Ant cues affect the oviposition behaviour of fruit flies (Diptera: Tephritidae) in Africa

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Abstract. Although most studies on fruit fly oviposition behaviour focus on horizontal interactions with competitors and cues from host plants, vertical interactions with predators are poorly documented. The present study provides direct evidence indicating that the oviposition behaviour of the two main mango fruit fly species, *Ceratitis cosyra* (Walker) and *Bactrocera invadens* Drew-Tsurata & White, is affected by secretions of the dominant arboreal ant *Oecophylla longinoda* (Latreille). When offered ant-exposed and unexposed mangoes in the absence of the ants, both fly species are reluctant to land on ant-exposed fruits and, when having landed, often take off quickly and fail to oviposit. The number of puparia collected from unexposed mangoes is approximately eight-fold higher than from ant-exposed ones. The results obtained from laboratory experiments and field observations confirm that adult fruit flies are more affected through repellence by ant cues than by direct predation. The use of cues by fruit flies in predator avoidance has implications for evolutionary ecology, behavioural ecology and chemical ecology.

Key words. Ant cues, frugivore deterrence, interspecific communication, *Oecophylla longinoda*, oviposition preference, predator avoidance, semiochemicals, Tephritidae, trophic interactions.

Introduction

Information flows within food webs influence the behaviour, ecology and population dynamics of animals (Abrams *et al.*, 1996; Krebs & Kacelnik, 1997; Vos *et al.*, 2006). The extrinsic information that plays a crucial role in the survival of animals includes cues on the availability of food and mates, oviposition sites, abiotic factors (e.g. temperature and shelter), as well as the presence of competitors and natural enemies (Dicke & Grostal, 2001). Fruit flies (Diptera: Tephritidae) use both visual and olfactory signals that function at various spatial scales to identify suitable host plants and fruits (Jang & Light, 1981; Fletcher & Prokopy, 1991; Brévault & Quilici, 2007). Because of their economic importance (Peña *et al.*, 2002; Vayssières *et al.*, 2009), there is extensive research available over the past century regarding the behaviour of fruit flies, with approximately 30% and 35% of the studies dealing with oviposition and sexual behaviour, respectively

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(Díaz-Fleischer & Aluja, 2000). Studies on substances that deter oviposition, albeit of great potential economic significance, are confined to botanical repellents, petroleum spray oils and host-marking pheromones that deter conspecifics from ovipositing on previously occupied hosts (Prokopy & Roitberg, 2001; Cook *et al.*, 2007).

Pupation of economically important fruit flies takes place in the soil, after final-instar larvae have left the infested fruit. Wong *et al.* (1984) provide an assessment of the extent of predation on the Mediterranean fruit fly *Ceratitis capitata* by the Argentine ant *Linepithema humile* (Hymenoptera: Formicidae). These ground-nesting ants cause a mortality rate of approximately 39% of puparia and teneral adult fruit flies (i.e. newly-emerged adults). They conclude that ant predation can be considerable but inadequate to regulate fruit fly populations. Aluja *et al.* (2005) report on the effect of predation by ants on fruit flies but, likewise, they limit their observations to the ground level. Because fruit fly population dynamics and fitness are influenced greatly by sexual, feeding and oviposition behaviours, which mainly take place in tree canopies, it is to be expected that arboreal ant species will directly and indirectly influence these behaviours.

Only two species of *Oecophylla* (Hymenoptera: Formicidae) exist, namely the Asian weaver ant *Oecophylla smaragdina* and the African weaver ant, *Oecophylla longinoda* (Latreille). The dominant arboreal *Oecophylla* colonizes a wide range of trees and effectively controls tree pests (Van Mele & Cuc, 2000; Van Mele, 2008). The ant workers hunt diurnally in groups, and prey detected visually from a relatively long distance is seized by an appendage and immobilized. This behaviour permits the ants to capture small and large insects and even other animals (Hölldobler & Wilson, 1990). In mango orchards in Benin, fruit fly damage is significantly reduced by *O. longinoda* as a function of the ant abundance level (Van Mele *et al.*, 2007). However, the mechanisms underlying ant protection of plants against herbivores and frugivores remain almost undocumented. Direct mechanisms include predation on, or deterrence of, organisms during direct encounters, whereas indirect mechanisms involve the detection by organisms of the territories of enemy ants (Dicke, 2000; Offenberg *et al.*, 2004).

Because adult flies are rarely observed being captured in the field, Van Mele *et al.* (2007) suggest that olfactory and/or visual cues related to *Oecophylla* could affect fruit fly behaviour. The present study investigates whether secretions of *O. longinoda* affect fruit fly oviposition behaviour.

Materials and methods

Mango fruits

In March 2008, fruits were collected from an orchard free of weaver ants in Ouidah (6°22′0.012″N; 2°4′59.988″E), southern Benin. Forty fruits of the same maturity and of similar size were collected from a single tree (cv. Gouverneur) of approximately 20 years of age. Ten additional fruits were picked and incubated separately to confirm that collected fruit had not been infested previously in the field.

Ant cues

The research was conducted at the International Institute of Tropical Agriculture (IITA), Cotonou, Benin. In the greenhouse, half of the fruits were put in a plastic basin (diameter 40 cm, depth 15 cm). One *Oecophylla* nest was collected from a mango tree near to the research station and placed in the same basin. The basin was covered with fine mesh and tightly closed to confine the ants. Ants, fed on sugar and water, were removed from the basin after 48 h. This method was conducted in accordance with the procedures of Jander & Jander (1979), who indicated that ants could be induced to deposit their pheromones on substrates. These mangoes were considered as ant-exposed mangoes, whereas the others were considered as unexposed mangoes.

Fruit fly species

The two most important fruit fly species in West African mango orchards, namely *Bactrocera invadens* Drew-Tsuruta &

White and *Ceratitis cosyra* (Walker) were obtained from the mass-rearing unit at IITA in Cotonou. Puparia collected with flexible tweezers were put into hatchery boxes. Water, sugar and protein (active ingredient: hydrolysate enzymatic, autolyzed brewers yeast) (ICN Biomedicals, Inc.) were provided for the newly emerged adults to feed on. Ten-day-old female flies (*Ceratitis*) and 12-day-old (*Bactrocera*) were checked to have mated before being considered ready for oviposition (Jang *et al.*, 1997). Three gravid females of each species were then collected carefully and moved into other screen cages (42 × 52 × 52 cm) to allow them to oviposit on mango fruits. Neither the fruit flies nor their parents had any prior experience with weaver ants. Temperature and humidity during the experiment averaged 28.6°C (range 25–30°C) and 77.5% (range 66–91%), respectively.

Fruit fly behaviour

Before being offered to *B. invadens* and *C. cosyra*, fruits were weighed and punctured through the skin (100 per fruit) to facilitate oviposition. In a preliminary test (comparing 24, 48, 72 and 96 h), no significant difference in oviposition took place between 72 and 96 h; thus, in subsequent trials, fruit flies were offered the treated mangoes for 3 days. One ant-exposed and one non-exposed mango were placed in the same cage and exposed to three gravid females of either *B. invadens* or *C. cosyra*. There were ten replicates for each fruit fly species.

The oviposition behaviour was observed three times a day for 3 days: 1, 5, 9, 25, 29, 33, 49, 53 and 57 h after flies and mango fruits were put together in the cage. Flies were observed for 5 min on each occasion. The number of fly landings and their time spent on the fruits were recorded during each observation. Additional observations were made on piercing behaviour.

Fruit fly damage in a choice test

After 72 h, fruits were removed from the cages. Each fruit was individually placed on mesh supports mounted on basins, covered with fine mesh to prevent larval escape, and then incubated. The bottom of the basins was covered with wet sand, into which larvae emerging from the fruits could drop and metamorphose into puparia. Once a week, over a period of 6 weeks, the sand of each container was sieved and the number of puparia recorded.

Fruit fly damage under field conditions

To investigate whether a gradient in oviposition deterrence could be observed under natural conditions, mango fruits were collected in early June 2008 from an orchard planted with different cultivars in Parakou, Borgou Department, Benin. Cultivars were selected, taking into consideration the possibility for a given cultivar to have trees harbouring ants,

as well as trees without ants. Three cultivars were selected, namely Kent, Smith and Ifac 3. It was assumed that *Oecophylla* releases less cues on fruits further away from their nest because the abundance and/or the frequency of patrolling activities would be less. In early June, three mango fruits were picked within 1 and 1–3 m distance from an ant nest from each ant-harbouring tree, and three fruits per ant-free mango tree, in five trees per variety, totalling 135 fruits from 30 trees. When brought to the laboratory, individual fruits were weighed, labelled and mounted for incubation as described earlier. All were monitored until adult emergence.

Statistical analysis

Log$_{10}$ ($x + 1$) transformation was used on count variables to stabilize the variance and normalize the data. Analysis of variance was performed using the general linear model procedure and mean separations were undertaken using the Student–Newman–Keuls test.

Results

Influence of ant cues on fruit fly oviposition behaviour

Interactions between treatment and fruit fly species *C. cosyra* and *B. invadens* were not significant ($P = 0.05$) (Table 1) and hence data for both species were pooled. The number of fly landings and time spent on the fruit was significantly lower for fruit with ant cues than for those without ($P = 0.029$ for both variables) throughout the 3 days of observation (apart from the initial few hours when fruit flies were becoming familiar with their new environment) (Fig. 1a,b). Fruit flies hardly attempted to oviposit in ant-exposed fruit and showed significant preference for the mangoes without ant cues ($P < 0.001$) (Fig. 1c). When landing on ant-exposed mangoes, fruit flies moved around initially but removed their ovipositor quickly as soon as they had inserted it. Generally, after two or more attempts, they flew away shortly afterwards.

Influence of ant cues on fruit fly damage in a choice test

Ant-exposed mangoes had 7.3 ± 1.5 puparia per kg fruit compared with 56.8 ± 2.7 for unexposed mangoes ($P < 0.05$).

Table 1. Number of fruit fly puparia (mean ± SE) per kg fruit from mangoes exposed and unexposed to *Oecophylla longinoda* in a choice test performed under laboratory conditions.

	Ceratitis cosyra	*Bactrocera invadens*
Ant-exposed mangoes	6.4 ± 2.0[a]	8.3 ± 2.3[a]
Non-exposed mangoes	52.3 ± 4.1[b]	61.2 ± 3.0[b]

Data were log$_{10}$ ($x + 1$) transformed before being analysed with the Student–Newman–Keuls test. Values in columns followed by different superscript letters are significantly different ($n = 10$, $P < 0.05$).

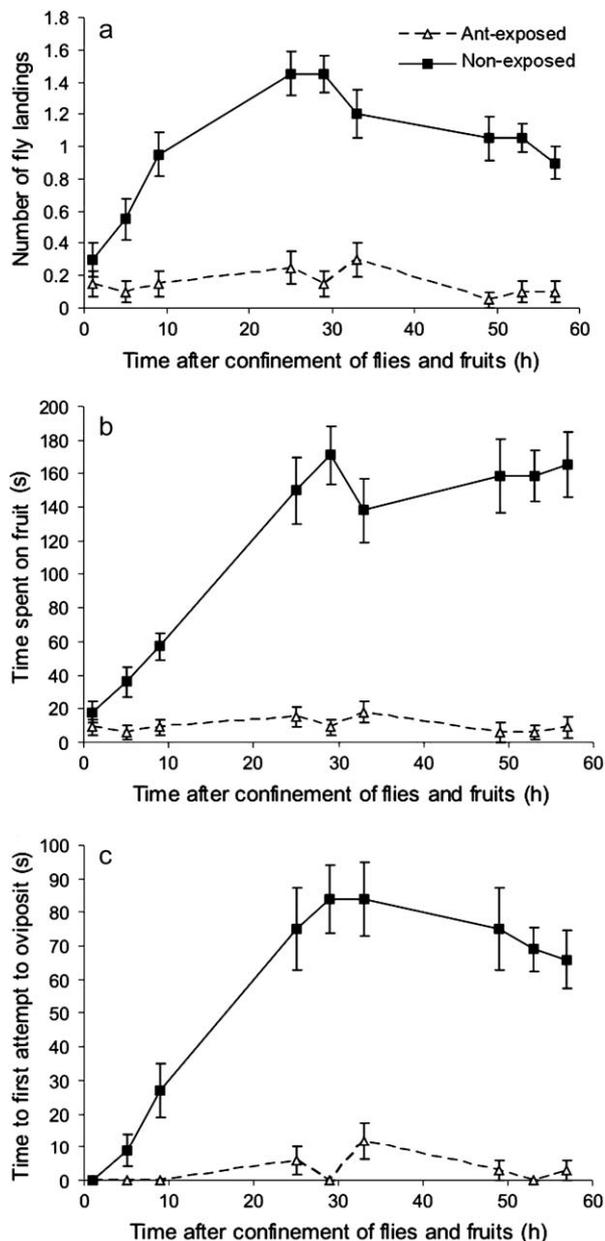The trend was similar for both *B. invadens* and *C. cosyra* (Table 1).

Fig. 1. Effects of cues of *Oecophylla longinoda* on fruit fly oviposition behaviour. Interactions between treatment and fruit fly species *Ceratitis cosyra* and *Bactrocera invadens* were not significant ($P = 0.05$), hence data were pooled. (a) Number of fruit fly landings on exposed and unexposed mangoes; (b) time spent per fruit fly walking on exposed and unexposed mangoes; (c) time to first attempt to oviposit by fruit flies on exposed and unexposed mangoes. Oviposition behaviour was significantly lower on mangoes on which predatory ants had patrolled (Student–Newman–Keuls test, $n = 20$, $P < 0.05$). Data are the mean ± SE.

Influence of ant cues on fruit fly damage under field conditions

Using the entire data set ($n = 135$), the Pearson correlation coefficient was -0.51 ($P = 0.008$), indicating a negative correlation between ant cues and fruit fly damage. All identified flies were *C. cosyra*, which is more prevalent than *B. invadens* during the time of year (Vayssières *et al.*, 2009) when the present study was conducted. Fruits from ant-harbouring trees showed a significantly lower infestation rate and contained significantly fewer puparia per kg fruit than fruits picked from ant-free trees ($P = 0.031$) (Table 2). The number of puparia (and hence eggs deposited) per kg of infested fruit was equally lower in trees where ants were present. No significant difference in fruit fly damage was found between fruit collected within 1 m and those collected at 1–3 m distance from *O. longinoda* nests. A similar positive effect of the presence of weaver ants was observed for each cultivar ($P = 0.142$).

Discussion

The receptivity of fruit repellents and deterrents influences the fruit flies' perceptual process of ovipositional acceptance (Jang & Light, 1981; Scarpati *et al.*, 1993). Besides changes in the landing behaviour of flies, oviposition repellence occurs. The results obtained in the present study suggest that cues deposited by *Oecophylla* on mangoes mask or supersede the attractancy of the fruit and act as strong predator avoidance cues, preventing fruit flies from ovipositing in mangoes on which ants had patrolled.

The fruit flies in this experiment were never exposed to the physical presence of *Oecophylla* ants. Therefore enemy avoidance by fruit flies is not attributable to learning, which plays a role in host selection (Mangel & Roitberg, 1989; Mayhew, 1997) but is the result of cues that trigger responses to potential predator risk. Other fruit flies (*Rhagoletis basiola*) delay oviposition when exposed to chemical information from their egg parasitoids (Hoffmeister & Roitberg, 1997).

The exact nature of the cues responsible for fruit fly oviposition repellence has yet to be identified because ants deploy a range of pheromones from exocrine glands (Hölldobler & Wilson, 1990; Billen & Morgan, 1998). As mangoes were exposed to *Oecophylla* in a relatively stress-free environment, alarm pheromones are unlikely causes of

the observed changes in fruit fly oviposition behaviour. *Oecophylla longinoda* defends chemically marked territories at both the intra- and interspecific levels (Hölldobler & Wilson, 1990; Dejean *et al.*, 2005). Dicke (2000) suggests that territorial ant pheromones may present reliable cues of ant presence and predation risk, and therefore can be exploited by potential prey. Offenberg *et al.* (2004) propose that the repellent effect on herbivores in Thai mangrove systems is a result of *Oecophylla* pheromones.

However, the study by Offenberg *et al.* (2004) is not conclusive regarding whether the cues are chemical or visual. Ants mark their entire territory and trails with visible cues produced in the rectal sac (anal spots) and with invisible trail pheromones produced in the rectal gland (Dejean & Beugnon, 1991). Anal-drop depositions have a dual function: territorial and orientational marking. These anal spots are not randomly distributed; they lead from the nest to a food source (Dejean & Beugnon, 1991). Although Offenberg *et al.* (2004) suggest that herbivores are repelled by ant pheromones, it is still unclear whether the visible or invisible cues (or a combination) are the principal cause.

The present study shows that ant cues have a suppressing effect on fly landings and time spent on fruit for both *C. cosyra* and *B. invadens*. Although *B. invadens* is reported to have entered the African continent only a few years ago (Vayssières *et al.*, 2005), it is receptive to the chemical cues emitted by the African weaver ant. The volatile exocrine secretions of the Asian weaver ant *O. smaragdina* and the closely-related African weaver ant *O. longinoda* are very similar (Keegans *et al.*, 1991). However, territorial pheromones also contain compounds that provide highly colony-specific cues. *Oecophylla* workers detecting the deposits of an alien colony respond with increased amounts of aversive and aggressive behaviour, and they later recruit nestmates to the area at a higher rate (Hölldobler & Wilson, 1990). At the same time, the present study suggests that more generic (noncolony-specific) cues play a role in interspecific communication. This claim is supported by the finding that different fruit fly species respond in a similar way to the cues.

Ant territorial pheromones may affect fruit fly searching behaviour for an appropriate fruit to oviposit once it has found an appropriate host tree. Considering their persistence and low volatility, it is unlikely that these same cues also influence fruit fly searching behaviour on a wider spatial scale (i.e. for habitat and host tree selection). Other volatiles, such as ant colony odour (formic acid) or other semiochemicals, may provide

Table 2. Effect of ant cues on fruit fly infestation and damage in the field (mean ± SE).

Distance of fruit from ant nest	Fruit fly infestation rate (%)	Number of fruit fly puparia per kg collected fruit	Number of fruit fly puparia per kg infested fruit
< 1 m	8.9 ± 2.2[a]	0.64 ± 0.45[a]	7.19 ± 4.22[a]
1–3 m	22.2 ± 6.1[a,b]	3.21 ± 1.85[a]	13.41 ± 6.94[a]
No ants	62.2 ± 6.7[b]	21.09 ± 7.64[b]	33.45 ± 10.74[b]

The amount of ant cues was assumed to be negatively correlated with distance from ant nests. Mango fruits were collected from trees with *Oecophylla* within 1 and 1–3 m distance from ant nests, as well as from trees without *Oecophylla*. Data on the number of puparia per kg fruit were $\log_{10}(x + 1)$ transformed before being analysed with the Student–Newman–Keuls test. Values in columns followed by different superscript letters are significantly different ($n = 45$, $P < 0.05$).

more important long-distance cues affecting fruit fly behaviour. Furthermore, frugivore mammals may be receptive to visual and olfactory cues released by ants. According to some Guinean mango farmers, fruit bats are deterred by the smell of *Oecophylla* and avoid colonized trees (Van Mele *et al.*, 2009). Major workers of *O. longinoda* emit venom from the tip of the abdomen as it is brought immediately above the head. The venom of *Oecophylla* elicits a mass attack response in other major workers, indicating that these compounds are more volatile than the ant trail pheromones. The sources of the venom comprise the poison gland, which contains formic acid, and Dufour's gland, which contains hydrocarbons, with undecane (characteristic of formicine species) being the most important one (Keegans *et al.*, 1991). Formic acid and undecane presented together experimentally proves to be more effective in triggering a mass attack than either compound tested separately (Bradshaw *et al.*, 1979). Different combinations of ant pheromones and other cues are likely to affect the behaviour of conspecifics, herbivores and frugivores at different spatio-temporal scales. The results obtained in the present study provide one more example of the extreme development of territorial behaviour and signal economy in *Oecophylla*.

Changes in fruit fly behaviour as a function of distribution of resources and natural enemies affects fitness. However, an accurate understanding of fruit fly sexual, feeding and oviposition behaviours requires a holistic approach, whereby mechanistic, visual and olfactory approaches to behavioural analysis are integrated. The interplay between *Oecophylla* and fruit flies offers an ideal opportunity to test such models and to assess trade-offs between optimal oviposition theory and enemy avoidance.

Acknowledgements

References

Abrams, P.A., Menge, B.A. & Mittelbach, G.G. (1996) The role of indirect effects in food webs. *Food Webs Integration of Patterns and Dynamics* (ed. by G. A. Polis and K. O. Winemiller), Chapman & Hall, New York, New York.

Aluja, M., Sivinski, J., Rull, J. & Hodgson, P.J. (2005) Behavior and predation of fruit fly larvae (*Anastrepha* spp.) (Diptera: Tephritidae) after exiting fruit in four types of habitats in tropical Veracruz, Mexico. *Environmental Entomology*, **34**, 1507–1516.

Billen, J. & Morgan, E.D. (1998) Pheromone communication in social insects: sources and secretions. *Pheromone Communication in Social Insects. Ants, Wasps, Bees, and Termites* (ed. by R. K. Vander Meer, M. D. Breed, K. E. Espelie and M. L. Winston), Westview Press, Boulder, Colorado.

Bradshaw, J.W.S., Baker, R. & Howse, P.E. (1979) Chemical composition of the poison apparatus secretions of the African weaver ant *Oecophylla longinoda* and their role in behavior. *Physiological Entomology*, **4**, 39–46.

Brévault, T. & Quilici, S. (2007) Influence of habitat pattern on orientation during host fruit location in the tomato fruit fly, *Neoceratitis cyanescens*. *Bulletin of Entomological Research*, **97**, 637–642.

Cook, S.M., Khan, Z.R. & Pickett, J.A. (2007) The use of push–pull strategies in integrated pest management. *Annual Review of Entomology*, **52**, 375–400.

Dejean, A. & Beugnon, G. (1991) Persistant intercolonial trunkroute-marking in the African weaver ant *Oecophylla longinoda* Latreille (Hymenoptera, Formicidae): Tom thumb's versus Adriane's orienting strategies. *Etiology*, **88**, 89–98.

Dejean, A., Le-Breton, J., Suzzoni, J.-P. *et al.* (2005) Influence of interspecific competition on the recruitment behavior and liquid food transport in the tramp ant species *Pheidole megacephala*. *Naturwissenschaften*, **92**, 324–327.

Díaz-Fleischer, F. & Aluja, M. (2000) Behavior of tephritid flies: a historical perspective. *Fruit Flies (Diptera: Tephritidae): Phylogeny and Evolution of Behavior* (ed. by M. Aluja and A. L. Norrbom), CRC Press, Boca Raton, Florida.

Dicke, M. (2000) Chemical ecology of host-plant selection by herbivorous arthropods: a multitrophic perspective. *Biochemical Systematics and Ecology*, **28**, 601–617.

Dicke, M. & Grostal, P. (2001) Chemical detection of natural enemies by arthropods: an ecological perspective. *Annual Review of Ecology and Systematics*, **32**, 1–23.

Fletcher, B.S. & Prokopy, R.J. (1991) Host location and oviposition in tephritid fruit flies. *Reproductive Behaviour of Insects. Individuals and Populations* (ed. by W. J. Bailey and J. Ridsdill-Smith), Chapman & Hall, U.K.

Hoffmeister, T.S. & Roitberg, B.D. (1997) To mark the host or the patch – decisions of a parasitoid searching for concealed host larvae. *Evolutionary Ecology*, **11**, 145–168.

Hölldobler, B. & Wilson, E.O. (1990) *The Ants*. Harvard University Press, Cambridge, Massachusetts.

Jander, R. & Jander, U. (1979) An exact field test for the fade-out time of the odor trails of the Asian weaver ants *Oecophylla smaragdina*. *Insectes Sociaux*, **26**, 165–169.

Jang, E. & Light, M.D. (1981) Olfactory semiochemicals of tephritids. *Fruit Fly Pest: A World Assessment of Their Biology and Management* (ed. by A. McPheron and G. J. Steck), St Lucia Press, Delray Beach, Florida.

Jang, E.B., Carvalho, L.A. & Stark, J.D. (1997) Attraction of female oriental fruit fly, *Bactrocera dorsalis*, to volatile semiochemicals from leaves and extracts of a nonhost plant, Panax (*Polyscias guilfoylei*) in laboratory and olfactometer assays. *Journal of Chemical Ecology*, **23**, 1389–1401.

Keegans, S.J., Billen, J. & Morgan, E.D. (1991) Volatile secretions of the green tree ant *Oecophylla smaragdina* (Hymenoptera: Formicidae). *Comparative Biochemistry and Physiology*, **100**, 681–685.

Krebs, J.R. & Kacelnik, A. (1997) Decision making. *Behavioural Ecology: An Evolutionary Approach* (ed. by J. R. Krebs and N. B. Davies), 4th edn. Blackwell Publishing, U.K.

Mangel, M. & Roitberg, B.D. (1989) Dynamic information and host acceptance by a tephritid fruit fly. *Ecological Entomology*, **14**, 181–189.

Mayhew, P.J. (1997) Adaptive patterns of host-plant selection by phytophagous insects. *Oikos*, **79**, 417–428.

Offenberg, J., Nielsen, M.G., Macintosh, D.J., Havanon, S. & Aksornkoae, S. (2004) Evidence that insect herbivores are deterred by ant pheromones. *Proceedings of the Royal Society of London Series B, Biological Sciences*, **271**, 433–435.

Peña, J.E., Sharp, J.L. & Wysoki, M. (2002) *Tropical Fruit Pests and Pollinators*. CABI Publishing, U.K.