PHEROMONAL COMMUNICATION IN EUROPEAN EARWIGS, *FORFICULA AURICULARIA L.* (DERMAPTERA: FORFICULIDAE)

by

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Abstract

European earwigs, *Forficula auricularia* L., are thought to use an aggregation pheromone but there is controversy about its source. Hence I investigated whether each developmental stage and sex produce and respond to this pheromone and what are its components. Laboratory experiments revealed that females, males and nymphs produce and respond to an airborne aggregation pheromone. Candidate pheromone components obtained from all potential sources were analyzed by gas chromatographic-electroantennographic detection (GC-EAD) and GC-mass spectrometry. A complex synthetic blend (SB) of 30 candidate pheromone components, including benzoquinones, acids, hydroquinone, vanillin, aldehydes, ketones and an acetal significantly arrested females and nymphs in laboratory experiments. In both laboratory and field experiments, the SB lacking benzoquinones elicited significant behavioral responses from nymphs, but not from adults, suggesting that adults, unlike nymphs, use an aggregation pheromone with benzoquinones as constituent components. Additional experiments are required to determine all essential components of the earwig aggregation pheromone.

Keywords:

European earwigs, aggregation pheromone, laboratory analyses, benzoquinones, olfactometer bioassays, field experiments

To my parents

O' human being, you are in the dark
Grope, grope towards light, take of your mark
Be bold and go ahead
Avoid evil and do the good
(Gurjeet Kaur, 1946-1985)

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Chapter 1 General Introduction

1.1 Biology and ecology

The common name "earwig" is derived from the Anglo-Saxon word "earwicga" denoting ear creature (Fulton, 1924a). Earwigs in different European languages are associated with the superstition that they crawl into the ears of sleeping humans. The origin of this superstition is not known (Fulton, 1924a; Crumb et al., 1941).

1.1.1 Distribution

The European earwig (EEW), *Forficula auricularia* L., is a native of Europe, western Asia and probably North Africa (Crumb et al., 1941; Clausen, 1978). It is an exotic species in North America with a wide distribution (Weems and Skelly, 2007). Chronological reports (Langston and Powell, 1975) indicate that it was first observed in 1909 in Portland (Oregon) (Fulton, 1924a), with subsequent observations in Newport, Rhode Island (1911; Jones, 1917; Stene, 1934), Seattle (1915; Jones, 1917), Vancouver (British Columbia) (1916; Treherne, 1923) and California (1923; Langston and Powell, 1975). In Canada, it is found in British Columbia, Manitoba, Newfoundland, Nova Scotia, Ontario, Quebec and Saskatchewan (Weems and Skelley, 2007). Thirty species of earwigs have been reported from the United States (Haas, 2006). In Canada, there are five species of earwigs: *Doru aculeatum* (Scudder) is native, and the little earwig, *Labia minor* (Linnaeus), might have been established a long time ago (Buckell, 1929). *Anisolabis maritima* (Géné), *Euborellia annulipes* (Lucas) and the EEW are exotic (Buckell, 1929). In North America, EEWs comprise two sibling species, which are reproductively isolated (Wirth et al., 1998). Populations in cold or continental

climates (e.g., Quebec, New York, Connecticut) mostly have one clutch per year, forming species A (Gingras and Tourneur, 2001), whereas those in warmer climates (e.g., coastal BC, Oregon) have two clutches per year, forming species B (Wirth et al., 1998; Guillet et al., 2000).

In Canada, EEWs were reported for first time from Vancouver in 1916, although they were suspected to have been introduced long before then (Treherne, 1923). Their population peaked in 1927-28 in Vancouver (Spencer, 1945) when they were considered pests (Lamb, 1974).

1.1.2 Morphological characters and taxonomic placement

Before the hemimetabolous EEWs were placed taxonomically in the Order Dermaptera, they were considered close relatives of grasshoppers, crickets and cockroaches (Fulton, 1924a). EEWs have an elongate flattened brownish colored body (Borror and White, 1970), with a shield-shaped pronotum (Buckell, 1929), two pairs of wings and a pair of forcep-like cerci (Fulton, 1924a). The second tarsal segment is lobed, extending distally below the 3rd tarsal segment (Helfer, 1963). The antenna consists of 11-14 segments, and the mouth parts are of the chewing type (Borror and White, 1970). Adult males are polymorphic in body weight and head width, as well as cercus length and width (Lamb, 1976b). Third instar or older nymphs that have lost one branch of cerci are capable of regenerating it in form of a straight structure. Males with such asymmetrical forceps are known as gynandromorphs or hermaphrodites because they resemble females (Behura, 1956).

1.1.3 Dispersal

EEWs spend the day time in cool, dark, inaccessible places such as flowers, fruits and wood crevices (Fulton, 1924a; Goe, 1925; Crumb et al., 1941). EEWs easily establish after

introduction to a new place (Essig, 1918) because they tolerate adverse conditions (Morgan, 1926). They rarely fly but have well developed wings (Jones, 1917; Goe, 1925 and Lamb, 1974). They disperse passively through transportation of commercial products, including lumber, ornamental shrubs and even newspaper bundles (Walker, 1997). Humans are concerned with their dispersal because they invade human dwellings (Fulton, 1924a; Crumb et al., 1941; Buxton, 1974; Weems and Skelley, 2007). EEWs are nocturnal, avoid strong light and hide during the day (Behura, 1956; Borror and White, 1970).

1.1.4 Microhabitat

EEWs survive well in cool and moist microhabitats with a mean temperature of 24°C (Crumb et al., 1941). Their daily abundance in a given year has been linked to temperature, wind velocity and the prevalence of easterly winds (Chant and McLeod, 1952). The development of EEWs also depends on temperature (Crumb et al., 1941; Behura, 1956). Thus, the occurrence of EEWs can be predicted based on weather parameters (Helsen et al., 1998). Hibernating adults can tolerate cool temperatures, but their survival is reduced in poorly drained soils such as clay (Crumb et al., 1941). To avoid excessive moisture, EEWs seek the southern sides of well drained slopes. Sometimes they also occupy the hollow stems of flowers where the soil is poorly drained (Behura, 1956; Weems and Skelly, 1989). Their eggs are capable of resisting damage from cold and heat (Chauvin et al., 1991).

1.1.5 Feeding habits

EEWs are reportedly omnivorous (Fulton, 1927; Beall, 1932; Buxton, 1974; Lamb, 1974; Walker, 1997) and necrophagous (Jones, 1917; Fulton 1924a), feeding on a wide variety of plants and insects (Crumb et al., 1941). Their favourite plants include the common crucifer *Sisymbrium officinale* L., white clover *Trifolium repens* L., and the dahlia *Dahlia*

variabilis (Beall, 1932). They also like to feed on molasses and lower forms of plants, such as lichens and algae (Crumb et al., 1941). They prefer meat or sugar to natural plant material even though plants are a major natural food source (Fulton, 1927). EEWs prefer aphids to the plant material, such as leaves and fruit slices of apple, cherry and pear (Asgari, 1966 cited in Carroll and Hoyt, 1984). Adults reportedly eat relatively more insects than do nymphs (Crumb et al., 1941).

1.1.6 Life cycle

The life cycle of EEWs has been studied in Edinburg, (UK) (Behura, 1956), Oregon, Washington and Virginia (USA) (Fulton, 1924a; Crumb et al., 1941; Walker, 1997) and in Vancouver (Beall, 1932 and Lamb and Wellington, 1975). EEWs can have a single brood per year (Burr, 1939 and Guillet et al., 2000) or two broods per year (Beall, 1932; Behura, 1956 and Lamb and Wellington, 1975). Many females and males hibernate in pairs in subterranean nests (2.5-5 cm below surface) (Fulton, 1924a; Behura, 1956), whereas others aggregate above ground (Lamb and Wellington, 1975). In Vancouver, females oviposit at the end of winter (Lamb and Wellington, 1975) and in United Kingdom from November to February (Behura, 1956). After oviposition, females expel the male from the nest (Behura, 1956; Lamb, 1974). Males then aggregate in cracks and crevices above ground (Fulton, 1924a), beginning typically in early spring (Lamb and Wellington, 1975). Many females oviposit a second time in the summer (Lamb, 1974). Each female lays on average 23-55 and 6-36 eggs during the first and second oviposition period, respectively (Behura, 1956). Freshly laid eggs are opaque and pale yellow or cream in colour and broadly elliptical (Fulton, 1924a), 1.9 × 0.9 mm in length and breadth, respectively (Behura, 1956). The incubation period is comparatively long in cold areas varying from 23-27 weeks (161-189 days) in Montreal (Quebec) (Gingras and Tourneur, 2001). In Washington, the mean incubation

period was 72.8 days for winter-laid eggs and 20 days for spring-laid eggs. The incubation period ranged from 56-85 days, depending upon the depth of the subterranean nest and the temperature of area (Crumb et al., 1941).

Eggs start to hatch in early May in British Columbia and Washington (Crumb et al., 1941; Lamb and Wellington, 1975) and in February-March in Edinburgh (UK) (Behura, 1956). In Vancouver, nymphs molt for the first time in their nests in mid-May and pass through four nymphal instars before turning into adults (Lamb and Wellington, 1975). The mean nymphal period at room temperature was 85.7 and 86.23 days for males and females, respectively (Behura, 1956).

The earwigs' life history has a distinct nesting phase and free-foraging phase (Lamb and Wellington, 1975). In the nesting phase, the population consists of family units, comprising at first a female and male pair and later the female and her offspring nymphs. They leave the nest for nocturnal foraging bouts and return to and stay in their own nest during the day. During the free-foraging phase, family units mingle, family members no longer return to their own nest and food-forage long distances. In this phase, they do not show any preference for a particular shelter. Some females mate and oviposit again (Lamb and Wellington, 1975).

The males use their forceps during copulation to lift a female's abdomen, and for defense (Fulton, 1924b; Crumb et al., 1941; Behura, 1956; Eisner, 1960) coupled with spray secretions from abdominal glands (Eisner, 1960). Studies of the EEW's sex-ratio revealed a slight female bias (Behura, 1956), pronounced female bias in field populations (Brindley, 1912, 1914; Beall 1932), but no female bias in laboratory populations (Crumb et al., 1941). Although there may be fewer males than females, a single male can fertilize many females, particularly considering that EEWs stay in aggregation (Callan, 1941 cited in Behura, 1956).

1.1.7 Mating behavior

A male finds prospective mates by olfaction. He then slips his cerci under the tip of the female's abdomen so that his and her ventral abdominal surfaces are in contact with each other, while both face in opposite directions. Pairs can stay in *copula* for many hours if not disturbed (Fulton, 1924a; Behura, 1956). Matings occurred frequently among clustered individuals particularly in locations that allow both partners to cling to a surface (Fulton, 1924a). The mating season peaked during August and September under laboratory conditions, and a single mating enabled females to lay fertilized eggs (Behura, 1956).

1.1.8 Oviposition and eclosion

Females construct a subterranean chamber in which they lay eggs during 2-3 days (Behura, 1956). During development, eggs double in size (Crumb et al., 1941). When eggs are close to hatching, the dark red eyes, mandibles, antennae, palps and legs of the first instars nymph are visible (Fulton, 1924a). During hatching, the head of the nymph ruptures the egg's chorion within five minutes and then the nymph crawls out (Fulton, 1924b; Behura, 1956), unassisted by their female parent (Fulton, 1924b and Crumb et al., 1941). After each molt, nymphs are white in colour and then turn grey in 10 hours. They pass through four instars before they become adults (Behura, 1956).

1.1.9 Maternal care

Maternal care includes nest construction, care of eggs and nymphs as well as defense and provision of food to newly hatched nymphs (Lamb, 1976a). Females use their mandibles to transform soil particles into small pellets for nest construction (Fulton, 1924b). They can also clean eggs with their mouth parts and move them from one place to another (Fulton, 1924b; Crumb et al., 1941; Lamb, 1976a). Eggs fail to hatch in the absence of female (Goe,

1925; Crumb et al., 1941; Guppy, 1947). In her absence, egg hatching can be stimulated by regular cleaning and rolling of eggs (Buxton and Madge, 1974). Females tend to eat their eggs if there is a disturbance or conditions are unfavorable (Crumb et al., 1941). They seal cracks of a nesting chamber for a few days after hatching of nymphs to prevent their escape (Fulton, 1924b). They also provision first instar nymphs with food which they carry to the nest or regurgitate (Crumb et al., 1941; Lamb, 1976a). Females are not able to discern between their own offspring and those of other females, and care for all offspring (Fulton, 1924b). Either fertilized or unfertilized eggs are laid in similar numbers and receive the same parental care (Behura, 1956). Because of this parental care EEWs are considered subsocial. They are not considered social insects because they don't have co-operative brood care, overlapping care between generations or reproductive castes (Lamb, 1976a).

1.1.10 Natural enemies

EEWs are affected by pathogens, such as (a) the fungus *Entomophthora forficulae* and the muscardine fungus *Metarrhizium anisopliae* Sorokin; (b) the gregarine protozoan *Clepsidrina ovata;* (c) the round worms *Mermis nigrescens* Dujardin and *M. subnigrescens* Cobb.; and (d) tyroglyphid mites (Crumb et al., 1941). They are also parasitized by the tachinid flies *Rhacodineura pallipes* Fallen and *Digonochaeta setipennis* Meigen (Phillips, 1983). They are commonly preyed upon by birds, such as the song thrush, *Turdus philomelos* (Collinge, 1913 cited in Brindley, 1918), whimbrel, *Numenius phaeopus,* green woodpecker, *Picus viridis*, nuthatch, *Sitta europaea*, chaffinch, *Fringilla coelebs*, great titmouse, *Parus major,* whinchat, *Saxicola rubetra* (Newstead, 1908 cited in Brindley, 1918), starling, *Sturnus vulgaris,* robin, *Turdus migratorius*, pheasant, *Phasianus colchicus*, cowbird, *Molothrus ater,* and brewer's blackbird, *Euphagus cyanocephalus* (Lamb, 1975).

1.2 Economic importance

The pest impact of EEWs appears exaggerated, although they can cause damage to a wide variety of plants (Lucas and Fes, 1906; Crumb et al., 1941), and have been rated as one of the six most important structural pests in California (Ebeling, 1978). They are household nuisance pests in residential areas (Jones, 1917; Fulton, 1924a; Dimick and Mote, 1934; Stene, 1934; Crumb et al., 1941; Lamb 1974; Ebeling, 1978; Flint, 2002) particularly at high population densities (Vickery and Kevan, 1985). In the past, the presence of EEWs could adversely affect property values (Gibson and Glendenning, 1925 cited in Buckell, 1929). They are also an important pest of flowers, fruits and vegetables (Fulton, 1924a; Tillyard, 1925; Lamb, 1974; Cranshaw, 2000; Flint, 2002; Anonymous, 2004a, b) and transmit the turnip yellow mosaic virus among crucifers under experimental conditions (Markham and Smith, 1949 cited in Behura, 1956). EEWs are capable of invading fruits through mechanical injuries and then cause secondary damage to soft fruits (Cranshaw, 2000; Flint, 2002; Anonymous, 2004a). On the other hand, EEWs are predators of insect pests, such as the aphid *Eriosoma lanigerum* (Carroll and Hoyt, 1984; Mueller et al., 1988; Nicholas et al., 2005) and the codling moth, *Cydia pomonella* (Glen, 1975), and are therefore valued as biological control agents (Anonymous, 2004b).

1.2.1 Trapping and detection

A phenological model was developed in the Netherlands to predict the occurrence of EEW populations. The model was based on a correlation between EEW growth and a degree day temperature summation > 6°C, indicating that EEWs require 880 degree days to develop from first instar nymphs to adults (Helsen et al., 1998). Older and alternative methods of EEW population assessments include soil-core spade sampling of hibernating or nesting insects (Wilson and Wilde, 1971), and collection from diurnal shelters such as cracks and

crevices of bark, rolled leaves (Lamb and Wellington 1974) or grooved board traps (Crumb et al., 1941; Chant and McLeod, 1952).

1.2.2 Baits

All literature concerned with trap baits dates back 50 years. Campaigns with poisonous baits were initiated in Oregon (Fulton, 1924a) and elsewhere along the Pacific Coast and were considered successful for EEW management (Buckell, 1929). Baits consisting of wheat bran, sodium fluosilicate and fish oil were recommended for earwig control (Crumb et al., 1941). Peanut butter used with whole wheat flakes in pitfall type traps was deemed better than fish oil and earwig scent (Legner and Davis, 1962). Grooved board traps were considered as effective as insecticide for EEW control and more effective than pitfall traps baited with bran flakes, honey, peanut butter, macerated earwigs or fish oil (Morris, 1965).

1.2.3 Biological control

In British Columbia, the dipteran parasite *Bigonicheta setipennis* (Fallen) was introduced in 1934-1939 as a biological control agent against EEWs (Spencer, 1947; McLeod, 1954 cited in Lamb, 1974). However, *B. setipennis* failed to cause long term decline of EEW populations (Lamb, 1974). In Vancouver, EEW populations stabilized at a level believed not to be pestiferous (Lamb, 1974).

1.3 Evidence for pheromonal communication and research objectives

EEWs are gregarious insects (Sauphanor and Sureau, 1993) that occupy diurnal shelters (Lamb, 1974) in groups ranging between 50-100 individuals per square meter (Lamb and Wellington, 1975). Prior occupancy enhanced the attractiveness of a shelter implying

pheromone-mediated aggregation behavior (Philips, 1981 cited in Helsen et al., 1998; Sauphanor, 1992). The source of the pheromone remains to be determined with certainty. It may be present in cuticular lipids of males and in fecal excreta (Walker et al., 1993) or in tibial glands (Brousse-Gaurry, 1983; Sauphanor, 1992). This controversy may be explained, in part, by the fact that fresh or old frass was bioassayed, and that tibial gland extracts were tested with nymphs (Sauphanor, 1992) and adults (Walker et al., 1993), respectively.

To further our understanding of the pheromone and its source I investigated, whether (1) both nymph and adult EEWs produce and respond to pheromone (Chapter 2); (2) the pheromone is perceived by olfaction or contact chemoreception (Chapter 2); (3) the pheromone is present in the effluvium, body parts, or feces of EEWs (Chapter 2); and (4) the pheromone is a complex blend of various components (Chapter 3).

1.4 References

Anonymous, 2004a. Earwigs (*Forficula auricularia*). http://www.agf.gov.bc.ca/cropprot/tfipm/earwigs.htm

Anonymous, 2004b. Effective control of earwigs.http://www.pmra-arla.gc.ca/english/consum/earwigs-e.html

Beall, G. 1932. The life history and behavior of the European earwig, *Forficula auricularia*, L. in British Columbia. *Proceedings of Entomological Society of British Columbia* 39:28-43.

Behura, B.K. 1956. The biology of common earwig, *Forficula auricularia. The Annals of Zoology* 1:117-142.

Borror, D.J. and White, R.E. 1970. A field guide to insects: America North of Mexico. Houghton Mifflin Company, Boston, New York pp 98-99.

Brindley, H.H. 1912. The proportion of sexes of *Forficula auricularia. Proceedings of the Cambridge Philological Society* 16:674-679.

Brindley, H.H. 1914. The proportion of sexes of *Forficula auricularia* in the Scilly Islands. *Proceedings of the Cambridge Philological Society* 17:326-334.

Brindley, H.H. 1918. Notes on certain parasites food and capture by birds of the common earwig (*Forficula auricularia*). *Proceedings of the Cambridge Philological Society* 19:167-177.

Brousse-Gaury, P. 1983. Sur l'existence d'une glande tibiale chez *Labidura riparia* Pallas et *Forficula auricularia* L. (Insectes: Dermapteres). *Comptes Rendus de l Academie des Sciences Paris* 297:203-208.

Buckell, E.R. 1929. The Dermaptera of Canada. *Proceedings of Entomological Society of British Columbia* 26:9-27.

Burr, M. 1939. Modern work on earwigs. *Science Progress* 34:20-30.

Buxton, J.H. 1974. The Biology of the European earwig *Forficula auricularia* L. with reference to its predatory activities on the Damson-hop aphid *Phorodon humuli* (Schrank). Ph.D. Dissertation. Wye College, University of London.

Buxton, J.H. and Madge, D.S. 1974. Artificial incubation of the Common eawig, *Forficula auricularia* (L.). *Entomological Monthly Magazine* 110:55-57.

Carroll DP and Hoyt SC 1984. Augmentation of European earwigs (Dermaptera: Forficulidae) for biological control of apple aphid (Homoptera: Aphididae) in an apple orchard. *Journal of Economic Entomol*ogy 77:738-740.

Chant, D.A. and McLeod, J.H. 1952. Effects of certain climatic factors on the daily abundance of the European earwig, *Forficula auricularia* L. (Dermaptera: Forficulidae), in Vancouver, British Columbia. *The Canadian Entomologist* 34:174-180.

Chauvin, G., Hamon, C., Vancassel, M. and Vannier, G. 1991. The eggs of *Forficula auricularia* L. (Dermapter: Forficulidae): ultrastructure and resistance to low and high temperatures. *Canadian Journal of Zoology* 69:2873-2878.

Clausen, C.P. 1978. Dermaptera Forficulidae, *in* Clausen, C.P. 1978 (ed.) Introduced parasites and predators of arthropod pests and weeds: A world review. Pp.15-18.

Cranshaw, W.S. 2000. European earwig: Habits and management. *Pest Alert* 17:4-5.

Crumb, S.E., Eide, P.M. and Bonn, A.E. 1941. The European earwig. *Technical Bulletin United States Department of Agriculture Washington, D.C.* 766:1-76.

Dimick, R.E. and Mote, D.C. 1934. The present status of the European earwig. *Monthly Bulletin of Department of Agriculture, State of California* 23:298-300.

Ebeling, W. 1978. Urban entomology. Divison of Agricultural Sciences, University of California, Berkeley. Pp. 560-565.

Eisner, T. 1960. The chemical and mechanical weapons of an earwig. *Psyche* 67:62-70.

Essig, E.O. 1918. The European earwig, *Forficula auricularia* Linn. *Journal of Economic Entomology* 11:338-339.

Flint, M.L. 2002. Earwigs: Integrated pest management for home gardeners. *Pest notes, University of California agriculture and natural resources publication,* 74102. Produced by IPM education and publications, UC Statewide IPM program, University of California, Davis, CA.

Fulton, B.B. 1924a. The European earwig. *Station Bulletin/ Oregon Agricultural College Experiment Station* 207:1-29.

Fulton, B.B. 1924b. Some habits of earwigs. *Annals of the Entomological Society of America* 17:357-367.

Fulton, B.B. 1927. Concerning some published statements on the habits of the European earwig (Orthoptera: Forficulidae). *Entomological News* 38:272-273.

Gibson, A. and Glendenning, R. 1925. European earwig - an undesirable pest. *Canada Department of Agriculture Circular-New Series* 24:1-4.

Gingras, J. and Tourneur, J. 2001. Timing of adult mortality, oviposition, and hatching during the underground phase of *Forficula auricularia* (Dermaptera: Forficulidae). *The Canadian Entomologist* 133:269-278.

Glen, D.M. 1975. The effects of predators on the eggs of codling moth *Cydia pomonella*, in a cider-apple orchard in south-west England. *Annals of Applied Biology* 80:115-135.

Goe, M.T. 1925. Eight months study of earwigs (Dermaptera). *Entomological News* 36:234-238.

Guillet, S., Josselin, N. and Vancassel, M. 2000. Multiple introductions of the *Forficula auricularia* species complex (Dermaptera: Forficulidae) in eastern North America. *The Canadian Entomologist* 132:49-57.

Guppy, R. 1947. Results of a season's study of the European earwig, *Forficula auricularia* (Dermaptera: Forficulidae). *Proceedings of the Entomological Society of British Columbia* 43:28-31.

Haas, F. 2006. Earwig research centre: country faunas and checklists. http://www.earwigs-online.de/faunas.html

Helfer, J.R. 1963. How to know the grasshoppers, cockroaches and their allies, pictured-keys for identifying many of the grasshoppers, crickets katydids, earwigs, termites, cockroaches, praying mantids, rock-crawlers, and walking-sticks occuring in North America, north of Mexico. Dubuque, IA: William C. Brown Co. Pp. 13-19.

Helsen, H., Vaal, F. and Blommers, L. 1998. Phenology of the common earwig *Forficula auricularia* L. (Dermaptera: Forficulidae) in an apple orchard. *International Journal of Pest Management* 44:75-79.

Jones, D.W. 1917. The European earwig and its control. Bulletin no. 566. *United States Department of Agriculture* 1-12.

Lamb, R.H. 1974. Earwig travel in relation to habitat. Ph.D. Dissertation. The University of British Columbia, Vancouver. 143pp.

Lamb, R.J. 1975. Effects of dispersion, travel and environmental heterogeneity on populations of the earwig, *Forficula auricularia* L. *Canadian Journal of Zoology* 53:1855-1867.

Lamb, R.J. 1976 a. Parental behavior in the Dermaptera with special reference to *Forficula auricularia* (Dermaptera: Forficulidae). *The Canadian Entomologist* 108:609-619.

Lamb, R.J. 1976 b. Polymorphism among males of the European earwig, *Forficula auricularia* (Dermaptera: Forficulidae). *The Canadian Entomologist* 108:69-75.

Lamb, R.J. and Wellington, W.G. 1974. Techniques for studying the behavior and ecology of the European earwig, *Forficula auricularia* (Dermaptera: Forficulidae). *The Canadian Entomologist* 106:881-888.

Lamb, R.J. and Wellington, W.G. 1975. Life history and population characteristics of the European earwig, *Forficula auricularia* (Dermaptera: Forficulidae) at Vancouver, British Columbia. *The Canadian Entomologist* 107:819-824.

Langston, R.L. and Powell, J.A. 1975. The earwigs of California (Order Dermaptera). *Bulletin of California Insect Survey* 20:1-25.

Legner, E.F. and Davis, D.W. 1962. Tests with attractants and a simple trap for the European earwig, *Forficula auricularia. Journal of Economic Entomology* 55:1006-1007.

Lucas, W.J. and F.E.S. B.A. 1906. Insecta: Orthoptera, *in* The wild fauna and flora of the royal botanic gardens, Kew. *Bulletin of Miscellaneous Information.* Pp. 22-23.

Markham, R. and Smith, K.M. 1949. Studies on the virus of turnip yellow mosaic. *Parasitology* 39:330-342.

McLeod, J.H. 1954. Statuses of some introduced parasites and their hosts in British Columbia. *Proceedings of the Entomological Society of British Columbia* 50:19-27.

Morgan, W.P. 1926. A note on the mode of distribution of earwigs. *Proceedings of the Indiana Academy of Science* 36:331-332.

Morris, R.F. 1965. Note on control of the European earwig, *Forficula auricularia* L. in Newfoundland with traps and attractants. *The Canadian Entomologist* 97:1075-1076.

Mueller, T.F., Blommers, L.H.M. and Mols, P.J.M. 1988. Earwig (*Forficula auricularia)* predation on the woolly apple aphid, *Eriosoma lanigerum. Entomologia Experimentalis et Applicata* 47:145-152.

Nicholas, A.H., Spooner-Hart, R.N. and Vickers, R.A. 2005. Abundance and natural control of the woolly aphid *Eriosoma lanigerum* in an Australian apple orchard IPM program. *BioControl* 50:271-291.

Phillips, M.L. 1983. Parasitism of the common earwig *Forficula auricularia* (Dermaptera: Forficulidae) by tachinid flies in an apple orchard. *Entomophaga* 28:89-96.

Sauphanor, B. 1992. Une Pheromone d'aggregation chez *Forficula auricularia L. Entomologia Experimentalis et Applicata* 62:285-291.

Spencer, G.J. 1945. On the incidence, density and decline of certain insects in British Columbia. *Proceedings of the Entomological Society of British Columbia* 42:19-23.

Spencer, G.J. 1947. The 1945 status of *Digonochaeta setipennis*, tachinid parasite of the European earwig, *Forficula auricularia* Linn. in West Point Grey, Vancouver BC *Proceedings of theEntomological Society of British Columbia* 43:8-9.

Stene, A.E. 1934. The European earwig as a pest in Rhode Island. *Journal of Economic Entomology* 27:566-569.

Tillyard, J. 1925. Insects in relation to the New Zealand food supply. *Mid-Pacific Magazine* 29:665-680.

Treherne, R.C. 1923. The European earwig in British Columbia. *Proceedings of the Entomological Society of British Columbia* 17:161-163.

Vickery, V.R. and Kevan, D.K.M. 1985. The insects and arachnids of Canada. Part 14. The grasshoppers, crickets and related insects of Canada and adjacent regions. Canadian Government Publishing Centre, Ottawa, Canada. Pp. 9-53.

Walker, K.A. 1997. Aggregation, courtship, and behavioural interactions in European earwigs, *Forficula auricularia* L. (Dermaptera: Forficulidae). Ph.D. Dissertation. Virginia Polytechnic Institute and State University.

Walker, K.A., Jones, T.H. and Fell, R.D. 1993. Pheromonal basis of aggregation in European earwig, *Forficula auricularia* L. (Dermaptera: Forficulidae). *Journal of Chemical Ecology* 19:2029-2038.

Weems , H.V. and Skelley, P.E. 1989. European earwig - *Forficula auricularia* Linnaeus (Dermaptera: Forficulidae). *Entomology Circular Number* 318:2.

Weems Jr, H.V. and Skelley, P.E. 2007. European earwig-*Forficula auricularia* Linnaeus. http://creatures.ifas.ufl.edu./veg/european_earwig.htm.

Wilson, W.A. and Wilde, W.H.A. 1971. Some effects of gamma radiation on earwigs in Ontario, Canada (Dermaptera: Forficulidae). *The Canadian Entomologist* 103:1201-1206.

Wirth T., Guellec R.L., Vancassel M. and Veuille M. 1998. Molecular and reproductive characterization of sibling species in the European earwig (*Forficula auricularia*). *Evolution* 52:260-265.

Chapter 2
Evidence for an Aggregation Pheromone

2.1 Introduction

European earwigs (EEWs) are nuisance insects in residential areas (Mason, 2000; Flint, 2002; Cranshaw, 2007), and have become garden pests, damaging leaves, fruits, vegetable and flowers (Lamb, 1974; Anonymous, 2004b). They are also considered pests in soft-fleshed tree fruit crops (Cranshaw, 2000; Flint, 2002; Anonymous, 2004a), but are valued as biological control agents in other crops, feeding on developmental stages of a wide range of insect species (Buxton, 1974; Glen, 1975; Carroll and Hoyt, 1984; Mueller et al., 1988).

EEWs are subsocial insects with complex behavior that includes nest construction and progeny care (Lamb, 1976). Late instar nymphs and adults exhibit distinctive gregarious behavior (Sauphanor and Sureau, 1993), seeking humid and dark crevices for shelter during the day. Prior occupancy of a shelter enhanced its attractiveness (Sauphanor, 1992), implying pheromone-mediated aggregation behavior. Shelters previously occupied by adults or nymphs elicited significant behavioral responses from nymphs (Sauphanor, 1992). However, whether each developmental stage and sex produce and respond to pheromone was not investigated.

The source of the pheromone remains to be determined with certainty. Extracts of amputated legs or leg trails caused EEW aggregations, leading Sauphanor (1992) to conclude that tibial glands (Brousse-Gaurry, 1983) are the source of the pheromones. In a subsequent

study, Walker et al. (1993) did not support this conclusion and argued instead that the aggregation pheromone was derived from, or associated with, fecal excreta and the male's integument.

My objectives were to determine whether (1) females, males and nymphs produce and respond to aggregation pheromone; (2) the pheromone is perceived by olfaction or contact chemoreception; and (3) whether the insects' integument or fecal excreta constitute the source of the pheromone.

2.2 Materials and methods

2.2.1 Collection and maintenance of experimental insects

EEWs were collected from apple orchards in Cawston BC in sheets of single-faced corrugated cardboard (45-60 × 15-20 cm wide; Shippers Supply Inc.; BC Canada) affixed to the base of trees in the evening and retrieved the next morning. Insects were kept in plastic cages (38 × 28.9 × 28.5 cm) with a chiffon cloth top for ventilation, and provisioned with dog food and corncob (Morallo-Rejesus et al., 2001). The cages were kept in Simon Fraser University's insectary at room temperature and a 17L:7D photoperiod (Wirth et al., 1998).

2.2.2 General bioassay procedure

EEWs naturally reside within confined spaces (Lamb and Wellington, 1974) and are very sensitive to air currents (Chant and McLeod, 1952). Thus, all experiments except 10-13 were conducted in still air three-chambered glass olfactometers (Takács and Gries, 2001), with treatment or control stimuli randomly assigned to side chambers. Experiments were conducted at 15-25°C and 35-55% RH. Considering that EEWs forage at night and seek shelter during the day (Lamb 1975), experiments 10-17, 22-29, 30-33 (conducted in 2004) were started 1-2 hours before the beginning of the 7-hour scotophase. For each replicate, an

insect isolated without food for 3-6 hours was released into the central chamber of the olfactometer and its position was recorded 21 hours later in the next photophase. In 2005 and 2006, the protocol for all other experiments was modified to expedite acquisition of data. Each insect isolated without food for 16-19 hours was released into the central chamber 5-6 hours into the scotophase and its position was recorded 3 hours into the following photophase. For each replicate, a thoroughly cleaned olfactometer (washed in hot water with Liqui-Nox Critical Cleaning Liquid Detergent, Alconox, Inc. New York, NY 10016 and oven dried), a new stimulus and a new insect were used. Unless otherwise stated, test stimuli were accessible to bioassay insects.

2.2.3 Preparation of test stimuli

Treatment paper towel discs (8 cm in diam., Embassy Scott Paper Towel Ltd. Ont.) were exposed for four days to either 20 male, 20 female or 20 nymphs in a Petri dish (2.5 × 8.5 cm diam.). Both treatment and control discs (the latter not exposed to insects) received 0.50-0.60 g of dog food (No Name® Special Dinner for Adult Dogs, Loblaws Inc., Montreal H4N 3L4 Canada) and braided cotton rolls (30 × 9.4 mm; Richmond Dental, P.O. Box 34276, Charlotte, NC 28205) soaked with water in a glass vial (2 dram, A VWR company, VW609 10A1, San Francisco, CA.). After three days, food and water were removed and discs transferred to olfactometers to test the response of bioassay insects.

2.2.4 Evidence for pheromone

Experiments 1-9 tested whether females, males and nymphs produce and respond to aggregation pheromone. Experiments 10-13 tested whether the aggregation pheromone is perceived by olfaction. Test stimuli were prepared as described above but were tested

inaccessible to bioassay insects in 2-choice olfactometers (Duthie et al., 2003). Experiments 14-17 served as positive controls, testing the same stimuli accessible to insects.

2.2.5 Source of pheromone

Experiments 18-29 tested whether fecal excreta (= frass) are the source of the aggregation pheromone. Fresh (0-48 hours old) frass (1 g) from separate groups of males and females was collected for bioassays, briefly (12 hours) stored in vials at -14°C or extracted with 4 ml of acetonitrile (MeCN) or methylene chloride ($MeCL_2$). After 0.5 hour of extraction, the supernatant was withdrawn and stored at -14°C. Aliquots of frass (experiments 18-21) or frass extract (experiments 22-29) were tested at 50 male or 50 female day equivalents (1 MDE = frass produced by 1 male during 24 hours). Aliquots were placed on paper towel discs (see above), whereas control discs received no frass (experiments 18-21) or the same amount and type of solvent (experiments 22-29).

Experiments 30-34 tested whether the insects' integument is the source of pheromone. Integument washes were obtained by immersing 150 males, 150 females or 150 nymphs in a vial with hexane (12-17 ml) kept on dry ice for 3 min and then at room temperature for 1 hour. Aliquots of 5 female, 5 male or 1 nymph equivalent of body wash (1 NE = Volatiles washed off the body surfaces of 1 nymph) were pipetted on paper towel discs and bioassayed for their ability to elicit behavioral responses from bioassay insects. Control discs received the equivalent amount of solvent.

2.2.6 Statistical analyses

Insects responding to treatment and control stimuli were analyzed with the $\chi 2$ goodness-of-fit test, using Yates correction for continuity ($\alpha = 0.05$) (Zar, 1999). Insects that

did not choose the treatment or control stimulus were considered nonresponders and were not included in statistical analyses.

2.3 Results

Unmated female, male and juvenile EEWs all exhibited significant behavioral responses to paper towel discs previously exposed for four days to either female, male or juvenile conspecifics (Figure 2.1). Females and males significantly responded to paper towel discs previously exposed to females or males whether test stimuli were accessible or inaccessible except response of females toward inaccessible female exposed paper towel discs (Figure 2.2). Neither fresh male frass nor fresh female frass at 50 insect-day-equivalents elicited significant responses from females or males (Figure 2.3, experiments 18-29). Similarly, neither MeCN nor $MeCL_2$ extracts of fresh female or fresh male frass elicited any behavioral responses from females or males (Figure 2.3). Finally, body washes of females at 5 FE failed to elicit responses from females or males (Figure 2.4; experiments 30, 31). Body washes of males were indifferent to females and deterrent to males (Figure 2.4; experiments 32, 33), and body washes of nymphs were indifferent to nymphs (Figure 2.4, experiment 34).

2.4 Discussion

My data support the conclusion that female, male and nymph EEWs produce an aggregation pheromone that elicits behavioral responses from conspecfics regardless of developmental stage or sex. Similar results have been obtained with insects of diverse taxa, including German cockroaches, *Blattella germanica* L., (Ishii and Kuwahara 1967, 1968), firebrats, *Thermobia domestica* (Packard) (Tremblay and Gries, 2003), silverfish, *Lepisma*

saccharina L., and giant silverfish, *Ctenolepisma longicaudata* Escherich (Woodbury and Gries, 2007).

Significant behavioral responses to both accessible and inaccessible test stimuli indicate that the pheromone is perceived by olfaction rather than contact chemoreception. Volatile pheromones mediating aggregation behavior may attract and/or arrest conspecifics. My still-air olfactometer experiments did not allow me to discern between attraction and arrestment responses. Anemotactic responses to pheromone would unambiguously demonstrate long-range attractiveness, but such responses would be difficult to obtain because EEWs avoid, and may even be disturbed by, moving air (personal observation). One might argue, however, that an attractive pheromone would be better suited than an arrestant pheromone to help EEWs find their familiar shelters after nocturnal foraging bouts. This argument is supported by reports that EEWs are attracted to shelters emanating pheromone (G. Karg, unpublished data, cited by Burnip et al., 2002).

The source of the aggregation pheromone remains unclear. Reportedly, the pheromone is associated with, or derived from, the males' cuticular lipids or from fecal excreta (Walker et al., 1993) or tibial glands (Sauphanor, 1992). In my experiments, neither fresh frass from males or females nor solvent extracts thereof elicited positive responses from males or females (Figure 2.3). Similarly, body washes of females, males or nymphs elicited no attraction or arrestment responses (Figure 2.4). Indeed, body washes of males were deterrent to males. The controversial conclusions in earlier studies with respect to the source of the pheromone may be explained, in part, by the bioassay procedure and developmental stage of the bioassay insects. Bioassays with groups of insects, as conducted by Walker et al. (1993), are more likely to yield a positive response to a test stimulus than bioassays with individual insects, as conducted in my study. Only one insect in a group needs to be

sufficiently sensitive to perceive and respond to trace pheromone components emanating from a test stimulus. Pheromone or other signals from the responding insect would then elicit a follow response from group members. Moreover, adults bioassayed by Walker et al. (1993) and nymphs bioassayed by Sauphanor (1992) may produce and respond to different aggregation pheromones.

It is also conceivable that the pheromone constitutes a multiple component blend, with individual components derived from various body parts, glands, or excreted metabolites. If so, strong responses from bioassay insects would be obtainable only when all pheromone components or all sources they originate from were present. Finally, the ratio of components extractable from the body surface or from glands may differ from that released by insects.

Considering that the aggregation pheromone is volatile (Figure 2.2), analyses of headspace volatiles either from aggregating EEWs or their frequently used shelters appear promising to determine the constituents of the aggregation pheromone. The identification and testing of candidate pheromone components will be reported in Chapter 3. Chapter 3 will also reveal whether nymphs and adults produce the same aggregation pheromone.

2.5 References

Anonymous, 2004a. Earwigs (*Forficula auricularia*). http://www.agf.gov.bc.ca/cropprot/tfipm/earwigs.htm

Anonymous, 2004b. Effective control of earwigs. http://www.pmra-arla.gc.ca/english/consum/earwigs-e.html.

Brousse-Gaury, P. 1983. Sur l'existence d'une glande tibiale chez *Labidura riparia* Pallas et *Forficula auricularia* L. (Insectes: Dermapteres). *Comptes Rendus de l Academie des Sciences Paris* 297:203-208.

Burnip, G.M., Daly, J.M., Hackett, J.K. and Suckling, D.M. 2002. European earwig phenology and effect of understorey management on population estimation. In: *55th Conference Proceedings of The New Zealand Plant Protection Society Incorporated* 390-395.

Buxton, J.H. 1974. The biology of the European earwig *Forficula auricularia* L. with reference to its predatory activities on the Damson-hop aphid *Phorodon humuli* (Schrank). Ph. D. Dissertation. Wye college, University of London.

Carroll, D.P. and Hoyt, S.C. 1984. Augmentation of European earwigs (Dermaptera: Forficulidae) for biological control of apple aphid (Homoptera: Aphididae) in an apple orchard. *Journal of Economic Entomology* 77:738-740.

Chant, D.A. and McLeod, J.H. 1952. Effects of certain climatic factors on the daily abundance of the European earwig, *Forficula auricularia* L. (Dermaptera: Forficulidae), in Vancouver, British Columbia. *The Canadian Entomologist* 34:174-180.

Cranshaw, W.S. 2000. European earwig: Habits and management. *Pest Alert* 17:4-5.

Cranshaw, W.S. 2007. European earwigs. Colorado State University Extension-Horticulture. http://www.ext.colostate.edu/Pubs/insect/055333.html

Duthie, B., Gries, G., Gries, R., Krupke, C., and Derksen, S. 2003. Does pheromone-based aggregation of codling moth larvae help procure future mates? *Journal of Chemical Ecology* 29:425-436.

Flint, M.L. 2002. Earwigs: Integrated pest management for home gardeners. *Pest notes, University of California agriculture and natural resources publication,* 74102. Produced by IPM education and publications, UC Statewide IPM program, University of California, Davis, CA.

Glen, D.M. 1975. The effects of predators on the eggs of codling moth, *Cydia pomonella*, in a cider-apple orchard in south-west England. *Annals of Applied Biology* 80:115-135.

Ishii, S. and Kuwahara, Y. 1967. An aggregation pheromone of the German cockroach *Blattella germanica* L. (Orthoptera: Blattellidae). I. Site of the pheromone production. *Applied Entomology and Zoology* 2:203-217.

Ishii, S. and Kuwahara, Y. 1968. Aggregation of German cockroach (*Blattella germanica*) nymphs. *Experientia* 24:88-89.

Lamb, R.H. 1974. Earwig travel in relation to habitat. Ph. D. Dissertation. The University of British Columbia, Vancouver.

Lamb, R.J. 1975. Effects of dispersion, travel and environmental heterogeneity on populations of the earwig, *Forficula auricularia* L. *Canadian Journal of Zoology* 53:1855-1867.

Lamb, R.J. 1976. Parental behavior in the Dermaptera with special reference to *Forficula auricularia* (Dermaptera: Forficulidae). *The Canadian Entomologist* 108:609-619.

Lamb, R.J. and Wellington, W.G. 1974. Techniques for studying the behavior and ecology of the European earwig, *Forficula auricularia* (Dermaptera: Forficulidae). *The Canadian Entomologist* 106:881-888.

Mason, S. 2000. Earwigs prove to be more than a nuisance. http://web.extension.uiuc.edu/champaign/homeowners/000701.html.

Morallo-Rejesus, B., Navasero, M., Javier, P. and Camarao, G. 2001. Mass rearing of earwigs. *BAR Chronicle, A monthly publication* **2**.

Mueller, T.F., Blommers, L.H.M. and Mols, P.J.M. 1988. Earwig (*Forficula auricularia)* predation on the woolly apple aphid, *Eriosoma lanigerum. Entomologia Experimentalis et Applicata.* 47:145-152.

Sauphanor, B. 1992. Une Pheromone d'aggregation chez *Forficula auricularia L. Entomologia Experimentalis et Applicata* 62:285-291.

Sauphanor, B. and Sureau, F. 1993. Aggregation behaviour and interspecific relationships in Dermaptera. *Oecologia* 96:360-364.

Takács, S. and Gries, G. 2001. Communication ecology of webbing clothes moth: evidence for male-produced aggregation signal (s). *The Canadian Entomologist* 133:725-727.

Tremblay, M.N. and Gries, G. 2003. Pheromone-based aggregation behaviour of the firebrat, *Thermobia domestica* (Packard) (Thysanura: Lepismatidae). *Chemoecology* 13:21-26.

Walker, K.A., Jones, T.H. and Fell, R.D. 1993. Pheromonal basis of aggregation in European earwig, *Forficula auricularia* L. (Dermaptera: Forficulidae). *Journal of Chemical Ecology* 19:2029-2038.

Wirth, T., Guellec, R.L., Vancassel, M. and Veuille, M. 1998. Molecular and reproductive characterization of sibling species in the European earwig (*Forficula auricularia*). *Evolution* 52:260-265.

Woodbury, N. and Gries, G. 2007. Pheromone-based arrestant behavior in the common silverfish *Lepisma saccharina* and giant silverfish *Ctenolepisma longicaudata.* Journal of Chemical Ecology. DOI 10.1007/s 10886-007-9303-4.

Zar, J.H. 1999. Biostatistical analysis. Prentice-Hall, New Jersey.

2.6 Figures

Figure 2.1. Number of female, male or juvenile *F. auricularia* responding to an inaccessible paper towel disc previously exposed to female, male or juvenile *F. auricularia*. An asterisk (*) indicates a significant preference for a particular test stimulus (X^2-test; $*P < 0.05$, $**P < 0.01$). Numbers in brackets indicate numbers of nonresponding insects.

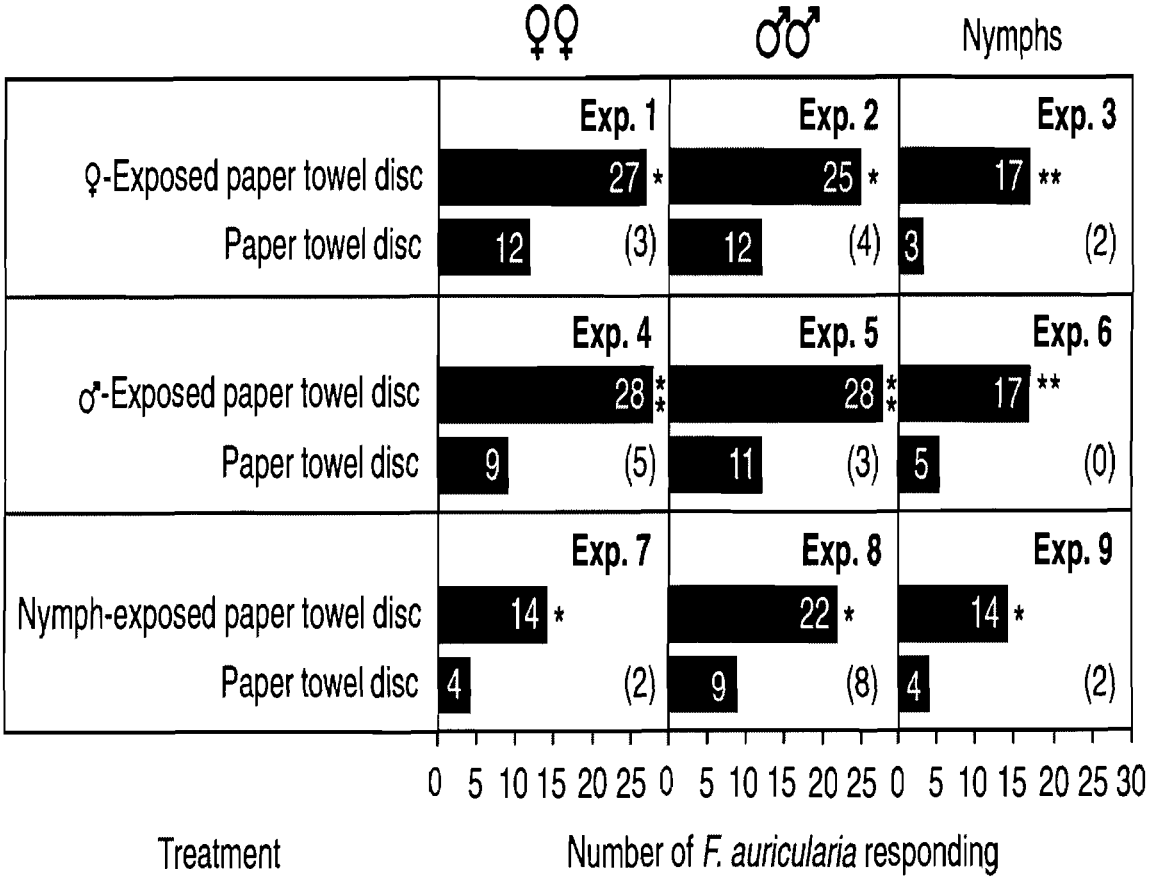

♀♀
♂♂
Nymphs
Exp. 1
Exp. 2
Exp. 3
♀-Exposed paper towel disc
27 *
25 *
17 **
Paper towel disc
12 (3)
12 (4)
3 (2)
Exp. 4
Exp. 5
Exp. 6
♂-Exposed paper towel disc
28 **
28 **
17 **
Paper towel disc
9 (5)
11 (3)
5 (0)
Exp. 7
Exp. 8
Exp. 9
Nymph-exposed paper towel disc
14 *
22 *
14 *
Paper towel disc
4 (2)
9 (8)
4 (2)
0 5 10 15 20 25 0 5 10 15 20 25 0 5 10 15 20 25 30
Treatment
Number of F. auricularia responding

Figure 2.2 Number of female or male *F. auricularia* responding to a paper towel disc previously exposed to female or male *F. auricularia* when stimulus contact was prohibited (top) or allowed. An asterisk (*) indicates a significant preference for a particular test stimulus (X^2-test; $*P < 0.05$, $**P < 0.01$). Numbers in brackets indicate numbers of nonresponding insects.

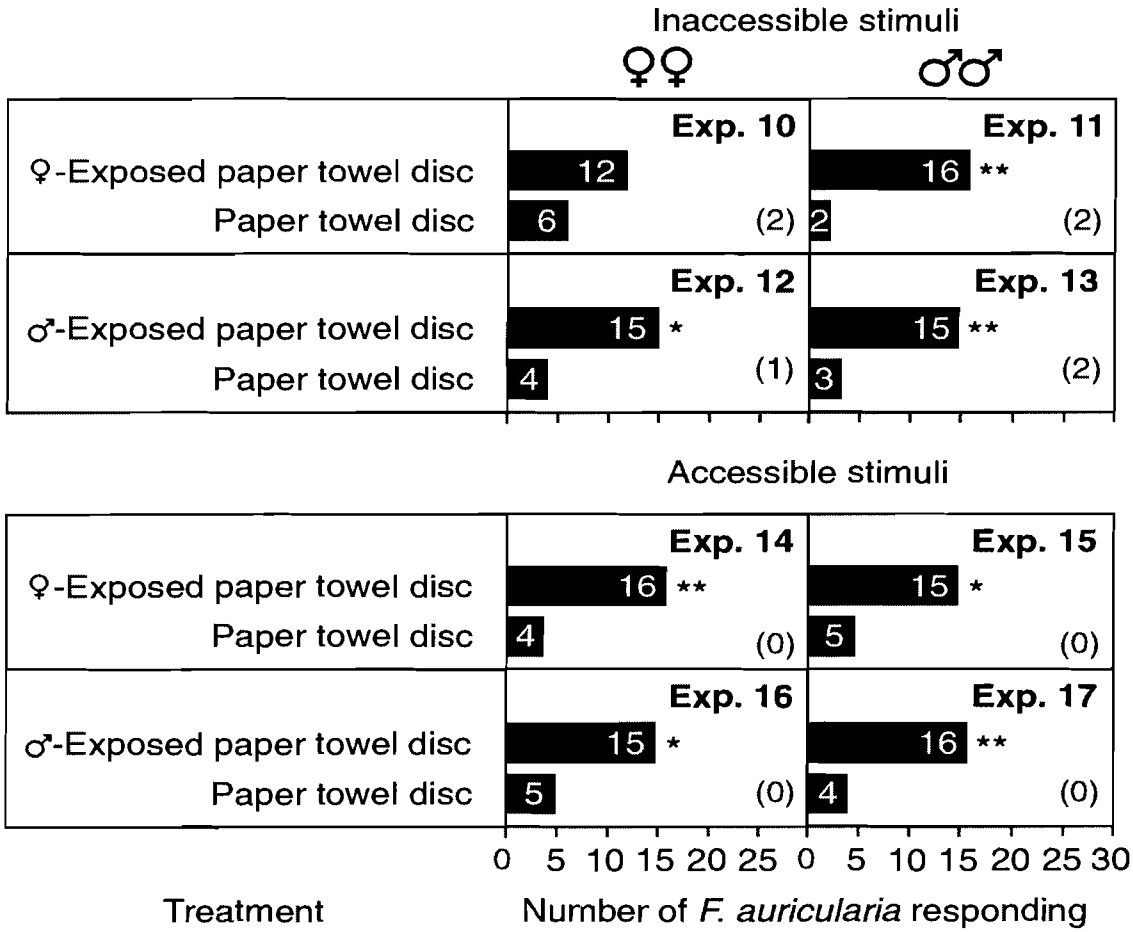

Inaccessible stimuli
♀♀
♂♂
Exp. 10
Exp. 11
♀-Exposed paper towel disc
12
16 **
Paper towel disc
6
(2)
2
(2)
Exp. 12
Exp. 13
♂-Exposed paper towel disc
15 *
15 **
Paper towel disc
4
(1)
3
(2)
Accessible stimuli
Exp. 14
Exp. 15
♀-Exposed paper towel disc
16 **
15 *
Paper towel disc
4
(0)
5
(0)
Exp. 16
Exp. 17
♂-Exposed paper towel disc
15 *
16 **
Paper towel disc
5
(0)
4
(0)
0 5 10 15 20 25 0 5 10 15 20 25 30
Treatment
Number of F. auricularia responding

Figure 2.3 Number of female or male *F. auricularia* responding to accessible fresh female or male fecal excreta (= frass) at 50 insect-day-equivalents (IDE) or solvent extracts thereof. In none of experiments 18-29 was the treatment stimulus preferred over the corresponding control stimulus (X^2-test; $^*P < 0.05$). Note: (1) Number in brackets indicate numbers of non-responding insects; (2) 1IDE = amount of frass produced by one insect during 24 hours; (3) MeCN = acetonitrile, $MeCl_2$ = methylene chloride; (4) control stimuli in experiments 22-29 received the same amount of solvent (41-79 µl) as treatment stimuli.

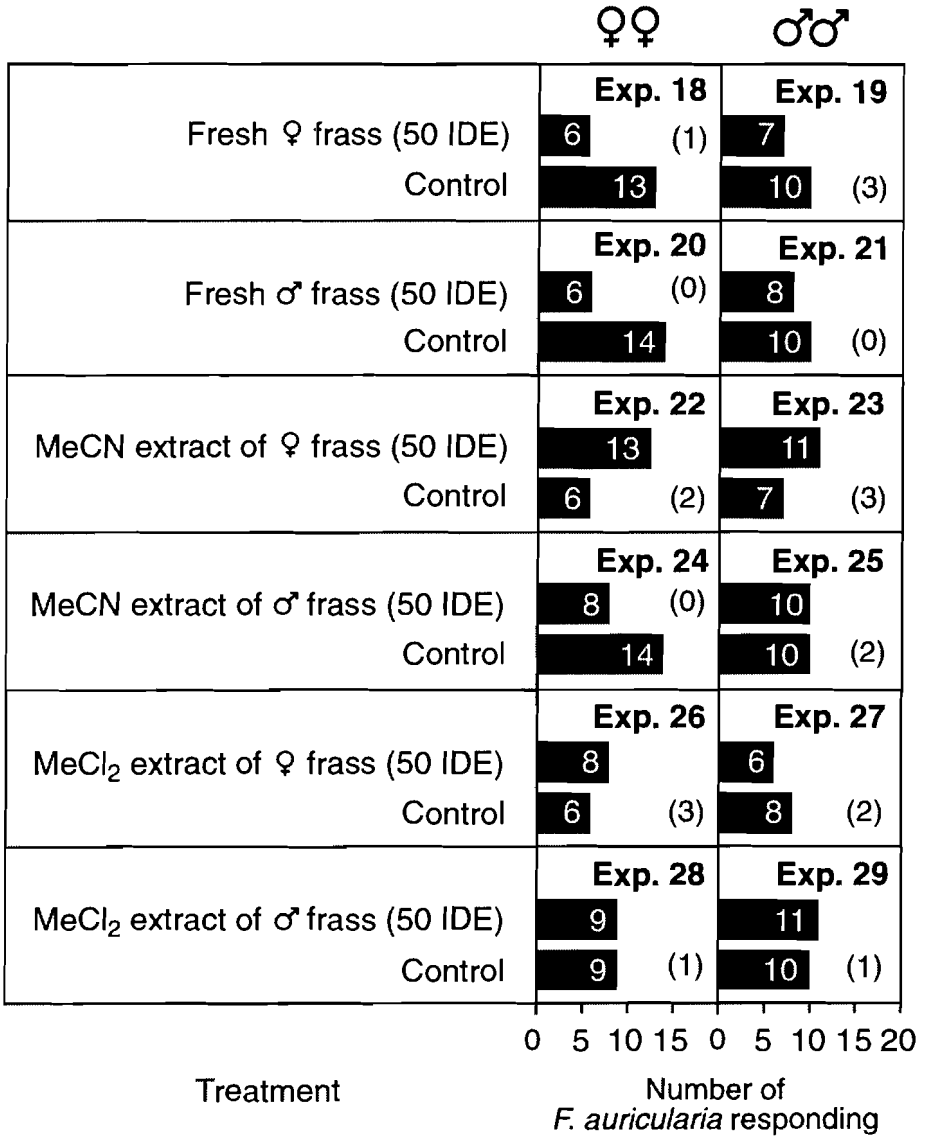
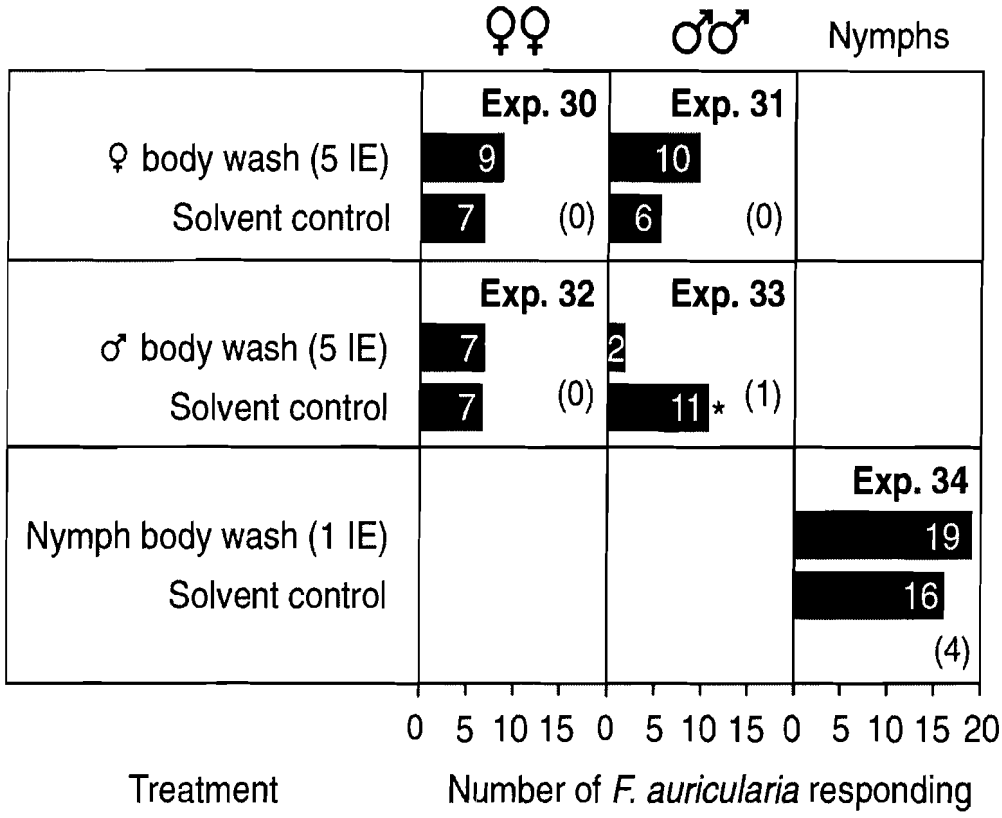

♀♀
♂♂
Exp. 18
Exp. 19
Fresh ♀ frass (50 IDE)
6
(1)
7
Control
13
10
(3)
Exp. 20
Exp. 21
Fresh ♂ frass (50 IDE)
6
(0)
8
Control
14
10
(0)
Exp. 22
Exp. 23
MeCN extract of ♀ frass (50 IDE)
13
11
Control
6
(2)
7
(3)
Exp. 24
Exp. 25
MeCN extract of ♂ frass (50 IDE)
8
(0)
10
Control
14
10
(2)
Exp. 26
Exp. 27
$MeCl_2$ extract of ♀ frass (50 IDE)
8
6
Control
6
(3)
8
(2)
Exp. 28
Exp. 29
$MeCl_2$ extract of ♂ frass (50 IDE)
9
11
Control
9
(1)
10
(1)
0 5 10 15 0 5 10 15 20
Treatment
Number of
F. auricularia responding

Figure 2.4 Number of female or male *F. auricularia* responding in experiments 30-33 to accessible body washes of conspecific females or males tested at five insect equivalents (IE), and number of nymph *F. auricularia* responding to body washes of conspecific nymphs tested at 1 IE. An asterisk (*) indicates a significant preference for a particular test stimulus (X^2-test; $*P < 0.05$). Numbers in brackets indicate the number of non-responding insects; 1 IE = chemical constituents washed off the surface of one insect; in all experiments, treatment and control stimuli received the same amount of solvent (40-68 µl).


♀♀
♂♂
Nymphs
Exp. 30
Exp. 31
♀ body wash (5 IE)
9
10
Solvent control
7
(0)
6
(0)
Exp. 32
Exp. 33
♂ body wash (5 IE)
7
2
Solvent control
7
(0)
11*
(1)
Exp. 34
Nymph body wash (1 IE)
19
Solvent control
16
(4)
0 5 10 15 0 5 10 15 0 5 10 15 20
Treatment
Number of F. auricularia responding

Chapter 3
Identification and Testing of Candidate Pheromone Components

3.1 Introduction

European earwigs (EEWs) are well known for their gregarious behavior in shelters they seek during daytime (Carillo, 1985; Sauphanor and Sureau, 1993). This behavior has been attributed to an aggregation pheromone that may be derived from, or associated with, tibial glands (Sauphanor, 1992), fecal excreta or the males' integument (Walker et al. 1993). The pheromone is produced by, and elicits aggregation responses from, each developmental stage and sex (Chapter 2). It is perceived by olfaction (Chapter 2) and thus may help EEWs relocate shelters after nocturnal foraging bouts. If so, synthetic aggregation pheromone could be deployed as a bait to capture EEWs and alleviate their damage to fruits, vegetables and flowers, or to enhance their concentration and predatory role in crops vulnerable to aphids (Albouy and Caussanel, 1991).

The defense compounds of EEWs, 2-methyl-1,4-benzoquinone and 2-ethyl-1,4-benzoquinone, were identified 47 years ago (Schildknecht and Weis, 1960), but the aggregation pheromone is still unknown. Twenty straight chain or methylated hydrocarbons were identified in cuticle washes of males and females, but none elicited significant behavioral responses in laboratory bioassays (Walker et al., 1993). Similarly, eight fatty acids were identified in fecal excreta, but only stearic and palmitic acids elicited behavioral responses at the high dose of 50 insect equivalents (IE) per day (Walker et al., 1993). In

Chapter 2, neither fresh EEW fecal excreta at 50 IE nor various solvent extracts thereof, elicited significant behavioral responses from bioassay insects.

With mounting evidence that the EEW aggregation pheromone may be complex (Sauphanor and Sureau, 1993) and associated with more than a single source, I extracted and captured (airborne) volatiles from many different potential pheromone sources. In Chapter 3, I report the identification as well as laboratory and field testing of complex blends of candidate pheromone components.

3.2 Materials and methods

3.2.1 Acquisition of candidate pheromone components from potential pheromone sources

Considering the controversy and uncertainty as to what constitutes the source of the EEW aggregation pheromone, volatiles from various potential sources of pheromone were acquired, including headspace volatiles from aggregating insects, extracts of fecal excreta and glands as well as washes of the insects' body surface.

3.2.1.1 Acquisition of headspace volatiles

Headspace volatiles were obtained by capture on Porapak Q and by solid-phase microextraction (SPME) (Millar and Haynes, 1998). For capture of volatiles on Porapak Q, 2170 males, 2100 females or 400 nymphs were placed in a glass chamber (15 x 27 cm) fitted with paper towels and provisioned with dog food (3-5 g) and water as in rearing cages. Charcoal-filter air was drawn at 1L/min for 1-9 days through the chamber and a glass tubing (15 x 1 cm) filled with Porapak Q (50-80 mesh, Water associates, Inc., Milford, Massachusetts). Volatiles were captured on Porapak Q and eluted with 3 ml of pentane.

Aerations of control stimuli were identical except that no EEWs were present in glass chambers.

To acquire volatiles by SPME, the exposed fiber coated with 100 μm of polydimethyl siloxane (Supelco, Bellefonte, PA, USA) was inserted for 30 minutes through a chiffon cloth opening into a plastic cage (38 × 28.9 × 28.5 cm) harbouring circa 170 males, 170 females or 170 nymphs. Volatiles were desorbed in the injection port (250°C) of a gas chromatograph (GC) or a GC mass spectrometer (MS).

3.2.1.2 Extraction of potential pheromone sources

Glands in the third and fourth abdominal segment of EEWs were considered another potential source of aggregation pheromone. These glands were excised from separate groups of 15-20 females, males and nymphs and extracted for 1 hour in separate vials containing 100 μl of hexane or methylene chloride. The supernatant was withdrawn and stored at -14°C until use.

The EEWs' integument and fecal excreta (= frass) are reported sources of aggregation pheromone (Walker et al., 1993). Fresh (0-48 hours old) frass (1 g) was collected from separate groups of females and males, and extracted for 30 minutes with 4 ml of methylene chloride. Integuments were extracted by immersing males, females or nymphs in separate vials with hexane kept on dry ice for 3 minutes and then at room temperature for 1 hour. The supernatant of frass extracts or integument washes was withdrawn and stored at -14°C until use.

3.2.2 Analyses of potential pheromone sources

Aliquotes of Porapak Q headspace, gland and frass extracts, integument washes as well as volatiles desorbed from SPME fibers were analyzed by coupled gas chromatographic-

electroantennographic detection (GC - EAD) (Arn et al., 1975; Gries et al., 2002), and GC-mass spectrometry (MS). GC-EAD analyses employed a Hewlett Packard (HP) 5890A gas chromatograph equipped with a GC column (30 m × 0.25 or 0.32 mm ID) coated with DB-5, DB-210 or DB-23 (J &W Scientific, Folsom, CA, USA). For GC-EAD recordings, the base of an antenna was carefully dislodged from an insect's head and placed into the opening of a glass capillary electrode filled with saline solution (Staddon and Everton, 1980). The tip of the antenna was then removed by spring microscissors (Fine Science Tools Inc., North Vancouver, British Columbia, CA) and the severed antenna placed into the opening of a second (indifferent) electrode.

Compounds that elicited antennal responses were analyzed by: (1) full-scan electron-impact mass spectrometry with a Varian Saturn 2000 Ion Trap GC-MS fitted with a DB-5 MS column (30 m × 0.25 mm) (J & W Scientific); (2) retention index calculations (Van den Dool and Kratez, 1963); and (3) micro-analytical treatments. The identification of EAD-active compounds was confirmed by comparing their GC retention times and mass spectra with those of authentic standards.

Synthetic standards were purified by high-performance liquid chromatography (HPLC), employing a Waters LC 626 HPLC equipped with a Waters 486 Variable-Wavelength UV visible detector set to 210 nm, HP Chemstation software (Rev. A. 07. 01), and a reverse-phase Nova-Pak C18 column (60 Å, 4 μm; 3.9 × 300 mm) eluted with 1 ml/min of 100% acetonitrile.

3.2.3 Procurement and syntheses of candidate pheromone components

Candidate pheromone components were purchased from various suppliers (Table 1) or were synthesized*, if they were not commercially available.

Ethyl-1,4-benzoquinone and propyl-1,4-benzoquinone were synthesised from 1,4-benzoquinone (Aldrich) by addition of ethyl- or propyl-free radicals generated from propionic or butyric acids in the presence of silver nitrate and ammonium persulfate in an aqueous solution, following the general method description by Jacobsen and Torssell (1972) and Jacobsen (1977). Similarly, mixtures of all three methylethyl-1,4-benzoquinones, diethyl-1,4-benzoquinones, methylpropyl-1,4-benzoquinones, and ethylpropyl-1,4-benzoquinones (see Table 1) were synthesized from monosubstituted methyl- or ethyl-1,4-benzoquinones, using the same synthetic approach. 2,3-dimethyl-1,4-benzoquinone and some ethyl-1,4-benzoquinone were synthesised from 2,3-dimethylphenol and 2-ethylanisole, respectively, using ceric ammonium nitrate [$Ce(NH_4)_2(NO_3)_6$] as an oxidating agent in an acetonitrile/water solution (Fisher and Henderson, 1985; Nojima et al., 2005). Ethyl-1,4-hydroquinone was synthesised from ethyl-1,4-benzoquinone by reducing it with sodium hydrosulfite in a water/methanol solution (Morrison and Boyd, 1983). 3,5,6-Trimethyl-2-cyclohexen-1-one was synthesized following the procedure by Aurell et al. (1994).

3.2.4 Laboratory and field experiments

3.2.4.1 General design of laboratory olfactometer experiments

EEWs usually reside within confined spaces with little or no air movement (Chant and McLeod, 1952; Lamb and Wellington, 1974). Thus, all experiments were conducted in still-air olfactometers. Considering that EEWs forage at night and seek shelter during the day

* Syntheses were conducted by Dr. Grigori Khaskin (Gries-Laboratory)

(Lamb 1975), experimental replicates were started 1-2 hours before the onset of the photophase and terminated 3 hours later by recording the insects' position. Experiments were conducted at 15-25°C, 33-55 % relative humidity and a 17L:7D photoperiod.

Three-chamber glass olfactometers (Takács and Gries, 2001) were deployed for experiments 1-41. For each replicate, treatment and control stimuli were randomly assigned to a paper towel disc in each of the side chambers. Aliquots of extracts (treatment) or equivalent amounts of solvent (control) were pipetted onto the paper towel disc (8 cm diam.), which was then covered with an inverted open-fluted corrugated cardboard disc (6 cm diam.), serving as a shelter for the single insect released per replicate. A thoroughly cleaned olfactometer (washed in hot water with Liqui-Nox Critical Cleaning Liquid Detergent; Alconox Inc., New York, NY 10016 and oven-dried at 100°C), a new stimulus and a new insect were used for each replicate. The insect was isolated for 16-19 hours prior to the onset of a replicate.

Plexiglas arena olfactometers (1.2 x 0.43 m) (Mistal et al., 2000) were deployed for experiments 42-44, maintaining the same abiotic conditions as in preceding experiments. For each replicate, a single insect isolated for 16-19 hours was released in centre of the arena 1-2 hours before the onset of the photophase and its position was scored 3 hours later. The treatment stimulus and the control stimulus (an equivalent amount of solvent;10 µl) were randomly assigned to one of two paper towel discs (8 cm diam.) placed on opposite sides of the arena 4-5 cm from the wall. Stimuli were pipetted onto the discs which were then covered with a single-faceted corrugated cardboard disc (6 cm diam.) with corrugations facing downwards. An insect found at the end of each replicate in or on a disc was considered a responder. For each replicate, a thoroughly cleaned (Purell hand sanitizer with moisturizers and Vitamin E) and air dried arena, new stimulus, and a new insect were used.

3.2.4.2 Stimuli tested in laboratory olfactometer experiments 1-41

Experiments 1 and 2 tested the response of females (experiment 1) and nymphs (experiment 2) to a synthetic blend (SB) (Table 1) of 30 candidate pheromone components identified in potential sources of aggregation pheromone. To determine the essential components in SB, experiments 3-16 tested solvent *versus* SBs lacking single or groups of organic chemicals, such as benzoquinones (experiments 3, 4), ketones (experiments 5, 6), aldehydes (experiments 7, 8), acids (experiments 9,10), ethyl hydroquinone (experiments 11, 12), vanillin (experiments 13, 14) or the acetal conophtorin (experiments 15, 16). Taking into account that nymphs appeared to respond best to the SB lacking benzoquinones (see results of experiment 4), experiments 17-19 re-tested the response of nymphs to SBs lacking benzoquinones (experiment 17) or containing them at a reduced or altered ratio (experiments 18, 19).

Considering that both females and nymphs significantly responded to SBs lacking or containing lower quantities of benzoquinones (see results of experiments 3, 4, 17, 18), follow-up experiments tested a new synthetic blend (NSB=SB without benzoquinones) and explored whether additional components could be deleted from the NSB without effecting its behavioral activity. Specifically, I tested whether NSBs would remain effective when lacking one of the three ketones (Fig. 3.4, experiments 20-27), one of the three aldehydes (Fig. 3.5, experiments 28-35), all saturated acids or the single unsaturated acid (Fig. 3.6, experiments 36-41).

Taken into account that blends less complex than NSB failed to be bioactive in experiments 20-41, (except NSB lacking 3,5,6-trimethyl-2-cyclohexen-1-one elicited attraction/arrestment responses by nymphs) arena olfactometer experiments 42-44 tested the NSB minus 3,5,6-trimethyl-2-cyclohexen-1-one for its effect on females, males and nymphs.

3.2.4.3 Field experiments

Field experiments were conducted in two orchards with 12- to 13-year-old apple trees near Cawston, British Columbia, from July until the end of August 2006. Traps were made from a sheet (13 × 8 cm) of single faceted corrugated cardboard which was folded longitudinally to form two identical halves. Between them was placed a white paper towel disc (8 cm diam.) impregnated with a test stimulus. The folded sheet was rolled up and tied with a rubber band, forming a cylindrical harborage. One treatment and one control harborage were then randomly assigned to opposite sides of a tree trunk (10-15 cm diam.) to which they were affixed with a metal wire 30-38 cm above ground. Experimental trees were separated by 10-12 m. Experiments were started at 18:00-21:00 hours and terminated noon the next day by carefully removing each harborage and placing it into a Ziploc® bag which was transferred into a deep freezer to kill insects before counting them.

Field experiments 45-58 were conducted in separate sets with individual experiments in each set run in parallel. Experiments 45-47, 48-51 and 52-53 all compared different doses of the NSB (stimuli S1–S4 in Table 3.2). Field-tested NSBs did not contain 3,5,6-trimethyl-2-cyclohexen-1-one which did not appear to contribute to the behavioral activity of the NSB in three-chamber olfactometer experiment 23. Considering that a dose of 10 NSB was sufficient to attract/arrest EEWs, and that adults did not appear to respond well to lures lacking benzoquinones, follow-up experiments 54-58 re-evaluated the role of the benzoquinones by testing 10 NSB (S2 in Table 3.2) 10 SB (having benzoquinones; S6 in Table 3.2), 10 SB with benzoquinones at 10% (S5 in Table 3.1) and 10 SB with benzoquinones at 10% and MBQ absent (S7 in Table 3.2).

3.2.4.4 Statistical analyses

In laboratory experiments, insects responding to treatment or control stimuli were analyzed with the $\chi 2$ goodness-of-fit test, using Yates correction for continuity ($\alpha = 0.05$) (Zar, 1999). Insects that did not choose the treatment or control stimulus were considered nonresponders and were not included in statistical analyses. In field experiments, number of insects captured in traps baited with treatment or control stimuli were analyzed with Wilcoxin paired-sample test ($\alpha = 0.05$) (Zar, 1999).

3.3 Results

GC-EAD and GC-MS analyses of all possible pheromone sources, including body washes (Figure 3.1), fecal excreta, leg and abdominal extracts as well as Porapak Q and SPME headspace volatiles of aggregating EEWs revealed 16 components that elicited responses from male or female antennae (Table 1). These included 2-methyl- and 2-ethyl-1,4-benzoquinos (MBQ, EBQ), four saturated acids, two unsaturated acids, one hydroquinone, three aldehydes, three ketones and one acetal. All except (*Z,Z*)-9,12-octadecadienoic acid, which was previously reported (Walker et al. 1993) and in my own preliminary experiments shown to be repellent, were considered candidate pheromone components and were included in a synthetic blend (SB). This blend also included 14 other benzoquinones which occurred in small or trace quantities. They were included in the blend because MBQ and EBQ are defense compounds and other benzoquinones could be attractive at small quantities. Some components, such as MBQ and EBQ, were detected in all sources, whereas other components, such as acids, were present only in extracts of fecal excreta or in cuticle washes (Table 1).

In three-chamber olfactometer experiments 1 and 2, the 30-component SB (Table 3.1) elicited significant behavioral responses from females, but not from nymphs (Figure 3.2). In

contrast, the SB lacking benzoquinones induced attraction or arrestment responses by both females and nymphs. SBs lacking ketones, aldehydes, acids, ethyl hydroxyquinone, vanillin or conophtorin were not behaviorally active (Figure 3.2). Even though vanillin did not appear to be bioactive in experiment 13 (Fig. 3.2), subsequent experiments (data not shown) revealed that it was an important constituent of SB. SBs lacking benzoquinones or containing them at a lower quantity elicited significant responses from nymphs, whereas the SB with an altered ratio of benzoquinones did not (Figure 3.3). Both the new synthetic blend (NSB = SB minus benzoquinones) and NSB lacking 3,5,6-trimethyl-2-cyclohexen-1-one induced significant responses from nymphs but not from females (Figure 3.4, experiments 20-23). NSBs lacking geranylacetone or sulcatone elicited no responses from females and nymphs (Figure 3.4, experiments 24-27). Both females and nymphs significantly responded to the NSB, but not to NSBs lacking phenylacetaldehyde, (*E*)-2-nonenal or (*E,E*)-2,4-nonenal (Figure 3.5, experiments 28-35). Similarly, both females and nymphs responded to the NSB but not to NSBs lacking either the four saturated acids or (*Z*)-9-hexadecenoic acid (Figure 3.6).

In arena olfactometer experiments 42-44, the NSB minus 3,5,6-trimethyl-2-cyclohexen-1-one elicited significant responses from nymphs, but not from females or males (Figure 3.7).

In early season (16-17 July) field experiments 45-47 (Table 3.3), numbers of EEWs in corrugated cardboard bands were too low to reveal treatment effects. In experiments 48-51, bands treated with 1 NSB or 10 NSB attracted/arrested significantly more nymphs than did unbaited control bands (Table 3.3). The higher doses of 100 NSB or 1000 NSB, in contrast, were not effective. No bait was significantly effective for adult females or adult males. In experiments 52-53, significantly more nymphs resided in bands treated with 10

NSB or 100 NSB than in untreated control bands. Significantly more males but not more females were present in bands baited with 10 NSB compared to control bands. Conversely, significantly more females but not more males were present in bands baited with 100 NSB. In experiments 54-58, when almost all nymphs had become adults, bands baited with 10 NSB had no significant effect on females or males. However, all baits containing benzoquinones attracted/arrested significantly more females and more males than did control bands.

3.4 Discussion

My data support the conclusion that the aggregation pheromone of the EEW is indeed complex. Seven groups of organic compounds (benzoquinones, ethylhydroquinone, fatty acids, vanillin, aldehydes, ketones, acetal) from one or more of five sources (headspace volatiles of aggregating insects, fecal excreta, tibial and abdominal glands, integument) appeared to contribute to the behavioral activity of the synthetic blend. However, whether each compound in each group, such as each of the 16 benzoquinones or each of the four saturated acids, is part of the pheromone blend will have to be determined in additional bioassays.

The presence or absence of compounds in various sources is to be interpreted with caution. For example, 2-methyl-1,4-benzoquinone and 2-ethyl-1,4-benzoquinone were detected in all sources, but due to their abundance in headspace volatiles may have "contaminated" some sources rather than originated from them. Conversely, fatty acids were not detected in headspace volatiles likely not because they were absent but because they tend to gas chromatograph poorly, rendering small quantities undetectable.

The role of the 1,4-benzoquinones in the EEW communication system seems to be amount- and context-specific. 2-Methyl-1,4-benzoquinone and 2-ethyl-1,4-benzoquinone are

reported as defense compounds (Schildknecht and Weis, 1960). Release of these components in my study could be provoked simply by exposing EEWs to an air current, even as faint as that required for Porapak capture of headspace volatiles. However, these benzoquinones and others (Table 3.1) were also present in SPME-acquired volatiles of EEWs that aggregated in enclosed shelters with no apparent disturbance. Moreover, synthetic blends lacking any benzoquinones failed to elicit: (1) consistent significant responses from females in three-chamber olfactometer experiments 3, 20, 28 and 36; (2) any significant responses from males and females in arena olfactometer experiments 42 and 44; and (3) consistent significant responses from females and males in field experiments 48-53. In contrast, all baits emitting benzoquinones in field experiments 54-58 (Tables 3.2, 3.3) were significantly more attractive/arrestant to females and males than were their corresponding controls. The one bait lacking benzoquinones in experiment 55 had no significant effect on females or males (Tables 3.2, 3.3). All this evidence suggests that benzoquinones are components of the aggregation pheromone that is attractive/arrestant to adult females and males. Whether their behavioral activity is attributable to moderate (instead of large) amounts of the defense compounds 2-methyl-1,4-benzoquinone and 2-ethyl-1,4-benzoquinone or to one or more of the other 14 benzoquinones is yet to be determined. At least 2-methyl-1,4-benzoquinone could be absent from a synthetic lure without affecting its behavioral activity (Table 3.3, experiment 57, 58).

The fact that benzoquinones are components of the EEW aggregation pheromone is not that surprising in light of prior findings of benzoquinones as pheromones. Gentisyl quinone isovalerate is the female-produced sex pheromone of the German cockroach, *Blattella germanica* (Nojima et al., 2005), and 1,4-benzoquinone is a sex pheromone produced by females of the forest cockchafer beetle *Melolontha hippocastani* which

enhances the attraction of males to plant volatiles induced by feeding females (Ruther et al., 2000; 2001).

Females, males and nymphs have been shown to produce and respond to aggregation pheromone (Chapter 2). In this chapter, I provide evidence that there could be nymph- and adult-specific pheromone blends. Unlike adult females and males, nymphs responded well in all laboratory experiments and in field experiments 48-53 to synthetic blends lacking benzoquinones. That they were not repelled by blends containing benzoquinones at a moderate dose (Figure 3.3, experiment 18) may allow them to co-inhabit shelters with adult females and males that seem to require benzoquinones as pheromone components. Stage-specific pheromonal communication has also been reported in the common bed bug, *Cimex lectularius*, where nymphs respond to nymph-produced contact aggregation pheromone and females and males respond to male-produced contact aggregation pheromone (Siljander et al., 2007). If EEWs indeed deploy stage-specific aggregation pheromones, this could explain, in part, why Sauphanor (1992) who bioassayed nymphs, and Walker et al. (1993) who bioassayed adults, arrived at different conclusions as to what constitutes the source of the aggregation pheromone.

In conclusion, this Chapter provides evidence that EEWs may have nymph- and adult-specific aggregation pheromones. As predicted (Sauphanor and Sureau, 1993), the pheromone(s) appears to be very complex, although many more laboratory and field experiments are needed to determine all essential components. The complexity of the "essential-component blend" will determine whether commercial lures will be affordable to trap EEWs or enhance their predatory role (Albouy and Caussanel, 1991).

3.5 References

Albouy, V. and Caussanel, C. 1991. Le perce-oreille: un insecte a double visage. *Nature et Progrès* 119:14-16.

Arn, H., Städler, E. and Rauscher, S. 1975. The elecetroantennographic detector-a selective and sensitive tool in the gas chromatographic analysis of insect pheromones. *Zeitschrift für Naturforschung* 30c:722-725.

Aurell M.J., Gaviña, P., Mestres R. 1994. Dienediolates of unsaturated carboxylic acids in synthesis. Synthesis of cyclohexenones and polycyclic ketones by tandem Michael-Dieckmann decarboxylative annulation of unsaturated carboxylic acids. *Tetrahedron* 50:2571-2582.

Carillo, J.R. 1985. Ecology of an aphid predation by the European earwig (*Forficula auricularia* L.) in grassland and barley. Ph.D. Dissertation. University of Southampton, Biology, United Kingdom. 198 pp.

Chant, D.A. and McLeod, J.H. 1952. Effects of certain climatic factors on the daily abundance of the European earwig, *Forficula auricularia* L. (Dermaptera: Forficulidae), in Vancouver, British Columbia. *The Canadian Entomologist* 34:174-180.

Fisher, A., and Henderson G.N. 1985. Oxidation of hydroquinones, catechols and phenols using ceric ammonium nitrate and ammonium dichromate coated with silica. *Synthesis* 641-643.

Gries, R., Khaskin, G., Gries, G., Bennet, R.G., King, G.G.S., Morewood, P., Slessor, K.N. and Morewood, W.D. 2002. (Z,Z)-4,7-Tridecadien-(*S*)-2-yL acetate: Sex pheromone of Douglas–fir cone gall midge, *Contarinia oregonensis*. *Journal of Chemical Ecology* 28:2283-2297.

Jacobsen, N. 1977. Free-radical Alkylation of Quinones: 2-Phenoxymethyl-1,4-benzoquinone. *Organic Syntheses* 56:68-71.

Jacobsen N. and Torssell K. 1972. Radikalishe Alkylierung von Chinonen: Erzeugung von Radikalen in Redoxreaktionen. *Liebigs Annalen der Chemie* 763:135-147.

Lamb, R.J. and Wellington, W.G. 1974. Techniques for studying the behavior and ecology of the European earwig, *Forficula auricularia* (Dermaptera: Forficulidae). *The Canadian Entomologist* 106:881-888.

Lamb, R.J. 1975. Effects of dispersion, travel and environmental heterogeneity on populations of the earwig, *Forficula auricularia* L. *Canadian Journal of Zoology* 53:1855-1867.

Millar, J. G. and Haynes, K.F. 1998. Methods in Chemical Ecology. Kluwer Academic Publishers. Vol 1. 390 pp.

Morrison, R.T. and Boyd R.N. Organic Chemistry. 1983. 4th Edition. Allyn and Bacon Inc., Boston, London, Sidney, Toronto. 1184 pp.

Nojima, S., Schal, C., Webster, F.X., Santangelo, R.G. and Roelofs, W.L. 2005. Identification of the sex pheromone of the German cockroach, *Blattella germanica*. *Science* 307:1104-1106.

Ruther, J., Reinecke, A., Thiemann, K., Tolasch, T., Francke, W. and Hilker, M. 2000. Mate finding in the forest cockchafer, *Melolontha hippocastani,* mediated by volatiles from plants and females. *Physiological Entomology* 25:172-179.

Ruther, J., Reinecke, A., Tolasch, T. and Hilker, M. 2001. Make love not war: a common arthropod defense compound as sex pheromone in the forest cockchafer *Melolontha hippocastani*. *Oecologia* 128:44-47.

Sauphanor, B. 1992. Une Pheromone d'aggregation chez *Forficula auricularia L.* *Entomologia Experimentalis et Applicata* 62:285-291.

Sauphanor, B. and Sureau, F. 1993. Aggregation behaviour and interspecific relationships in Dermaptera. *Oecologia* 96:360-364.

Schildknecht, V.H. and Weis, K.H. 1960. VI Mitteilung über Insektenabwehrstoffe, Zur Kenntnis des Pygidialdrüsen-Sekretes vom gemeinen Ohrwurm, *Forficula auricularia*. *Zeitschrift für Naturforschung* 15:755-757.

Siljander, E., Penman, D., Harlan, H. and Gries, G. 2007. Evidence for male- and juvenile-specific contact pheromones of the common bed bug, *Cimex lectularius* Linnaeus (Hemiptera: Cimicidae). *Entomologia Experimentalis et Applicata. Entomologia Experimentalis et Applicata in press.*

Staddon, B.W. and Everton, I.J. 1980. Haemolymph of milkweed bug *Oncopectus fasciatus* (Heteroptera: Lygaeidae): inorganic constituents and amino acids. *Comparative Biochemistry and Physiology* 65:371-374.

Takács, S. and Gries, G. 2001. Communication ecology of webbing clothes moth: evidence for male-produced aggregation signal (s). *The Canadian Entomologist* 133:725-727.

Van den Dool. H. and Kratz, P.D. 1963. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *Journal of Chromatography* 2:463-471.

Walker, K.A., Jones, T.H. and Fell, R.D. 1993. Pheromonal basis of aggregation in European earwig, *Forficula auricularia* L. (Dermaptera: Forficulidae). *Journal of Chemical Ecology* 19:2029-2038.

3.6 Tables

Table 3.1 Name, source and supplier of candidate aggregation pheromone components of *F. auricularia*, and the composition of a synthetic blend for laboratory experiments.

Chemical name	Abbreviations	Ref #[a]	Synthetic blend (SB)[b]	MW[c]	Source[d]	Supplier	HPLC conditions MeCN:H_2O:MeOH (ml/min)
2-Methyl-1,4-benzoquinone	MBQ	1	2	122	1, 2, 3, 4, 5, 8,10, 12	Aldrich	RP 80:20:0 (1)
2-Ethyl-1,4-benzoquinone	EBQ	2	4	136	1, 2, 3, 4, 5, 8, 10, 12	SFU[e]	
2,3-Dimethyl-1,4-benzoquinone	2,3-DBQ	3	0.04	136	1, 2, 3, 4, 5, 10, 12	SFU	RP 50:50:0 (0.5)
2,3-Methylethyl-1,4-benzoquinone	MeEtBQs	4	0.04	150	1, 2, 3, 4, 5, 10, 12	SFU	RP 50:45:5 (0.5)
2,5-Methylethyl-1,4-benzoquinone			0.04		1, 2, 3, 10, 12		
2,6-Methylethyl-1,4-benzoquinone		5	0.04		1, 2, 3, 10, 12		
2-Propyl-1,4-benzoquinone	PBQ	6	0.02	150	4, 10, 12	SFU	RP 60:40:0 (0.6)
2,3-Diethyl-1,4-benzoquinone	DEBQs	7	0.04	164	1, 2, 3, 4, 5,10,12	SFU	RP 60:40:0 (0.9)
2,5-Diethyl-1,4-benzoquinone			0.04		1, 3, 10, 12		
2,6-Diethyl-1,4-benzoquinone		8	0.04		1, 3, 4, 10, 12		
2-Methyl-3-propyl-1,4-benzoquinone	MPBQs	9	0.02	164	1, 2, 3, 10, 12	SFU	RP 50:50:0 (0.6)
2-Methyl-5-propyl-1,4-benzoquinone			0.02		1, 2, 3, 10, 12		
2-Methyl-6-propyl-1,4-benzoquinone			0.02		1, 2, 3, 10, 12		
2-Ethyl-3-propyl-1,4-benzoquinone	EPBQs	10	0.04	178	3	SFU	RP 60:40:0 (0.9)
2-Ethyl-5-propyl-1,4-benzoquinone			0.04		2, 3, 4, 10, 12		
2-Ethyl-6-propyl-1,4-benzoquinone			0.06		1, 2, 3, 4, 10,12		
Nonanoic acid	9 Acid	11	0.2	158	8	Sigma	
Dodecanoic acid	12 Acid	12	0.2	200	7, 8	Sigma	
Tetradecanoic acid	14 Acid	13	0.2	228	7, 8	Sigma	
Hexadecanoic acid	16 Acid	14	0.2	256	7, 8	Sigma	
(*Z*)-9-Hexadecenoic acid	*Z*9-16 Acid	15	2	254	4, 5, 7, 8	Sigma	
(*Z,Z*)-9,12-Octadecadienoic acid	*Z*9*Z*12 18Acid[f]	16	-	280	4, 5, 7, 8		

Chemical name	Abbreviations	Ref #[a]	Synthetic blend (SB)[b]	MW[c]	Source[d]	Supplier	HPLC conditions MeCN:H_2O:MeOH (ml/min)
2-Ethyl-1,4-hydroquinone	E-OH-Q	17	0.4	138	2, 3, 4, 5, 8, 10, 12	SFU	
Vanillin		18	0.4	152	13	BDH	
(E)-2-nonenal	*E*2-9Ald	19	0.06	140	1, 2, 3	Bedoukian	
(*E*,*E*)-2,4-nonadienal	*E*2*E*4-9Ald	20	0.04	138	1, 2, 3	Bedoukian	
Phenylacetaldehyde		21	0.25	120	7	Aldrich	
3,5,6-Trimethyl-2-cyclohexen-1-one		22	0.02	152	2, 3, 4, 5	SFU	
6-Methyl-5-hepten-one = sulcatone		23	0.04	126	1, 2, 3	Aldrich	
Geranylacetone		24	0.04	194	1, 2, 3, 10, 12	Aldrich	
Conophtorin		25	0.04	156	2, 3, 4, 5	SFU	

Footnotes:

[a] Reference number as referred to in figure 1

[b] Numbers indicate the amount in nanograms of each chemical in the synthetic blend

[c] MW = molecular weight

[d] "Source" indicates where the compound was detected, as follows:

1=aeration of males
2=aeration of females
3=aeration of nymphs
4=body wash of males
5=body wash of females
6=body wash of nymphs
7=combined body wash of males and females
8=extract of frass from a mixed group of females, males and nymphs
9= extract of gland in 3rd abdominal segment of males
10=extract of gland in 4th abdominal segment of males
11=extract of gland in 3rd abdominal segment of females
12=extract of gland in 4th abdominal segment of females
13=Headspace volatiles of males obtained by solid phase microextraction (SPME)

[e] All compounds supplied by SFU were synthesized by Dr. G. Khaskin except compound 25 which was synthesized Dr. H.D. Pierce Jr.

[f] This compound was excluded from the synthetic blend because it was reported (Walker et al., 1993) and in preliminary experiments shown to be repellent.

Table 3.2 Stimuli tested in field experiments 43-58.

		Stimuli (S) tested							
Class of compounds	**Chemical name**	**S1**	**S2**	**S3**	**S4**	**S5** [a]	**S6**	**S7**	**S8**
Benzoquinones	2-Methyl-1,4-benzoquinone	-	-	-	-	2	20	-	-
	2-Ethyl-1,4-benzoquinone	-	-	-	-	4	40	4	40
	2,3-Dimethyl-1,4-benzoquinone	-	-	-	-	0.04	0.4	0.04	0.4
	2,3-Methylethyl-1,4-benzoquinone	-	-	-	-	0.04	0.4	0.04	0.4
	2,5-Methylethyl-1,4-benzoquinone	-	-	-	-	0.04	0.4	0.04	0.4
	2,6-Methylethyl-1,4-benzoquinone	-	-	-	-	0.04	0.4	0.04	0.4
	2-Propyl-1,4-benzoquinone	-	-	-	-	0.02	0.2	0.02	0.2
	2,3-Diethyl-1,4-benzoquinone	-	-	-	-	0.04	0.4	0.04	0.4
	2,5-Diethyl-1,4-benzoquinone	-	-	-	-	0.04	0.4	0.04	0.4
	2,6-Diethyl-1,4-benzoquinone	-	-	-	-	0.04	0.4	0.04	0.4
	2-Methyl-3-propyl-1,4-benzoquinone	-	-	-	-	0.02	0.2	0.02	0.2
	2-Methyl-5-propyl-1,4-benzoquinone	-	-	-	-	0.02	0.2	0.02	0.2
	2-Methyl-6-propyl-1,4-benzoquinone	-	-	-	-	0.02	0.2	0.02	0.2
	2-Ethyl-3-propyl-1,4-benzoquinone	-	-	-	-	0.04	0.4	0.04	0.4
	2-Ethyl-5-propyl-1,4-benzoquinone	-	-	-	-	0.04	0.4	0.04	0.4
	2-Ethyl-6-propyl-1,4-benzoquinone	-	-	-	-	0.04	0.4	0.04	0.4
Saturated acids	Nonanoic acid	0.2	2	20	200	2	2	2	2
	Dodecanoic acid	0.2	2	20	200	2	2	2	2
	Tetradecanoic acid	0.2	2	20	200	2	2	2	2
	Hexadecanoic acid	0.2	2	20	200	2	2	2	2
Unsaturated acid	(Z)-9-Hexadecenoic acid	2	20	200	2000	20	20	20	20
Hydroquinone	2-Ethyl-1,4-hydroxyquinone	0.4	4	40	400	4	4	4	4
	Vanillin	0.4	4	40	400	4	4	4	4
Aldehydes	(E)-2-nonenal	0.06	0.6	6	60	0.6	0.6	0.6	0.6
	(E,E)-2,4-nonadienal	0.04	0.4	4	40	0.4	0.4	0.4	0.4
	Phenylacetaldehyde	0.25	2.5	25	250	2.5	2.5	2.5	2.5
Ketones	6-Methyl-5-hepten-one = Sulcatone	0.04	0.4	4	40	0.4	0.4	0.4	0.4
	Geranylacetone	0.04	0.4	4	40	0.4	0.4	0.4	0.4
Acetal	Conophtorin	0.04	0.4	4	40	0.4	0.4	0.4	0.4

[a]S5 = 10 SB

Table 3.3 Stimuli tested and number of females, males, and nymphs responding to treatment and control stimuli in field experiments 45-58.

Exp.#	Date (2006)	N	Stimuli (S) tested[a,b,c,d]	Number of insects responding[e,f]							
				Females		Males		Nymphs		All	
				S:	C	S:	C	S:	C	S:	C
45	July 16	15	1 NSB (S1)	5:	10	5:	8	11:	8	21:	26
46	July 16	15	10 NSB (S2)	5:	6	3:	3	12:	7	20:	16
47	July 16	15	100 NSB (S3)	13:	8	9:	1	16:	9	38:	18*
48	July 30	16	1 NSB (S1)	24:	12*	13:	10	126:	55*	163:	77*
49	July 30	16	10 NSB (S2)	20:	7	24:	8	136:	65*	180:	80*
50	July 30	16	100 NSB (S3)	18:	8	15:	6	119:	74	152:	88**
51	July 30	16	1000 NSB (S4)	29:	13	16:	8	70:	51	115:	72
52	Aug. 6	31	10 NSB (S2)	27:	17	24:	6*	55:	25*	106:	48**
53	Aug. 6	31	100 NSB (S3)	36:	16*	16:	15	53:	29 *	105:	60*
54	Aug. 18	30	10 SB with BQ at 10% (S5)	36:	11**	28:	8*	1:	0	65:	19***
55	Aug. 18	30	10 NSB (S2)	35:	26	18:	18	1:	1	54:	45
56	Aug. 18	30	10 SB (S6)	51:	20***	34:	11*	3:	1	88:	32**
57	Aug. 18	30	10 SB with BQ at 10% *minus* MBQ (S7)	52:	16*	40:	12**	2:	1	94:	29**
58	Aug. 18	30	10 SB *minus* MBQ (S8)	57:	21**	51:	37**	0:	3	108:	61*

[a]SB = Synthetic blend (see Table 3.1)
[b]NSB = SB minus benzoquinones (BQ)
[c]Composition of treatment (T) stimuli 1-8 as listed in Table 2
[d]The equivalent amount of solvent served as control stimulus in all experiments
[e]Most nymphs had become adults in experiments 54-58
[f]$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$

3.7 Figures

Figure 3.1 Flame ionization detector (FID) and electroantennographic detector (EAD: female *F. auricularia* antenna) responses to aliquots of body washes of male and female *F. auricularia*. Chromatography: Hewlett Packard 5890A gas chromatograph equipped with a DB-5 coated column (30 m x 0.25 mm ID); linear flow velocity of carrier gas: 35 cm/sec; injector and FID temperature: 220°C; temperature program: 2 min at 50°C, 10°C/min to 280°C. Numbers of components in the FID trace refer to compounds listed in Table 3.1.

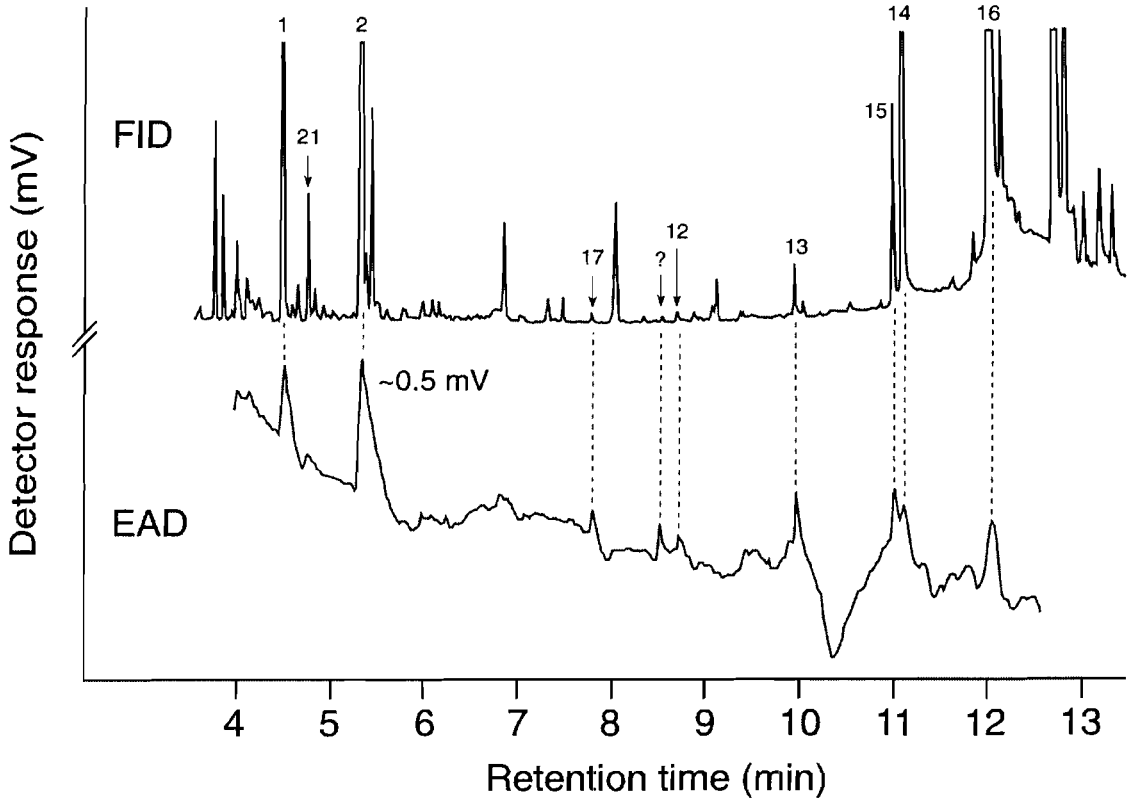

Detector response (mV)
FID
EAD
1
2
21
17
?
12
13
15
14
16
~0.5 mV
4
5
6
7
8
9
10
11
12
13
Retention time (min)

Figure 3.2 Number of female and nymph *F. auricularia* responding in three-chamber olfactometers to a synthetic blend (SB; see Table 3.1) or to SBs from which specific groups of organic compounds had been deleted. An asterisk (*) indicates a significant preference for a particular test stimulus (X^2-test; $^*P < 0.05$). Number in brackets indicate number of nonresponding insects.

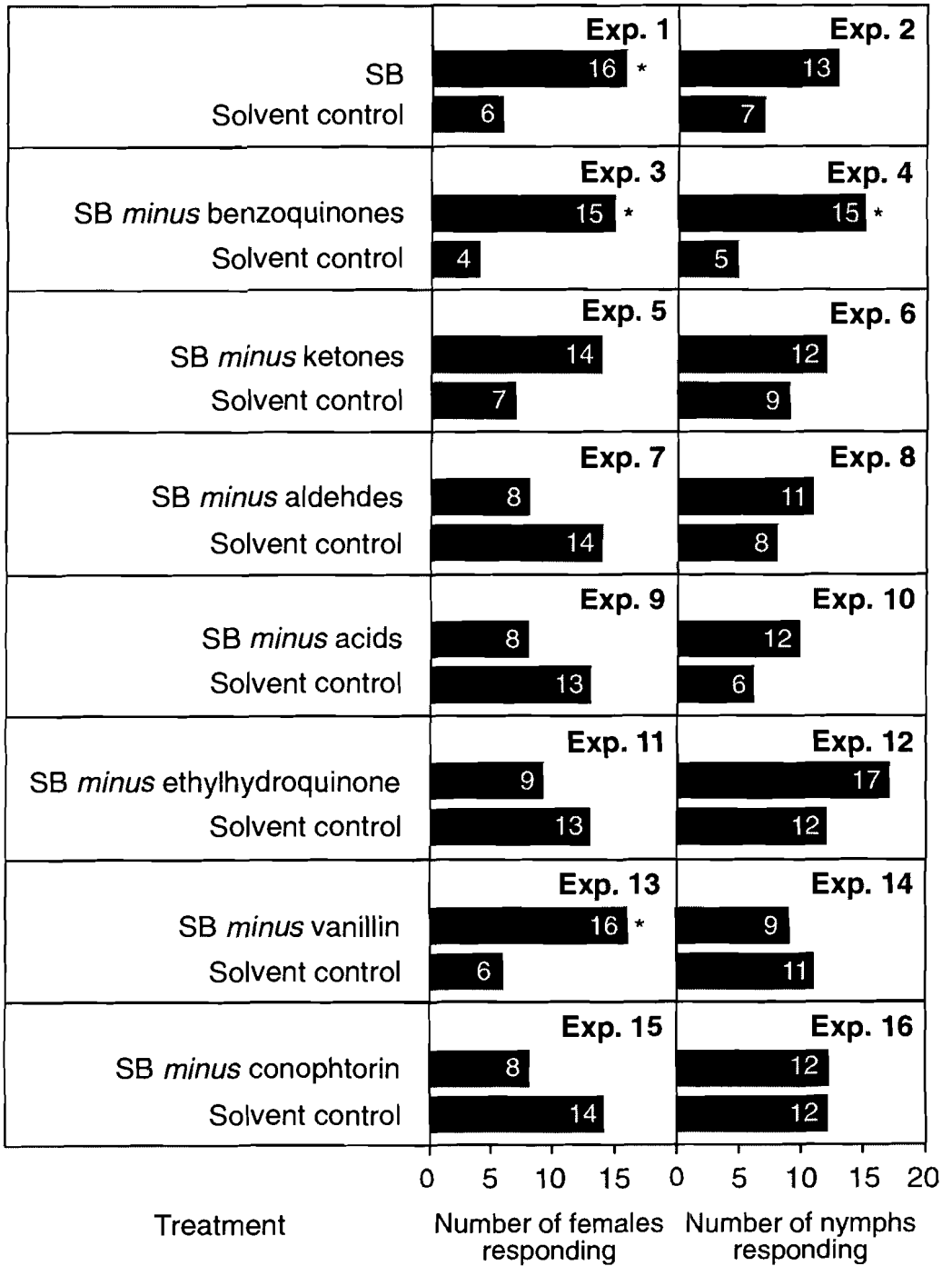

Exp. 1
SB
16 *
Solvent control
6
Exp. 2
13
7
Exp. 3
SB minus benzoquinones
15 *
Solvent control
4
Exp. 4
15 *
5
Exp. 5
SB minus ketones
14
Solvent control
7
Exp. 6
12
9
Exp. 7
SB minus aldehdes
8
Solvent control
14
Exp. 8
11
8
Exp. 9
SB minus acids
8
Solvent control
13
Exp. 10
12
6
Exp. 11
SB minus ethylhydroquinone
9
Solvent control
13
Exp. 12
17
12
Exp. 13
SB minus vanillin
16 *
Solvent control
6
Exp. 14
9
11
Exp. 15
SB minus conophtorin
8
Solvent control
14
Exp. 16
12
12
0 5 10 15
0 5 10 15 20
Treatment
Number of females responding
Number of nymphs responding

Figure 3.3 Number of nymph *F. auricularia* responding in three-chamber olfactometers to a synthetic blend (SB; see Table 3.1) with benzoquinones absent, at reduced amount or altered ratio. An asterisk (*) indicates a significant preference for a particular test stimulus (X^2-test; $^*P < 0.05$). Numbers in brackets indicate numbers of nonresponding insects.

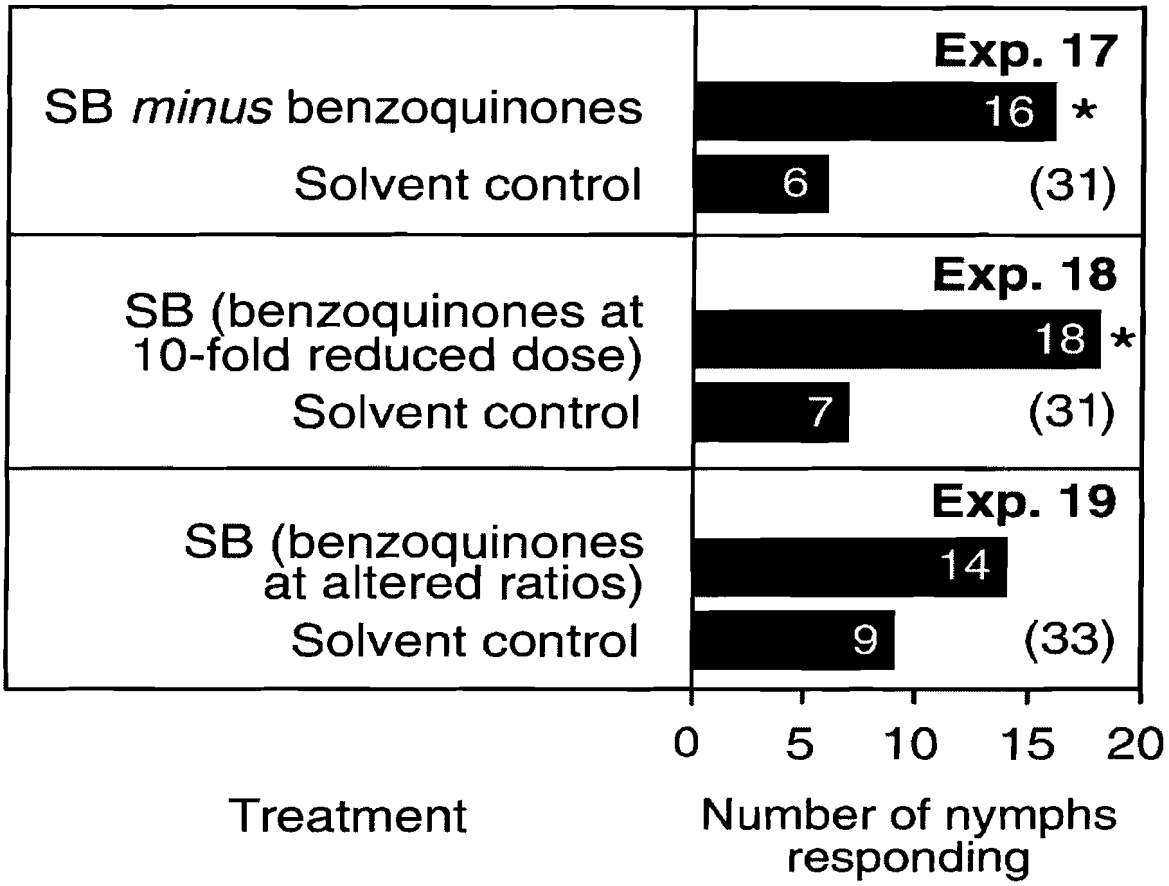

Exp. 17
SB *minus* benzoquinones
16 *
Solvent control
6
(31)
Exp. 18
SB (benzoquinones at 10-fold reduced dose)
18 *
Solvent control
7
(31)
Exp. 19
SB (benzoquinones at altered ratios)
14
Solvent control
9
(33)
0 5 10 15 20
Treatment
Number of nymphs responding

Figure 3.4 Number of female and nymph *F. auricularia* responding to a new synthetic blend [NSB = SB (see Table 3.1) *minus* benzoquinones] or NSBs lacking one of the three ketones. An asterisk (*) indicates a significant preference for a particular test stimulus (X^2-test; $*P < 0.05$). Numbers in brackets indicate numbers of nonresponding insects.

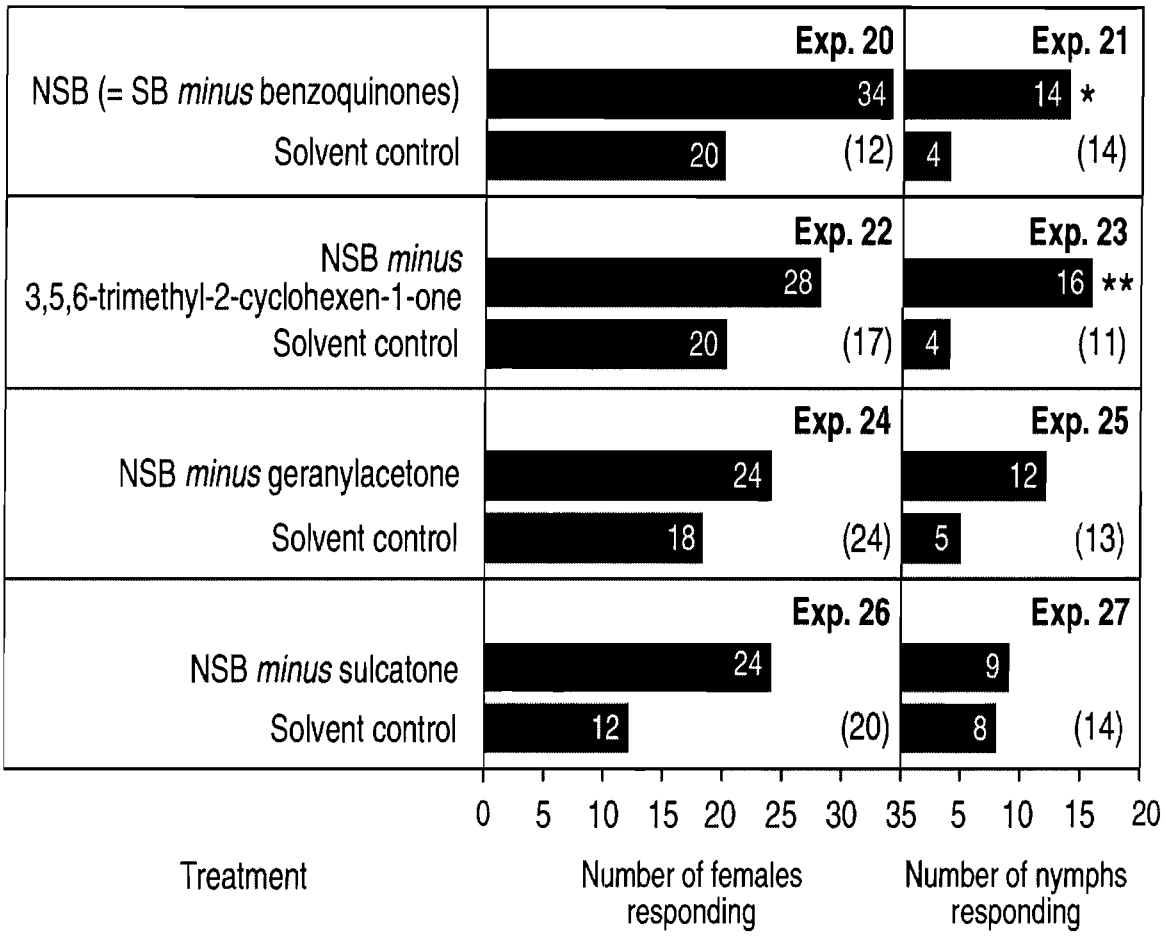

Exp. 20
Exp. 21
NSB (= SB *minus* benzoquinones)
34
14 *
Solvent control
20
(12)
4
(14)
Exp. 22
Exp. 23
NSB *minus* 3,5,6-trimethyl-2-cyclohexen-1-one
28
16 **
Solvent control
20
(17)
4
(11)
Exp. 24
Exp. 25
NSB *minus* geranylacetone
24
12
Solvent control
18
(24)
5
(13)
Exp. 26
Exp. 27
NSB *minus* sulcatone
24
9
Solvent control
12
(20)
8
(14)
0 5 10 15 20 25 30 35 5 10 15 20
Treatment
Number of females responding
Number of nymphs responding

Figure 3.5 Number of female and nymph *F. auricularia* responding in three-chamber olfactometers to a new synthetic blend [NSB = SB (see Table 3.1) *minus* benzoquinones] or NSBs lacking one of the three aldehydes. An asterisk (*) indicates a significant preference for a particular test stimulus (X^2-test; $*P < 0.05$). Numbers in brackets indicate numbers of nonresponding insects.

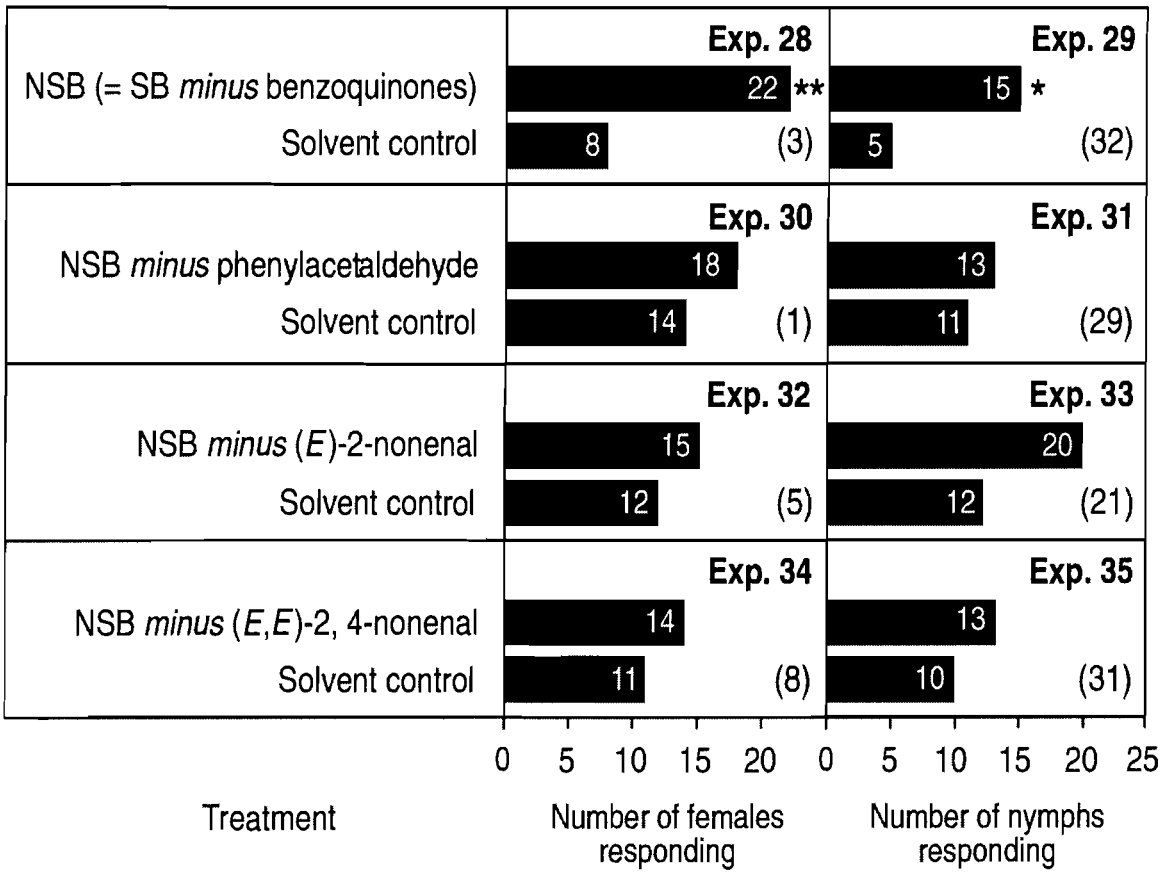

Exp. 28
Exp. 29
NSB (= SB minus benzoquinones)
22 **
15 *
Solvent control
8
(3)
5
(32)
Exp. 30
Exp. 31
NSB minus phenylacetaldehyde
18
13
Solvent control
14
(1)
11
(29)
Exp. 32
Exp. 33
NSB minus (E)-2-nonenal
15
20
Solvent control
12
(5)
12
(21)
Exp. 34
Exp. 35
NSB minus (E,E)-2, 4-nonenal
14
13
Solvent control
11
(8)
10
(31)
0 5 10 15 20
0 5 10 15 20 25
Treatment
Number of females responding
Number of nymphs responding

Figure 3.6 Number of female and nymph *F. auricularia* responding in three-chamber olfactometers to a new synthetic blend [NSB = SB (see Table 3.1) *minus* benzoquinones] or NSBs lacking the four saturated acids or the unsaturated acid (*Z*)-9-hexadecenoic acid. An asterisk (*) indicates a significant preference for a particular test stimulus (X^2-test; $^*P < 0.05$). Numbers in brackets indicate numbers of nonresponding insects.

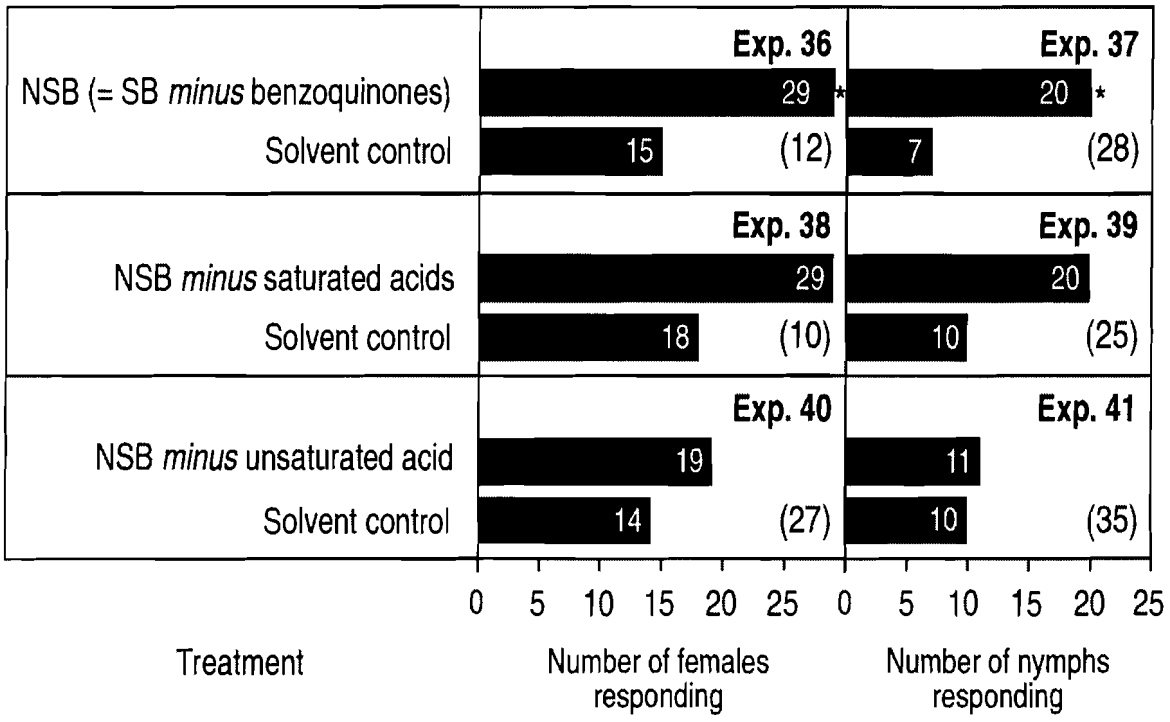
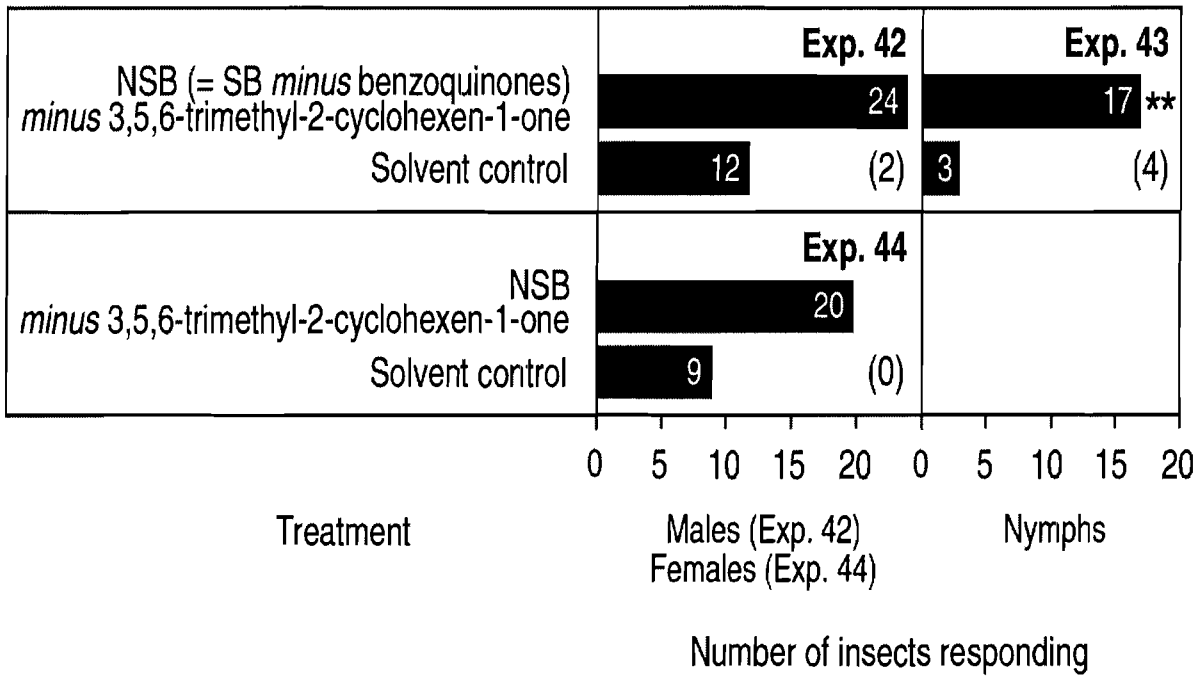

Exp. 36
Exp. 37
NSB (= SB minus benzoquinones)
29
*
20
*
Solvent control
15
(12)
7
(28)
Exp. 38
Exp. 39
NSB minus saturated acids
29
20
Solvent control
18
(10)
10
(25)
Exp. 40
Exp. 41
NSB minus unsaturated acid
19
11
Solvent control
14
(27)
10
(35)
0 5 10 15 20 25
0 5 10 15 20 25
Treatment
Number of females responding
Number of nymphs responding

Figure 3.7 Number of adult female, adult male and nymph *F. auricularia* responding in arena olfactometers to a new synthetic blend [NSB = SB (see Table 3.1) *minus* benzoquinones minus 3,5,6-trimethyl-2-cyclohexen-1-one]. The asterisks (**) indicate a significant preference (X^2-test; $*P < 0.01$) for the test stimulus. Numbers in brackets indicate the numbers of nonresponding insects.


Exp. 42
Exp. 43
NSB (= SB *minus* benzoquinones) *minus* 3,5,6-trimethyl-2-cyclohexen-1-one
24
17 **
Solvent control
12
(2)
3
(4)
Exp. 44
NSB *minus* 3,5,6-trimethyl-2-cyclohexen-1-one
20
Solvent control
9
(0)
0 5 10 15 20 0 5 10 15 20
Treatment
Males (Exp. 42)
Females (Exp. 44)
Nymphs
Number of insects responding

Chapter 4
Concluding Summary

European earwigs reportedly use an aggregation pheromone but there is controversy about its source and which are its constituents. Hence, I investigated whether each developmental stage and sex produce and respond to this pheromone and analyzed its chemical constituents. Based on these laboratory analyses, and laboratory and field experiments the following conclusions can be drawn:

1) Dual-choice still-air olfactometer experiments revealed that females, males and nymphs produce and respond to an aggregation pheromone.

2) The aggregation pheromone is airborne and perceived by olfaction.

3) Neither frass nor cuticle are the source of the aggregation pheromone.

4) Because of uncertainty and controversy as to what constitutes the source of the aggregation pheromone, candidate pheromone components were obtained from all potential sources, including headspace volatiles (collected by Porapak Q capture and solid phase microextraction), extracts of abdominal and tibial glands, and of frass as well as washes of the insects' integument.

5) Aliquots of all extracts were analyzed by gas chromatographic-electroantennographic detection (GC-EAD) and GC-mass spectrometry.

6) A complex synthetic blend (SB) of 30 candidate pheromone components, including 16 benzoquinones, four saturated acids, one unsaturated acid, one hydroquinone, vanillin,

three aldehydes, three ketones and one acetal significantly attracted/arrested females and nymphs in still-air dual-choice three-chamber olfactometer experiments.

7) To determine the essential compounds in the SB, SBs that lacked a specific group of organic chemicals, such as acids or aldehydes, were tested in three-chamber olfactometers.

8) The SB lacking benzoquinones still elicited significant behavioral responses from nymphs in three-chamber and arena olfactometers experiments as well as field experiments.

9) SBs lacking benzoquinones elicited inconsistent responses from females in three-chamber olfactometers, no responses from females and males in arena olfactometers, and inconsistent or no responses from females and males in field experiments.

10) Results described under points 8 and 9 suggest that nymphs and adults may use different aggregation pheromones and that one or more benzoquinones are constituents only of the adult-specific aggregation pheromone.

11) Additional elaborate experiments are required to determine the essential components of the aggregation pheromone deployed by nymphs and adults. Results of these experiments will determine whether a synthetic blend may become commercially viable as a trap bait to help control earwig populations or to enhance their predatory role as biological control agents.