

The effect of sex and maturation on cuticular semiochemicals in *Monochamus scutellatus* (Coleoptera: Cerambycidae)

Bekka S. Brodie,¹ Jacob D. Wickham, Stephen A. Teale

Abstract—Contact pheromones are a subset of a complex mixture of hydrocarbons secreted on the cuticle and play an important role in the mating behaviour of several cerambycid species (Coleoptera: Cerambycidae). In this study, we investigated the relationship between maturation (newly eclosed and sexually mature) adult *Monochamus scutellatus* (Say) beetles and sex and the composition of the cuticular hydrocarbon blend to determine if this information is encoded in the blend and potentially available for communication purposes. Whole-body extracts of unfed females, and both mature females and males were analysed by gas chromatography mass spectrometry to identify and quantify the components of the cuticular hydrocarbons. There were no unique compounds present in any of the three groups, but discriminant analysis indicated that the relative proportions of the cuticular hydrocarbon components were unique for each group.

Résumé—Les phéromones de contact sont un sous-ensemble du mélange complexe d'hydrocarbures sécrétés par la cuticule; elles jouent un rôle important dans le comportement sexuel de plusieurs espèces de cérambycides. Dans cette étude, nous explorons la relation entre la maturité (récemment éclos ou sexuellement matures), le sexe et la composition du mélange d'hydrocarbures cuticulaires afin de déterminer si ces informations sont contenues dans le mélange et pourraient être utilisées comme élément de communication. Des extractions du corps de femelles non nourries, et de femelles et de mâles matures ont été analysées par CPG/SM afin d'identifier et de quantifier les composants des hydrocarbures cuticulaires. Il n'y avait aucun élément unique à chacun des trois groupes, mais une analyse discriminante montre que la proportion relative des différents hydrocarbures cuticulaires était spécifique à chaque groupe.

Introduction

The white-spotted sawyer, *Monochamus scutellatus* (Say) (Coleoptera: Cerambycidae), is widely distributed in North America and damage forest resources in several ways. The larvae mine in the cambium, phloem, and xylem of host trees, causing substantial saw-log degradation and economic loss due to the large, deep holes in the xylem and the introduction of wood-staining fungi. For example, cerambycids, including *Monochamus*

Dejean species, cause degradation losses that total \$1.8–4.8 million United States of America dollars (USD) in the province of British Columbia, Canada alone (Allison *et al.* 2001). If these values were extrapolated to encompass all interior mills, annual losses would be \$293 million USD, \$43.6 million USD of which would be attributable to large wood-borers (Allison *et al.* 2001). *Monochamus* species are considered forest pests in other countries where they vector the pinewood nematode, *Bursaphelenchus xylophilus*

Received 27 February 2012. Accepted 20 June 2012.

B.S. Brodie,¹ College of Environmental Science and Forestry, State University of New York, 1 Forestry Drive, Syracuse, New York 13210, United States of America; and Simon Fraser University, 8888 University Drive, Burnaby, British Columbia V5A 1S6, Canada

J.D. Wickham, College of Environmental Science and Forestry, State University of New York, 1 Forestry Drive, Syracuse, New York 13210, United States of America; and Institute of Chemistry, Chinese Academy of Sciences, Zhongguancun, Beijing 100190, P.R. China

S.A. Teale, College of Environmental Science and Forestry, State University of New York, 1 Forestry Drive, Syracuse, New York 13210, United States of America

¹Corresponding author (e-mail: bbrodie@sfu.ca).
doi:10.4039/tce.2012.82

(Aphelenchida: Parasitaphelenchidae); the causal agent of pine-wilt disease (Linit 1989; Vallentgoed 1991). *Bursaphelenchus xylophilus* is indigenous to the United States of America and causes little damage to native North American pine species but has devastated pine forests in eastern Asia (Cram and Hanson 2004) and killed 90% of planted Scots pine, *Pinus sylvestris* Linnaeus (Pinaceae), in the mid-western United States of America (Gleason *et al.* 2000). Due to the economic significance of *Monochamus* species in countries that receive North American softwood products, significant resources have been dedicated to monitoring and controlling these insects in order to protect North American softwood exports. Monitoring strategies based on chemical attractants are generally efficient and cost-effective, but have not yet been developed for *M. scutellatus*.

Semiochemicals in the cuticular wax layer of insects have important roles communicating information about species, sex, and kinship (Blomquist *et al.* 1996; Howard and Blomquist 2005). Cuticular waxes are composed of complex mixtures that may include long-chain fatty acids, methyl esters, aliphatic alcohols, aldehydes, ketones, and hydrocarbons, which also protect the organism from desiccation (Edney 1967; Neville 1975; Jackson and Blomquist 1976; Gibbs 1998). The hydrocarbon composition of the insect cuticle is not constant throughout the lifespan of an insect and can change due to the environment or physiological factors, including but not limited to age, ovarian activity, or nutritional condition and habitat (Dillwith *et al.* 1983; Wakonigg *et al.* 2000; D'Etorre *et al.* 2006). Males of many species increase their probability of successfully mating by choosing mates based on the females' reproductive status (Thomas 2011).

Behavioural studies of cerambycid beetles showed that contact cuticular hydrocarbons play an important role in mate recognition (Hanks *et al.* 1996; Ginzel and Hanks 2003). Male long-horn beetles locate females on bark or foliage of host trees using other cues, and then use cuticular hydrocarbons and contact chemoreception to recognise the female as a potential mate. This behavioural mechanism has been documented in the subfamilies Prioninae (Barbour *et al.* 2007; Spikes *et al.* 2010), Cerambycinae (Ginzel and Hanks 2003), and Lamiinae (Wang 1998; Ginzel

and Hanks 2003; Zhang *et al.* 2003). Components of the contact sex pheromones may be unique to the wax layer of females (Ginzel *et al.* 2003a, 2003b; Lacey *et al.* 2008), or alternatively, the uniqueness may be encoded as sexual dimorphism in the relative abundance of a subset of the cuticular hydrocarbons (Howard and Blomquist 2005).

Here, we test the hypothesis that cuticular hydrocarbons encode information about sex and maturation status of females in *M. scutellatus*. Specifically, our objectives were: (1) to determine if information about sex and maturation is encoded as unique components in the hydrocarbon blends and (2) to determine if information about sex and maturation is encoded in the relative proportions of cuticular hydrocarbons.

Materials and methods

Collection and rearing

Red pine, *Pinus resinosa* Soland (Pinaceae), infested with *M. scutellatus* larvae were felled at Pratts Falls County Park in Pompey, New York, United States of America in September 2005 and logs were transported to the State University of New York College of Environmental Science and Forestry (SUNY-ESF) and stored at 4.5°C for 2 months to force larvae into diapause. Logs were then placed in emergence chambers at ~23°C.

Adult beetles emerged after 2–4 weeks and were placed in environmental chambers at 20–24°C with a 16:8 light/dark photoperiod. The date and sex of each beetle were recorded upon emergence. Beetles were allowed to feed on white pine, *Pinus strobus* Linnaeus (Pinaceae), shoots for ~1 week in environmental chambers before they were extracted.

Extraction of cuticular hydrocarbons

Cuticular hydrocarbons were individually extracted in pentane by the method of Ginzel *et al.* (2003b). Unfed females were separately extracted within 1 day of emergence with no access to food. We individually extracted cuticular hydrocarbons of three groups of virgin beetles: maturation-fed females ($n = 20$), unfed females ($n = 18$), and maturation-fed males ($n = 20$) of *M. scutellatus*.

Hydrocarbon identification

Cuticular hydrocarbon samples were analysed with a gas chromatography mass spectrometry (Series II 5890 gas chromatograph and HP 5971 MSD, Hewlett Packard, Palo Alto, California, United States of America) with the temperature program at 40°C for 1 minute, then 10°C/minute to 300°C for 10 minutes. Injector and quadrupole mass spectrometer detector temperatures were 300°C and 280°C, respectively. Electron impact (70 eV) mass spectra were obtained with a scan range of 40–500 *m/z*. For each sample analysis, a 1.0 µl aliquot was injected. Samples were also analysed using chemical ionisation mass spectrometry with isobutene (Hewlett Packard Model 5989B GC/MS, Agilent, Santa Clara, California, United States of America) under the same temperature program as described above. In comparing the ratio of both maturation-fed females and unfed females an internal standard of heptadecane was used to quantify hydrocarbons.

Data analyses

The relative quantities of cuticular hydrocarbons of maturation-fed males, fed females, and unfed females were analysed using discriminant analysis to determine if the hydrocarbon signatures of the three treatment groups were distinct. Statistical analyses were performed using program Statistica (Statsoft, Inc., Tulsa, Oklahoma, United States of America). Structure coefficients (correlations between the discriminating variables and the discriminate groups) were used to assess the importance of individual compounds in the different groups. To avoid limitations inherent to the analysis of compositional data, peak areas were transformed prior to the analysis using the formula of Aitchinson (1986):

$$Z_{ij} = \ln \left[\frac{Y_{ij}}{g(Y_j)} \right],$$

where Z_{ij} is the transformed area of peak i for beetle j ; Y_{ij} is the area of peak i for beetle j ; and $g(Y_j)$ is the geometric mean of the areas of all peaks for beetle j (Aitchinson 1986; Steiner *et al.* 2007). Peeters *et al.* (1999) reduced the number of compounds by excluding peaks with small relative amounts. However, relative peak sizes may not be an appropriate selection criterion as small peaks have the potential to encode information. Rather, peaks with the highest variation between treatment groups should be identified and included. Selection

of peaks for this analysis was based on the Kruskal–Wallis test statistic H (Zar 1996). H -values were calculated for each compound and compounds with the highest H -values, those exhibiting the highest inter-group variability, were chosen and included (Steiner *et al.* 2007) in the discriminant analysis. Eighteen compounds were selected based on the H -values from the original 33 peaks that occurred regularly in the samples of all three groups (totalling 58 individuals). Four additional compounds were also included as univariate t -test comparisons of peak areas for these compounds from maturation-fed male and female beetles were significantly different ($P < 0.05$).

Results

Hexane extracts of female and male *M. scutellatus* consisted principally of saturated and unsaturated hydrocarbons, and there were consistent, sex-specific differences in male and female hydrocarbon total ion chromatograms (Table 1, Fig. 1). While the same compounds appeared in both male and female extracts, there were significant differences in relative quantity between the two sexes (one-tailed t -test, $P < 0.05$) in the two C_{25} monoenes, the methyl-branched C_{25} , nC_{26} , the two C_{27} monoenes, and nC_{27} (Table 2). C_{28} monoene was a dominant compound in males, and there were no compounds that were specific to males or females (Table 1, Fig. 1).

There were significantly different relative amounts of 20 hydrocarbons in the cuticular extracts of *M. scutellatus* of different sex and feeding status (Wilks' $\lambda = 0.062$, $F_{34,78} = 196.943$; $P < 0.00001$). Discriminant analysis showed that the first root separates all three groups, while the second root separates fed females from the other two groups (Fig. 2). Although the majority of hydrocarbons are present in both groups of females, the quantity is approximately four times greater in maturation-fed females than in unfed females (Table 1, Fig. 1). During maturation, some cuticular hydrocarbons may be produced in a high quantity, and it is very possible that new cuticular hydrocarbons are produced.

Discussion

Chemical communication a very old and widespread form of communication (Wyatt 2003).

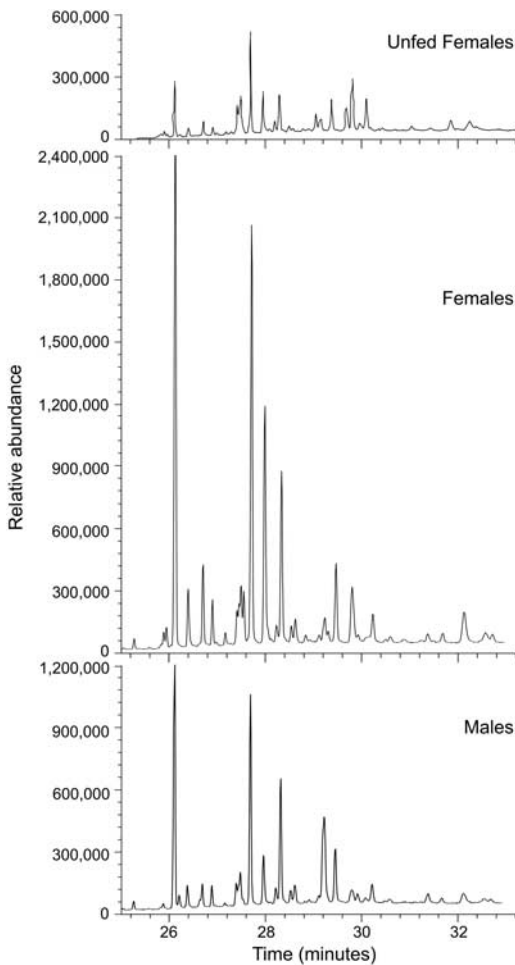
Table 1. Relative quantities of cuticular hydrocarbons from female and male *Monochamus scutellatus* after 14 days of maturation feeding.

Peak number	Ret. indices	% Area \pm SE			Hydrocarbon	Diagnostic ions
		Male	Female	Unfed females		
1	2400	0.03827 \pm 0.0026	0.0071 \pm 0.0258	0.0008 \pm 0.0014	nC ₂₄	338 (M+)
2	2467	0.0003 \pm 0.0013	0.0016 \pm 0.0034	0.0011 \pm 0.0013	C ₂₅ Diene	348 (M+)
3**	2473	0.0016 \pm 0.0027	0.0050 \pm 0.0048	0.0028 \pm 0.0002	C ₂₅ Monoene ₁	350 (M+)
4**	2481	0	0.0021 \pm 0.0034	0.0010 \pm 0.0002	C ₂₅ Monoene ₂	350 (M+)
5	2500	0.1469 \pm 0.0562	0.1740 \pm 0.0492	0.0243 \pm 0.0001	nC ₂₅	352 (M+)
6	2534	0.0163 \pm 0.0067	0.0178 \pm 0.0073	0.0021 \pm 0.0033	11-MeC ₂₅	366 (M+), 168, 224
					13-MeC ₂₅	366 (M+), 196
7**	2576	0.0155 \pm 0.0088	0.0235 \pm 0.0095	0.0024 \pm 0.0024	3-MeC ₂₅	366 (M+), 337
8**	2600	0.0137 \pm 0.0058	0.0184 \pm 0.0051	0.0086 \pm 0.0045	nC ₂₆	366 (M+)
9	2637	0.0050 \pm 0.0074	0.0035 \pm 0.0049	0.0005 \pm 0.0094	Unknown	
10	2663	0.0136 \pm 0.0098	0.0146 \pm 0.0091	0.0070 \pm 0.0021	2-MeC ₂₆	380 (M+), 365, 337
11	2668	0.0123 \pm 0.0134	0.0126 \pm 0.0141	0.0013 \pm 0.0010	C ₂₇ Diene	376 (M+)
12*	2675	0.0061 \pm 0.0100	0.0150 \pm 0.0157	0.0004 \pm 0.0006	C ₂₇ Monoene ₁	378 (M+)
13**	2680	0.0047 \pm 0.0081	0.0197 \pm 0.0133	0.0039 \pm 0.0001	C ₂₇ Monoene ₂	378 (M+)
14**	2700	0.1487 \pm 0.0513	0.1991 \pm 0.0297	0.0599 \pm 0.0094	nC ₂₇	380 (M+)
15	2734	0.0763 \pm 0.0346	0.0904 \pm 0.0007	0.0100 \pm 0.0130	11-MeC ₂₅	379 (M-15), 168, 252
					13-MeC ₂₅	379 (M-15), 196, 224
16	2763	0.0153 \pm 0.0141	0.0111 \pm 0.0092	0.0014 \pm 0.0042	13-MeC ₂₇	379 (M-15), 168, 196
					15-MeC ₂₇	379 (M-15), 239
					17-MeC ₂₇	379 (M-15), 267
17	2776	0.0776 \pm 0.0299	0.0801 \pm 0.0259	0.0067 \pm 0.0013	3-MeC ₂₇	394 (M-15), 365
18	2800	0.0179 \pm 0.0083	0.0249 \pm 0.0242	0.0018 \pm 0.0008	nC ₂₈	394 (M+)
19	2810	0.0141 \pm 0.0098	0.0166 \pm 0.0088	0.0008 \pm 0.0013	10-MeC ₂₆	394 (M+), 127, 155
					14-MeC ₂₆	394 (M+), 224
20	2863	0.0081 \pm 0.0084	0.0084 \pm 0.0076	0.0005 \pm 0.0004	2-MeC ₂₈	408 (M+), 365, 393
21*	2882	0.0183 \pm 0.0419	0.0126 \pm 0.0165	0.0032 \pm 0.0027	C ₂₈ Monoene	406 (M+), 83, 97, 111
22	2900	0.0908 \pm 0.0394	0.0759 \pm 0.0303	0.0127 \pm 0.0001	nC ₂₉	408 (M+)
23	2938	0.0463 \pm 0.0217	0.0413 \pm 0.0185	0.0043 \pm 0.0003	11-MeC ₂₉	422 (M+), 168, 280/282
					13-MeC ₂₉	422 (M+), 224
					15-MeC ₂₉	422 (M+), 196, 252
24	2949	0.0421 \pm 0.0006	0.0227 \pm 0.0304	0.0190 \pm 0.0012	10-MeC ₂₉	422 (M+), 155, 253
25	2978	0.0339 \pm 0.0124	0.0287 \pm 0.0104	0.0095 \pm 0.0073	3-MeC ₂₉	422 (M+), 407, 393

* $P < 0.05$ and ** $P < 0.005$ for sexually dimorphic compounds.

Peak numbers correspond to those in Figure 1. Molecular weights were confirmed by chemical ionisation with isobutene.

Fig. 1. Representative total ion chromatograms of hexane extracts of representative virgin, maturation fed, and unfed adult females, and virgin, maturation fed, adult male *Monochamus scutellatus*. Numbered peaks are sexually dimorphic compounds found in Table 1. Females are of varying ages: unfed are <1 day, 2 days and fed are aged 2 weeks and 1 month.



Males of many species increase their probability of successfully mating by choosing mates based on the females' reproductive status (Thomas 2011). One of the ways males discriminate is by using female reproduction status cues, such as female age, as a proxy. For example, male bush-crickets (*Requena verticalis* Walker; Orthoptera: Tettigoniidae), can differentiate females based on their age and preferentially mates with younger females (Simmons *et al.* 1994). By choosing young females, males reduce the probability of copulating with mated females, thereby increasing

Table 2. Degrees of freedom and test value for one-tailed *t*-test of hydrocarbons Z9:C₂₅, Z7:C₂₅, 3-MeC₂₅, nC₂₆, Z9:C₂₇, Z7:C₂₇, nC₂₇, Z9:C₂₈, and (*P* = 0.05).

Peak number ^a	DF	<i>P</i> (<i>T</i> ≤ <i>t</i>) one-tail	Hydrocarbon
3**	37	0.0030	C ₂₅ monoene ₁
4**	23	0.0026	C ₂₅ monoene ₂
7**	38	0.0038	MeC ₂₅
8**	34	0.0049	nC ₂₆
12*	39	0.0155	C ₂₇ monoene ₁
13**	39	0.0000	C ₂₇ monoene ₂
14**	25	0.0005	nC ₂₇
21*	30	0.0070	C ₂₈ monoene

^aPeak numbers correspond to those in Figure 1.

P* < 0.05 and *P* < 0.005 for sexually dimorphic compounds.

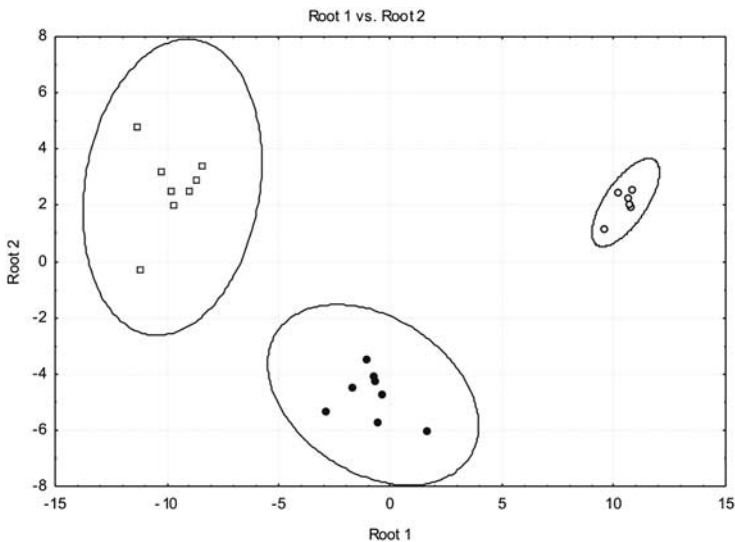
the likelihood that they will sire offspring. However, it is not clear if odour is used as the cue to discriminate female ages in this species. An example where age-dependent chemical signals have been demonstrated and, most likely, used by males is in *Drosophila virilis* (Sturtevant) (Diptera: Drosophilidae). In this case, the average chain length of hydrocarbons decreases with age (Jackson and Bartelt 1986).

Our analysis of *M. scutellatus* male and female cuticular hydrocarbons indicates that hydrocarbons are sexually dimorphic (Table 2 and Fig. 1). Some or all of these compounds may be contact pheromones used for mate recognition, as in other cerambycids (Hanks 1999; Ginzl and Hanks 2003; Ginzl *et al.* 2003a, 2003b; Barbour *et al.* 2007).

This investigation demonstrates that in *M. scutellatus*, maturation feeding is associated with a change in the relative proportion of cuticular hydrocarbons in females. The majority of compounds found were the same in maturation-fed females compared with unfed females; however, there were significant differences in the quantities and ratios of these compounds (Fig. 1). These beetles have a required maturation-feeding period of 7 days (Rose 1957) prior to mating, and our observations indicate that beetles do not discriminate the sexes until maturation feeding has occurred.

The sexually dimorphic compounds that show highly significant differences between males and females (Table 2) are likely contact pheromones for *M. scutellatus*. Due to their

Fig. 2. *Monochamus scutellatus* males (open squares), females (solid diamonds), and newly emerged females (open circles) clustered in distinct groups based on discriminate analysis of 18 cuticular hydrocarbons isolated from 58 beetles. Ellipses represent 95% confidence limits.



molecular weight they may have potential to be somewhat volatile and thus, may act as close range pheromones. Additionally, some of these same compounds have also been identified as contact sex pheromones in other cerambycid species. For example, (Z)-9-pentacosene has been identified as a contact pheromone in both the locust borer, *Megacyllene robiniae* (Ginzl *et al.* 2003), and the Asian longhorn beetle, *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae) (Zhang *et al.* 2003). One of the known contact pheromones of the rustic borer, *Xylotrechus colonus* (Fabricius) (Coleoptera: Cerambycidae), is 3-methylpentacosane (Ginzl *et al.* 2003a).

The ontogenetic changes in cuticular hydrocarbons in social insects have been widely studied. In these insects, factors such as habitat and nutritional conditioning induce changes in cuticular hydrocarbon composition. A few examples include the arrangement of hydrocarbons revealing age-related changes in honey-bee drones (Wakonigg *et al.* 2000), vespid wasps (Panek *et al.* 2001), and ants (Cuvillier-Hot *et al.* 2001). However, similar changes in ontogenetic variation in cuticular hydrocarbon compositions in non-social organisms have yet to be addressed and are currently poorly understood. Investigating these mechanisms

may provide important information regarding non-social insects' ecology and communication pathways.

Acknowledgements

The authors thank Francis Webster and Dave Kiemle for their advice in developing the experiment and chemistry expertise. They also thank Melissa Fierke for her support, guidance, and helpful comments on an early version of the manuscript. This work was supported, in part, by a grant from the Alphawood Foundation to S.A.T.

References

- Aitchinson, J. 1986. The statistical analysis of compositional data: monographs in statistics and applied probability. Chapman and Hall, London, New York, United States of America.
- Allison, J.D., Borden, J.H., McIntosh, R.L., DeGroot, P., and Gries, R. 2001. Kairomonal response by four *Monochamus* species (Coleoptera: Cerambycidae) to bark beetle pheromones. *Journal of Chemical Ecology*, **27**: 633–646.
- Barbour, J.D., Lacey, E.S., and Hanks, L.M. 2007. Cuticular hydrocarbons mediate mate recognition in a species of longhorned beetle (Coleoptera: Cerambycidae) of the primitive subfamily Prioninae. *Annals of the Entomological Society of America*, **100**: 333–338.

- Blomquist, G.J., Tillman-Wal, J.A., Guo, L., Quilici, D.R., Gu, P., and Schal, C. 1996. Hydrocarbon and hydrocarbon derived sex pheromones in insects: biochemistry and endocrine regulation. University of Nebraska Press, Lincoln, Nebraska, United States of America.
- Cram, M. and Hanson, J. 2004. How to identify and manage pine wilt disease and treat wood products infested by the pinewood nematodes [online]. Available from http://www.na.fs.fed.us/spfo/pubs/howtos/ht_pinewilt/pinewilt.htm [accessed 24 September 2012].
- Cuvillier-Hot, V., Cobb, M., Malosse, C., and Peeters, C. 2001. Sex, age and ovarian activity affect cuticular hydrocarbons in *Diacamma ceylonese*, a queenless ant. *Journal of Insect Physiology*, **47**: 485–493.
- D'Ettorre, P., Wenseleers, T., Dawson, J., Hutchinson, S., Boswell, T., and Ratnieks, F.L.W. 2006. Wax combs mediate nestmate recognition by guard honeybees. *Animal Behavior*, **71**: 773–779.
- Dillwith, J.W., Adams, T.T., and Blomquist, G.J. 1983. Correlation of housefly sex-pheromone production with ovarian development. *Journal of Insect Physiology*, **29**: 377–386.
- Edney, E.B. 1967. Water balance in desert arthropods. *Science*, **156**: 1059–1066.
- Gibbs, A.G. 1998. Water-proofing properties of cuticular lipids. *American Zoologist*, **38**: 471–482.
- Ginzl, M.D., Blomquist, G.J., Millar, J.G., and Hanks, L.M. 2003a. Role of contact pheromones in mate recognition in *Xylotrechus colonus*. *Journal of Chemical Ecology*, **29**: 533–545.
- Ginzl, M.D. and Hanks, L.M. 2003. Contact pheromones as mate recognition cues of four species of longhorned beetles (Coleoptera: Cerambycidae). *Journal of Insect Behavior*, **16**: 181–187.
- Ginzl, M.D., Millar, J.G., and Hanks, L.M. 2003b. (z)-9-pentacosene-contact sex pheromone of the locust borer, *Megacyllene robiniae*. *Chemoecology*, **13**: 135–141.
- Gleason, M., Linit, M., Narjess, Z., Donald, P., Tisserat, N., and Giesler, L. 2000. Pine wilt: a fatal disease of exotic pines in the Midwest [online]. Available from <http://www.extension.iastate.edu/Publications/SUL9.pdf> [accessed 24 September 2012].
- Hanks, L.M. 1999. Influence of the larval host plant on reproductive strategies of cerambycid beetles. *Annual Review of Entomology*, **44**: 483–505.
- Hanks, L.M., Millar, J.G., and Paine, T.D. 1996. Mating behavior of the eucalyptus longhorned borer (Coleoptera: Cerambycidae) and the adaptive significance of long “horns”. *Journal of Insect Behavior*, **9**: 383–393.
- Howard, R.W. and Blomquist, G.J. 2005. Ecological, behavioral, and biochemical aspects of insect hydrocarbons. *Annual Review of Entomology*, **50**: 371–393.
- Jackson, L.L. and Bartelt, R.J. 1986. Cuticular hydrocarbons of *Drosophila virilis* – comparison by age and sex. *Insect Biochemistry*, **16**: 433–439.
- Jackson, L.L. and Blomquist, G.J. 1976. Insect waxes. *In Chemistry and biochemistry of natural waxes. Edited by P.E. Kolatukudy.* Elsevier, Amsterdam, The Netherlands. pp. 201–233.
- Lacey, E.S., Ginzl, M.D., Millar, J.G., and Hanks, L.M. 2008. 7-Methylheptacosane is a major component of the contact sex pheromone of the cerambycid beetle *Neoclytus acuminatus acuminatus*. *Physiological Entomology*, **33**: 209–216.
- Linit, M.J. 1989. Temporal pattern of pinewood nematode exit from the insect vector *Monochamus carolinensis*. *Journal of Nematology*, **21**: 105–107.
- Neville, A.C. 1975. *Biology of the arthropod cuticle.* Springer-Verlag, Berlin.
- Panek, L.M., Gamboa, G.J., and Espelie, K.E. 2001. The effect of a wasp's age on its cuticular hydrocarbon profile and its tolerance by nest-mate and non-nestmate conspecifics (*Polistes fuscatus*, Hymenoptera: Vespidae). *Ethology*, **107**: 55–63.
- Peeters, C., Monnin, T., and Malosse, C. 1999. Cuticular hydrocarbons correlated with reproductive status in a queenless ant. *Proceedings of the Royal Society London Series B-Biological Sciences*, **266**: 1323–1327.
- Rose, A.H. 1957. Some notes on the biology of *Monochamus scutellatus* (Say) (Coleoptera: Cerambycidae). *The Canadian Entomology*, **89**: 547–553.
- Simmons, L.W., Llorens, T., Schinzig, M., Hosken, D., and Craig, M. 1994. Sperm competition selects for male mate choice and protandry in the bush-cricket, *Requena verticalis* (Orthoptera, Tettigoniidae). *Animal Behavior*, **47**: 117–122.
- Spikes, A.E., Paschen, M.A., Millar, J.G., Moreira, J.A., Hamel, P.B., Schiff, N.M., et al. 2010. First contact pheromone identified for a longhorned beetle (Coleoptera: Cerambycidae) in the subfamily Prioninae. *Journal of Chemical Ecology*, **36**: 943–954.
- Steiner, S., Peschke, K., Francke, W., and Muller, J.K. 2007. The smell of parents: breeding status influences cuticular hydrocarbon pattern in the burying beetle. *Proceedings of the Royal Society B – Biological Sciences*, **274**: 2211–2220.
- Thomas, M.L. 2011. Detection of female mating status using chemical signals and cues. *Biological Reviews*, **86**: 1–14.
- Vallentgoed, J. 1991. Some important woodborers related to export restrictions. *Forest Pest Leaflet*, Pacific Forestry Centre, **74**: 1–14.
- Wakonigg, G., Eveleigh, L., Arnold, G., and Crailsheim, K. 2000. Cuticular hydrocarbon profiles reveal age-related changes in honey bee drones (*Apis mellifera carnica*). *Journal of Apicultural Research*, **39**: 137–141.
- Wang, Q. 1998. Evidence for a contact female sex pheromone in *Anoplophora chinensis* (Forster) (Coleoptera: Cerambycidae: Lamiinae). *Coleopterists Bulletin*, **52**: 363–368.

- Wyatt, T.D. 2003. Pheromones and animal behavior: communication by smell and taste. Cambridge University Press, Cambridge, United Kingdom.
- Zar, J.H. 1996. Biostatistical analysis, 3rd ed. Prentice Hall, Saddle River, New Jersey, United States of America.
- Zhang, A.J., Oliver, J.E., Chauhan, K., Zhao, B.G., Xia, L.Q., and Xu, Z.C. 2003. Evidence for contact sex recognition pheromone of the Asian longhorned beetle, *Anoplophora glabripennis* (Coleoptera: Cerambycidae). *Naturwissenschaften*, **90**: 410–413.