Published in M. Kazimirová, M. Labuda, P. A. Nuttall, P. A. (Eds)
Proceedings of the 3rd International Conference
"Ticks and Tick-borne Pathogens : Into the 21st Century", pp. 199-204, 2000
which should be used for any reference to this work

INFLUENCE OF DOSE AND RATIO OF MIXTURES OF PHEROMONE COMPONENTS ON THE ATTRACTION OF THE TROPICAL BONT TICK, *AMBLYOMMA VARIEGATUM*

C. McMahon & P.M. Guerin

Institute of Zoology, University of Neuchâtel, Rue Emile-Argand 11, Neuchâtel 2007, Switzerland;
e-mail: Patrick.Guerin@zool.unine.ch

Abstract. The responses of *A. variegatum* adults to constituents of their aggregation-attraction pheromone presented in an air stream were studied using a locomotion compensator. The responses to vapours from two mixtures were compared, the first a 1:1 mixture of *o*-nitrophenol and methyl salicylate, the second a four component mixture ® containing *o*-nitrophenol, methyl salicylate, 2,6 dichlorophenol and phenyl acetaldehyde in the ratio 10:10:2:1, respectively. No difference was found between the kineses responses of *A. variegatum* adults for either mixture: both elicited an increase in angular velocity and a reduction in speed. Moreover, attraction to vapours of either mixture was reduced significantly as the source dose was increased: both mixtures were good attractants at a source dose of 10 μg of the main components, but were not attractive at source doses 100 times higher. As with many other arthropods, *A. variegatum* adults are responsive to the ratio, as well as to the dose, of constituents in an attractant mix. We suggest that the quantity and ratios of compounds in attractive mixtures released from commercial dispensers should be measured before use in any control programme.

Key words: Pheromone, *Amblyomma*, behaviour.

Introduction

Dose and ratios of products are critical to the performance of attractants for arthropods. To date, the best studied models are those of moths to constituents of their multicomponent pheromone blends (Witzgall & Arn 1990, Vickers et al. 1991, Rauscher et al. 1984). Among non-flying arthropods, much information has been collected on the aggregation-attachment pheromone (AAP) of the tropical bont tick, *Amblyomma variegatum*, following its discovery by Norval & Rechav (1979). This pheromone serves to attract conspecifics to the feeding site (Yunker et al. 1990), but its presence is also a prerequisite for the attachment of adult females (Barré et al. 1998). The AAP contains *o*-nitrophenol and methyl salicylate as major components released from dermal glands type 2 of adult males after feeding for some days on a host (Diehl et al. 1991). Although mixtures containing *o*-nitrophenol (ONP) and methyl salicylate (MS) attracted this species when presented off the host in the field (Norval et al. 1992; Barré et al. 1997), no investigations on either dose or ratio of these two compounds in mixtures on the behaviour of *A. variegatum* had been conducted until recently (McMahon & Guerin 2000).

The purpose of this contribution is to compare the responses of *A. variegatum* adults to vapours of a 1:1 ONP:MS mixture with those from a four component mixture ® containing ONP and MS as major constituents (Allan et al. 1998).

Material and methods

The behavioural responses of arthropods to attractants can be studied in detail using the locomotion compensator (Fig. 1) developed by Kramer (1976). The compensator, or servosphere, is particularly suited to studies on walking arthropods and has been used to study the behaviour of a diverse range of these such as the mite *Varroa jacobsoni* (Rickli et al. 1992), the Colorado beetle *Leptinotarsa decemlineata* (Thiéry & Visser 1987) and the cockroach *Periplaneta americana* (Bell & Kramer 1980). The apparatus has also been used to study the responses of some insects after immobilisation of their wings, such as the honeybee (Kramer 1976) and triatomines (Taneja & Guerin 1995, 1997). Briefly, the compensator serves to maintain the animal, in our case an *A. variegatum* adult, at the apex of a perspex sphere by compen-

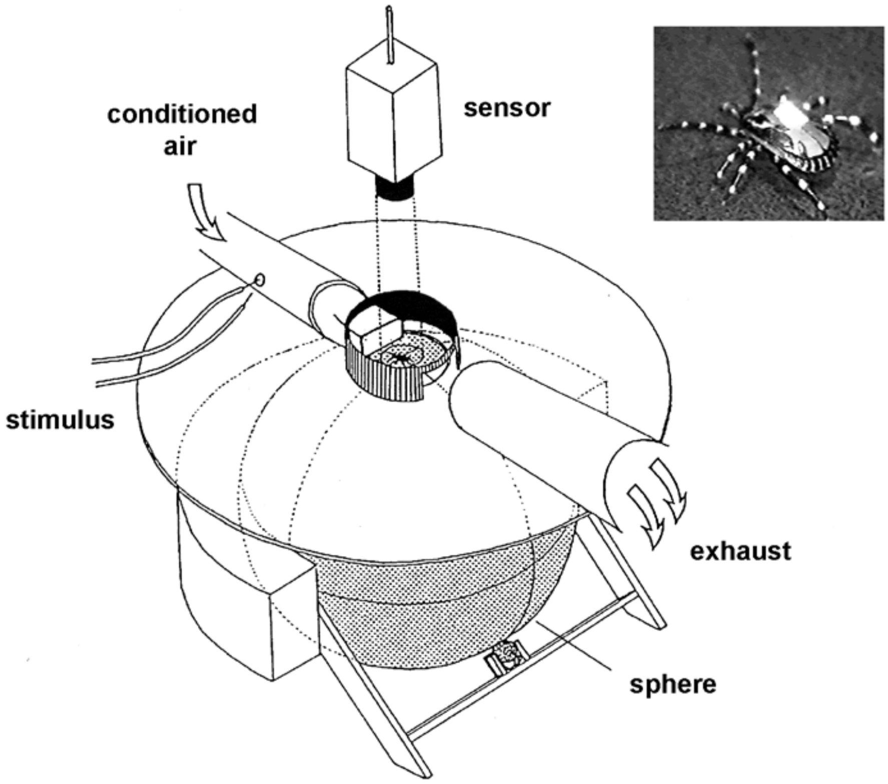

Fig. 1. The set-up for behavioural studies consisting of a locomotion compensator (a perspex sphere linked to a position sensor) and a horizontal air stream delivery with stimulus injection and exhaust.

sating for any displacement made by the walking arthropod. As the position of the tick is fixed in space, it is possible to control the conditions of the air stream in which the tick walks and change it in time, e.g., by addition of a chemical. This is not usually feasible in other behavioural assays or in field tests. Furthermore, due to the controlled conditions of the air stream used to deliver the test chemicals (Taneja & Guerin 1995) the concentration of odour molecules can be specified.

The displacements of the sphere caused by the walking animal are recorded at regular intervals using pulse generators (Kramer 1976). This sampling allows the reconstruction of the tracks described by the arthropod and permits an evaluation of several parameters such as speed, angular velocity and the duration of the walk in any given direction. The latter parameter can be used to calculate upwind responses to volatiles. In our study, an index of attraction for a treatment was calculated from the percentage time spent walking in a cone 60° either side of due upwind by the ticks in the presence of vapours from synthetic AAP mixtures relative to the time spent in this cone by the same ticks in the preceeding control period of equal duration (1 min). The significance of the attraction index for any given treatment was determined using the Wilcoxon signed rank test (two-tailed). To determine if treatments differed in attractivity to ticks, the indices of attraction were compared using the Wilcoxon-Mann-Whitney test (two-tailed). Walks of the ticks were also monitored following the withdrawal of test volatiles (end control period). Continuous macro-video recording of the tick to monitor other behavioural elements was also undertaken, facilitated by this experimental setup which maintains the tick at the same place.

Responses of ticks were recorded to vapours of ONP and MS presented as binary mixtures. Treatments were prepared by making solutions of the mixtures in dichloromethane and applying them to filter paper. After the evaporation of the solvent, the filter paper was introduced into a gas wash bottle and clean air passing through the bottle served to transport the vapours in the flask to the main air stream flowing over the tick. Furthermore, by entraining volatiles present in the gas wash flask onto a porous polymer (Porapak) we estimated that 2.5% of the quantity of either ONP or MS applied to filter paper entered the main air stream during the 1 min exposure of the tick (McMahon & Guerin 2000).

Results

The effect of dose was examined by comparing the response to the 1:1 ONP:MS mixture at source doses of 10 µg, 100 µg and 1 mg of both ONP and MS. The ONP:MS mixture at 10 µg each provides a good attractant (Fig. 2a). A significant reduction in attraction occurs as the dose is increased and there is no attraction at 1 mg source doses of ONP and MS (Fig. 2a). Responses to different doses of a four component mixture ® used as a lure (Allan et al. 1998) were also recorded. This lure consists of 10 parts ONP, 10 parts MS, 2 parts 2,6-dichlorophenol and 1 part phenylacetaldehyde. Dichloromethane solutions containing these proportions (g/v) of the four compounds at different doses were made up and the volatiles delivered from filter paper in gas wash

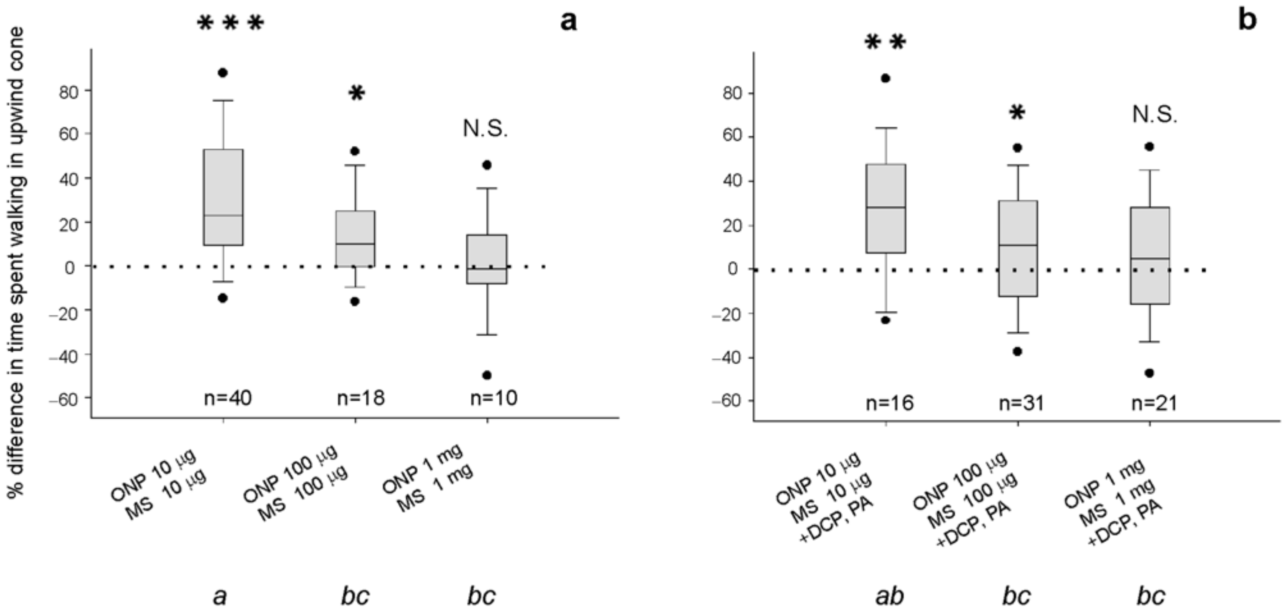

Fig. 2. Box plots of the change in time spent walking in a cone 600 either side of due upwind on a locomotion compensator by *A. variegatum* adults in the presence of vapours from increasing source doses from **(a)** a 1:1 mixture of o-nitrophenol (ONP) and methyl salicylate (MS) and **(b)** a four component (10:10:2:1) mixture consisting of ONP, MS, 2,6 dichlorophenol (DCP) and phenylacetaldehyde (PA) after Allan et al. (1998). The line within a box marks the median, the lower and upper boundaries of a box indicate the 25th and 75th percentiles, error bars below and above a box indicate the 10th and 90th percentiles, and the 5th and 95th percentiles are shown as circles. Asterisks above a treatment indicate the significance of the response (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; Wilcoxon signed rank test, two-tailed). Treatments not sharing the same letter (*a*, *b*, *c*) are significantly different (Wilcoxon-Mann-Whitney test, two-tailed). The trend towards loss of attraction at higher doses of both the binary **(a)** and four component mixture **(b)** was significant ($P < 0.025$, Jonckheere test).

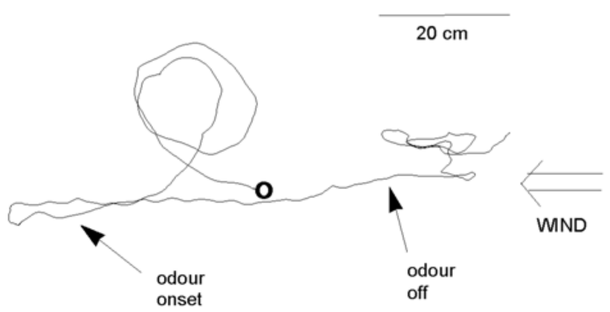

Fig. 3. Response of an *A. variegatum* adult female on the servosphere to vapours of a 1:1 mixture of *o*-nitrophenol and methyl salicylate (source dose 10 mg each). The track commenced (**o**) with the tick circling during the control period (1 min) followed by a one minute walk in the presence of the aggregation-attachment pheromone vapour during which the tick proceeded towards the source. In the 1 min record of the walk after odour off (end control) the tick undertook a random walk at reduced speed.

bottles as described above for the binary ONP:MS mixtures. The response profile to different doses of this four component mixture was found to be the same as to the 1:1 binary mixture, i.e., loss of attractivity above 100 µg ONP and MS on the filter paper (Fig. 2b). To test the effect of blends of ONP and MS, a comparison was made between the 3:1 ratio of ONP:MS found over feeding males (Diehl et al. 1991) and a 1:1 mixture. The natural ratio is significantly less attractive ($P < 0.05$, Wilcoxon-Mann-Whitney test, two-tailed) than the 1:1 mixture of the same compounds (McMahon & Guerin 2000). Vapours from the AAP mixtures elicit an increase in angular velocity and a reduction in speed which persists into the end control (Figs 3, 4). This slower walk is accompanied by a reaching behaviour where the tick raises its forelegs to sample the air. However, this may not represent the behavioural response induced by tick semiochemicals released from the host.

The responses to the major pheromone component, ONP, in the presence of host (steer hair) odour were also evaluated. Cleaned air passed through a gas wash bottle containing 25 g of steer hair did not attract the walking ticks ($P > 0.6$; Wilcoxon signed rank test, two-tailed, $n = 33$). Admixture of ONP (source dose 10 ng) to this host odour, however, was attractive ($P < 0.0001$, Wilcoxon signed rank test, two-tailed, $n = 33$; McMahon & Guerin 2000). Moreover, the attraction to this combination occurred in the absence of any kineses effects typical of synthetic AAP mixtures (above), i.e., there was no

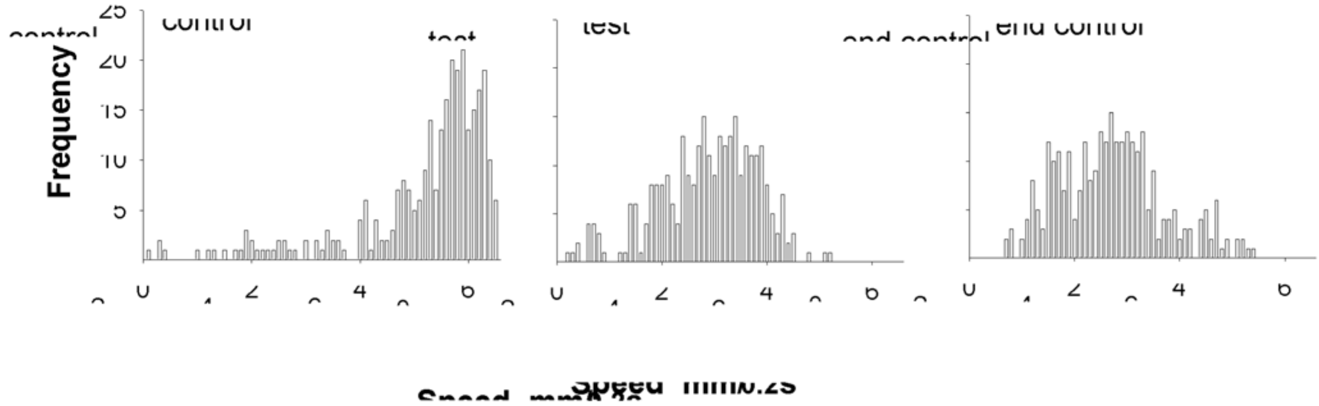

Fig. 4. Frequency distribution of the instantaneous speed based on the displacement during 0.2 s components of the path described by the *A. variegatum* female presented in Fig. 3. Vapours of the aggregation-attachment pheromone induced a reduction in speed in the test period compared to the control period, an effect that continued after the withdrawal of the attractant (end control period). For further explanation see legend to Fig. 3.

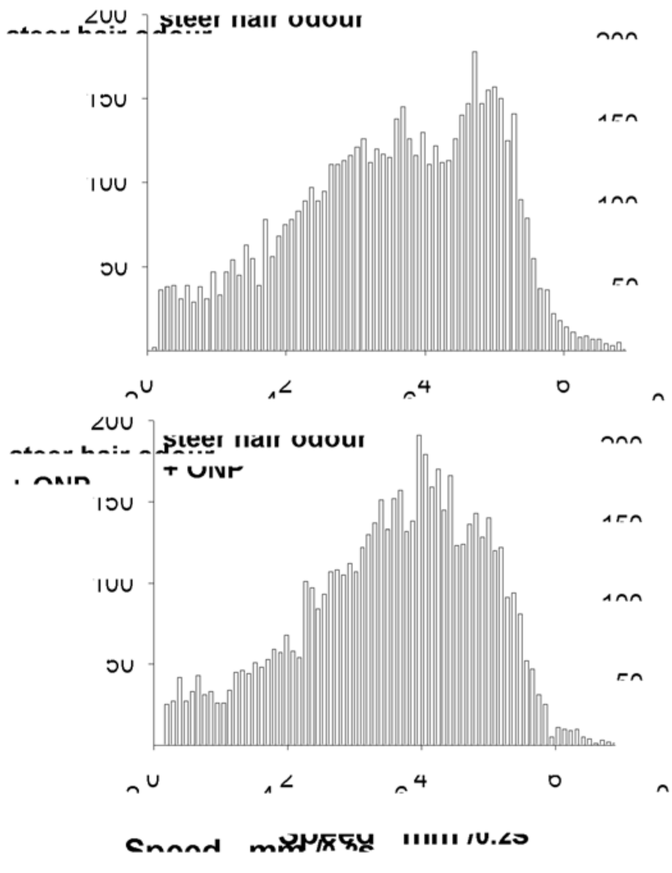

Fig. 5. Frequency distributions of instantaneous speed based on the displacement during 0.2 s components of the paths described by twenty *A. variegatum* adults presented with unattractive vapours from steer hair (upper bar diagram, see Material and Methods) for one minute followed by an attractive combination of ONP vapour (source dose 10 ng, lower bar diagram) added to vapours of steer hair for a further minute. No significant change in speed was observed ($P > 0.15$, $n > 5400$; two-tailed t-test, for unpaired samples of equal variance).

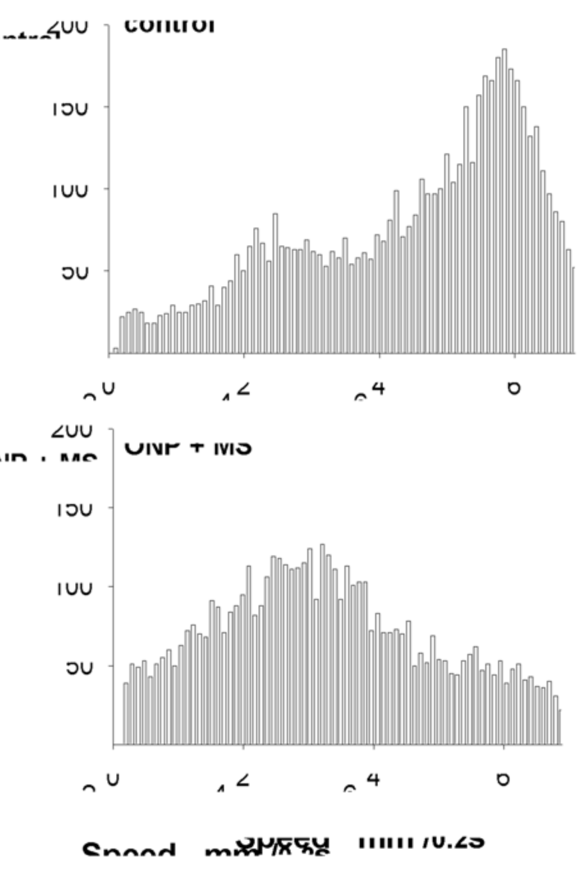

Fig. 6. Frequency distributions of instantaneous speed based on the displacement during 0.2 s components of the paths described by twenty *A. variegatum* adults. Animals were presented with vapours from a 1:1 mixture of *o*-nitrophenol and methyl salicylate (source dose10 mg of each, lower bar diagram) for one minute preceded by a 1 min control (upper bar diagram) in clean air. The reduction in speed (25%) in the test period (lower bar diagram) due to the attractant was significant ($P < 0.0001$, $n > 5400$; two-tailed t-test for unpaired samples of unequal variance).

increase in angular velocity or reduction in speed (Fig. 5) observed to the pheromone alone (Fig. 6). This suggests that the more complete blend of host compounds plus pheromone evokes a more efficient walk towards the source.

Conclusions

A. variegatum adults can distinguish between varying doses and blends of synthetic attractants. Nonetheless, all synthetic AAP mixtures induce the same kineses in these ticks, an increase in angular velocity and reduction in speed. This is in marked contrast to the attraction induced by the admixture of ONP to steer hair odour, the normal context in which the AAP is perceived by the ticks.

We found that source doses higher than 100 µg of MS and ONP in binary mixtures and a mixture ® containing 1 mg of both these compounds as major components do not attract *A. variegatum*. Based on known release rates of ONP from feeding adult males (Diehl et al. 1991) and the known evaporation rate of these compounds in our experimental design (McMahon & Guerin 2000), we calculate that 10 µg, 100 µg and 1 mg source doses of ONP on filter paper release amounts equivalent to that released in one minute from 8–48, 80–480 and 800–4800 feeding males, respectively. Applying this data to the release rates from decoys developed by Norval et al. (1996) and Allan et al. (1998), their ONP release rates are equivalent to that from 320–1920 and 560–3360 feeding males, respectively. The data for pheromone evaporation from dispensers in both the Norval et al. (1996) and Allan et al. (1998) studies were taken at weekly intervals and so do not reflect circadian fluctuations in release rate due to temperature that may influence their attraction. Furthermore, the widely fluctuating release rates of methyl salicylate between the Norval et al. (1996) and Allan et al. (1998) studies suggest that the ratio of methyl salicylate to ONP varied considerably.

This study shows that when the aim is to attract ticks to a point source (the orifice of the delivery tube on a servosphere in our case), the dose and ratio of products delivered is critical. This is consistent with what has been established for other arthropods (e.g., Arn et al. 1985, 1986). With a lure incorporating an acaricide for the "attract and kill" strategy for tick control, careful attention must therefore to be paid to the release rate from whatever dispenser is used. Our results show that an upper limit of biologically active products in the air exists for attracting *A. variegatum*. We hope that due consideration of this factor will prevail, in recognition of the pioneering contribution made by Norval et al. (1989) who, by demonstrating that ticks are preferentially attracted to hosts infested by feeding males, first revealed the potential of lures containing AAP constituents to control these important pests.

Acknowledgements. This research was supported by the Swiss National Science Foundation (Grant Nos. 31-37420.93 and 31-50658.97 entitled "Studies on the chemical ecology, sensory physiology and behaviour of ticks"). We are indebted to the Hasselblad, Roche, and Sandoz Foundations, the Ciba-Geigy-Jubilaeums-Stiftung and the Swiss Office for Education and Science for funding studies on tick sensory physiology and behaviour at Neuchâtel. We thank Messrs. P. Bula and J. Jonczy of Novartis Centre de Recherche Santé Animale S.A., 1566 St. Aubin, Switzerland for supplying us with ticks. Our thanks to Dr. M. de Bruyne for his drawing of the locomotion compensator setup.

References

ALLAN, S.A., BARRÉ, N., SONENSHINE, D.E. & BURRIDGE, M.J. 1998. Efficacy of tags impregnated with pheromone and acaricide for control of *Amblyomma variegatum*. *Medical and Vetinary Entomology* **12:** 141–150.

ARN, H., GUERIN, P.M. BUSER, H.R., RAUSCHER, S. & MANI, E. 1985. Sex pheromone blend of the codling moth, *Cydia pomonella*: evidence for a behavioural role of dodecan-1-ol. *Experientia* **41:** 1482–1484.

ARN, H., RAUSCHER, S., BUSER, H.R. & GUERIN, P.M. 1986. Sex pheromone of *Eupoecilia ambiguella* female: analysis and male response to ternary blend. *Journal of Chemical Ecology* **11:** 1417–1429.

BARRÉ, N., GARRIS, G.I. & LORVELEC, O. 1997. Field sampling of the tick *Amblyomma variegatum* (Acari: Ixodidae) on pastures in Guadeloupe; attraction of CO_2 and/or tick pheromones and conditions of use. *Experimental and Applied Acarology* **21:** 95–108.

BARRÉ, N., NAVES, M., APRELON, R., FARGETTON, M. & L'HOSTIS, M. 1998. Attractivity of cattle infested by *Amblyomma variegatum* (Acari: Ixodidae) for conspecific adult ticks from the field in Guadeloupe. *Experimental and Applied Acarology* **22:** 297–308.

BELL, W. J. & KRAMER, E. 1980. Sex pheromone-stimulated orientation of the American Cockroach on a servosphere apparatus. *Journal of Chemical Ecology* **6:** 287–295.

DIEHL, P.A., GUERIN, P.M., VLIMANT, M. & STEULLET, P. 1991. Biosynthesis, production site, and emission rates of aggregation-attachment pheromone in males of two *Amblyomma* ticks. *Journal of Chemical Ecology* **17:** 833–847.

KRAMER, E. 1976. The orientation of walking honeybees in odour fields with small concentration gradients. *Physiological Entomology* **1:** 27–37.

MCMAHON, C. & GUERIN, P.M. 2000. Responses of the tropical bont tick, *Amblyomma variegatum* (Fabricius), to its aggregation-attachment pheromone presented in an air stream on a servosphere. *Journal of Comparative Physiology A Sensory Neural and Behavioural Physiology.* [In press]

NORVAL, R.A.I., ANDREW, H.R. & YUNKER, C.E. 1989. Pheromone-mediation of Host-Selection in Bont Ticks (*Amblyomma hebraeum* Koch). *Science* **243:** 364–365.

NORVAL, R.A.I., PETER, T., SONENSHINE, D.E. & BURRIDGE, M.J. 1992. Responses of the ticks *Amblyomma hebraeum* and *Amblyomma variegatum* to known or potential components of the aggregation -attachment pheromone. III. Aggregation. *Experimental and Applied Acarology* **16:** 237–245.

NORVAL, R.A.I. & RECHAV, Y.H. 1979. An assembly pheromone and its perception in the tick *Amblyomma variegatum* (Acarina, Ixodidae). *Journal of Medical Entomology* **16:** 507–511.

NORVAL, R.A.I., SONENSHINE, D.E., ALLAN, S.A. & BURRIDGE, M.J. 1996. Efficacy of pheromone-acaricide-impregnated tail-tag decoys for controlling the bont tick, *Amblyomma hebraeum* (Acari: Ixodidae), on cattle in Zimbabwe. *Experimental and Applied Acarology* **20:** 31–46.

RAUSCHER, S., ARN, H. & GUERIN, P.M. 1984. Effects of dodecyl acetate and Z-10-tridecenyl acetate on attraction of *Eupoecilia ambiguella* males to the main sex pheromone component, Z-9-dodecenyl acetate. *Journal of Chemical Ecology* **10:** 253–264.

RICKLI, M., GUERIN, P.M. & DIEHL, P.A. 1992. Palmitic acid released from honeybee worker larvae attracts the parasitic mite *Varroa jacobsoni* on a servosphere. *Naturwissenschaften* **79:** 320–322.

TANEJA, J. & GUERIN, P.M. 1995. Oriented responses of the triatomine bugs *Rhodnius prolixus* and *Triatoma infestans* to vertebrate odours on a servosphere. *Journal of Comparative Physiology A Sensory Neural and Behavioural Physiology* **176:** 455–464.

TANEJA, J. & GUERIN, P.M. 1997. Ammonia attracts the haematophagous bug *Triatoma infestans:* behavioural and neurophysiological data on nymphs. *Journal of Comparative Physiology A Sensory Neural and Behavioural Physiology* **181:** 21–34.

THIÉRY, D. & VISSER, J. H. 1987. Misleading the Colorado potato beetle with a odor blend. *Journal of Chemical Ecology* **13:** 1139–1146.

VICKERS, N.J., CHRISTENSEN, T.A., MUSTAPARTA, H. & BAKER, T.C. 1991. Chemical communication in heliothine moths. III. Flight behavior of male *Helicoverpa zea* and *Heliothis virescens* in response to varying ratios of intra- and interspecific sex pheromone components. *Journal of Comparative Physiology A Sensory Neural and Behavioural Physiology* **169:** 275–280.

WITZGALL, P. & ARN, H. 1990. Direct measurement of flight behaviour of male moths to calling females and synthetic pheromones. *Zeitschrift für Naturforschung* **45c:** 1067–1069.

YUNKER, C.E., ANDREW, H.R., NORVAL, R.A.I. & KEIRANS, J.E. 1990. Interspecific attraction to male-produced pheromones of two species of *Amblyomma* ticks (Acari: Ixodidae). *Journal of Insect Behaviour* **3:** 557–565.