compared with the undamaged willow (Table 1). There were three major emissions from all the undamaged plants ($n = 3$), these being benzaldehyde and the green leaf volatiles cis-3-hexenyl acetate and cis-3-hexenol. These chemicals increased to varying degrees in the headspace emissions of all the damaged plants, the first and last being significantly higher in the damaged plants, whereas the acetate was higher at $P = 0.087$. This lack of significance for cis-3-hexenyl acetate may be due to the variability between plants, especially for the relatively higher quantity of this compound upon damage in the first plant tested (67 ng compared with 15 and 21 ng for the other plants). The large differences in the quantity of this chemical between undamaged and damaged plants does suggest that this compound increases upon beetle-feeding damage similar to the other two compounds (Table 1). There were a number of other volatiles recorded in the collections from damaged plants that were not detectable from undamaged plants (Table 1).

Discussion

Phratora vulgatissima has been shown to be clustered on a per plant basis throughout the growing season (Peacock et al., 1999), a spatial pattern typical of the Chrysomelidae, a family of insects that often forms aggregations on its food plants (Rowell-Rahier & Pasteels, 1986). A preliminary laboratory study revealed that *P. vulgatissima* males were attracted to a favourable willow genotype, this attraction synergistic with the addition of five females (Ansell, 1996). The present study shows a comparable synergistic effect of willow beetle feeding-damage and presence of conspecifics under field conditions.

Significantly more *P. vulgatissima* overall (and females) were attracted to plants with conspecific beetles confined on plants with 2-day old or current feeding damage than to plants with beetles alone or feeding damage alone or neither present. The necessity of a combination of beetles and damage to elicit this female aggregative response could explain the lack of significant results (yet close at $P = 0.066$ and $P = 0.069$) for the first two experiments in which beetles were not allowed to feed. These results are similar to those of other researchers who suggest that insects are attracted to pheromones, plant kairomones, or a combination of these and even to feeding-induced kairomones to help guide them to a preferred host on which to feed, mate or oviposit (Loughrin et al., 1996; Loughrin et al., 1997; Schütz et al., 1997; Rojas, 1999a). For instance, the chrysomelid Colorado

Potato Beetle (Schütz et al., 1997) and female cabbage moth (*Mamestra brassicae*) (Rojas, 1999b) have been shown to be strongly attracted to green leaf volatiles (GLVs) which greatly increase upon damage. One of the two GLVs which elicited upwind flight and landing in mated females (as opposed to virgin females and males) of *M. brassicae*, was cis-3-hexenyl acetate, a possible attractant for oviposition (Rojas, 1999b). The Colorado Potato Beetle has recently been shown to be attracted to the GLV cis-3-hexenyl acetate with cis-3-hexenol attractive to its natural enemies (Coghlan, 2000). These mirror our results of a higher level and complexity of GLVs from damaged, as compared with undamaged, willow leaves and the same two GLVs being identified.

The attraction of the willow beetle (especially female) to a combination of conspecifics and feeding-damage can be explained, in part, by their behavioural ecology. As the willow beetle overwinters away from willow plantings, it is necessary each spring to re-colonise willows in order to find a suitable host and mate, thus may have evolved to respond to pheromones and kairomones. Response to these stimuli could lead the beetle to an area of a suitable host and mate which would reduce the time wasted in random chance landings. Once in the plantation, hosts and mates would be easier to find, with the likely result of a lowering of aggregative behaviour. This was found to occur as beetles were strongly aggregated in early spring, with weakly aggregated distributions recorded as the season progressed (Peacock et al., 1999). Our study was performed with adult beetles during the peak of emergence from overwintering sites (early May), mating and ovipositing. This is the most likely time of insect response to pheromones and kairomones. Female willow beetles were more attractive and responsive than were male willow beetles as colonisation was greatest on plants containing females, significantly so by conspecific females. While there are several species of beetles that use female sex attractant pheromones (Landolt, 1997), generally there is more intersex attraction within insect species, with the attraction of both sexes usually resulting from male-produced aggregation pheromones (Landolt, 1997). However, some studies do show the attraction of both sexes of an insect species to females and female volatiles (Hardie et al., 1996; Warren et al., 1996).

In conclusion, the aggregative behaviour of *P. vulgatissima* under field conditions was influenced by the presence of conspecifics and willow beetle feeding

damage. Preliminary results suggest that willow beetles may be responding to the combination of feeding-induced GLVs and beetle pheromones. Further studies are currently underway to simultaneously measure plant volatiles and willow beetle antennal responses to test these compounds on beetle behaviour.

Acknowledgements

Many thanks to Judy Harris for valuable systematic field assistance, David Glen, Angela Karp and three anonymous referees for helpful comments and to The Ministry of Agriculture, Fisheries and Food (project code NF0406) for funding this work. IACR receives grant-aided support from the Biotechnology and Biological Sciences Research Council of the United Kingdom.

References

- Ansell, A., 1996. *P. vulgatissima* olfactory cues: how does it locate a mate? Unpublished Placement report, IACR-Long Ashton Research Station, Bristol, UK.
- Armstrong, A., 1999. Establishment of short rotation coppice. Practice Note FCPN7, Forestry Commission, Edinburgh, UK.
- Bach, C. E. & D. S. Carr, 1990. Aggregation behaviour of a willow flea beetle, *Altica subplicata* (Coleoptera: Chrysomelidae). The Great Lakes Entomologist 23: 65–76.
- Cerda, H., K. Mori, T. Nakayama & K. Jafe, 1998. A synergistic aggregation pheromone component in the banana weevil *Cosmopolites sordidus germar* 1824 (Coleoptera: Curculionidae). Acta Cientifica Venezolana. 49: 201–203.
- Coghlan, A., 2000. Double-crossed. A potato pest's favourite fragrance could be its undoing. New Scientist 156: 14.
- European Commission, 1997. Energy for the future. Renewable sources of energy. White paper for a community strategy and action plan, COM (97) 599 final (26/11/97).
- Fadamiro, H. Y., I. Gudrups & R. J. Hodges, 1998. Upwind flight of *Prostephanus truncatus* is mediated by aggregation pheromone but not food volatiles. Journal of Stored Products Research 34: 151–158.
- Giblin-Davis, R. M., A. C. Oehlschlager, A. Perez, G. Gries, R. Gries, T. J. Weissling, C. M. Chinchilla, J. E. Pena, R. H. Hallett, H. D. Pierce & L. M. Gonzalez, 1996. Chemical and behavioural ecology of palm weevils (Curculionidae: Rhynchophorinae). Florida Entomologist 79: 153–167.
- Hardie, J., J. R. Storer, F. J. Cook, C. A. M. Campbell, L. J. Wadhams, R. Lilley & L. Peace, 1996. Sex pheromone and visual trap interactions in mate location strategies and aggregation by host-alternating aphids in the field. Physiological Entomology 21: 97–106.
- Hunter, T., D. J. Royle & G. M. Arnold, 1996. Variation in the occurrence of rust (*Melampsora* spp.) and other diseases and pests, in short-rotation coppice plantations of *Salix* in the British Isles. Annals of Applied Biology 129: 1–12.
- Kendall, D. A. & C. W. Wiltshire, 1996. An applied study of clonal resistance to willow beetle attack in SRC willows. Report for ETSU (DTI) Biofuels Study B/M4/00532/27/REP.

- Kendall, D. A., C. W. Wiltshire & M. Butcher, 1996. Phenology and population dynamics of willow beetles (Coleoptera: Chrysomelidae) in short-rotation coppiced willows at Long Ashton. Report for ETSU (DTI) Biofuels Study B/M4/00487/14.
- Landolt, P. J., 1997. Sex attractant and aggregation pheromones of male phytophagous insects. *American Entomologist* 43: 12–22.
- Loughrin, J. H., D. A. Potter, T. R. Hamilton-Kemp & M. E. Byers, 1996. Role of feeding-induced plant volatiles in aggregative behaviour of the Japanese Beetle (Coleoptera: Scarabaeidae). *Environmental Entomology* 25: 1188–1191.
- Loughrin, J. H., D. A. Potter, T. R. Hamilton-Kemp & M. E. Byers, 1997. Diurnal emission of volatile compounds by Japanese Beetle-damaged grape leaves. *Phytochemistry* 45: 919–923.
- Peacock, L., S. Herrick & P. Brain, 1999. Spatio-temporal dynamics of willow beetle (*Phratora vulgatissima*) in short-rotation coppice willows grown as monocultures or a genetically diverse mixture. *Agricultural and Forest Entomology* 1: 287–296.
- Pickett, J. A., 1998. Pest semiochemicals in arable crop protection. *Pesticide Science* 54: 290–299.
- Rojas, J. C., 1999a. Influence of host plant damage on the host-finding behaviour of *Mamestra brassicae* (Lepidoptera: Noctuidae). *Environmental Entomology* 28: 588–593.
- Rojas, J. C., 1999b. Electrophysiological and behavioural responses of the cabbage moth to plant volatiles. *Journal of Chemical Ecology* 25: 1867–1883.
- Rowell-Rahier, M. & J. M. Pasteels, 1986. Economics of chemical defense in Chrysomelinae. *Journal of Chemical Ecology* 12: 1189–1203.
- Schütz, S., B. Weißbecker, A. Klein & H. E. Hummel, 1997. Host plant selection of the Colorado Potato Beetle as influenced by damage induced volatiles of the potato plant. *Naturwissenschaften* 84: 212–217.
- Warren, C. E., D. L. Wood, S. J. Seybold, A. J. Storer & W. E. Bros, 1996. Olfactory responses of *Ips plastographus maritimus* Lanier (Coleoptera: Scolytidae) to insect and host-associated volatiles in the laboratory. *Journal of Chemical Ecology* 22: 2299–2316.