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# 6.12 Pheromones: Function and Use in Insect Control

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## 6.12.1. Introduction

### 6.12.1.1. Basic and Applied Research on Pheromones

During the 45 years since the identification of the first pheromone by Butenandt *et al.* (1959), insect pheromones have achieved significant stature in agricultural and urban pest management systems worldwide. If a pest species is known to use a sex pheromone to communicate, one of the most important initial efforts in trying to establish a workable pest management strategy is to explore that pheromone system to try to develop a useful synthetic pheromone attractant.

Since the mid-1960s, basic research has advanced our understanding of the biochemical, neurophysiological, and behavioral underpinnings of insect pheromone communication systems to a degree that none of us in the field could have years ago imagined. However, these advances have to this point not played a significant role in the development of useful and commercially successful applied pheromone tools for pest management. Basic pheromone research has provided valuable after-the-fact insight into why certain pest management applications of pheromones may be working better than others. This insight is now ready to be transformed into predictive knowledge that can, and should, be used to improve existing applied pheromone technologies and to create new ones.

The applied pheromone technologies and products currently in use were developed by applied entomologists using repeated trial-and-error field trials that have shown what works and what does not work. Improved pheromone dispenser technologies resulted from engineering and manufacturing advances that were made mainly by research and development teams at commercial companies. Chemical ecology techniques, which are at the heart of any initial investigations aimed at isolating

and identifying pheromones, have advanced in sophistication and sensitivity over the years, and have played an essential role in identifying several thousand pheromone components. Most pheromone identifications, however, have been initiated by a need for pest management tools and not for basic exploration of communication systems.

Basic research on insect pheromone olfaction has provided knowledge about animal olfaction that arguably has been as valuable as similar lines of research performed on vertebrates (Hildebrand and Sheppard, 1997). This research has been illuminating and exhilarating; now, many of the new insights that have emerged need to be reconnected with efforts toward application.

There are many reasons why insects have received such intense focus for research on pheromones. Certainly a major reason, apart from the simplicity and accessibility of their behavioral, olfactory, and pheromone production systems, is that so many insect species are pests. Also, society's awareness of the overuse of insecticides and the environmental damage they were imparting accelerated during the mid-1960s, and along with it emerged a hope that pheromones might be a safe and effective alternative to insecticides. It is important in this chapter to bring awareness to readers of the successful commercial use and grower acceptance of pheromone-based products. Many who have had the privilege to perform basic pheromone research realize that to a large degree we have been able to do so because of the exciting promise that pheromones presented to society early on that they might replace existing insecticides.

The purpose of this chapter is, however, not to merely summarize the many commercial successes that pheromones have had or to note the inroads they have made into being accepted as reliable tools for population suppression of key pests. Rather, it is to bring together some of the basic knowledge about insect behavior and olfaction from the past 30-plus years of knowledge the authors have gained from the successful field uses of pheromones over the same period. A few new ideas are also suggested for increasing the efficacy and economic competitiveness of pheromones in some pest management arenas.

Successful use of pheromones in insect control depends upon successfully controlling insect behavior through the emission of synthetic pheromone blends. The behaviors that are most amenable to manipulation are those that involve long-distance attraction. There is more power over the target insects with long-range attraction because human control over the behavior reaches far out into the field from every pheromone emission source. Thus, our focus is mainly on sex pheromones in this chapter. It is evident that basic and applied research on sex pheromones historically has involved mainly two groups, the Lepidoptera and Coleoptera, and therefore dwelt mostly on these two taxa. Although this emphasis is necessary, many of the principles developed from research on these two insect orders should be transferable to the pheromone systems of other groups.

## 6.12.2. Brief Overview of Insect Sex Pheromone Communication Systems

Sex pheromones in insects include a variety of molecules from large, but volatile, aliphatic molecules to small cyclic monoterpenoids (**Figure 1**). Although the vapor pressures of many of these compounds are very low (**Table 1**), many behavioral and electrophysiological experiments over the years have shown that insects do indeed smell these compounds even at extremely low concentrations (Kaissling, 1974, 1987, 1990; Priesner *et al.*, 1986; Szöcs *et al.*, 1989; Cowles *et al.*, 1996; Minor and Kaissling, 2003). Much of the information to follow has come more or less directly from an excellent recent book edited by Hardie and Minks (1999) that reviews nonlepidopteran pheromones.

### 6.12.2.1. Lepidoptera

Lepidopteran pheromones were the first to be widely studied and include a huge collection of mostly female-based pheromones. Female moths typically produce long-range, fatty acid-derived molecules (e.g., (**11**) in **Figure 1**) that function over long distances, whereas male moths tend to produce close-range courtship compounds that are often very similar in structure to plant secondary metabolites (e.g., (**3**) in **Figure 1**) (Baker, 1989a; Birch *et al.*, 1990). In contrast to moths, butterflies tend to use color and motion cues more than pheromones to find mates. Butterfly pheromones are generally restricted to close-range or contact pheromones (Baker, 1989a). Moth pheromones have been cataloged in The Pherolist, a free database.

The majority of moth pheromones identified thus far are blends of 10- to 18-carbon-long straight-chain primary alcohols, acetates, or aldehydes. However, one interesting second major class of moth pheromone components is found in noctuids, geometrids, arctiids, and lymantriids comprises polyene hydrocarbons and epoxides (e.g., (**10**) and (**13**) in **Figure 1**) (review: Millar, 2000). For the majority of moth species, after synthesis of the fatty acyl chain to either 16 or 18 carbons in length, these pheromone

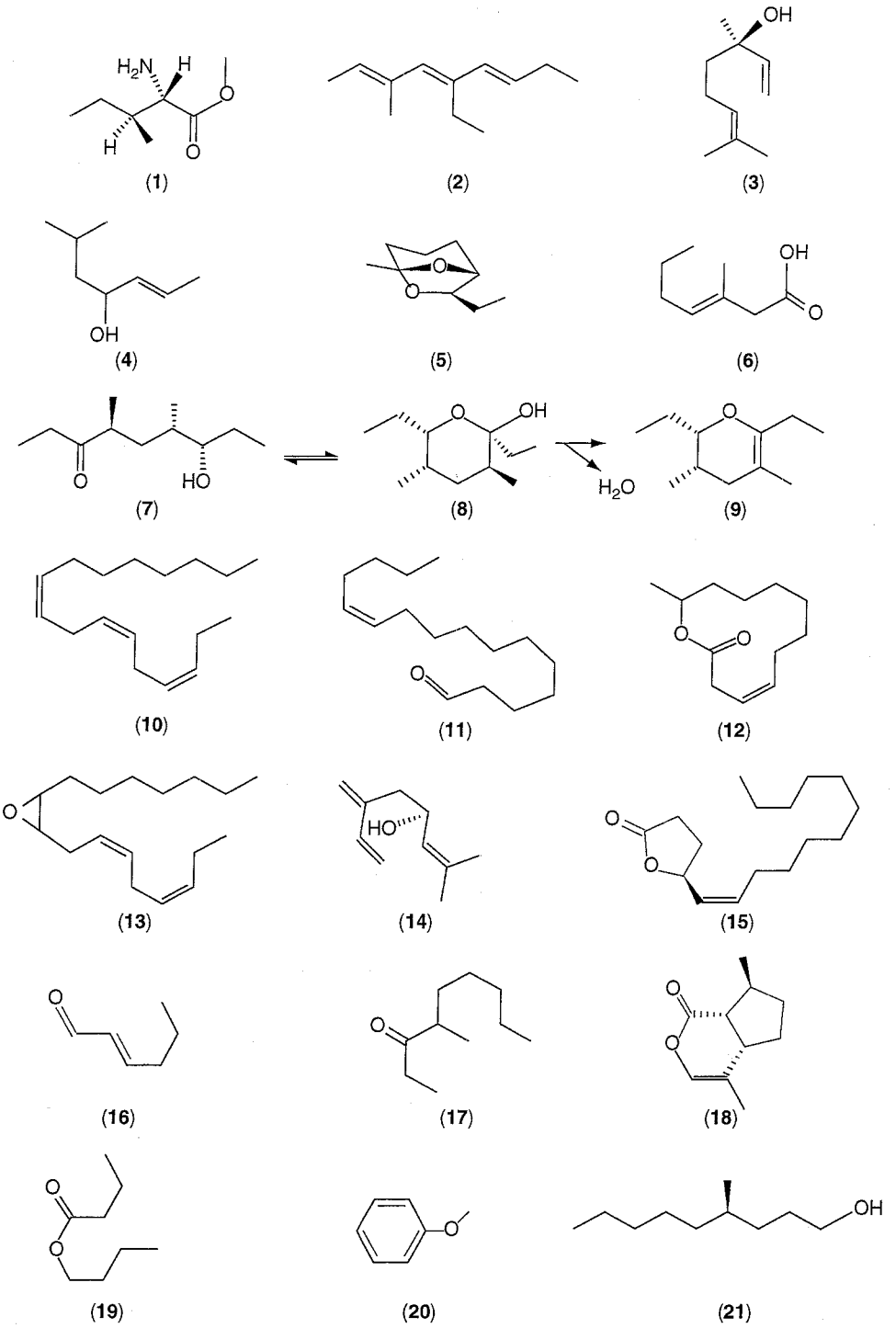


**Figure 1** Pheromone component structures for various insects of the orders Lepidoptera, Trichoptera, Coleoptera, and Hemiptera. (**1**), L-isoleucine methyl ester; (**2**), (2*E*,4*E*,6*E*)-5-ethyl-3-methyl-2,4,6-nonatriene; (**3**), *R*-linalool; (**4**), (2*E*,4*S*)-6-methyl-4-heptenol; (**5**), (1*R*,5*S*,7*R*)-*exo*-brevicomin; (**6**), (*E*)-3-methyl-3-heptenoic acid; (**7**), (4*S*,6*S*,7*S*)-serricornin; (**8**), serricornin (cyclic); (**9**), anhydroserricornin; (**10**), (*Z*,*Z*,*Z*)-3,6,9-heptadecatriene; (**11**), (*Z*)-11-hexadecenal; (**12**), (*Z*)-3-dodecen-11-olide; (**13**), (*Z*,*Z*)-3,6-*cis*-9,10-epoxyheptadecadiene; (**14**), (*S*)-(+)-ipsdienol; (**15**), (*S*,*Z*)-5-(+)-(1-decenyl)oxacyclopentan-2-one; (**16**), (*E*)-2-hexenal; (**17**), 4-methylnonan-3-one; (**18**), (+)-(4a*S*,7*S*,7a*R*)-nepetalactone; (**19**), butyl butyrate; (**20**), anisole; (**21**), (*R*)-4-methyl-1-nonanol.

**Table 1** Vapor pressures of some insect pheromone components; vapor pressures of some plant-related volatiles are also listed

| *Name*[a] | *Vapor pressure at 25° C*[b] | *Registry number*[c] | *Insect order* |
|---|---|---|---|
| (*E,Z*)-3,13-18:OH | 0.000000557 | 66410-28-4 | Lepidoptera |
| (*Z*)-11-16:COOH | 0.00000187 | 2271-34-3 | Lepidoptera, precursor |
| (*Z*)-9-16:COOH | 0.00000282 | 373-49-9 | Lepidoptera, precursor |
| Linoleic acid | 0.00000354 | 60-33-3 | |
| (*Z*)-11-16:Ac | 0.0000493 | 34010-21-4 | Lepidoptera |
| (*Z*)-11-16:OH | 0.0000597 | 56683-54-6 | Lepidoptera |
| (*Z*)-9-14:Ac | 0.000136 | 16725-53-4 | Lepidoptera |
| (*Z*)-11-16:Ald | 0.000253 | 53939-28-9 | Lepidoptera |
| (*Z*)-11-14:OH | 0.000445 | 34010-15-6 | Lepidoptera |
| (*Z*)-9-14:Ald | 0.00135 | 53939-27-8 | Lepidoptera |
| (*Z*)-11-14:Ald | 0.00213 | 35237-64-0 | Lepidoptera |
| Linalool | 0.0905 | 78-70-6 | Coleoptera, Heteroptera |
| Hexanoic acid | 0.158 | 142-62-1 | Coleoptera |
| Pentanoic acid | 0.452 | 109-52-4 | Coleoptera |
| (*Z*)-3-hexenol | 1.04 | 928-96-1 | Lepidoptera |
| Limonene | 1.54 | 138-86-3 | |
| β-Ocimene | 1.56 | 13877-91-3 | |

[a]Abbreviated chemical names, e.g., "(*E,Z*)-3,13, 18:OH" is shortened form of (*E,Z*)-3,13-octadecadien-1-ol. Ac, acetate; OH, alcohol; Ald, aldehyde.
[b]Vapor pressures were taken from Scifinder Scholar.
[c]Registry numbers are given to allow searching for additional properties in chemical abstract services.

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 (Tillman *et al.*, 1999; Roelofs and Rooney, 2003). The desaturation step is, depending on the species, either preceded or followed by β-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 pheromone component structures in moths (Roelofs and Rooney, 2003). 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 key neuropeptide named pheromone biosynthesis activating neuropeptide (PBAN) has been found in females of all pheromone-producing moth species thus far. PBAN stimulates both the behavioral release of pheromone (i.e., calling behavior) and the biosynthesis of the pheromone components (Nation, 2002). A receptor for PBAN has recently been characterized in *Helicoverpa zea* by Choi *et al.* (2003) and is a membrane-bound heptahelical G protein-coupled receptor (i.e., a seven-transmembrane GPCR). The PBANs of most species are extremely similar in structure, and injection of one species' PBAN into the female of a second species often causes enhanced pheromone production in the injected female, even across families (Raina and Menn, 1987).

### 6.12.2.2. Trichoptera

Trichopterans are closely related to Lepidoptera. In fact, on a family level phylogeny they form a monophyletic group with the lepidopterans; that is, they are the sister group to Lepidoptera (Ross, 1964). Trichopteran pheromones, like their lepidopteran counterparts, are produced by females and attract males from a distance. However, the limited number of trichopteran sex pheromone components that have been identified do not share the most common features of their lepidopteran counterparts. Trichopteran pheromone components are, however, similar in structure to those of some primitive lepidopterans of the family Eriocraniidae (Zhu *et al.*, 1995; Kozlov *et al.*, 1996). In only three species have putative pheromones been shown to have behavioral activity in the trichopterans (Löfstedt *et al.*, 1994; Bjostad *et al.*, 1996) and all three are produced by the paired set of sternal glands that secrete their products onto the fifth abdominal sclerite through a dorsolateral opening on each side of the sclerite (Ivanov and Melnitsky, 1999). Bjostad *et al.* (1996) identified 6-methylnonan-3-one (similar to (**17**) in **Figure 1**) as the major component of the sex pheromone of *Hesperophylax occidentalis*, and Löfstedt *et al.* (1994) found nonan-2-one and heptan-2-one to be the major components of the sex pheromones of *Hydropsyche angustipennis* and *Rhyacophila fasciata*, respectively.

Although nothing is known yet about the behavioral impact of the differences in pheromone

chirality that these trichopteran pheromone components have, chirality has been shown to be important to the activity of receptor neurons in individual olfactory sensilla (Larsson and Hansson, 1998). In some species these compounds had been implicated as being defensive secretions (see references in Ivanov and Melnitsky, 1999), but the first behavioral studies definitively demonstrated their roles as sex pheromones (Wood and Resh, 1984; Resh and Wood, 1985). No information is available regarding biosynthesis of these pheromones or endocrine regulation. Work on this group may not proceed as quickly as it has in others because trichopterans are not agricultural pests. However, they are important indicators of environmental quality (Brigham and Sadorf, 2001) and monitoring their presence with pheromone traps may be more convenient and efficient than stream sampling.

#### 6.12.2.3. Coleoptera

Coleopteran pheromones have mainly been identified for scarabs (Scarabaeidae) (Leal, 1999), sap beetles (Nitidulidae) (Bartelt, 1999a), scolytids (Scolytidae) (Schlyter and Birgersson, 1999), weevils (Curculionidae) (Bartelt, 1999b), and stored-product beetles (Anobiidae, Dermestidae, Bruchidae, Tenebrionidae, Bostrichidae, Cucujidae, and Silvanidae) (Plarre and Vanderwel, 1999). Recently, much of the literature regarding beetle pheromones and all other insect pheromones has been compiled into one site (Pherobase). This site is likely the most extensive database of pheromones of insects currently freely available.

**6.12.2.3.1. Scarabaeidae** Scarab pheromone components are generally of four types: fatty acid-derived lactones ((15), **Figure 1**); straight-chain unsaturated aldehydes, alcohols, ketones, and esters (similar to 16, but with nine carbon atoms; **Figure 1**); terpenoids ((3), **Figure 1**); amino acid derivatives ((1), **Figure 1**); or phenolics ((20), **Figure 1**). They are produced by either unicellular epidermal glands on the posterior ventral sclerites or by an everted soft tissue sac on the last few segments of the abdomen, similar to the glands of higher lepidopterans. There seems to be an interesting segregation of chemistry among these two gland types that is also manifested at the subfamily level. The everted soft tissue sacs produce mainly terpenoids or amino acid-derived compounds and are associated with the Melolonthinae, whereas the unicellular glands produce the fatty acid derived compounds and are mainly associated with the Rutelinae (Leal, 1999). Not all the Melolonthinae use eversible glands; females in the genus *Macrodactylus* have never been observed everting a gland from the tip of their abdomens (Heath *et al.*, 2002). The lack of obvious calling behavior as well as the lack of cuticular pores in this genus may present a unique opportunity to study the evolution of sex pheromones in melolonthine beetles. In some scarabs the pheromone seems to be synergized by the presence of plant volatiles. For example, the Japanese beetle pheromone (Tumlinson *et al.*, 1977) ((15), **Figure 1**) captures twice as many beetles when selected plant compounds are blended and coemitted with it (Klein *et al.*, 1981).

**6.12.2.3.2. Nitidulidae** Sap beetle (Nitidulidae) pheromones are mainly known as a result of a series of studies conducted by Bartelt and colleagues (Bartelt, 1999a) on species in the genus *Carpophilus*. The pheromones, which attract both sexes, are produced by male glandular cells in the posterior of the abdomen that secrete their contents into tracheal tubes. The chemical structures of the pheromone molecules are of polyunsaturated, multibranched hydrocarbons ((2), **Figure 1**). Seventy-five times more *Carpophilus hemipterus* beetles are attracted to the blend of pheromone plus food odor than to either pheromone or food odor alone (Bartelt *et al.*, 1994). In fact, a high level of attraction synergism between food and pheromone odors exists throughout the genus *Carpophilus* (Bartelt, 1999a). These pheromone components seem to be biosynthesized through the use of acetate, propionate, and butyrate acyl units (Petroski *et al.*, 1994; Bartelt and Weisleder, 1996). The initial condensation reaction seems to differ significantly from the typical fatty acid synthesis with malonyl CoA (Petroski *et al.*, 1994). In some methyl-branched pheromones, the branched units are derived ultimately from amino acids (Tillman *et al.*, 1999). Possible hormonal and neurohormonal mechanisms regulating initiation and termination of biosynthesis remain unexplored at present.

**6.12.2.3.3. Curculionidae** A significant number of weevil (Curculionidae) pheromones have been identified (review: Bartelt, 1999b), and many of these have contributed to highly successful integrated pest management (IPM) of weevil pest species through their effectiveness in mass trapping (see Section 6.12.5.3.1). The majority of the known systems involved are male-emitted and the pheromones are often strong enough to attract both sexes from tens of meters away (Bartelt, 1999b and references therein). The structures of the pheromone molecules seem very diverse. However, they share a common mode of action in that, as in the *Carpophilus* sap beetles, the attraction of weevils by the pheromone is synergized by coemission of plant compounds. For example, all six palm weevil pheromones of

*Rhynchophorus* spp., (e.g., (**4**), **Figure 1**), are synergized by the presence of plant material such as sugarcane stalks (Oehlschlager *et al.*, 1993). In the Andean potato weevil, *Premnotrypes suturicallus*, a severe pest in the Peruvian Andes, field-collected *P. suturicallus* females must be actively feeding on a host plant to attract males (J.J. Heath *et al.*, unpublished data). It is not well known how or where weevil pheromones are produced, but there is evidence from *R. palmarum* males that they are synthesized in glands in the prothorax and exuded from the mouth (Sánchez *et al.*, 1996). Studies on the mechanisms of biosynthesis and regulation are also lacking.

**6.12.2.3.4. Scolytidae** Bark beetles (Scolytidae) pheromones were among the earliest studied and identified in insects (Silverstein *et al.*, 1966, 1968; Kinzer *et al.*, 1969; Bedard *et al.*, 1970). They were also, along with lepidopterous pheromones, the group of pheromones that showed the most promise in large-scale field tests to be effective in suppressing or manipulating populations of the devastating pests present in this family (Bedard and Wood, 1981; Lanier, 1981).

Recent work on this group (review: Schlyter and Birgersson, 1999) has not completely resolved a long-standing discussion of whether bark beetle pheromones are biosynthesized *de novo* or are just slightly modified (detoxified) host compounds. In at least two genera, *Tomicus* and *Dendroctonus*, it is the females that locate a host tree, begin boring galleries for oviposition, and in the process produce a long-distance pheromone that results from detoxified host compounds and host resin constituents. *Dendroctonus ponderosae* females release (−)-trans-verbenol, which attracts males. Once males arrive they produce (+)-*exo*-brevicomin ((**5**), **Figure 1**), which acts as an aggregation pheromone (i.e., both sexes are attracted). *Tomicus minor* females use a similar system.

Females of *Dendroctonus brevicomis*, alternatively, start producing (+)-*exo*-brevicomin as the first-arriving beetles burrow into the host tree to initiate galleries. Once they have reached the phloem tissue a resin containing myrcene, a hydrocarbon similar in structure to linalool ((**3**), **Figure 1**), begins to flow. The blend of these two compounds is attractive to males. Once males arrive they begin producing (−)-frontalin. This three-component blend is now attractive to both males and females. It has also been shown that myrcene is converted to (+)-ipsdienol ((**14**), **Figure 1**) simply by passing myrcene-laden air over *Ips paraconfusus* (Byers, 1995 and references therein). Male *D. brevicomis* and many other *Ips* spp. also produce (+)-ipsdienol, which has an antagonistic effect in field studies with *D. brevicomis* (Bertram and Paine, 1994).

Regardless of whether bark beetle pheromones are synthesized entirely *de novo* or their the mode of production involves the beetle making relatively small modifications of existing plant-derived compounds, one feature remains common throughout the bark beetles: pheromone-based attraction is synergized by coemission of plant volatiles. This is true for species in the well-studied genera *Dendroctonus*, *Ips*, and *Scolytus* as well as for species in other genera such as *Conophthorus coniperda* in which pioneering females produce 2*R*,5*S*-pityol while burrowing into the tree. Attraction is synergized by α-pinene and other monoterpenes (Schlyter and Birgersson, 1999).

**6.12.2.3.5. Stored-product beetle pests** Stored-product pests, including the families Anobiidae, Dermestidae, Bruchidae, Tenebrionidae, Bostrichidae, Cucujidae, and Silvanidae, have an interesting array of pheromones aptly reviewed by Plarre and Vanderwel (1999). These include both female-produced pheromones that only attract males, and also male-produced aggregation pheromones. Anobiid females produce cyclic compounds that can rapidly interconvert between different forms. For example, the acyclic form of serricornin ((**7**), **Figure 1**) is in equilibrium with the cyclic form ((**8**), **Figure 1**), which can then lose water to form anhydroserricornin ((**9**), **Figure 1**) (Mori *et al.*, 1984). Dermestid females produce unsaturated fatty acids and derivatives thereof that serve as pheromone components in this group (Silverstein *et al.*, 1967). The dermestid pheromone components are similar to those of moths ((**11**), **Figure 1**). Bruchid females emit short-chain, branched, unsaturated acids as their pheromone components that attract males ((**6**), **Figure 1**).

In most of the species of stored-product beetle pests, pheromones are produced by glands on or between abdominal segments. The genus *Tribolium* may be an exception. The male-produced aggregation pheromone, 4,8-dimethyldecanal (Plarre and Vanderwel, 1999 and references therein), in *T. castaneum* is apparently produced in glands located on the ventral side of the prothoracic femurs (Faustini *et al.*, 1981). The production of the *Tenebrio molitor* female-produced pheromone, (*R*)-4-methyl-1-nonanol ((**21**), **Figure 1**), is regulated by juvenile hormone III (Menon, 1976). The bostrychids use branched esters very similar in structure to the pheromones of some heteroptera (e.g., (**19**) in **Figure 1**). Cucujidae and Silvanidae use macrocyclic lactones likely produced by fatty acid biosynthesis ((**12**), **Figure 1**).

#### 6.12.2.4. Homoptera

Knowledge of homopteran sex attractant pheromones comes from two agriculturally important groups, the scale insects and the aphids. Most of the species of aphids studied thus far use a blend of nepetalactone and nepetalactol compounds as their sex pheromone (Pickett *et al.*, 1992; Hardie *et al.*, 1994). For instance, the sex pheromone of the aphid *Aphis fabae* is a 29 : 1 blend of (+)-(4a*S*,7*S*,7a*R*)-nepetalactone and (−)-(1*R*,4a*S*,7*S*,7a*R*)-nepetalactol (Pickett *et al.*, 1992). These are monoterpenoids in the cyclopentanoid series ((**18**), **Figure 1**) and are produced by females in glands on the tibiae of the hind legs (Pettersson, 1971; Hardie *et al.*, 1999). Although the nepetalactones and related compounds are found in plants of the genus *Nepeta*, biosynthesis and regulation are not well understood in either kingdom (Hardie *et al.*, 1999). There is considerable research effort at present being made to explore the pheromone systems of an increasing number of aphid species for use in pest management. There is usually only one sexually communicating generation, and so this generation represents a vulnerable target for aphid control via pheromones. If mating and hence egg-laying by the sexually reproducing females could be disrupted with pheromones, there could possibly be significant population suppression effects.

One other aspect of aphid pheromone communication is their use of alarm pheromones. Interestingly, the main component of most aphid alarm pheromones is the branched chain sesquiterpene (*E*)-β-farnesene, whereas the alarm pheromone for the spotted alfalfa aphid is the cyclic sesquiterpene (−)-germacrene-A (Hardie *et al.*, 1999). Dawson *et al.* (1987) found that (*E*)-β-farnesene, the alarm pheromone of the turnip aphid, *Lipaphis erysimi*, was not effective unless isothiocyanates were added to the blend, suggesting a host-plant volatile, context-dependent effect of (*E*)-β-farnesene.

#### 6.12.2.5. Heteroptera

Heteropterans produce a wide array of compounds, often in unusually large quantities compared with pheromones of other groups of insects (reviews: Aldrich, 1995; McBrien and Millar, 1999). These compounds are produced by a number of different glands that can be found in at least six different parts of the body, including metathoracic glands, dorsal abdominal glands, sternal glands, unicellular glands, subrectal glands, and ventral abdominal glands (Aldrich, 1995). Metathoracic glands are mostly noted for their role in production of defensive secretions in adult bugs, but these glands are known to be sexually dimorphic and do produce pheromones in some species. The secretions from this paired set of glands most often contaminate pheromone extractions. Dorsal abdominal glands most often produce the defensive secretions of nymphs and then they disappear by adulthood. However, these have sometimes been shown to remain functional in adults where they then are involved in pheromone production. The sex pheromone systems of heteropterans are generally male-emitted, but there are a few examples of female-emitted systems. Aldrich (1995), in his comprehensive review of the diversity of heteropteran pheromones, has highlighted the need for focused research in this group that includes many important pest species as well as beneficial predatory species that are useful biological control agents.

Heteropteran pheromones tend to be either a blend of esters (e.g., butyl butyrate, (**19**), **Figure 1**) or an ester and a short-chain aldehyde or alcohol (Smith *et al.*, 1991; Leal *et al.*, 1996). These esters, aldehydes, and alcohols (e.g., (**16**) and (**19**) in **Figure 1**) are also often combined with a terpenoid ((**3**), **Figure 1**) to form a pheromone. For example, McBrien *et al.* (2002) showed that a conjugated triunsaturated methyl ester, when combined with one or more of three sesquiterpenes, was attractive to the stink bug *Thyanta pallidovirens*. There is little known about pheromone biosynthesis in this group, or of the hormones involved in the regulation of production in the Heteroptera.

### 6.12.3. Pheromone-Mediated Behavior

#### 6.12.3.1. Research on Olfaction and Orientation

Two major basic lines of investigation have been taken to understand how pheromones cause insects to be "attracted" and arrive at the source. One has focused on orientation movements that are evoked by exposure to the correct pheromone blend for a particular species. By necessity, studies of insect pheromone-mediated orientation have been concerned with flight control, which ultimately needs to be understood with regard to how a flying insect stabilizes its flight in wind in all three possible planes of movement in space (David, 1982, 1986). The second major line has focused on studies of insect olfaction. It is the exposure to the correct blend of pheromone components, and not partial blends or suboptimal blend ratios, that most intensely turns on programs that change an insect's movements and maximize attraction toward the source. Because the reception and integration of the pheromone blend's quality modulates these programs, insect pheromone

olfaction has been an equally important and rewarding line of inquiry, and one that has received much funding support from federal funding agencies.

### 6.12.3.2. Understanding Pheromone-Mediated Orientation

**6.12.3.2.1. Importance of the fine-scale structure of pheromone plumes to orientation behavior** Wright (1958) recognized, in contrast to Bossert and Wilson's time-averaged "active space" depiction and modeling (Bossert and Wilson, 1963), that an odor plume is composed 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, 1997). 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 (Kennedy *et al.*, 1980, 1981; Baker *et al.*, 1985) and subsequently when moths were shown to have the ability to react fast enough during upwind flight to the subsecond changes in pheromone concentration (Baker and Haynes, 1987) that one began to understand that these individual pheromone strands and pockets of clean air are what are producing the sustained upwind flight behavior that is called attraction (Mafra-Neto and Cardé, 1994; Vickers and Baker, 1994). Thus, the major advance in basic understanding of insect pheromone-mediated behavior over the past 20 years is that with but one exception (Justus and Cardé, 2002), 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.

Concerted attempts to apply this new knowledge have not yet been made, but this is an area of research that may offer the best chance for improving the efficacy and economic competitiveness of pheromones in pest control. Wyatt (1997) reviewed the advances in basic and applied pheromone research that had been made through the mid-1990s and provided a series of suggestions as to the types of research and extension efforts that should be undertaken to facilitate the more widespread commercial use of pheromones for direct control of insect pests. One objective of this chapter is to provide an updated snapshot of what has been learned from the continued successful commercial use of pheromones in recent years and also to review some recent advances in basic knowledge about the behavior and neurophysiology of insect olfaction to suggest new ways to improve and optimize the field use of insect pheromones for pest control. Biochemical and molecular aspects of insect olfaction are reviewed in depth by Vogt (see **Chapter 3.15**), and so attempt is not made to cover the many advances made in this important area.

The focus is mainly on sex pheromones because these have achieved the widest commercial utility. The treatment of the basic neuroethological mechanisms involved in olfaction and attraction is restricted mainly to the Lepidoptera, because research on this group has provided the most comprehensive and probably the most useful model by which to understand odor-mediated attraction of all insects. Based on current knowledge about olfactory pathways and flight responses of insects in other orders, there is no reason to suspect that the mechanisms of olfaction or in-flight orientation generally used by insects will be significantly different from those used by Lepidoptera.

**6.12.3.2.2. Optomotor anemotaxis and self-steered counterturning: two indirect reactions to pheromone** The insight and experience of John S. Kennedy began to free pheromone researchers of their preconceptions about attraction being a chemo-orientation process involving steering with regard to odor gradients (Kennedy and Marsh, 1974; Kennedy, 1977, 1983, 1986; Marsh *et al.*, 1978; Kennedy *et al.*, 1980, 1981). This allowed a better understanding of insects' responses to pheromones and other odors as being an *indirect* response to odor exposure that turns on steering programs with respect to *wind*, and *not* to odor. It was also discovered that a second indirect response is also turned on in moths by pheromone, an internally generated program of side-to-side oscillations that is switched on and takes place even in the absence of wind, persisting for many seconds after pheromone is removed (Baker and Kuenen, 1982; Kuenen and Baker, 1983).

Since those initial landmark studies by Kennedy's group it has been possible to learn more precisely how, when a flying insect encounters a series of odor strands of the right quality, these two programs are performed. In the wind-steering program, a visually mediated response called optomotor anemotaxis, the moth compensates for any off-line displacement due to wind direction, or for any changes in progress over the ground that arise due to changes in wind 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; Kuenen and Baker, 1983) is a "counterturning" pattern of rapid left–right reversals of direction (Kennedy, 1983, 1986) which 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 that the clean air causes the anemotactic program to allow more crosswind, transverse optical image flow, to result in slower-reversing, greater amplitude crosswind casting flight (Kennedy, 1983, 1986; Baker, 1985, 1989b, 1989c).

**6.12.3.2.3. Altitude control** Wind is the horizontal motion component of a moving air mass, and optomotor anemotaxis only explained steering with respect to wind to result in attraction (upwind displacement). A flying insect traversing pheromone plume strands also needs to navigate in a third dimension, i.e., with regard to its altitude. David (1982, 1986) provided experimental evidence that altitude control is accomplished via optomotor compensation for the apparent up or down movement of objects. He showed that these responses are performed by *Drosophila hydei* by changing their wing beat frequency to regulate the total flight forces that the wings generate when moving visual cues give them the sensation that they are rising or sinking. This vertical flight-force response system is intimately coupled with changes in body pitch angle that the insect performs in response to changes in the horizontal component of optomotor feedback that arise from changes in total flight force (e.g., wing beat frequency) (David, 1982, 1986). Studies of flight tracks of several moth species, which represent the total pheromone-mediated flight system involving both counterturning and anemotaxis, have shown that when a moth is casting widely following loss of pheromone, its vertical (up-and-down) excursions are correspondingly greater (VonKeyserlingk, 1984; Baker, 1989c). When the insect is surging upwind following repeated contact with pheromone strands, its vertical motion is much reduced as is its side-to-side movement (VonKeyserlingk, 1984; Baker, 1989c) due to high-frequency counterturning, with more thrust being generated to carry the insect upwind at a faster rate (David, 1986).

**6.12.3.2.4. Subsecond reaction times of the two programmed responses to pheromone** Intermittency of stimulation from the strands of pheromone and pockets of clean air that comprise a pheromone plume was shown to be required for at least two species of moths to promote sustained upwind flight of males (Kennedy *et al.*, 1980, 1981; Baker *et al.*, 1985). Soon after, it was found that male oriental fruit moths were able to react within 1/6 of a second to changes in pheromone concentration (Baker and Haynes, 1987). It was now possible to hypothesize that each encounter with an individual pheromone strand or pocket of clean air produces subsecond reactions in male moth behavior that, when reiterated during multiple encounters with strands and pockets of clean air in a plume, promote successful sustained upwind flight to the pheromone source (Baker and Haynes, 1987; Baker, 1990; Kaissling and Kramer, 1990).

The research progress resulting from many experiments aimed at understanding behavioral responses of moths to pheromone (reviews: Kennedy, 1983, 1986; Baker, 1985, 1986, 1989b; Willis and Arbas, 1991, 1997; Arbas, 1997; Baker and Vickers, 1997; Cardé and Mafra-Neto, 1997) brought the field to the point of being able to embrace a comprehensive understanding of how male moths locate sources of sex pheromone. A series of experiments using two different lepidopteran species from different families (Mafra-Neto and Cardé, 1994; Vickers and Baker, 1994) confirmed that the split-second reactions to individual odor strands are, in fact, the hypothesized upwind surges (Baker, 1990; Kaissling and Kramer, 1990) that are strung together during repeated contacts with strands in a natural plume to produce sustained upwind flight in response to pheromone. The intermittency provided by pheromone strands has been shown, in three of the four moth species that have been experimentally examined for this, to be essential to producing reiterative upwind surges that result in attraction of moths (Kennedy *et al.*, 1980, 1981; Baker *et al.*, 1985; Justus and Cardé, 2002).

The timescale over which the moths' reactions to pheromone strands and clean air occur is remarkably small. The behavioral responses to both the onset and loss of filaments can be as fast as 0.15 s (*Grapholita molesta*: Baker and Haynes, 1987), but usually are between 0.3 and 0.6 s (*Heliothis virescens*: Vickers and Baker, 1996, 1997; *Helicoverpa zea*: Quero *et al.*, 2001; and *Antheraea polyphemus*: Baker and Vogt, 1988). 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).

The speed of the reaction of moths to encounters with a pheromone strand or clean air pocket is not limited apparently by the speed of olfactory pathway processing; the same latency to a change in

movement occurs when visual cues related to wind-shift are experimentally generated instead of odor-loss cues (Baker and Vickers, 1994). The current evidence thus indicates that for responses to pheromone or host odor, the shape of the insect's flight track is determined by the frequency of encounters with filaments relative to encounters with pockets of clean air, and these encounters determine the relative proportions of upwind surging and casting, respectively (Baker, 1990; Kaissling and Kramer, 1990; Mafra-Neto and Cardé, 1994; Vickers and Baker, 1994; Kaissling 1997).

There is no evidence from other insect species in other orders that their in-flight response mechanisms to pheromone or other odors is different from that of moths. But further research examining the behavioral responses of other groups will be enlightening and essential to testing just how universally applicable these mechanisms are to insect odor-mediated flight.

**6.12.3.2.5. Relevance of fast pheromone-strand reaction times to field attraction** The rapid response to an odor filament 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 (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 when it smells the odor: the insect can locate the odor source by steering into and progressing upwind when it smells the correct odor. However, it is just as important for the insect to respond quickly to clean air as it is to an odor filament, because large-scale turbulence produces large swings in the wind direction that dictates along which lines the strands are flying away from the source (David *et al.*, 1982). If the insect continues to move upwind into these wind-swing-produced large pockets of clean air for any length of time, it will more rapidly distance itself from the odor plume it has just lost contact with, as well as steer more off-line relative to the odor source (David *et al.*, 1982; David and Kennedy, 1987; David and Birch, 1989; Baker, 1990).

A rapid cessation of upwind progress in response to odor loss, coupled with rapid and increasingly lengthy side-to-side oscillations in response to odor loss, allows the insect to increase its ability to regain contact quickly with the odor strands that have swung to one side or the other of its body. Because the insect has no way of telling to which side the odor has gone, this casting flight provides an unbiased response to one side of the wind line or the other in clean air, either of which could be the wrong side and result in failure to relocate the plume. The cessation of upwind progress during casting also prevents the insect from plunging too deeply and off-line from the source into a large pocket of clean air that is now moving off-line from the insect's previous toward-source line.

There is experimental evidence in which odor exposure to the insect or ground pattern movement is manipulated that odor-mediated optomotor anemotaxis is used by a variety of species besides Lepidoptera during odor-mediated upwind flight, including flies (*Drosophila*: David, 1982; *Tsetse*: Gibson and Brady, 1988, 1991). Similarities between male moths' reactions to gain and loss of sex pheromone odor and female moths' reactions to gain and loss of host volatile odor are striking (Haynes and Baker, 1989). Thus, knowledge about pheromone-mediated orientation mechanisms should help us better understand how to manipulate not only pheromone-mediated behavior of moths, but also odor-mediated behavior of male and female insects of all types, for better insect control.

### 6.12.3.3. Pheromone Blend Quality and Upwind Flight Behavior

The blend ratio of pheromone components for evoking optimal attraction and source contact by male insects is the one that most closely approximates the natural ratio emitted by females. Linn *et al.* (1986a) convincingly demonstrated for several species that males' behavioral thresholds for the optimal blend are lower than they are for partial blend compositions or suboptimal ratios. They also showed in the field 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 that from which some components are missing (Linn *et al.*, 1986b), indicating that the threshold for upwind flight attraction to the optimal blend is lower than that for suboptimal or partial blends. As pointed out by Linn *et al.* (1986a, 1986b) and reiterated by Baker (1989b), these results showed that we must discard a previous view of how blends are used by males. The previous model proposed that there was a stepwise use by males during upwind flight of first one component in a female's blend when they are far downwind, and then two, three, and successively more components as they get closer to the source (Cardé *et al.*, 1975; Nakamura, 1981; Bradshaw *et al.*, 1983).

Recent studies have demonstrated that blend-quality reactions take place on a strand-by-strand basis, within fractions of a second. Vickers and Baker (1997) showed for *H. virescens* and Quero *et al.* (2001) using *H. zea*, that the duration and

length of males' upwind surging reactions to single strands of pheromone depend on their quality. 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 pheromone blend alone.

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. This was demonstrated when male *H. virescens* were shown to be able to discriminate experimentally generated incompletely mixed strands of their major and minor pheromone components from strands that were completely mixed (Vickers and Baker, 1992). They can also discriminate experimentally generated strands of pheromone that are incompletely mixed with heterospecific antagonist from those in which each and every strand contains both antagonist and pheromone (Fadamiro and Baker, 1997; Baker *et al.*, 1998a; Fadamiro *et al.*, 1999a). This level of plume-strand quality temporal resolution was first suspected in other species using two natural and confluent plumes of pheromone and antagonist placed only tens of centimeters apart (Witzgall and Preisner, 1991; Liu and Haynes, 1992; Rumbo *et al.*, 1993).

The propensity of insects to be attracted optimally to pheromone blends most closely approximating those emitted by the opposite sex is therefore attributable to their repeated strong surging reactions to individual strands. Thus, the higher quality surging that occurs in response to each strand of pheromone that is of optimal blend quality will, during repeated contact with natural strands in a pheromone plume, promote more sustained and intense upwind flight and displace a male farther upwind at a faster rate from greater distances downwind than will the surges in response to suboptimal blend strands. Presumably, the long-lasting casting response in a large pocket of clean air will also be of longer duration after contact with optimal blend-strands rather than suboptimal ones.

## 6.12.4. Pheromone Olfaction

### 6.12.4.1. Optimizing Flux Detection

It has become clear over the past 20 years that, as emphasized by Kaissling (1998), the insect olfactory system is designed to optimize flux detection and not to measure concentration. New understandings of the biochemical, neurophysiological, and molecular processes that contribute to the propagation of behavior after contact with airborne pheromone plumes show that the emphasis is on processing speed related to temporal resolution of the strand encounters. All the steps in the olfaction process work rapidly in succession to report pheromone strand encounters to higher centers (Vickers *et al.*, 2001), from the biochemistry of transduction (see **Chapter 3.15**) to action potential propagation, to integration of the signal at the antennal lobe level, and finally to the output patterns sent to higher central nervous system (CNS) levels. Along at least some specialized pathways in this succession of synapses, the reporting of changes in flux is preserved and seems necessary to promote repeated neuronal excitation and quickly modulate behavior.

### 6.12.4.2. Elements of Pheromone Olfactory Pathways in Insects

The pheromone olfactory systems of insects are exemplified by that which exists in the Lepidoptera. Decades of research on moths has allowed knowledge about animal olfaction in general to be developed that rivals what is known in the vertebrates (Hildebrand and Shepard, 1997). Although the receptor organs used by different orders of insects to begin transducing pheromone molecules into action potentials vary in type, shape, and size (Kaissling, 1974, 1987; Steinbrecht, 1987, 1999), the integrative pheromone olfactory pathways after transduction that lead to motor output are strikingly similar to those in the Lepidoptera in the most well-studied groups such as cockroaches (e.g., Boeckh and Ernst, 1983; Boeckh *et al.*, 1984). Thus, pheromone olfaction in the Lepidoptera at length to provide an overview of a typical insect pheromone olfaction system is discussed. A thorough review of all aspects of the pathways involved in insect olfaction is provided by many authors in Hansson (1999).

**6.12.4.2.1. Olfactory receptor neurons and their perireceptor environments** In the Lepidoptera, pheromone molecules in a plume-strand contact the thousands of extra-long receptor hairs (trichoid sensilla) on the antennae of male moths that are the primary pheromone receptor organs (Kaissling, 1974, 1987; Steinbrecht, 1987, 1999). In some families in the Coleoptera such as the Scarabaeidae, the pheromone-component-sensitive receptor neurons apparently reside in other types of olfactory receptor organs. The word "apparently" must be used here because it is virtually impossible to identify from which type of sensillum one is recording when using the tungsten-type electrodes that are requisite for recording from these tiny and often densely packed sensilla. For scolytid bark beetles, short trichoid or basiconic sensilla (short stubby hairs) have been implicated in sex pheromone reception (Dickens and Payne, 1978; Dickens, 1979). In

scarab beetles, coeloconic (pit-peg) and placoid (flat, plate-like) sensilla that are arrayed in various patterns on the lamellar hair surfaces are suspected to house the pheromone-sensitive receptor neurons (Mustaparta, 1984; Leal and Mchizuki, 1993; Kim and Leal, 2000; Ochieng *et al.*, 2002).

After landing on a hair's surface, the pheromone molecules enter the interior of the hair (or other types of sensilla in other groups) through pores and associated tubules and traverse a perireceptor gel comprised mainly of pheromone binding protein until they arrive at the membrane-bound pheromone receptor on the dendrite of an olfactory receptor neuron (ORN). Usually there are two, but sometimes three, pheromone-component-sensitive ORNs that are cocompartmentalized within one sensillum. For a thorough review of the biochemical events involving binding proteins, pheromone degrading enzymes, and the receptors themselves (see **Chapter 3.15**). A depolarization of the dendritic membrane of the ORN results if the pheromone component molecule has the correct geometry and electron distribution due to various double bonds and functional groups (e.g., acetate, alcohol, aldehyde, or ketone) to allow it to fit optimally into the protein pocket that is the receptor.

It is a point of debate as to whether the binding-protein-plus-pheromone-component molecule complex or the pheromone-component molecule itself binds to the "pheromone receptor" (see **Chapter 3.15**). A G protein coupled system cascade results in ion channel changes that result in dendritic depolarization and action potential generation down the axon to the brain.

This process is occurring simultaneously in the receptor hairs that contain ORNs that are specifically tuned to the other components of the pheromone blend. Often, as in the Tortricidae, the ORNs tuned to the minor pheromone components reside in the same hairs as those tuned to the major component (Akers and O'Connell, 1988). The minor components arrive on the antenna in the same plume-strands as the major component, and the pheromone components blended in each strand at particular ratios are immediately transduced to action potentials by their respective, component specifically tuned ORNs. The blend composition in each strand is therefore broken down into its component parts and the composition reported to neurons farther down the line by the differential activities of the different types of ORNs responding selectively to their own optimal ligands (Todd and Baker, 1999). The process is remarkable in that the across-antennal response of differentially tuned ORNs to each odor strand is basically a split-second gas chromatographic readout of the odor strand composition that is repeated two or three or more times a second as long as the insect remains in contact with the plume (Vickers *et al.*, 2001).

The differential responsiveness of the ORNs to compounds is imparted by the peculiar receptor site proteins on the dendrites and perhaps even by the selectivities of binding proteins that bathe the ORNs as a gel (Stengl *et al.*, 1999) (see **Chapter 3.15**). The result is that the ORNs respond optimally only to the molecules in the blend to which they are differentially tuned (and also to which the binding protein binds and delivers to the ORN's dendrite), even though the complete blend of compounds has adhered to and possibly entered the hair lumen with its own binding protein milieu. The abundance of each pheromone component in each strand is thus first represented in the relative activities of the neurons specifically tuned to them. The action-potential activities of these thousands of neurons responding to their specific portion of the two- or three-component blend then stream down in separate lines, apparently without cross-communication with other axons in the antennal nerve, to finally arrive at the macroglomerular complex (MGC) in the antennal lobe located at the base of the antenna (Matsumoto and Hildebrand, 1981; Christensen and Hildebrand, 1988; Christensen, 1989; Hildebrand and Shepard, 1997; Hansson and Christensen, 1999).

**6.12.4.2.2. Glomeruli in the MGC** The MGC comprises varying numbers of glomeruli, which are knots of neuropil made up primarily of axonal branches from antennal sensory neurons but also the fibers and arborizations of interneurons that synapse with them and with each other. The MGC is found only in males and receives information exclusively from sex-pheromone-sensitive antennal neurons (Matsumoto and Hildebrand, 1981; Boeckh and Ernst, 1983; Boeckh *et al.*, 1984; Hildebrand and Shepard, 1997). Antennal neurons that respond to plant- and flower-derived compounds terminate in what are termed ordinary glomeruli located more ventrally in the antennal lobes of both males and females (Hansson, 1995; Hildebrand and Shepard, 1997; Anton and Homberg, 1999; Hansson and Christensen, 1999).

Studies by Hansson *et al.* (1992) using activity-dependent uptake of cobalt to stain ORNs and following them to the MGC revealed that each MGC subcompartment in the antennal lobe of the turnip moth, *Agrotis segetum*, exclusively handles incoming information from the antenna about only one pheromone component or a known behavioral antagonist. Earlier, proof that there is an orderly and

reliable position of ordinary glomeruli in the insect brain from individual to individual was known from anatomical work on a moth and butterfly species (Rospars, 1983). However, it was the discovery that there are functionally, as well as spatially, distinct MGC subcompartments that receive pheromone-component-specific information from the antennal ORNs that was a breakthrough for understanding animal olfactory processing (Hansson *et al.*, 1991, 1992). Results using cobalt staining of ORNs on other moth species mirrored those of Hansson *et al.* (1992), Berg *et al.* (1995), Christensen *et al.* (1995a), Ochieng *et al.* (1995), Todd *et al.* (1995); review: Hansson *et al.* (1995).

Using a new technique involving calcium-sensitive dye developed by Galizia and colleagues for the honeybee, *Apis mellifera* (Galizia *et al.*, 1997, 1998, 1999; Sachse *et al.*, 1999) allowed for the dramatic visualization of the activities of populations of whole populations of lepidopteran ORNs projecting their pheromone-component-specific excitations to their target glomeruli (Galizia *et al.*, 2000; Carlsson *et al.*, 2002; Carlsson and Hansson, 2003). These studies confirmed, on an ORN population-wide stimulation basis, the results of cobalt staining of individual ORNs that showed projections of physiologically identified ORNs tuned to one pheromone component or antagonist projecting to a single glomerulus in the MGC. The interpretation of the calcium dye excitation as being due mostly to the predominance of signal being provided by calcium released in the glomeruli from the cholinergic ORN terminals was at first a matter of debate. But the correspondence of target glomeruli from calcium dye visualization (Galizia *et al.*, 2000; Carlsson and Hansson, 2003) with the target glomeruli to which individual cobalt-stained ORNs had been shown to project to (Ochieng *et al.*, 1995; Berg *et al.*, 1998) was confirmed.

The complete blend of odor in each pheromone plume-strand is represented by a particular balance of neuronal activity across the glomerular subcompartments of the MGC. Early researchers in olfaction and taste studies realized that odor quality discrimination must result from the ratios of activity being weighed across the many different types of receptor neurons (Boeckh, 1967), a phenomenon called "across-fiber patterning" (Dethier, 1972). With recent advances, we can, however, now understand that across-fiber patterning can be considered to be an across-glomerular pattern (Galizia *et al.*, 1998), as anticipated by Rospars (1983).

**6.12.4.2.3. Across-glomerular pattern enhancement by local interneurons in the MGC** The across-glomerular pattern of relative intensities is initially enhanced by the activities of the first level of interneurons that reach into these glomerular pools of pure, component-specific ORN activity. These first interneurons are called "local" interneurons (Matsumoto and Hildebrand, 1981; Boeckh and Ernst, 1983; Anton and Hansson, 1999; Anton and Homberg, 1999; Hansson and Christensen, 1999) because they spread locally across the antennal lobe like a net, sending no axons out of the antennal lobe. They interconnect the glomerular subcompartments within the MGC and also to ordinary glomeruli receiving input from ORNs about nonpheromone-related volatiles (Matsumoto and Hildebrand, 1981; Anton and Homberg, 1999).

The integrative, discrimination-enhancing ability of local interneurons is indicated by the fact that for most of them, their neurotransmitter is γ-aminobutyric acid (GABA), which imparts inhibition on neurons upon which they synapse (Anton and Homberg, 1999). A greater acetylcholinergic excitation of a local interneuron by antennal ORN synapses in one component-specific glomerulus will therefore impart a greater amount of GABA-ergic inhibition that spreads to the other, neighboring subcompartments (Anton and Homberg, 1999).

Research on the American cockroach (*Periplaneta americana*) MGC synaptic interconnections clearly indicates that inhibition also apparently can be imposed by local interneuron fibers onto the incoming signals from ORN axons, as well as onto projection interneurons and to other local interneurons within one glomerulus (Distler and Boeckh, 1996, 1997a, 1997b; Anton and Homberg, 1999). Whatever pattern across glomeruli that would have occurred due to the inputs to the antennal lobe glomeruli from the ORNs responding to the blend components might now be enhanced by lateral inhibition imparted by these local interneurons (Christensen *et al.*, 1993). Interestingly, the various local interneuron synapses occurring within and between glomeruli might result in some excitation exiting the antennal lobe via projection interneurons due to local interneuron-imposed disinhibition of previously inhibited interneurons (Christensen *et al.*, 1993, 1998a, 1998b; Anton and Homberg, 1999). The glomeruli from which excitatory projection interneuron output emerges can also apparently be shifted by the inhibitory–disinhibitory activities of local interneurons so that the output locations do not necessarily correspond to the input locations of ORNs (Anton and Hansson, 1994, 1999).

**6.12.4.2.4. MGC projection interneurons** In the male moth (and other insects), pheromone odor

blend information emerges from the MGC and travels downstream to locations deeper in the brain through a very small number of interneurons (Boeckh *et al.*, 1984). These are called projection interneurons, because they project out of the MGC and arborize with interneurons in higher brain centers. Unlike local interneurons, projection interneurons usually arborize only in one or a limited number of subcompartments in the MGC and generally travel to the far back of the brain where they synapse with neurons in the mushroom body (Christensen, 1989; Hildebrand and Shepard, 1997; Hansson and Christensen, 1999) before continuing on to synapse with other neurons in the extreme lateral portion of the protocerebrum. Protocerebral interneurons themselves can respond in enhanced manner to optimal blends (Kanzaki *et al.*, 1991; Kanzaki and Shibuya, 1992).

The interneuronal level in the MGC at which blends have been shown most frequently to be uniquely responded to is at the projection interneuron level (but see below how mixture interactions of ORNs might confound these interpretations). However, there are many projection interneurons that respond only when a particular individual component is present, regardless of whether it is part of a blend (Christensen, 1989; Christensen *et al.*, 1991, 1995b, 1996; Hansson *et al.*, 1991; Hildebrand and Shepard, 1997; Hansson and Christensen, 1999; Vickers and Christensen, 2003). But then again there are even slight differences in pheromone blend ratio, not just composition, that are responded to selectively by blend-sensitive interneurons within the antennal lobe (Wu *et al.*, 1996).

Blend-integrating responses carried by projection interneurons are known to be of at least two different types. One type involves an increased initial spike frequency plus a long-lasting excitation that occurs in response only to the presence of a blend of two components on the antenna and not to each component presented individually. Such blend-dependent excitation responses have now been discovered in antennal lobe interneurons of several species of moths, including *Manduca sexta*, *H. virescens*, and *H. zea* (Christensen, 1989; Christensen *et al.*, 1991; Hansson *et al.*, 1991; Berg *et al.*, 1998; Hansson and Christensen, 1999).

However, another very important type of blend-integrating response also is known from *M. sexta* and other moths, in which the presence of the blend on the antenna sharpens the onset and offset of the train of spikes emerging from these interneurons, resulting in a more highly phasic burst than would otherwise occur in response to either component alone. The temporal sharpening occurs due to a combination of inhibition from neuronal elements responding to one of the two pheromone components and excitation from those responding to the other (Christensen and Hildebrand, 1988; Christensen, 1989; Christensen *et al.*, 1991, 1998a, 1998b). This suggests that the blend uniquely sharpens the time courses of the plume-strand related successive trains of action potentials streaming toward the mushroom body and lateral protocerebrum. Thus two different types of blend-integrating information, based on the temporal aspects of the response – either highly phasic-sharpened, or more long-lasting and tonic – travel out through the projection interneurons to the brain.

As discussed above, it is just as behaviorally important for males to respond to the pockets of clean air between pheromone filaments as to the pheromone filaments themselves because clean wind no longer points toward the source, and the moth continuing upwind for too long upon flying into clean wind can find itself flying off-line from the source. The interneuronal pathways in the insect pheromone olfaction system do seem to provide ample ways to integrate and send highly phasic information related to pheromone filament contact all the way through to higher centers (Christensen and Hildebrand, 1988; Anton and Homberg, 1999; Vickers *et al.*, 2001; Vickers and Christensen, 2003). Therefore, the need to quickly turn off the excitation caused by a plume-strand to allow a male to change its behavior at the first hint of clean air is reflected in the highly irregular, plume-filament correlated responses of interneurons in the MGC of the antennal lobe when placed in natural pheromone plumes (Vickers *et al.*, 2001).

**6.12.4.2.5. Mushroom body–antennal lobe-derived oscillations** There is also evidence that inhibitory feedback loops between the mushroom body and the antennal lobe can cause oscillations to occur that may impose repetitive and very short time-windows within which the ratios of activities within glomeruli can be most accurately reported by the integrative circuits out of which projection interneuron excitation occurs (Laurent, 1996; Laurent *et al.*, 1996; Stopfer *et al.*, 1997, 1999). These oscillations have been demonstrated to facilitate discrimination of odor blend quality in the honeybee (Stopfer *et al.*, 1997).

**6.12.4.2.6. Protocerebral interneuron activity** The blend integration already occurring in the MGC does not mean that the sensation of blend cannot also be promoted by integration farther downstream by protocerebral interneurons (Kanzaki *et al.*, 1991; Kanzaki and Shibuya, 1992), or even by descending interneurons, which could weigh the

ratios of activity of component-specific projection interneurons and also integrate them with other inputs such as visual flow-field information (Olberg and Willis, 1990). Because ORNs are now known to be capable of responding to blends either through mixture suppression (Nikonov and Leal, 2002) (see Section 6.12.4.4) or mixture enhancement (O'Connell *et al.*, 1986; Ochieng *et al.*, 2002), the possibility cannot be ruled out that at least a portion of what seem to be blend-sensitive projection interneurons may merely be those same interneurons relaying an ORN-derived mixture response that originated farther upstream at the antennal level.

### 6.12.4.3. Pheromone-Sensitive ORNs as High-Fidelity Reporters of Plume-Strand Encounters

Based on the need for fast behavioral reaction times to individual strands of pheromone and pockets of clean air between the strands, the peripheral olfactory receptor neurons of flying insects should be built to report information to the brain about the odor blend such that it can rapidly discriminate odor quality on a strand-by-strand basis. At least some populations of ORNs, therefore, should be built to very rapidly detect and respond to the onset of each odor strand, and interneurons in the antennal lobe farther downstream should preserve and even enhance the reporting of the temporal aspects of the signal so as to be able to report this information to higher centers and then to motor neurons. But there also should be some kind of long-lasting response in the olfactory pathways that persists for many seconds after an encounter with a pheromone strand to promote sustained casting flight in clean air that will optimize an insect's chances of bridging the long clean-air gaps between odor strands that occur during large, sudden swings in wind direction. Another function of the fast response time of the ORNs would be to report sufficiently fine-grained information that the insect can discriminate a plume in which every filament contains a complete blend of odor from one in which the blend is partitioned into separate filaments (Baker *et al.*, 1998a; Todd and Baker, 1999).

**6.12.4.3.1. Importance of fast-phasic response for reporting changes in flux** Insect sensory physiologists early on noted the phasic–tonic nature of the time course of typical ORN-generated action potential spike trains after stimulation with pheromone components (Kaissling, 1974, 1986, 1987). The phasic–tonic profile is characterized by the first portion of action potential output being a sharp burst that is shorter than, and does not correlate with, the longer-lasting stimulus (Kaissling, 1974, 1986, 1987). The remainder of the response is a tonic, residual low level of above-background firing that may not mirror the stimulus, especially at the highest dosages. The lack of correspondence of both the phasic and tonic portions of the receptor response have been shown not to be artifacts resulting from the techniques used to deliver the odorants (Kaissling, 1987).

Pheromone-sensitive ORNs do, however, display a wide variation in the phasic or tonic elements of their responses to pheromone components (Kaissling, 1974, 1986, 1987; Almaas *et al.*, 1991; Berg *et al.*, 1995; Berg and Mustaparta, 1995). There are examples of both highly phasic and highly tonic ORNs in the same species. For example, Berg *et al.* (1995) found that the receptors on male *H. virescens* antennae that were sensitive to (*Z*)-9-tetradecenal (*Z*9-14:Ald) responded in a more phasic manner than those sensitive to (*Z*)-11-tetradecenal (*Z*11-14:Ald). Rumbo (1983) and Rumbo and Kaissling (1989) found differences in the durations of response of antennal neurons tuned to different components of the sex pheromones of the light brown apple moth, *Epiphyas postvittana*, and *A. polyphemus*, respectively. The differences were usually related to the type of ORN, with the ORN tuned to the major component being the more phasic, and the ORN tuned to one of the minor components firing more tonically.

The propensity of insect olfactory receptor neurons to fire in a phasic versus a tonic pattern is related to their relative rates of adaptation and disadaptation (Kaissling, 1987). In general, phasic neurons are fast to disadapt and are able to track (respond to) the presence of pheromone that is repeatedly and rapidly pulsed at 10 Hz (Almaas *et al.*, 1991) or even more than twice that (Bau *et al.*, 2002). More tonically firing neurons are slower to disadapt, sometimes taking minutes or tens of minutes to do so, and cannot respond to a new pulse of pheromone during this time (Rumbo, 1983; Rumbo and Kaissling, 1989). Even for fast adapting–disadapting receptor neurons, disadaptation is not necessarily absolute, and the tracking response to rapidly repeated stimuli does not report accurate information about the concentration. The number of action potentials in response to each pulse, reflecting the concentration of odorant in each pulse, is significantly reduced under rapid stimulation, but the fidelity of response with regard to the temporal presence or absence of the pheromone is accurate. Thus, the sensitivity of a receptor unit in response to an odorant is not absolute and is subject to adaptation–disadaptation mechanics (Kaissling, 1986,

1987; Kaissling and Kramer, 1990). As emphasized by Kaissling (1998), it is the change in flux, which has a temporal element, and not concentration, which does not, that ORNs are specialized to report.

Contact with the strands of odorant within a natural plume is on a highly irregular basis in time, as shown both from the work of Murlis (1986, 1997) by using an idealized receptor (ion detector) and odor mimic (ionized air) as well as from the recordings of real odor plumes in the field and laboratory by using insect antennae or their ORNs as detectors (Baker *et al.*, 1988; Haynes and Baker, 1989; Hansson and Baker, 1991; Vickers and Baker, 1994; Willis *et al.*, 1994; Vickers *et al.*, 2001; Bau *et al.*, 2002). The adaptation–disadaption characteristics of receptors are concentration- and time-dependent (Kaissling, 1986, 1987; Almaas *et al.*, 1991; Hansson and Baker, 1991; Berg *et al.*, 1995), and these two aspects affect receptors' abilities to report to the CNS the presence of all of the strands of odorant that are contacted in a natural plume.

Murlis (1986, 1997) emphasized that a form of temporal averaging of the fine structure of an odor plume would occur when two equally concentrated strands contact the receptor too rapidly, resulting in the second strand evoking no action potentials from the already adapted ORN that had not recovered from responding to the first strand. Alternately, contact with a low-concentration strand could follow a high-concentration strand after an appropriately long time interval, but due to adaptation from the highly concentrated first strand, again evoke no action potentials. The adaptation–disadaptation related propensity of the ORN to adjust to both concentration and frequency thus ensures that the CNS receives high-fidelity inputs concerning plume-strand arrival over a wide dynamic range in turbulence-induced plume structures and reports them to higher centers (Vickers *et al.*, 2001). The integrative mechanisms in the CNS (e.g., for odor-quality discrimination and enhancement of phasic inputs) (Christensen and Hildebrand, 1988; Christensen, 1989) thus is buffered from outside variance in the plume, and behavioral response likewise attains a higher fidelity over such wide-ranging differences in plume-strand flux.

Limits in the dynamic range of one type of pheromone-component-specific ORN to report the arrival of strands containing the component to which it is tuned compared with the capabilities of others tuned to other components has been shown to be important in natural pheromone plumes and has been correlated with changes in behavior (Baker *et al.*, 1988; Hansson and Baker, 1991). Excessive concentrations of the three-component pheromone blend of *A. segetum* were shown to differentially impair the ability of only the (*Z*)-5-dodecenyl acetate (*Z*5-10:Ac) receptor neurons to disadapt and fire in response to repeated contact with filaments in a natural plume (Baker *et al.*, 1988; Hansson and Baker, 1991). The competencies of neurons tuned to both *Z*7-12:Ac and *Z*9-14:Ac to fire in this very same plume were completely unaffected. Thus, the excessive plume concentration caused a distorted ratio of firing from the three receptor types, with now only two types firing when there should have been three. The responses of all three neuronal types to either a 10- or 100-fold lower pheromone-source loading were correlated with high levels of completed upwind flights to the source. Both *Z*7-12:Ac and *Z*9-14:Ac would be emitted at lower concentrations than *Z*5-10:Ac due to their lower volatility, and this would explain in part the ability of the receptors tuned to these two components to avoid becoming adapted to the very same frequency of filament contacts that produced adaptation of the *Z*5-10:Ac-tuned neuron (Hansson and Baker, 1991).

The importance of a phasic reporting of stimulus arrival by ORNs to the promotion of sustained upwind movement was further emphasized in experiments that directly manipulated the outputs of ORNs (Kaissling *et al.*, 1989; Kramer, 1992). Kaissling *et al.* (1989) found that a hydrocarbon mimic of bombykol, the pheromone of the silkworm, *Bombyx mori*, caused highly tonic firing of the antennal receptor neurons tuned to bombykol that lasted several minutes, and that this tonic response was correlated with no upwind walking toward the source. Kaissling *et al.* (1989) then found that the plant-related compound linalool effectively hyperpolarized this neuron and prevented action potentials from occurring whenever it was puffed over the sensilla. When Kaissling *et al.* (1989) first stimulated the receptor neurons with the pheromone analog to induce high levels of tonic firing, and then interrupted the firing with intermittent puffs of linalool, they could create repeated, regular bursts of firing from the bombykol neurons that was indistinguishable from repeated puffs of bombykol itself. They reasoned that this highly artificial stimulus that does not contain the pheromone but mimics a reiterative pattern of phasic reporting of pheromone stimulation from the pheromone-sensitive neuron should be a behaviorally effective stimulus that should result in upwind walking by intact male moths. Kramer (1992) found that was indeed the case. High levels of upwind walking to the source occurred in response to this unnatural stimulus and not to the uninterrupted tonic stimulus.

#### 6.12.4.4. Mixture Interactions between ORNs Cocompartmentalized within Sensilla

There has been emerging evidence in recent years that insects can discriminate between two overlapping odor plumes due to the incomplete mixing of their intertwined strands (Witzgall and Preisner, 1991; Liu and Haynes, 1992; Vickers and Baker, 1992; Rumbo *et al.*, 1993; Baker *et al.*, 1998a). Wind-tunnel bioassays using experimentally generated, pulsed plumes have demonstrated that two pheromone-related odorants are not as effective when they are presented in staggered manner (in every other pulse) compared with when they are coincident in every pulse (Vickers and Baker, 1992; Fadamiro and Baker, 1997). *Helicoverpa zea* males demonstrate a startling ability to distinguish completely coincident strands of pheromone and heterospecific antagonist from those whose strands are separated by only 1 mm (Baker *et al.*, 1998a; Fadamiro *et al.*, 1999a). Baker *et al.* (1998a) and Todd and Baker (1999) pointed out that cocompartmentalization of the ORNs that are differentially tuned to these odorants (Cossé *et al.*, 1998) should optimize both the spatial and the temporal odor quality resolution ability of the animal and that on-site processing of mixtures within each sensillum would be the most likely mechanism to explain this high resolution of odor strand arrival.

Neurophysiological support for this hypothesis comes from a recent report by Nikonov and Leal (2002) in which the firing of pheromone-component-sensitive ORNs on the antennae of the Japanese beetle, *Popillia japonica*, was shown to be suppressed when a known behavioral antagonist was puffed in a perfect mixture simultaneously from a single odor cartridge across the antenna. They recorded from sensilla that always house two cocompartmentalized ORNs, one being tuned to the pheromone component and the other tuned to the antagonist (the opposite enantiomer of the pheromone component). Mixture suppression of firing by the pheromone component sensitive ORN was not observed, however, when the "mixture" of the two enantiomers consisted of simultaneous puffs from two different pipettes separated by only 1–2 mm, creating a time lag of only 1.5–3 ms (Nikonov and Leal, 2002). On-site processing of the mixture was thus found to occur in the Japanese beetle sex pheromone olfaction system and would result in reduced reporting of strands of odor containing both pheromone and antagonist to the antennal lobe.

Pheromone component sensitive ORNs on *H. zea* antennae exhibit distinct and significant responses to blends of the pheromone component mixed with certain plant volatiles (Ochieng and Baker, 2002). These plant volatiles mixed with the major *H. zea* pheromone component, (*Z*)-11-hexadecenal, enhance firing frequency during the phasic as well as tonic portions of the response of ORNs tuned to this component (Ochieng and Baker, 2002). The simultaneous arrival of the two odorants is necessary for the mixture-enhanced firing to occur (Ochieng and Baker, unpublished data). These results suggested the possibility that males' behavioral sensitivity and upwind flight response to pheromone might be elevated by the coemission of certain plant volatiles with the pheromone (Ochieng and Baker, 2002). However, a wide range of plant-related compounds as well as pheromone-related compounds mixed with (*Z*)-11-hexadenal are able to enhance firing of this ORN (Ochieng and Baker, unpublished data), and so it is unclear what role such an unspecific mixture response could play in the real world.

Until recently, little attention has been paid to the possibility of mixture interactions occurring at the ORN level, even with regard to their responses to combinations of sex pheromone components that are known to be behaviorally agonistic (O'Connell *et al.*, 1986; Akers and O'Connell, 1988). Likewise, a few sensory physiological studies have involved pheromone sensitive ORN responses to mixtures of pheromones and plant volatiles (Van der Pers and Den Otter, 1978; Van der Pers *et al.*, 1980). The knowledge that enhanced firing can occur on-site by primary olfactory receptor neurons in response to mixtures should provide a note of caution to researchers working at the antennal lobe level. It can no longer automatically be concluded that mixture interactions recorded from projection neurons are due entirely to blend integration occurring within the antennal lobe. Many of these "blend sensititive" interneurons may be merely relaying to higher centers the unprocessed, blend-enhanced activity that has already taken place far upstream, within the sensilla on the antennae (Todd and Baker, 1999).

#### 6.12.4.5. Background Noise and Resolution of Odor Strands in Overlapping Plumes

Under natural field conditions, strands of pheromone are transported in air masses that are comprised also of complex mixtures of other volatile chemicals, including those originating from surrounding vegetation. Plant-related volatiles are an integral part of the pheromone systems of the majority of beetle species that have been investigated thus far (Byers, 1995; Bartelt, 1999a, 1999b; Leal, 1999) (see Section 6.12.2.3). Also, plant-derived volatiles are

used in the courtship pheromones of moths (Birch *et al.*, 1990; Landolt and Philips, 1997). The question of how mixtures of pheromone components or plant volatiles are reported by ORNs to higher centers is of increasing importance to understanding how insects' olfactory systems can discriminate pheromone plus plant odor plume strands and respond behaviorally to natural pheromone blend signals.

In moths, a few field-trapping studies have demonstrated that certain groups of plant volatiles added to sex pheromone can increase the capture of males, including *H. zea*, in sticky traps (Dickens *et al.*, 1990; Light *et al.*, 1993). However, it is not clear whether the enhancement of male attraction to the traps was due to separate enhanced responses of male moths to either the sex pheromone or the plant volatile portion, or to a response to the total blend that may in its entirety represent a unique odor quality.

The behavioral and evolutionary implications of fine-grained spatiotemporal resolution of odor strands first of all with respect to pheromone-related compounds such as pheromone components emitted by nonconspecific females that antagonize upwind flight have been considered (Baker *et al.*, 1998a; Fadamiro *et al.*, 1999a; Todd and Baker, 1999). Strands of heterospecific antagonistic pheromone components should antagonize upwind flight only if they occur as a complete mixture with conspecific pheromone in each and every strand, which would indicate that the strands originated from a single point source, i.e., a nonconspecific female. They should not be as effective in antagonizing upwind flight if they are not perfectly admixed in every filament, with some filaments containing only pure pheromone, others containing antagonist as well. But what about strands of plant volatiles, or more uniform clouds of plant volatile background odor?

If certain plant volatiles do play a role in modulating responses of males to female-emitted pheromone, it is not so clear what the olfactory system would be doing to optimize these responses. Plant odors in the field would seem to be ubiquitous and serve as a rather tonic fog of average chemical "noise" in which pheromone strands in a plume would travel and be detected by the male ORNs. However, there are many ways in which stronger point sources of plant volatiles can occur, the most obvious being flowers, fruits, and seeds (also corn silk) as strong point source plume generators. Other strong point sources can be generated by direct larval insect feeding damage to leaves (Turlings *et al.*, 1995; Alborn *et al.*, 1997; De Moraes *et al.*, 1998), and still other strong sources can be entire plants that have been induced to produce bursts of volatiles over the their entire surface by the systemic effect of insect saliva "elicitors" on the plants' biochemical machinery (Turlings *et al.*, 1995; Alborn *et al.*, 1997; De Moraes *et al.*, 1998).

There are many ways that any of these point source plumes of plant volatiles could be mixed with strands of pheromone from an adult insect. Some of these situations would see pheromone-plus-plant volatiles issuing as fully mixed strands from relatively strong point sources such as from bark beetle gallery holes, or from weevil feeding-wounding (J.J. Heath *et al.*, unpublished data). It would seem that the simultaneous emission of plant volatiles and pheromone from small, adult-generated point sources corresponds with the enhanced pheromone-plant-volatile attraction seen in the majority of studies of species of Scolytidae and Curculionidae. With moths, it is not as clear that there can be many situations where females can generate fully mixed strands of pheromone and plant volatiles from a small point source. One major difficulty here is that for most species in nature we do not know where females go to call. We do know that they will call from almost any surface, natural or unnatural, and in the presence of plant odors as well as in their absence. With no strong requirement having been observed for female moth calling in the presence of plant volatiles, it makes sense that only a very few studies have shown a significant enhancement of male attraction to pheromones by adding plant volatiles.

In natural situations, regardless of whether the calling female is sitting directly on a flower, fruit, or damaged leaf, it seems unlikely that perfectly mixed strands of pheromone plus plant volatiles having the same temporal flux dynamics will arrive simultaneously on males' antennae. Thus, it is questionable as to whether there is the same need in moths for fine-grained resolution of plant odor and pheromone odor strands as there might be in the Coleoptera, where plant-odor–pheromone mixture enhanced attraction is prevalent and strong point sources emitting plant volatiles plus pheromone are prevalent. But the tolerances of olfactory systems and behavioral responses for synchronous versus asynchronous plant-odor-plus-pheromone plume-strand arrival in different species' communication systems have not been explored. In future neuroethological studies of insect pheromone–plant-volatile mixture interactions, attention needs to be paid to the fine-grained resolution of odor strands and the natural plant-plus-pheromone odor plume generation occurring for each species to understand how these communication systems may have been evolutionarily shaped.

#### 6.12.4.6. Malleability of Receptor Tuning in Evolution of Sex Pheromones

That the relative abundance of differentially tuned ORNs on insect antennae can change over time is graphically illustrated by the findings of George and Nagy (1984), who found that after 20 years of rearing the same culture of oriental fruit moth, the number of sensilla trichodea housing pheromone ORNs on male antennae decreased to one-half of the original, wild-type number. In the noctuid moth species *A. segetum*, over a wide geographic range encompassing Europe and Africa, variation in the ratios of pheromone components emitted by females from different regions is mirrored by the ORN system of males (Löfstedt *et al.*, 1986; Hansson *et al.*, 1990). In *A. segetum*, the proportional abundance of ORNs tuned to *Z*5-10:Ac, *Z*7-12:Ac, and *Z*9-14:Ac is at least coarsely directly proportional to the proportions of these compounds found in gland extracts of females (Löfstedt *et al.*, 1986; Hansson *et al.*, 1990; Löfstedt, 1990). If the vapor pressures of the components were to be considered as well, the degree of correspondence between emitted proportions of components and the proportion of ORNs tuned to these components would certainly be even more highly correlated. Thus, there seems to be a good degree of ability of the ORNs of moths that are tuned to different components to change their abundance on insect antennae fairly quickly in ecological time. There is also evidence that ORN response profiles can be modified by factors from the brain, as suggested by the cross-species antennal transplant studies of Ochieng *et al.* (2003).

How this malleability is accomplished across a population of differentially tuned ORNs on antennae is unclear, but mutations in some insect olfactory communication systems have provided some insight. Mutations in the ORNs of *Drosophila* have been correlated with changes in olfactory mediated behavior (Carlson, 1996; deBryune *et al.*, 1997). These results and others in the area of the neurogenetics of olfaction are discussed thoroughly in the recent review chapter by Stocker and Rodriguez (1999). The *Drosophila* findings show that mutations affecting an ORN can involve the silencing of the ORN, in addition to the altering of the response spectra of other neurons (deBryune *et al.*, 1997). The changes in ORN response profiles were discovered after behavioral evidence in the mutants indicated that significant olfactory changes must have occurred.

Recently, it has been hypothesized that major shifts in moth pheromone blends might occur when previously unexpressed genes controlling pheromone component double bond position become expressed in some females in a population (Roelofs *et al.*, 2002). The new, altered blend might be attractive to a very few males in the population that have the capability of responding to both this new blend and the old blend, and through a process of asymmetric tracking over generations by males (Phelan, 1997), a new population of males and females communicating with this new blend would be formed (Baker, 2002; Roelofs *et al.*, 2002; Roelofs and Rooney, 2003). The degree to which pheromone-sensitive ORNs would be able to shift their tuning properties to correspond to and track, over generations, the new pheromone components is important for such a shift in pheromone blend communication to occur.

It does seem that in some moth species that, compared with the rest of the species in their group, use unusual pheromone components (Löfstedt *et al.*, 1991; Löfstedt, 1993), the male pheromone-sensitive ORNs display correspondingly unusual response spectra (Löfstedt *et al.*, 1990). The examination of pheromone-sensitive ORN tuning properties within species as it relates to the ability to potentially accept odd pheromone molecules should be informative in future studies after the exciting findings of Roelofs *et al.* (2002). The degree to which pheromone blends can suddenly shift via expression of pseudogenes (Roelofs *et al.*, 2002) followed by asymmetric tracking by responding males (Phelan, 1997) can be first ascertained by studying the variation in pheromone-sensitive ORN tuning curves within, and then between, two or more targeted species within a group (Löfstedt *et al.*, 1990). Attention also needs to be paid to ORNs that are responsive to heterospecific antagonists, because these are the ones that may be involved in completing the asymmetric tracking process by preventing the continued attraction of broadly tuned males to females of the parent population over generations (Baker, 2002).

### 6.12.5. Use of Pheromones in Insect Control

#### 6.12.5.1. Monitoring Established Populations

The most widespread and nearly ubiquitous use of pheromones has been for the monitoring of pest species' adult populations. Comprehensive IPM programs that were initiated in New York State and Michigan during the early 1970s were based on good, species-specific monitoring traps for the complex of tortricid moth pests that impose direct and indirect damage to fruit. Tree fruit IPM was

impacted positively, resulting in elimination of prophylactic calendar-date spray schedules and the introduction of timed applications of short-residual insecticides. During the mid-1970s to late 1980s, insecticide applications were reduced by more than 50% in New York State, Michigan, and the Pacific Northwest due to monitoring programs that improved decision-making about the need to spray insecticides (Madsen, 1981) as well as their timing. Such programs have become even more refined over the years, with concomitant further reductions in insecticide applications being documented (Agnello *et al.*, 1994).

Monitoring of leafroller pests coupled with computer assisted degree-day models (Riedl and Croft, 1974; Riedl *et al.*, 1976) allowed sprays to be timed for optimum efficacy against eggs and first instars on such key pests as the codling moth, *Cydia pomonella*, and oriental fruit moth. Spray-or-no-spray decisions based on abundance of adults in monitoring trap grids were also made possible by effective, standardized monitoring traps and deployment schemes developed against some pests such as the codling moth (Madsen, 1981). On other crops in the United States as well as around the world, pheromone monitoring traps have asserted themselves into IPM programs as essential elements to the success of these programs. Their use is such an integral and accepted part of IPM that we do not further review their use.

### 6.12.5.2. Detection and Survey Programs for "Exotic" Pests

Whereas we usually think of exotic pest species as being those originating from overseas, oftentimes the more distinct threat to agricultural crops comes from immigrating populations from one geographic area within a country into a different location. Pheromone traps have played a large role in detecting influxes 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 so routinely to report and track the yearly arrival of migrating adult populations of insects such as the black cutworm, *Agrotis ipsilon*, in the Midwest (Showers *et al.*, 1989a, 1989b) or the spread of expanding populations such as the gypsy moth (Elkinton and Cardé, 1981) that it has become an essential part of our arsenal of tools for tracking and ameliorating pest movement related threats. A few examples of how pheromone trapping survey-detection systems have provided essential information that has aided in the suppression of migrating or expanding populations are reviewed.

**6.12.5.2.1. Pink bollworm** The pink bollworm, *Pectinophora gossypiella*, is a key worldwide pest of cotton. In the southern California desert valleys and in Mexico, its presence has altered IPM schemes since the 1960s, necessitating the application of 10–15 extra insecticide sprays per hectare per season. In the San Joaquin (Central) Valley in California, growers do not have to apply insecticides against this pest because it is not endemic, and therefore they enjoy much greater profit than their southern California counterparts. Indeed, many have attributed the near complete demise of cotton agriculture in southern California over the past two decades to the presence of the pink bollworm. The pink bollworm has not yet become established in the San Joaquin Valley, in large part due to an intensive grid of detection-survey traps that is deployed every year in a program run by the California Department of Food and Agriculture (CDFA) and supported by bale-tax "check-off" receipts from the grower group, the Cotton Pest Control Board.

Every summer, adult pink bollworms are blown into the Central Valley from Mexico and the southern California desert regions on tropical storm systems. The threat of these "blow-ins" mating and depositing eggs is huge; that they could survive and flourish to become established in the 284 000 ha of cotton grown in the valley is not disputed. The CDFA monitors an extensive grid of pheromone traps averaging approximately one trap per square mile, but extending at various densities throughout the Central Valley (Baker *et al.*, 1990). When a male moth is discovered in a trap, an airdrop of sterile moths is implemented, with the plane being guided to the affected set of cotton fields, and the jettisoning of sterile moths from the plane all precisely recorded and verified by global positioning system computer-monitored tracking systems back at a home base. Sterile males are reared at the USDA–APHIS sterile pink bollworm moth-rearing facility in Phoenix. The sterile moths are dyed pink by the diet they ingest at the rearing facility, and so the many sterile males captured in the pheromone traps are easily distinguished from nondyed blow-ins. Quality control is also implemented by project leaders to confirm the vigilance of the many scouts who are employed each summer to check the traps. At predetermined and random times a nondyed male is placed in a trap and the discovery of this planted blow-in by the scout is awaited.

It is assumed that the presence of a nondyed male moth in a trap is indicative of females in the same area, and that the threat of an increasing endemic population of pink bollworms becoming established is real. The airdrops of sterile moths are aimed at

creating a ratio of at least 60:1 pink:normal-colored (sterile:wild) male captures in the pheromone traps. This has proven to be an effective ratio for the sterile insect technique to eventually eliminate the nascent endemic population in location after location, year after year. When the 60:1 ratio cannot be attained, either by too many wild males present or too few sterile individuals being produced at a particular time by the sterile moth rearing facility, the CDFA then applies pheromone mating disruption. In the late 1980s, up to 2025 ha of cotton each year in the Central Valley received mating disruptant formulation applications in this context (Baker *et al.*, 1990). Monitoring and sanitation of this exotic pest under this program has provided a significant savings to growers and to the environment, allowing cotton to continue to be grown in central California with minimal insecticide load and maximum profits. The program is still operating successfully.

**6.12.5.2.2. Boll weevil** The boll weevil, *Anthonomus grandis*, has historically been one of the most economically damaging pests of agriculture in the United States. Its invasion into all of the southeastern states by 1917 from Mexico through Texas (Ridgway *et al.*, 1990) is widely considered to have caused the severe decline that occurred in cotton production in this region through the 1960s. Even now, it continues to be considered one of the major agricultural insect pests in the United States.

The identification of the male-emitted sex pheromone of the boll weevil by J.H. Tumlinson and colleagues in the late 1960s (Hardee *et al.*, 1967a, 1967b; Tumlinson *et al.*, 1968, 1969, 1970, 1971) resulted in the isolation and identification of a four-component pheromone (Tumlinson *et al.*, 1969, 1970, 1971). The components were identified as: (1*R*-*cis*)-1-methyl-2-(1-methylethenyl)cyclobutaneethanol; (*Z*)-2-(3,3-dimethylcyclohexylidene)ethanol; (*Z*)-(3,3-dimethylcyclohexylidene)acetaldehyde; and (*E*)-(3,3-dimethylcyclohexylidene)acetaldehyde. This blend was named (thankfully!) "grandlure." Numerous field trapping tests determined the requirement that all four components need to be present for optimal activity. The range of ratios that could be tolerated in achieving optimum trap capture of weevils was also determined (Coppedge *et al.*, 1973; Hardee *et al.*, 1974). An effective trap design was developed (Mitchell and Hardee, 1974) that allowed survey-detection and mass trapping studies to be initiated.

A program was subsequently initiated in Texas to begin large-scale efforts at suppressing boll weevil populations with stalk destruction, whose efficacy at reducing populations is monitored and confirmed by widespread deployment of pheromone traps (Ridgway *et al.*, 1990). However, the most widespread and effective use of the boll weevil pheromone traps has been in the highly successful population suppression program in the southeastern states. Initial trials were begun in the late 1970s in Virginia and northern North Carolina, consisting of surveillance and suppression through widespread pheromone trapping. 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). Over the years of this program in this initial region, boll weevil capture levels declined to near-zero.

The success of the program in Virginia and northern North Carolina allowed the "boll weevil eradication" program to be expanded to the remainder of North Carolina and all of South Carolina in 1983. In 1987, the program was expanded further to include Georgia and parts of Alabama and Florida. The intensive dependence on pheromone traps as the key tool used in this program is indicated by the numbers of traps and lures used. In 1988 alone, approximately 590 000 traps were deployed and more than 8.25 million pheromone dispensers were used in these southeastern states (Ridgway *et al.*, 1990).

The pheromone traps served as a guide for application of insecticides, and this use of traps predominated during the initial years of the program when weevil densities and trap captures were high (**Table 2**). Beginning in year 2, if trap captures exceeded an average of 0.1 weevil per trap, insecticides were applied. However, if capture levels were lower than this, mass-trapping alone was considered adequate for population suppression (Ridgway, 1990). During the last 2 years of the second phase of the project, mass trapping was nearly exclusively used, due to the capture of so few weevils in most of the hectarage (**Table 2**). The effect of the program on weevil population densities over the first 4 years of the program was dramatic (**Table 2**). Whereas in the first year virtually no fields out of the 2800 fields sampled captured zero weevils, by year 4 more than 99.9% of the fields had zero captures. During the first year more than 95% of the fields had pheromone trap captures exceeding five weevils per trap, but by year 4 only 0.6% of the fields had trap captures exceeding this level (**Table 2**).

The impact of the program was significant, in terms of allowing cotton production to once again expand and flourish, in terms of the reduction in insecticides applied per hectare (savings of \$69–74 $ha^{-1}$ in Virginia, North Carolina, and

**Table 2** Boll weevil captures in successive years of the expanded boll weevil eradication zones in North Carolina and South Carolina

| Year | Area (ha) | Number of fields | Percentage of fields capturing indicated numbers of weevils | | |
|---|---|---|---|---|---|
| | | | 0 | 1–5 | >5 |
| *1983* | | | | | |
| North Carolina | 6600 | 800 | 0 | 1 | 99 |
| South Carolina | 21240 | 2000 | 2 | 5 | 93 |
| *1984* | | | | | |
| North Carolina | 9200 | 1000 | 21 | 35 | 44 |
| South Carolina | 32000 | 2300 | 22 | 25 | 53 |
| *1985* | | | | | |
| North Carolina | 9400 | 1000 | 90 | 7 | 3 |
| South Carolina | 37000 | 4300 | 81 | 12 | 7 |
| *1986* | | | | | |
| North Carolina | 8600 | 1000 | >99.9 | 0 | 0 |
| South Carolina | 34400 | 4200 | 97.3 | 2.3 | 0.4 |

South Carolina), and in terms of the increased yield value of the harvested cotton (increase of $\$85\,ha^{-1}$) (Ridgway *et al.* 1990).

### 6.12.5.3. Mass Elimination of Insects from the Population by Using Pheromones

Early research in the applied uses of pheromones experimented with mass trapping (Roelofs *et al.*, 1970). When experiments, mostly with adult moths, resulted in little or no suppression of populations, explanations as to the lack of effect focused on the fact that only males were being trapped, and various mathematical models were offered that showed the potential futility in using mass trapping with female-produced pheromones that attract and trap less than 95% of the males (Roelofs *et al.*, 1970). It was, however, recognized that mass-trapping could be an effective method if good attractants for females could be identified (Lanier, 1990). Such attractants were already known for bark beetle species of the genus *Ips*, in which it is the males that locate the host trees, build galleries, and then emit pheromone to attract females. This male-produced sex pheromone also coincidentally attracts other (opportunistic) males, who fly to the trees on which any newly arriving females might be able to be mated with before they locate the male producing the pheromone (Borden, 1985).

Although this approach to using pheromones for direct control was largely ignored by many groups of pheromone researchers as a tenable population suppression method, Borden (1994) stated, "I contend that mass trapping has acquired an unjustifiably bad reputation." Borden, along with several other groups of bark beetle pheromone researchers were instrumental in exploring various forms of mass trapping to come up with successful demonstration plot programs for a variety of bark beetle pest species (Bedard and Wood, 1981; Borden and McLean, 1981; Lanier, 1981). Commercially successful programs then evolved from some of these programs.

Only a few moth sex pheromone researchers continued to explore another form of mass trapping (attract-and-kill) involving attracting only males to synthetic female sex pheromone sources laced with small amounts of insecticide (Charmillot *et al.*, 1996; Charmillot and Hofer, 1997). This technique has begun to enjoy entry into the marketplace in recent years.

Finally, research and development of mass trapping systems for a variety of highly damaging tropical weevil species by using male-produced sex pheromones has resulted in highly successful commercial mass-trapping systems for suppressing populations of these species (Oehlschlager *et al.*, 1992a, 1993, 1995, 2002). A few examples of the development of research successes into successful commercial products or governmental programs that have utilized various forms of mass trapping, including attract-and-kill will be discussed.

**6.12.5.3.1. Mass trapping** In the late 1970s populations of the spruce bark beetle, *Ips typographus*, were increasing yearly and threatening the very existence of the paper and lumber industries of Sweden and Norway. Because of the existence of a highly effective synthetic pheromone blend and the desperation of the industry, the governments of these two countries agreed to subsidize a mass-trapping program that would include virtually all the hectares of forest land affected or that could be affected by this pest. In 1979, more than 600 000 traps baited with the male-produced sex pheromone ("aggregation pheromone") were deployed in Norway, and more than 300 000 traps were deployed in Sweden, often with the help of local citizens who agreed to place them in the forests near their summer cottages in the northern areas of the countries. More than 4.5 billion beetles were trapped during the summer of 1979. The following year, the number of trees killed by *I. typographus* in Norway remained the same as during 1979 or was slightly reduced, and the program was deemed highly successful because a large increase had been expected. In Sweden, there was a great reduction in the number of trees killed (Lie and Bakke, 1981). In both countries, this was the first year in

many that tree mortality had not continued to increase. The forest industry was saved, and populations declined in the succeeding years. No replication or check plots could be used to assess this program due to the areawide (country-wide) deployment of the traps; however, this was not a scientific experiment, it was a desperate measure whose implementation correlated with the first nonincrease in the population in years.

The American palm weevil, *Rhynchophorus palmarum*, is a highly damaging pest of oil and coconut palms in the American tropics, and in Central and South America. A related species, *R. ferrugineus* is a major pest of oil and other palms in the Middle East. Larvae of *R. palmarum* cause direct damage when they bore into the trunks of the trees, but they also are a vector of red ring disease, which is caused by a nematode, *Rhadinaphelenchus cocophilus*. In the early 1990s, losses of trees either due to the weevil itself or to red ring disease (which necessitates removal of the infected trees) often routinely reached 15%. Due to the threat to the oil palm industry posed by this weevil in Costa Rica, experiments aimed at mass trapping the adult weevils were undertaken. An effective male-emitted sex pheromone (Rochat *et al.*, 1991; Oehlschlager *et al.*, 1992b) plus food attractant bait had been developed (Chinchilla and Oehlschlager, 1992; Oehlschlager *et al.*, 1993) which attracts primarily females but also males, that are trapped in inexpensive bucket-type traps. The traps are baited with the pheromone, 2-methylhept-5-en-4-ol (Oehlschlager *et al.*, 1992b) plus a stalk of sugarcane laced with carbofuran. Previous experiments had shown that neither sugarcane alone nor pheromone alone attracted significant numbers of weevils, whereas the combination attracted and trapped large numbers (Oehlschlager *et al.*, 1992a, 1993).

In 1991 in a 30 ha stand of oil palms in a highly infested plantation in Costa Rica, 219 bucket traps baited with pheromone plus insecticide-laced sugarcane were placed at eye level on the trunks of oil palm trees in a 30 ha plot within the 1256 ha plantation. Over the course of the year, the number of weevils captured per trap per week declined from an initial 15 per trap to fewer than two per trap (Oehlschlager *et al.*, 1995, 2002). In contrast, in the surrounding survey areas of the plantation, the number of weevils per trap per week increased from approximately 15 to an average of about 60 for the subsequent months, and ending at more than 20 per trap per week at sampling termination. Incidence of red ring disease in the mass trapping plot decreased in the year after initiation of the mass trapping, to level approximately only 20% of the previous levels and one-half that in the surrounding untreated stands (Oehlschlager *et al.*, 1995, 2002).

Plantation-wide mass trapping was undertaken at the same time on two major oil palm plantations in Costa Rica, one comprising 6514 ha and the other comprising 8719 ha (Oehlschlager *et al.*, 2002). Four-liter bucket traps baited as described above with pheromone plus insecticide-laced sugarcane stalks were deployed at chest level at a density of only one trap per 6.6 ha. The results in the two plantations were virtually identical (**Figure 2**). Before the mass trapping program was implemented, normal sanitation procedures involving removal of red ring disease-infected palms had failed to reduce the incidence of this disease. Once mass trapping was added to the system, after 1 year the incidence of diseased trees needing to be removed dropped by nearly 80%, from about 10 000 diseased trees removed to 2000 (Oehlschlager *et al.*, 2002) (**Figure 2**). During that first year (1992–93) more than 200 000 weevils were trapped. In each successive year, the incidence of diseased trees declined until in 2001 only 50 diseased trees needed to be removed through sanitation (Oehlschlager *et al.*, 2002) (**Figure 2**).

Subsequent commercial development of the *R. palmarum* mass trapping system has seen it be transformed into one of the most important pest management tools for weevil pests worldwide. Approximately 25 000 ha of palm plantings is estimated to be under *R. palmarum* mass trapping control yearly in Central and South America (A.C. Oehlschlager, personal communication). The density of traps now used is now only one trap per 7 ha. In addition to the ability of this male-based pheromone to attract females, and males, the reasons given for the success of mass trapping against *R. palmarum* (Oehlschlager *et al.*, 2002) are first, that although the weevils cause great direct and indirect damage and induce tree mortality, they are present in relatively small numbers. Second, they have a long adult life, and so the capture of even low numbers steadily throughout the year can remove a large proportion of a generation and have a significant impact on population growth. Third, they are strong flyers, which allows the pheromone traps to be widely spaced (Oehlschlager *et al.*, 2002).

These characteristics favoring success of mass trapping also seem to pertain to the other tropical pest weevil species. Other highly successful and effective commercial mass trapping systems using male-emitted sex pheromones are in place for these other species. For *R. ferrugineus*, more than 35 000 ha of palm plantings are treated with mass trapping every

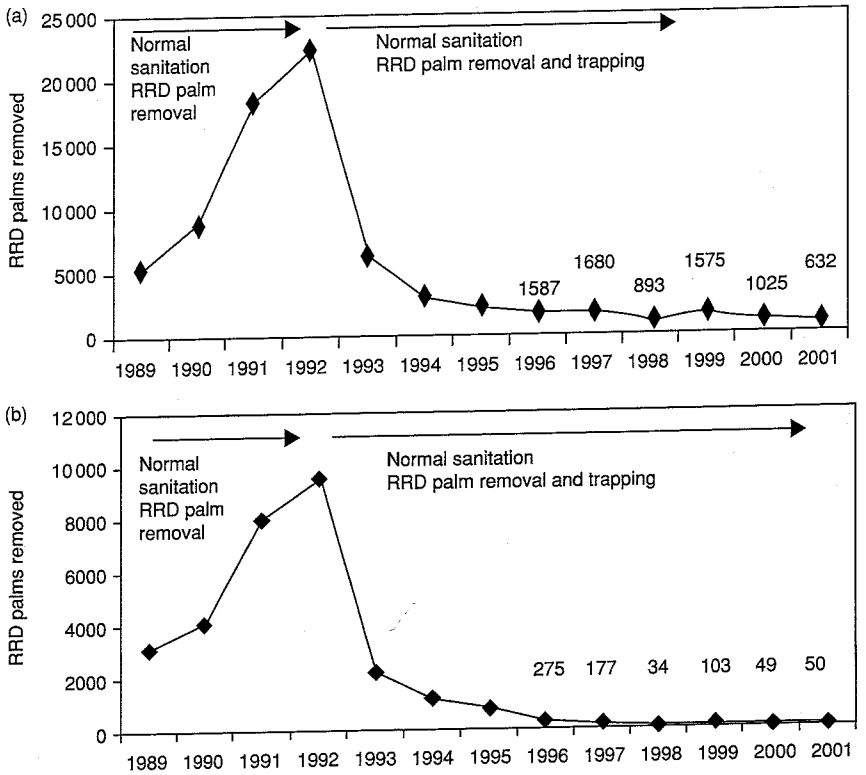


**Figure 2** Incidence of red ring disease (RRD) vectored by the *Rynchophorus palmarum* weevil in oil palms in Costa Rica, before and after mass trapping of the weevils, as measured by the number of diseased trees that had to be removed each year from two plantations, (a) and (b), from 1989 to 2001. Plantation (a) comprised 6514 ha and plantation (b) totaled 8719 ha. From 1989 to 1992, before the implementation of mass trapping of the weevils, disease incidence steadily rose. During this period only normal sanitation and diseased palm tree removal were used to try to control the weevils. After 1992, when pheromone mass trapping was added to the program, the incidence of red ring disease declined dramatically. (Adapted from Oehlschlager, A.C., 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.)

year in the Middle East. For another pest of palm, *Oryctes rhinoceros*, more than 50 000 ha yearly are under a mass trapping program in the Middle East (A.C. Oehlschlager, personal communication).

An estimated 10 000 ha of commercially grown bananas in the American tropics is under mass trapping programs every year against the banana weevil, *Cosmopolites sordidus* (A.C. Oehlschlager, personal communication). A simple and inexpensive pheromone trap plus this species' pheromone is used in only about four traps per hectare, but here the traps are placed on the ground because the weevils must walk into them to be captured in pit-fall manner. The traps are moved in a line week by week through the banana plantations to trap-out the weevils, and so the actual density of traps per hectare throughout the entire plantation that accomplishes weevil trap-out is extremely small.

Mass trapping also is being used extensively in palmito palm, which produces a delicacy, palm hearts. In palmito palm plantations, a combination lure for *R. palmarum* and the West Indian sugarcane weevil, *Metamasius hemipterus*, is used; the pheromones of the two species, 2-methylhept-5-en-4-ol and 2-methyl-4-heptanol (Perez *et al.*, 1997), do not interfere significantly with attraction and significantly lower the incidence of damage when placed in the same bucket traps at a density of four traps per hectare in this crop (Alpizar *et al.*, 2002). Hence, this "combination trap" kind of mass trapping program aimed at two species at once is now showing success at trapping and removing two damaging species of weevils from the population (Bulgarelli, 2002).

The ambrosia beetle, *Trypodendron lineatum*, is a worldwide pest in industrial timber processing areas, which, along with two other such beetles, *Gnathotrichus sulcatus* and *G. retusus*, causes degradation in lumber value that in the early 1990s was estimated in to be more than $100 million per year in British Columbia (Lindgren and Fraser, 1994). This estimate ignores other losses, including export restrictions and modified harvesting and processing needed for the damaged timber. A major pheromone component of *T. lineatum*, 3,3,7-trimethyl-2,

9-dioxatricyclo [3.3.1.0$^{4,7}$] nonane ("lineatin"), had been isolated and identified from female frass in 1977 (MacConnell *et al.*, 1977) and its field activity confirmed (Borden *et al.*, 1979). The pheromone of *G. sulcatus* was identified from the volatiles of boring males as a 65 : 35 ratio of (*S*)-(+)- and (*R*)-(−)-6-methyl-5-hepten-2-ol (sulcatol) (Byrne *et al.*, 1974), and the blend of both enantiomers was requisite to attraction (Borden *et al.*, 1976). *Gnathotrichus retusus*, in contrast, was found to use only the (*S*)-(+)-enantiomer, which was identified from the dust of boring males (Borden *et al.*, 1980a). Small amounts of the *R*-(−)-enantiomer in the blend were found to reduce attraction to the (*S*)-(+)-enantiomer (Borden *et al.*, 1980a).

With the addition of host volatiles, synthetic versions of this female-emitted pheromone were highly effective at attracting males and females as well (Borden *et al.*, 1980b). Research efforts towards developing a mass trapping program for keeping lumber yard operations sanitized from attack by all three ambrosia beetle species demonstrated the efficacy of this approach (Borden and McLean, 1981). Before the pheromone-based mass trapping program was available, many timber processing facility managers had used a trap-crop approach in protecting their yards, placing log piles in strategic locations around the yards and then collecting and destroying them (and the beetles) in pulp-chippers (Borden, 1990). The pheromone-based mass trapping IPM strategy evolved first from baiting log piles with pheromones, and then to enhancing beetle attraction to the piles, and finally to a pheromone-trap-only system that was made possible by the development of the easily used and highly effective multiple-funnel trap (Lindgren, 1983; Lindgren and Borden, 1983; Lindgren *et al.*, 1983; Borden, 1990).

Several companies successfully marketed this ambrosia beetle mass trapping system, which is still in use commercially. One evaluation of the history of its use in one timber-sorting area on Vancouver Island showed that in more than 12 years, at least 16.5 million beetles had been captured, while keeping infestations to a minimum at an acceptable cost and economic benefit (Lindgren and Fraser, 1994). A key element to the companies' success, and the efficacy of the mass trapping programs, was that clients were required to purchase a service contract to accompany the sales of the traps and lures for the three species. This ensured that the appropriate vigilance and consistency in trap efficacy were maintained (Borden *et al.*, 2001).

It is clear that the once-discounted technique of mass trapping using either male-produced or female-produce pheromones has become a newly appreciated, highly effective, environmentally friendly, and relatively inexpensive means of suppressing populations of certain pest species whose pheromone communication systems and bionomical characteristics make them susceptible to this approach. The next section dealing with attract-and-kill is yet another example of possible ways to gain control over pest populations with sex-attractant pheromones.

**6.12.5.3.2. Attract-and-kill** In the early 1980s many cotton growers in the southern California desert valleys who had been successfully using the hollow-fiber sex pheromone mating disruption system against the pink bollworm started experimenting with reducing the amount of active ingredient (a.i.) per acre, often by one-half or more, and adding small amounts of pyrethroid to the sticky glue in the tank mix that adheres the fibers to the cotton leaves when flung from the specialized aerial application mechanism (Baker *et al.*, 1990). The apparent success of this technique in keeping damage low with reduced amounts of a.i. signaled the beginning of the concept of "attract-and-kill." Observations showed that males indeed were attracted all the way to the pyrethroid-impregnated stickum on the fibers (Haynes *et al.*, 1986; Baker *et al.*, 1990; Miller *et al.*, 1990) and that they were killed or else rendered incapable of flying upwind to subsequent sources in the following days due to sublethal toxic effects (Haynes *et al.*, 1986).

Pierre Charmillot and colleagues, after years of meticulous experiments in codling moth mating disruption with small apple orchards with irregular borders and high population hot spots (Charmillot, 1990), began experimenting with attract-and-kill in isolated apple orchard blocks (Charmillot *et al.*, 1996; Charmillot and Hofer, 1997). In their studies, they used a formulation called Sirene CM (Ciba Corporation) that consisted of very small viscous gel droplets (0.05 ml) containing small amounts of permethrin (3 mg) and 0.08 mg of codling moth pheromone. Droplets were applied at between 1600 and 5000 drops ha$^{-1}$ and after either one of the treatments, damage at harvest was 0.24%, 0.32%, 1.1%, and 1.14% in four orchards.

Two orchards that received a second year of attract-and-kill had negligible larval attack and very low hibernating larval levels on the trunk (less than one larva per tree). These experiments showed that this technique is effective in keeping damage from this pest at acceptable levels using attract-and-kill alone. The technique works especially well in small (ca. 1 ha) blocks of apple that are not amenable to

treatment with standard mating disruption formulations due to their large border-length-to-area characteristics. Proof that the attract-and-kill mechanism and not just mating disruption was the cause of the population suppression came from a second set of experiments in which the pheromone-impregnated droplets either did or did not contain small amounts of insecticide. The plots to which the insecticide-containing droplets were applied showed significantly reduced trap capture and tethered female mating reduction, whereas plots that received droplets containing pheromone without insecticide exhibited virtually no monitoring trap capture reduction and no reduction in tethered female mating (Charmillot *et al.*, 1996; Charmillot and Hofer, 1997).

One major conclusion that can be drawn from these successful studies using the codling moth is that it is possible to use mass trapping (attract-and-kill) against only males and achieve successful population suppression. The innovative bent of certain cotton growers and at least one determined codling moth researcher showed that the initial negative conclusions of early pheromone researchers concerning mass trapping of male moths were premature, or at least based on incompletely understood assumptions. At present, a commercially successful attract-and-kill product against the codling moth based on Charmillot's work and through substantial development efforts by CIBA Corporation is being marketed as "Last Call" in North America by IPM Technologies, Inc. Other attract-and-kill Sirene products using the pheromones of other species are being developed against other pests.

For attract-and-kill to work, obviously an optimally attractive pheromone blend formulation is required that will attract the males of the target species all the way to the source so that they contact the insecticide-laced matrix. This, and the need for so many droplets to be applied and retain their contact toxicity for a long period, are some of the exacting requirements necessary to be met for attract-and-kill to successfully take its place as a useful and successful pheromone-mediated population suppression tool.

#### 6.12.5.4. Mating Disruption

**6.12.5.4.1. Evolution of mating disruption technique** 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. In the earliest days of mating disruption trials, 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. Most trials concentrated on the use of microcapsules sprayed through conventional pesticide sprayers to achieve uniform coverage. Then, the first mating disruption formulation to be registered by the Environmental Protection Agency (EPA), 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 on and stuck to the cotton leaves, emitted volatilized pheromone by means of an open capillary tube technique and were effective when applied at an estimated density of one fiber $m^{-2}$. 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 polyethyelene 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 1000 $ha^{-1}$ was achieved with fewer but higher strength point sources with no loss of efficacy.

Still, researchers had not paid attention to the results of an important set of experiments conducted by Farkas *et al.* (1974), who followed up on initial intriguing results from Shorey *et al.* (1972) which showed that the number of point sources could be reduced to only a few per hectare if they were made to emit at very high rates so that the total amount of pheromone emitted per hectare remained at as high a rate as when many more, but weakly emitting, point sources were deployed. Some researchers started experimenting with this concept in the 1990s, and formulations using only 10–15 dispensers per hectare are now available commercially. The initial research on these involved the use of "active" emitters, which were machines that could be programmed to project aerosolized pheromone solution into the air and onto absorbent pads, which would then emit high amounts of pure pheromone of the target insect (Mafra-Neto and Baker, 1996a; Shorey and Gerber, 1996a, 1996b; Shorey *et al.*, 1996; Baker *et al.*, 1997; Fadamiro *et al.*, 1999b).

The initial concept was that the machines could be programmed to emit pheromone only during the time of activity of the target (moth) insect pest's sexual activity. Efficacy in disrupting sex pheromone communication of a number of different species was clearly demonstrated (Mafra-Neto and Baker, 1996a; Shorey *et al.*, 1996; Shorey and Gerber, 1996a, 1996b; Baker *et al.*, 1997a, 1997b;

Fadamiro *et al.*, 1998, 1999b). Then, it became evident that the emission of pheromone, whether from the pads or from surrounding foliage onto which the pheromone solution absorbed, was a constant, passive emission phenomenon. The MSTRS Technologies, Inc., dispensers that had previously been of the active-spray type using aerosolized pheromone evolved into high-release-rate passive-emitting dispensers. The MSTRS system is currently commercially available for use against several pest species, and dispensers are deployed at densities ranging from 10 to 20 $ha^{-1}$. One original machine-driven aerosolized dispenser system Suterra LLC's is also commercially available and in use against the coding moth.

**6.12.5.4.2. Behavioral mechanisms involved in successful mating disruption** A very few important studies have provided behavioral observations that have helped understand how moths' abilities to locate sources of female sex pheromone are diminished by the application of mating disruption dispensers. At least four mechanisms have been implicated when the disruptant used is an effective synthetic blend mimicking the natural blend (Bartell, 1982; Cardé, 1990; Cardé and Minks, 1995):

1. Attraction-competition, in which males are attracted some distance toward the disruptant dispensers and waste time flying around in response to these plumes.
2. Sensory adaptation or habituation, in which exposure to the synthetic disruption blend raises the threshold of response (lowers sensitivity) to subsequent plumes.
3. Camouflage of natural female pheromone plumes by the strong plume-strands emanating from the synthetic dispensers.
4. Advancement of the periodicity of activity of males such that they begin responding to the synthetic disruptant dispensers before females begin to call.

These mechanisms are not thought of as operating independently of one another, but rather most of the time they act in concert, depending on the dispenser type that is used and the environmental conditions (Cardé, 1990; Cardé and Minks, 1995).

There is evidence from many studies that what has now been more aptly termed "competition" (Cardé and Minks, 1995) but had previously been labeled "confusion" or "false trail-following," does in fact occur in mating disruption-treated fields, especially those receiving discrete point sources of dispensers such as hollow fibers or ropes. It is unknown, but it is regarded as unlikely, that male moths in fields treated with sprayable microcapsules, creating a nearly uniform fog of pheromone from the closely spaced, weak point source emitters, would be subject to the competition mechanism. The majority of species that have been tested for their responses to uniform clouds of pheromone quickly habituate when released in the pheromone fog, and cease upwind flight after only 1 or 2 s, reverting to cross-wind casting within the cloud (Kennedy *et al.*, 1980, 1981; Willis and Baker, 1984; Baker *et al.*, 1985; Justus and Cardé, 2002).

However, for disruptant formulations that use slightly more widely spaced and stronger point sources, attraction competition imposed by these sources on males should be more likely to come into play, and has been observed in the field (Miller *et al.*, 1990; Cardé *et al.*, 1993). The flight tracks of pink bollworm males have been recorded (Cardé *et al.*, 1997) in large wind tunnels placed over disruptant-treated cotton fields, as they flew upwind in response to the pheromone emitted by Shin-Etsu ropes that had been in the field for 7 days (as well as to those that had been in the field for only 24 h). Males that had been preexposed to the ropes within disruptant-treated fields for 24 h, as well as those males having had no preexposure, flew upwind to the rope dispensers, with the only difference being that the percentage of the 24 h preexposed males doing so was significantly reduced (Cardé *et al.*, 1997). The flight tracks of males flying upwind in response to the ropes appeared similar to the tracks of males flying upwind to much lower strength rubber septum monitoring trap lures, with the tracks of males flying to the ropes exhibiting a slower ground speed and more tortuous paths. The males were observed to land on or near (within 50 cm of) the rope dispensers and then become quiescent for long periods, implicating a second mechanism, habituation of the olfactory pathways, as having occurred after attraction competition.

Habituation is not an all-or-nothing phenomenon. The degree to which the olfactory pathways are habituated depends on the dose of pheromone applied during preexposure. Mafra-Neto and Baker (1996b) demonstrated, using a graded series of preexposure dosages of the pheromone of the almond moth, *Cadra cautella*, that lower habituating dosages reduced males' ability to respond subsequently to normal-strength pheromone plumes but that the usual percentage of these males could fly upwind and contact the source if stronger loadings of pheromone were used on the test lure. Thus, the degree of habituation depends on the concentration of the preexposure regime, as well as on the subsequent

attractant lure, demonstrating that habituation behaves in a graded manner. The habituating concentration elevates the subsequent response threshold, and the highest habituating concentrations elevated the threshold of tested males to the greatest degree, requiring extremely strong attractant lure dosages to achieve any subsequent attraction whatsoever (Mafra-Neto and Baker, 1996b).

Habituation can occur without attraction-competition being evoked at all. Female pink bollworm produce and emit approximately a 50:50 ratio of (*Z,E*)-7,11-hexadecadienyl acetate and (*Z,Z*)-7,11-hexadecadienyl acetate (Hummel *et al.*, 1973). The 1:1 ratio of the two isomers captures the most males in field trapping experiments, and neither the (*Z,Z*) nor the (*Z,E*) isomer alone traps significant numbers of males (Flint *et al.*, 1979). The pure (*Z,Z*) isomer does not even elicit significant levels of upwind flight in laboratory wind tunnels (Linn and Roelofs, 1985). In a series of experiments, Flint and colleagues experimented with applying disruptant formulations, either Hercon laminated flakes or Shin-Etsu rope dispensers emitting only the pure (*Z,Z*) isomer (Flint and Merkle, 1983, 1984; Flint *et al.*, 1988). Monitoring traps containing lures emitting a series of blend ratios from 0:100 to 100:0 of the two isomers were deployed throughout the disruptant-treated fields. Trap catch was totally shut down in the fields treated with the 50:50 ratio-emitting hollow fibers. Likewise, trap catch was also nearly totally suppressed by the pure (*Z,Z*) isomer-emitting dispensers. However, in these (*Z,Z*) isomer-treated fields there was a significant capture of males, albeit small, but the captures were significantly shifted to the monitoring traps emitting a 90:10 ratio of (*Z,Z*) to (*Z,E*). Males apparently were becoming habituated to the pure (*Z,Z*) isomer alone, and the monitoring lures emitting the highly enriched (*Z,Z*) to (*Z,E*) blend were now able to attract pink bollworm males as if the blend now was perceived as being more similar to 50:50 in these disrupant-treated fields.

Because the dispensers emitting the pure (*Z,Z*) isomer will not have been able to cause any attraction (and therefore no competition), the conclusion must be that sensory adaptation or habituation of the sensory pathways alone must be the mechanism acting to cause the near-elimination of trap capture in the (*Z,Z*) isomer treated disruption plots. Importantly, even though strong point source dispensers were used (especially the Shin-Etsu ropes), enough pheromone wafted through the fields from such sources that habituation alone without attraction significantly affected male behavior. Linn and Roelofs (1981) had demonstrated, using the oriental fruit moth, the ability of single components to exert a component-specific habituation on a particular sensory pathway specific for that component. The result was, as in the pink bollworm mating disruption experiments, that males that had been preexposed and habituated to only one (nonattractive) component were subsequently optimally attracted to multicomponent blends that needed to be enriched with excessive amounts of that component. The artificially enriched blend would now be perceived as the normal unenriched blend to which nonhabituated males are optimally attracted.

A third mechanism, "plume camouflage," has also been implicated as a mechanism involved in mating disruption (Cardé *et al.*, 1993; Cardé and Minks, 1995). The amount of pheromone in the plumes from the disruption dispensers is thought either to overwhelm the strength of the signal from the female-equivalent source or possibly to dampen the amplitude of ups-and-downs in plume-strand flux over the antenna; the plume's fine-scale structure has been shown to be essential in eliciting reiterative upwind surges from strand to strand that promote sustained upwind flight (Mafra-Neto and Cardé, 1994; Vickers *et al.*, 1994). Using their large field wind tunnels situated in cotton fields, Cardé *et al.* (1993) concluded that the reduced ability of male pink bollworms to fly upwind to and be trapped in monitoring traps in some of their experimental regimes in the tunnels was due to the ambient emitted amounts of pheromone from pheromone dispensers present in the fields that camouflaged the weaker plumes from the monitoring traps. As they pointed out (Cardé *et al.*, 1997), it is difficult to prove that such a mechanism is working independently of habituation and also competition, but the measurements of pheromone concentration by means of field electroantennograms seemed to support their conclusions that camouflage was indicated as the most likely dominant mechanism in some special experimental regimes (Cardé *et al.*, 1993). Camouflage using uniform clouds of pheromone does not seem sufficient for preventing upwind flight of moths in response to the intermittent flux provided by point source plumes (Kennedy *et al.*, 1980, 1981; Willis and Baker, 1984; Schofield *et al.*, 2003).

Finally, it has been well known since the early days of pheromone research that in a large number of moth species, the diel periodicity of male responsiveness to pheromone is broader than the female calling period (Cardé and Baker, 1984). For pink bollworm, this includes an advanced responsiveness of males to synthetic pheromone lures that precedes the normal time of sexual activity to females in the field

(Breasley and Adams, 1994; Schouest and Miller, 1994). The observations of Cardé *et al.* (1997) in their large field wind tunnels also seemed to indicate that not only were freshly released males able to respond in a precocious burst of activity just after sunset but also males that had been exposed to field levels of mating disruptant for 24 h exhibited an advanced period of responsiveness compared with native males. Such behavior would work to the advantage of mating disruptant formulations, causing males to be exposed or to expose themselves through attraction competition with habituating amounts of pheromone before females would even begin calling.

Cardé and Minks (1995) and Cardé *et al.* (1997) suggested that combinations of mechanisms will likely be operating in concert to various degrees, depending on the dispersion of pheromone by a particular formulation, to reduce males' abilities to respond to pheromone plumes from their females. For example, when males are being "confused" and flying upwind in the plumes of synthetic pheromone emitted by hollow fibers (Haynes *et al.*, 1986; Miller *et al.*, 1990) or Shin-Etsu ropes (Cardé *et al.*, 1997), they are receiving high-concentration contacts with the strands of pheromone in those plumes, and habituation of the sensory pathways is occurring as a result of the male remaining in upwind flight and continuing to maintain contact with those strong pheromone strands.

This combination of attraction and habituation has been invoked as the mechanisms explaining why the very high concentration, low point source density dispensers such as the "puffers" and MSTRS dispensers work despite their very wide spacing (Baker *et al.*, 1998b). The very highly concentrated pheromone strands in a plume from a single such dispenser can be effective at great distances downwind, causing males that contact them to begin flying upwind while maintaining contact with the plume. As the males proceed upwind, they continue to "dose" themselves with highly concentrated pheromone strands, and at some point after a prolonged upwind flight to the source or near the source, the males become habituated to the pheromone and cease upwind flight, as has been observed for males flying upwind to the lower emission rate, less widely spaced Shin-Etsu ropes (Cardé *et al.*, 1997).

As formulations use increasingly widely spaced, high emission rate means of emitting pheromones, optimal attraction using the blends most closely approximating the natural female blend will become necessary to prolong the time a male spends locked onto the plume, dosing himself with high amounts of pheromone to become habituated. At the other extreme, sprayable microcapsule formulations will likely depend very little, if at all, on the attraction mechanism, relying almost exclusively on habituation or plume camouflage. Regardless of the type of formulation, it is expected that all should be able to take advantage of the advanced period of sexual responsiveness that males exhibit before females begin to emit pheromone.

**6.12.5.4.3. Mechanisms by which mating disruption suppresses population growth** It used to be assumed, and is still assumed by many people working in chemical communication, 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, in all but a handful of the huge number of mating disruption field trials that have been conducted over the years, mating disruption itself has never been directly assessed. That is, the mating success of feral, freely flying females has rarely been evaluated. The use of tethered females or clipped-wing females placed on "mating tables" or at stations deployed throughout the disruption plot and then dissected for the presence (mated) or absence (unmated) of spermatophores does not assess what is really happening to feral females that have the ability to fly freely throughout the area. The tethered female technique gives the illusion of being a robust, real-world assessment of mating disruption efficacy, and although it is one of many good indicators, it is especially deficient when the insects distribute themselves unevenly within the habitat due to environmental factors such as heat, humidity, and wind. Unless the researcher knows ahead of time the locations where adults typically are most densely clumped and can tether the females there, the ability of the disruptant formulation to keep males from finding females will be overestimated.

The results of the few studies that have assessed the mating success of freely flying feral females are illuminating. For the highly successful and grower-accepted Shin-Etsu rope formulation used against the oriental fruit moth, Rice and Kirsch (1990) found that in plot after plot treated with mating disruptant, females' abilities to mate at least once in disruption-treated plots was only suppressed at most 50% relative to check plots during a flight. Just as commonly, this ability was as little as 15–20% suppressed, despite the reduction of fruit damage in these plots to acceptable levels comparable with those when the standard insecticide regime was used (Rice and Kirsch, 1990). Thousands of females were captured in terpinyl acetate bait pails

and analyzed for the presence or absence of spermatophores in their bursae copulatrices in both the disruptant treated plots and the check plots. This study was the first indication that for successful population suppression and damage reduction, it is not necessary to keep females unmated throughout their adult lives. Vickers *et al.* (1985) reported similar results for oriental fruit moth feral female mating success in Australian mating disruptant treated plots (23% mated females in mating disruption plots versus 90% in check plots).

Knight (1997) released six batches of 6000 sterilized codling moth females into disruption treated and check orchards and recaptured them in light traps or passive interception traps to dissect them and to look for the presence of male spermatophores to assess females' success at mating. The disruption formulation used was Isomate-C+, a Shin-Etsu rope-type dispenser system that had already been showing success at suppressing populations of codling moths in the Pacific Northwest. Again, as for the oriental fruit moth, after several days, more than 40% of codling moth females from the disruptant treated plots contained spermatophores, showing that they had mated, despite the application of this highly successful formulation. However, Knight (1997) noticed that females took longer to reach this level of mating success (4 days) in the disruption plots than they did in the check plots (1 day), and introduced the concept that perhaps successful population suppression through the application of mating disruptants requires only that the formulation delay mating, not suppress it entirely. Laboratory studies that prevented codling moth females from gaining their first mating by from 1 to 10 days showed that females whose first mating was delayed by as little as 3 days had reduced fertility and fecundity compared with those who had gained their first mating by day 1 or 2. When female first mating was delayed by 8 to 10 days, the number of fertile eggs laid was only 20% of that of females that had mated on day 1 or 2 (Knight, 1997).

The delayed mating concept was again implicated in studies on European corn borer *Ostrinia nubilalis* mating disruption (Fadamiro *et al.*, 1999b). In these studies, analyses were made of the bursae copulatrices of more than 2400 feral females that were captured by hand-netting during the daylight hours as they were flushed from their grassy aggregation areas. The data showed that 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 (Fadamiro *et al.*, 1999b). What was interesting was that at the beginning of the first flight ca. 50% of the females remained virgin in the mating disruption plots, but the mating success of females in these plots eventually reached 100% during the ensuing weeks as the flight proceeded (**Figure 3**). They attained this 100% mated status more slowly than did females in the check plots, which were 100% mated beginning at day 1 (**Figure 3**).

Analysis of the frequency of mating by the European corn borer females captured in the disruptant treated plots versus those from the check plots showed that throughout the entire flight females from the disruption plots were attaining matings at a significantly lower rate than those from the check plots (Fadamiro *et al.*, 1999b) (**Figure 4**). The mating disruptant was impairing the ability of females to attract and mate with males on a constant, daily basis but it did not completely eliminate mating. The application of this MSTRS formulation has subsequently been shown to reduce damage to corn by an average of 50–70% in various trials (Baker, unpublished data). Thus, as demonstrated in studies on the oriental fruit moth (Rice and Kirsch, 1990) and codling moth (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 attain their first or even subsequent matings. Thus, we must now expect to only retard, not eliminate, mating by females as a benchmark for successful population suppression through the application of mating disruption formulations.

How would delayed mating produce such significant population reductions? A follow-up study by Fadamiro and Baker (1999) investigated the effects of several manipulations on the timing and frequency of European corn borer females' matings during their lifetimes. With regard to delaying the first mating of these females, a delay of first mating to day 4 compared with day 1 produced a greater than 30% overall reduction in the number of fertile eggs laid during their 16-day lifetimes. Further delaying first mating until day 7 resulted in no fertile eggs laid at all, even though these females lived for 16 days total (Fadamiro and Baker, 1999). These results mirrored those of Knight (1997) that experimentally delayed the first matings of codling moth females (see above).

Fadamiro and Baker (1999) also investigated the effects of reducing multiple mating, because the majority of females from the check plots in the field experiments had mated two or more times (two or more spermatophores), implying that there is an advantage for feral females to obtain a second mating. Therefore, another set of experiments was performed to examine the effects of allowing

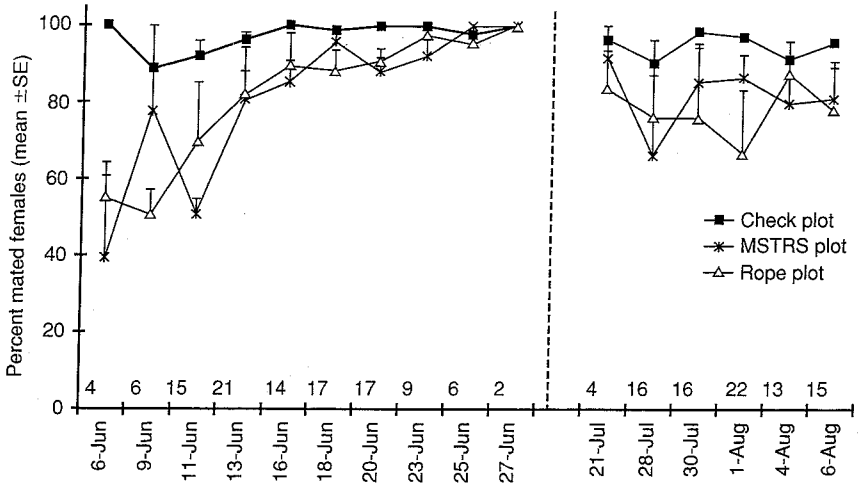


**Figure 3** Delayed mating imposed by mating disruption of the European corn borer (*Ostrinia nubilalis*) in Iowa as measured by the percentage of feral, free-flying European corn borer females that were found to have mated at least once on the indicated dates in mating disruptant treated plots or in untreated check plots. Females were captured by hand-netting on the dates shown in grassy areas within and between corn fields and examined for the presence of male spermatophores. During the first of the two flight periods (left panel) 50% of the first females to emerge were kept unmated by the mating disruption treatments, either MSTRS aerosol-based spray machines (MSTRS plot), or by Shin-Etsu polyethylene tubes (rope plots). Over the course of the flight, the percentage of mated females increased to finally reach 100% by the end of the flight period. In check plots the proportion of mated females was nearly 100% from the first days of the flight and continued at that level throughout. During the second flight (right panel), hand-netting did not begin early enough to measure mating success at the beginning of the flight and ended before the flight period ceased. (Reproduced from Fadamiro, H.Y., Cossé, A.A., Baker, T.C., **1999b**. 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.)

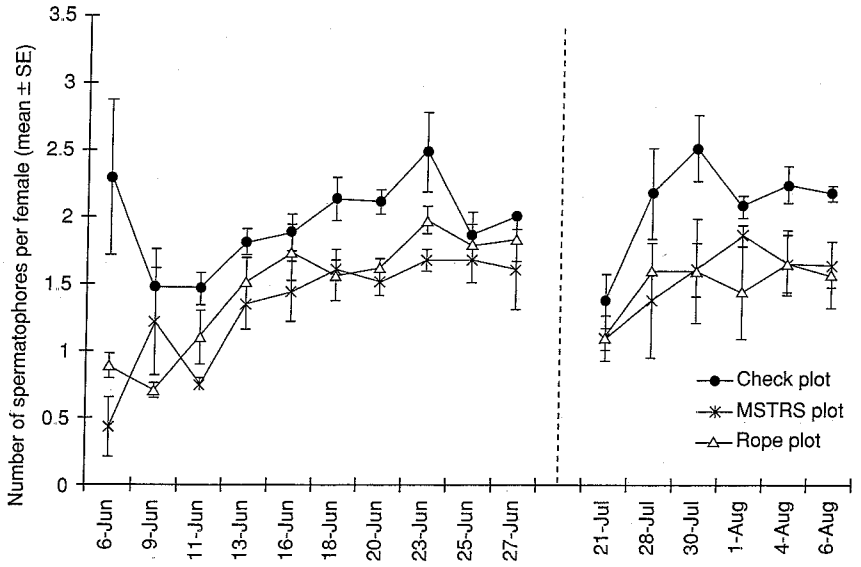


**Figure 4** The frequency of mating by European corn borer females in disruptant-treated fields ("MSTRS plot" or "rope plot") is lower than in untreated check plots, as measured by the mean number of male spermatophores found in females collected by hand-netting on the indicated dates. Females' ability to attain matings on any date during the first (left panel) or second flights (right panel) was impaired continuously throughout the flights by the presence of mating disruption dispensers. Thus not only is a female's ability to mate for the first time delayed by mating disruptant dispensers, but so is the second mating. The propensity of females in check plots to mate more than once also suggests that one mating is not sufficient to lay a full complement of fertile eggs. (Reproduced from Fadamiro, H.Y., Cossé, A.A., Baker, T.C., **1999b**. 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.)

females to mate more than once (Fadamiro and Baker, 1999). Females allowed to mate only once at day 2 laid only 50% as many fertile eggs during their lifetimes as those that had been allowed to mate twice. Thus, another mechanism by which mating disruption of this species can help suppress populations is by significantly reducing fertile egg production by impeding females' ability to achieve a second mating. When one considers other factors such as mortality of females over time due to predation and other natural causes, these ancillary factors could conceivably augment the fertility effects produced by delayed mating and result in even more profound levels of population reduction.

#### 6.12.5.4.4. Assessing the efficacy of mating disruption

*6.12.5.4.4.1. Damage reduction* Of primary importance to growers and to companies marketing mating disruption products is the ability of a formulation to reduce crop damage to acceptable levels. In this context, "successful" mating disruption means assessing crop damage in pheromone treated plots versus check plots and finding that damage in mating disruption plots is lower. This process is problematic, but considered by many to be a major outcome. The assessment is relatively straightforward, but it is essential that plots be large enough to reduce the probability that significant numbers of gravid females can fly in from nearby untreated plots and confound the damage data in the pheromone treated plots.

The choice of an appropriately large size for the plots depends on the propensity of females of a particular species to move long distances . It also depends on the damage acceptance levels for a particular pest, with direct-damage pests such as codling moth having lower damage tolerances than indirect-feeding pest species that may have higher damage acceptance levels. As a starting point for all species, 2 ha plots could be considered to be a minimum size for assessing damage reduction. But for direct-feeding pests and perhaps also a greater tendency of females to fly far, 10 ha would be a safer plot size for such testing. True replication, with check plots having to be many hundreds of meters away from the treated plots to reduce the possibility of drift effects, is difficult to attain, if not questionable with regard to ideal statistical design. However, these are the constraints under which such experiments need to be designed.

For tree fruit crops such replication is achievable and less scientifically debatable, but for field crops such as corn, it is virtually impossible to meet all the objections that arise when trying to compare damage and crop yield. This is because, for instance for corn, finding plots planted to identical cultivars is not the entire problem; differences in planting date and crop phenology can confound comparisons among identical cultivars. In corn, oviposition by European corn borer females is notoriously affected by the height of the corn, which in turn is affected by planting date or temperature differences between fields located even a few miles apart. A difference of only 2 or 3 days in planting date can produce 0.3 or 0.6 m differences in plant height, and so even if two fields are chosen for an experiment that have been planted to the same cultivar, damage may differ significantly between the two fields merely because the taller plants in the earlier planted field are targeted preferentially by ovipositing females.

Being an indirect measure, damage assessment evaluates the end result of many processes that are of interest to those operating in commercial integrated pest management and agronomic arenas. Conclusions that mating disruption was "successful" in the context of suppressing damage and being cost-effective can be arrived at without knowing exactly to what degree the formulation affected the behavior of males to reduce mating by freely flying feral females.

*6.12.5.4.4.2. Assessing reduction of mating by use of tethered females* Considered over many years to be the ultimate test of mating disruption efficacy, tethering females either on a thread or by clipping their wings and placing them on open arenas so that they cannot move from the location at which they are placed has been a good tool in assessing mating disruption efficacy (Evendon *et al.*, 1999a, 1999b). However, although it looks as if it is a direct and appropriate assessment tool, it is actually only one of several other indirect measures, and it can be argued that these other measures may be even better indicators than are tethered females as to what is happening to the actual female population in the field.

Tethered females are placed on stations in the field and allowed to remain overnight to determine whether they are able to attract and mate with males either in disruptant treated fields or in check fields. They are retrieved and dissected in the laboratory to look for the presence of spermatophores, which, if present, would mean that one or more males had been able to detect, locate, and mate with the female by means of her pheromone. One limitation to this technique is that tethering or otherwise restraining females limits their ability to find the best location in the foliage to try to attract males. It is true that a relative difference in mating by tethered females in check plots compared with disruptant treated

plots can be valuable, but translating this directly into conclusions about the absolute ability of a disruption formulation to similarly reduce the matings of feral females is inadvisable.

For example, if the adult moths in the natural population reside in a more clumped distribution in some preferred substructure of the vegetative habitat, placing females on artificial stations outside of each of these clumps will overestimate the efficacy of the disruption formulation with regard to preventing mating of feral females. The formulation in effect will only be being assessed for its ability to prevent the tethered females from attracting males out of their aggregation sites, and this long distance attraction will be easier to disrupt than will be the disruption of males from locating females that are within the same clumps as the males.

Another limitation to this technique is that researchers only get an all-or-nothing, yes-or-no indication from each female as to how well the disruptant is working in the field. Once a female mates with the first male arriving at her station, she emits no more pheromone and the ability to assess disruption of communication using that female ends. The data are binomial, with no opportunity for a graded assessment from individual females as to how many males they could have attracted had they been calling for the entire activity period that night and not mated.

*6.12.5.4.4.3. Trap capture of males by using caged, calling females* In this technique, a few virgin females are placed in small screen cages within which is a sugar water source to keep the females alive and hydrated (Baker *et al.*, 1997b). The cage is situated within a sticky trap, and so males that are attracted to the calling virgin females become ensnared before reaching the females. Males that do manage to land on the cage containing the females without getting trapped will still not be able to mate with any female in the cage. This technique has many advantages over other indirect measurement techniques such as the use of tethered females.

First, as with tethered females, the caged females are emitting their natural blend at its natural emission rate. However, unlike tethered females, the caged females in traps provide a graded assessment of their ability to attract males. If males are able to get close enough to the calling females that they can be trapped, they certainly would have mated if given that opportunity at such close range. The final step, mating, is an unnecessary one to examine because if the disruptant was not able to prevent a male's long distance orientation to the female's plume, certainly it will not be sufficient to stop the male's orientation to the female over the last 10–20 cm or so. Thus, this technique allows a more robust assessment of females' ability to attract males in disruption treated plots than is available from seeing whether tethered females mate.

*6.12.5.4.4.4. Trap capture of males by using lures emitting synthetic pheromone blends* The most widely used technique for assessing the disruption of mate-finding communication by disruptant formulations is the use of standard pheromone monitoring traps in which are placed synthetic pheromone lures (Rice and Kirsch, 1990; Knight, 1997; Staten *et al.*, 1997; Baker *et al.*, 1998b; Fadamiro *et al.*, 1998, 1999b). This technique can be as informative as the use of caged calling females if the lure that is used has been shown previously in untreated check plots to be able to attract equivalent numbers of males as do caged calling females. Using synthetic lures that are either too strong or too weak (or emit a suboptimal blend composition) will provide data that are not relevant to what is happening with calling females' ability to attract males. However, if a synthetic blend composition emitted at levels that approximate the attraction ability of caged females is used, then conclusions about the ability of a formulation to disrupt attraction to calling females can be valid.

Again, the advantage of assessing trap capture reduction is that it provides a robust, graded data set from these continuously emitting sources. The disruption formulation is challenged throughout the attraction period each night or evening for its ability to continuously suppress the ability of males to locate "females." Using monitoring traps baited with a good synthetic lure is also significantly easier and less problematic than is the use of live, calling females.

Although assessing mating disruption efficacy by means of synthetic pheromone baited traps is an optimal technique when the appropriate synthetic monitoring lures have been developed, it does not mean that there will always be concomitant demonstration of damage reduction when successful trap shutdown occurs. There are many other factors that play into the latter, and there are several species of moths, including the obliquebanded leafroller, *Choristoneura rosaceana*, and the peach twig borer, *Anarsia lineatella*, where trap capture is routinely reduced by 95% or more, and little to no effect is seen on the damaging population. Perhaps this has something to do with some of these species having aggregation or mating site habits that have been overlooked and the monitoring traps only measure the ability of the disruptant to prevent males from flying out of their aggregated area out to the

monitoring traps. Alternatively, perhaps the disruption plots have not been large enough to prevent immigration of gravid females, whose propensity to move great distances perhaps may have been underestimated in these species.

Regardless, trap capture data have proven to be useful indicators in the early tests performed in the development of disruption formulations; they measure the potential of a formulation to be effective and provide graded data sets that are more informative and easier to obtain. In addition, such tests are useful in determining the longevity of a formulation with regard to its effect on male behavior under natural temperature and wind conditions, which is more relevant to measuring the longevity of a formulation than are emission rate data.

*6.12.5.4.4.5. Wind tunnel and other laboratory tests* Although there have been many attempts to assess the efficacy of a potential mating disruption blend or formulation in the wind tunnel over the years, the major contribution of such studies has been to elucidate, after the fact, possible behavioral mechanisms that are at work in producing successful disruption. The wind tunnel enables researchers to observe and record individual male behavior in response to disruptant dispensers, or the dispensers' ability to interfere with responses to normal strength pheromone plumes. A key example is the work of Cardé *et al.* (1993, 1997) in investigating the behavior of pink bollworm males when exposed to hollow fibers or Shin-Etsu ropes (see Section 6.12.5.4.2). Evendon *et al.* (2000) were able to deduce from a series of laboratory wind tunnel experiments the possible mechanisms that could be at work in disrupting *C. rosaceana* sex pheromone communication in the field.

As useful and informative as these experiments can be, however, it is impossible in a laboratory wind tunnel to create a spatial array of dispensers that mimics the plume dynamics and plume flux exposures that a male would encounter farther downwind under shifting wind conditions in the field. Disruption dispenser arrays that in the field need to be spaced even as moderately widely as the ropes (and especially with yet more widely spaced, extremely high emission rate dispensers such as MSTRS and "puffers") cannot be scaled down to laboratory wind tunnel size. Wind tunnel tests have been useful to explore some of the physiological and behavioral limits that certain kinds of close-range exposure to plumes of various strengths and structures impose on males' olfactory pathways.

**6.12.5.4.5. Pheromone component blend composition in the disruptant formulation** In considering the cost of formulating and registering pheromone blends for commercial use, it is crucial to balance efficacy of the registered blend with the cost of the product. If an effective product is too expensive and is not able to be competitive with other available products, it will not be purchased and will "fail," despite its good biological activity and despite all the applied research that has been performed. In general, the fewer components that need to be formulated and still retain good efficacy, the less expensive and more competitive the final product will be. However, the cost of the product is also dependent on the amount of a.i. that needs to be applied per hectare, and so a blend formulation that is more effective at lower amounts of a.i. for a longer period under field conditions may prove to be more economically competitive, despite the inclusion of more pheromone components. For a multicomponent pheromone, how many of the minor components play a significant role in effective disruption and how many can therefore be left out of a final formulated product must be decided.

Minks and Cardé (1988) and Cardé and Minks (1995) reviewed results from many key sex pheromone communication disruption experiments and concluded that for a given species, the synthetic blend compositions and ratios most closely mimicking the natural blend for that species should be the most effective mating disruptants at a given dose per hectare because they will be able to make use of more of the mechanisms (see Cardé and Minks, 1995) that result in disruption than will suboptimal, partial blends or off-ratios. Although the majority of field experimental evidence supports this conclusion, this does not mean that one or more of the more minor components, due to their more subtle effects, might be able to be eliminated from the final formulated product and still retain sufficient efficacy.

Also, there are a few apparent exceptions to Cardé and Minks' general rule. Evendon *et al.* (1999a, 1999b, 1999c, 2000) have thoroughly explored sex pheromone communication in the western population of the obliquebanded leafroller. They performed experiments exploring the underlying mechanisms likely involved in disruption and also conducted field tests in small plots by using different blend compositions that affect both *C. rosaceana* and a sympatric leafroller pest, the threelined leafroller, *Pandemis limitata*. Their results and insightful discussions have been in instructive in helping to conceptually and experimentally guide researchers as to how to pick

apart the many mechanisms that may, depending on the species, play larger or smaller roles in successful sex pheromone communication disruption.

Evendon *et al.* (1999a) found in small plot (0.1 ha) field experiments that the four-component sex pheromone blend of *C. rosaceana* emitted from Conrel hollow fiber tapes deployed at a density of 500 dispensers per ha was effective in reducing successful mating of tethered females to a level of only 5% mating, but it was no more successful than a partial three-component or even a two-component blend containing the major component, (*Z*)-11-tetradecenyl acetate plus (*E*)-11-tetradecenyl acetate emitted at the same rate per hectare. Even at emission rates that they determined were at threshold levels for suppressing mating with tethered females or by deploying disruptant dispensers at reduced densities, the two-component blend was as effective as the four-component blend in suppressing mating. Tests of the competition attractiveness of the disruptant dispensers themselves via tests of attraction of males into traps showed that the two- and three-component hollow fiber tapes were ineffective in attracting males to traps, whereas the four-component fiber tapes resulted in high male trap captures.

Other studies (Evendon *et al.*, 1999b, 1999c, 2000) had demonstrated, in detailed wind tunnel and field experiments, that the attraction competition mechanism as well as long-term habituation were likely not very significant contributors to communication disruption in this species. Rather, sensory adaptation plus plume camouflage seemed to be implicated as playing more dominant roles in thwarting attraction to females. Importantly, even disruptant blends that contained the large amount of the heterospecific antagonistic compound, (*Z*)-9-tetradecenyl acetate, that is emitted by *P. limitata* females were as effective as conspecific blends in disrupting attraction to *C. rosaceana* females. Even these, too, were unattractive to *C. rosaceana* males and would therefore not be able to work via the competition mechanism (Evendon *et al.*, 1999b, 1999c). The ability of *P. limitata* males to locate and mate with tethered conspecific females is likewise disrupted as effectively by *C. rosaceana*-related blends emitting no (*Z*)-9-tetradecenyl acetate, and these are not effective for attracting *P. limitata* males.

The results emphasize again that even with moderately strongly emitting and moderately widely spaced dispensers (Evendon *et al.*, 1999b, 1999c), mechanisms other than attraction competition seem to have been dominating in these studies to result in disruption of sex pheromone communication in these two species. The inclusion of heterospecific antagonists in disruption dispensers was similarly found to be as effective as the conspecific blend in mating disruption studies of the pea moth, *Cydia nigricana* (Bengtsson *et al.*, 1994; Witzgall *et al.*, 1997). Because in Evendon *et al.*'s studies (Evendon *et al.*, 1999a, 1999b, 1999c, 2000), sensory adaptation and also perhaps plume camouflage were shown to be so strongly implicated as the dominant disruption mechanisms, the vulnerability of *C. rosaceana* males to sensory adaptation alone (Evendon *et al.*, 2000) may explain why nonoptimal, nonattractive blends can be so effective at sex pheromone communication disruption. Indeed, other studies using partial and completely unattractive blends dispensed in moderately strong and widely spaced dispensers have shown that effective disruption can be attained (Flint and Merkle, 1983, 1984); presumably, this comes from mostly sensory adaptation or habituation to the ambient pheromone that the unattracted males are exposed to.

Previous studies on a different, eastern population of *C. rosaceana* (Roelofs and Novak, 1981) contrast sharply with results from the western population (Evendon *et al.*, 1999a). Roelofs and Novak (1981) found that the optimal, natural female-emitted three-component blend for eastern *C. rosaceana* imposed better disruption than did partial blends. Evendon *et al.* (1999a) used tethered females for their assessment of disruption and as the authors themselves pointed out, Roelofs and Novak used the "more robust" (Evendon *et al.*, 1999a) assessment of measuring the numbers of males captured. As discussed above, tethered females provide a binomial, mated–unmated measure that does not allow for a graded assessment of how many more males would have been attracted to the females had they not mated with the first one arriving.

The mechanisms affecting mating disruption will operate to different degrees in different species under different environmental conditions and pheromone disruptant deployment schemes (Bartell, 1982; Minks and Cardé, 1988; Cardé, 1990; Cardé and Minks, 1995). There do seem to be differences in the behavior and neurophysiology of the various species that have been targeted and these may allow us to use surprisingly unattractive blends for effective mating disruption. Whether this is always related to each species' vulnerability to sensory adaptation and plume camouflage as opposed to other mechanisms, the studies discussed above suggest that this is one possible conclusion to be made. The only way to optimize a disruptant formulation with regard to the blend used as the a.i., the dispenser loading and longevity, dispenser deployment density, and the overall cost and efficacy, is with extensive trial-and-error field testing.

**6.12.5.4.6. Successful mating disruption in population control by using commercial dispensers**

*6.12.5.4.6.1. Codling moth* In the final two decades of the 1900s, apple and pear growers in California and the Pacific Northwest were facing a variety of factors making untenable the continued use of conventional broad spectrum insecticides. Among these factors were the increasing development of resistance of the codling moth to organophosphate insecticides, and also new federal legislation, the Food Quality Protection Act (FQPA), that would require review and reregistration of traditional broad spectrum insecticides such as the organophosphates (Brunner *et al.*, 2001). During this same period, beginning with initial small plot studies (Cardé *et al.*, 1977; Charmillot, 1990) using hollow fiber and other dispensers, research on mating disruption of the codling moth had proceeded to the point where effective population suppression using improved, long-lasting mating disruption formulations such as Shin-Etsu ropes seemed feasible (Knight, 1996; Gut and Brunner, 1998).

Due to the reduced efficacy of traditional insecticide applications, the need for more insecticide applications to gain effective control, and the looming specter of FQPA legislation, growers in the early 1990s began using commercially available mating disruption formulations. The number of hectares that was treated with mating disruptants against the codling moth grew from a few hundred in 1990 to approximately 5000 in 1994. Due in part to the increasing grower acceptance of this population management tool, a Codling Moth Areawide Management Program (CAMP) was proposed and then approved. Five sites in the Pacific Northwest were identified, with three of them in Washington State and one each in California and Oregon. A total of approximately 1240 ha was included for codling moth mating disruption application over all five sites.

One overall goal of this study was to achieve an 80% or greater reduction of the use of broad spectrum conventional insecticides by the end of the 5-year program. A subsidy was provided to all participating growers of \$125 $ha^{-1}$ for the first 3 years of the project to help defray the cost (\$275 $ha^{-1}$) of the mating disruption treatments (Brunner *et al.*, 2001). For the final 2 years, growers had to pay the full cost of the treatments themselves. Standard high-dose codling moth monitoring traps were used to assess trap capture reductions, and damage assessments were made for all blocks within the CAMP project and compared with fruit from non-CAMP participating grower blocks.

The largest site in the project was at Howard Flat, Washington, more than 440 ha. Data from this site are representative of the trends in the other four CAMP sites (Brunner *et al.*, 2001), although their differences in weather, topography, and horticultural practices were many, including the fact that the California and Oregon sites were planted mainly to pears. In 1995, the first year of the CAMP program, codling moth mating disruption formulations (mostly the Shin-Etsu ropes, IsoMate CM) were applied to the CAMP hectarage, and at the end of the year in Howard Flat, trap capture of males had been reduced by 80% and fruit damage at harvest averaged 0.55% for the entire hectarage of the project. The previous year's at-harvest damage had averaged 0.8%. Moreover, the number of conventional insecticide sprays targeting the codling moth averaged only 1.2 $ha^{-1}$, whereas during the previous, non-CAMP year it had averaged 2.9 $ha^{-1}$ (Brunner *et al.*, 2001).

During 1996, the average trap capture of males was reduced further from the levels in 1995, with only 1.5 moths captured per trap compared with 8.8 per trap in 1995. Damage at harvest was reduced to only 0.2% and most of the damage (sometimes reaching 25%) occurred in the few blocks of trees at Howard Flat in which one or two growers had refused to participate in the CAMP program. Higher damage also occurred in mating disruption treated blocks of trees of CAMP participants that bordered these untreated, high-infestation sources. In subsequent years these blocks of trees were leased by neighboring growers who wanted to eliminate these last remaining codling moth infestation foci, and these hot spot infestations were subsequently fully reduced. During 1996, the number of insecticide applications against codling moth fell further to only 1.1 application per hectare (Brunner *et al.*, 2001).

During the next 2 years, trap capture fell further to exceptionally low levels, with 90% of the traps capturing zero males during the entire 2 years. During 1998, the average number of insecticide applications fell to only 0.5 application per ha and crop damage at harvest reached an all-time low of between 0.01% and 0.03% damaged fruit. These levels were accomplished in 1998 with one-half the density of dispensers per hectare, because this was the first year without a cost subsidy from the program. Also, mating disruption had been so effective in reducing codling moth populations the first 3 years that there was little perceived threat of incurring increased damage by reducing dispenser density. By doing so, growers kept their expenses the same as during the previous 3 years.

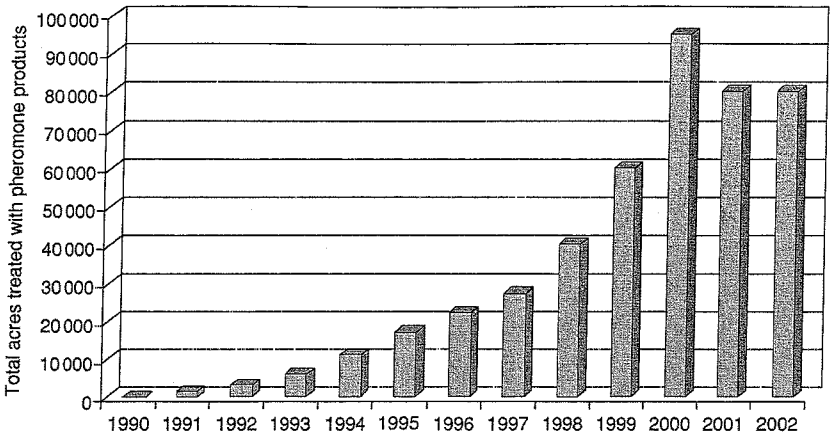


**Figure 5** The number of acres treated each year with codling moth (*Cydia pomonella*) commercial mating disruption products in Washington State, USA from 1990 to 2002 (Brunner *et al.*, 2001; Years 2001 and 2002 provided by Brunner, personal communication). In 1995 the Codling Moth Areawide Management Project (CAMP) began, with subsidized mating disruption applications provided to growers. The three CAMP sites in Washington State comprised a constant 760 ha during the 5 years of the program (1995–99).

It is clear from the continued increase in acreage using commercial codling moth mating disruption formulations in Washington State (**Figure 5**), that growers statewide were satisfied with the population suppression and their economic balance sheets involved in using codling moth mating disruption. The CAMP program served as a focal point for providing mating disruption efficacy data that guided growers statewide as to what was happening on their own mating disruption treated acreage. Not only did the growers in the CAMP program achieve a 75% reduction in insecticide applications while reducing damage to unprecedented levels, but also secondary pests did not arise with the reduced insecticide pressure, as had initially been feared would occur. On the contrary, comparison plots under conventional practice experienced higher levels of secondary pests and often lower levels of beneficial insects and mites than did the CAMP program plots (Brunner *et al.*, 2001).

*6.12.5.4.6.2. Pink bollworm* A similar history of successful area wide use of sex pheromones for mating disruption exists for another key agricultural pest, the pink bollworm (Staten *et al.*, 1997). The Parker Valley of Western Arizona, planted to more than 10 000 ha of cotton, was overrun with pink bollworms that had developed resistance to every insecticide on the market. The grower groups in the valley mandated an areawide program of using mating disruption against this pest, and the entire valley had various commercial mating disruption formulations applied from first moth emergence through to August. The formulations included the Shin-Etsu ropes and (the formerly Conrel, now Scentry) hollow fibers. The fibers had been commercially available since 1978, and growers were already knowledgeable about them and about the ropes as well. However, before the start of the program in 1989, virtually no hectarage in Parker Valley had had mating disruptants applied, even though their use had been prevalent in neighboring southern California desert valley cotton hectarage (Staten *et al.*, 1997).

As in the codling moth CAMP program, damage diminished year by year in the Parker Valley with continued area wide application of pheromone mating disruption formulations. By 1993, the average season-long capture of males in monitoring traps in Parker Valley was reduced to only 0.5 moth per trap, whereas the statewide average in conventional practice farms grew to greater than 30 per trap (averaging 23 moths per trap for all 3 years). Most importantly, whereas the percentage of infested bolls out of the tens of thousands that were sampled each year was more than 25% during mid-August of year 1, during the same August period in year 2 (1990) damage was only 5.9% (Staten *et al.*, 1997) (**Table 3**). At the mid-August point of year 3 damage was only 0.03% and by 1993, out of more than 22 000 bolls sampled season-long, not a single infested boll was found (0% damage) (**Table 3**). In contrast, the central Arizona average infestation rate for conventionally insecticide treated hectarage in 1993 was 9–10% by mid-August (Staten *et al.*, 1997).

*6.12.5.4.6.3. Other successes and some limitations* Growers of peaches have had mating disruption successes similar to the above, by deploying Shin-Etsu polyethylene tubes over a wide area against the oriental fruit moth in Australia (Vickers,

**Table 3** Pink bollworm damage to cotton bolls in Parker Valley mating disruption project, Arizona, USA

| | *Larvae per 100 bolls* | | | | |
|---|---|---|---|---|---|
| *Week of* | *1989* | *1990* | *1991* | *1992* | *1993* |
| 9 July | | 0.3 | | 0.19 | 0 |
| 16 July | | 0.6 | 0.6 | 0.09 | |
| 23 July | 3.6 | 1.4 | 0.03 | 0.95 | |
| 30 July | 7.7 | 2.7 | 0.09 | 0.61 | 0 |
| 6 Aug. | 17.9 | 1.5 | 0.03 | 0 | 0 |
| 13 Aug. | 25.9 | 5.9 | 0.03 | 2.45 | 0 |
| 27 Aug. | 36.4 | 20.6 | 1.6 | 1.19 | 0 |
| 3 Sept. | 34.5 | 10.9 | 1.9 | 1.6 | 0 |
| 10 Sept. | 21.6 | 10.4 | 3.7 | 1.78 | 0 |
| 17 Sept | 28.4 | 33.3 | 6.6 | | 0 |
| Total bolls sampled per year | 23 847 | 31 630 | 21 675 | 25 603 | 22 852 |

1990), in California (Rice and Kirsch, 1990), and in South Africa (Barnes and Blomefield, 1997). The above results from areawide mating disruption against three different species and cropping systems indicate that efficacious mating disruption formulations applied on large areas over a 3-year period can result in the near elimination of pest population problems. During year 1 of a program, high pest populations may not appear to be well controlled, but during year 2, the mating disruption effects from year 1 become evident. Lower populations are present at the beginning of the season, and mating disruption during year 2 is better able to exert its full effect. By years 3 and 4, populations have often been reduced to such low levels that growers decide that they can apply reduced rates of the disruption formulations. This has its dangers in that it can result in less effective mating disruption, allowing populations to build back up again.

Charmillot (1990) summed up lessons he and his coworkers learned concerning when it is that mating disruption becomes less effective for codling moth population suppression. His lessons regarding codling moth are applicable to mating disruption efforts on other pests as well. First, mating disruption works best when applied on an area wide basis and is not advisable on very small hectarage (less than 1 ha). The crop borders represent vulnerable edges for immigration of mated females from adjoining untreated crop areas, and they also serve as a zone that concentrates females along the borders when there is no nearby hectarage of the same crop. Due to geometry, small hectarage accentuates the problem because the edge-to-area ratio becomes greater. Also, the use of more widely spaced dispensers requires that borders be given special attention to reduce the presence of pheromone-free clean air "holes" along the borders; this can be accomplished either by decreasing the spacing between dispensers, or else through the use of different dispenser technology along borders, such as sprayable microcapsules. Mating disruption will be more problematic when crop borders are especially irregular or when the fields are too elongated; in these cases again the crop's edge-to-area ratio becomes too high. This important effect of field geometry is especially exaggerated for very widely spaced high-release-rate disruption formulations.

### 6.12.5.4.7. Constraints to successful adoption of mating disruption

*6.12.5.4.7.1. Upfront cost* A primary obstacle to the use of pheromone mating disruption on any crop is that it must be applied no later than the start of the first adult flight period, that is, before a grower knows for sure that there is even going to be a pest problem that season. The requirement of this upfront investment in pheromone puts mating disruption at a disadvantage compared with curative pest management tools that provide a wait-and-see option. Insecticide applications can be made after a preliminary assessment of the population density during the first flight of adults or even after oviposition when larval damage can be assessed. Pheromone mating disruption cannot be used curatively that way.

In the areawide mating disruption successes discussed above, growers had no other recourse other than mating disruption because their usual curative insecticide tools had been rendered ineffective due to pest resistance. Here, mating disruption no longer represented an upfront cost, but rather became a viable option that growers readily embraced and accepted.

*6.12.5.4.7.2. Appearance of secondary pests* Except for a few small groups or pairs of species, pheromones are extremely species-specific. The replacement of broad spectrum insecticides in some IPM systems with mating disruption that targets only one species in a pest complex can lead to increases in the populations of species that had been only secondary pests before mating disruption was used (Rice and Kirsch, 1990). No pheromone disruption formulation has been created thus far that functions effectively as a "broad-spectrum" formulation. Evendon *et al.* (1999b, 1999c) experimented with single blend formulations targeting *C. rosaceana* and *P. limita*, with some success. Perhaps there are other situations where effective multispecies blend formulations can be developed, although there are still none that have attained commercial success.

*Choristoneura rosaceana* disruption seems to depend less on attraction competition and habituation, and more on sensory adaptation (Evendon *et al.*, 2000). It remains to be seen whether development of multispecies disruptant formulations can be successful on more sets of species. Certainly when widely spaced, high-emission-rate dispenser systems are used there should be less of a chance for this to work, because attraction competition that facilitates habituation seems to be more essential for success with these formulations (Baker *et al.*, 1998b).

*6.12.5.4.7.3. Cost of mating disruption formulations* Pheromone mating disruption formulations have to this point been expensive compared with curative applications of insecticides. Although growers of many crops are now well aware that mating disruption "works" as a pest management tool, pheromones' expense plus the upfront nature of the cost has put them at a disadvantage relative to curative pest management tools. The a.i., the pheromone itself, is the most expensive part of a formulation. Costs per gram of even the least expensive pheromone components are approximately \$1.00, and many other more expensive major pheromone components cost \$3–20 $g^{-1}$. Formulating the a.i. into specialized dispensers adds to the cost, but nevertheless, if cheaper organic synthetic routes to the a.i.s can be developed, costs of the final formulated products could be reduced substantially.

Unfortunately the best and cheapest routes for large scale syntheses have apparently been discovered and are already the ones in use by pheromone manufacturers. Therefore, finding other ways to emit behaviorally effective amounts of the active ingredients more judiciously while maintaining efficacy and extending the longevity of dispensers under field conditions is a much-needed area of research. Below, we present some ideas for how to do this as we try to bring together some of the advances in recent basic knowledge about male moth upwind flight behavior with applied aspects of pheromone use in the field.

## 6.12.6. New Frontiers

### 6.12.6.1. New Ideas for Reducing the Cost of Pheromone Mating Disruption

**6.12.6.1.1. Widely spaced, high emission rate dispensers** Widely spaced, high emission rate dispensers offer an advantage for future cost-saving measures to make mating disruption more economically competitive with other tools such as insecticides. Because these dispensers can be deployed at very low densities, from one to 15 dispensers per ha (Shorey and Gerber, 1996a, 1996b; Shorey *et al.*, 1996; Baker *et al.*, 1997b, 1998b; Fadamiro *et al.*, 1998, 1999b), the labor cost for their deployment is lower than for other hand-applied dispenser types. Importantly, widely spaced dispensers are the only type whose density per hectare goes down as the area of crop they are applied to goes up, regardless of starting grid-spacing distance. This is at first not easy to comprehend because it is counterintuitive.

In the example shown in **Figure 6**, high emission rate dispensers are hypothetically deployed on a 1 ha orchard, using a 25 m spacing between dispensers (center square, large dots). The density needed to treat the 1 ha orchard calculates out to be 25 dispensers per ha. However, if a 9 ha orchard needed to be treated using this same 25 × 25 m grid pattern, the cost per hectare is reduced, because over the 9 ha plot the density has been reduced to 18.8 dispensers per ha (**Figure 6**, second square, small dots). For a 25 ha orchard, the cost per hectare is reduced further as the density falls to 17.6 dispensers per ha, still keeping this same grid spacing (**Figure 6**, outermost square, no dots); and to treat 100 ha the dispenser density goes down to 16.8 dispensers per ha.

If the starting dispenser grid pattern is less dense than this, the reduction to low densities upon scaling up to very large hectarage is even more striking. Starting with a dispenser spacing twice the above (50 × 50 m), treating the 1 ha orchard with a grid pattern requires a density of 9 dispensers per ha. Treating a 9 ha orchard reduces the density to 5.4 per ha, and treating 25 ha requires only 4.8 dispensers per ha. The 100 ha orchard needs only 4.4 dispensers per ha.

In the Farkas *et al.* (1974) initial experiments demonstrating that widely spaced, high dispenser loadings work as well as closely spaced, low loadings,

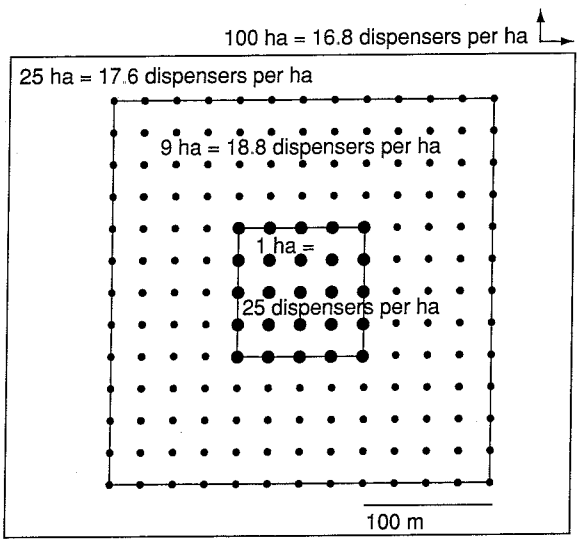


**Figure 6** Widely spaced, high emission rate dispensers are the only type of dispenser whose density per hectare diminishes significantly as the area of crop they are applied to increases. In this example, such dispensers are deployed with a 25 m spacing on 1 ha (interiormost square, large dots); here dispenser density calculates to 25 dispensers per ha. Treating 9 ha (intermediate square, smaller dots) while keeping the same 25 m dispenser spacing results in a reduced density of 18.8 dispensers per ha. Enlarging the treated area still further to 25 ha (outer square) and 100 ha (beyond outer square) while keeping the same grid spacing of dispensers results in even lower dispenser densities of 17.6 $ha^{-1}$ and 16.8 $ha^{-1}$, respectively.

they emphasized that the amount of active ingredient per hectare should be maintained. This aspect needs to be explored more with many more trials, because if this turned out to be a strict requirement, then no cost savings from reduced dispenser number per hectare would be gained from this surprising phenomenon of geometry that provides a numerical advantage to wide spacing. But as emphasized in the discussions above, it is not the overall concentration of pheromone that moths respond to, it is the changes in flux provided by the pheromone plume-strands. Therefore there may be previously unrealized opportunities to further reduce the cost of pheromone per hectare by designing dispensers that optimize, with regard to plume-strand strength, what is emitted and not what is applied.

**6.12.6.1.2. Strong plume strand emitting dispensers** The existing dispensers that have been used for insect mating disruption have emphasized emission aspects related to field longevity that are calculated on the total amount of pheromone emitted per hectare per unit time. Work on improving these previous devices has largely ignored recent findings that have shown that males fly upwind in response to individual strands of pheromone comprising the fine structure of the plume.

An insect's antennae can only sample a small volume of an odor plume, and the strands and pockets of clean air give the olfactory neurons on the antenna rapid fluctuations in stimulation (2–10 Hz). Mating disruption dispensers and deployment spacing schemes that can maximize changes in flux will be able to elicit attraction competition from farther downwind than will less well-designed types. Olfactory pathway habituation, which accompanies attraction competition (Cardé *et al.*, 1997), is known to be optimally imparted by strong pulses of pheromone and not by continuous stimulation, which only will produce sensory adaptation (Bartell and Lawrence, 1977a, 1977b; Bartell, 1982).

It became apparent while working on optimizing the emission rate from MSTRS high release rate pads that, within a few days, the pads became loaded with an amount of pheromone beyond which adding more pheromone did not further increase the emission rate. If the size of the pad were to be increased, the pad's total emission rate would be raised proportionately. However, we also realized that because the insects' antennae are so small, any increase in the diameter of the pad will not increase the strength of the individual pheromone strands that contact the antennae. The amount emitted per dispenser, or per hectare per unit time, will seem to researchers to create greater and more powerful amounts of pheromone to affect behavior, but in fact it will not at all increase the amount that strikes an insect's antenna.

It became clear during our studies that smaller point sources will be better at generating large plume-strand flux, but only if the emission rate of the small point source can be kept the same as the rate that large surface area dispensers emit across their total area. The effect will be to pack more molecules into a smaller space, thereby increasing pheromone strand flux. One way to accomplish this would be to add energy to the small point source that would generate heat and increase the evaporation rate. Energy could also be used to generate ultrasonic vibration of the point source and increase evaporation rate by increasing air movement over the source. However, both such types of dispenser might be unnecessarily expensive and subject to technical malfunction.

The authors propose that pheromone flux of individual strands can be increased very simply by using an entirely passive technique that allows strands to be enriched before they are sheared from the downwind end of the dispenser surface. The dispenser needs to be asymmetrically shaped and either of a

planar or cylindrical design. It then needs to be suspended so that it is allowed to align itself parallel to (along) the wind line. Each parcel of air that passes over the dispenser–surface air boundary will progressively accumulate more molecules as it traverses along more of the dispenser surface, enriching the parcel with increasingly higher concentrations of pheromone before the resulting odor strand is shed from the dispenser's downwind end.

Measurements of plume-strand flux downwind of a rectangular planar dispenser taken in our laboratory using a solid phase microextraction (SPME) fiber approximately the size of a moth antenna have confirmed the large strand enrichment effect of orienting the plane along the wind line (parallel to the wind) (Baker and Park, unpublished data). A greater than 40-fold increase in flux across the SPME fiber (across a moth antenna) resulted, compared with a cross-wind orientation (perpendicular to the wind line). As expected, there were no apparent differences in emission rate from the dispenser when the overall release rates of the planar dispensers were measured using the older method of drawing air over the dispensers housed in a wide tube and collecting the entire volume of the tube onto a cartridge filled with Tenax adsorbent beads (Baker and Park, unpublished data).

From this concept, it follows that if such a planar dispenser (or a cylindrical one) is made much longer than it is wide and its long axis is then aligned along the wind line, pheromone strand concentration and plume flux will be proportionately increased according to dispenser length. Therefore, this method for passively creating the strongest possible odor strands anticipates that odor strand flux can be maximized by using a more linear shaped dispenser, and then keeping the longest axis of its release surface (planar, cylindrical) aligned parallel to the wind. Under variable wind directions, as occur in the field, this wind alignment can be done simply by the use of a wind vane that rotates the emission source to follow all the changes in wind flow direction to keep the surface perfectly aligned to allow odor concentration to occur along the long axis (**Figure 7b**). Alternatively, the long axis of a planar dispenser itself can be allowed to swing in the wind to act as a wind vane, by using single attachment points to a stable surface and either wire, string, or swivel-type rotating fasteners to allow the dispenser to swivel freely in the wind (**Figure 7a**).

The authors emphasize that this concept for increasing plume flux by increasing odor strand concentration differs from the multitude of previous studies over the years that have used planar filter paper dispensers aligned cross-wind versus parallel

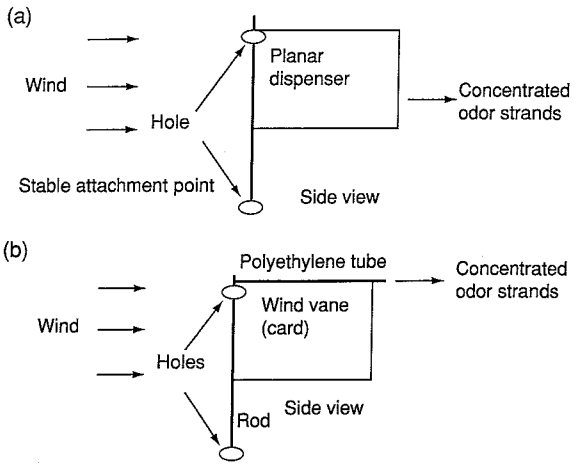


**Figure 7** An illustration of how to easily and passively (without the addition of heat or other energy) enrich the strength of the pheromone signal (odor strand) that emanates from a dispenser. The dispenser must be planar-shaped (a) or cylindrical–tubular-shaped (b). The dispenser should be allowed to rotate freely so that the dispenser's long axis will align along the wind line. This can be accomplished by attaching the tubular dispenser to a wind vane (b), or else by allowing the planar dispenser to function as a wind vane (a). It follows from this priniciple that the amount of enrichment for a given evaporative surface area will be further optimized by making the dispenser much long than it is wide, with the increase in strand concentration being proportional to the increase in dispenser length that is aligned along the wind line.

to the wind to widen or narrow the plume (Mafra-Neto and Cardé, 1994), or else have used obstacles upwind of the source (Willis *et al.*, 1994) to create different plume structures or plume concentrations. In such previous studies, the effort was only to preserve the plume-strand structure and flux *after* the strands had already been shed from the dispenser. Previous work on optimizing plume shape in the field downwind of pheromone traps likewise focused on minimizing turbulence and preserving plume structure (Lewis and Macaulay, 1976), and not on enriching pheromone strand concentration before pheromone leaves the dispenser surface. This present method suggests a novel way to enrich odor strands *before* they are shed from the dispenser, thereby maximizing the flux of pheromone in individual odor strands and hence maximizing the resulting changes in flux across the antenna stationed within a plume.

It is realized that the highly concentrated plume-strands from such wind-aligned dispensers are vulnerable to break-up by the turbulent mixing imparted as they travel through surrounding foliage, and their ability to affect male behavior far downwind might be compromised. Nevertheless, there may be ways to take advantage of this system, especially in the deployment of mating disruption

dispensers above canopy level against pests of field crops. Over short grass, the ability of such small point source generated strands to stay highly concentrated as they travel downwind has been demonstrated; very strong strands of ionized air do occur at great distances from the source, but not as frequently as they do close to the source (Murlis, 1986, 1997). It is possible that extremely long length, strong plume-strand emitting dispensers can be deployed on wind vanes from very few locations outside very large fields to protect the interiors.

The high emission rate, small point source concept also is instructive with regard to the evolution of lepidopterous female pheromone glands. Female moths likely have, as hypothesized by Greenfield and Karandinos (1979), been selected to emit very little pheromone per unit time because judicious emission of small amounts of pheromone is a form of sexual selection that allows females to discriminate for only the fittest (most sensitive) males that are able to locate females from far downwind.

We would propose in addition that females may be able to economize and use such low amounts of pheromone because they emit it from an extremely small piece of tissue situated on the tip of their abdomens. Although the amount of time-averaged pheromone emitted seems to be very small, the plume-strands that are shed are perhaps optimized with regard to the flux over the male's antenna that they produce. Studies aimed at measuring plume-strand flux from calling female moths on various plant species and foliage substrates may be very informative in investigating this idea further.

The confluence of applied and basic pheromone research and the synergy of ideas is apparent from new lines of inquiry such as these. We must continue to create new blends in this manner.

## References


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