PHEROMONAL AND KAIROMONAL ATTRACTION OF *ASCOGASTER QUADRIDENTATA* WESMAEL (HYMENOPTERA: BRACONIDAE), A PARASITOID OF *CYDIA POMONELLA* L. (LEPIDOPTERA: TORTRICIDAE)

by

Naomi Catherine DeLury

B.Sc., Simon Fraser University, 1994

THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in the Department

of

Biological Sciences

SIMON FRASER UNIVERSITY

JULY 1998

ABSTRACT

Ascogaster quadridentata Wesmael is an egg-larval endoparasitoid of the codling moth, *Cydia pomonella* L., a key pest of apples worldwide. Little is known about the chemical ecology of *A. quadridentata.*

Porapak Q-collected extracts of female *A. quadridentata*-produced volatiles bioassayed in Y-tube olfactometers attracted male, but not female, *A. quadridentata.* Coupled gas chromatographic-electroantennographic detection (GC-EAD) analysis of the bioactive extracts revealed three compounds that elicited responses by male *A. quadridentata* antennae. GC-mass spectra (MS) indicated and comparative analyses of standards confirmed that these compounds were *Z*9,*Z*12-octadecadienal, *Z*9-hexadecenal and 3,7,11-trimethyl-6*E*,10-dodecadienal. *Z*9,*Z*12-Octadecadienal alone attracted laboratory-reared male *A. quadridentata* in Y-tube olfactometers and field-cage bioassays, and attracted wild male *A. quadridentata* in a field experiment. This sex pheromone could be used to help delineate natural distributions of *A. quadridentata*, investigate why *A. quadridentata* survives in some areas and not others and determine potential sources of parasitoids for capture and release.

Host location by *A. quadridentata* was shown to be mediated by kairomones associated with its *C. pomonella* host. In Y-tube olfactometer bioassays, female *A. quadridentata* were attracted to Porapak Q-collected volatile extracts from female *C. pomonella* scales and eggs, but not to *C. pomonella* sex pheromone. Only the scales of female *C. pomonella* were attractive to male *A. quadridentata.* Antennally-active compounds in scale volatile extracts included heptanal, octanal, nonanal, decanal, undecan-2-one, dodecanal, pentadecan-2-one, *Z*6-pentadecen-2-one, *Z*9-hexadecenal, *Z*6-heptadecen-2-one, and 3,7,11-trimethyl-2*E*,6*E*,10-dodecatrien-1-ol acetate. A synthetic blend of these compounds at quantities and ratios equivalent to Porapak Q scale extract was attractive to female *A. quadridentata* in a Y-tube olfactometer bioassay. Identification and deployment of the behaviourally active compounds may augment *A. quadridentata* management in target orchards, increasing immigration while decreasing emigration of the parasitoid.

ACKNOWLEDGEMENTS

This research would not have been possible without the support, advice and assistance of many people. I am especially grateful to the following individuals: Dr. Gerhard Gries, my senior supervisor, for his thorough supervision and for providing me with the opportunity and guidance to make this thesis possible; Dr. Gary Judd, for his intellectual and financial support, for his critical review of my thesis, for presenting me with the idea of studying kairomones of codling moth parasitoids and for getting me started; Dr. Manfred Mackauer, for his advice and encouragement throughout my research, for his guidance in the sexing of my insects and for his critical and prompt review of this manuscript; Ms. Regine Gries, for her patience and expertise while training me to perform electroantennographic detector analyses and for her seemingly endless assistance with chemical investigations; Ms. Nicole Jeans, for a summer of dedicated assistance and thoughtful advice; Dr. John Brown, for providing me with *Ascogaster quadridentata* and field sites when I most needed them; Dr. Grigori Khaskin and Dr. Skip King, for synthesizing a number of chemicals for my research; Dr. Harold Pierce, Jr., for his assistance in volatile collections and for his chemical expertise; Ms. Maya Evenden, for assistance and advice over the course of my research, for providing me with a place to stay during many of my field trips and for exposing me to the world of chemical ecology; Ms. Lila DeLury, for her much needed help with laboratory colonies and field experiments during the last two summers, as well as for giving me a roof over my head last field season; Mr. Mark Gardiner, for his exceptional photography, his attention to detail and his assistance throughout this study; Dr. Tom Unruh, for leading me to *A. quadridentata* when the codling moth parasitoid I initially planned to work with was unavailable; Dr. M. Sharkey, for providing advice on how to sex *A. quadridentata*; Ms. Pam Javan-Sehati, for assistance in rearing and bioassays; Dr. Heather McBrien, for locating field sites for me and for smoothing out the rough edges of my first paper presentation; Dr. Ken Bloem, for his willingness to provide funding; Dr. Stephanie Bloem, for providing me with field cages last summer and for taking them down; Mr. Les Wakida, for construction of the glass Y-tubes; Mr. Robert DeLury, for construction of a growth chamber when none were available; Ms. Mary Margaret DeLury, for laboratory assistance; Mr. Ian Bercovitz, for statistical advice; Dr. Rick

Routledge, for an excellent course on statistics; Dr. John Borden, for a valuable forest-pest-management course and for his advice and inspiration; Mr. Greg Owen, for mass spectrometry; Ms. Elizabeth Carefoot, for preparation of diagrams and slides; Dr. Michael Weis, for preparation of the photographs and scanning electron micrograph; Ms. Catherine DeLury for editing; and my parents, Nancy and Robert, for their emotional support and occasional financial contributions.

I thank my colleagues in the Gries, Borden, Winston, and Roitberg labs, not only for helpful discussions but for their friendship and humour, which have made my graduate experience memorable.

My special thanks go to my husband, Mr. Trevor Robinson, who not only patiently provided me with much needed support and encouragement but also served as my laboratory assistant, field assistant and editor at various times throughout the course of my thesis.

I would also like to thank the organizations that made this research possible through funding: the Science Council of British Columbia (GREAT Award), the Washington Tree-Fruit Research Commission, the Okanagan-Kootenay Sterile Insect Release Program, Phero Tech Inc. and Simon Fraser University (Graduate Fellowship).

TABLE OF CONTENTS

PAGE

LIST OF TABLES

TABLE **PAGE**

LIST OF FIGURES

I. GENERAL INTRODUCTION

1. ODOUR COMMUNICATION IN HYMENOPTERAN PARASITOIDS

"The behavioral manipulation of natural enemies of pest insects has been a long standing dream among the biological control practitioners" (Vinson, 1977). Before this dream becomes a reality, however, the behaviour of beneficial insects and factors that mediate that behaviour must be understood (Tumlinson *et al.*, 1993). Many intraspecific and interspecific parasitoid interactions are mediated by chemical cues (Lewis and Martin, 1990). These semiochemicals [chemical signals that convey information between organisms (Law and Regnier, 1971)] are believed to serve an important role as cues to aid parasitoids in locating mates (Eller *et al.*, 1984; van den Assem, 1986; Godfray, 1994; Pompanon *et al.*, 1997) and in guiding them to potential hosts (Vinson, 1977; Powell, 1986; Lewis and Martin, 1990; Godfray, 1994).

Many female insects release sex pheromones that attract mates from long distances (Godfray, 1994). Although much of the research on insect sex pheromones has focussed directly on "pest" insects (Eller *et al.*, 1984), there is a growing body of research demonstrating that chemical cues play an important role in mate location and recognition by "beneficial" hymenopterous parasitoids (van den Assem, 1986; Godfray, 1994). Evidence for sex pheromones has been found in the Aphelinidae, Chalcididae, Cynipidae, Pteromalidae, Scelionidae, Braconidae, Ichneumonidae (reviewed in Eller *et al.*, 1984) and in the Trichogrammatidae (Pompanon *et al.*, 1997). Typically, female parasitic wasps release pheromones to attract males (Boush and Baerwald, 1967; Cole, 1970; Vinson, 1972, 1978;

Matthews, 1974; Obara and Kitano, 1974; Gordh and de Bach, 1978; Browning and Oatman, 1985; Kamano *et al.*, 1989; Kainoh *et al.*, 1991; Field and Keller, 1993; Pompanon *et al.*, 1997), although either sex may signal as males have been reported to form swarms that are attractive to females, perhaps via chemical signals (van den Assem, 1986). In the presence of the female's pheromone extract and/or the isolated pheromone, male wasps are stimulated to search and/or are attracted over a relatively short range; there are very few demonstrations of long-range sex pheromones among parasitic wasps (Godfray, 1994).

Host-selection behaviour in parasitoids has been studied intensively since Salt (1935) proposed a conceptual model discriminating between host location and host acceptance. A revised and generally accepted model proposes a hierarchical sequence of four major stages: location of host habitat, location of host, acceptance of host and determination of host suitability (Flanders, 1953; Doutt, 1959, 1964; Vinson, 1976, 1981, 1984, 1985; Vinson and Iwantsch, 1980; van Alphen and Vet, 1986; Wellings, 1991). Many studies in this area are being conducted to provide information that will enable pest managers to deploy semiochemical stimuli for manipulation of parasitoids in target environments (Godfray, 1994).

Female parasitoids use diverse information to locate hosts and to assess their suitability. They exploit cues which reliably indicate the presence of suitable hosts (Vet *et al.*, 1995). These include chemical, visual, physical (size, shape, texture, movement and heat) and physiological signals of host condition (Godfray, 1994). The search strategies and stimuli they exploit reflect the host stage being attacked, as host-habitat and host-derived stimuli change throughout the host's development.

Egg parasitoids utilize cues from the habitat as well as those directly associated with

the eggs, however as host eggs are sessile and have limited interaction with their environment there are very few additional cues available (Vet *et al.*, 1995). Egg parasitoids tend to use semiochemicals, such as sex pheromones (Lewis *et al.*, 1982; Noldus, 1989), volatiles from egg-adhesives or from scales deposited on eggs by ovipositing females of the adult host (reviewed in Vet *et al.*, 1995) to direct them toward host eggs. Once an egg parasitoid is in close proximity to host eggs (Laing, 1937), visual cues, such as colour and shape of eggs, become important (Vet *et al.*, 1995).

Parasitoids attacking the herbivorous larval stage of their host have the additional advantage of utilizing cues derived from their hosts' interaction with the environment. Some use long-range cues, such as plant-derived odours, particularly those from injured plants (Vet *et al.*, 1995), as well as host larval-derived stimuli, such as frass, webbing and mandibular secretions (Weseloh, 1981).

Pupal parasitoids are generally unable to rely on plant-derived cues, as host pupae may not be present on plants and do not cause plants to emit wound responses (Vet *et al.*, 1995). Pupal parasitoids may exploit odours from the preceding larval stage, however, such as feeding damage or webbing (Vet *et al.*, 1995), or they may use general visual environmental cues (Schmidt *et al.*, 1993) to locate appropriate microhabitats of host pupae (Drost and Carde, 1992; Vet *et al.*, 1995). Once a pupal parasitoid is in the appropriate microhabitat, many of the reliable indicators of host presence come from the host itself, as in egg parasitoids (Vet *et al.*, 1995); however, as pupae are usually removed in time from the previous adult generation, pupal parasitoids are unlikely to use cues associated with the previous adult stage to locate their hosts.

The importance of host-derived kairomones, interspecific chemical signals that induce a beneficial behavioural response in the recipient (Nordlund and Lewis, 1976), in attracting and arresting parasitoids appears universal (Vinson, 1977; Weseloh, 1981; Vet *et al.*, 1995). As host-derived kairomones reliably indicate the host's presence (Vet et al., 1995), it is not surprising that kairomones have frequently been found to play a significant role in host location by parasitoids (Vinson, 1977).

Most kairomones promote area-restricted and intensified searching behaviour in receptive parasitoids (Vinson, 1977; van Alphen and Vet, 1986), unlike sex pheromones that guide the receiver to the emitter. Parasitoids can use kairomones, by responding to either a series of independent cues or a hierarchy of cues, to reduce the distance between them and their hosts, moving from host-habitat cues (synomones) to host-derived cues (kairomones) in a step-wise manner (Vinson, 1977). Because kairomones form part of a noncontinuous volatile trail, their broadcast release should not disrupt behaviour but should enhance parasitoid efficiency with respect to a particular orchard (Vinson, 1977, 1981).

Knowledge of semiochemicals and their impact on parasitoid behaviour could enhance the efficacy of parasitoids as biological control agents (Lewis and Martin, 1990; Tumlinson *et al.*, 1993). In this thesis, I present the identification of a sex pheromone produced by a female egg-larval parasitoid, *Ascogaster quadridentata* Wesmael (Hymenoptera: Braconidae) and the identification of host-derived kairomones, demonstrate attraction of parasitoids to synthetic pheromones and kairomones, and discuss potential uses of these semiochemicals for the enhancement of *A. quadridentata* as a biological control agent for one of its hosts, the codling moth, *Cydia pomonella* L. (Lepidoptera: Tortricidae).

2. DISTRIBUTION AND PEST STATUS OF *C. POMONELLA*

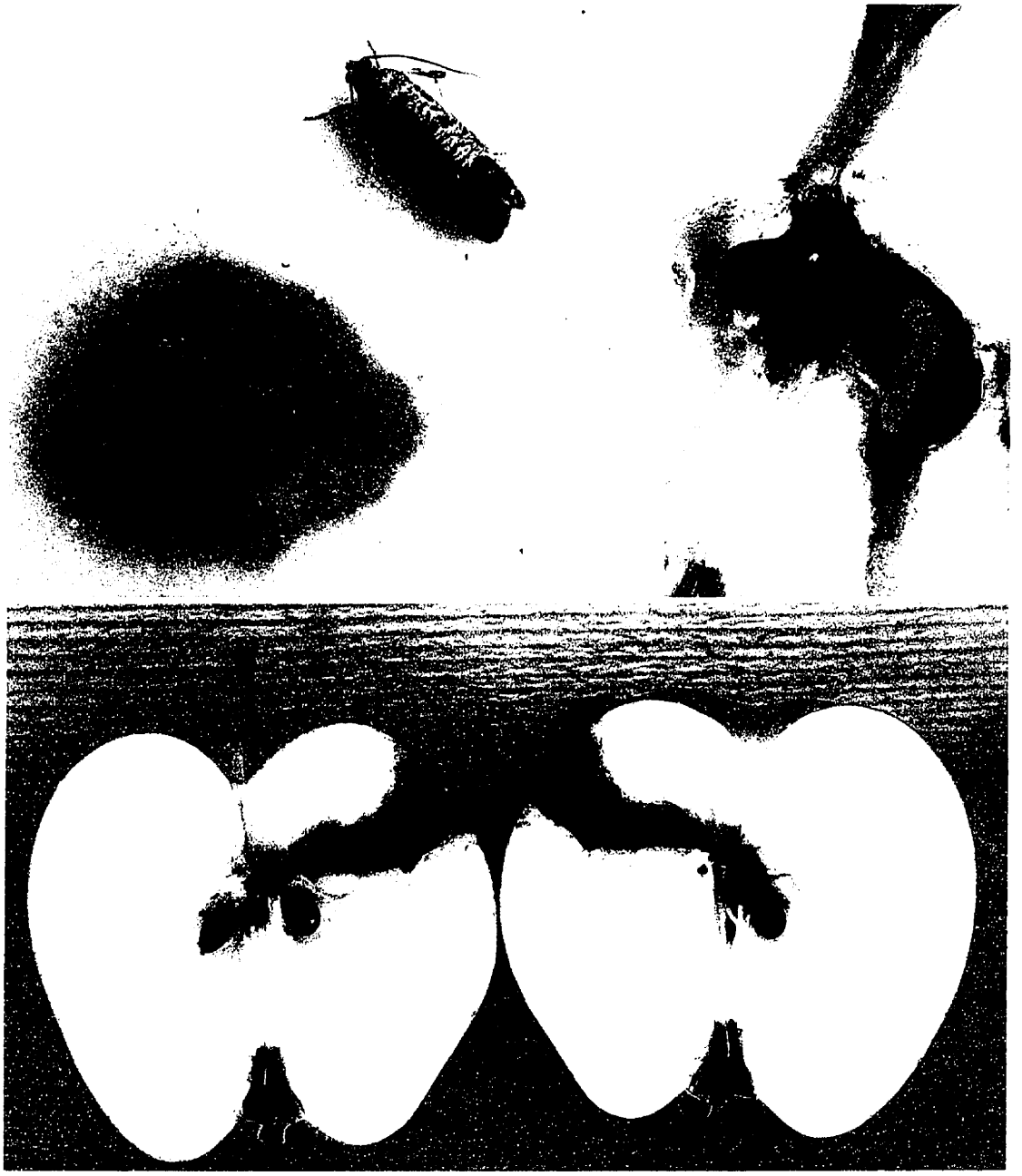
C. pomonella was chosen as the host system for this research because it is a severe pest of apple crops throughout the world (Putman, 1963). Originating in Kazakhstan, the codling moth has spread to all temperate regions where apples are grown except for Japan, parts of China, India and Western Australia (Putman, 1963; Clausen, 1978; Pedigo, 1989). In 1750, *C. pomonella* was first recorded in New England, USA. By 1868, it was present throughout Ontario (Putman, 1963; Le Roux, 1971), and by 1905, it had spread to the west coast of Canada (Putman, 1963; Le Roux, 1971).

C. pomonella commonly colonizes apple and pear, but also attacks crabapple, quince, apricot, peach, plum, cherry, walnut and hawthorn (Putman, 1963; Clausen, 1978). While not all varieties of apple are equally susceptible to attack by *C. pomonella*, none is resistant (Cutright and Morrison, 1935; Putman, 1963).

C. pomonella is considered the "key" insect pest in most apple-growing regions. Damage is inflicted as a larva burrows into the apple fruit, eating seeds and vacating the fruit thereafter (Dolstad, 1985). The fruit are left with holes surrounded by frass, making them unmarketable (Putman, 1963; Dolstad, 1985) (Figure 1). Even if first-instar larvae begin but do not continue feeding on the fruit, they leave superficial penetrations ("stings"), resulting in apples that will be downgraded (Vakenti, 1977). Damage to the fruit as a result of larval feeding also renders the apples more susceptible to secondary rot and deformity (Dolstad, 1985; Winston, 1997).

Damage from *C. pomonella* can be extensive. In untreated orchards with more than one generation of this moth, 75-95% of all apples may be infested (Putman, 1963; Clausen,

FIG. 1. Damaged apple caused by larva of *C. pomonella*: (top left) Adult *C. pomonella* beside a larval entrance hole with frass; (top right) *C. pomonella* larva feeding on seeds in the apple core; and (bottom) A cross section of an apple showing larval damage. (Photographs courtesy of M. Gardiner, Agriculture and Agri-Food Canada, Summerland, BC).



1978; Dolstad, 1985). A great deal of effort is therefore expended to control this "worm in the apple." Traditionally, insecticides have been employed to accomplish this, but the development of resistance in *C. pomonella* to heterocyclic organophosphates, such as Guthion® (Brown, 1993), the deregistration of many insecticides and the detrimental impact of insecticides on beneficial insects and the environment (Putman, 1963; Dolstad, 1985; Brown, 1993; Riddick and Mills, 1994) have generated great interest in alternative controls for *C. pomonella*. Major efforts to eradicate *C. pomonella* using the sterile-male technique (Dyck and Gardiner, 1992) or pheromone-based mating disruption (reviewed in Riddick and Mills, 1994) are currently under way. Biological control agents could serve as important components in an integrated management system for *C. pomonella* (Brown, 1993) when organophosphates are replaced by more benign controls.

3. *A. QUADRIDENTATA* AS A BIOLOGICAL CONTROL AGENT OF *C. POMONELLA*

A. quadridentata is a biological control agent that shows promise for reducing *C. pomonella* populations. By ovipositing into eggs of *C. pomonella*, *A. quadridentata* is able to circumvent the protection the larvae receive once they are inside the apple fruit and are no longer accessible to attack. In Eurasia, where *C. pomonella* is believed to have originated, *A. quadridentata* is one of the most common parasitoids collected from overwintering *C. pomonella* larvae (Brown and Reed-Larsen, 1991). Introduced into North America together with *C. pomonella* (Brown and Reed-Larsen, 1991), *A. quadridentata* has become common, and even abundant, throughout the eastern part of the continent (Dozier and Butler, 1929;

Cox and Daniel, 1935). However, it did not follow *C. pomonella* west (Boyce, 1936; Clausen, 1978; Brown and Reed-Larsen, 1991). Following mass releases of *A. quadridentata* in Washington (Johansen, 1957), Idaho and California (Clausen, 1978), *A. quadridentata* has become established in all release areas. Releases of *A. quadridentata* in British Columbia between 1933 and 1939 resulted in establishment of the parasitoid but not in control of *C. pomonella* (Boyce, 1936; McLeod, 1954).

A. quadridentata was also released in South Africa in the 1920s, and was recovered in select locations as late as 1936 (Clausen, 1978). *A. quadridentata* was released in New Zealand, Australia, Peru and West Pakistan between the 1930s and 1960s with undetermined or no success (Rao *et al.*, 1971; Clausen, 1978).

A. quadridentata currently occurs throughout Europe and eastern North America (Clausen, 1978) and has "embraced the whole Eurasian continent to China and Japan, as well as northern Africa, North America and Australia" (Shumakov, 1977). Local distributions of *A. quadridentata* are uncertain within any of these areas, partly because monitoring involves labour-intensive sampling of the overwintering host population.

Pheromone-baited traps can be used to evaluate parasitoid populations (Lewis *et al.*, 1971a). Due to the scattered distribution of their hosts, emergent male *A. quadridentata* must often search over long distances for females. It is likely that sex pheromones, signals which have the ability to summon mates over relatively long distances, are involved in mate-finding. Monitoring *A. quadridentata* with pheromone-baited traps could conceivably replace the current labour-intensive sampling procedure.

Where monitoring has been conducted, as in Washington (Brown and Reed-Larson,

1991), populations of *A. quadridentata* were detected only in unmanaged apple trees, possibly because *A. quadridentata* is sensitive to organophosphates used for control of *C. pomonella* in commercial orchards (Brown and Reed-Larsen, 1991). In these areas, *A. quadridentata* may contribute to the area-wide control of *C. pomonella* populations by reducing the number of adult moths that potentially immigrate into managed orchards from wild orchards (Brown and Reed-Larsen, 1991).

Achieving economic control of *C. pomonella* by simply establishing *A. quadridentata* in an orchard has been elusive. To date, *A. quadridentata* alone has not been effective in exerting control of *C. pomonella*. Its lack of effectiveness may stem from detrimental effects of insecticides on *A. quadridentata* populations, or it may be attributed to the impact of hyperparasitoids, such as *Perilampus* spp., *P. fulvicornis* Ashm., *P. tristis* Mayr and occasionally *Dibrachys cavus* (Wlk) (Boyce, 1940; Putman, 1963). In some studies, hyperparasitoids have parasitized up to 72% of *A. quadridentata* larvae (Boyce, 1940; Putman, 1963), although <1% parasitism by *Perilampus* spp. may be more common (Brown *et al.*, 1988). Inundative and regular releases of *A. quadridentata* might, however, greatly enhance levels of parasitism and could become part of an area-wide integrated management programme.

To succeed as biological control agents, laboratory-reared *A. quadridentata* must be able to locate wild *C. pomonella* eggs in the field. Lack of success in establishing parasitoids in target orchards has often been attributed to their rapid dispersal from release sites (Vinson, 1977). Host kairomones applied close to the site of parasitoid release have, however, made some parasitoids forage for hosts in the immediate area (Lewis *et al.*, 1975b). In commercial

orchards with low incidence of *C. pomonella*, low levels of natural kairomones could be enhanced by broadcast release of synthetic kairomones, thereby reducing dispersal and stimulating host-seeking behaviour of released parasitoids, enhancing their chance of encountering hosts and reducing economic damage (Vinson, 1977).

Lewis *et al.* (1990) and Vet *et al.* (1990) have suggested that parasitoids are born "with an innate set of 'response potentials' to different stimuli: a parasitoid presented with a number of stimuli will react to the one with the highest response potential" (Godfray, 1994). The ranking of the stimuli would determine the parasitoid's efficiency in finding a host (Godfray, 1994). Parasitoids are not static in their use of cues to locate and exploit hosts, however (Tumlinson et al., 1993). As plasticity in parasitoid responses has been recognized, it has become clear that cues to which parasitoids respond can be influenced by learning; a parasitoid can alter the ranking of stimuli, increasing the rank of those stimuli associated with the host (Taylor, 1974; Lewis *et al.*, 1990; Vet *et al.*, 1990). Thus, the chain of cues responsible for host location may be continuously altered with the experience of an individual parasitoid. Once a parasitoid has located and parasitized a host, its motivation to continue searching for additional hosts should be enhanced (Tumlinson *et al.*, 1993). In this way, an individual parasitoid may be "primed" to respond more strongly to cues that are associated with the host (Tumlinson *et al.*, 1993).

Because parasitoids can recognize stimuli through associative learning (Arthur, 1971; Vinson *et al.*, 1977; Vet *et al.*, 1995), rearing them in the presence of kairomones may make them more sensitive to synthetic kairomones in the field and may allow researchers to (re)direct them to previously unacceptable or less favourable hosts (Vinson, 1977). The first

step in testing this hypothesis is the identification of kairomones and their potential role in host-searching behaviour.

4. OBJECTIVES

My first objective was to identify the sex pheromone of *A. quadridentata.* Once identified, it could be used for pheromone-based monitoring to help delineate natural distributions of *A. quadridentata,* investigate why *A. quadridentata* survives in some areas and not others and determine potential sources of parasitoids for capture and release.

My second objective was to identify the source of kairomones that attract female *A. quadridentata* to their host, the eggs of *C. pomonella.* The ability to attract *A. quadridentata* to, and retain them in, target orchards could greatly enhance the impact of *A. quadridentata* as a biological control agent.

II. INSECT LIFE HISTORIES, CULTURES AND GENERAL BIOASSAY PROCEDURE

1. LIFE HISTORY

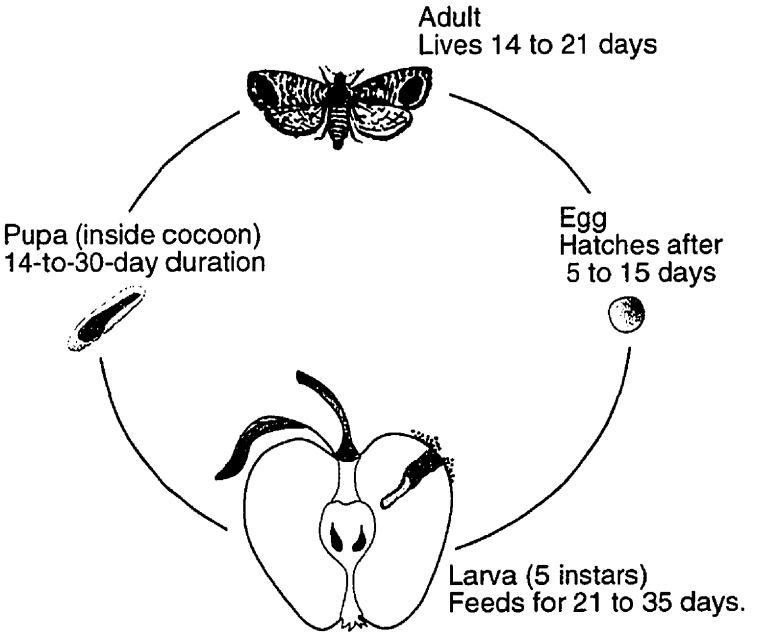
1.1. *C. pomonella*

Female codling moth, *C. pomonella*, generally lay their eggs singly, on or near an apple (Swan, 1964; Putman, 1963; Clausen, 1978; Thiery *et al.*, 1995). On average, females oviposit 30-40 eggs (Pedigo, 1989) (Figure 2a), predominantly in the upper crown of apple trees (Summerland and Steiner, 1943; Putman, 1963; Richardson and Du Chanois, 1950). Eggs are ca. 0.98-1.25 mm in length (Putman, 1963), initially disc-shaped (Dolstad, 1985) and closely appressed to the substrate (Putman, 1963). Freshly laid eggs appear colourless and translucent, but when incubation is ca. one-third complete, a transient red ring surrounds the embryo, and finally, 1 or 2 days before hatching, the black head capsule becomes visible (Putman, 1963). Neonates hatch ca. 5-15 days after oviposition, depending on temperature (Dozier and Butler, 1929; Clausen, 1978; Pedigo, 1989). The codling moth has five larval instars (Putman, 1963; Geier, 1963; Brown and Friedlander, 1995). The first instar is ca. 1.5 mm long, whitish in colour with a black head capsule, while the mature fifth instar is ca. 20 mm long and pinkish, with a brown head capsule and cervical shield (Putman, 1963; Dolstad, 1985).

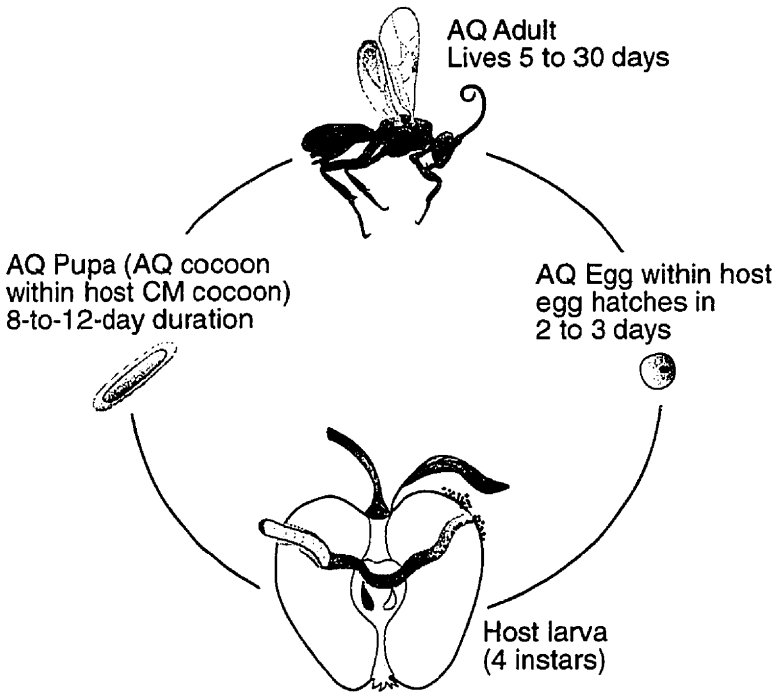
First-instar larvae locate the fruit and usually burrow into the calyx, blemishes or unbroken epidermis (Putman, 1963). Larvae feed on the pulp of the fruit until they reach the core, usually by the third instar, and then they attack the seeds (Clausen, 1978; Dolstad, 1985). *C. pomonella* larvae are unusual because they are able to digest seeds, despite the

FIG. 2. Life cycle of (a) the codling moth, *C. pomonella*, and (b) its egg-larval parasitoid, *A. quadridentata*.

a) Codling Moth, *Cydia pomonella* (CM)



b) Parasitoid, *Ascogaster quadridentata* (AQ)



CM Feeds for 14 to 35 days, then AQ moults into 2nd instar, and 2 to 3 days later, into its 3rd and final instar.

high level of cyanide, because of specialized digestive enzymes that enable them to overcome the apple's chemical defence (Winston, 1997). Third-instar larvae are aggressive, often attacking and even cannibalizing conspecific larvae, thereby limiting the number of larvae that can survive within a fruit (Putman, 1963; J.J. Brown, personal communication). Fifth-instar larvae burrow out of the apple, cease feeding and search for pupation sites under the tree bark, beneath debris on the ground or in other shelters where they will spin a cocoon (Putman, 1963; Clausen, 1978).

Adult male *C. pomonella* eclose ca. 1 week before females (Dolstad, 1985). The moths are 6-7 mm long, with a wingspan of 15-20 mm (Putman, 1963; Dolstad, 1985). Their greyish brown forewings are striated with light transverse bands and a large, copper-brown coloured spot on each tip. The hind wings are brown (Putman, 1963; Dolstad, 1985; Pedigo, 1989). Some adults are of lighter colour described as "buff" (Putman, 1963). Adults live for an average of 14-21 days (Hagley, 1972; Dolstad, 1985; Pedigo, 1989).

C. pomonella fly during low light intensity, with peak flight occurring within 20 min before and after sunset (Borden, 1931; Dolstad, 1985; Howell *et al.*, 1990). Although the flight is most intense during dusk, some moths have sporadically been recorded flying during sunrise (Putman, 1963). Under favourable conditions, *C. pomonella* may extend their flight over 2 h. There is no flight at high (33°C) or low (< 12 - 15°C) temperatures or when it is too windy (Borden, 1931; Dolstad, 1985). Mating occurs during the evening flight. Females "call" the males by releasing a sex pheromone which they disperse by fluttering their wings (Borden, 1931; Putman, 1963). The sex pheromone, *E*8,*E*10-dodecadien-1-ol, codlemone (Roelofs, 1971), is a long-distance attractant for males. Visual stimuli become important only

when the males are within close range (Weissling and Knight, 1994). Mating pairs will remain *in copula* for at least 20 min (Borden, 1931). Oviposition occurs mainly during sunset and requires 1-2 min for each egg (Borden, 1931; Putman, 1963). There is evidence of a marking pheromone or oviposition deterrent left on or in the eggs (Thiery *et al.*, 1995).

The number of generations *C. pomonella* produces each year is dependent on the climate, ranging from one to five generations in most northern and southern distributions, respectively (Schoene *et al.*, 1928; van Leeuwen, 1929; Putman, 1963). There are two complete generations of *C. pomonella* in the Okanagan Valley of British Columbia (where part of the field research was conducted), with a partial third generation in some locations during warm years (Vakenti, 1977).

1.2. *A. quadridentata*

A. quadridentata is a solitary egg-larval koinobiont endoparasitoid. *A. quadridentata* parasitizes a large number of micro-Lepidoptera, primarily from the family Tortricidae (Clausen, 1978). Hosts of *A. quadridentata* include striped peach worm (*Gelechia confusella* Cham.), strawberry leafroller (*Ancylis comptana* (Froelich)), grape berry moth (*Endopiza viteana* Clemens), oriental fruit moth (*Grapholita molesta* (Busck)) (Rosenberg, 1934; Boyce, 1936), lesser appleworm (*Grapholita prunivora* (Walsh)) (Tang and Marsh, 1994), eye-spotted bud moth (*Spilonota ocellana* (Denis and Schiffermuller)) (Rosenberg, 1934), plum moth (*Cydia funebrana* (Treitschke)) (Rosenberg, 1934; Shumakov, 1977), pea moth (*Cydia nigricana* (Fabricius)) (Rosenberg, 1934; Clausen, 1978) and codling moth (*C. pomonella*).

Female *A. quadridentata* place a single egg just under the chorion or into the yolk of a *C. pomonella* egg (Brown *et al.*, 1988) (Figure 2b). Age of host eggs does not appear to influence success of parasitism (Reed-Larsen and Brown, 1990). Parasitized and unparasitized eggs of *C. pomonella* are indistinguishable with the unaided eye. While parasitism does not affect the incubation time of eggs, parasitized *C. pomonella* complete four, rather than five larval instars (Brown *et al.*, 1988; Brown and Friedlander, 1995), are one-quarter to one-third smaller than normal and are less strikingly pink (Cox and Daniel, 1935). *A. quadridentata* also causes "embryonic castration" (Reed-Larsen and Brown, 1990), as the codling moth is left devoid of internal reproductive organs. This may be vital to the parasitoid's successful development, because it may compete with the host's reproductive organs for nutrients (such as glycogen and lipids) and for ecdysone, originally intended for the host's testes but possibly necessary for *A. quadridentata* to interpret the developmental state of the host (Reed-Larsen and Brown, 1990).

Two to 3 days after oviposition, the larva of *A. quadridentata* hatches and moves into the developing host embryo (Swan, 1964; Brown *et al.*, 1988). It remains as a first-instar as the host hatches and develops through three apparently normal instars (Swan, 1964; Brown *et al.*, 1988; Brown and Friedlander, 1995), with the notable exception of a lack of aggression in the third instar, which allows multiple *C. pomonella* to develop within a single apple (J.J. Brown, personal communication). The fourth-instar host larva ceases development and initiates precocious behaviour equivalent to that of a healthy fifth-instar larva, vacating the fruit in search of a pupation site (Brown *et al.*, 1988; Brown and Friedlander, 1995).

If the fourth-instar *C. pomonella* host initiates diapause, *A. quadridentata* overwinters

as a first-instar within the host (Brown *et al.*, 1988). In a non-diapausing host larva, *A. quadridentata* moults to a second-instar at the same time as a healthy *C. pomonella* larva would normally moult to the fifth-instar. *A. quadridentata* remains a second-instar for 2 or 3 days (Brown and Friedlander, 1995), which gives the host larva time to spin a cocoon. After moulting to a third-instar, *A. quadridentata* orients head to head within the cocooned prepupal *C. pomonella* and chews its way out of the host (Boyce, 1936; Brown, 1996). *A. quadridentata*, acting as an ectoparasite, consumes the entire *C. pomonella* host except the cuticle (Boyce, 1936; Brown and Friedlander, 1995). Finally, *A. quadridentata* spins a cocoon within the existing *C. pomonella* cocoon and pupates, emerging as an adult 8-12 days thereafter (Boyce, 1936). Unparasitized *C. pomonella* will eclose at approximately the same time.

Male *A. quadridentata* emerge before females. Adults are ca. 4.5 mm long, black, with mostly brown ca. 3 mm long antennae and transparent wings tinged with brown. Dorsally, the abdomen has only one visible segment and is concave ventrally. Males and females can be distinguished only by examining their reproductive organs through a microscope. Adults live from ca. 5-30 days (Boyce, 1936).

Females and males may (Boyce,1936), or may not, mate almost immediately after emerging from their cocoons. Females do not exhibit any obvious calling behaviour. Males, however, rapidly vibrate their antennae and vibrate or flutter their wings in a raised position, referred to as wing fanning, when in pursuit of a female.

A. quadridentata are arrhenotokous (Rosenberg, 1934). Females commence oviposition when finding host eggs or within 24 h of fertilization if host eggs are available,

with each female laying several hundred eggs (Boyce, 1936; Swan, 1964). Females have been observed ovipositing into any stage of the host egg and even attempting to oviposit into shells of hatched eggs or where host eggs have been removed (Boyce, 1936), suggesting the presence of kairomones associated with eggs. Initially, females avoid eggs that have been parasitized. After continued exposure to host eggs, however, they will superparasitize the host which ultimately prevents host eggs from hatching (Boyce, 1936; J.J. Brown, personal communication). The period from egg to adult takes 35-45 days at 27°C and 70% RH (Cox and Daniels, 1935). *A. quadridentata* cycles through as many generations per year as does *C. pomonella* (Brown *et al.*, 1988).

2. LABORATORY CULTURES

C. pomonella was reared on an artificial diet (modified from Brinton *et al.*, 1969) under a 16:8 L:D photoregime, 25°C and 65% RH. Eclosed and mated female moths oviposited on sheets of wax paper. Each week, one sheet was sent from the Agriculture and Agri-Food Canada Research Station in Summerland, BC, to Simon Fraser University, where it was stored at ca. 4°C.

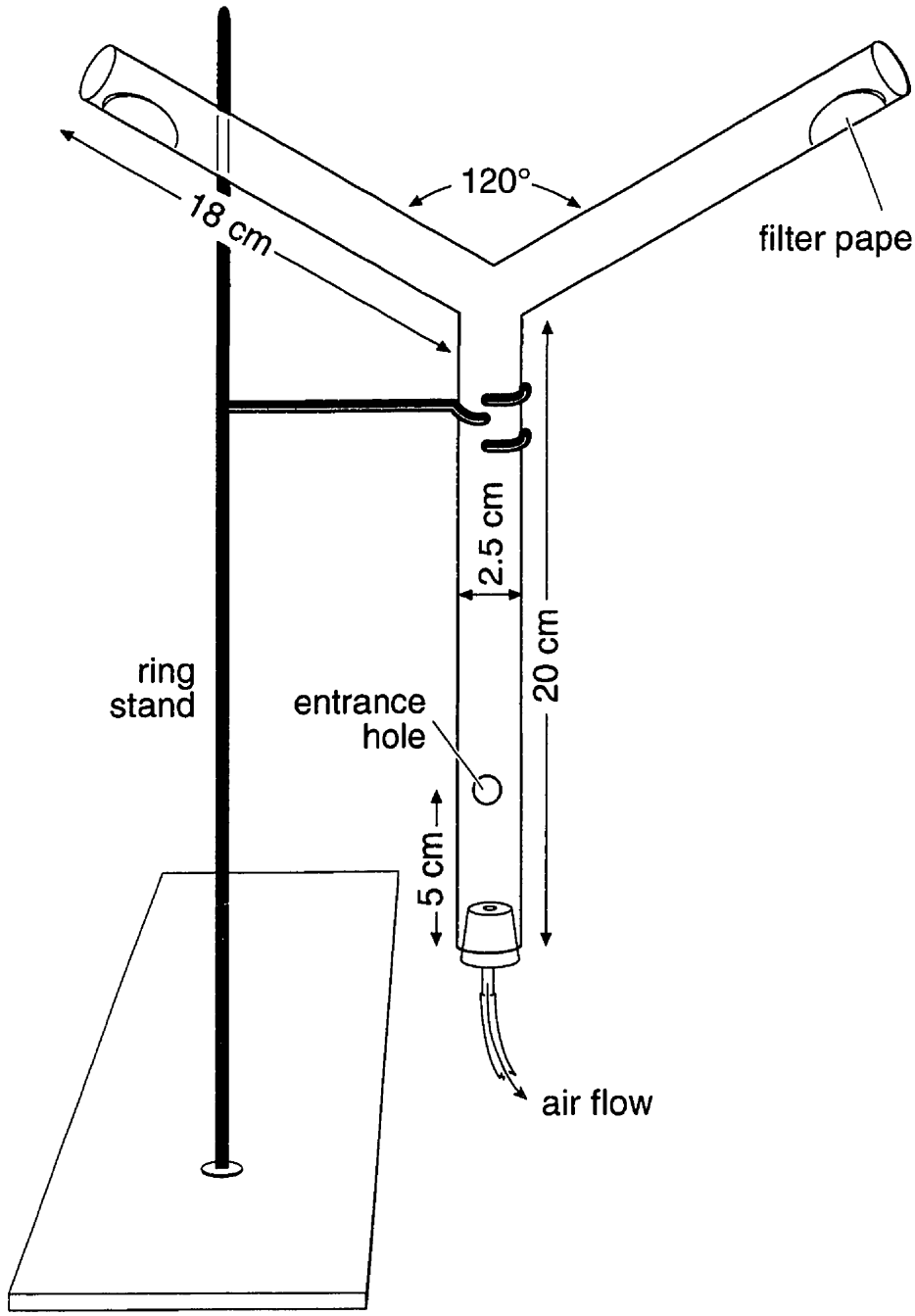
The colony of *A. quadridentata* was established, and periodically augmented, with specimens from Dr. John Brown's colony at Washington State University, Pullman, Washington. Adult *A. quadridentata* were maintained in mesh cages (30 x 30 x 45 cm) with a Plexiglass™ front under a 16L (27°C): 8D (15°C) photoregime and 70% RH. Insects were provided with a 10% honey-water solution, distilled water and honey-covered filter paper *ad libitum*. Strips of wax paper with eggs of *C. pomonella*, at a ratio of 10 eggs per adult *A.*

quadridentata, were placed into cages for ca. 24 h. Sheets with parasitized eggs were transferred to covered plastic drinking cups (400 mL) with moist filter paper. Upon hatching, pairs of two neonates were transferred to, and encased within, smaller plastic cups (30 mL) provisioned with two cubes (ca. 1.5 x 1.5 x 1.5 cm) of artificial diet (Bio-Serv, Inc., Frenchtown, NJ). Male and female parasitoids were transferred to mesh cages as they emerged.

3. Y-TUBE OLFACTOMETER BIOASSAY

Anemotactic responses of walking or flying *A. quadridentata* to odour sources were assessed at 25-27°C and 50-70% RH in a vertical Y-shaped Pyrex® glass olfactometer (Figure 3). Bioassays were conducted between 2-12 h of the insects' photophase (8 am-8 pm). Because *A. quadridentata* is positively phototactic, a single light source composed of tubes of fluorescent "daylight" (Osram Sylvania Ltd., ON, Canada; F40D H5b8) and "wide spectrum grow light" (Osram Sylvania Ltd., ON, Canada; F40GRO WS6 H568) at a 1:1 ratio (Shields, 1989) was centred above the vertical olfactometer. The density of light (radiometric irradiance) at the top and base of the olfactometer was respectively 8.0 W/m^2 and 3.8 W/m^2 (Radiometer model # IL1400A, International Light Inc.). Visual cues were standardized by enclosing the olfactometer on three sides with white poster board. Treatment and control odour sources were micropipetted onto Whatman #1 filter paper (4.25 cm) placed near the orifice of each side arm. For each replicate, a new (cleaned and oven-dried) Y-tube, insect and filter paper were used, with test stimuli randomly assigned to side arms. Air drawn through the apparatus at 2.4 - 3.3 L/min with a water aspirator carried volatiles from odour

FIG. 3. Vertical Y-tube olfactometer used for testing response of *A. quadridentata* to various stimuli.


120°
18 cm
filter paper
2.5 cm
20 cm
ring
stand
entrance
hole
5 cm
air flow

sources through the stem of the Y-tube. Thirty seconds after placement of stimuli, a parasitoid was released through the entrance hole of the olfactometer (Figure 3). Parasitoids that reached an odour source within 15 min were classed as responders; all others were classed as nonresponders and were not included in statistical analyses.

Numbers of parasitoids responding to stimuli in olfactometer bioassays (>85%) were analyzed with the χ^2 goodness-of-fit test using Yates correction for continuity ($\alpha = 0.05$) to determine whether observed frequencies deviated significantly from expected frequencies, under the null hypothesis that sampled *A. quadridentata* did not prefer either treatment or control odours (Zar, 1996).

III. SEX PHEROMONE OF *A. QUADRIDENTATA*

1. INTRODUCTION

Searching for mates poses a challenge for solitary parasitoids such as *A. quadridentata*, because hosts are well spaced and mates may not be available at emergence sites (van den Assem, 1986). With a limited life span and only a narrow window of opportunity in which to reproduce, it is unlikely that adult *A. quadridentata* would rely on random search to locate mates. It is more conceivable that they produce chemical signals such as pheromones to recruit mates over relatively long distances.

My objectives were as follows: (1) to obtain behavioural evidence that pheromones mediate mate-seeking behaviour of *A. quadridentata*; (2) to identify the pheromone(s); and (3) to demonstrate behavioural activity of identified compounds in laboratory and field experiments.

2. MATERIALS AND METHODS

2.1. Behavioural evidence for the presence of sex pheromones

To test the hypothesis that *A. quadridentata* release pheromones to attract mates, 12-to-168-h-old unmated females and males were placed separately into horizontal cylindrical Pyrex® glass aeration chambers (2.5 cm in diam. x 18.5 cm in length). A water aspirator drew humidified, charcoal-filtered air at a rate of 1.2 L/min for 5 days through each chamber and corresponding glass column (14 cm x 1.3 cm OD) downwind filled with Porapak Q (50-80 mesh, Waters Associates, Inc., Milford, MA 01757). Volatiles from female and male *A. quadridentata* were eluted separately from respective Porapak Q volatile traps, each with 2

mL of redistilled pentane.

Five experiments (Table 1) were conducted in Y-tube olfactometers (chapter II, section 3) to determine attractiveness of male or female *A. qudridentata*-produced volatiles. Exp. 1 and 2 tested response of males to 2 doses of female-produced volatiles to provide evidence for male-attractive pheromones. Exp. 3 determined whether mating altered the attractiveness of female-produced volatiles to males. To assess whether males produce pheromones, volatiles from male *A. quadridentata* were tested with female (Exp. 4) and male (Exp. 5) *A. quadridentata*. With the exception of Exp. 3, pentane served as the solvent control.

2.2. IDENTIFICATION OF CANDIDATE PHEROMONES

2.2.1. Analyses of *A. quadridentata* volatiles

Aliquots of ca. 1 female h equivalent (FHE) of Porapak Q-captured volatiles were subjected to analysis by coupled gas chromatographic-electroantennographic detection (GC-EAD) (Arn *et al.*, 1975), employing a Hewlett Packard (HP) 5890A gas chromatograph equipped with a fused silica column (30 m x 0.25 or 0.32 mm ID) coated with DB-5, DB-23, or DB-210 (J & W Scientific, Folsom, CA 95630). For GC-EAD recordings, an antenna was gently pulled from an insect's head; the distal segment was removed, and it was then suspended between glass capillary electrodes filled with insect Ringer's solution (6.5 g/L NaCl, 1.4 g/L KCl, 0.12 g/L $CaCl_2$, 0.1 g/L $NaHCO_3$, 0.01 g/L Na_2HPO_4, in 1 L distilled H_2O). EAD-active compounds were identified using the following procedures: (1) full-scan electron-impact (EI) and chemical-ionization (isobutane) (CI) mass spectra (MS) obtained

Table 1. Stimuli tested in Y-tube olfactometer bioassays and numbers of male or female *A. quadridentata* (AQ) bioassayed.

Exp.	Test stimuli		Parasitoids tested	
	Treatment	Control[a]	Number[b]	Sex
1	1 female hour equivalent of female AQ volatiles in pentane	pentane	28 (28)	Male
2	10 female hour equivalents of female AQ volatiles in pentane	pentane	31 (30)	Male
3	10 female hour equivalents of mated female AQ volatiles	10 female hour equivalents of unmated female AQ volatiles	37 (37)	Male
4	1 male hour equivalent of male AQ volatiles in pentane	pentane	20 (19)	Female
5	1 male hour equivalent of male AQ volatiles in pentane	pentane	30 (27)	Male

[a] The volume of pentane in controls was identical to corresponding treatment volumes, ranging from 1-12 μL.

[b] Number of responding insects given in parentheses.

from a Varian Saturn II Ion Trap GC-MS and a HP 5985B, respectively, each fitted with the DB-5 or DB-210 column referred to above; (2) retention index calculations (van den Dool and Kratz, 1963); and (3) microanalytical treatments (acetylation, hydrogenation and borohydride reactions). Differential diagnosis (GC and GC-MS) of volatiles from female and male *A. quadridentata* allowed determination of volatiles specifically produced by female or male parasitoids. Elucidations of chemical structures were confirmed by comparative GC, GC-MS and GC-EAD analyses of insect-produced compounds and authentic standards. Purity and source of synthetic standards are given in Table 2.

2.2.2. Testing of candidate pheromone components

2.2.2.1. Laboratory experiments

In Y-tube olfactometer bioassays (Table 3; chapter II, section 3), I tested the response of *A. quadridentata* to synthetic female-specific candidate pheromone components [3,7,11-trimethyl-6,10-dodecadienal (dihydrofarnesal), *Z*9-hexadecenal (*Z*9-16:Ald) and *Z*9,*Z*12-octadecadienal (*Z*9,*Z*12-18:Ald)]. Exp. 6-12 tested response of males to these compounds singly at various doses. Exp. 13 tested response of female *A. quadridentata* to *Z*9,*Z*12-18:Ald to ascertain whether this compound is a sex or aggregation pheromone. In order to determine potential synergism between compounds, Exp. 14-17 tested candidate pheromone components singly against binary (Exp.14, 15) and ternary (Exp. 16, 17) combinations. Exp. 18 tested *Z*9,*Z*12-18:Ald *versus* a volatile extract from female *A. quadridentata*, containing *Z*9,*Z*12-18:Ald at the equivalent quantity. Unless otherwise specified, hexane was used for solvent controls.

Table 2. Name, purity and source of synthetic test stimuli.

Compound	Chemical purity (%)	Source
3,7,11-Trimethyl-6*E*,10-dodecadienal (Dihydrofarnesal)	95	Synthesized by G.G.S. King, SFU.
*Z*9-Hexadecenal (*Z*9-16:Ald)	95	Sigma Chem. Co., St. Louis, MO, USA.
*Z*9,*Z*12-Octadecadienal (*Z*9,*Z*12-18:Ald)	97	Linoleic acid obtained from Sigma Chem. Co., St. Louis, MO, USA; reduced to alcohol, then oxidized to aldehyde by R. Gries, SFU.

Table 3. Stimuli tested in Y-tube olfactometer experiments and numbers of male or female *A. quadridentata* (AQ) bioassayed.

Exp.	Test stimuli		Parasitoids tested	
	Treatment[a]	Control[b]	Number[c]	Sex
6	10 ng dihydrofarnesal in hexane	hexane	15 (15)	Male
7	100 ng dihydrofarnesal in hexane	hexane	18 (17)	Male
8	10 ng Z9-16:Ald in hexane	hexane	56 (51)	Male
9	100 ng Z9-16:Ald in hexane	hexane	34 (29)	Male
10	1 ng Z9,Z12-18:Ald in hexane	hexane	34 (29)	Male
11	1ng Z9,Z12-18:Ald in hexane	hexane	30 (28)	Male
12	10 ng Z9,Z12-18:Ald in hexane	hexane	49 (45)	Male
13	10 ng Z9,Z12-18:Ald in hexane	hexane	22 (22)	Female
14	10 ng dihydrofarnesal & 0.2 ng Z9,Z12-18:Ald in hexane	10 ng dihydrofarnesal in hexane	36 (35)	Male
15	10 ng Z9,Z12-18:Ald & 1 ng Z9-16:Ald in hexane	10 ng Z9,Z12-18:Ald in hexane	22 (21)	Male
16	0.1 ng dihydrofarnesal, 0.1 ng Z9-16:Ald, 0.1 ng Z9,Z12-18:Ald, all in hexane	0.1 ng Z9,Z12-18:Ald in hexane	41 (39)	Male
17	1 ng dihydrofarnesal, 1 ng Z9-16:Ald, 1 ng Z9,Z12-18:Ald, all in hexane	1 ng Z9,Z12-18:Ald in hexane	36 (36)	Male
18	1 ng Z9,Z12-18:Ald in pentane and hexane	10 female hour equivalents of female AQ volatiles, containing 1 ng Z9,Z12-18:Ald in pentane and hexane	41 (39)	Male

[a] Compound abbreviations as in Table 2.

[b] The volume of hexane or pentane in controls was identical to corresponding treatment volumes, ranging from 1-24 μL.

[c] Number of responding insects given in parentheses.

2.2.2.2. Field-cage experiments

To demonstrate pheromonal attractiveness of *Z*9,*Z*12-18:Ald in the field, 48-to-168-h-old laboratory-reared male *A. quadridentata* were released into field cages (3.60 x 3.60 x 3.60 m), each encompassing a single apple tree (Figure 4a). Cages were located in the Entomology orchard of the Agriculture and Agri-Food Canada Research Station in Summerland, BC. Each tree received a treatment and a control trap, randomly assigned to respective north or south faces and suspended ca. 1.7 m above ground.

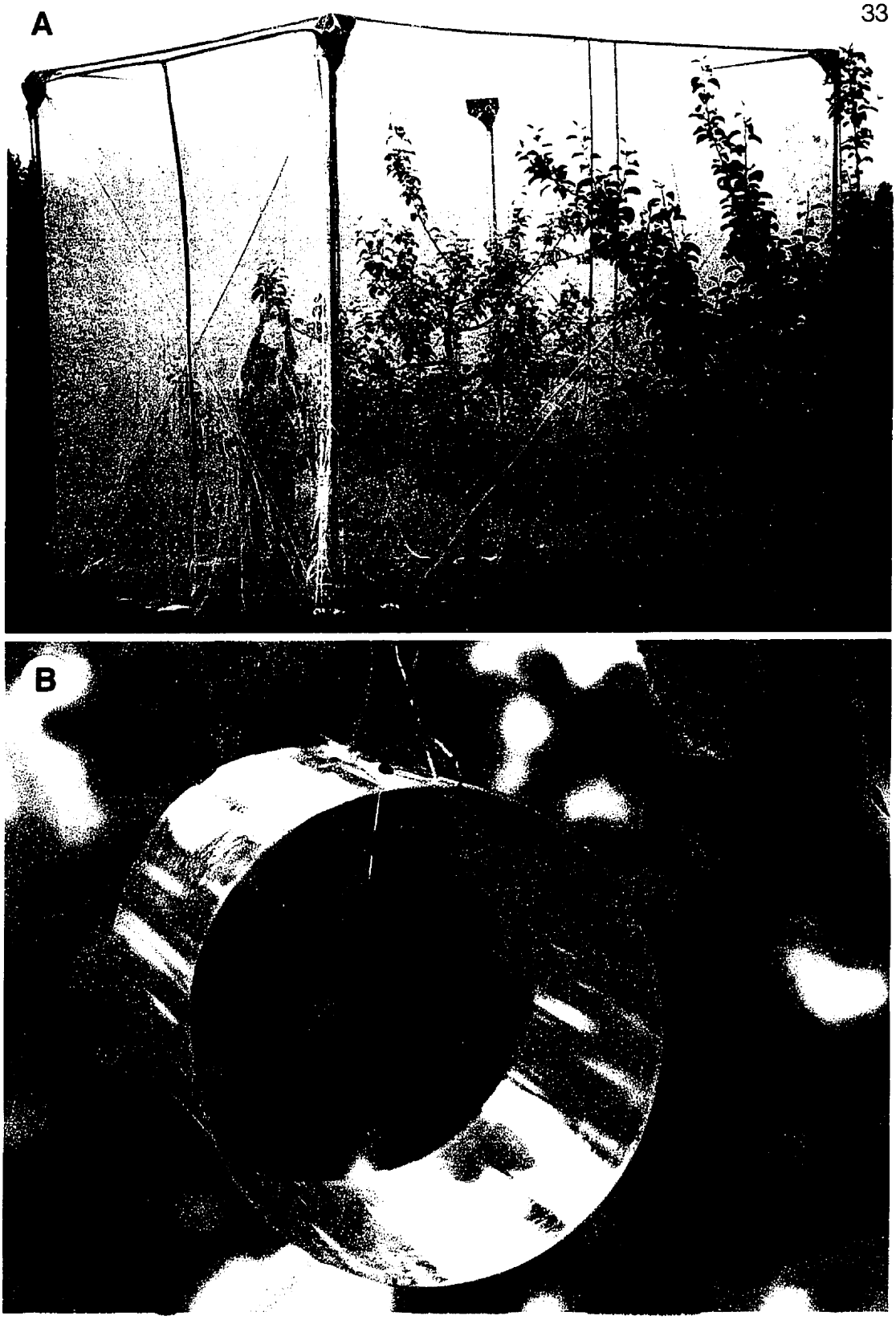
*Z*9,*Z*12-18:Ald (97% purity) in hexane was micropipetted (10 μg: Exp. 19; 100 μg: Exp. 20) into rubber septa (The West Company, Lionville, PA 19341) just before the start of each replicate, while control septa contained an equivalent volume of hexane. Lures were suspended in acetate cylinders (ca. 12 cm ID and 12.5 cm wide) fitted with an equal-sized acetate insert covered with Stickem Special (Phero Tech Inc., BC) (Figure 4b).

Between 9 and 32 laboratory-reared male *A. quadridentata* were released in each cage for each replicate from plastic cups (400 mL) with perforated lids, provisioned with honey-covered filter paper and a dental wick moistened with distilled water. Fifteen min after placement of release devices on the east side of each caged tree ca. 0.5 m from the tree bole and the ground, experimental replicates were initiated by removing the cup lids.

The one-tailed Wilcoxon paired-sample test ($\alpha = 0.05$) was used to test the null hypothesis that numbers of male *A. quadridentata* caught in treatment traps were less than or equal to numbers caught in control traps, as the difference values obtained were roughly symmetrical about the median but could not be assumed to come from a normal distribution (Zar, 1996). Because proportions of male *A. quadridentata* caught in Exp. 20 did fit the

FIG. 4. a) Representative field cage used to test attraction of male *A. quadridentata* to candidate pheromone *Z*9,*Z*12-octadecadienal. (Photograph courtesy of M. Gardiner, Agriculture and Agri-Food Canada, Summerland, BC).

b) Acetate trap (12 cm in diam. x 12.5 cm in width) used to capture male *A. quadridentata* in field-cage and field experiments. A Stickem-covered insert of identical size was added to retain the attracted parasitoids. (Photograph courtesy of M. Gardiner, Agriculture and Agri-Food Canada, Summerland, BC).


A
B

normal approximation, data were analyzed by a one-tailed paired-sample *t* test ($\alpha = 0.05$) (Zar, 1996) to assess whether the difference in the proportion of insects caught in treatment traps was less than or equal to the proportion caught in control traps.

2.2.2.3. Field experiment

A 10-replicate field experiment (Exp. 21) tested attractiveness of *Z*9,*Z*12-18:Ald in a documented wild population of *A. quadridentata* (J.J. Brown, personal communication) at Steptoe Butte State Park in Washington. *Z*9,*Z*12-18:Ald (97% purity) in hexane was micropipetted at four doses (0, 1, 10, or 100 μg) into rubber septa just before their placement into acetate traps (Figure 4b). Position of treatments was completely randomized, with one trap hung in the upper third of each tree.

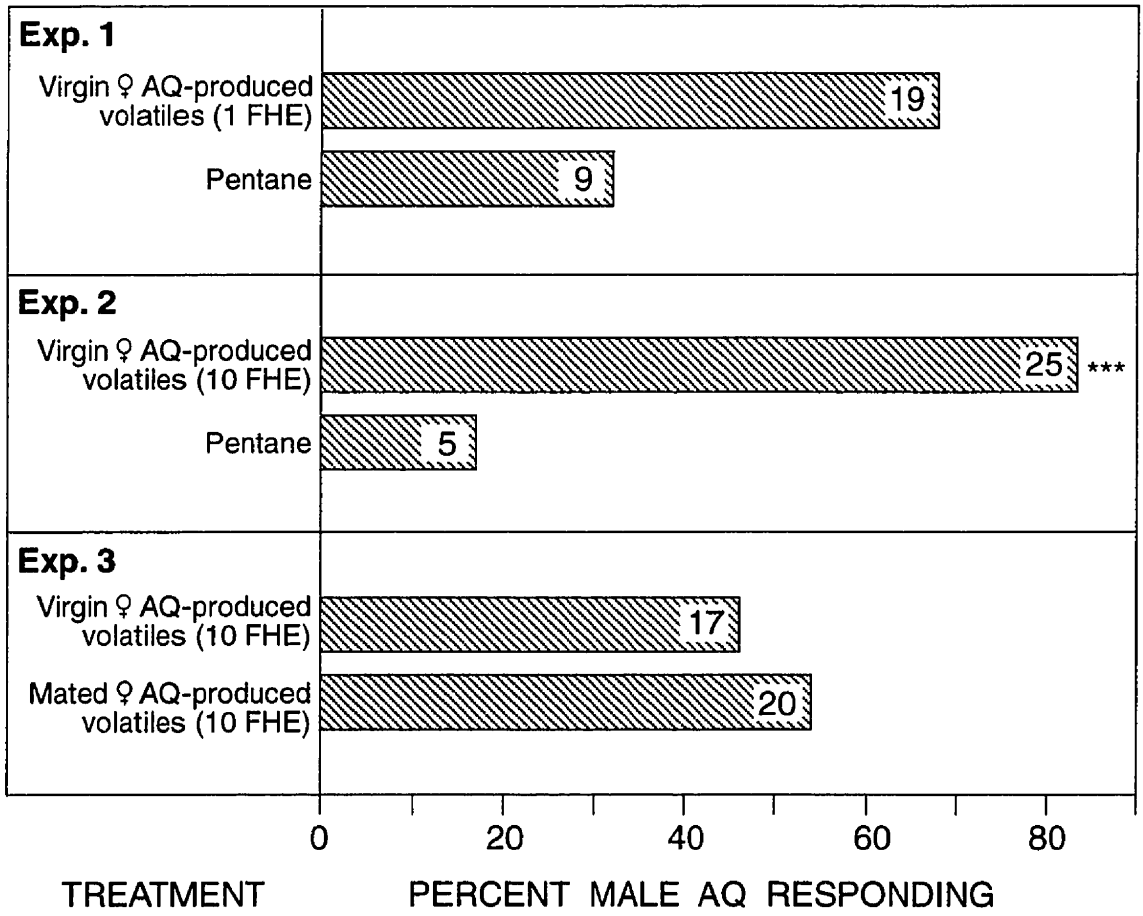
Results of Exp. 21 were analyzed with a single-factor analysis of variance (Model 1 [fixed-effects]) ($\alpha = 0.05$) under the null hypothesis that mean insect captures for all four treatments were equal. This was followed by the Tukey test ($\alpha = 0.05$) to determine which means were significantly different (Zar, 1996).

3. RESULTS

3.1. Behavioural evidence for the presence of sex pheromones

A dose of 10, but not 1, FHE elicited significant behavioural response from male *A. quadridentata* (Figure 5, Exp. 1 and 2). Volatiles (10 FHE) from virgin and mated females were equally attractive to males (Figure 5, Exp. 3). Wing fanning was induced in all three bioassays, and males were often arrested by the female-produced volatiles.

FIG. 5. Response of male *A. quadridentata* (AQ) to volatiles produced by virgin or mated female AQ (Table 1: Exp. 1-3) or solvent controls in a Y-tube olfactometer. Volatiles were tested at 1 or 10 FHE [female h equivalent] (Exp. 1-2). Numbers of males responding to each stimulus given within bars for each experiment; bars with asterisks indicate a significant preference for a particular treatment; χ^2 test with Yates correction for continuity, treatment *versus* control; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.


Exp. 1
Virgin ♀ AQ-produced volatiles (1 FHE)
19
Pentane
9
Exp. 2
Virgin ♀ AQ-produced volatiles (10 FHE)
25
***
Pentane
5
Exp. 3
Virgin ♀ AQ-produced volatiles (10 FHE)
17
Mated ♀ AQ-produced volatiles (10 FHE)
20
0
20
40
60
80
TREATMENT
PERCENT MALE AQ RESPONDING

Male *A. quadridentata*-produced volatiles at 1 male h equivalent (MHE) attracted male, but not female, *A. quadridentata* (Figure 6, Exp. 5, 4).

3.2. Identification of candidate pheromones

3.2.1. Analyses of *A. quadridentata* volatiles

Coupled GC-EAD analyses of female *A. quadridentata*-produced volatiles, using an antenna of a male or female *A. quadridentata* as an electroantennographic detector, revealed three compounds that consistently elicited a response from male antennae (Figure 7). Of these, only the first compound elicited antennal response from female *A. quadridentata*.

Retention indices (RI) of EAD-active compound 1 on three fused silica columns (DB-23: RI=2078; DB-5: RI=1620; DB-210: RI=1929) were indicative of an aldehyde functionality. Both reduction with $NaBH_4$ and unsuccessful acetylation with pyridine and acetic anhydride of the HPLC-isolated compound 1 supported an aldehyde functionality. Mass spectra of reduced compound 1 and of 3,7,11-trimethyl-2*E*,6*E*,10-dodecatrien-1-ol or *EE*-farnesol were similar, except fragmentation ions of compound 1 indicated two – instead of three – unsaturations of the molecule. Selective hydrogenation (House, 1972) of 3,7,11-trimethyl-2*E*,6*E*,10-dodecatrienal (*EE*-farnesal) resulted in 3,7,11-trimethyl-6*E*,10-dodecadienal (dihydrofarnesal), which had identical mass spectral (Figure 8) and retention characteristics to EAD-active compound 1, completing the identification procedure.

Quantities of EAD-active compound 2 were insufficient to obtain a mass spectrum. Retention indices of this compound on DB-5, DB-23 and DB-210 columns (1805, 2280 and 2153, respectively) were indicative of a hexadecenal. Of all possible monounsaturated

FIG. 6. Response of female (Exp. 4) or male (Exp. 5) *A. quadridentata* (AQ) to male AQ-produced volatiles; MHE = male h equivalent (Table 1). Numbers of individuals responding to each stimulus given within bars. For each experiment, bars with asterisks indicate a significant preference for a particular treatment; χ^2 test with Yates correction for continuity, treatment *versus* control; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

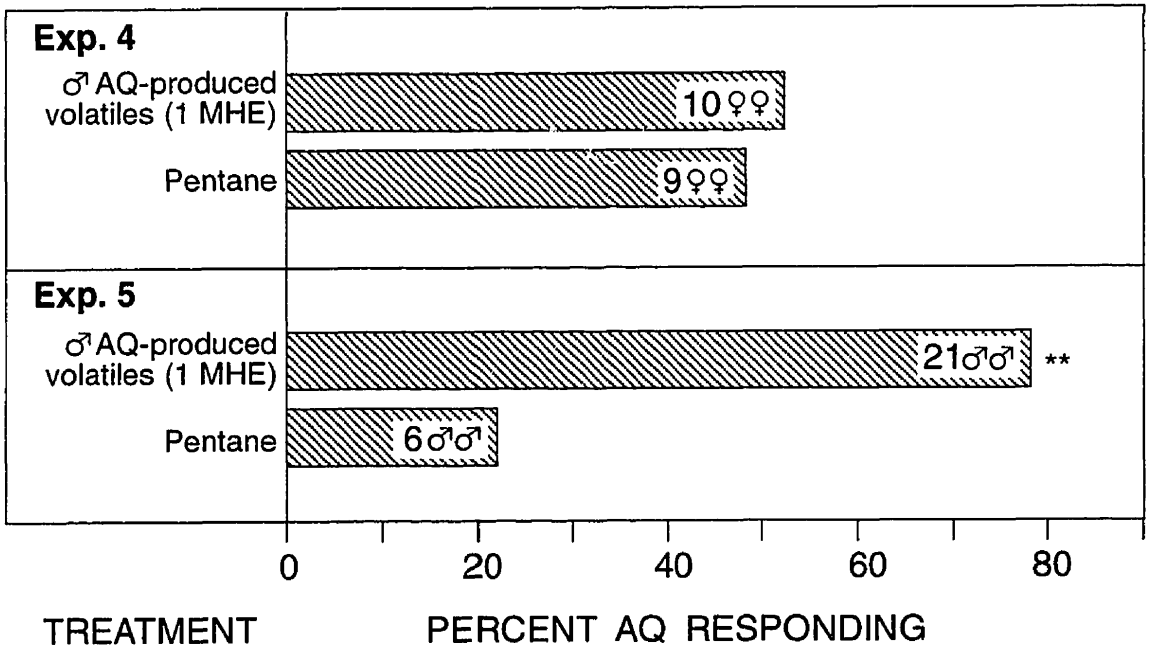

Exp. 4
♂ AQ-produced volatiles (1 MHE)
10♀♀
Pentane
9♀♀
Exp. 5
♂ AQ-produced volatiles (1 MHE)
21♂♂
**
Pentane
6♂♂
0
20
40
60
80
TREATMENT
PERCENT AQ RESPONDING

FIG. 7. Flame ionization detector (FID) and electroantennographic detector (EAD: *A. quadridentata* (AQ) antenna) responses to aliquotes of 1 female h equivalent (FHE) of volatiles from female AQ. Chromatography: Hewlett Packard 5890A equipped with a DB-5 coated column (30 m x 0.25 mm ID); linear flow velocity of carrier gas: 35cm/sec; injector and FID detector temperature: 240°C; temperature program: 1 min at 50°C, 10°C/min to 240°C.

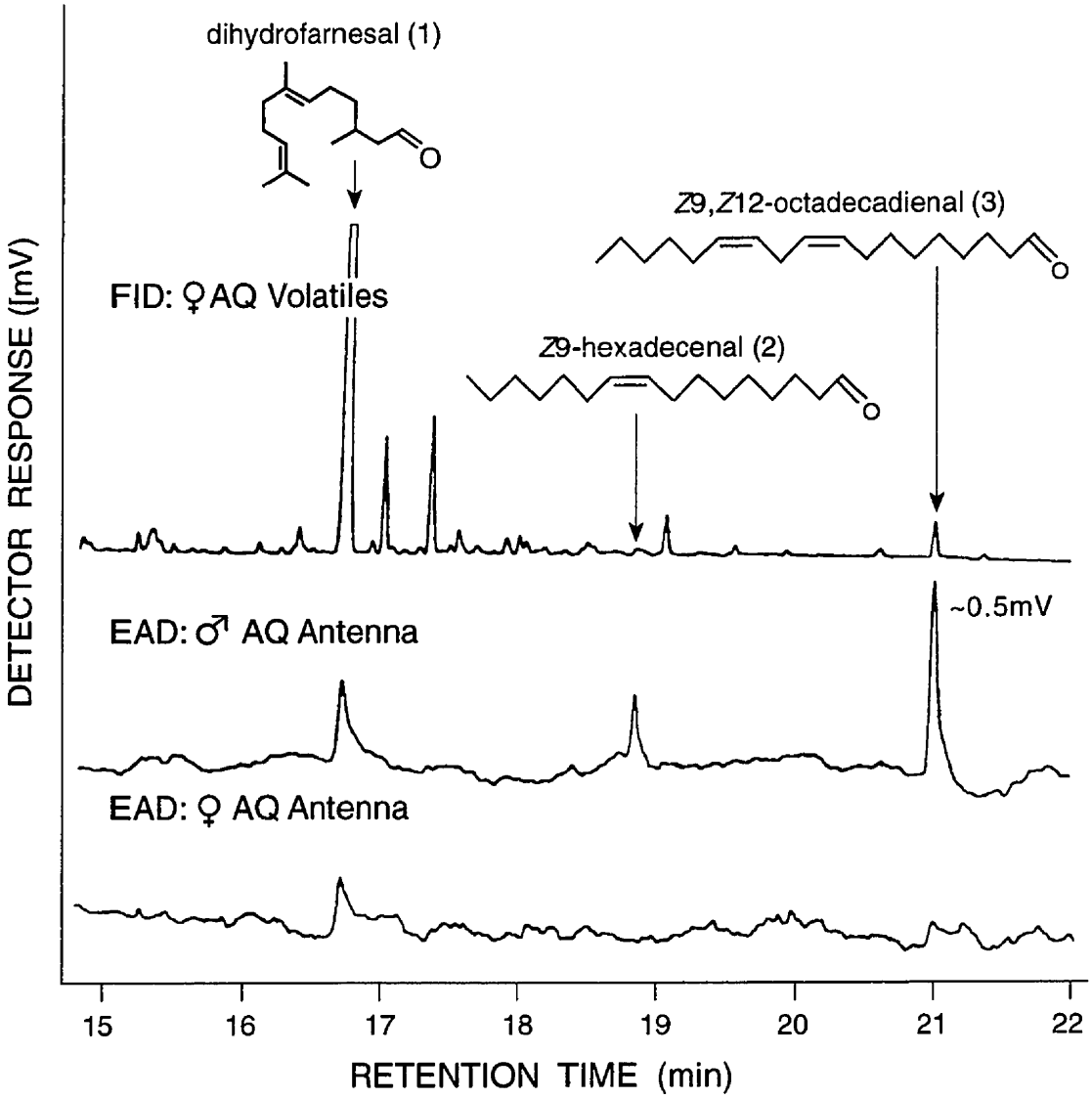
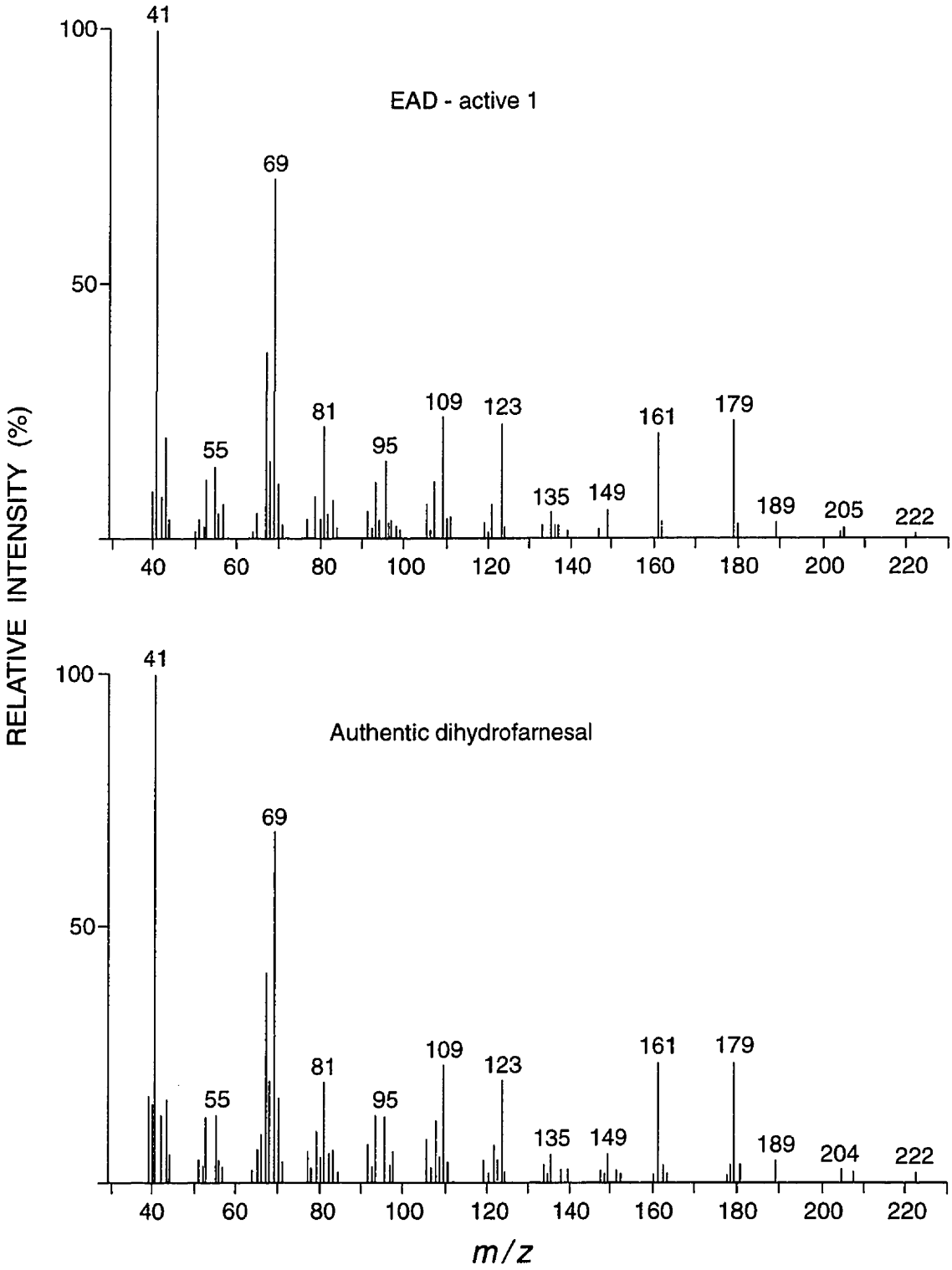

dihydrofarnesal (1)
Z9,Z12-octadecadienal (3)
Z9-hexadecenal (2)
FID: ♀ AQ Volatiles
EAD: ♂ AQ Antenna
~0.5mV
EAD: ♀ AQ Antenna
DETECTOR RESPONSE ([mV)
15
16
17
18
19
20
21
22
RETENTION TIME (min)

FIG. 8. Mass spectra of the EAD-active compound 1 (Figure 7) and of authentic dihydrofarnesal. Chromatography: Hewlett Packard 5985 GC-MS equipped with a DB-5 coated column (30 m x 0.25 mm ID); linear flow velocity of carrier gas: 35cm/sec; injector and FID detector temperature: 240°C; temperature program: 1 min at 50°C, 10°C/min to 280°C.


EAD - active 1
Authentic dihydrofarnesal
RELATIVE INTENSITY (%)
m/z
100
50
41
69
55
81
95
109
123
135
149
161
179
189
205
204
222
40
60
80
100
120
140
160
180
200
220

hexadecenals with *E*- or *Z*- double bond configurations, only *Z*9-hexadecenal co-chromatographed with EAD-active compound 2 on each of the three columns referred to above.

The mass spectrum of EAD-active compound 3 (Figure 9), with strong molecular ion *m/z* 264 and evidence for two double bonds (*m/z* 67, 81, and 95), suggested an octadecadienal. With *Z*9,*Z*12-octadecadienoic (linoleic) acid as a conceivable precursor, EAD-active compound 3 was hypothesized and, through comparative GC-MS with an authentic standard, confirmed to be *Z*9,*Z*12-octadecadienal (Figure 9).

3.2.2. Testing of candidate pheromone components

At a 10 or 100 ng dose, dihydrofarnesal (Figure 10, Exp. 6, 7) or *Z*9-16:Ald (Figure 10, Exp. 8, 9) singly was no more attractive to male *A. quadridentata* than a hexane control. Attractiveness of *Z*9,*Z*12-18:Ald increased dose dependently (Figure 10, Exp. 10-12). In contrast, female *A. quadridentata* were not attracted to *Z*9,*Z*12-18:Ald (Figure 10, Exp. 13).

There was no synergistic effect between *Z*9,*Z*12-18:Ald and dihydrofarnesal (Exp. 14) nor *Z*9-16:Ald (Exp. 15) (Figure 11). A ternary blend of synthetic *Z*9,*Z*12-18:Ald, *Z*9-16:Ald and dihydrofarnesal at ratios [doses (ng)] of 1:1:1 or 0.1:0.1:0.1 was as attractive as *Z*9,*Z*12-18:Ald singly at the equivalent quantity (Figure 11, Exp. 16, 17). Synthetic *Z*9,*Z*12-18:Ald and female *A. quadridentata*-produced volatiles, containing the equivalent amount of *Z*9,*Z*12-18:Ald, were equally attractive to male *A. quadridentata* (Figure 11, Exp. 18).

In field-cage bioassays, *Z*9,*Z*12-18:Ald at 10 μg (Figure 12, Exp. 19) or 100 μg (Exp.

FIG. 9. Mass spectra of EAD-active compound **3** (Figure 8) and of authentic *Z*9,*Z*12-octadecadienal. Chromatography: Hewlett Packard 5985 equipped with a DB-5 coated column (30 m x 0.25 mm ID); linear flow velocity of carrier gas: 35cm/sec; injector and FID detector temperature: 240°C; temperature program: 1 min at 50°C, 10°C/min to 280°C.

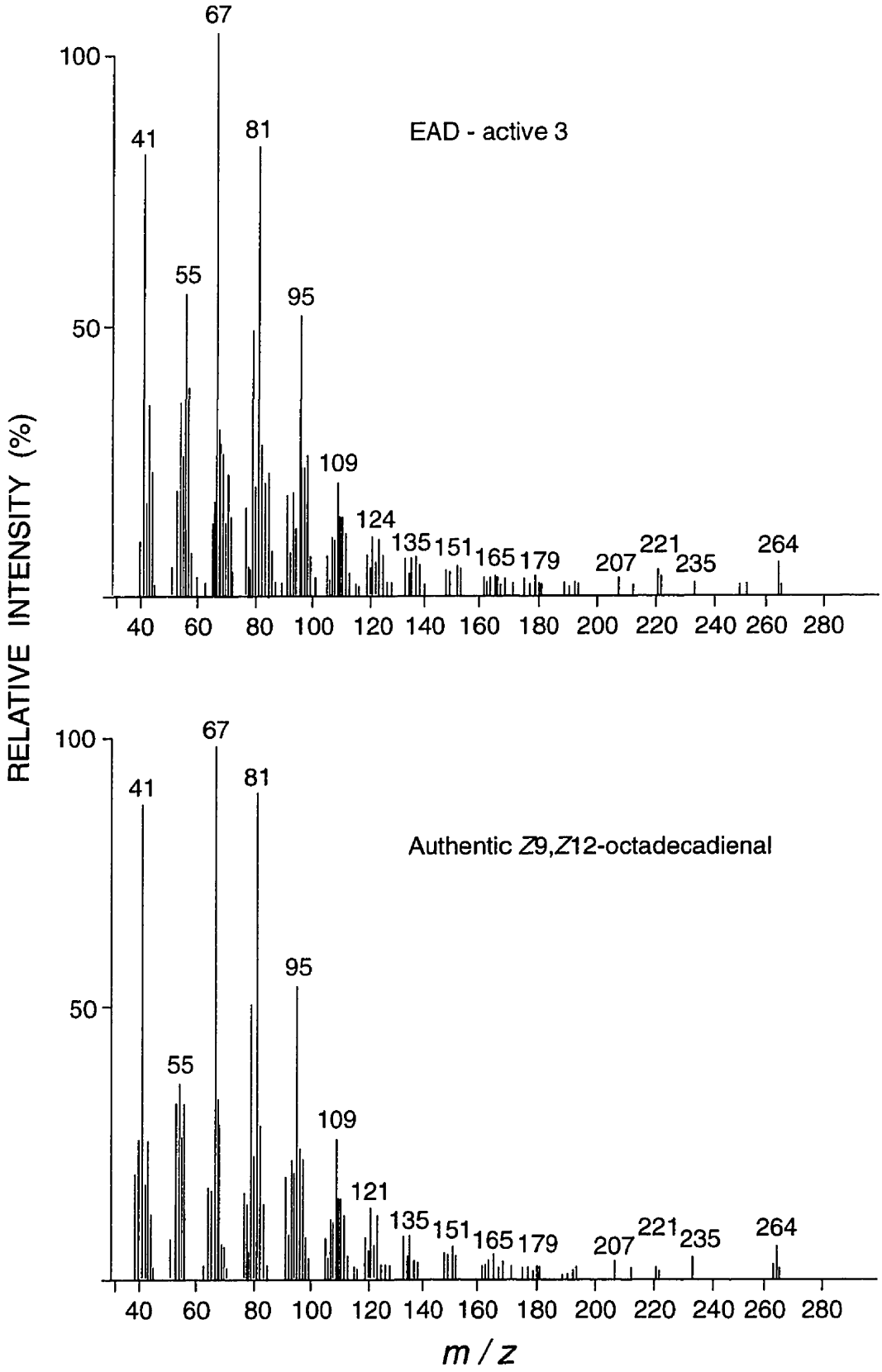

EAD - active 3
Authentic Z9,Z12-octadecadienal
RELATIVE INTENSITY (%)
m / z
100
50
40 60 80 100 120 140 160 180 200 220 240 260 280
41 55 67 81 95 109 124 135 151 165 179 207 221 235 264
41 55 67 81 95 109 121 135 151 165 179 207 221 235 264

FIG. 10. Response of male (Exp. 6-12) or female (Exp. 13) *A. quadridentata* (AQ) to synthetic candidate pheromone components (Table 3). Numbers of insects responding to each stimulus given within bars. Asterisks indicate a significant preference for a particular treatment; χ^2 test with Yates correction for continuity, treatment *versus* control; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

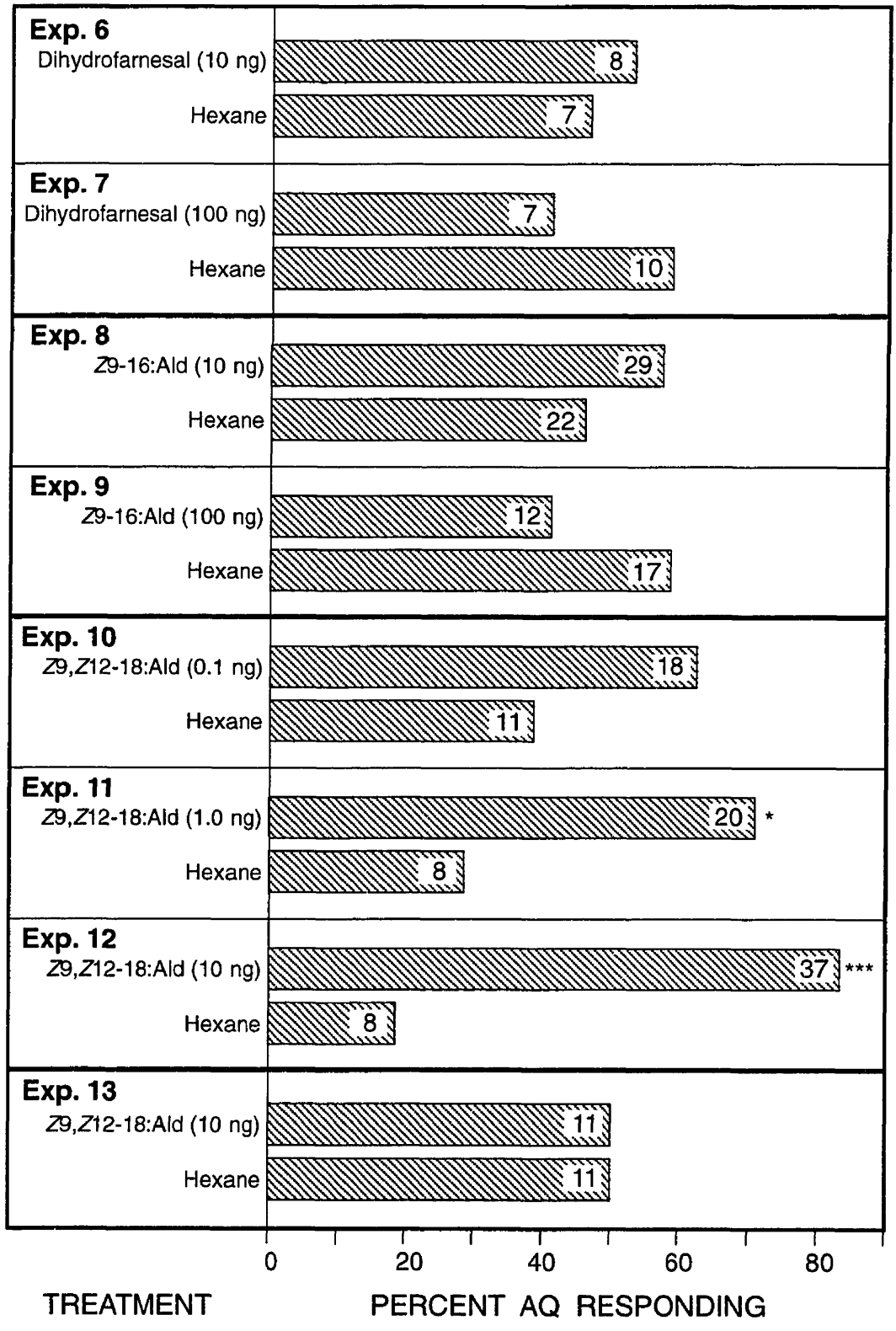

Exp. 6
Dihydrofarnesal (10 ng)
8
Hexane
7
Exp. 7
Dihydrofarnesal (100 ng)
7
Hexane
10
Exp. 8
Z9-16:Ald (10 ng)
29
Hexane
22
Exp. 9
Z9-16:Ald (100 ng)
12
Hexane
17
Exp. 10
Z9,Z12-18:Ald (0.1 ng)
18
Hexane
11
Exp. 11
Z9,Z12-18:Ald (1.0 ng)
20
*
Hexane
8
Exp. 12
Z9,Z12-18:Ald (10 ng)
37
***
Hexane
8
Exp. 13
Z9,Z12-18:Ald (10 ng)
11
Hexane
11
0
20
40
60
80
TREATMENT
PERCENT AQ RESPONDING

FIG. 11. Response of male *A. quadridentata* (AQ) to candidate pheromone components singly and in combinations (Table 3). Numbers of insects responding to each stimulus given within bars. Asterisks indicate a significant preference for a particular treatment; χ^2 test with Yates correction for continuity, treatment *versus* control; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

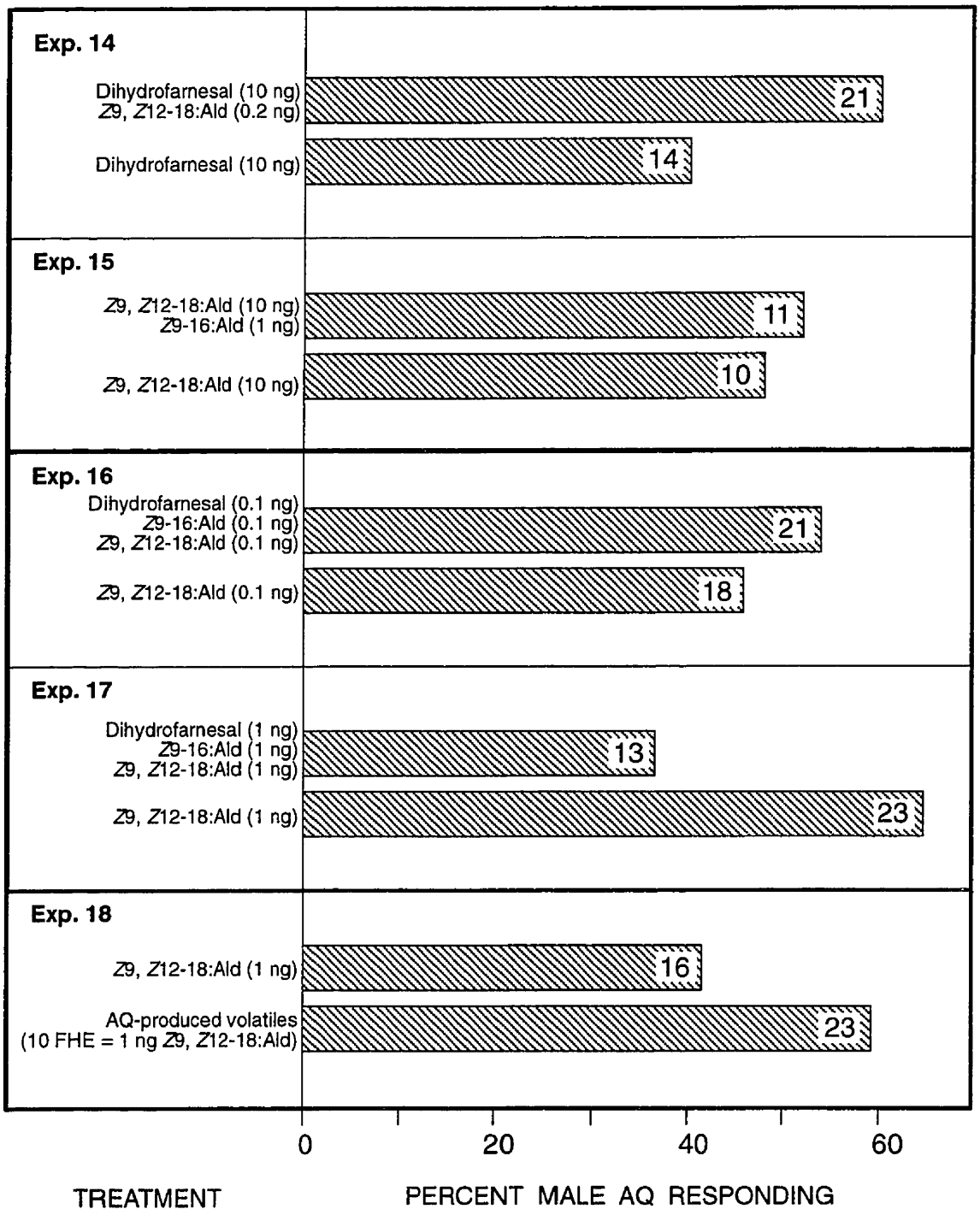
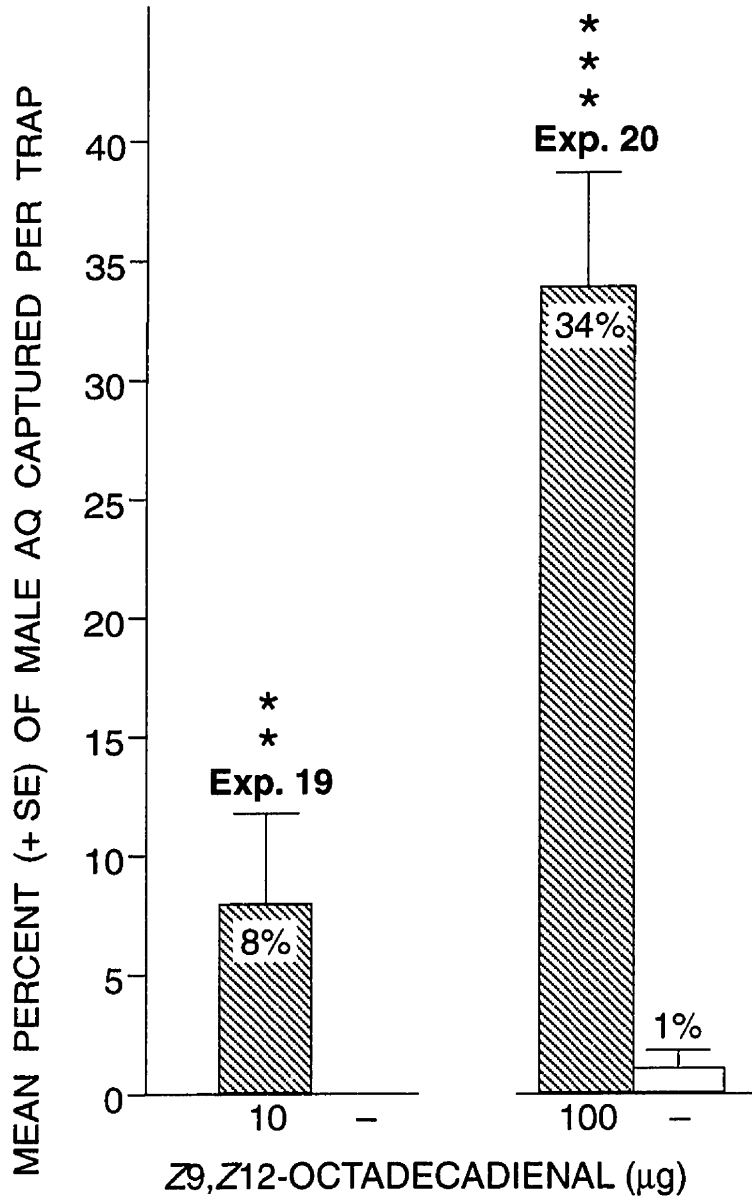

Exp. 14
Dihydrofarnesal (10 ng)
Z9, Z12-18:Ald (0.2 ng)
21
Dihydrofarnesal (10 ng)
14
Exp. 15
Z9, Z12-18:Ald (10 ng)
Z9-16:Ald (1 ng)
11
Z9, Z12-18:Ald (10 ng)
10
Exp. 16
Dihydrofarnesal (0.1 ng)
Z9-16:Ald (0.1 ng)
Z9, Z12-18:Ald (0.1 ng)
21
Z9, Z12-18:Ald (0.1 ng)
18
Exp. 17
Dihydrofarnesal (1 ng)
Z9-16:Ald (1 ng)
Z9, Z12-18:Ald (1 ng)
13
Z9, Z12-18:Ald (1 ng)
23
Exp. 18
Z9, Z12-18:Ald (1 ng)
16
AQ-produced volatiles
(10 FHE = 1 ng Z9, Z12-18:Ald)
23
0
20
40
60
TREATMENT
PERCENT MALE AQ RESPONDING

FIG. 12. Mean percent capture of released male *A. quadridentata* (AQ) in acetate cylinder traps (Figure 4b) baited with 10 μg (Exp. 19: n=8) and 100 μg (Exp. 20: n=13) of *Z*9,*Z*12-octadecadienal in field-cage bioassays (Figure 4a), Summerland, British Columbia. For each experiment, bars with asterisks indicate a significant preference for a particular treatment; Exp. 1: one-tailed Wilcoxon paired-sample test (α=0.05); Exp. 2: one-tailed paired-sample *t* test on proportions (α=0.05), treatment *versus* control; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.


MEAN PERCENT (+ SE) OF MALE AQ CAPTURED PER TRAP
0
5
10
15
20
25
30
35
40
*
*
Exp. 19
8%
*
*
*
Exp. 20
34%
1%
10
–
100
–
Z9,Z12-OCTADECADIENAL (µg)

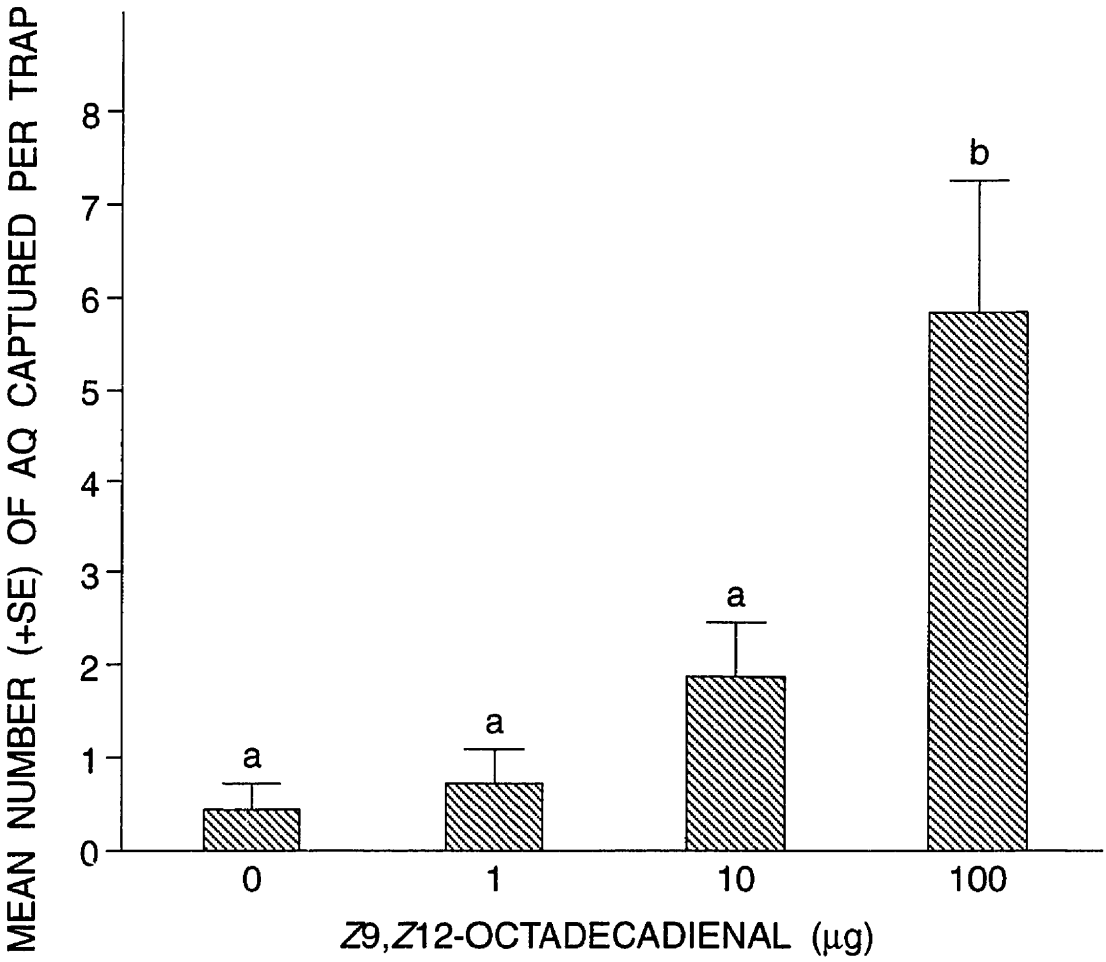
20) captured a significantly higher number of male *A. quadridentata* than did solvent controls. Captures of male *A. quadridentata* in the field experiment increased with increasing doses of *Z*9,*Z*12-18:Ald (Figure 13, Exp. 21).

4. DISCUSSION

Evidence that *Z*9,*Z*12-18:Ald is a pheromone component of female *A. quadridentata* includes the following: (1) *Z*9,*Z*12-18:Ald was identified in the effluvia of female but not male *A. quadridentata*; (2) synthetic *Z*9,*Z*12-18:Ald elicited strong antennal responses from male *A. quadridentata* in GC-EAD recordings (Figure 7); (3) it induced amplified antennal movement, wing vibrations and accelerated walking in male *A. quadridentata*; and (4) it dose-dependently attracted male *A. quadridentata* in Y-tube olfactometer bioassays (Figure 10, Exp. 10-12), field-cage bioassays (Figure 12, Exp. 19-20) and in a field experiment (Figure 13, Exp. 21).

Although eliciting antennal activity, neither *Z*9-16:Ald nor dihydrofarnesal singly were attractive to male *A. quadridentata* (Figure 10, Exp. 6-9), nor did they enhance the attractiveness of *Z*9,*Z*12-18:Ald (Figure 11, Exp. 14-17). Electrophysiological activity of *Z*9-16:Ald may be interpreted as olfactory recognition of a pheromone component from a congener. In *A. reticulatus* Watanabe, *Z*9-16:Ald serves as a contact pheromone that may provide information about potential mates at close range (Kainoh *et al.*, 1991). Whether or not *Z*9-16:Ald plays a similar role in *A. quadridentata* remains to be investigated.

FIG. 13. Mean numbers (+SE) of *A. quadridentata* (AQ) captured in acetate cylinder traps (Figure 4b) baited with *Z*9,*Z*12-octadecadienal at increasing doses (Exp. 21). Steptoe Butte State Park, Washington, USA, n=10. Bars with the same letter superscript are not significantly different, Tukey's test, $\alpha = 0.05$.


MEAN NUMBER (+SE) OF AQ CAPTURED PER TRAP
0
1
2
3
4
5
6
7
8
a
a
a
b
0
1
10
100
Z9,Z12-OCTADECADIENAL (µg)

Lack of behavioural activity from dihydrofarnesal may be attributed to one or more of several factors: (1) dihydrofarnesal is also produced by male parasitoids and may indeed not be a pheromone component of female *A. quadridentata*; (2) female *A. quadridentata* may stereoselectively biosynthesize only one enantiomer (or a specific ratio of enantiomers) of dihydrofarnesal, and response by male *A. quadridentata* may have been inhibited by the unnatural enantiomer (or incorrect ratio) in enantiomeric synthetic dihydrofarnesal that was bioassayed (Figure 10, Exp.6-7); (3) because dihydrofarnesal was the only compound in GC-EAD recordings that also elicited responses from female antennae (Figure 7), this compound may mediate chemical communication between female parasitoids rather than attracting mates. Similar attractiveness of synthetic *Z*9,*Z*12-18:Ald and of volatiles from female *A. quadridentata*, containing the equivalent amount of *Z*9,*Z*12-18:Ald (Figure 11, Exp. 18), suggests that female *A. quadridentata* may indeed employ *Z*9,*Z*12-18:Ald as a single-component sex pheromone to attract mates.

Comparable responses of male *A. quadridentata* to volatiles from virgin and mated females (Figure 5, Exp. 3) suggests that mating does not affect pheromone production in females, which is consistent with observations of repetitive matings by female and male *A. quadridentata*. When male parasitoids perceive the female's pheromone, they increase antennal movement, vibrate their wings and follow the female in an accelerated manner. Pulling air over their body through wing vibrations may help males orient toward the pheromonal source (Vinson, 1972). Sound associated with wing vibrations likely resonates from the chitinous wall of the thorax (van den Assem and Putters, 1980) and seems to be

common during courtship in parasitic wasps (Miller and Tsao, 1974; van den Assem and Putters, 1980; van den Assem, 1986; Sivinski and Webb, 1989; Field and Keller, 1993). Wing vibration and sound production by male parasitoids appear to occur primarily in response to female-produced sex pheromones and in some species are necessary for the female to permit continuation of courtship (Obara and Kitano, 1974; van den Assem and Putters, 1980).

The role of the male-produced and male-attractive pheromone is not clear: (1) males responding to male-produced volatiles (Figure 6, Exp. 5) and detecting other males in search of females may enhance their probability of encountering females; (2) males attracted to low levels of male-produced pheromone may form leks, as found in other parasitoids (van den Assem, 1986), collectively produce more pheromone and thus become more attractive to foraging females; or (3) they may release pheromone which, together with wing vibration, induces mating receptivity in females rather than directional response (van den Assem and Putters, 1980; van den Assem, 1986) (Figure 6, Exp. 4).

IV. KAIROMONAL ATTRACTION IN *A. QUADRIDENTATA*

1. INTRODUCTION

Parasitoids use diverse chemical cues to locate their hosts. These cues may originate from the habitat of the host or from the host itself (Shaw and Huddleston, 1991; Godfray, 1994). Parasitoids, such as *A. quadridentata*, attacking the eggs of moths might be attracted to sex pheromone components of the moth (Lewis *et al.*, 1982), to volatiles from scales deposited by female moths during oviposition (Lewis *et al.*, 1972; Chiri and Legner, 1986; Kainoh *et al.*, 1990) and/or to volatiles from the host egg itself (Vinson, 1975).

My objectives were as follows: (1) to determine whether host location by *A. quadridentata* is mediated by kairomones; (2) to determine the source(s) of kairomones; (3) to identify and bioassay kairomones for their attractiveness to *A. quadridentata*.

2. MATERIALS AND METHODS

2.1. Behavioural evidence for kairomone-mediated host location

2.1.1. Acquisition of volatile stimuli from potential kairomonal sources

Volatile test stimuli consisted of the following: (1) synthetic *C. pomonella* pheromone (codlemone); (2) pheromone gland extract of female *C. pomonella*; (3) volatiles from *C. pomonella* eggs; and (4) volatiles from body and wing scales of female *C. pomonella*.

Synthetic codlemone (98% chemical purity) was obtained from Shin-etsu (Fine Chemical Dept., Chemical Co. Ltd., 2-6-1 Ohtemochi Chiyoda-Ku, Tokyo). Pheromone

gland extracts of female *C. pomonella* were obtained by removing abdominal tips with pheromone glands from 108 48-to-72-h-old, virgin female *C. pomonella* and extracting them for ca. 10 min in HPLC-grade hexane. The supernatant was withdrawn and stored at ca. -10°C before use. One μL aliquots of this extract contained 1 female equivalent of pheromone gland extract (1 FEPGE).

Egg volatiles were collected from a wax-paper sheet (1950 cm^2) on which ca. 5000 eggs had been oviposited by female *C. pomonella.* The egg sheet was placed in a cylindrical Pyrex® glass chamber (ca. 155 mm ID x 280 mm in height) and was aerated for 72 h. A water aspirator drew charcoal-filtered air at 2 L/min through the chamber and a connected glass column (14 cm x 1.3 mm OD) filled with Porapak Q. Volatiles were eluted from the Porapak Q with 3 mL of redistilled pentane. One μL of extract was equivalent to ca. 120 egg hour equivalents (120 EHE; i.e., volatiles released from 120 eggs of *C. pomonella* during 1 h).

To collect volatiles from body and wing scales of female *C. pomonella*, 500 chilled female moths were placed into the upper half of two Petri dishes (8.5 cm in diam.) separated by a fine wire mesh (1 mm^2). The insects were then vigorously shaken, and displaced scales collected in the bottom Petri dish were transferred to a cylindrical Pyrex® glass aeration chamber (2.5 cm in diam. x 18.5 cm in length). A water aspirator drew charcoal-filtered air through the chamber and a volatile trap containing Porapak Q (see above) at a rate of 1.2 L/min for 142 h. Captured volatiles were eluted from the Porapak Q with 3 mL of redistilled pentane. A 1 μL aliquot contained the equivalent of volatiles emitted for 1 h from the scales

displaced from 24 female *C. pomonella* [24 female scale h equivalents (SHE)].

2.1.2. Testing of potential kairomonal sources

Potential kairomone sources were tested for attractiveness to *A. quadridentata* in Y-tube olfactometer experiments (chapter II, section 3). Pheromone gland extract (1 FEPGE) was tested with females (Table 4, Exp. 22) and males (Table 5, Exp. 31). Synthetic codlemone at 10 ng (Exp. 23, 32), 1 ng (Exp. 24, 33) and 0.1 ng (Exp. 25, 34) was tested with females (Table 4, Exp. 23-25) and with males (Table 5, Exp. 32-34). Volatiles from eggs of *C. pomonella* at 120 EHE were tested with unprimed (Table 4, Exp. 26) and primed (Table 4, Exp. 27) female *A. quadridentata*. "Priming" lowers the behavioural response threshold to stimuli (Kaiser and Carde, 1992; Harris and Foster, 1995) and ensures that females not interested in oviposition are excluded from experiments. Egg volatiles at 120 EHE (Table 5, Exp. 35) and 1200 EHE (Table 5, Exp. 36) were also tested with male *A. quadridentata*. Volatiles from body and wing scales of female *C. pomonella* were tested at 24 SHE with female *A. quadridentata* (Table 4, Exp. 28) and at 240 SHE with male *A. quadridentata* (Table 5, Exp. 37). Controls consisted of either hexane or pentane.

Table 4. Stimuli tested in Y-tube olfactometer experiments and numbers of female *A. quadridentata* bioassayed.

Exp.	Test stimuli		Parasitoids tested	
	Treatment[a]	Control[b]	Status[c]	Number[d]
22	1 FEPGE in hexane	hexane	Mated Unprimed	28 (27)
23	10 ng codlemone in hexane	hexane	Virgin Unprimed	13 (12)
24	1 ng codlemone in hexane	hexane	Virgin Unprimed	11 (10)
25	0.1 ng codlemone in hexane	hexane	Mated Unprimed	20 (20)
26	120 EHE in pentane	pentane	Mated Unprimed	20 (18)
27	120 EHE in pentane	pentane	Mated Primed	20 (19)
28	24 SHE in pentane	pentane	Mated Primed	16 (15)
29	Synthetic scale volatiles (24 SHE) in hexane	hexane	Virgin Primed	26 (23)
30	Synthetic scale volatiles (240 SHE) in hexane	hexane	Virgin Primed	20 (20)

[a] Abbreviations and explanations:
1 FEPGE = Female *C. pomonella* Equivalent of Pheromone Gland Extract (= volatiles extracted from the pheromone gland of 1 female *C. pomonella*).
1 EHE = Egg Hour Equivalent (= volatiles released from 1 egg of *C. pomonella* during 1 hour).
1 SHE = Female *C. pomonella* Scale Hour Equivalent (= volatiles released from body and wing scales [displaced from 1 female *C. pomonella*] during 1 hour).
Codlemone = *E*8,*E*10-dodecadienol – sex pheromone of female *C. pomonella*.

[b] The volume of solvent in the controls was equivalent to corresponding treatment volumes, ranging between 0.5-10 μL.

[c] Pre-test conditioning of parasitoids:
virgin = unmated females.
mated = females that have been exposed to males and have copulated.
unprimed = no previous contact with *C. pomonella* eggs, or egg-laying sheets, prior to the start of the experiment.
primed = exposed to *C. pomonella* egg-laying sheets and oviposited once prior to the start of the experiment.

[d] Number of responding insects given in parentheses.

Table 5. Stimuli tested in Y-tube olfactometer experiments and numbers of male *A. quadridentata* bioassayed.

Exp.	Test stimuli		Parasitoids tested[c]
	Treatment[a]	Control[b]	
31	1 FEPGE in hexane	hexane	12 (12)
32	10 ng codlemone in hexane	hexane	30 (30)
33	1 ng codlemone in hexane	hexane	33 (32)
34	0.1 ng codlemone in hexane	hexane	20 (20)
35	120 EHE in pentane	pentane	20 (18)
36	1200 EHE in pentane	pentane	18 (17)
37	240 SHE in pentane	pentane	18 (17)

[a] Abbreviations and explanations:
1 FEPGE = Female *C. pomonella* Equivalent of Pheromone Gland Extract (= volatiles extracted from the pheromone gland of 1 female *C. pomonella*).
1 EHE = Egg Hour Equivalent (= volatiles released from 1 egg of *C. pomonella* during 1 hour).
1 SHE = Female *C. pomonella* Scale Hour Equivalent (= volatiles released from body and wing scales [displaced from 1 female *C. pomonella*] during 1 hour).
Codlemone = *E*8,*E*10-dodecadienol – sex pheromone of female *C. pomonella*.

[b] The volume of solvent in the controls was equivalent to corresponding treatment volumes, ranging from 1-10 μL.

[c] Number of responding insects given in parentheses.

2.2. Identification of candidate kairomones

Aliquots of 1 FEPGE, 120 EHE and 24 SHE of volatile extracts were subjected to GC-EAD and GC-MS analyses conducted as previously described (chapter III, section 2.2.1.). Structural assignments of EAD-active scale volatiles were confirmed by comparative GC, GC-MS and GC-EAD analyses of insect-produced and authentic chemicals.

Synthetic equivalents of the 11 EAD-active compounds identified from scale volatile extract of female *C. pomonella* were combined in quantities and ratios as found in the scale volatile extract. This synthetic blend was tested at 24 SHE (Table 4, Exp. 29) and 240 SHE (Table 4, Exp. 30) with female *A. quadridentata* in a Y-tube olfactometer bioassay (chapter II, section 3) *versus* hexane controls.

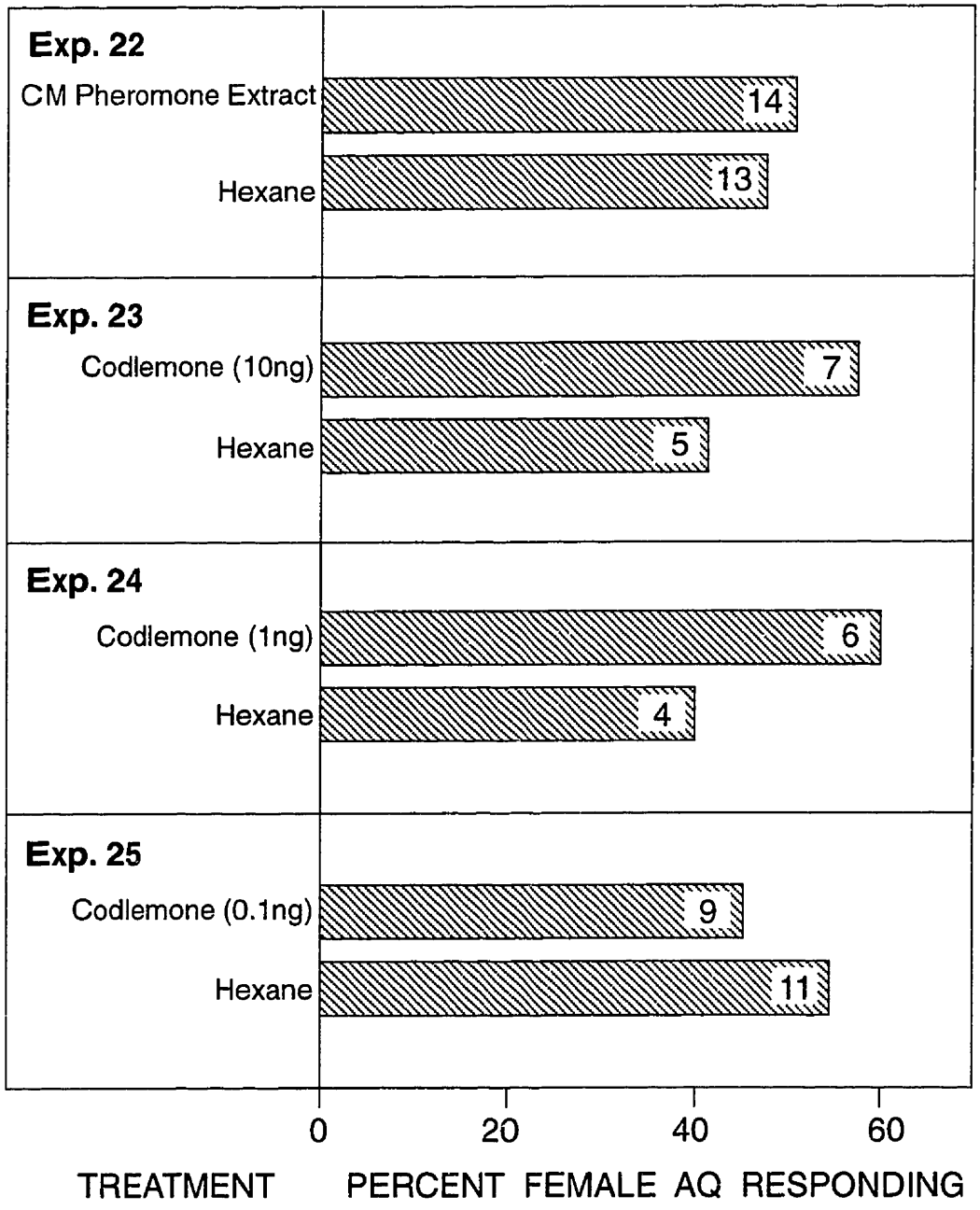
3. RESULTS

3.1. Behavioural evidence for kairomone-mediated host location

In olfactometer bioassays, pheromone gland extracts of female *C. pomonella* failed to attract female *A. quadridentata* (Figure 14, Exp. 22). Codlemone at doses of 10, 1 or 0.1 ng was no more attractive to female *A. quadridentata* than the hexane control (Figure 14, Exp. 23-25).

In contrast, volatiles from eggs of *C. pomonella* were attractive to primed female parasitoids (Exp. 27: χ^2 test, P=0.00591) and marginally insignificantly attractive to unprimed

FIG. 14. Percent response of female *A. quadridentata* (AQ) to 1 female equivalent of *C. pomonella* extract (Table 4, Exp. 22) and to 3 doses of codlemone (10, 1 and 0.1 ng) (Exp. 23-25). Numbers of females responding to each stimulus given within bars. Asterisks indicate a significant preference for a particular treatment; χ^2 test with Yates correction for continuity, treatment *versus* control; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.


Exp. 22
CM Pheromone Extract
14
Hexane
13
Exp. 23
Codlemone (10ng)
7
Hexane
5
Exp. 24
Codlemone (1ng)
6
Hexane
4
Exp. 25
Codlemone (0.1ng)
9
Hexane
11
0
20
40
60
TREATMENT
PERCENT FEMALE AQ RESPONDING

female parasitoids (Exp. 26: χ^2 test, P=0.09896) (Figure 15). In the presence of egg volatiles, female parasitoids exhibited behaviours such as wing fanning and occasional antennal tapping (antennae contact the substrate in a tapping motion). Volatiles from scales of female *C. pomonella* (Figure 15, Exp. 28) were attractive to female *A. quadridentata*, elicited wing fanning and regularly induced antennal tapping.

Neither pheromonal extract of female *C. pomonella*, synthetic codlemone (Figure 16, Exp. 31-34), nor egg volatiles (Figure 16, Exp. 35 and 36) were attractive to male *A. quadridentata* in Y-tube olfactometers. However, volatiles from female *C. pomonella* scales at a high dose did attract male *A. quadridentata* (Figure 16, Exp. 37). Males wing-fanned occasionally in the presence of codlemone, egg or scale volatiles.

3.2. Identification of candidate kairomones

In coupled GC-EAD recordings, codlemone elicited only a very weak response from female *A. quadridentata* antennae, while other components of female *C. pomonella* pheromone gland extract were not EAD-active (Figure 17). In contrast, male *A. quadridentata* responded strongly to codlemone (Figure 17).

In GC-EAD recordings with volatiles from eggs and wing and body scales of *C. pomonella* (Figure 18), numerous volatiles elicited antennal responses from both female and male *A. quadridentata*. EAD-active compounds were further subjected to GC-MS and their identification (Table 6) confirmed by comparative GC, GC-MS and GC-EAD analyses of

FIG. 15. Percent response of unprimed (Exp. 26, 27) and primed (Exp. 28-30) female *A. quadridentata* (AQ) to candidate kairomone sources (Table 4). Numbers of females responding to each stimulus given within bars. Asterisks indicate a significant preference for a particular treatment; χ^2 test with Yates correction for continuity, treatment *versus* control; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

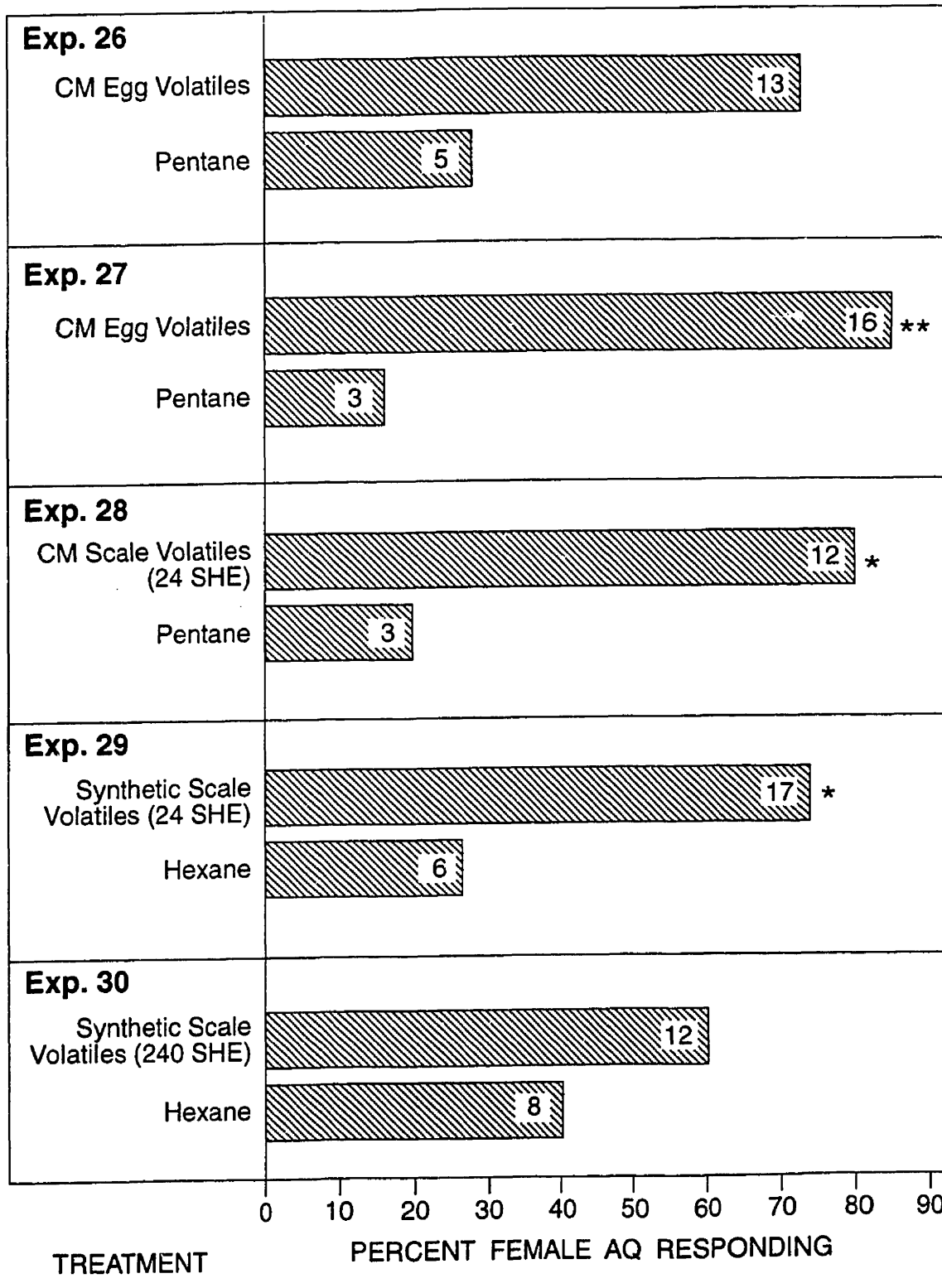

Exp. 26
CM Egg Volatiles
13
Pentane
5
Exp. 27
CM Egg Volatiles
16
**
Pentane
3
Exp. 28
CM Scale Volatiles (24 SHE)
12
*
Pentane
3
Exp. 29
Synthetic Scale Volatiles (24 SHE)
17
*
Hexane
6
Exp. 30
Synthetic Scale Volatiles (240 SHE)
12
Hexane
8
0 10 20 30 40 50 60 70 80 90
TREATMENT
PERCENT FEMALE AQ RESPONDING

FIG. 16. Percent response of male *A. quadridentata* (AQ) to potential kairomone sources (Table 5). Numbers of males responding to each stimulus given within bars. Asterisks indicate a significant preference for a particular treatment; χ^2 test with Yates correction for continuity, treatment *versus* control; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

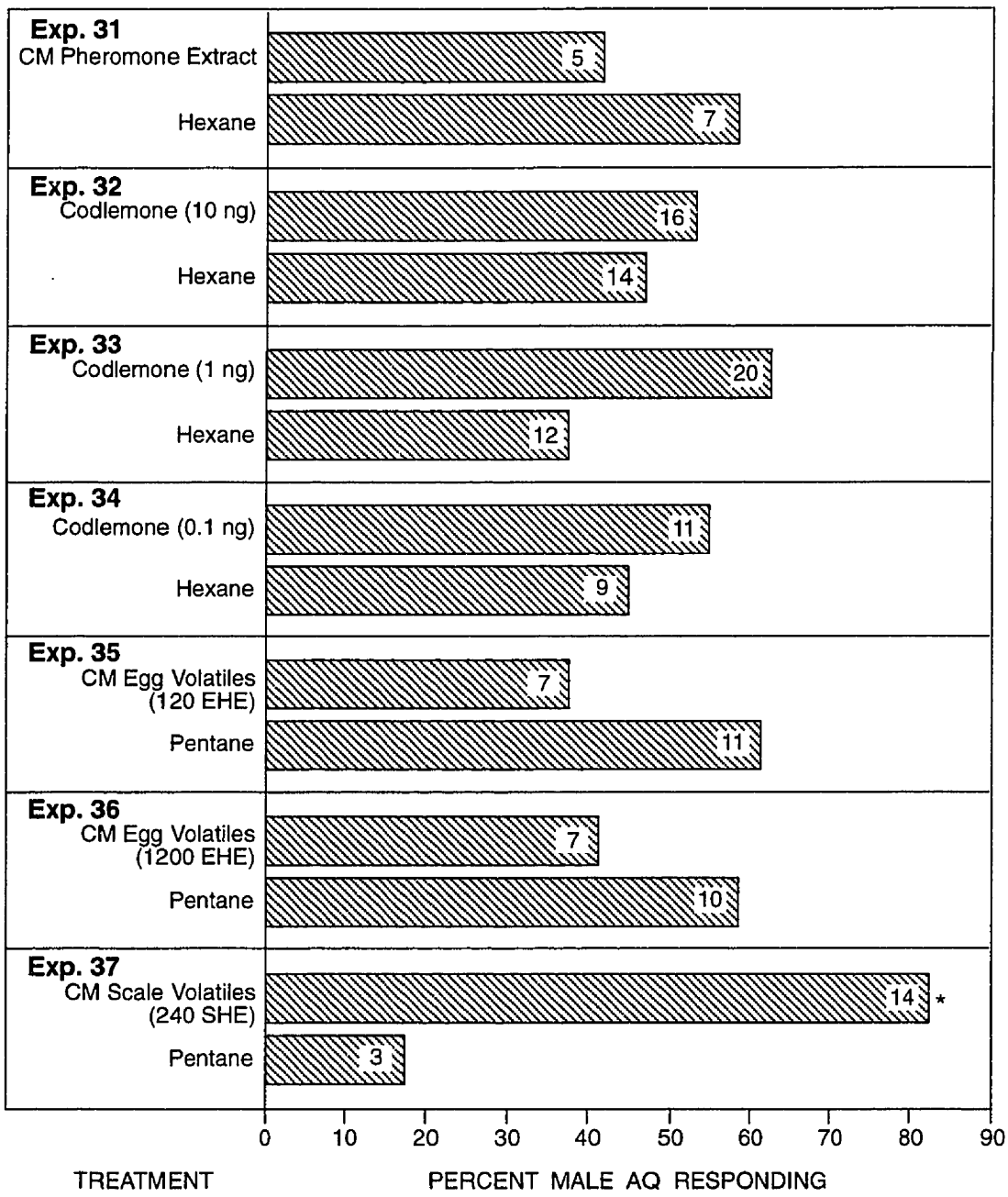

Exp. 31
CM Pheromone Extract
5
Hexane
7
Exp. 32
Codlemone (10 ng)
16
Hexane
14
Exp. 33
Codlemone (1 ng)
20
Hexane
12
Exp. 34
Codlemone (0.1 ng)
11
Hexane
9
Exp. 35
CM Egg Volatiles (120 EHE)
7
Pentane
11
Exp. 36
CM Egg Volatiles (1200 EHE)
7
Pentane
10
Exp. 37
CM Scale Volatiles (240 SHE)
14
*
Pentane
3
0
10
20
30
40
50
60
70
80
90
TREATMENT
PERCENT MALE AQ RESPONDING

FIG. 17. Flame ionization detector (FID) and electroantennographic detector (EAD: *A. quadridentata* (AQ) antenna) responses to aliquotes of 1 female equivalent of pheromone gland extract from female *C. pomonella*. Chromatography: Hewlett Packard 5890A equipped with a DB-5 coated column (30 m x 0.25 mm ID); linear flow velocity of carrier gas: 35cm/sec; injector and FID detector temperature: 240°C; temperature program: 1 min at 50°C, 10°C/min to 240°C.

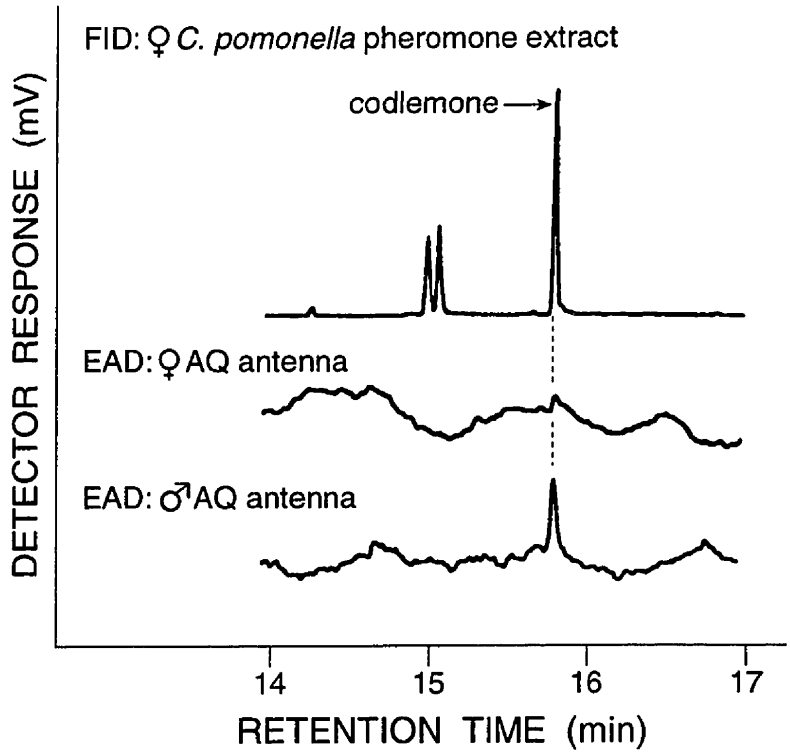

FID: ♀ C. pomonella pheromone extract
codlemone
EAD: ♀ AQ antenna
EAD: ♂ AQ antenna
DETECTOR RESPONSE (mV)
14
15
16
17
RETENTION TIME (min)

FIG. 18. Flame ionization detector (FID) and electroantennographic detector (EAD: *A. quadridentata* (AQ) antenna) responses to aliquotes of 24 female scale h equivalents (SHE) of volatiles from *C. pomonella*. Chromatography: Hewlett Packard 5890A equipped with a DB-5 coated column (30 m x 0.25 mm ID); linear flow velocity of carrier gas: 35cm/sec; injector and FID detector temperature: 240°C; temperature program: 1 min at 50°C, 20°C/min to 280°C.

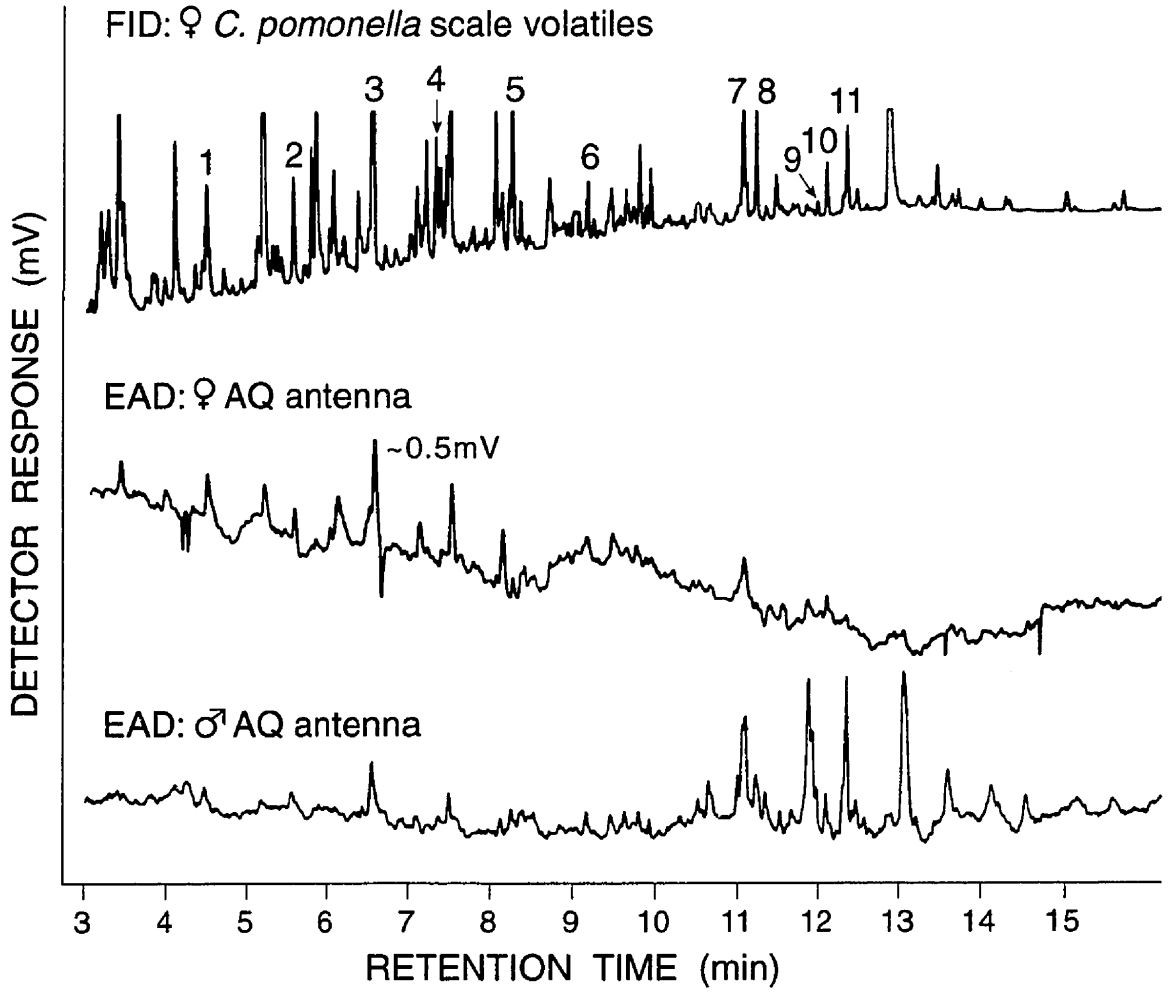
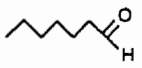
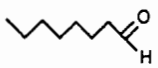
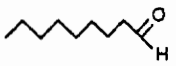
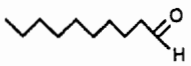
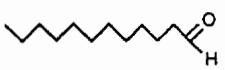
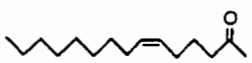
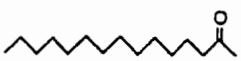
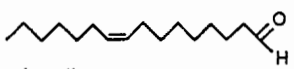
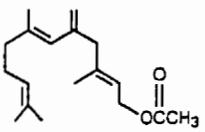
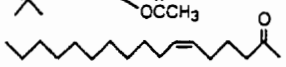

FID: ♀ C. pomonella scale volatiles
1
2
3
4
5
6
7 8
9
10
11
EAD: ♀ AQ antenna
~0.5mV
EAD: ♂ AQ antenna
DETECTOR RESPONSE (mV)
3 4 5 6 7 8 9 10 11 12 13 14 15
RETENTION TIME (min)

Table 6. Name, structure, purity and source of chemicals identified in volatiles of body and wing scales from female *C. pomonella* that elicited antennal responses by females and males of *A. quadridentata*.

Compound[ab]	Structure	Chemical purity (%)	Source[c]
Heptanal (1) 1.5 ng		>95	Heptanol from Sigma, oxidized to aldehyde by R. Gries, SFU.
Octanal (2) 1.3 ng		>95	Sigma
Nonanal (3) 4.5 ng		>95	Nonanol from Sigma, oxidized to aldehyde by R. Gries, SFU.
Decanal (4) 4 ng		99	Sigma
Undecan-2-one (5) 2.8 ng		>99	Fluka
Dodecanal (6) 0.6 ng		92	Sigma
*Z*6-pentadecen-2-one (7) 1.7 ng		95	Synthesized by G. Khaskin, SFU.
Pentadecan-2-one (8) 1.3 ng		>97	Synthesized by G.G.S. King, SFU.
*Z*9-hexadecenal (9) 0.1 ng		95	Sigma
EE-Farnesyl acetate (10) 0.7 ng		95	Aldrich
*Z*6-heptadecen-2-one (11) 1 ng		>91	Synthesized by G. Khaskin, SFU.

[a] Number in brackets refers to EAD-active volatiles in GC-EAD recordings of scale volatile extract (Figure 18).
[b] Quantities of chemicals as found in 24 scale hour equivalents (see text for details).
[c] Sigma-Aldrich Canada Ltd., 2149 Winston Park Drive, Oakville, Ont. L6H 2J8
Fluka Chemika-Biochemika, CH-9470 Buchs, Switzerland
Aldrich Chemical Co., Inc., 1001 West Saint Paul Avenue, Milwaukee, WI 53233 USA

insect-produced and authentic standards.

Attractive to female *A. quadridentata* at 24 SHE (Figure 15, Exp. 29) but not at 240 SHE (Figure 15, Exp. 30), synthetic scale volatiles elicited wing fanning and regularly induced antennal tapping in Y-tube olfactometer bioassays.

4. DISCUSSION

Female moth sex pheromones retained by foliage on which calling and mating have occurred serve as kairomones in several parasitoids (egs. Lewis *et al.*, 1982; Noldus and van Lenteren, 1985; Noldus, 1989). Insignificant antennal responses from female *A. quadridentata* to synthetic codlemone (Figure 17), and no behavioural response to codlemone (Figure 14, Exp. 23-25) or hexane extract of *C. pomonella* pheromone glands (Figure 14, Exp. 22), provided strong evidence that the sex pheromone of female *C. pomonella*, on its own, is not a kairomone for foraging female *A. quadridentata*. Because mated female *C. pomonella* widely distribute their eggs, rarely depositing two or more eggs on the same pome fruit or leaf (Borden, 1931), pheromone adhering to the mating site may indeed not be a reliable semiochemical for foraging female *A. quadridentata*.

Volatiles emanating from eggs of *C. pomonella* elicited strong behavioural response by female parasitoids (Figure 15, Exp. 26-27). Egg volatiles also induced wing fanning by male and female *A. quadridentata*. While wing fanning in response to female pheromone is part of many hymenopteran male parasitoid courtship displays (van den Assem, 1986), it has

not been reported previously for male or female parasitoids in response to host kairomones. Vinson (1972) hypothesized that wing fanning causes air movements that help parasitoids orient to the semiochemical source; the behaviour observed for female *A. quadridentata* supports this hypothesis as females increased their frequency of wing fanning in response to lower doses of attractive kairomones. Female *A. quadridentata* search for host eggs following semiochemical-mediated location of host habitat. They continuously tap the plant surface with their antennal tips until they have contacted a host egg (Rosenberg, 1934). The contact-kairomone(s) associated with host eggs is water-soluble, but its chemical structure has not yet been identified (Kromer, 1986).

Peculiar oviposition behaviour by female *C. pomonella*, including brushing the surface of the leaf or fruit with the tip of her abdomen during oviposition (Borden, 1931), suggests that female moths may inadvertently deposit body and wing scales on plants or eggs during the process of oviposition. Scanning electron micrographs revealed many scales from female moths attached to the surface of eggs from *C. pomonella* (Figure 19).

Scales and their volatiles may constitute a reliable cue for foraging female *A. quadridentata* as they adhere to the egg surface, even when subjected to a fine air stream. Because scales have been reported as a kairomonal source for other egg parasitoids (e.g., Lewis *et al.*, 1972; Chiri and Legner, 1986), including *A. reticulatus* (Kainoh *et al.*, 1990), I tested the hypothesis that scales from female *C. pomonella* complement attractiveness of egg volatiles or are the exclusive source of kairomonal attraction for female *A. quadridentata.* Antennal responses from female and male *A. quadridentata* to scale volatile extract (Figure

FIG. 19. Scanning electron micrograph of *C. pomonella* eggs, depicting moth scales embedded in the surface of the egg. Eggs were sputter-coated with gold, then viewed with a Hitachi S-2500 scanning electron microscope at 10 kV accelerating voltage. (Photograph courtesy of M. Weis and M. Gardiner, Agriculture and Agri-Food Canada, Summerland, BC).


0.1 mm

18), wing fanning in the presence of scale volatiles followed by antennal tapping by females and attraction of parasitoids to both natural (Figure 15, Exp. 28; Figure 16, Exp. 37) and synthetic scale volatile blends (Figure 15, Exp. 29) all support the hypothesis that scale volatiles provide an important kairomonal cue for foraging female *A. quadridentata.*

Response of female parasitoids to scale volatiles is likely of adaptive significance, focussing searching behaviour on areas with high probabilities of encountering host eggs (Chiri and Legner, 1982). By contrast, attraction of male parasitoids to scale volatiles (Figure 16, Exp. 37) may have evolved to enhance the probability of encountering ovipositing female parasitoids who do not require fertilization to lay eggs.

Several volatiles in the synthetic kairomonal blend (Table 6) attractive to female *A. quadridentata* (Figure 15, Exp. 30) have also been reported as (potential) semiochemicals for other parasitic wasps. Z9-16:Ald, for example, is a sex pheromone component of bollworm, *Heliothis zea* (Boddie), mediating kairomonal attraction of its egg parasitoid *Trichogramma pretiosum* Riley (Lewis *et al.*, 1982). Heptanal elicits antennal responses from both male and female braconid *Microplitis croceipes* (Cresson) (Li *et al.*, 1992). Habitat-derived nonanal is a synergistic kairomone for *Apanteles carpatus* (Say), a parasitoid of clothes moth larvae (Takacs *et al.*, 1997). Nonanal in leaf volatiles of chestnut (*Castanea sativa* Miller) also elicits antennal responses from female *A. quadridentata*, although behavioural activity has not been established (Rotundo and Tremblay, 1993). Finally, octanal, nonanal and decanal in abdominal gland secretions from stink bugs (Pentatomidae) may serve as kairomones for parasitic Tachinidae (Diptera) and Scelionidae (Hymenoptera) (Aldrich *et al.*, 1995).

Sometimes, combinations of semiochemicals from various sources are necessary to attract a parasitoid (Nordlund *et al.*, 1977; Godfray, 1994). For example, kairomonal attractiveness of *Trichogramma maidis* Pint. et Voeg., an egg parasitoid of the corn borer, *Ostrinia nubilalis* (Hubner), is based upon a complex mixture of odours comprising the corn borer's sex pheromone and volatiles from both the host egg and host plant (Kaiser *et al.*, 1989). While scale volatiles of *C. pomonella* act alone as a single kairomonal attractant for male and female *A. quadridentata*, it should be investigated whether sex pheromone of *C. pomonella*, and/or apple volatiles, enhance the attractiveness of scale-derived kairomones.

V. PRACTICAL IMPLICATIONS

Semiochemical-mediated communication of *A. quadridentata* was studied because this parasitoid shows promise as a potential biological control agent against *C. pomonella*, a severe pest of apples. Findings described in my thesis further our understanding about the parasitoid's communication ecology and have relevance for integrated management of *C. pomonella*. Identification of insect-produced pheromone and host-derived kairomones may allow the monitoring of parasitoid populations in the field and enhance the effectiveness of their control of *C. pomonella*.

Traps baited with synthetic *A. quadridentata* sex pheromone have the following potential uses: (1) detecting the presence or absence of *A. quadridentata* in target orchards; (2) determining the seasonal phenology of parasitoid activity; and (3) measuring the parasitoid:host ratio. Because *A. quadridentata* sex pheromone is not attractive to female *A. quadridentata*, future work needs to be done to correlate capture of male parasitoids in pheromone-baited traps with densities of female parasitoid populations. If sex ratios of *A. quadridentata* in the field are not stable (mating affects the sex ratio in this species), information on abundance of female parasitoids may possibly be obtained using synthetic host kairomone-baited traps (Powell, 1986).

The use of monitoring traps to capture parasitoids may have adverse effects on existing *A. quadridentata* populations. Capture of male parasitoids, for example, may result in fewer matings of females and ultimately – as unmated females produce male offspring –

may cause a male-biased parasitoid population with decreased impact on *C. pomonella*. Sustainable monitoring of parasitoid populations may require development of special traps that allow captured *A. quadridentata* to be released after their numbers have been recorded.

At a low parasitoid:host ratio, inundative release of parasitoids may provide the means for substantial parasitism of *C. pomonella* (Knipling, 1992; Newton, 1993). However, in order to be effective, mass-released *A. quadridentata* must be retained in the target area and be able to locate and recognize the target host. Deployment of synthetic kairomones in commercial orchards may initiate intensive host-searching by mass-released parasitoids, thereby retaining them within, as well as attracting feral parasitoids to, the target site (Lewis *et al.*, 1971, 1972, 1975a; Vinson, 1977; Nordlund *et al.*, 1981; Beevers *et al.*, 1981). While the behavioural effect of kairomones on female *A. quadridentata* needs to be investigated in the field, laboratory bioassays (Figure 15, Exp. 27, 28 and 29) and observations suggest that field attraction and retention of *A. quadridentata* may be achieved through the use of kairomones.

The potential of synthetic host-scale volatiles to manipulate *A. quadridentata* depends on how they mediate host location. If kairomones induce a semi-random search within a host-infested localized area without interfering with the parasitoid's ability to find or move efficiently between host eggs, the potential for enhanced *C. pomonella* control exists (Vinson, 1977). However, if scale volatiles retain a female parasitoid in an area not infested with host eggs and inhibit her from locating or moving freely between host eggs, deployed kairomones may confuse the parasitoid and reduce control of *C. pomonella* (Vinson, 1977; Beevers *et*

al., 1981). Synthetic kairomones must retain the parasitoid without inhibiting her movement from one ovipositional site to the next (Lewis *et al.*, 1979; Beevers *et al.*, 1981), stimulating the parasitoid to search rather than guiding her directly to the host (Vinson, 1977).

Inundative release of *A. quadridentata* in target orchards would involve prior laboratory rearing of many generations of parasitoids. Although laboratory-reared parasitoids may receive all of their dietary requirements, the cues they learn upon emergence may not necessarily be those associated with the target host, compromising efficient host location (Godfray, 1994). "Feral" parasitoids, in contrast, may experience traces of host chemicals at the site of emergence, thereby learning about the host before they begin to search for it (Corbet, 1985; Vet and Groenewold, 1990; Turlings *et al.*, 1992). The identification and application of essential host kairomones could, however, allow laboratory-reared parasitoids to be "taught" target-specific search modes before their release (Gross *et al.*, 1975; Wardle and Borden, 1985; Lewis *et al.*, 1990; Vet *et al.*, 1990).

From a biological control perspective, chemical guidance systems hold a great opportunity for behavioural manipulation of parasitoids. While further research is required before the current findings can be applied, my research indicates that there is exciting potential for semiochemical-based monitoring and management of *A. quadridentata*.

VI. REFERENCES

ALDRICH, J.R., ROSI, M.C., and BIN, F. 1995. Behavioral correlates for minor volatile compounds from stink bugs (Heteroptera: Pentatomidae). *J. Chem. Ecol.* 21(12): 1907-1920.

VAN ALPHEN, J.J.M. and VET, L.E.M 1986. An evolutionary approach to host finding and selection, pp. 23-61, *in* J.K. Waage and D. Greathead (eds). Insect Parasitoids. Academic Press, London.

ARN, H., STADLER, E., and RAUSCHER, S. 1975. The electroantennographic detector – a selective and sensitive tool in the gas chromatographic analysis of insect pheromones. *Z. Naturforsch.* 30c: 722-725.

ARTHUR, A.P. 1971. Associate learning by *Nemeritis canescens* (Hymenoptera: Ichneumonidae). *Can. Entomol.* 103: 1137-1141.

VAN DEN ASSEM, J. 1986. Mating behaviour in parasitic wasps, pp. 137-167, *in* J.K. Waage and D. Greathead (eds.). Insect Parasitoids. Academic Press, London.

VAN DEN ASSEM, J., and PUTTERS, F.A. 1980. Patterns of sound produced by courting Chalcidoid males and its biological significance. *Entomol. Exp. Appl.* 27: 293-302.

BEEVERS, M., LEWIS, W.J., GROSS, H.R. Jr., and NORDLUND, D.A. 1981. Kairomones and their use for management of entomophagous insects: X. Laboratory studies on manipulation of host-finding behavior of *Trichogramma pretiosum* Riley with a kairomone extracted from *Heliothis zea* (Boddie) moth scales. *J. Chem. Ecol.* 7(3): 635-648.

BORDEN, A.D. 1931. Some field observations on codling moth behavior. *J. Econ. Entomol.* 24: 1137-1145.

BOUSH, G.M., and BAERWALD, R.J. 1967. Courtship behavior and evidence for a sex pheromone in the apple maggot parasite, *Opius alloeus* (Hymenoptera: Braconidae). *Ann. Entomol. Soc. Am.* 60(4): 865-866.

BOYCE, H.R. 1936. Laboratory breeding of *Ascogaster carpocapsae* Vier. with notes on biology and larval morphology. *Can. Entomol.* 68: 241-246.

BOYCE, H.R. 1940. Biological control of the codling moth in Ontario. *Entomol. Soc. Ontario Ann. Rpt.* 71: 40-44.

BRINTON, F.E., PROVERBS, M.D., and CARTY, B.E. 1969. Artificial diet for the mass production of the codling moth, *Carpocapsa pomonella* (Lepidoptera: Olethreutidae). *Can. Entomol.* 101: 577-584.

BROWN, J.J. 1993. Codling moth integrated pest management. *Proc. Wash. State Hort. Assoc.* 89: 68-71.

BROWN, J.J. 1996. The compatibility of Tebufenozide with a laboratory Lepidopteran host/Hymenopteran parasitoid population. *Biol. Cont.* 6: 96-104.

BROWN, J.J., AHL, J., and REED-LARSEN, D. 1988. Endocrine communication between a host and its endoparasitoids in relationship to dormancy, pp. 443-447, *in* F. Sehnal, A. Zabza, and D.L. Denlinger (eds.). Endocrinological Frontiers in Physiological Insect Ecology. Wroclaw Technical University Press, Wroclaw.

BROWN, J.J., and FRIEDLANDER, M. 1995. Influence of parasitism on spermatogenesis in the codling moth, *Cydia pomonella* L. *J. Insect. Physiol.* 41(11): 957-963.

BROWN, J.J., and REED-LARSEN, D. 1991. Ecdysteroids and insect host/parasitoid interactions. *Biol. Cont.* 1: 136-143.

BROWNING, H.W., and OATMAN, E.R. 1985. Reproductive biology of *Microplitis brassicae* (Hymenoptera: Braconidae), parasite of the cabbage looper, *Trichoplusia ni* (Lepidoptera: Noctuidae). *Ann. Entomol. Soc. Am.* 78(3): 369-372.

CHIRI, A.A., and LEGNER, E.F. 1982. Host-searching kairomones alter behavior of *Chelonus* sp. nr. *curvimaculatus*, a hymenopterous parasite of the pink bollworm, *Pectinophora gossypiella* (Saunders). *Environ. Entomol.* 11: 452-455.

CHIRI, A.A., and LEGNER, E.F. 1986. Response of three *Chelonus* (Hymenoptera: Braconidae) species to kairomones in scales of six Lepidoptera. *Can. Entomol.* 118: 329-333.

CLAUSEN, C.P. 1978. Olethreutidae, pp. 211- 218, *in* C.P. Clausen (ed.). Introduced parasites and predators of arthropod pests and weeds: A world review. U.S. Dept. Agr., Agri. Handbook No. 480.

COLE, L.R. 1970. Observations on the finding of mates by male *Phaeogenes invisor* and *Apanteles medicaginis* (Hymenoptera: Ichneumonidae). *Anim. Behav.* 18: 184-189.

CORBET, S.A. 1985. Insect chemosensory response: a chemical legacy hypothesis. *Ecol.*

Entomol. 10: 143-153.

COX, J.A., and DANIEL, D.M. 1935. *Ascogaster carpocapsae* Viereck in relation to arsenical sprays. *J. Econ. Entomol.* 28: 113-120.

CUTRIGHT, C.R., and MORRISON, H.E. 1935. Varietal susceptibility to codling moth injury. *J. Econ. Entomol.* 28: 107-109.

DOLSTAD, K.D. 1985. Biology and control of the codling moth in the Pacific Northwest. M.P.M. Professional Paper. Simon Fraser University, Burnaby.

VAN DEN DOOL, H., and KRATZ, P.D. 1963. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *J. Chromatog.* 2: 463-471.

DOUTT, R.L. 1959. The biology of parasitic Hymenoptera. *Ann. Rev. Entomol.* 4: 161-182.

DOUTT, R.L. 1964. Biological characteristics of entomophagous adults, pp. 145-167, *in* P. DeBauch (ed.). Biological Control of Insect Pests and Weeds. Reinhold Publishing Co., New York.

DOZIER, H.L., and BUTLER, H.G. 1929. Notes on the rearing of *Ascogaster carpocapsae*, a braconid parasite of the codling moth. *J. Econ. Entomol.* 22: 954-957.

DROST, Y.C., and CARDE, R.T. 1992. Use of learned visual cues during habitat location by *Brachymeria intermedia*. *Entomol. Exp. Appl.* 64: 217-224.

DYCK, V.A., and GARDINER, M.G.T. 1992. Sterile-insect release program to control the codling moth *Cydia pomonella* (L.) (Lepidoptera: Olethreutidae) in British Columbia, Canada. *Acta Phytopathol. et Entomol. Hungarica* 27(1-4): 219-222.

ELLER, F.J., BARTELT, R.J., JONES, R.L., and KULMAN, H.M. 1984. Ethyl (*Z*)-9-hexadecenoate, a sex pheromone of *Syndipnus rubiginosus*, a sawfly parasitoid. *J. Chem. Ecol.* 10(2): 291-300.

FIELD, S.A., and KELLER, M.A. 1993. Courtship and intersexual signaling in the parasitic wasp *Cotesia rubecula* (Hymenoptera: Braconidae). *J. Insect Behav.* 6(6): 737-750.

FLANDERS, S.E. 1953. Variations in susceptibility of citrus-infesting coccids to parasitization. *J. Econ. Entomol.* 46: 266-269.

GEIER, P.W. 1963. The life history of codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), in the Australian capital territory. *Aust. J. Zool.* 11: 323-367.

GODFRAY, H.C.J. 1994. *Parasitoids: Behavioral and Evolutionary Ecology*. J.R. Krebs and T. Clutton-Brock (eds.). Princeton Univ. Press, Princeton, New Jersey.

GORDH, G., and DE BACH, P. 1978. Courtship behavior in the *Aphytis lingnanensis* group, its potential usefulness in taxonomy, and a review of sexual behavior in the parasitic Hymenoptera (Chalcidoidae: Aphelinidae). *Hilgardia*. 46(2): 37-75.

GROSS, H.R. Jr., LEWIS, W.J., JONES, R.L., and NORDLUND, D.A. 1975. Kairomones and their use for management of entomophagous insects: III. Stimulation of *Trichogramma achaeae, T. pretiosum,* and *Microplitis croceipes* with host-seeking stimuli at time of release to improve their efficiency. *J. Chem. Ecol.* 1(4): 431-438.

HAGLEY, E.A.C. 1972. Observations on codling moth longevity and egg hatchability. *Environ. Entomol.* 1: 123-125.

HARRIS, M.O., and FOSTER, S.P. 1995. Behavior and integration, pp. 3-46, *in* R.T. Carde and W.J. Bell (eds.). Chemical Ecology of Insects 2. Chapman and Hall, Toronto.

HOUSE, H.O. 1972. Modern Synthetic Reactions, second edition. p. 93. W.A. Benjamin, California.

HOWELL, J.F., SCHMIDT, R.S., HORTON, D.R., KHATTAK, S.U.K., and WHITE, L.D. 1990. Codling moth: male moth activity in response to pheromone lures and pheromone-baited traps at different elevations within and between trees. *Environ. Entomol.* 19(3): 573-577.

JOHANSEN, C.A. 1957. History of biological control of insects in Washington. *Northwest Sci.* 31: 57-92.

KAINOH, Y., NEMOTO, T., SHIMIZU, K., TATSUKI, S., KUSANO, T., and KUWAHARA, Y. 1991. Mating behavior of *Ascogaster reticulatus* Watanabe (Hymenoptera: Braconidae), an egg-larval parasitoid of the smaller tea tortrix moth, *Adoxophyes* sp. (Lepidoptera: Tortricidae) III. Identification of a sex pheromone. *Appl. Ent. Zool.* 26(4): 543-549.

KAINOH, Y., TATSUKI, S., and KUSANO, T. 1990. Host moth scales; a cue for host location for *Ascogaster reticulatus* Watanabe (Hymenoptera: Braconidae). *Appl. Entomol. Zool.* 25(1): 17-25.

KAISER, L., and CARDE, R.T. 1992. In-flight orientation to volatiles from the plant host complex in *Cotesia rubecula* (Hymenoptera: Braconidae): increased sensitivity through olfactory conditioning. *Physiol. Entomol.* 17: 62-67.

KAISER, L., PHAM-DELEGUE, M.H., BAKCHINE, E., and MASSON, C. 1989. Olfactory responses of *Trichogramma maidis* Pint. et Voeg.: effect of chemical cues and behavioral plasticity. *J. Insect Behav.* 2: 701-712.

KAMANO, Y., SHIMIZU, K., KAINOH, Y., and TATSUKI, S. 1989. Mating behavior of *Ascogaster reticulatus* Watanabe (Hymenoptera: Braconidae), an egg-larval parasitoid of the smaller tea tortrix, *Adoxophyes* sp. (Lepidoptera: Tortricidae) II. Behavioral sequence and a role of sex pheromone. *Appl. Entomol. Zool.* 24(4): 372-378.

KNIPLING, E.F. 1992. Principles of insect parasitism analyzed from new perspectives: Practical implications for regulating insect populations by biological means. U.S. Department of Agriculture, Agriculture Handbook No. 693, 337 pp.

KROMER, V.F. 1986. The kairomone of the codling moth *Laspeyresia pomonella* L. and its influence on the host-finding behavior of the braconid wasp *Ascogaster quadridentatus* Wesm. *Zool. Anz.* 216: 271-278.

LAING, J. 1937. Host finding by insect parasites. I. Observations on the finding of hosts by *Alysia manducator*, *Mormoneilla vitripennis* and *Trichogramma evanescens*. *J. Anim. Ecol.* 6: 298-317.

LAW, J.H., and REGNIER, F.E. 1971. Pheromones. *Ann. Rev. Biochem.* 40: 533.

VAN LEEUWEN, E.R. 1929. Life history of the codling moth in northern Georgia. *U.S. Dept. Agr. Bull.* 90.

LE ROUX, E.J. 1971. Biological control attempts on pome fruit (apple and pear) in North America, 1860-1970. *Can. Entomol.* 103: 963-974.

LEWIS, W.J., BEEVERS, M., NORDLUND, D.A., GROSS, H.R. Jr., and HAGEN, K.S. 1979. Kairomones and their use for management of entomophagous insects: IX. Investigation of various kairomone-treatment patterns for *Trichogramma* spp. *J. Chem. Ecol.* 5(5): 673-680.

LEWIS, W.J., JONES, R.L., NORDLUND, D.A., and GROSS, H.R. Jr. 1975b. Kairomones and their use for management of entomophagous insects: II. Mechanisms causing increase in rate of parasitization by *Trichogramma* spp. *J. Chem. Ecol.* 1: 349-360.

LEWIS, W.J., JONES, R.L., NORDLUND, D.A., and SPARKS, A.N. 1975a. Kairomones and their use for management of entomophagous insects: I. Utilization for increasing rate of parasitism by *Trichogramma* spp. *J. Chem. Ecol.* 1: 343-347.

LEWIS, W.J., JONES, R.L., and SPARKS, A.N. 1972. A host-seeking stimulant for the egg parasite *Trichogramma evanescens*: its source and a demonstration of its laboratory and field activity. *Ann. Entomol. Soc. Am.* 65: 1087-1089.

LEWIS, W.J., and MARTIN, W.R. Jr. 1990. Semiochemicals for use with parasitoids: status and future. *J. Chem. Ecol.* 16(11): 3067-3089.

LEWIS, W.J., NORDLUND, D.A., GUELDNER, R.C., TEAL, P.E.A., and TUMLINSON, J.H. 1982. Kairomones and their use for management of entomophagous insects. XIII. Kairomonal activity for *Trichogramma* spp. of abdominal tips, excretion, and a synthetic sex pheromone blend of *Heliothis zea* (Boddie) moths. *J. Chem. Ecol.* 8(10): 1323-1331.

LEWIS, W.J., SNOW, J.W., and JONES, R.L. 1971a. A pheromone trap for studying populations of *Cardiochiles nigriceps*, a parasite of *Heliothis virescens*. *J. Econ. Entomol.* 64: 1417-1421.

LEWIS, W.J., SPARKS, A.N., and REDLINGER, L.M. 1971b. Moth odor: a method of host finding by *Trichogramma evanescens*. *J. Econ. Entomol.* 64: 557-558.

LEWIS, W.J., VET, L.E.M., TUMLINSON, J.H., VAN LENTEREN, J.C., and PAPAJ, D.R. 1990. Variations in parasitoid foraging behavior: essential element of a sound biological control theory. *Environ. Entomol.* 19: 1183-1193.

LI, Y., DICKENS, J.C., and STEINER, W.W.M. 1992. Antennal olfactory responsiveness of *Microplitis croceipes* (Hymenoptera: Braconidae) to cotton plant volatiles. *J. Chem. Ecol.* 18(10): 1761-1773.

MATTHEWS, R.W. 1974. Biology of Braconidae. *Ann. Rev. Entomol.* 19: 15-32.

MC LEOD, J.H. 1954. Statuses of some introduced parasites and their hosts in British Columbia. *Proc. Entomol. Soc. British Columbia*, 50: 19-27.

MILLER, M.C., and TSAO, C.H. 1974. Significance of wing vibration in male *Nasonia vitripennis* (Hymenoptera: Pteromalidae) during courtship. *Ann. Entomol. Soc. Am.* 67(5): 772-774.

NEWTON, P.J. 1993. Increasing the use of trichogrammatids in insect pest management: a case study from the forests of Canada. *Pestic. Sci.* 37: 381-386.

NOLDUS, L.P.J.J. 1989. Semiochemicals, foraging behavior and quality of entomophagous insects for biological control. *J. Appl. Entomol.* 108: 425-451.

NOLDUS, L.P.J.J., and VAN LENTEREN, J.C. 1985. Kairomones for the egg parasite *Trichogramma evanescens* Westwood. I. Effect of volatile substances released by two of its hosts, *Pieris brassicae* L. and *Mamestra brassicae* L. *J. Chem. Ecol.* 11(6): 781-791.

NORDLUND, D.A., and LEWIS, W.J. 1976. Terminology of chemical releasing stimuli in intraspecific and interspecific interactions. *J. Chem. Ecol.* 2(2): 211-220.

NORDLUND, D.A., LEWIS, W.J., GROSS, H.R. Jr., and BEEVERS, M. 1981. Kairomones and their use for management of entomophagous insects: XII. The stimulatory effects of host eggs and the importance of host-egg density to the effective use of kairomones for *Trichogramma pretiosum* Riley. *J. Chem. Ecol.* 7(6): 909-917.

NORDLUND, D.A., LEWIS, W.J., TODD, J.W., and CHALFANT, R.B. 1977. Kairomones and their use for management of entomophagous insects VII. The involvement of various stimuli in the differential response of *Trichogramma pretiosum* Riley to two suitable hosts. *J. Chem. Ecol.* 3(5): 513-518.

OBARA, M., and KITANO, H. 1974. Studies on the courtship behavior of *Apanteles glomeratus* L. I. Experimental studies on releaser of wing-vibrating behavior in the male. *Kontyu.* 42(2): 208-214.

PEDIGO, L.P. 1989. Entomology and Pest Management. Macmillan Publishing Co., NY.

POMPANON, F., DE SCHEPPER, B., MOURER, Y., FOUILLET, P., and BOULETREAU, M. 1997. Evidence for a substrate-borne sex pheromone in the parasitoid wasp *Trichogramma brassicae*. *J. Chem. Ecol.* 23(5): 1349-1360.

POWELL, W. 1986. Enhancing parasitoid activity in crops, pp. 319-340, *in* J.K. Waage and D. Greathead (eds.). Insect Parasitoids. Academic Press, London.

PUTMAN, W.L. 1963. The codling moth, *Carpocapsa pomonella* (L.) (Lepidoptera: Tortricidae): A review with special reference to Ontario. *Proc. Entomol. Soc. Ont.* 93: 22-60.

RAO, V.P., GHANI, M.A., SANKARAN, T., and MATHUR, K.C. 1971. Codling moth – *Cydia pomonella* (L.) (Lepidoptera, Olethreutidae), pp. 3-4, *in* Review of the biological control of insects and other pests in South-East Asia and the Pacific region. Technical communication no. 6. Commonwealth institute of biological control, Trinidad, West Indies. Commonwealth Agricultural Bureaux, Farnham

Royal, England.

REED-LARSEN, D.A., and BROWN, J.J. 1990. Embryonic castration of the codling moth, *Cydia pomonella* by an endoparasitoid, *Ascogaster quadridentata*. *J. Insect Physiol.* 36(2): 111-118.

RICHARDSON, C.H., and DU CHANOIS, F.R. 1950. Codling moth infestation of the tops of apple trees. *J. Econ. Entomol.* 43(4): 466-470.

RIDDICK, E.W., and MILLS, N.J. 1994. Potential of adult Carabids (Coleoptera: Carabidae) as predators of fifth-instar codling moth (Lepidoptera: Tortricidae) in apple orchards in California. *Environ. Entomol.* 23(5): 1338-1345.

ROELOFS, W., COMEAU, A., HILL, A., and MILICEVIC, G. 1971. Sex attractant of the codling moth: characterization with electroantennogram technique. *Science (Washington)*. 174: 297-299.

ROSENBERG, H.T. 1934. The biology and distribution in France of the larval parasites of *Cydia pomonella* L. *Bull. Entomol. Res.* 25: 201-256.

ROTUNDO, G., and TREMBLAY, E. 1993. Electroantennographic responses of chestnut moths (Lepidoptera: Tortricidae) and their parasitoid *Ascogaster quadridentatus* Wesmael (Hymenoptera: Braconidae) to volatiles from chestnut (*Castanea sativa* Miller) leaves. *Redia.* 76(2): 361-373.

SALT, G. 1935. Experimental studies in insect parasitism. III. Host selection. *Proc. Royal Soc. London B*, 117: 413-435.

SCHMIDT, J., CARDE, R.T., and VET, L.E.M. 1993. Host recognition by *Pimpla instigator* F. (Hymenoptera: Ichneumonidae): preferences and learned responses. *J. Insect Behav.* 6: 1-11.

SCHOENE, W.J., HOUGH, W.S., STEARNS, L.A., CAGLE, L.R., WILLEY, C.R., and WOODSIDE, A.M. 1928. Life history of the codling moth in Virginia. *Virginia. Agr. Expt. Sta. Bull.* 261.

SHAW, M.R., and HUDDLESTON, T. 1991. Classification and biology of braconid wasps (Hymenoptera: Braconidae). W.R. Dolling and R.R. Askew (eds.). Handbooks for the Identification of British Insects. Vol. 7, part 11, Royal Entomological Society of London, London.

SHIELDS, E.J. 1989. Artificial light: Experimental problems with insects. *Bull. Entomol. Soc. Am.* Summer: 40-44.

SHUMAKOV, E.M. 1977. Ecological principles associated with augmentation of natural enemies, pp. 39-78, *in* R.L. Ridgway and S.B. Vinson (eds.). Biological control by augmentation of natural enemies. Plenum Press, New York.

SIVINSKI, J., and WEBB, J.C. 1989. Acoustic signals produced during courtship in *Diachasmimorpha* (=*Biosteres*) *longicaudata* (Hymenoptera: Braconidae) and other Braconidae. *Ann. Entomol. Soc. Am.* 82(1): 116-120.

SUMMERLAND, S.A., and STEINER, L.F. 1943. Codling moth oviposition and fate of eggs. *J. Econ. Entomol.* 36(1): 72-75.

SWAN, L.A. 1964. Insect parasites: the Braconids, pp. 156-158, *in* L.A. Swan (author). Beneficial insects. Harper & Row, Publishers, New York.

TAKACS, S., GRIES, G., and GRIES, R. 1997. Semiochemical-mediated location of host habitat by *Apanteles carpatus* (Say) (Hymenoptera: Braconidae), a parasitoid of clothes moth larvae. *J. Chem. Ecol.* 23(2): 459-472.

TANG, Y., and MARSH, P.M. 1994. A taxonomic study of the genus *Ascogaster* in China (Hymenoptera: Braconidae: Cheloninae). *J. Hym. Res.* 3: 279-302.

TAYLOR, R.J. 1974. Role of learning in insect parasitism. *Ecol. Monogr.* 44: 89-104.

THIERY, D., GABEL, B., FARKAS, P., and JARRY, M. 1995. Egg dispersion in codling moth: influence of egg extract and of its fatty acid constituents. *J. Chem Ecol.* 21(12): 2015-2026.

TUMLINSON, J. H., TURLINGS, T.C.J., and LEWIS, W.J. 1993. Semiochemically mediated foraging behavior in beneficial parasitic insects. *Arch. Insect Biochem. and Physiol.* 22: 385-391.

TURLINGS, T.C.J., WACKERS, F.L., VET, L.E.M, LEWIS, W.J., and TUMLINSON, J.H. 1992. Learning of host-location cues by hymenopterous parasitoids, pp. 51-78, *in* W.J. Lewis and D.R. Papaj (eds.). Insect Learning: Ecological and Evolutionary Perspectives. Chapman and Hall, New York.

VAKENTI, J.M. 1977. Utilization of synthetic codling moth pheromone in apple pest management systems. M.Sc. Thesis. Simon Fraser University, Burnaby.

VET, L.E.M., and GROENEWOLD, A.W. 1990. Semiochemicals and learning in parasitoids. *J. Chem. Ecol.* 16(11): 3119-3135.

VET, L.E.M, LEWIS, W.J., and CARDE, R.T. 1995. Parasitoid foraging and learning, pp.

65-101, *in* R.T. Carde and W.J. Bell (eds.). Chemical Ecology of Insects 2. Chapman and Hall, Toronto.

VET, L.E.M., LEWIS, W.J., PAPAJ, D.R., and VAN LENTEREN, J.C. 1990. A variable response model for parasitoid foraging behavior. *J. Insect Behav.* 3: 471-91.

VINSON, S.B. 1972. Courtship behavior and evidence for a sex pheromone in the parasitoid *Campoletis sonorensis* (Hymenoptera: Ichneumonidae). *Environ. Entomol.* 1(4): 409-414.

VINSON, S.B. 1975. Source of material in the tobacco budworm which initiates host-searching by the egg-larval parasitoid, *Chelonus texanus*. *Ann. Entomol. Soc. Am.* 68: 381-384.

VINSON, S.B. 1976. Host selection by insect parasitoids. *Ann. Rev. Entomol.* 21: 109-133.

VINSON, S.B. 1977. Behavioral chemicals in the augmentation of natural enemies, pp. 237-279, *in* R.L. Ridgeway and S.B. Vinson (eds.). Biological control by augmentation of natural enemies: insect and mite control with parasites and predators. Plenum Press, New York.

VINSON, S.B. 1978. Courtship behavior and source of sexual pheromone from *Cardiochiles nigriceps*. *Ann. Entomol. Soc. Am.* 71(6): 832-837.

VINSON, S.B. 1981. Habitat location, pp. 51-78, *in* D.A. Nordlund, R.L. Jones and W.J. Lewis (eds.). Semiochemicals: Their Role in Pest Control. Wiley, New York.

VINSON, S.B. 1984. How parasitoids locate their hosts: a case of insect espionage, pp. 325-348, *in* W.J. Lewis (ed.). Insect Communication. Academic Press, London.

VINSON, S.B. 1985. The behavior of parasitoids, pp. 417-469, *in* G.A. Kerkut & L.I. Gilbert (eds.). Comprehensive Insect Physiology, Biochemistry and Pharmacology Volume 9. Pergamon Press, New York.

VINSON, S.B., BARFIELD, C.S., and HENSON, R.D. 1977. Oviposition behavior of *Bracon mellitor*, a parasitoid of the boll weevil (*Anthonomus grandis*). II. Associative learning. *Physiol. Entomol.* 2: 157-164.

VINSON, S.B., and IWANTSCH, G.F. 1980. Host suitability for insect parasitoids. *Ann. Rev. Entomol.* 25: 397-419.

WARDLE, A.R., and BORDEN, J.H. 1985. Age-dependent associative learning by *Exeristes roborator* (F.) (Hymenoptera: Ichneumonidae). *Can. Entomol.* 117: 605-616.

WEISSLING, T.J., and KNIGHT, A.L. 1994. Passive trap for monitoring codling moth (Lepidoptera: Tortricidae) flight activity. *J. Econ. Entomol.* 87(1): 103-107.

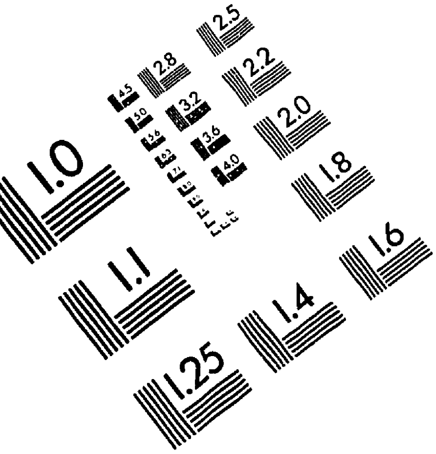
WELLINGS, P.W. 1991. Host location and oviposition on animals, pp. 75-107, *in* W.J. Bailey and J. Ridsdill-Smith (eds.). Reproductive Behavior of Insects: individuals and populations. Chapman & Hall, Melbourne.

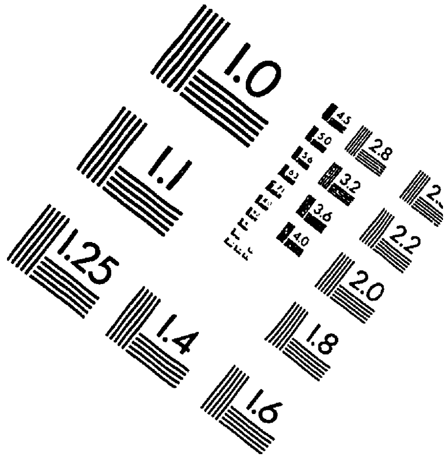
WESELOH, R.M. 1981. Host location by parasitoids, pp. 79-95, *in* D.A. Nordlund, R.L. Jones and W.J. Lewis (eds.). Semiochemicals: their Role in Pest Control. Wiley, New York.

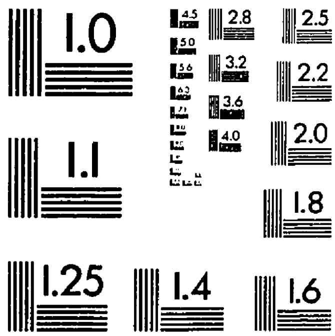
WINSTON, M.L. 1997. The worm in the apple, pp. 80-99, *in* M.L. Winston (author). Nature wars: people vs. pests. Harvard University Press, Cambridge.

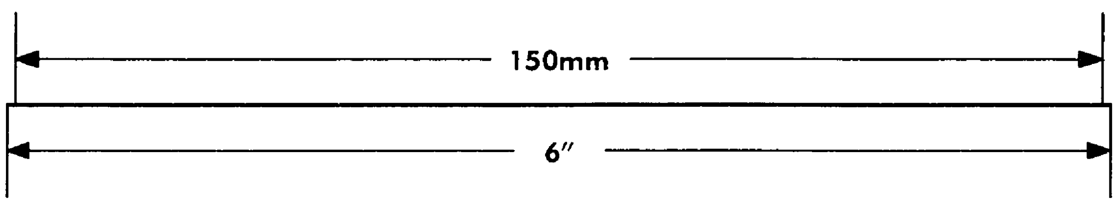
ZAR, J.H. 1996. Biostatistical Analysis. Prentice-Hall, Upper Saddle River, New Jersey.

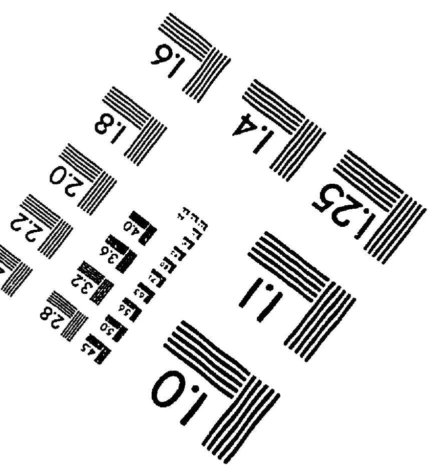
IMAGE EVALUATION TEST TARGET (QA-3)

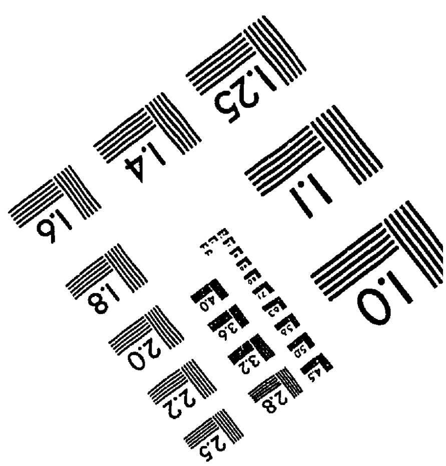












APPLIED IMAGE, Inc
1653 East Main Street
Rochester, NY 14609 USA
Phone: 716/482-0300
Fax: 716/288-5989