Chapter 27
Insect Pheromones: Useful Lessons for Crustacean Pheromone Programs?

Thomas C. Baker

Abstract Insect pheromones, especially sex pheromones, have successfully contributed to pest management programs around the world since the 1970s. In this chapter I examine some of the ways in which pheromones have been used in insect management programs and introduce some of the real-world issues that have promoted, and hindered, their adoption for use against different species. These include biological differences in the mate-finding behaviors of different species, the chemistries of the pheromones that they use, the successful engineering of controlled-release dispensers for different compounds, as well as the different political, economic, and use-pattern-related situations that exist even just in the United States to more strictly regulate the use of some types of pheromones and techniques than others. The experiences of entomologists who have witnessed insect pheromones finding their place in integrated pest management systems over the past four decades should be instructive in helping crustacean biologists develop crustacean pheromone systems into useful management tools. By far the greatest use of insect pheromones has been for monitoring existing populations and detecting the presence of invasive species. Monitoring with pheromone traps allows for other, curative measures such as insecticide applications or cultural/biological controls to be implemented intelligently. Mating disruption has taken its place in different cropping systems around the world, especially in fruit orchards. Research has now shown that pheromone does not need to completely shut off mating, but merely impede it to the point of delaying first and second matings in females to reduce their fecundity by ca. 50%. Mass trapping by deploying large numbers of pheromone traps regularly spaced in the cropping area has reemerged as a viable and effective technique for using pheromones directly for population suppression. This use of pheromones has been most effective in male-emitted pheromone systems such as in pest species of weevils (snout beetles). In these systems females can be trapped out using the synthetic version of the male-emitted pheromone, and thus egg-laying is

T.C. Baker (✉)
Department of Entomology, 105 Chemical Ecology Laboratory, Penn State University, University Park, PA 16802, USA
e-mail: tcb10@psu.edu

T. Breithaupt and M. Thiel (eds.), *Chemical Communication in Crustaceans*,
DOI 10.1007/978-0-387-77101-4_27,

directly reduced by mass trapping of females. Although the experimental, technological, and legal hurdles of developing pheromones for widespread use in the field can seem daunting, experience has shown that with determination and a real need, these hurdles can be overcome.

27.1 Introduction

As has been articulated by many authors in other chapters of this volume, relatively few crustacean pheromones have been isolated and identified, and their behavioral effects demonstrated. The reasons for this are manifold, involving methodological difficulties inherent in working in aquatic environments and availability of sufficient numbers of animals in the proper physiological state to allow the acquisition of sufficient amounts of pheromone and bioassaying their activities.

At this point, the field of crustacean pheromone research seems to be appropriately focused on the necessities of developing basic techniques for chemically identifying pheromone components. Ideas for practical application of these pheromones have only recently begun to be proposed, for instance, in the trapping of invasive species (Hardege and Terschak, Chap. 19), preventing fouling of ships by barnacles (Clare, Chap. 22), and reducing fish parasitization in aquaculture (c.f. Mordue Luntz and Birkett 2009). But if identifications can be successful, there likely will follow many actual efforts and field tests to put pheromones to use. These uses, if crustacean applied pheromone research is to proceed along the successful path that insect pheromone application followed, will depend on a thorough understanding of the behaviors of the animals in their natural environments. Knowledge of how individuals move in response to pheromone signals carried by currents along sandy bottoms or among complex reef structures will allow the most effective strategies to be designed and tested for manipulating crustacean behavior.

Over 35 years ago, I became interested in learning more about arthropod chemical communication while working as a research technician in Wendell Roelofs' laboratory at Cornell University. I was struck by the precision of insect sex pheromones and their ability to evoke full-blown, hard-wired sexual responses so reliably that if one had the correct blend of compounds, it could be used to sensitively attract and trap males of the target species and determine whether or not suppressive action needed to be taken against them. I was involved in the electrophysiological and behavioral bioassaying of prospective new pheromone blends for scores of pest species of moths and saw the power of sex pheromones, first in laboratory assays and then under field conditions in upstate New York apple orchards. My sticky traps sometimes overflowed with male moths in a seemingly magical process by which the males could not help but be lured to their demise from hundreds of meters away.

Successful use of pheromones in insect control depends upon successfully controlling insect behavior through the emission of synthetic pheromone blends. The behaviors that have turned out to be most amenable to and important for

manipulation are those that involve long-distance attraction. In this chapter I will outline some of the lessons we have learned from decades of research on insect sex pheromones and the intense efforts that have been made to develop successful integrated insect pest management systems with them. Such systems now utilize insect sex pheromones for monitoring, mass trapping, and mating disruption. My goal is to set the stage (an aquatic one) for efforts to develop and use crustacean pheromones in a similarly successful manner. I believe that the concepts developed for insect systems can provide bright illumination for many successful underwater pheromone applications.

27.2 Pheromone Emission and Pheromone Identity

In moths, sex pheromone communication occurs at a particular time of the day or night depending on the species. Females, usually more than 1 day old, take up a "calling" posture (Fig. 27.1a) by raising the abdomen and everting glandular tissue at the end of the abdomen associated with the ovipositor from which pheromone is emitted at a rate of a few tens of picograms per second.

Sex pheromones in insects include a variety of molecules from large, but volatile, aliphatic molecules to small cyclic monoterpenoids. Moth pheromones are the first pheromones identified and are the most widely studied of all the insect pheromones. They include a huge collection of mostly female-emitted chemical blends that are composed of typically fatty acid-derived molecules that function

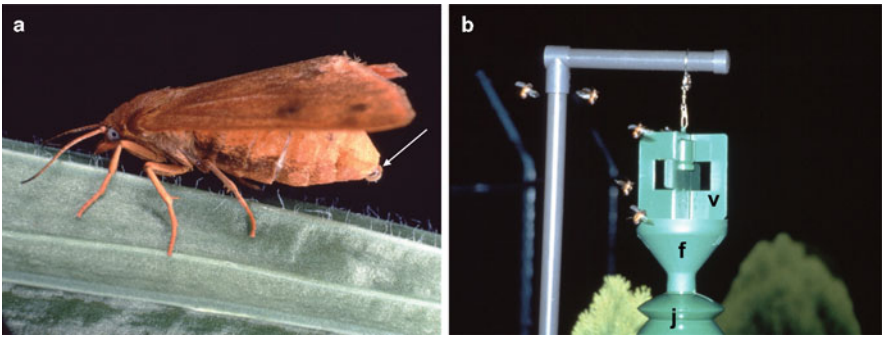

Fig. 27.1 (**a**) Photograph of a calling female arctiid moth. Notice the everted pheromone gland tissue at the end of the abdomen (*arrow*) associated with the ovipositor. (**b**) An example of a typical pheromone trap for male scarab beetle pests of turf and ornamentals, including cranberries and blueberries. The trap has intercepting vanes (v) designed to stop flight and cause the males to fall into a funnel (f) and finally into a collecting jug (j) containing a small amount of ethylene glycol-water from which they cannot escape. Males can be seen here being attracted to the trap by the synthetic female-emitted sex pheromone of this species. (**a**) From Roelofs, W.L. and Cardé, R.T., 1971, Science 171:648–686. Reprinted with permission of AAAS; (**b**) From Leal, W.S., Sawada, M., Matsuyama, S., Kuwahara, Y., and Hasegawa, M., 1993, J. Chem. Ecol. 19:1381–1391. Reprinted with permission of Springer Science & Business Media

over long distances (10–100 m). Recently, the literature regarding moth pheromone blends as well as other insect pheromones and chemical structures has been compiled into one website, (http://www.pherobase.com), an extremely useful free information resource.

The majority of moth pheromones identified thus far are blends of 10–18-carbon-long straight-chain primary alcohols, acetates, or aldehydes (Fig. 27.2a, b). However, one interesting second major class of moth pheromone components is found in many noctuids, geometrids, arctiids, and lymantriids and is composed of blends of polyene epoxides (Fig. 27.2c) and hydrocarbons (Fig. 27.2d). For the majority of moth species, after synthesis of the fatty acyl chain to either 16 or 18 carbons in length, these pheromone component precursors begin to achieve some species-specific structural differences that are imparted by one or more desaturases that place double bonds at specific locations in the fatty acyl chain. The desaturation step is, depending on the species, either preceded or followed by enzymatic beta-oxidation to reduce the chain length of the molecule.

Merely reversing the two-step sequence in which these two enzyme systems work creates most of the major differences in monounsaturated pheromone component structures in moths. Diunsaturated compounds with double bonds in two

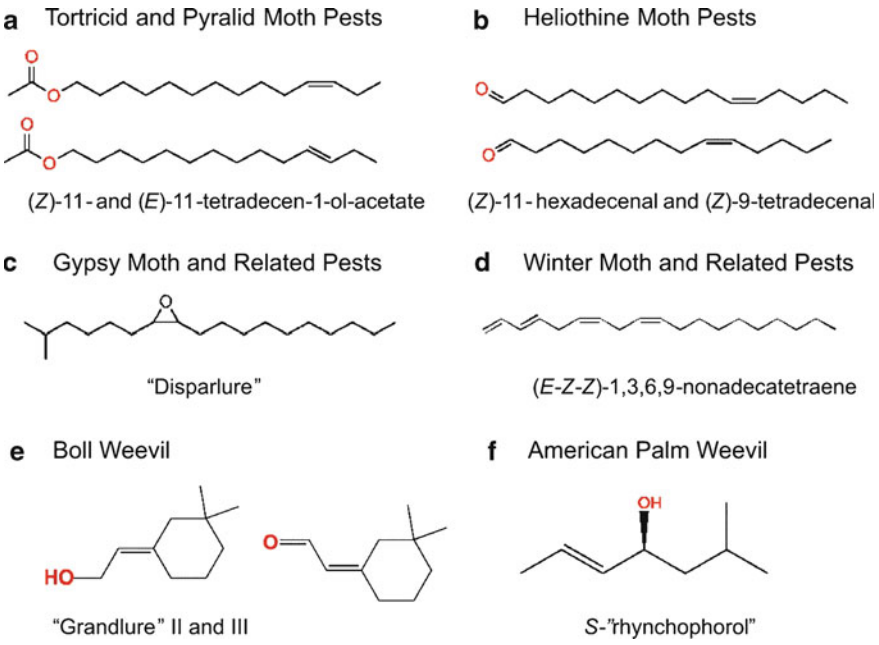

Fig. 27.2 Pheromone structures of some groups of moth and beetle pests. The names of the chemical structures in the blend of boll weevil pheromone components (2e) are (*Z*)-2-(3,3,-dimethyl)-cyclohexylideneethanol (grandlure II) and (*Z*)-(3,3-dimethyl)-cyclohexylideneacetaldehyde (grandlure III). The chemical name of *S*-rhynchophorol (2f) is 4S-(*E*)-6-methyl-2-hepten-4-ol. From "The Pherobase" (http://www.pherobase.com)

locations on the chain are created by the desaturase acting twice, both before and after the chain-shortening step. Final species-specific structural differences result from the final biosynthetic step, conversion of the fatty acyl molecule to its active functional group by reductases and oxidases. A subset of two or three compounds from the collection of precursors of different chain lengths and double bond positions may all be converted as a group to comprise the unique blend of that particular species. Precise blend ratios of such two- and three-component sex pheromones are the rule in moth systems, and deviations from these ratios elicit lower levels of upwind flight in males (Baker 1989; Linn et al. 1986a,b; see below).

27.3 Behavioral Responses to Sex Pheromones are Indirect and Depend On Flow-Detection

Males of each species are synchronized in their daily activity to the behavior of females and are optimally responsive to pheromone at the same time of the day as females emit pheromone. They are capable of responding to pheromone by flying upwind in the plume from tens, even hundreds of meters downwind to locate the source (Fig. 27.1b).

Steering with respect to a time-averaged pheromone concentration gradient has often been erroneously invoked as an orientation mechanism used by pheromone-responding insects. This type of *direct* chemical-gradient steering (chemotaxis) is in fact NOT used by moths to locate females. Rather, two *indirect* reactions to sex pheromone, "optomotor anemotaxis" combined with "self-steered counterturning," are the mechanisms that result in the successful location of sex pheromone sources by insects. The anemotactic response (steering with respect to the wind) in response to flow information is performed via optical feedback and not wind mechano-sensors. Detection of wind-induced motion across the eyes results in compensatory motor responses to stabilize this motion; hence, the name optomotor anemotaxis. The result is a pheromone-*modulated* (not pheromone-steered) upwind displacement of the insect toward the pheromone source (Kennedy 1983).

We have learned in more detail since the initial landmark studies by J.S. Kennedy's group (Kennedy and Marsh 1974) how these two programs are performed when a flying insect encounters a series of pheromone strands of the right quality. In optomotor anemotaxis, the moth compensates with changes in its direction of thrust and corrections in its airspeed for any off-course displacement or groundspeed maintenance difficulties caused by changes in wind direction or velocity. During pheromone stimulation, the optomotor anemotactic response keeps the insect heading and progressing more or less directly upwind. The other, nonanemotactic program (Baker and Kuenen 1982), is a "counterturning" oscillatory motor pattern of rapid left–right reversals of direction (Kennedy 1983) that is performed concurrently with anemotaxis. Counterturning frequency increases upon

contact with pheromone and coincides with more upwind-oriented optomotor anemotactic steering (Baker and Haynes 1987). Counterturning frequency slows down upon loss of pheromone at the same time as clean air causes the anemotactic program to relax and allow more cross-wind, transverse optical image flow. The result in clean air is a slower-reversing, greater amplitude cross-wind left–right "casting" flight (Kennedy 1983; Baker 1989).

For crustaceans walking or swimming in a plume of sex pheromone, we should anticipate that a similar set of indirect responses (i.e., rheotaxis and looping or side-to-side zigzagging) might be switched on by pheromone that help the animal execute the optimal set of maneuvers to steer up the flow to the sex pheromone source. For instance, swimming male copepods have been shown, like moths, to "cast" cross-current when their pheromone odor is lost (Yen and Lasley, Chap. 9). Benthic crustaceans like lobsters, crabs, and crayfish walk along the substratum to locate an odor source. We can expect that for such animals flow direction can be discerned by using mechanoreceptors on the body or involving the antennules allowing them to orient by odor-gated rheotaxis (Weissburg, Chap. 4).

For macroscopic crustaceans such as shrimp that swim in response to sex pheromones, we might expect that optical feedback would be essential in allowing up-current progress to be made toward the pheromone emitter via optomotor rheotaxis. Obviously sufficient light and light-gathering abilities of crustacean eyes would be needed for sufficient edge- and motion-detection to occur. Over shorter distances, other mechanisms not known in insects might be employed. For instance, in some copepod species (e.g., *Acartia tonsa*) males have been shown to be able to locate females by using their wake structure, which is facilitated by the viscuous properties of water (Yen and Lasley, Chap. 9).

Some differences in orientation behavior between crustaceans and flying insects are due to the slower ambulatory speeds of some of the animals that have been researched thus far (lobsters and crabs). In addition, the narrowness of close-range food-odor plumes that crustaceans respond to along the ocean bottom creates opportunities for left–right chemotaxis decisions that do not exist for tiny insects flying in very wide and fast-shifting pheromone plumes far from the source.

Many of the potential differences between the orientation mechanisms used by crustaceans compared to insects will have to do with fluid dynamics that dictate plume dispersion directions as well as microturbulence that determines fine plume structure due to the shedding and shredding of pheromone strands from the source. Whether or not individual strands can be detected and resolved by a crustacean's receptor system will determine whether the animal can only time-average the odor concentration at a particular distance from the source. Some crustaceans can use "temporal sampling" of odor flux in strands with chemosensor responses of up to 5 Hz (Gomez and Atema 1994). However, as pointed out by Weissburg (Chap. 4), time-averaging may be all that is needed for slower-moving, large crustaceans such as lobsters.

27.4 Insect Sex Pheromone Olfaction Systems: Flux Detection and Mixtures

Wright (1958) recognized that an odor plume consists of strands of odor that issue from the surface of the odor source. The strands are sheared from the release surface and drift downwind where they become stretched, twisted, and more tortuous as they are ripped apart into substrands during their journey farther downwind (Murlis 1986). These disjointed substrands interspersed with pockets of clean air comprise what we call the odor plume. However, it was not until intermittency of stimulation from the plume was shown to promote sustained upwind flight (Baker et al. 1985) and subsequently when moths were shown to have the ability to react during upwind flight to the subsecond changes in pheromone concentration (Baker and Haynes 1987) that we began to understand that these individual pheromone strands and pockets of clean air are what are producing the sustained upwind flight behavior that we call attraction (Vickers and Baker 1994; Mafra-Neto and Cardé 1994).

It has become clear that, as emphasized by Kaissling (1998), the insect olfactory system is designed to optimize flux detection and not to measure concentration. This is a major reason why chemotaxis is not used by pheromone-responding insects. Rapid increases in flux from individual plume strands and decreases from the clean-air pockets between strands are essential to producing reiterative upwind surges that result in the attraction of male moths to pheromone sources. The speed of the reaction to pheromone ON and pheromone OFF is related to the need to change behavior according to swings in the wind direction to prevent erroneous upwind movement during zero odor conditions. In crustaceans we might expect behavioral reaction time to pheromone ON and OFF to match the shifts occurring in current flow direction and speed (see Fig. 4.2 in Weissburg, Chap. 4) and the walking or swimming speeds of the respective species.

27.4.1 Subsecond Reaction Times of the Two Programmed Responses to Pheromone

The timescale over which the moths' reactions to pheromone strands and clean air occur is remarkably small. The behavioral responses to both the onset (upwind surge) and loss of filaments (cross-wind casting) can be as fast as 0.15 s in *Grapholita molesta* (Busck) (Baker and Haynes 1987), but usually are between 0.3 and 0.6 s; Vickers and Baker 1997). In studies of host-odor responses by flying female moths, only the latency of the casting flight response to loss of the odor has been measured, and its time course is similar to that of the latency for pheromone loss, 0.7 s (Haynes and Baker 1989).

Recent findings with terrestrial crustaceans seem to indicate that their olfactory systems have adapted to detect and process airborne volatiles (and thus are quite similar to insect olfactory systems), while maintaining the general olfactory

pathways of aquatic crustaceans (Schmidt and Mellon, Chap. 7; Hansson et al, Chap. 8). Crustaceans' peripheral receptor neurons appear to be flux detectors, just as in insects, with fast on–off responses. The giant robber crab's aesthetascs on its antennules respond with high-speed depolarizations similar to insects' electroantennograms when challenged with puffs of airborne odor from odor cartridges (Stensmyr et al. 2005). Terrestrial hermit crabs possess pathways in their olfactory systems that appear to parallel those of insects in terms of both high-speed odor processing and odor classification (see Hansson et al., Chap. 8). Marine crustaceans such as the American lobster or spiny lobsters are known to flick their aesthetascs to increase flux and increase detection sensitivity (Koehl, Chap. 5).

27.4.2 Relevance of Fast Pheromone-Strand Reaction Times to Field Attraction

The rapid response to an odor strand is important because the strand has been shed from the odor source, and in higher velocity airflow, continues to travel in a more or less straight line away from the source (Fig. 27.3) (David et al. 1982; Elkinton and Cardé 1987). This trajectory allows the responding insect to move in a straight line toward the source if it flies upwind each time it detects an odor strand: the insect can eventually locate the source by steering into and progressing upwind through reiterative upwind surges in response to strands (Fig. 27.3b). It is, however, just as important for the insect to go into cross-wind casting quickly when it encounters clean air between pheromone strands as it is to surge upwind in response to the strands, because any such clean-air pocket can turn out to be a very large expanse of clean air due to large swings in the wind direction (Baker 2008; David et al. 1983) (Fig. 27.3b). If the insect continues to move upwind into a large pocket of clean air for any length of time, it will, to its detriment, distance itself rapidly from the odor plume that it has just lost contact with while steering increasingly off-line away from the odor source (David et al. 1982).

A subsecond, rapid cessation of upwind progress in response to odor loss and a shift to a more cross-wind track (usually after only one left-to-right reversal of flight direction), and a coupling of these anemotactic reactions to an internal motor program of increasingly lengthy side-to-side oscillations, allows the insect to increase its ability to quickly regain contact with the odor strands that have swung to one side or the other of its body (Figs. 27.3b and 27.4). In some species of moths, the surge reaction in response to pheromone strands and the initiation of casting in response to clean air can be fast enough that the moths can often "scoot" over in response to a shifting wind-line plume, using a sawtoothed-shaped succession of left–right surge-cast tracks (Fig. 27.4). Normally the subsecond alternation between surging and casting in response to strands and clean-air pockets in a plume is seen as "zig-zagging upwind flight" (Figs. 27.3b, 27.4, and 27.5). When the insect encounters a large pocket of clean air due to a wind-swing, the long-duration

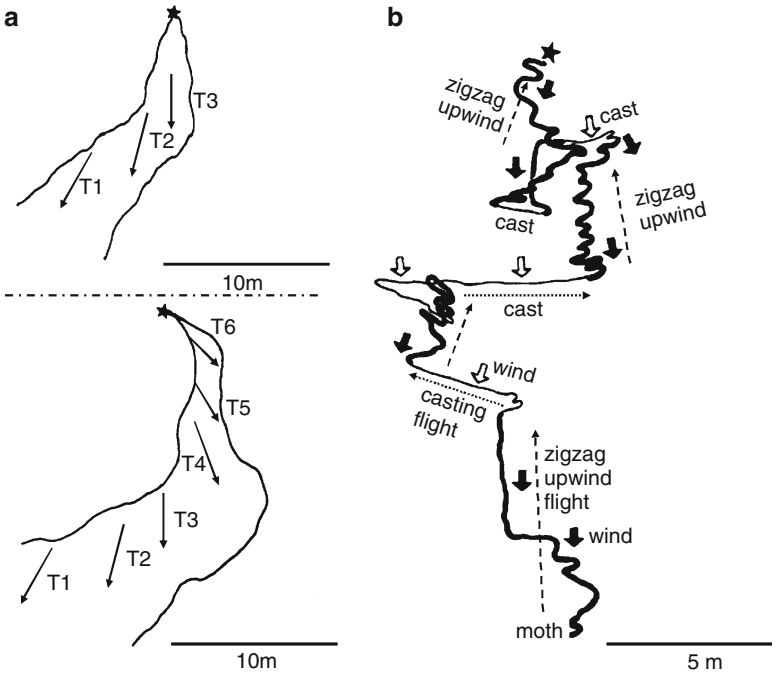

Fig. 27.3 (**a**) *Top*: The different directions of wind within a smoke plume in the field recorded on videotape by David et al. (1982), illustrating how odor-bearing air parcels have been sheared from the source (*black star*) and are transported away in straight lines (*arrows*) at different time periods (T1–T3), regardless of the instantaneous shape of the time-averaged plume (*black lines* outlining the sinuous snapshot-image of the plume). *Bottom*: Depicted here, ca. 5 s later, the wind has completed a swing so that new odor-bearing air parcels are flying away in straight lines as they were sheared from the source at times T4–T6. The air parcels sheared at times T1–T3 continue on along their previous trajectories. Adapted from David et al. (1982). (**b**) Fifty-second-long flight track of a male gypsy moth that was video-taped by David et al. (1983) as it approached a source of sex pheromone (*black star*) in a wind-field (0.8–2.0 m/s) that shifted to a similar degree as the wind in (**a**). Thick black flight tracks indicate periods when the moth was in contact with pheromone, with wind directions at these times shown as *thick black arrows*. *Thin-lined flight tracks* indicate periods when the male was not in contact with pheromone, with wind directions shown as *hollow arrows*. Progress directly towards the source was due to upwind flight in the pheromone plume (*dashed lines*) and not due to cross-wind casting (*dotted lines*). Adapted from David et al. (1983)

left–right oscillations of casting flight provide a behavioral bridge over clean air that helps position the moth in an unbiased left–right fashion to recontact pheromone strands. Thus, the more typical pheromone-mediated flight track of an insect exhibits an alternation between long-duration casting in response to large clean air pockets and upwind zigzagging (rapid surge-casts) in response to the finely structured plume with its intermittent pheromone strands (Figs. 27.3b and 27.5). The result is a halting succession of upwind flight tracks that gets the insect to the source in a shifting wind-field (Figs. 27.3b and 27.5).

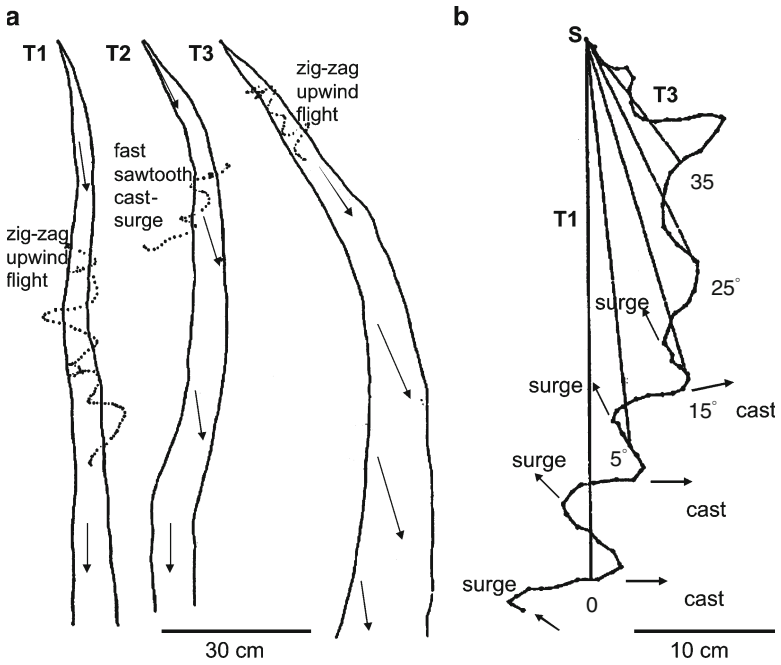

Fig. 27.4 (**a**) Snapshots at three times ca. 0.5 s apart, T1, T2, and T3, of the flight track (*dotted lines*) of a male oriental fruit moth flying upwind in response to pheromone in a wind-field that is shifting in direction (*arrows*) by 35° in 2 s. The time-averaged outline of the pheromone plume is shown as *solid wavy lines*. *Dots* indicate the location of the male every 1/60 s. This male "scoots" over with a sawtooth track during T2, alternating between upwind surges and cross-wind casting on a left–right basis every 1/6 s, approximately. (**b**) Flight track (enlarged) of another male during the T2–T3 section that reacted every 1/6 s to pheromone and then clean air on each successive asymmetric track reversal. Dots indicate the male's position every 1/30 s. Long straight lines indicate each 5° shift in the direction of the wind line. "S" denotes the location of the pheromone source (adapted from Baker and Haynes 1987)

27.4.3 Precise Pheromone Blend Composition Determines Intensity of Upwind Flight Behavior

In insects, the optimal blend ratio of synthetic pheromone components for attraction and source contact by males has been shown to be the one that most closely approximates the natural female-emitted ratio. Linn et al. (1986a, b) convincingly demonstrated in field experiments that the optimal blend causes the initiation of upwind flight in more males from greater distances from the source than do blends comprised of suboptimal ratios or those from which some components are missing. The duration and length of males' upwind surging reactions to single strands of pheromone depend on their quality (Vickers and Baker 1997; Quero et al. 2001). Strands of the correct pheromone blend tainted with small amounts of heterospecific antagonist are poorer in intensity than are the surges to strands of the pure

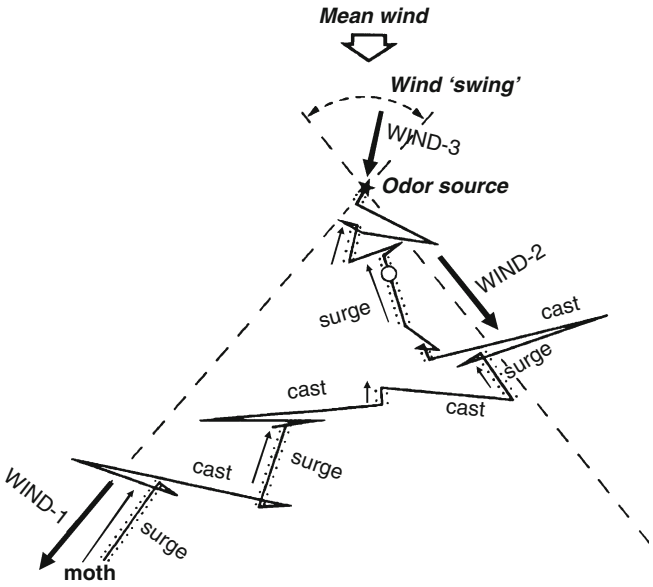

Fig. 27.5 A depiction of the average types of changes in flight track movements of gypsy moths flying upwind to a pheromone source (*black star*) over short grass in a plume that swings nearly 90° in a matter of seconds due to shifts in wind direction (*solid arrows* labeled Wind-1 and Wind-2). The distance from the source to the start of the flight track at lower left is ca. 15 m. Upwind surges by the moth (*dashed arrows*) in response to pheromone-bearing wind (small stippling on either side of the flight track) occur periodically and are interspersed with short periods of left–right casting across the wind line in clean air (no stippling). Wind direction at the time the male locates the source is Wind-3 (adapted from David et al. 1982)

pheromone blend alone (Vickers and Baker 1992). The blend of pheromone molecules must arrive simultaneously on the male's antenna, i.e., they must be perfectly blended in each strand, to have their optimal effect. Plumes from females of two different heliothine moth species (Fig. 27.2b) calling from ca. 5 cm lateral spacing apart can reduce the successful attraction of males by 80% or more, even though only 50% of the plume strands from the females are "mixed", i.e., arriving on the male antenna within 120 ms of each other (Lelito et al. 2008). For crustaceans (e.g., crayfish), in habitats populated by closely related species, males may need to discriminate between odor plumes from conspecific and heterospecific females.

27.5 Pheromone Dispenser and Trap Design: Much Technological Development Needed

Decades of experience with attempts to develop insect sex pheromones as effective pest management tools can be instructive as to the possible ways crustacean pheromones might be used. Strong plumes of the correct blend of components

that create above-threshold plume strands far downwind of the pheromone source have been the key to the optimal use of pheromones in detection, mass trapping, and mating disruption. It should be anticipated that crustacean pheromones will find their optimal uses when pheromone plumes in aquatic environments can be optimized to be above behavioral thresholds at maximal distances downstream for months at a time. In large-scale monitoring/detection programs involving sometimes thousands of traps, the visiting of traps by human scouts and replacing their lures every week or two is prohibitively expensive.

For such plumes to be created and maintained for long time periods, much work on controlled release systems in aquatic environments will be needed. In the insect world, dispensers have had to be optimized on a trial-and-error basis, based on behavioral reactions (trap catch) to various dispenser types and pheromone loading rates. Optimization has had to be assessed on a species-by-species trial system because each species has different pheromone components comprised of differing molecular weights and functionalities such as aldehydes, alcohols, and acetates. The field longevity of these same dispensers had to be similarly assessed based on trap capture levels over time.

In aquatic environments the challenges will be great in designing effective, long-lasting pheromone dispensers for emitting possibly highly labile molecules of varying solubilities and migration rates through dispenser walls. The experimentation needed to achieve such optimization will involve, just as it has for insect pheromones, relatively thankless trial-and-error experimentation that will be viewed as "engineering," not science, by many crustacean pheromone researchers' colleagues.

As with the experimentation needed for optimizing dispenser-controlled emissions technology, much experimentation will be needed to create the most effective pheromone traps for each targeted crustacean species. For insects, optimizing the pheromone blends and dosages to create the best lures for different pest species has only been part of the task at hand to make pheromones useful for monitoring and detection. The efficacies of traps cannot be predicted, because even within genera, different species of insects respond differently to different trap shapes, sizes, and colors (c.f., Fig. 27.1b). All of these variables must be tested independently for each new species. In addition, for insects, further testing always needed to be done to optimize deployment schemes for traps to optimize capture rates. These assays involve extensive testing to identify seasonal trap attractiveness, the most efficient trap density, and favorable trap positions. The latter includes habitat differences, e.g., in sun or shade as well as higher vs. lower positions above the substratum or the vegetation canopy. For optimizing uses of pheromones in crustacean systems, similar methodical experimentation will no doubt need to be performed that relates to such real-world issues as how the availability of natural shelters and harborages can affect trap capture levels and also how capture can be affected by other complexities of the underwater terrain (see Aggio and Derby, Chap. 12).

These are time-consuming and difficult-to-publish types of studies, but have proven necessary for optimal decision-making involving curative measures to be taken that depend on the correct interpretation of trap catch levels. Crustacean

pheromone researchers should be ready to perform similar types of experimentation in order to use crustacean pheromones most effectively. They should be aware of their colleagues' potential disdain for these types of applied, nonhypothesis-driven studies.

27.6 Pheromones for Monitoring, and for Detection and Eradication of Invasive Species

Monitoring the population densities of endemic pests with pheromone traps has been done successfully for decades against scores of species worldwide. When pheromone traps are used for monitoring, they are widely spaced and are at such a low density that they have no effect on reducing population density by themselves. They only serve as sentinels to trigger, at some threshold capture level, the application of other pest control techniques such as insecticide sprays against larvae at some later date that is often based on accumulated thermal units (Baker 2008).

Insect sex pheromone traps also have played a large role in detecting movements of adult pest species from one region to another and also even from noncrop areas into crop areas. Survey programs involving grids of pheromone traps are used routinely to report and track the yearly arrival of migrating adult populations of insects such as the black cutworm *Agrotis ipsilon* (Hufnagel) in the Midwest (Showers et al. 1989) or expanding populations such as the gypsy moth *Lymantria dispar* L. (Fig. 27.2c) (Elkinton and Cardé 1981). This approach has become an essential part of our arsenal of tools for tracking and mitigating pest-movement-related threats.

As an example, boll weevil pheromone traps were used in a highly successful boll weevil population suppression program in the southeastern United States (Fig. 27.2e). Release of sterile boll weevils and also diapause control procedures (insecticide applications to kill adult weevils before cotton begins fruiting) were triggered if pheromone trap captures exceeded two to five weevils per trap (Ridgway et al. 1990; Baker 2008). Over the years of this program across six southeastern states, boll weevil capture levels declined to near zero and it was economically advantageous to grow cotton again in this region (Baker 2008). The program was extensive and not cheap. In 1988 alone, approximately 590,000 traps were deployed, in which more than 8.25 million dispensers were used (Ridgway et al. 1990). Many more lures than traps were needed because the dispensers were replaced every 2 weeks over the year in order to keep lure attractiveness at optimal levels. The sensitive and reliable detection capabilities provided by pheromones for insect monitoring and detection should also be found in crustacean pheromones. Even if only one sex is attracted, the extra sensitivity of responders to these pheromones compared to food and other general odors should make them a preferred lure to use.

27.7 Pheromone Mass Trapping: Successful Rebirth of an Old Idea

Mass trapping is the deployment of a sufficiently high density of pheromone traps that ensnares and eliminates enough adults from the population and reduces subsequent larval damage. Mass trapping differs from trapping for monitoring or detection in that mass trapping alone directly diminishes the pest population. Although in the early days of sex pheromone research mass trapping for control of pest populations was discounted as impractical on many fronts, a few decades of further experience have shown that this technique can be highly successful against certain species. For instance, research and development of mass trapping systems using male-produced sex pheromones for a variety of highly damaging tropical weevil species has resulted in successful commercial mass trapping systems for suppressing populations of these species (Oehlschlager et al. 2002; Baker 2008). Successive years of mass trapping using pheromone (Fig. 27.2f) dispensed in inexpensive bucket traps at a density of only one trap per 7 ha reduced the damage to trees from the American palm weevil to near zero, as illustrated on thousands of hectares of oil palms on two plantations (Fig. 27.6).

If mass trapping for direct population control is to be used against crustacean pests, the target species likely will need to have some of the same attributes as these weevil species (Oehlschlager et al. 2002). First, the crustacean species should use a sex pheromone system that attracts females, the egg-layers, so that mass trapping will directly reduce egg-laying. Secondly, the adults of the target species should be present in relatively small numbers, live a long time before egg-laying, and lay a small number of eggs whose emerging larvae cause a relatively large amount of damage. Thus, as in the case of the weevils, trapping out females steadily over a period of weeks will remove a large proportion of the egg-layers in any generation and have a large impact on subsequent populations. Trapping males will be less likely to reduce populations because male insects are usually capable of mating once per day and females only once or twice per lifetime. Thus, a single male can mate with multiple females over his lifetime (for crustaceans see, e.g., Gosselin et al. 2005), and this feature allows any remaining males to "replace" the missing inseminations that would have been achieved by the other males had they not been trapped out. Third, if the individuals are sufficiently vagile and the attractive power of the pheromone strong enough that the females can be trapped over long distances, the traps can be widely spaced and thus be deployed at economically favorable numbers in terms of both traps and the pheromone lures that are needed throughout the control program (Oehlschlager et al. 2002).

27.8 Pheromone Mating Disruption Delays Mating

Mating disruption is the technique by which pheromone is dispensed into a pest habitat in sufficient amounts to reduce the ability of males to find females, or vice versa. Here the searching sex is attracted toward extra-high release-rate synthetic

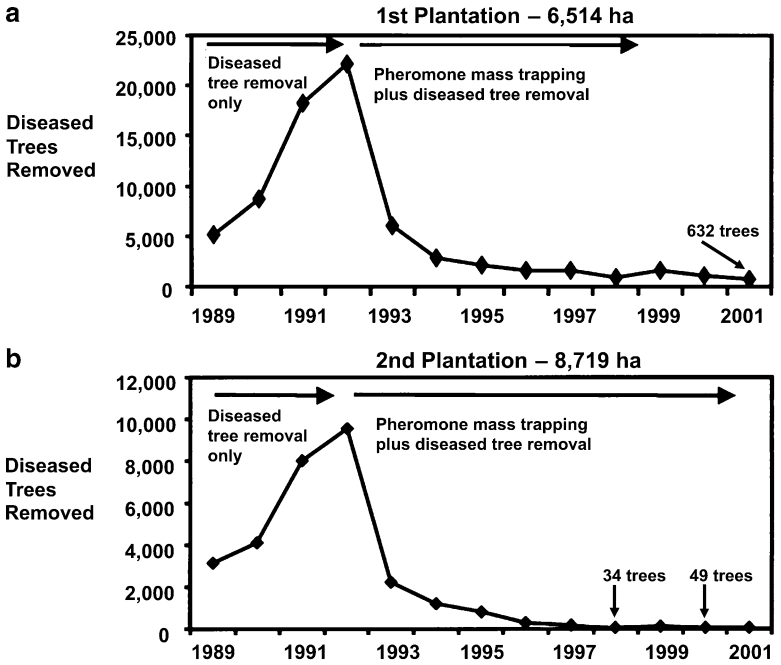

Fig. 27.6 Incidence of *R. palmarum* weevil-vectored red ring disease in oil palms as indicated by the number of diseased palms that had to be removed in two large plantations in Costa Rica before and after nine successive years of mass trapping of the weevils. From 1989 to 1992 with no mass trapping, disease incidence steadily rose and tremendous economic losses were incurred due to the removal of mature fruit-bearing trees. After pheromone mass trapping was begun in 1992, the incidence of tree removal due to red ring disease declined dramatically due to the elimination of adult weevils from the population and near-elimination of egg-laying, larval damage, and disease spread (adapted from Oehlschlager et al. 2002)

pheromone dispensers, and in the process, habituated by a prolonged exposure to the strong flux in these strands, rendering these individuals incapable of smelling (and finding) their mates. In the earliest mating disruption trials (early 1970s), it was thought that it was essential to dispense the pheromone such that a ubiquitous cloud of pheromone pervaded every cubic meter of habitat to disrupt mate-finding communication. The earliest trials concentrated on the use of microcapsules sprayed through conventional pesticide sprayers to achieve uniform coverage. These trials were mostly failures because the tiny microcapsules expended their allotment of pheromone from their small reservoirs in only a few days because the capsule membranes allowed pheromone to escape too fast. Making the walls thicker or a slower-releasing matrix resulted in too slow release rates again causing failure to impede mating.

Then, the first EPA-registered mating disruption formulation, the Conrel hollow fibers, was developed in 1978. It used hollow microfibers ca. 2 cm in length. These fibers were distributed over cotton fields by using airplanes equipped with

specialized applicator technology. These dispensers, once they landed, stuck to the cotton leaves, from where they emitted volatilized pheromone at sufficiently high rates in the desert heat for over 2 weeks by means of an open capillary tube-type technique. The fibers were effective at disrupting mating when applied at an approximate density of one fiber per square meter of cotton crop. The amount of active ingredient (pheromone) applied per acre was only 15 g/acre applied every 2 weeks (Cardé and Minks 1995; Baker 2008).

The evolution of dispensers into a yet stronger point-source-emission mode and further away from the original uniform fog mode occurred in the mid-1980s with the commercial appearance of the Shin-Etsu "ropes." These were made up of the pheromone of the target species residing in sealed polyethylene tubes that were hand-applied in a twist-tie manner around plant stems. The reduction in the number of point sources per hectare from tens of thousands (fibers) to now only 1,000 or even 500/ha was achieved with fewer, but higher-strength point sources with no loss of efficacy.

Finally, new types of aerosol dispensers ejecting pheromone at very high rates onto pads were developed so that formulations were developed in which the number of dispensers could be reduced to only 10–15 dispensers per hectare (Mafra-Neto and Baker 1996; Shorey and Gerber 1996; Shorey et al. 1996; Baker et al. 1997; Fadamiro et al. 1999). Efficacy in disrupting sex pheromone communication of a number of different species was clearly demonstrated.

27.8.1 Successful Mating Disruption Involves both Attraction and Habituation

Why could much higher-dose dispensers deployed at a much wider spacing turn out to be more successful at disrupting female–male communication than the uniform fog created by microcapsules? The main reason turns out to be that a fog only can cause habituation (desensitization) up to a certain background level, and not higher. A strong plume, on the other hand, causes males to be attracted and spend lots of time in the plume, while at the same time they become habituated to the very highly concentrated plume strands of pheromone (Cardé and Minks 1995; Cardé et al. 1997; Stelinski et al. 2004, 2005). They get huge ups and downs in flux on a subsecond basis from the strands interspersed with pockets of clean air and this optimally stimulates the central nervous system synapses to weaken them more than would a constant low-level fog.

27.8.2 Delayed (not Elimination of) Mating is Key to Mating Disruption Success

It had routinely been assumed that successful mating disruption can only occur if the majority of females in a population are prevented from mating after the application of a mating disruption formulation. In reality, the females' abilities to

obtain their first or second matings merely needs to be impaired and delayed (Knight 1997). Delayed mating was directly confirmed in studies on European corn borer *Ostrinia nubilalis* (Hübner) using very high release rate aerosol mating disruption dispensers (Fadamiro et al. 1999). During each of the two summer flights, 100% of the females eventually became mated despite the application of high release rate, low-point-source density dispensers of the two-component sex pheromone (Fig. 27.2a) (Fadamiro et al. 1999). Damage, however, was reliably reduced by 50% every time the disruptant was used at a dispenser density of only 1 per acre of corn (Baker, unpublished). Population effects of perturbing reproductive timing could be achieved in highly seasonal crustacean species (see Breithaupt, Chap. 13; Hardege and Terschak, Chap. 19; Kamio and Derby, Chap. 20).

Analyses involving spermatophore counts showed that the mating disruptant was impairing the ability of females to attract and mate with males on a constant, daily basis (Fadamiro et al. 1999). Thus, as demonstrated in the oriental fruit moth (Rice and Kirsch 1990) and codling moth studies (Knight 1997), mating disruption success does not require keeping the population of females virgin, but rather just needs to impede females' ability to attract males and retard the dates at which they achieve their first or even second matings. Retarding the dates at which first or second matings are achieved significantly reduces fecundity in the European corn borer and codling moth by over 50% (Fadamiro and Baker 1999; Knight 1997).

27.9 Outlook and Conclusions

There is a good deal to be optimistic about the prospects for successful implementation of crustacean pheromones in population management programs involving trapping for monitoring and detection, and possibly even for mass trapping. Careful studies of behavioral responses, plume dispersion, and dispenser performance/longevity will help inform the decision-making needed for choosing optimal trap deployment locations and trap density. For mating disruption to be considered a useful tool against crustacean pests, some key aspects that are important in insect mating disruption programs should be kept in mind. First, mating disruption works best when applied on an area-wide basis. There is a larger surface-area-to-edge ratio in larger disruption plots, which is advantageous in preventing gravid females from moving in from untreated areas. Second, intense testing of dispenser technology for optimal lifetime and efficiency of the pheromone is critical. Emission rates of the target species' particular pheromone blend need to persist for as long a period as possible while economically using up as much of the active ingredient in the dispenser as possible. Third, knowledge of the behavior of the target species will help in developing strategies for deployment grid or other spacing patterns of the dispensers to ensure that most of the potential responders are contacted by the pheromone plumes from dispensers.

Mating disruption is not a curative procedure used like pesticides that can be applied to immediately reduce population levels. Like many insect pheromone

products, crustacean pheromone mating disruption may tend to get outcompeted by pesticides that can be applied on a wait-and-see, curative basis. With no other control measures taken such as insecticide sprays, it often takes three generations of successive use of disruption in order to get populations back to acceptable levels (Baker 2008). Another factor to anticipate for crustacean pheromone usage is that the biggest and most consistent successes involving insect pheromone mating disruption in North America have been in federally supported programs that have subsidized the cost of pheromone for large "area-wide" grower participation programs over periods of several years. Thus, for future crustacean systems, government agencies may have to fund the large-scale initiatives that are needed for area-wide trapping or mating disruption successes. Finally, pheromone usage for insect mating disruption has had to meet environmental regulations that have caused frustration in small companies and been daunting in the scope of requirements such as toxicity testing and label restrictions. Thus, for future uses of crustacean pheromones, registering these compounds and slow-release formulations with government agencies may be a long and difficult process, depending on the country involved, even though these are naturally occurring compounds that are emitted at extremely low rates. Despite this somewhat sobering outlook regarding pheromone regulatory issues, it is exciting to look ahead and envision the many ways in which crustacean pheromones may possibly be used to manipulate behavior and have an impact on populations for the benefit of society.

References

Baker TC (1989) Sex pheromone communication in the Lepidoptera: new research progress. Experientia 45:248–262

Baker TC (2008) Use of pheromones in IPM. In: Radcliffe T, Hutchinson B (eds) Integrated Pest Management. Cambridge University Press, Cambridge, pp 273–285

Baker TC, Haynes KF (1987) Manoeuvres used by flying male oriental fruit moths to relocate a sex pheromone plume in an experimentally shifted wind-field. Physiol Entomol 12:263–279

Baker TC, Kuenen LPS (1982) Pheromone source location by flying moths: a supplementary non-anemotactic mechanism. Science 16:424–427

Baker TC, Willis MA, Haynes KF, Phelan PL (1985) A pulsed cloud of sex pheromone elicits upwind flight in male moths. Physiol Entomol 10(2):57–265

Baker TC, Mafra-Neto A, Dittl T, Rice MA (1997) Novel controlled release device for disrupting sex pheromone communication in moths. IOBC/wprs Bulletin 20:141–149

Cardé RT, Minks AK (1995) Control of moth pests by mating disruption: successes and constraints. Annu Rev Entomol 40:559–585

Cardé RT, Mafra-Neto A, Staten RT, Kuenen LPS (1997) Understanding mating disruption in the pink bollworm moth. IOBC/wprs Bulletin 20:191–201

David CT, Kennedy JS, Ludlow AR, Perry JN, Wall C (1982) A re-appraisal of insect flight towards a point source of wind-borne odor. J Chem Ecol 8:1207–1215

David CT, Kennedy JS, Ludlow AR (1983) Finding of a sex pheromone source by gypsy moths released in the field. Nature 303:804–806

Elkinton JS, Cardé RT (1981) The use of pheromone traps to monitor distribution and population trends of the gypsy moth. In: Mitchell ER (ed) Management of insect pests with semiochemicals. Plenum, New York, pp 41–55

Elkinton JS, Cardé RT (1987) Pheromone puff trajectory and upwind flight of the male gypsy moth in a forest. Physiol Entomol 12:399–406

Fadamiro HY, Baker TC (1999) Reproductive performance and longevity of female European corn borer, *Ostrinia nubilalis*: effects of multiple mating, delay in mating, and adult feeding. J Insect Physiol 45:385–392

Fadamiro HY, Cossé AA, Baker TC (1999) Mating disruption of European corn borer, *Ostrinia nubilalis* by using two types of sex pheromone dispensers deployed in grassy aggregation sites in Iowa cornfields J Asia-Pacific Entomol 2:121–132

Gomez G, Atema J (1994) Frequency filter properties of lobster chemoreceptor cells determined with high-resolution stimulus measurement. J Comp Physiol 174:803–811

Gosselin T, Sainte-Marie B, Bernatchez L (2005) Geographic variation of multiple paternity in the American lobster, *Homarus americanus*. Mol Ecol 14:1517–1525

Haynes KF, Baker TC (1989) An analysis of anemotactic flight in female moths stimulated by host odour and comparison with the males' response to sex pheromone. Physiol Entomol 14:279–289

Kaissling KE (1998) Flux detectors versus concentration detectors: two types of chemoreceptors. Chem Senses 23:99–111

Kennedy JS (1983) Zigzagging and casting as a programmed response to wind-borne odour: a review. Physiol Entomol 8:109–120

Kennedy JS, Marsh D (1974) Pheromone-regulated anemotaxis in flying moths. Science 184:999–1001

Knight AL (1997) Delay of mating of codling moth in pheromone disrupted orchards. IOBC/wprs Bulletin 20:203–206

Leal WS, Sawada M, Matsuyama S, Kuwahara Y, Hasegawa M (1993) Unusual periodically of sex pheromone production in the large black chafer *Holotrichia parallela*. J Chem Ecol 19:1381–1391

Linn CE Jr, Campbell MG, Roelofs WL (1986a) Male moth sensitivity to multicomponent pheromones: critical role of female-released blend in determining the functional role of components and active space of the pheromone. J Chem Ecol 12:659–668

Linn CE Jr, Campbell MG, Roelofs WL (1986b) Pheromone components and active spaces: what do moths smell and where do they smell it? Science 237:650–652

Mafra-Neto A, Baker TC (1996) Timed, metered sprays of pheromone disrupt mating of *Cadra cautella* (Lepidoptera: Pyralidae). J Agric Entomol 13:149–168

Mafra-Neto A, Cardé RT (1994) Fine-scale structure of pheromone plumes modulates upwind orientation of flying moths. Nature 369:142–144

Mordue Luntz AJ, Birkett MA (2009) A review of host finding behaviour in the parasitic sea louse, *Lepeophtheirus salmonis* (Caligidae: Copepoda). J Fish Dis 32:3–13

Murlis J (1986) The structure of odor plumes. In: Payne TL, Kennedy CEJ, Birch MC (eds) Mechanisms in Insect Olfaction. Clarendon Press, Oxford, pp 27–39

Oehlschlager AC, Chinchilla C, Castillo G, Gonzalez L (2002) Control of red ring disease by mass trapping of *Rhynchophorus palmarum* (Coleoptera: Curculionidae). Fla Entomol 85:507–513

Quero C, Fadamiro HY, Baker TC (2001) Responses of male *Helicoverpa zea* to single pulses of sex pheromone and behavioural antagonist. Physiol Entomol 26:106–115

Rice RE, Kirsch P (1990) Mating disruption of oriental fruit moth in the United States. In: Ridgway RL, Silverstein RM, Inscoe MN (eds) Behavior-Modifying Chemicals for Insect Management. Marcel Dekker, New York, pp 193–212

Ridgway RL, Inscoe MN, Dickerson WA (1990) Role of the boll weevil pheromone in pest management. In: Ridgway RL, Silverstein RM, Inscoe MN (eds) Behavior-modifying chemicals for insect management. Marcel Dekker, New York, pp 437–472

Roelofs WL, Cardé RT (1971) Hydrocarbon sex pheromone in tiger moths. Science 171:684–686

Shorey HH, Gerber RG (1996) Use of puffers for disruption of sex pheromone communication of codling moths (Lepidoptera: Tortricidae) in walnut orchards. Environ Entomol 25:1398–1400
Shorey HH, Sisk CB, Gerber RG (1996) Widely separated pheromone release sites for disruption of sex pheromone communication in two species of Lepidoptera. Environ Entomol 25:446–451
Showers WB, Whitford F, Smelser RB, Keaster AJ, Robinson JF, Lopez JD, Taylor SE (1989) Direct evidence for meteorologically driven long-range dispersals of an economically important moth. Ecology 70:987–992
Stelinski LL, Gut LJ, Pierzchala AV, Miller JR (2004) Field observations quantifying attraction of four tortricid moth species to high-doage pheromone rope dispensers in untreated and pheromone-treated apple orchards. Entomol Exp Et Appl 113:187–196
Stelinski LL, Gut LJ, Epstein D, Miller JR (2005) Attraction of four tortricid moth species to high dosage pheromone rope dispensers: observations implicating false plume following as an important factor in mating disruption. IOBC/wprs Bulletin 28:313–317
Stensmyr MC, Erland S, Hallberg E, Wallén R, Greenaway P, Hansson BS (2005) Insect-like olfactory adaptations in the terrestrial giant robber crab. Curr Biol 15:116–121
Vickers NJ, Baker TC (1992) Male *Heliothis virescens* sustain upwind flight in response to experimentally pulsed filaments of their sex-pheromone. J Insect Behav 5:669–687
Vickers NJ, Baker TC (1994) Reiterative responses to single strands of odor promote sustained upwind flight and odor source location by moths. Proc Natl Acad Sci USA 91:5756–5760
Vickers NJ, Baker TC (1997) Chemical communication in heliothine moths. VII. Correlation between diminished responses to point-source plumes and single filaments similarly tainted with a behavioral antagonist. J Comp Physiol 180:523–536
Wright RH (1958) The olfactory guidance of flying insects. Can Entomol 98:81–89