Chapter 3

# Wind Tunnels in Pheromone Research

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## I. Introduction

During the dramatic growth in research on insect sex pheromones over the past 10 years, it has been demonstrated that both the chemical signal and the precopulatory behaviors exhibited by males and females are much more complex than originally thought. Chemical studies of sex pheromones have shown that, with few exceptions, females release a blend of several chemical components in a specific ratio and release rate. This specificity of the chemical signal is in part a result of the behavioral reactions of males under "natural" conditions: flight through space from a distance of several meters or more in shifting wind fields. Upwind progress in the plume of chemicals continues if the blend is that of a conspecific female, and the maintenance of contact with the plume is one of the most complex behavioral responses, involving visual feedback, a chemically modulated self-steered program of zigzags, and changes in linear velocity of flight, all performed as the insect samples the odor environment sequentially (Kennedy, 1983; Kuenen and Baker, 1983).

Because the responses made in-flight are so highly integrated, bioassays utilizing flight in wind are probably the most discriminating in pheromone research. Field bioassays involving the capture of males, discussed in Chapter 4 by Cardé and Elkinton, are the ultimate tests of a pheromone blend's "activity," due in a

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large part to the complexity of the environment and of the shifting wind fields. Sexual communication systems evolved in the field, and one would expect that insects brought indoors and placed in more simplified environments would encounter less demanding conditions. Performance indoors, then, could result in less "specificity" of response compared to field behavior.

In-flight bioassays in wind are performed indoors using wind tunnels, or "sustained-flight" tunnels, and they have some definite advantages over outdoor flight bioassays involving field capture of insects. First and most important, the flight tunnel is a physical model of the environment, allowing the experimental manipulation of one variable at a time. Temperature, humidity, wind velocity, and chemical plume conditions can be reproduced day after day, and the experimenter does not encounter the daily variation in results common to field tests that must be factored out by replication and experimental design (see Cardé and Elkinton, Chapter 4). Cause-and-effect relationships from these variables can be gained more easily in a wind tunnel than in the field.

Although wind tunnels cannot duplicate the combination of wide plume dimensions and low concentration that occur in the field at great distances from a source, the use of low, as well as high, emission rates in a wind tunnel can shed light on the responses that occur there, at both ends of the active space (Baker et al., 1981; Linn and Roelofs, 1981). The prolonged (sustained) flight over a distance of many tens or hundreds of meters can be mimicked in the wind tunnel by rotating a visual floor pattern beneath the insects as they fly. The insects compensate for this higher velocity of optomotor stimulation by reducing their airspeed and hence can be made to fly for many minutes, even hours, at zero net up-tunnel ground speed while in the pheromone plume. This can increase discrimination among treatments (Miller and Roelofs, 1978a,b). Another key advantage of wind tunnels over field tests is that experiments can be performed throughout the year, and especially for pheromone identifications, much progress can be made under inclement conditions in preparation for the time when field tests can ultimately be performed.

Because flying while maintaining contact with pheromone while progressing upwind is such a highly integrated behavior and is sensitive to even tiny changes in thrust and attack angle, wind tunnels have come to be used for a wide variety of studies requiring detection of subtle behavioral changes. They are currently being used for pheromone identifications, blend quality testing, in the design of pheromone traps, in tests for habituation, for orientation studies, and most recently to detect sublethal, behavioral effects from insecticide intoxication (Linn and Roelofs, 1984; Haynes and Baker, unpublished).

Wind tunnels are not strictly an invention of researchers in the field of sex pheromones. Rather, their use grew out of studies on orientation responses of insects to visual feedback from the environment (Kennedy, 1940), and to host odors (Kellogg and Wright, 1962; Kellogg et al., 1962; Daykin, 1967). These early studies are well documented by Kennedy (1977a). With the increased interest in, and identification of, sex pheromones, they became used for investigating orientation mechanisms used by male moths flying to sex pheromone (Traynier, 1968; Farkas and Shorey, 1972; Kennedy and Marsh, 1974).



The scientific exchange of ideas between Farkas and Shorey (1972) and Kennedy and Marsh (1974) (also Shorey, 1973; Kennedy, 1977a; Farkas and Shorey, 1976) concerning the relative importance of anemotaxis and chemotaxis in the pheromone-mediated upwind flight of male moths was a direct result of experiments performed in wind tunnels, and these brought the technique to the attention of researchers in the field of pheromone identification and chemistry. Realizing its potential value in pheromone research, Miller and Roelofs (1978a) designed a sustained-flight tunnel and demonstrated its versatility and utility in discriminating among blends of incipient pheromone components.

The need for more discriminating bioassay techniques and for a more complete knowledge of the behavioral reactions to pheromones was also stressed at the same time in reviews by Kennedy (1977a,b, 1978). These papers set a standard for future studies of pheromone-mediated behaviors and occurred slightly after a dramatic increase had begun in the number of pheromone identifications involving multiple components (Roelofs and Cardé, 1977).

This chapter will discuss a number of pheromone-related studies involving wind tunnels and emphasizing design features and techniques that are necessary for achieving optimal sensitivity, discrimination, and experimental flexibility. We will illustrate the diversity of uses for wind tunnels in trying to dissect the behavioral responses that result in the displacement outcomes "attraction," "arrestment," and "repellency," and that contribute to the evolutionary outcome, reproductive isolation.

## II. Wind Tunnel Design and Operation

We find it helpful to categorize wind tunnels by mode of air movement. As described in Chapter 2 on bioassays by Baker and Cardé, the most common form of wind tunnel in pheromone research involves horizontally moving air, and horizontal displacement using anemotaxis (steering with respect to wind direction) is possible. But just as it is possible for bioassay tubes and chambers to employ moving air that does not permit anemotaxis by moving the air perpendicular to the plane in which displacement is permitted, so too can wind tunnels measure movements of flying insects that are not steered anemotactically. Such a situation is found in vertical wind tunnels, in which air moves either straight up or down and the insect's horizontal displacement in or out of pheromone has no wind-induced drift component. Also, air movement can be stopped in a horizontal tunnel to observe pheromone-mediated movements lacking an anemotactic component. Finally in at least one tunnel, horizontal air flow has been superimposed on vertical air flow to create an elaborate assay for a pheromone's behavioral effects (Phelan and Miller, 1982).

## 2.1. Moving the Air

The two fundamental decisions to make regarding wind tunnels are these: how to get the air to move and how to keep the air in the room and in the tunnel clean and free of contamination. Contamination is bad because the insects waiting to be tested can slowly become habituated, or they may respond poorly because the air in the tunnel can become tainted with an antagonistic compound. The second decision is somewhat related to the first in that if you choose to pull the air through the tunnel rather than push it, you can accomplish air cleansing by having the exhaust fan (centrifugal blower) pull the entire volume of the tunnel's air out of the room, and usually, out of the building. This may be the easiest way to ensure that all the pheromone, especially from a tunnel permeated with a cloud of pheromone (see below), is exhausted from the room. If a chemical exhaust hood is present in the laboratory, usually it is easy to adapt it for air pulling by building boxes to fit it to the end of the tunnel or by running a tube from its duct to hook up to the end of the tunnel.

Other than for pheromone permeation tunnels, however, we do not recommend using a fan to pull the air. It is better to push it, for the simple reason that access to the inside of the tunnel can be gained through doors, holes, slits, etc., without worrying about disturbing the pheromone plume's position or structure. With titanium tetrachloride-generated smoke, one quickly sees that even with access doors to push-fan-type tunnels left open, actions such as sticking hands, head, arms, etc., into the tunnel do not disturb the plume, unless of course they are placed directly upwind of the smoke source. If the tunnel is housed in a small room, though, walking rapidly past the open doors can push the plume off course momentarily, and so it is always wise to operate the tunnel with the doors closed if the operator is expected to do much moving around while the insects are in flight.

With pulling-type tunnels, however, even without observer movement any crack, hole, pinhole leak, or especially any open tunnel door creates visible, severe perturbations of the (smoke) plume. Therefore these tunnels must always be operated completely sealed along the side, and this makes them less adaptable for photography and for in-flight experimental manipulations of all types. This is true even during the act of releasing an insect into a plume, because the turbulence caused by opening a door at the downwind end creates uncertainty as to where the plume has moved.

Our experience with designing and constructing wind tunnels has led us to formulate the "First Law of Wind Tunnels:" *Do not follow the advice of engineers.* In our view, engineers have been taught to be concerned about wind tunnel features that have no bearing on their use as an instrument to study insect flight. For one thing, they have traditionally used wind tunnels to study the behavior of objects in winds of very high velocities, usually 100-640 km/h. Under these velocities, they have learned to be concerned about events occurring near the walls of the tunnel and at the intake end, such as friction, shearing, and



turbulence. Entomologists are interested in the center of the tunnel, near which the insects fly, and the ultralow velocities needed for such flights are not only quite foreign to engineers, but also they make many of the wall-wind interactions insignificant.

Engineers nearly always will recommend building a pull-type tunnel, because this is what they are used to using, and for their studies at high velocities this type has been most reliable. We are aware of three out of three cases in which entomologists have first consulted engineers about constructing a tunnel and have been advised to pull, rather than push, air. The two who followed the advice have had many problems and eventually changed over to push-type tunnels. In the third case, R. T. Cardé and T. C. Baker at Michigan State University went ahead and constructed a push-type from the start, and were extremely happy with it.

A smoke source of some type is essential for making sure of the placement of pheromone sources and insect release cages, demarcating time-averaged plume boundaries, checking for efficacy of wind-stopping procedures, and for visualizing the movement of air throughout all areas of the tunnel. Smoke plume movement is also the easiest and most accurate way to measure the wind velocity, as pointed out by Kellogg and Wright (1962). The observer only needs to time with a stopwatch the passage of a smoke filament down a known length of the tunnel. J. S. Kennedy and his colleagues (personal communication) light up a few cigarettes and place them in a stoppered flask, waiting a bit for the smoke to build up, and then turn on a gentle flow of air through the flask. The smoke flows out of the flask through flexible tubing whose tip can be positioned easily anywhere in the tunnel.

Another way of generating smoke is by using an airstream laden with the combined vapors from concentrated HCl and ammonium hydroxide. Kellogg and Wright (1962) found that ammonium acetate smoke, generated by mixing the vapors from $NH_4OH$ and acetic acid, was the smoke least irritating to flying insects that they tested, and so they could use it combined with a host attractant to visualize the passage of flying insects through and up the plume.

Titanium tetrachloride-generated smoke, made by placing a small quantity of this material straight out of the bottle onto a cotton wick or rubber septum, is mildly irritating to flying moths. We have been able to combine the smoke with a pheromone plume, and oriental fruit moths will fly in apparently normal fashion upwind in the plume until they reach a critical point near the source and drop to the ground, apparently dead. At least they do not move any more. We find this white smoke to be the thickest and easiest to produce. Very thick black smoke can be produced by burning acetylene from a tank, but this produces much heat and seems best to be used outdoors (Von Keyserlingk, 1983).

There are many ways to generate wind to push air down a tunnel, but perhaps the simplest way is with a simple, rotary-blade fan. Whether the tunnel is round or rectangular, air can be easily conducted from a fan to the tunnel by means of a duct constructed from thick, flexible plastic sheeting, such as plastic

bag material, taped together and sealed to form a tube. Usually the duct must expand from the smaller fan's size to the larger tunnel's opening.

The duct of R. T. Cardé's tunnel at Michigan State (Fig. 1) was made of Plexiglas and expanded from a smaller, round fan opening to the larger rectangular opening of the tunnel. At the tunnel's opening, some sort of mixing chamber is needed to dampen the turbulence created by the fan's blades and to balance the wind velocities throughout the tunnel's middle, sides, top and bottom. The chamber, usually a box shaped like the tunnel, creates resistance by means of several layers of narrow-mesh cloth or screening, or both.

Miller and Roelofs' tunnel used several layers of cheesecloth stretched tightly across the opening and separated from each other by several centimeters or more. A final layer of silk was the last layer the air passed through as it entered the tunnel. The mixing chamber of one of the author's (TCB) wind tunnels is a box 125 X 110 X 31 cm made of plywood, and inside there are three particle board panels that slide into the box from the top along grooved tracks. Although the chamber and the panels are rectangular and the tunnel is U shaped, made from bowed Plexiglas (Miller and Roelofs, 1978a), the panels have a U-shaped section carved out of their center so air can pass through them in that configuration. Both a single layer of cheesecloth and an aluminum window screening (to support the cloth) are stretched and stapled across each panel's U-shaped opening. The panels are easily slid out and removed when the cloth and screens need cleaning to remove moth scales and dirt (see Section 2.3 below). A final layer of brushed nylon is stretched across the box where air exits the box and enters the tunnel.

The mixing chambers have usually been described as essential so that laminar flow can be created. We do not think that laminar flow is necessary to get successful insect flights to a pheromone source. Indeed air that is too smooth has been cited at least once as a hindrance to successful orientation (Marsh et al., 1978), and special "spoilers" had to be installed to create a little turbulence and

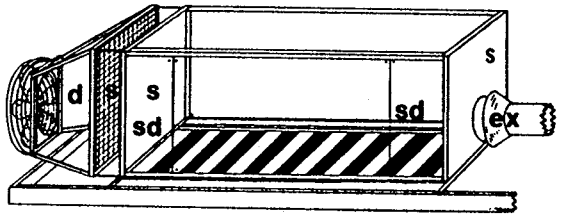


**Figure 1.** Rectangular, Plexiglas wind tunnel with push-type fan. Plexiglas duct (d) expands from circular fans' size to larger, rectangular tunnel's size, and conducts air to screens (s) which smooth the airflow. Access to tunnel is gained by two sliding doors (sd), and pheromone plume is scavenged from tunnel by exhaust tube (ex). Insects fly from right to left up a pheromone plume, and moving striped cloth floor pattern located beneath the Plexiglas floor can be used to sustain flight (from Cardé and Hagaman, 1979).



make the plume wider and choppier. Notwithstanding the fact that laminar flow is not essential, we think that it is desirable for most pheromone studies because it creates a simplified, moving air environment.

As experimental biologists, we prefer fewer variables to worry about during analysis and interpretation of results. Of course, during studies of in-flight orientation mechanisms, air blowing predictably in one direction at a known velocity and laminar structure is essential in interpreting steering in wind, but for most studies where tunnels are used, as when comparing different blends and dosages of pheromone, getting the flow to be laminar is of less concern. It is more important to be able to pinpoint where the plume is so that every insect can be placed in it without a doubt that it is in fact receiving optimal exposure to the pheromone plume. We have both had the experience of getting suboptimal responses from male moths and then upon checking the plume's location with smoke (which we should have done beforehand), found that the release-cage platform had been moved slightly so that the moths were not really in the plume's center. We cannot stress this enough: consistency of the plume's position and that of the release station for insects are of the utmost importance regardless of the tunnel design that is being used.

## 2.2. Cleaning the Air

Given that a push-type fan is the best way to move air and that it allows the most freedom in experimentation, how best to clean the air? If it is not likely that you will ever be able to install an exhaust blower to remove air from the room in which your wind tunnel is housed, the air can be scrubbed by means of a bed of activated charcoal. This technique was demonstrated by Kellogg and Wright (1962) in their tunnel (Fig. 2), and later used with much success by J. S. Kennedy and his colleagues at Imperial College, Silwood Park, England, for many years. The advantages are that the tunnel can be aligned anywhere in the room without having the worry about fighting exhaust tubes and hoods. Probably more importantly, the entire room's air is recirculated and therefore it is relatively easy to keep the temperature and humidity constant.

In wind tunnel rooms having exhaust tubes and hoods, large volumes of air are continually being lost, and efforts to heat or moisturize the air often are made futile. If the climate-controlling capabilities are great, the room is large, and the volume of exhausted air is relatively small, then the problem of climate control versus exhausting of pheromone is minimized, as in the case of Wendell Roelofs' tunnel at Geneva, New York.

Disadvantages to the bed of activated charcoal are that it can have high resistance to wind flow, and it may be difficult to force enough air through the bed to create wind velocities other than the very lowest. Kellogg and Wright's charcoal bed was 5 cm thick but of unspecified width, height, and weight. Because it was housed in the inlet tube to the tunnel, it was likely no more than 30 cm in diameter, which would have reduced the weight considerably. The

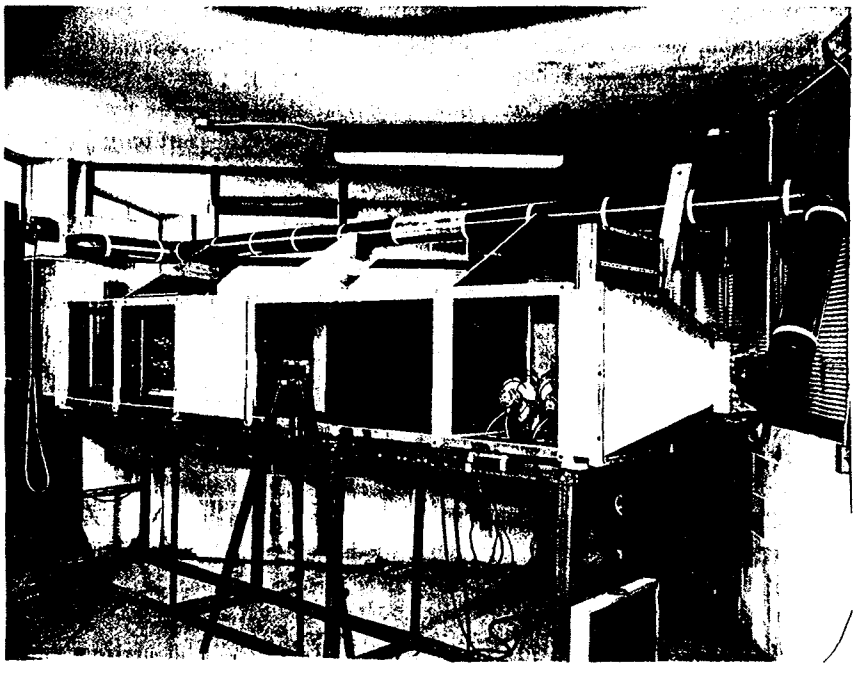

**Figure 2.** Kellogg and Wright's wind tunnel, which used a 5 cm-thick bed of activated charcoal at the inlet end (right) to cleanse the air. Air was drawn through the tunnel by a centrifugal fan at downwind end (left), and air could be recirculated into the tunnel's inlet end, if necessary, by the return pipe shown on top of the tunnel. Tunnel is made of plywood, with front and rear walls made of glass. Access to tunnel's interior was through either the top or the bottom of the center, working section. Stereo cameras are positioned horizontally for photographing tracks of flying insects against a black background (from Kellogg and Wright, 1962).

charcoal bed described by Kennedy and Marsh (1974) and Marsh et al. (1978) was contained between two 2.4 X 1.2 X 0.45 m vertical screens and was 0.5 cm thick. One problem with a vertical bed of charcoal such as this is that the material tends to settle toward the bottom and create a higher density (and more wind resistance) at the bottom of the bed compared to the top (J. S. Kennedy and C. T. David, personal communication).

A later modification reduced the charcoal bed's thickness to facilitate higher wind velocities, and positioned the bed horizontally, not vertically, nearer the fan. The horizontal placement solved the charcoal settling problem, and even this relatively thin layer of charcoal has been sufficient to adsorb all the gypsy moth pheromone that would have recirculated into the tunnel over several years. There has been no apparent sign of "breakthrough" of pheromone due to saturation of the active sites in the bed (J. S. Kennedy and C. T. David, personal communication).



When plumes are the usual mode of using pheromone, the best way to remove the pheromone using an exhaust system is the technique described by Miller and Roelofs (1978a). A wide exhaust tube connected to the outside of the building, ca. 20–40-cm diameter with an exhaust flow much faster than the tunnel's wind speed, is placed just outside the end of the tunnel at the plume's height (Fig. 1). The pheromone is effectively scavenged and the rest of the tunnel's (pheromone-free) air is allowed to recirculate without filtering. One advantage to removing only the plume-air from the room is that wind-induced pressure changes at the exhaust outlet outside the building will affect only the rate at which pheromone is scavenged, not the tunnel's wind velocity. We know of at least two cases where exhaust fans pulling wind through the tunnel have been affected significantly by outdoor wind, so much so that in one case the wind flow in the tunnel would reverse direction every so often!

## 2.3. Contamination of Surfaces

Another type of contamination concerns surfaces within the tunnel. All surfaces that may possibly come in contact with the pheromone source or the plume should be of a material able to withstand rinsing with a solvent. Glass and metal are best, but a tunnel made of glass is extremely heavy and fragile. Most types of Plexiglas (polycarbonate, acetate, etc.) can withstand rinsing with ethanol: acetone is usually not worth the risk, although it is the solvent of choice for rinsing all metal surfaces such as release cages for insects, the platform on which the source rests, etc.

Most of the working wind tunnels in pheromone research are constructed of Plexiglas and metal. The table on which our wind tunnel rests and which is not likely to ever be exposed to much pheromone, is made of wood. For studies using point source plumes, pheromonal adsorption onto the sides of the tunnel can be minimized by positioning the plume so that even with lateral and vertical spreading, it does not touch the sides. Special care should be taken if an end-of-tunnel screen is used, as described by Miller and Roelofs (1978a), to keep moths from exiting the back of the tunnel. This arrangement, which employs a hole in the end screen at plume height into which the release cages for moths are inserted, has a special problem in that the area of end screen in the plume can become contaminated from previous plumes (J. R. Miller, personal communication). Thus, even if the release cages are cleaned with solvent after each use, anomalous results can occur if the screening immediately surrounding the release cage is not also rinsed whenever a new treatment is tested.

The surface most likely to be contaminated is the one on which the pheromone source rests. Certainly the rubber septum, filter paper, glass rod, etc., can be suspended by a thread (see Baker and Cardé, Chapter 2, for types of pheromone sources). However, most often it is easiest to place it on some type of platform which will need rinsing after every change of treatment.

We have found that a simple stand with a sheet-metal base and four sheet-metal legs oriented with their edges parallel to the wind line is a very workable

setup for quickly changing pheromone sources. The stand creates minimal turbulence because of the edge-on orientation to the wind, and the plume is thus minimally disturbed by mechanical turbulence. A clean sheet-metal plate is placed on this stand whenever a new source is to be tested and the base and legs of the stand remain in the tunnel throughout the experiment. This plate becomes the table top of the platform when in place, there being just four legs sticking into the air when the plates are absent. The entire stand is rinsed with acetone after each experiment, and the plates are rinsed before each use. If a permanent table top to the stand is left in place, even if clean plates are placed on top of it the bottom plate can become contaminated, presumably due to slight turbulence at the airspace between the plates, and can affect responses to subsequent treatments.

One final source of contamination should be mentioned, but it is not clear whether it is pheromonal or not. Over time, enough escaped males and their scales enter the mixing box's intake, and scales and dirt can build up on the layers of cloth stretched across the mixing chamber. In several laboratories, we have experienced and heard reports of poor flights correlated with scale-laden screens and cloths. When the screens are washed with acetone and the cloths are cleaned or replaced, the responses improve significantly, usually back up to their normal levels.

In these instances airflow as checked by smoke plumes appeared to be normally laminar but possibly the velocity was slightly reduced. The scales and dirt may possibly interfere chemically with optimal behavioral responses, although this is pure conjecture. When poor responses occur day after day, we suggest checking the screens for scales and cleaning them for good luck.

### 2.4. Movable Visual Patterns

To have stands of any type resting on the tunnel's floor would seem to prohibit moving the floor. It may be important for some studies to have the ability to rotate a ground pattern beneath a flying insect to make use of the optomotor compensatory responses they perform. Long-duration flights of zero net up-tunnel ground speed can then be executed for increased discrimination among treatments. We use a movable floor pattern stationed just below a clear Plexiglas floor, and the insects can see the pattern because they respond nicely to it by changing their airspeeds when it moves beneath them. Depending on the species, lighting, and the height above the floor that the plume is stationed and at which moths are forced to fly, the moving floor pattern will have varying degrees of effect on flying insects.

For instance, Sanders et al. (1981) found that their spruce budworm males responded better to the striped pattern's motion after it was mounted on the ceiling of the tunnel and the glass floor was given a black backing, making it into a mirror to reflect the pattern upward. Earlier they had suspected that the stripes, when mounted below the glass floor, were being obscured by the fluorescent lights' reflection from the floor, because it was often difficult to slow males' progress by moving the pattern. Kennedy and Marsh (1974) stationed their plume ca. 15 cm above the floor pattern to obtain good optomotor responses to its movement. Kuenen and Baker (1982a) found that for *Grapholita molesta* and *Heliothis virescens* males, increasing the height of the plume to 40 cm or more above the floor pattern eliminated detectable optomotor response to the floor's movement. Above 30 cm the males seemed to be increasingly watching other cues in the wind tunnel room.



Interestingly, Farkas and Shorey (1972) might not have discarded optomotor anemotaxis and embraced chemotaxis alone as an orientation mechanism in wind had they stationed their pink bollworm pheromone plume closer to the floor. They reported in a footnote that they had tried rotating the floor beneath the males, and because there was no observable effect on the males' flight speeds they concluded optomotor anemotaxis was not being used. After inspecting their 0.6 × 0.6 × 1.5 m tunnel, we conclude that it is likely that they had stationed their plume 30 cm or so above the rotating pattern. The males were using visual cues in their optomotor reactions to wind-induced drift, but unknown to Farkas and Shorey, at that height, the cues must have been predominantly from the stationary objects in the room.

Because Kennedy and Marsh (1974) used a treadmill-type floor pattern with transverse (cross-tunnel) stripes, most others that have been used since then have also been striped treadmills, e.g., Miller and Roelofs (1978a) who used alternating black and orange 10-cm-wide transverse stripes. R. T. Cardé used alternating green and white stripes (Fig. 1) (Michigan State U.'s colors) and all species of insects tested, even a fungus gnat *Bradysia impatiens* (Alberts et al., 1981) and Oriental fruit moths originating from Cornell, responded nicely to its movement.

There are some problems with moving treadmill-type cloth patterns (see below), and the first patterns used successfully were projected onto the floor (Fig. 3) (Kennedy, 1940; Kellogg and Wright, 1962). These clearly evoked optomotor compensation in both free-flying insects and those flying in odor, and in the future perhaps more use will be made of this method of moving a pattern beneath, above, or beside insects.

Kennedy (1940) used a slatted cylinder rotating around a light (Fig. 3) and Kellogg and Wright (1962) used a continuous loop of film through which light was transmitted. One advantage to projected patterns is that very small changes in velocity or direction of the pattern's flow result in large changes on the tunnel's floor. It is therefore much easier to play with these variables to observe movement changes in flying insects than for cloth treadmills. This is especially true if the moving pattern needs to be rotated to flow at some oblique angle with respect to the wind.

One disadvantage of such patterns would seem to be that for diurnal species it would be difficult to get the pattern bright enough to compete with the room lights that are at daylight intensities. For nocturnal insects, too, the problem of

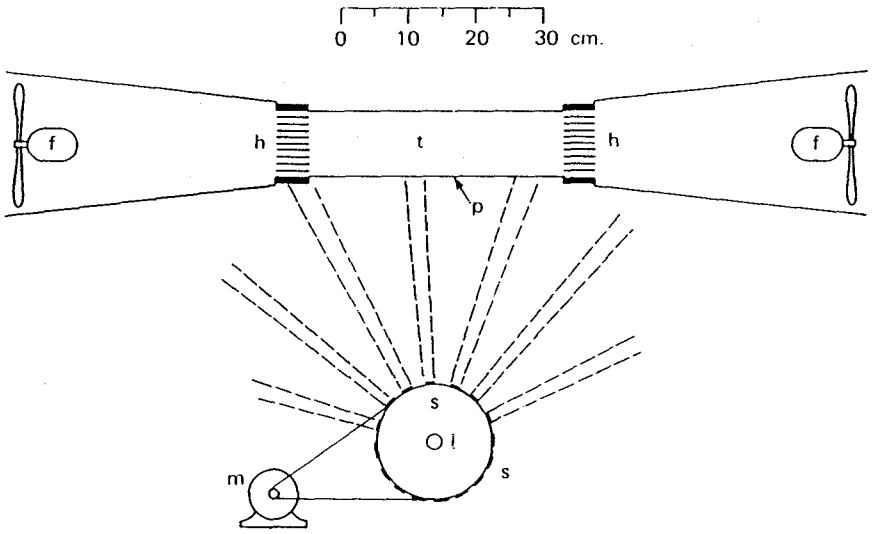


**Figure 3.** Kennedy's (1940) method for projecting moving, visual striped pattern onto the floor of his wind tunnel, 38 cm long, 7.5 cm wide, and 10 cm high. Strips of metal (ss) driven by motor (m) rotate around light bulb (l) and project a pattern onto waxed paper sheet (p) on the floor of the tunnel. Fan (f) can draw air in either direction through honeycombs (h) which smooth the air.

balancing the intensities, especially for floor-projected patterns, would seem to be critical. If the light below is too bright, the insects' dorsal light response can cause them to end up diving toward the floor rather than flying up-tunnel (K. F. Haynes, personal communication).

Regardless of whether the pattern is projected or not, recent findings indicate it might be safer not to use transverse stripes, but instead use spots or some other pattern lacking long, straight lines. This caution would seem to be applicable mainly for orientation studies in which track and course angles are being measured. J. S. Kennedy, whose tunnel originally had transverse stripes on the floor, recently switched to a pattern of solid pink circles on a yellow background (Kennedy et al., 1980, 1981).

Charles David, also at Silwood Park, recently demonstrated that stripes can have some unexpected effects on the orientation of flying insects, in this case free-flying *Drosophila* (David, 1982a). He recommended that for orientation studies, spotted instead of striped patterns be used because of these findings and because long straight lines are seldom found in nature.

The colors of the patterns should be chosen carefully, especially if photography of the insects' flight tracks is to be performed. In our tunnel (TCB's), we started with black and white 10-cm-wide transverse stripes, but quickly found that in video recordings, the oriental fruit moths disappeared whenever they were over black. Cardé and Hagaman (personal communication) had the same problem, and they analyzed only the one-half of the data where their gypsy



moth males were over the white stripes, not the green (Cardé and Hagaman, 1979). To tackle our problem, we then softened the contrast by placing a layer of cheesecloth on the Plexiglas over the floor, so that now the moths could be seen over the grayish stripes. They continued to respond to the stripes' movement (Baker and Kuenen, 1982).

To make it even easier to track our moths in frame-by-frame playback and to remove possible bias caused by stripes, we have recently converted the pattern to 10-cm-diameter red circles on a white background. The red pattern influences the males' movements because the red should appear fairly dark to them against the white background (insects in general are not as sensitive to long wavelengths as to short ones).

On our black and white video recordings we obtain a nearly uniform white background because we place a red filter over the lens. A similar effect can be gained by illuminating the tunnel with red lights. Making spots of any color appear white on black and white video tape is possible by using a filter or room lighting of the spots' color.

One of the problems with cloth-treadmill patterns is that they frequently come off-center, sag, or wrinkle. It is best to make at least one of the two roller-axels capable of being adjusted backward or forward in tiny increments so that the belt can be realigned when necessary. It is extremely frustrating to have to stop repeatedly in the middle of an experiment and yank the tightly seated cloth or canvas back into position to keep it from slipping off the side of the roller or binding and wrinkling at the sides. If you are lucky, a good axel alignment can last many months and many revolutions of the pattern. For tautness, J. S. Kennedy and colleagues use a third spring-loaded roller below one of the end rollers that presses on the lower fabric and takes up the slack that will always occur there.

The belts can be driven in a variety of ways, but it is always best to have a motor or drive system capable of a continuum of speeds without the motor burning up. We (TCB) use a sewing machine motor which was built for variable speeds, whereas Miller and Roelofs scavenged a motor from a counter-current distillation apparatus that had rarely been used. Their variable speeds resulted from the use of a worm-gear-clutch arrangement controlled by a joy stick. The motor runs at a constant speed, and another nice feature of their pattern (and J. S. Kennedy's and R. T. Cardé's) is that its direction can be reversed at the flip of a switch.

## 2.5. Creating Pheromone "Clouds"

For some studies, such as those in which the researcher wants to make sure that the insect is in continual contact with pheromone, point source-generated plumes are not adequate. Rather, the pheromone needs to be diffused into a nearly homogeneous cloud by creating turbulence around the pheromone source before the pheromone-air mixture is made laminar by the layers of fine-mesh screening. Traynier (1968) was the first to attempt this with a sex pheromone as

the odor, although in vertical wind tunnels Daykin and Kellogg (1965), Daykin (1967), and Daykin et al. (1965) generated air uniformly permeated with water vapor, $CO_2$, or repellents. Two separate columns of permeated air could be formed, one in each half of the tunnel, and the tracks of insects between the halves of the "choice-chamber" were recorded and analyzed for the nonanemotactically guided horizontal movements modulated by the odors.

Traynier (1968) first used pheromone-permeated air in the horizontal tunnel of Kellogg and Wright (1962) (Fig. 2). He placed the abdominal tips of 30 *Anaghasta kuhniella* females into the air-inlet tube of the tunnel and then recirculated the air. The upwind tracks of males flying in air permeated with pheromone certainly appeared different from those flying to a point source, but they were not quantified, smoke was not used to visualize the cloud, and it is not clear how uniform the air really was.

This simple experiment, though, stimulated much thought on the anemotactic–anemomenotactic model of orientation to a pheromone source (Kennedy et al., 1980, 1981; Kuenen and Baker, 1983; Kennedy, 1983). Kennedy et al. (1980, 1981) created a cloud of pheromone uniformly permeating the air by means of a grid of turbulence-producing strips of masking tape immediately downwind of a grid of pheromone sources, all affixed to a brushed nylon screen. The strips of tape were 2.5 cm wide and applied to the upwind side of the screen at 6.0-cm intervals vertically and horizontally, leaving seven rows of 20 squares of uncovered nylon screen, each 3.5 X 3.5 cm. Every alternate square was then covered with tape, leaving a total of 70 unoccluded squares. The grid of polyvinyl chloride rod pheromone sources was formed by attaching the 0.4 X 1.0 cm rods to the center of each of the 70 occluded tape squares on the upwind side. A second, "smoothing" screen was located 50 cm further downwind, effectively creating a mixing section for the cloud before the flow was smoothed before entering the tunnel's working section (Fig. 4).

The ability of this system to permeate the air uniformly with pheromone was checked with smoke. Side "corridors" of air uniformly permeated with pheromone were neatly created by placing only a partial grid of pheromone sources at one side of the tunnel. Smoke source visualization confirmed that there would have been a sharp edge between the clean air and the pheromone cloud. Although Kennedy et al. used their clouds to investigate orientation movements, Sanders (1982) used a nearly identical system of creating clouds to examine habituation and background odor "noise" effects on males' ability to locate successfully a point source.

Further orientation studies in uniformly permeated air were performed by Willis and Baker (1984) using a system of turbulence strips again similar to Kennedy et al.'s (1980, 1981). Their system differed slightly, however, in that only vertically oriented 4.5-cm-wide sheet-metal strips separated from each other by 0.5 cm were used, and the pheromone sources were located downwind of the strips. Experimentation with cardboard prototypes revealed that the distance between the grid of rubber septum pheromone sources and the strips



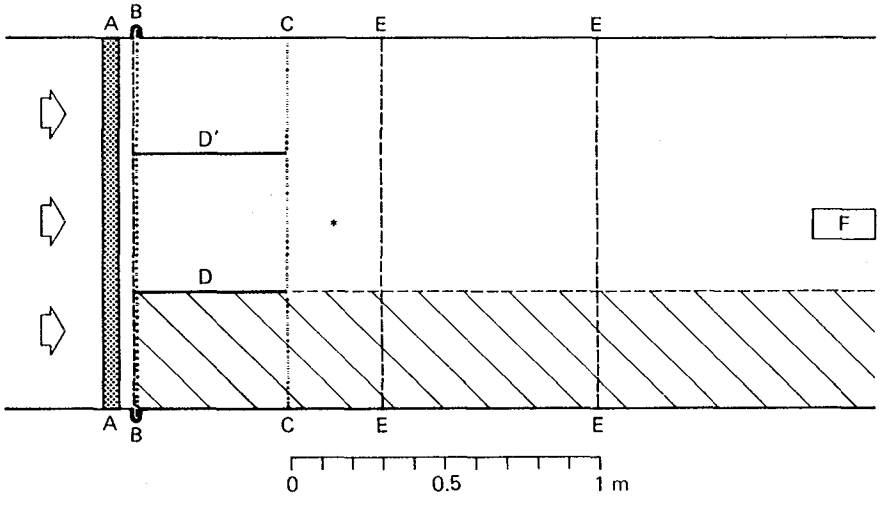


**Figure 4.** Wind tunnel used by Kennedy et al. (1980, 1981) to create uniform, permeated clouds of pheromone to study flight tracks of *Adoxophyes orana* males (this tunnel was same one used by Marsh et al., 1978). In this depiction, a side corridor of pheromone (hatched lines) was created by lowering a "spoiler" grid of tape affixed to a nylon screen into the airstream with pheromone pellets attached to its upwind side only along the one section in which the corridor was to appear. A, charcoal filter; B, brushed nylon fabric screen carrying the "spoiler" grid of tape and pheromone-containing PVC pellets; C, plain brushed nylon smoothing screen; D, partition preventing cross-tunnel mixing of the pheromone-bearing air (D' serving the same purpose when the pheromone was released on the other side of the tunnel); E, video-recording zone; F, release cage for moths.

was critical, with the strips and grid of sources needing to be securely fastened in place with a separation of 1.5 cm. Another feature which aided in homogenizing the pheromone and air was two layers of fine-mesh brass screening located farther downwind in the sheet-metal mixing box just before the pheromone entered the Plexiglas working area of the tunnel. A side corridor of pheromone was created by only partially filling the grid with pheromone sources, plus a sheet-metal partition in the mixing box kept the clean air and pheromone from mixing before reaching the tunnel's working section.

Another way of premeating the air with pheromone is to coat a fabric screen uniformly with pheromone solution. There is at least one drawback to this method, though. Over the course of an experiment, the concentration of the cloud is likely to drop very rapidly due to the rapid loss of pheromone from the screen. Therefore the movements of insects even an hour after the start of observations are likely to be caused by an unknown, lower concentration than that used at the start. Also, it will be very difficult to be sure of the initial concentra-

tion actually adsorbed onto an area of screening. If the screen is dunked into the solution, the loading rate may depend on the duration of the dunking.

This way of applying the pheromone will also require a large volume of solution, and is likely to be quite messy with regard to contamination of the person doing the dunking and of the room. If the solution is sprayed through an atomizer or other such device, the pattern of application must be quite carefully performed to prevent overlapping layers of higher concentration strips, and again the initial amount actually absorbed by the fabric's fibers compared to the quantity passing through the holes will vary from application to application. On the positive side, this method probably is the one most likely to create the most uniform cloud of pheromone.

### 2.6. Stopping the Wind

For some experiments, usually for studies of orientation, the researcher may need to stop the wind to create various chemical stimulus situations in which the insect must move without the benefit of wind drift. Over the years, several methods have arisen for stopping wind. Kennedy (1940) used a system of louvers at the downwind and upwind ends of the tunnel which were snapped shut quickly. Farkas and Shorey (1972) used two solid panels which were abruptly slid into place along grooved tracks simultaneously at the upwind and downwind ends of the tunnel. Smoke visualization confirmed that the wind stopped at the instant the panels closed, and only lateral spreading and gradual dissipation of the plume's filaments were observed without significant up- or downwind displacement.

Baker and Kuenen (1982) used a leather cinch attached to the axel of their rotary-blade fan to stop the rotation of the blades after the power had been switched off. However, it took over 2 sec for the blades to stop rotating and the wind (as visualized by smoke) to stop. A cardboard cover was placed over the exhaust tube simultaneously with turning off the fan in order to keep a vortex from rolling up the tunnel and disturbing the plume.

Baker et al. (1983) later improved on this technique by using two window shades, one at each end of the tunnel, which on cue were lowered simultaneously. The one at the upwind end was located inside the mixing chamber, and its cord for lowering it exited the chamber's bottom through a small hole. It was essential that it be pulled down while the fan was still on, because the air pushed the shade against the downwind end of the chamber, sealing it against the sides and completely blocking air flow. A piece of weather stripping was also essential to seal the bottom of the shade against the bottom of the box. The air was now diverted out through the chamber's open top and into the room housing the wind tunnel. The shade at the downwind end was necessary to keep slight air movements of all types from entering the tunnel and disturbing the plume.

Smoke was extensively used outside the tunnel to find out where air movements that might cause within-tunnel disturbances were originating, and this was



how it was discovered that the down-tunnel shade was needed. Smoke was also used to check for the complete cessation of up- or down-tunnel displacement of the plume within the tunnel, which in actuality could be limited to less than 0.5 cm/sec. Smoke also revealed another important feature of wind stoppage. If the tunnel's doors were not completely sealed, even just a tiny crack would result in a puff of turbulence at the moment the shades were pulled down, causing the plume to snake sideways and dissipate. The plume remained completely intact when the doors were closed during stoppage (Baker et al., 1983).

Kennedy, David, and Ludlow (personal communication) found another simple way to stop the wind suddenly. They created a small door on the duct leading from the fan to the bed of activated charcoal before the entrance to the tunnel. When the door was abruptly opened by the operator, who pulled on a long cord from his position near the observation area several meters away, the air now dumped out into the room. The wind in the tunnel stopped immediately, and the fan was then switched off. One key feature which allowed this technique to work was the relatively high resistance to airflow by the charcoal, and the very low resistance through the open door.

### 2.7. Recording and Analyzing the Data

As discussed in the previous chapter on bioassays by Baker and Cardé, data can be recorded in wind tunnels by a variety of methods, depending on the objectives of the experiment and the speed with which the data need to be analyzed, conclusions drawn, and new experiments designed. If only the presence or absence of a behavior needs to be scored, a pencil and paper often are the only required tools. For testing responses to different blends of components, we often have a data sheet composed of a checklist in our wind tunnel, where, after each moth is tested, the observer checks off the behaviors performed by the moth and notes the closest approach to the source.

If temporal features are of interest, such as latencies and duration of response, then audio recording of the events as described by the observer will likely be most useful. A multichannel strip chart event recorder can also be useful here for gaining an immediate "hard copy" of the timing and sequence of behaviors (Baker et al., 1981). The recent availability and low cost of microcomputers have made these more appealing as event-recording tools. Different keys can code for different behaviors, and the advantage is that the data are immediately stored on disc for further analysis by programs written for the needs of the experiment. These may be increasingly used for wind tunnel studies and other behavioral experiments.

Studies of the orientation of flying insects, including a fine-grained dissection of the movements, requires more complex instruments, such as multi-image, still-frame photographic techniques, movie cameras, or video-recording devices. Video recording is probably the most popular device at present and for good reasons. A video recording gives immediate feedback to the researcher as to

whether the images that were recorded are of good enough quality to be analyzed.

Films require much more time to develop. Video tapes can also be reused, and much expense is saved over a long period of time. Finally, there are now many low-light-level-sensitive video cameras available that make recording under moonlight levels or lower very easy, without requiring any special alterations. In contrast, film photography under low-light intensities requires expensive, fast lenses and special films.

One of the disadvantages to video has been, until recently, that motion resolution has been low compared to that of films. One video scan occurs in 1/60 sec (1/50 sec in Europe) and thus a fast-moving flying insect in still-frame will often appear as a faint streak, or worse, become invisible, over that span of time. For slower-moving species, the ordinary video camera has proven to be adequate for single-frame tracking of the insect's motion.

Baker and Kuenen (1982) found, however, that a special rotary-shutter camera combined with a special video disc playback device improves motion resolution significantly. They used a Sony RSC 1050 camera that comes equipped with a high-sensitivity Newvicon tube to record moths' tracks onto ordinary cassette tape. The internal, rotating shutter provided 1/500 sec "snap shots" of the moth on the tape, and then when rerecorded onto the Sony SVM 1010 motion analyzer's video disc and replayed, discrete point images of the moths, not streaks, were visible in still-frame. This system is now used routinely in TCB's lab.

The Newvicon tube is advisable because much light is lost when the shutter is rotating and the more sensitive tube allows low levels of light to be used even with the shutter rotating (another model, RSC 1010, equipped with a less sensitive tube is also available). If slower insects are used, one advantage to the Newvicon-equipped camera is that the shutter can be turned off and ultralow levels of light used successfully. This type of tube is very sensitive to the near-infrared, and recordings can be made under (visible) light levels that are too low for human eyes if even an incandescent bulb covered with an I.R. filter is used. By viewing the monitor, the system can double as a night-vision device for watching live behavior. We see no need to use color cameras for motion recording and analysis; the wide array of black and white high-sensitivity cameras currently available gives them the best flexibility for all sorts of conditions under which insects may be responding to sex pheromone.

Of course, the positioning of the camera(s) in the wind tunnel depends on the experiment being performed. We (TCB) place ours right on top of the tunnel on a tripod, at the back of the tunnel looking up the plume toward the source (Von Keyserlingk, 1983), or from the side, depending on the experiment. J. S. Kennedy and his colleagues and R. T. Cardé's group have successfully used a large mirror mounted on top of the tunnel at an angle such that the video camera can obtain a straight-down image of the moth while shooting from the side while mounted on the wind tunnel room's floor, or aimed horizontally at



the mirror while fixed to the tunnel. The reversed images obtained by this technique need correcting only if the absolute, not relative, directions of movement are of importance in the study.

Analysis of the recorded tracks is another matter entirely. The minimal requirement for orientation studies is a video deck that is capable of slow-motion and still-frame playback of the recordings. The tracks may be traced by hand from the monitor onto clear acetate sheets for a hard copy, and then analyzed by hand for velocity and angular features of the movement. But clearly this is an extremely slow and tedious method, prohibitively so for large numbers of tracks and experiments.

The advent of microcomputers and X, Y digitizing and plotting devices has made the job of analyzing such tracks not quite as hopeless as it once was. We (TCB) increased our analysis speed and accuracy by taking our acetate-sheet tracings and digitizing them onto a microcomputer by means of a HiPad digitizer pad (Houston Instruments). The coordinates were stored on disc on a Radio Shack TRS-80 microcomputer and analyzed for turning and velocity features by means of a program written especially for our purposes by a number of graduate students and postdoctorates.

We next eliminated the time-consuming step of tracing onto acetate by using a T-bar type of digitizer from Radio Shack whose cursor can be maneuvered on the video screen's surface. As the operator advances the video image frame by frame, he also moves the cursor over the images on the screen and enters the moths' coordinates onto the computer disc with each advance. A hard copy of the track is immediately obtained and examined by having the track plotted by a Radio Shack flatbed plotter. We believe that it is important to have an intimate knowledge of each recording, as gained by the experimenter physically digitizing the moths' positions and entering the coordinates.

Instruments will soon be available that can electronically track and digitize a moving image, and this may be fine for future studies where we already know much about the orientation of flying insects. But for right now, we feel that the time spent over at least one frame-by-frame playback of a track is extremely valuable in producing new ideas and questions about orientation. On the other hand, too much time can be spent, as in hand measuring and hand tracing of tracks. Some degree of computer assistance is definitely needed for this type of research.

## 2.8. Optimizing Stimulus and Response Variables

We will not spend time in this chapter outlining the techniques for optimizing the responsiveness (internal state) of the insects to be tested, or with matters concerning the reliable, consistent emission of pheromone from the source. In these regards, wind tunnels are just like any other type of bioassay, and these techniques are covered in the previous chapter by Baker and Cardé. Suffice it to say, that because wind tunnels are more discriminating, sensitive types of assays,

any inconsistencies in the performance of such techniques would be more likely to result in inconsistencies in the flight responses in a wind tunnel than in other types of assays.

A number of studies have shown, though, that apart from the usual isolation of males from females and optimization of diel responsiveness, best results were achieved when males were acclimated to flight tunnel conditions for at least 15-30 min before testing (Linn and Gaston, 1981; Turgeon and Linn, unpublished). In the case of the nocturnally active cabbage looper moth, acclimation to the tunnel's light intensity was most important. In the absence of acclimation moths seldom flew more than 30 cm from the release point. In addition, "spontaneous" flight activity inside the cages was very high; many of these males could not be tested (Linn and Gaston, 1981). Thus for some insects proper acclimation and careful handling to avoid mechanical and auditory disturbance may be critical to obtaining optimum, consistent results in the wind tunnel.

## III. Uses of Wind Tunnels in Pheromone Research

### 3.1. Pheromone Component Isolation and Identification

Horizontal wind tunnels have proven to be very useful for sex pheromone identifications, either at the stage where gas-liquid chromatography fractions are recombined, or when synthetic compounds purported to be components need to be validated for their ability to attract flying insects all the way to the source. Usually the latter is needed either because field trapping is impossible due to the time of year, or evaluation of the mixture's activity is needed before involving a lot of people in a large, expensive field test.

Hill and Roelofs (1981) identified three chemical components from the salt marsh caterpillar moth, *Estigmene acrea,* using Miller and Roelofs' horizontal flight tunnel to make sure complete activity was present in the recombined fractions and synthetic components ((*Z,Z*)-9,12-octadecadienal, (*Z,Z,Z*)-9,12,15-octadecatrienal, and (*Z,Z*)-3,6-*cis*-9,10-epoxyheneicosadiene. Males fanned their wings and began walking to the epoxide alone, but successful upwind flight to the source 1.5 m away occurred only to binary or tertiary combinations, not to any component presented alone. At the filter paper source, males hovered for long periods, and extended their claspers while wing fanning on the paper. More examples of pheromone identifications which utilized horizontal wind tunnels are Hill et al. (1979, 1982) and Roelofs et al. (1982).

### 3.2. Behavioral Roles of Pheromone Components and Blends

The sex pheromone of the gypsy moth, *Lymantria dispar,* is (+)(*Z*)-7,8-epoxy-2-methyl octadecane (Bierl et al., 1970). Although the (+) enantiomer is produced by females and is very active, addition of the (−) enantiomer significantly reduced captures of males in traps in the field (Cardé et al., 1977; Miller et al., 1977). The reduction in trap capture could not be explained by wing-fanning levels of males exposed to (+) and (±) enantiomers (Yamada et al., 1976). Using their horizontal wind tunnel for choice tests between (+) and (±) disparlure, Miller and Roelofs (1978b) found no significant differences in the number of in-flight orientations to either source. It was only in paired, flight duration tests in which sustained flights in one location were induced by moving the floor pattern beneath the males that significant differences in the behavior began to unfold. Males flew for considerably shorter periods of time to the (+) enantiomer when exposed first to the (−) enantiomer. Under optimal conditions, males exhibited continuous flight for 30 min to the (+) enantiomer alone, and significantly shorter durations were recorded for flights to the (±) mixture.



The cabbage looper moth, *Trichoplusia ni,* was also the subject of a study in a horizontal wind tunnel (Linn and Gaston, 1981) to determine the behavioral effect of a recently identified secondary component, dodecyl acetate. Bjostad et al. (1980) determined that females release a 93:7 ratio of *Z*7-12:OAc and 12:OAc. The males were found to exhibit a sequence of behaviors similar to that described by Baker and Cardé (1979) in wind tunnel studies of *G. molesta,* and this was utilized as a framework for comparing different treatments. The results supported two important points concerning the behavioral function of pheromone components. First, the minor component, 12:OAc, did not elicit any behavior when presented alone. Second, the optimal attraction was obtained to the synthetic blend and dosage ($10^{-3}$ μg of 93:7 ratio *Z*7-12:OAc/12:OAc) that most closely mimicked the natural blend and emission rate. With the optimum blend and dosage, significantly more males flew upwind to the source and spent significantly longer periods within 25 cm of the source.

Choice tests similar to those of Miller and Roelofs for the gypsy moth (1978b) were performed to determine the interaction of the two pheromone components. Two copper disc pheromone emitters were placed upwind and separated by either 12 or 8 cm. Smoke plume visualization showed that pheromone component plumes should have merged 85 or 35 cm downwind, respectively. Males flew upwind in the merged, two-component plume, and never flew in the 12:OAc plume when they reached the "choice" point. They did fly in the *Z*7-12:OAc plume after that point, but not nearly as close to the source as when both components were present on one of the discs as the choice (Fig. 5). Therefore, 12:OAc could not be called a "close-range" component, because it did not elicit upwind flight by itself at close range. Rather, it was clear that the two-components together was a good close-range blend compared to *Z*7-12:OAc, which alone evoked optimal levels of upwind flight from long-range, with or without the addition of 12:OAc.

### 3.3. Response Profiles to Blends and Concentrations

In studies by Baker and Cardé (1979), Baker et al. (1981), and Linn and Roelofs (1981a, 1983a), the flight behavior of individual male *G. molesta* in a horizontal wind tunnel was observed. In some of the studies, the ratio of components and

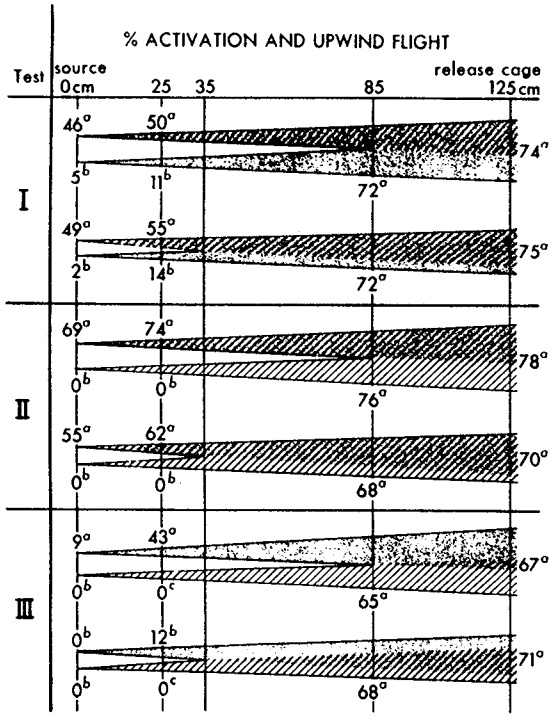


**Figure 5.** Percentage of male *T. ni* flying upwind in choice tests between two sources positioned in a wind tunnel so that a common plume formed 35 or 85 cm downwind of the sources. Time-averaged plumes are drawn to show the presumed boundaries (as determined by $NH_4Cl$ smoke) and the contents of the two plumes (12:OAc = slashed lines; Z7-12:OAc = shading). For each test, $n = 65$ for each pair of sources (from Linn and Gaston, 1981).

the dosage were varied around the natural levels emitted by females (Baker et al., 1980). Males were found to be very sensitive to changes in blend and concentration, with optimal attraction to the source occurring to a narrow range of concentrations of the natural 6% *E*8-12:OAc blend containing 3 to 10% of *Z*8-12:OH. The ability of the experimenter to discriminate among the treatments was clearly increased when moths were required to fly close to the source (Fig. 6) (Linn and Roelofs, 1983; Baker et al., 1981). Males became activated and took flight in nearly equal numbers to a wide range of blends and dosages, but only a narrow range of treatments elicited high levels of completed flights to the source. Increasing the proportion of (*E*) isomer from 6 to 10% (*E*), caused significant arrestment of upwind flight within the plume apparently due to increased turning and decreased linear velocity. This suggested that the (*E*) isomer may function as a "turning" component, which may explain what happens when the "arrestment threshold" is reached (Baker and Roelofs, 1981) either with too



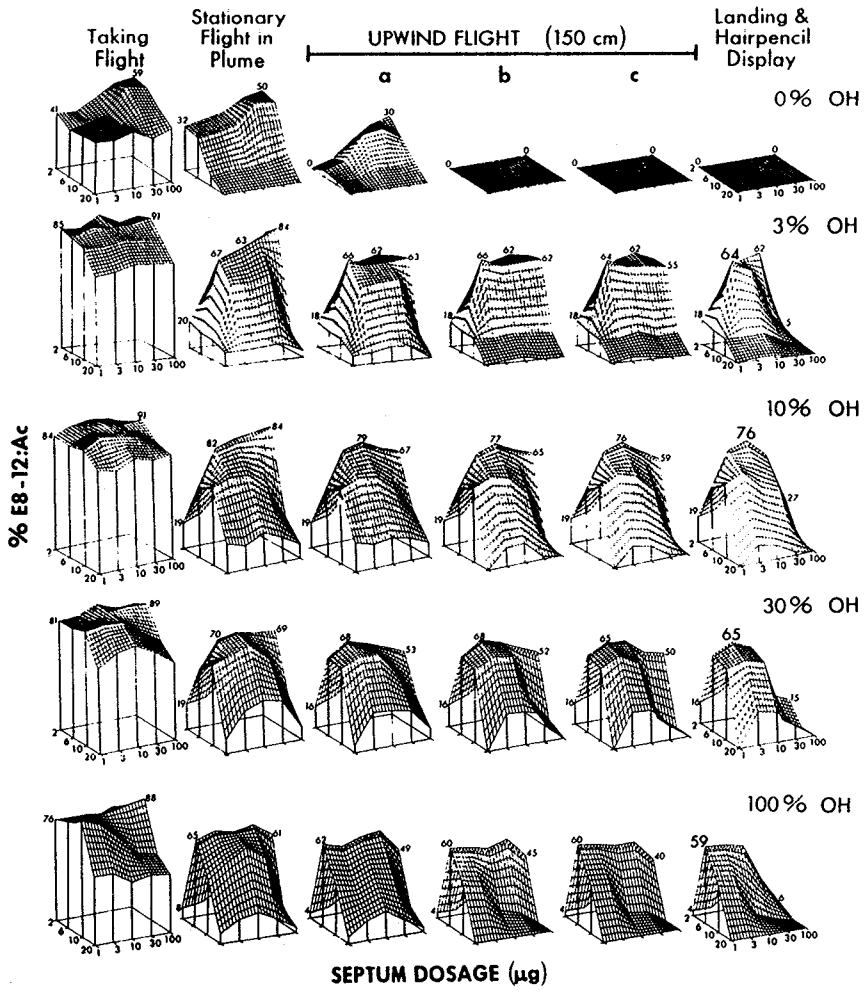


**Figure 6.** Response profiles (percentage response) of male *G. molesta* to 20 blend–dosage combinations of *Z*8- and *E*8-12:OAc and five proportions of *Z*8-12:OH. Response surfaces for each behavior in the sequence are based on the number of responding males to each treatment ($N = 100$). Response values for selected treatments are shown along with peak values for the number of hairpencil displays at the source within each percentage of *Z*8-12:OH (from Linn and Roelofs, 1983).

much (*E*) in the blend or concentrations of the optimal blend that are too high. In the latter case we now know that arrestment is not only a function of lower flight velocity and higher turning frequency, but also the single most important change involved in within-plume arrestment is that the males steer more obliquely across the wind, allowing more lateral drift (Kuenen and Baker, 1982b).

In a subsequent study, Linn and Roelofs (1983) varied all three components. Using hierarchical clustering techniques, they were able to define an area of optimal response around the natural blend ratio and emission rate, partitioned by "threshold" regions affecting specific behaviors in the sequence.

### 3.4. Habituation, Sensory Adaptation, Disruption

Habituation and sensory adaptation to sex pheromone are phenomena that have long interested researchers, but the traditional ways of measuring them have been by means of observing "key" responses (see Baker and Cardé, Chapter 2; Bartell and Shorey, 1969). In two instances, habituation has now been measured in horizontal wind tunnels, and new unexpected results have come from the greater ability of the wind tunnels to monitor subtle behavioral changes.

Kuenen and Baker (1981) found that a pulsed pheromonal preexposure of cabbage looper males did not reduce their wing-fanning reaction in a small chamber over the course of an hour. However, this same regime reduced the percentage of males that flew all the way to the pheromone source in horizontal wind tunnels. Continual preexposure, however, of males in the chamber did not result in as severe a reduction of flight to the source in the tunnel, but nevertheless the decrease was significant compared to those receiving no preexposure whatsoever. Because electroantennogram responses of males preexposed to both pulsed and continual pheromone recovered within minutes after the exposure, habituation rather than sensory adaptation was proposed as the mechanism for reducing flights in the tunnel. This corresponded well to bioassay studies and predictions by Bartell and Lawrence (1977), but contradicted the results of Farkas et al. (1975), who thought pulsed exposure did not affect subsequent responses. Farkas et al.'s responses were measured in small bioassay chambers, and in this respect, their lack of reduced wing-fanning responses was similar to that found by Kuenen and Baker.

Linn and Roelofs (1981) preexposed male *G. molesta* to *E*8-12:OAc and were able to demonstrate a prolonged effect (likely habituation) on the males' pheromone quality perception. After such preexposure, males readily flew all the way to sources containing high percentages of the (*E*) isomer, something they would not do without being habituated to (*E*). A combination of (*E*)-isomer preexposure duration and dosage directly determined the subsequent tendency to fly all the way to sources emitting as high as 20% (*E*).

Rather than preexposing moths to pheromone, simultaneously presenting pheromone point sources in the midst of other pheromone sources has resulted in a greater understanding of the mechanisms or disruption of communication in air permeated with pheromone. Sanders (1982) created a grid-array of nine synthetic spruce budworm (*Choristoneura fumiferana*) pheromone point sources at the upwind end of his horizontal wind tunnel and placed a cage of calling females at various points within that grid. He also created a cloud of uniformly permeated air by the tape-grid-turbulence method of Kennedy et al. (1980, 1981), against which he placed the cage of calling females allowed to create a discrete plume. He found that the uniform cloud was less effective in preventing location of the females than the grid of discrete point sources of comparable overall emission concentration. He hypothesized that two different mechanisms were responsible for reduced location of females under the two regimes. The cloud produced habituation or adaptation which could be overcome by the higher concentration bursts of pheromone in the filamentous plume from the females, whereas the discrete point sources often elicited upwind flight of males, who switched over from the female's plume. The fact that the discrete plumes caused a greater reduction of orientation to females could also be due to a higher overall effective concentration of preexposure caused by the peak concentrations within filaments and the condensed nature of the narrow plumes compared to diffuse clouds.



Disruption of upwind flight to sex pheromone was also performed in a horizontal wind tunnel by Baker and Cardé (1978), only here the disruption was created by ultrasound. Gypsy moth males (*L. dispar*) were allowed to begin flying upwind in a plume of their synthetic sex pheromone and then an ultrasonic burst was generated that caused males to fly out of the plume (Fig. 7). Males experimentally deafened on one side nearly always turned away from the plume toward their deaf side. The wind tunnel provided a nice experimental environment to study the interaction between sex pheromone-stimulated flight and

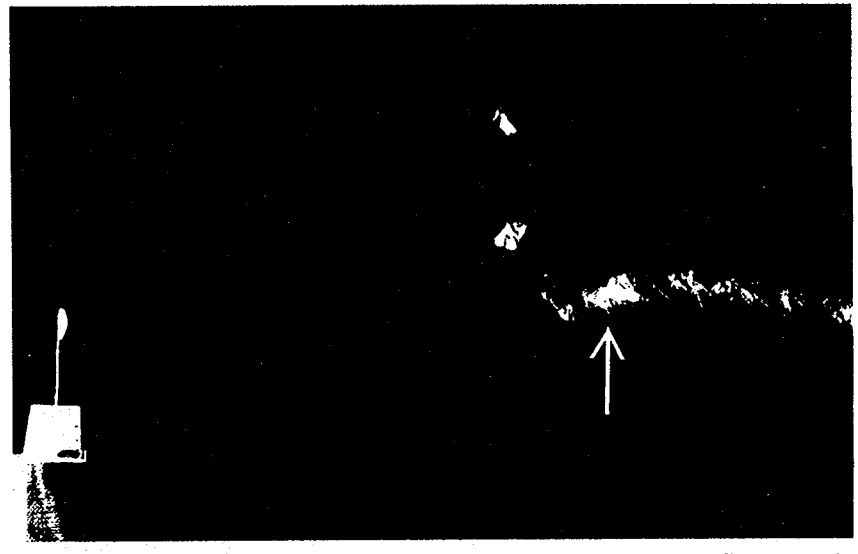

**Figure 7.** Stroboscopically photographed track of male *L. dispar* flying upwind to synthetic sex pheromone emitted from filter paper disc (left). When a burst of ultrasound was generated outside the wind tunnel, the male abruptly changed course and flew out of the plume (from Baker and Cardé, 1978).

evasive flight from ultrasound, which apparently evolved as a defense against predation by bats and overrides mate location. For studies of in-flight responses to sex pheromone by noctuoid moths, sources of ultrasound in wind tunnel rooms such as machinery that "clicks" on and off, or any metal-on-metal impact or friction, should be silenced for greatest experimental consistency.

An elaborate wind tunnel utilizing horizontal and vertical wind was used by Phelan and Miller (1982) to try to disrupt landing on host plants or host plant models by aphids by introducing clouds of the aphids' alarm pheromone over the plants (Fig. 8). The part of the tunnel with vertically moving air was

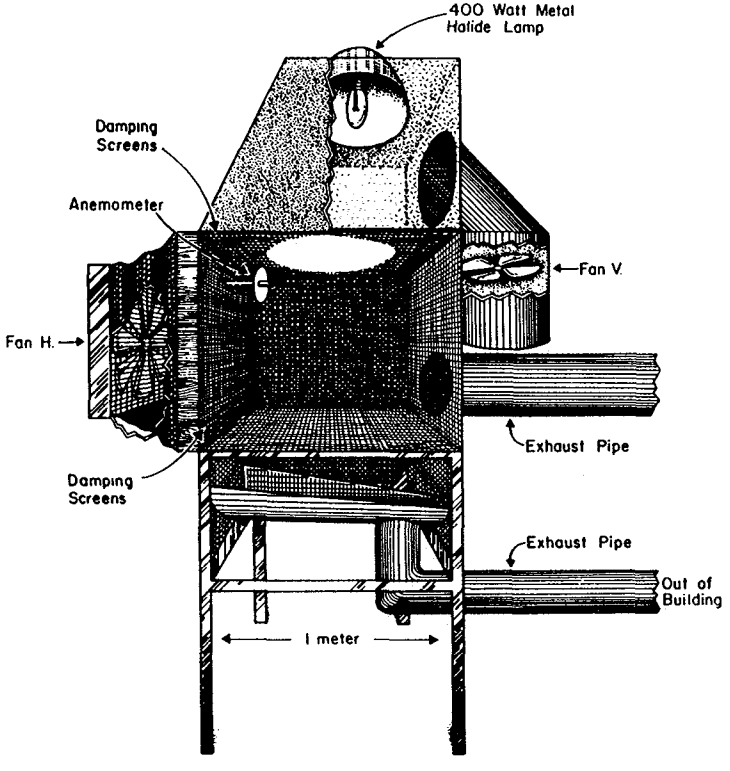


**Figure 8.** Diagram of wind tunnel used by Phelan and Miller (1982) to generate both vertical and horizontal wind to study the responses of flying *Myzus persicae* aphids to their alarm pheromone and various fatty acids. Aphids fly toward light in the ceiling, but the operator counteracts their upward movement with downward-moving air generated by fan at upper right. After aphids have flown for awhile, their tendency to land on a green plate introduced at the bottom is heightened, and potentially repellent or deterrent odors preventing landing and feeding are introduced as a cloud moving horizontally from left to right in bottom third of tunnel by means of second fan at left. Odor is removed by exhaust tube at right.



modeled after that of Kennedy (1966), in which the velocity of downward-moving air could be increased quickly by the experimenter to counteract the aphid's upward flight toward a light in the tunnel's center, placed there to elicit such flight. Phelan and Miller's tunnel was used precisely like Kennedy's, to elicit prolonged dispersal flight toward the light, and then, when the aphids were most responsive to vegetative stimuli (plants), they would tend to fly instead toward a green plate placed below them in the tunnel. Phelan and Miller's tunnel enabled them to introduce over the plate a horizontally moving layer of air permeated with (*E*)-β-farnesene, the aphid's alarm pheromone, and to observe the possible repellent or deterrent effects of this pheromone on landing and feeding. Although they found no reduction in landing to the alarm pheromone, behavioral reductions were observed when the certain fatty acids were presented, and this unusual tunnel demonstrates that two columns of air moving in different directions can be superimposed for pheromone studies of flying insects.

### 3.5. Orientation Studies

Entomologists constructed the first wind tunnels in order to determine the mechanisms of orientation used by insects flying in wind with or without odor. However, it took a long time, along with the growth of the pheromone field, for their use to become widespread. Kennedy (1940) developed several models for steering with respect to wind after constructing a horizontal wind tunnel and observing the odor-free flight of yellow fever mosquitos in wind and in still air, plus visually imposed "drift" through ground pattern movement. Kellogg and Wright (1962) developed a horizontal wind tunnel to study the in-flight maneuvers of insects flying to various odors, and these studies really marked the beginning of quantitative, detailed studies of olfactory-mediated flight. Wright, Kellogg, and colleagues not only developed sophisticated, three-dimensional photographic imaging techniques involving stereoscopic cameras or simultaneous side and plan photography but also demonstrated an awareness of the need to simplify the odor environment. They were the first to present odor as a homogeneous cloud and, using smoke sources, demonstrated repeatedly the highly complex nature of an odor plume. They were able to find a less irritating smoke, ammonium acetate, that could be presented along with an attractant with minimal effect on flying insects. They also demonstrated an awareness of the role of visual wind-drift information on the orientation of insects flying to odors, by experimenting with projected, moving floor patterns and through experiments in which wind was absent. Finally, they were the first to use vertically moving air to study the nonanemotactic, odor-mediated horizontal movements of insects flying in choice chambers of odor-permeated columns of air. A pheromone orientation study by Traynier (1968) in a horizontal wind tunnel developed earlier for the host-odor work now set the stage for the growth of wind tunnels in pheromone research.

Farkas and Shorey (1972) used a horizontal tunnel to create a pheromone

plume in wind, induce males to fly within a pheromone plume, and then stop the wind in order to prove, so they believed, that only chemotaxis was used for pheromone source location in wind. Kennedy and Marsh (1974) used their tunnel to negate neatly part of this hypothesis, that anemotaxis was not used, by demonstrating the optomotor-anemotactic response to a moving floor pattern. But they did not address the part of Farkas and Shorey's hypothesis concerning a chemotactic mechanism for maintaining contact laterally with the plume. Support for a chemotactic mechanism superimposed on anemotaxis came from wind tunnel studies by Baker and Kuenen (1982) and Kuenen and Baker (1983) in which they repeated and extended Farkas and Shorey's experiment of stopping the wind and observing whether males could locate the source. However,

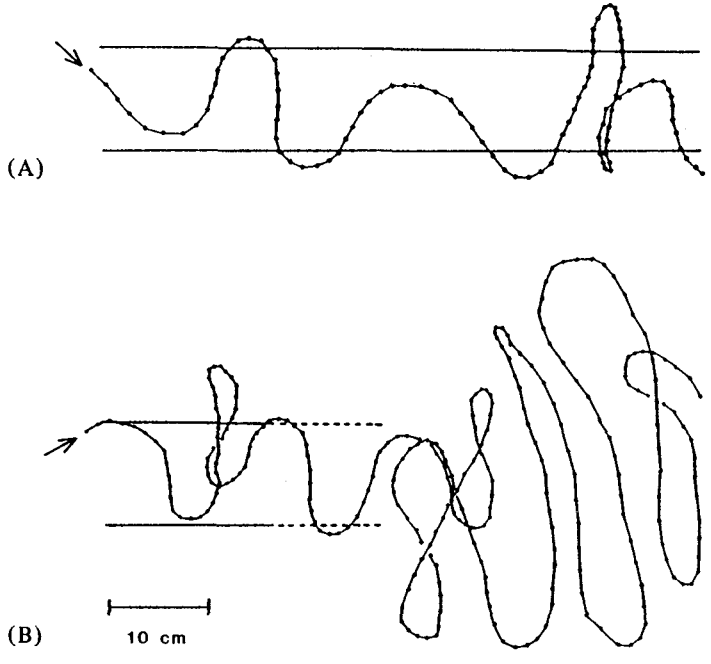


**Figure 9.** Plan view (from above) of male Oriental fruit moths flying through a 65-cm-long section wind tunnel. Dots represent each 1/60 sec of elapsed time. Solid straight lines denote the boundaries of the time-averaged pheromone plume as visualized by smoke. The pheromone source was 105 cm from the right edge in each illustration, and the wind, before stoppage, was from the right. Arrows denote the direction of flight in each track. (A) The wind stopped 0.25 sec before the male entered the field of view, and the male continued to the septum. (B) The pheromone plume was removed, and the wind stopped just as the male entered the field of view; the approximate up-tunnel end of the plume (by smoke visualization) is indicated by the dashed, straight lines; on entering clean air, the male's track changed significantly, with a closest approach to the septum of only 90 cm (from Baker and Kuenen, 1982).



they made more detailed measurements of the flight tracks before and after wind stoppage, discovering that the tracks up the plume with no wind remained quite similar to those in wind. The definitive experiment demonstrating a chemotactic program of zigzagging in zero wind was one in which the pheromone source was removed and the wind then was stopped to create a truncated plume in zero wind. Males zigzagging along the plume in still air changed the amplitude, frequency, and angle of their zigzags upon entering clean air as they flew out the end of the plume, showing that their movements were in fact pheromone mediated, independent of anemotaxis (Fig. 9).

Later it was found that in-flight experience with wind-induced drift tended to polarize the zigzags of males in the toward-source direction, even when wind was later stopped when males were part way to the source. Males released into a stationary plume in zero wind performed their zigzag flight movements, but the zigzags meandered with no consistent directional component as they had in the cases where the wind was stopped after they had launched themselves in the plume (Fig. 10) (Baker et al., 1984). This was more evidence for a self-steered program of zigzagging mediated by pheromone concentration.

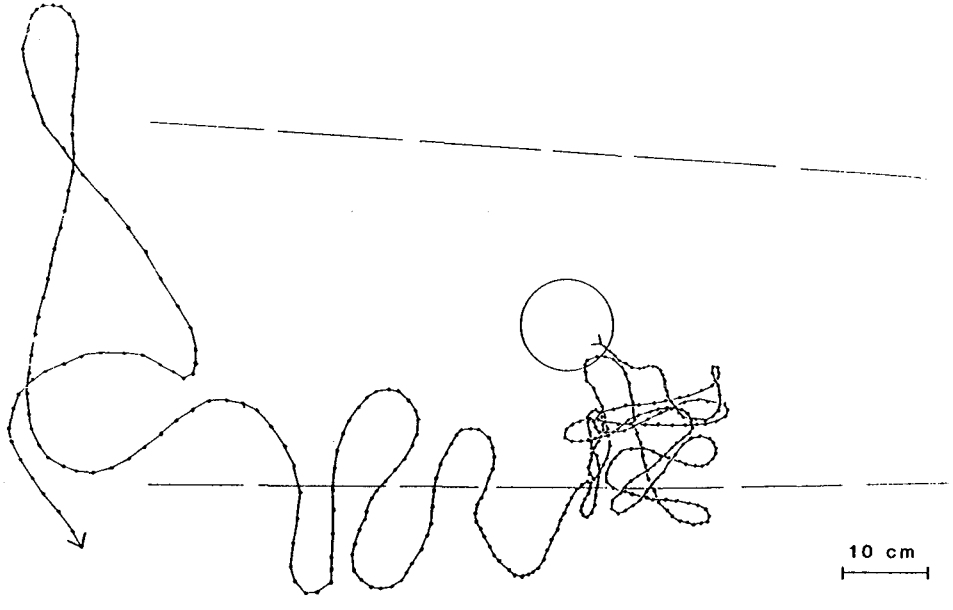


**Figure 10.** Top view of *G. molesta* male, introduced into plume in zero wind, taking flight from release cage (circle) 5 sec after wind had stopped. Zigzagging flight began immediately in the plume, but meandered in the down-tunnel direction (source is to right, 1.5 m away from release cage). With no pheromone in zero wind, males seldom take off, but when they do, no such zigzagging occurs. Dashed, straight lines indicate plume boundaries 5 sec after wind stoppage, and dots on moth's track indicate 1/60 sec elapsed time (from Baker et al., 1984).

Horizontal wind tunnels provided even more support for a chemotactic program of zigzagging. Using a tunnel uniformly permeated with a cloud of pheromone or with only a side corridor-cloud, Kennedy et al. (1980, 1981) showed that for male *Adoxophyes orana* casting in clean air, upon encountering the cloud males decreased the width and increased the frequency of their zigzagging. However, after several seconds of this tonic stimulation, the reversals became wide again and the surge of upwind progress that had accompanied the narrow zigzags now ceased and the moths became arrested once more in wide casting flight.

Their hypothesis was that adaptation to the constant stimulus caused the narrow reversals to wane, because the superpositioning of a point source plume of pheromone in the cloud readily elicited rapid upwind zigzagging to the source. This demonstrated that the cloud's concentration had not been too high, but rather adaptation may have developed, which the phasic arrival of higher peak concentrations then overcame.

These results were supported with another species, *G. molesta,* using a similar set of pheromone clouds in a horizontal tunnel (Willis and Baker, 1984). Males casting in clean air briefly narrowed their zigzags upon entering a homogeneous cloud of pheromone, but rapid upwind zigzagging was elicited at the "edge" of a side corridor of pheromone. Evidence came from another experiment that it was the phasic stimulation from the non-uniform mixing of air and pheromone at the edge that initiated the self-steered program, as well as the males' excursions and incursions from the corridor that perpetuated turning responses along the corridor's edge. The tunnel was rotated 90° so that the side corridor now became a corridor filling the bottom half of the tunnel, with clean air above. The males zigzagged up the tunnel at the horizontal edge of the bottom corridor in a manner similar to males zigzagging up the vertical edge of the side corridor (Fig. 11) (Willis and Baker, 1984).

The mechanisms of counterturning in walking insects that may be analogous to zigzagging in flying insects has also been studied in horizontal wind tunnels (Tobin, 1981; Tobin et al., 1981). Using a plume of periplanone-B positioned 2 cm off the ground at the upwind end of a 2.4 × 1.2 × 0.6 m tunnel, the tracks of *Periplaneta americana* were recorded from above. The males walked upwind in the plume by means of a combination of anemotaxis, endogenously triggered counterturns, and counterturns back into the plume triggered by a decrease in concentration at the boundary of the time-averaged plume. Thus again an integrated system of chemically mediated movements and anemotaxis was implicated, this time for a walking insect.

New advances in the understanding of the orientation of insects flying in response to pheromone will be gained through the use of wind tunnels in which flight tracks are recorded and analyzed in three dimensions. R. H. Wright and his colleagues in the later 1950s and early 1960s realized the importance of three-dimensional analysis, although until recently technical and data-handling procedures have been major deterrents to quantitative rather than descriptive



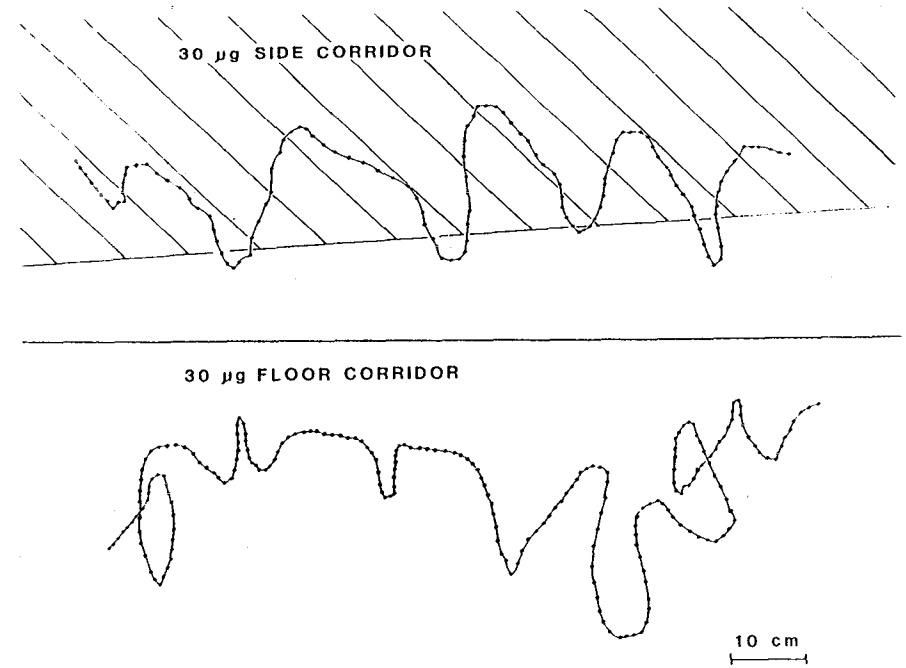


**Figure 11.** (Top): Top view of male *G. molesta* flying along the vertical edge of a side corridor (hatched lines) of air moving left to right uniformly permeated with pheromone emitted from a grid of rubber septa each loaded with 30 µg. (Bottom): Same cloud, except now it has been rotated so it occupies bottom half of tunnel along the floor. Male zigzags upwind left to right, maintaining an altitude consistent with the horizontal edge's location. Distance between dots denotes 1/60 sec elapsed time (from Willis and Baker, 1984).

studies. Advances will be made on the vertical components of flight movements and how they are integrated with the horizontal zigzagging components. Height control is obviously integrated into the zigzagging component, but how this is accomplished remains to be discovered. Certainly more ingenious studies involving wind tunnels will need to be performed such as C. T. David's (1982b) (Fig. 12) in which a visual surround of "barber pole" stripes was made to appear to move past flying insects. In this case the *Drosophila* adult was allowed to fly in a plume of banana odor, and the angular velocity of apparent image movement, the frequency, and the pitch of the stripes in the barber pole cylinder were varied systematically to determine the effect upon upwind flight speed. The advantage of the moving visual surround is that contradictory visual information from stationary objects is absent, unlike when moving floor or ceiling patterns are used. In order to relate wind tunnel effects to those in the field, further adaptations will have to be made on existing tunnels once the simpler questions are answered. Wind in the field can shift direction and velocity very

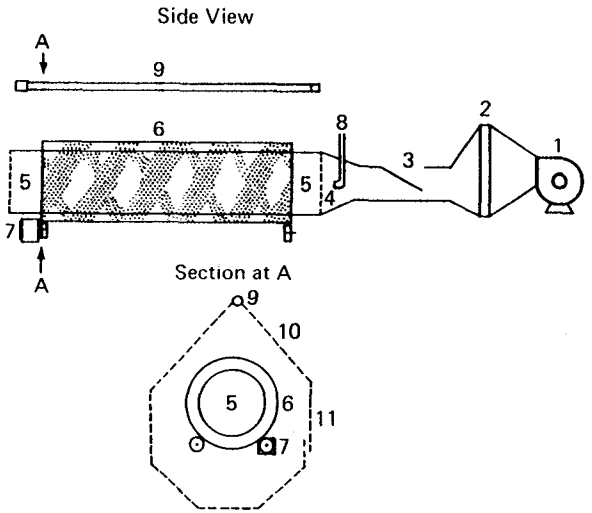


**Figure 12.** "Barber's pole" wind tunnel of David (1982b), with cylindrical cellulose acetate working section 1.4 m long X 0.25 m diameter. 1, fan; 2, charcoal filter; 3, wind speed control valve; 4, brushed nylon air-smoothing screen; 5, working section; 6, outer cylinder with helical pattern; 7, stepper motor to rotate cylinder; 8, tube for introducing attractant odors; 9, fluorescent light; 10, brushed nylon screen; 11, muslin screen.

rapidly (David et al., 1982, 1983), and tunnels will need to have some of these features incorporated into their design.

Certainly the use of wind tunnels in pheromone research will continue to expand and, as this is done, further understanding of odor perception and orientation will be gained. In the meantime, wind tunnels will retain their utility to entomologists as useful tools in which to test potential field formulations of pheromones.

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Chapter 4

# Field Trapping with Attractants: Methods and Interpretation

*Ring T. Cardé* [1] *and J. S. Elkinton* [1]

## I. Introduction

Numerous insects communicate with pheromones that induce attraction or directed movement toward the pheromone source. Documentation of orientation to such chemicals and their use to monitor insect pests typically require development of traps and a trapping protocol. Field trapping also has served as the bioassay in the characterization and identification of many pheromones. Among the attributes sought in a trapping system are low cost, sensitivity to and specificity for the target species and user convenience.

Our review will not attempt to summarize all the approaches tested, nor will it suggest that there are 'generic' solutions to the development of a trapping system. Indeed, the behavioral reactions, nonchemical cues, and the trapping system all potentially differ from one species to another. Even though each species must be examined individually, it will be useful to describe some of the successful approaches and define areas where principles remain elusive.

## II. Verification of Pheromone Identity

Trapping experiments are commonly used to compare the behavioral activity of synthetic compounds to natural pheromone, typically emitted by caged insects. If the magnitude of trap catch in the synthetic pheromone-baited traps matches or exceeds the catch evoked by the natural source, then the identification of the pheromone is assumed to be correct and complete with certain reservations.

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