

BEYOND DISCOVERY[®]

THE PATH FROM RESEARCH TO HUMAN BENEFIT

INSECT PHEROMONES MASTERING COMMUNICATION TO CONTROL PESTS

With pride and satisfaction, a farmer eyes the glistening red globes of the tomatoes he has just harvested. A few years ago he had been ready to abandon tomato farming because the destructive tomato pinworm was ruining as much as two-thirds of his crop. Despite his zealous use of insecticides, these worms would tunnel into his tomatoes, leaving telltale pinholes and unsightly black blotches that destroyed the crop's marketability.

But now, spiraling around the stems of many of his tomato plants, are dispensers of a potent chemical guardian. These hollow plastic tubes emit a chemical that interferes with the ability of the pinworm moth to find mates, and breaks the cycle of infestation. Thanks to pinworm birth control, the farmer was able to bring three-quarters of his crop to market this year.

The farmer's success story is the result of more than a century's worth of investigation by entomologists and chemists bent on solving such mysteries as how a moth lures mates from far and wide, or how an ant lets her whole colony know the location of a food source. Scientists looking for new methods of pest management then expanded on this basic research. As a result, farmers of many kinds of crops now have highly effective weapons for their perennial battle against insect pests.

The new weapons use chemical substances generated by the insects themselves. Unlike conventional pesticides, the chemicals, known as pheromones, do not damage other animals, nor

do they pose health risks to people. Pheromones specifically disrupt the reproductive cycle of harmful insects. They also can be used to lure the pests into traps that help farmers track insect population growth and stages of development. In this way, farmers can reduce the amount of insecticide they need—spraying only when the insects are in a vulnerable stage or when their numbers exceed certain levels.

The following article explores the trail of research that ultimately led to the design of pheromone-based pest management. The story behind the measures that are beginning to transform agriculture provides a dramatic example of how science works, by illustrating how basic research produces knowledge that can lead to practical results of human benefit.

A Seductive Scent

One May morning in the 1870s, the French naturalist Jean-Henri Fabre was pleased when a female great peacock moth emerged from a cocoon on a table in his laboratory-study. He put her under a wire-gauze bell-jar



A plastic pheromone dispenser spirals around the stem of a tomato plant. The dispenser is intended to prevent damage to tomatoes and other crops infested by tomato pinworms by disrupting their mating. (photo courtesy of Maggie Sliker)



Fabre concluded that male great peacock moths, like the one illustrated above, are attracted by an odor released by female peacock moths. (Photo courtesy Dr. Thomas Eisner, from Röseler, August Johann, 1746. Insekten Belustigung. Johann Joseph Fleischmann, publisher, Nürnberg.)

and left her to spread her wings to dry. Around nine o'clock that evening Fabre's pleasure turned to amazement as dozens of male great peacocks, with striking eyespots on wings as much as 6 inches across, floated in through the open doors and windows of the house. "Coming from every direction and appraised I know not how," Fabre wrote, "here are forty lovers eager to pay their respects to the marriageable bride born that morning amid the mysteries of my study." Over the following week Fabre caught more than 150 males. No matter where in the house he moved the female, the male moths made directly for her. What was drawing them, he wondered?

Over the next several years Fabre carried out painstaking experiments to learn the moths' secret. Eventually, he concluded that, even though no human nose could detect it, the female moth must release an odor that is powerfully attractive to the opposite sex of her species.

New York entomologist Joseph A. Lintner came to the same conclusion a short time later when he created a spectacle by placing a female spicebush silk moth on his office window sill. Within minutes a crowd of large brown male spicebush silk moths, with wingspans of up to 4 inches, began making their way toward the window sill. Fifty male moths were drawn to the female, in turn attracting a large crowd of amazed people on the sidewalk below.

But Lintner took Fabre's musings further. He not only assumed that the female releases a chemical substance to which the male is exquisitely sensitive; he foresaw that people might be able to harness such chemicals as a means to control insect pests. Noting the chemicals' "irresistible and far-reaching force," Lintner asked, "Cannot chemistry come to the aid of the economic entomologist in furnishing at moderate cost the odorous substances needed?"

Finding Hidden Chemicals

Given the techniques available to chemists at the end of the nineteenth century and the beginning of the twentieth, the mysterious substances remained elusive. Then, in the 1930s, a persistent German chemist at the Kaiser Wilhelm Institute for Biochemistry in Germany decided to tackle the problem.

Adolph Butenandt had already made a name for himself by discovering the human sex hormones estrone, testosterone, and progesterone. Branching out into a different arena, he aimed to discover the substance that female moths use to attract males. Butenandt thought the work would open up an entirely new field of research, and like Lintner, he envisioned this research creating a new way to control insect pests.

Butenandt pursued his goal throughout the years of Hitler's regime, World War II, and Germany's long recovery after the war. The task was difficult. He began by snipping off the abdominal tips of virgin female silkworm moths and grinding them up. Then, using analytical chemistry techniques, he separated the moth slurry into various extracts and tested each one on male silkworm moths. The domesticated silkworm moth has lost its ability to fly. But the male will flutter his wings when excited by a nearby female—and when fooled by one of Butenandt's extracts.

Working over the course of nearly three decades, Butenandt ground up about half a million female silkworm moths in his quest to identify their alluring perfume. At last in 1959, he announced success: The



Adolf Butenandt at work in his laboratory. (Archiv zur Geschichte der Max-Planck-Gesellschaft, Berlin-Dahlem)



substance was a kind of alcohol that Butenandt christened bombykol, after the moth's Latin name, *Bombyx mori*.

That same year German biochemist Peter Karlson and Swiss entomologist Martin Lüscher introduced the term “pheromone” (Greek for “carrier of excitement”). The researchers were working on identifying the chemicals that maintain the elaborate caste system of termites, and they coined the word to describe a substance that an animal gives off to trigger a specific behavioral or developmental reaction in another member of the same species.

Butenandt's successful characterization of an insect pheromone inspired others to undertake the tedious effort required to seek out the pheromones made by other insects.

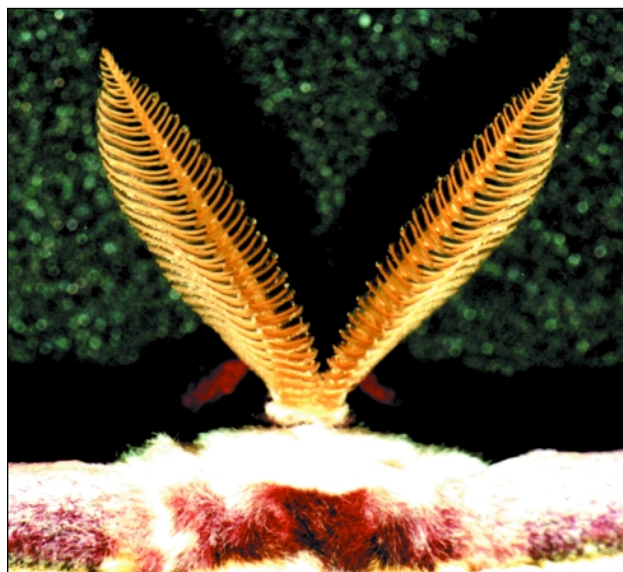
Behavioral assays, such as the wing-fluttering response used by Butenandt, remained key to identification of pheromones throughout the 1960s. For example, in 1961, Colin G. Butler at the Rothamsted Experimental Station in London used a behavioral assay to identify a pheromone that regulates the physiological development of an insect, specifically the honey bee. Scientists knew that queen bees emit a substance that stops worker bees from rearing other queens. Butler tested mandibular gland secretions to determine whether they inhibited worker bees from constructing specialized queen rearing chambers. Through this behavioral assay he identified a pheromone produced by the queen bee that would not only suppress the rearing of queens, but also halt the development of the worker bees' ovaries.

Scientists quickly turned their attention from studying beneficial insects, such as the silk moth and the honey bee, to investigating pestiferous insects. Using behavioral assays, researchers identified the pheromones used as attractants by the black carpet beetle, the California 5-spined engraver beetle, the western pine beetle, the cabbage looper moth, and a leaf-cutting ant, among others.

Many scientists were frustrated in their search for pheromones. Further progress would depend on the development of new methods and approaches. Was there, for example, another more general test for pheromones?

Researchers had been pondering the question for some time. As early as 1953, Peter Karlson had suggested to his neighbor, biologist Dietrich Schneider that he use his expertise in electrophysiology to develop an electrical means of detecting pheromones.

Schneider took up the challenge. At the time biologists suspected that the large furry antennae



Close up of adult luna moth male antennae showing the feathery appearance. Scientists can detect the burst of electrical activity produced by antennae such as these when they are exposed to pheromones. (photo courtesy of Jacalyn Loyd Goetz)

of many moth species enabled them to detect pheromone molecules in the air. Schneider came up with the brilliant idea that he could use the antennae as “sniffers” for pheromones, reasoning that they might respond to a relevant chemical with a small burst of electrical activity, a characteristic response of nerve cells when stimulated.

Schneider removed an antenna from a male silk moth, bathed it in a saline solution to keep its cells fresh, and lodged it between two electrodes, devices that sense electrical activity. He then gave the antenna a whiff of air that swept past an extract containing bombykol (graciously provided by Butenandt's lab). The biologist was thrilled to note a peak of electrical activity in the antenna corresponding to exposure to the extract. Schneider named this odor-prompted electrical response of an insect antenna an “electroantennogram” (EAG). He reported his technique in 1957.

A Vanishing Act

Despite these successes, pheromone research still proved frustrating for many. Extracts that were highly attractive to male insects when left in their crude form mysteriously lost their allure when purified into their various components. And in many cases, synthetic compounds that passed the pheromone test in the lab failed abysmally to attract male moths in the field.

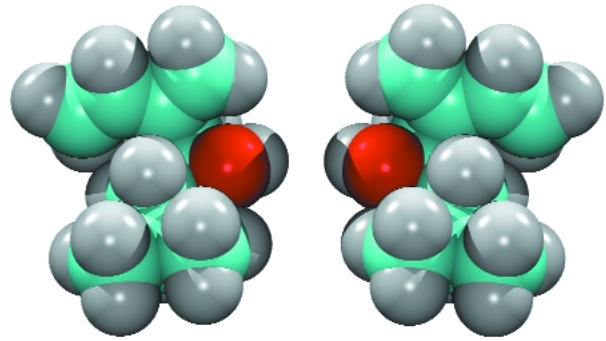


Clues to why this was so began surfacing in the mid 1960s in the laboratory of chemist Robert Silverstein, then at Stanford Research Institute in California. Silverstein was collaborating with entomologist David Wood at the University of California, Berkeley, to identify the pheromone that spurs both male and female bark beetles (specifically, *Ips confusus*) to colonize specific pine trees en masse. As the beetles tunnel through the bark of trees this “aggregation” pheromone draws ever larger crowds of beetles to overwhelm the tree’s defenses, such as the resin that oozes from wounds.

Wood determined that the pheromone lurked in the sawdust-like mixture of wood borings and fecal pellets the beetle expels out of its excavation tunnel. He sent almost ten pounds of this potent mixture, known as “frass,” to Silverstein, who set out to analyze its components.

Silverstein and Wood assessed which portion of the frass contained the pheromone by seeing which extract spurred beetles to walk upwind toward it. When they broke down that attractive portion into its three main chemical compounds, they found that each individually had no effect on the beetles; however, when they combined two of the components, the attractiveness to beetles was restored in laboratory tests.

Encouraged, Silverstein and Wood tested the two-component mixtures out in the field. In a surprising



The chemical structure of two forms of Ipsdienol, a bark beetle pheromone. Molecules of this type are called chiral because they are mirror images of each other. Even if you flip or turn one form they are not identical, which enables some insects to distinguish between the two forms. S stands for sinister or left and R stands for rectus or right. (Courtesy of Mark Midland, University of California, Riverside)

development, their tests failed to attract the intended bark beetle but instead attracted another species, *Ips latidens*. But when they re-combined all three components and used that mixture as a lure, they trapped

Timeline

This timeline outlines the chain of events have led to use of pheromones in pest management.

1870s

French naturalist Jean-Henri Fabre notices a female peacock moth is able to attract 150 male peacock moths from miles away.

1957

German biologist Dietrich Schneider develops the electroantennogram (EAG), a method for using the antenna of a moth to detect pheromones electrically.

1959

German biochemist Peter Karlson and Swiss entomologist Martin Lüscher coin the term “pheromone” to describe a compound an animal gives off that triggers a specific behavioral or developmental reaction in a member of the same species.

1960s

Pheromone researchers begin to use gas chromatography, mass spectrometry, and nuclear magnetic resonance along with EAG to identify insect pheromones.

1870s

New York entomologist Joseph A. Lintner suggests the chemical scents emitted by insects could be used to control insect pests.

1959

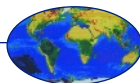
German chemist Adolf Butenandt isolates and characterizes the first insect pheromone, that of the domestic silkworm moth.

1960

U.S. Department of Agriculture chemist Morton Beroza reports his idea of using sex pheromones to disrupt insect mating.

1961

Colin G. Butler identifies the pheromone of the honey bee, the first pheromone that regulates the development of an insect.



as many of the intended bark beetles as they did when using live beetles as bait. This mixture was no longer attractive to *Ips latidens*, demonstrating an interruption of attraction response by the addition of the third component.

The findings were a revelation to pheromone researchers. Although the notion of testing every fraction of a mixture in combination with every other fraction made pheromone research more complex, it also helped to explain many failures of the past. During the 1970s, several scientists reanalyzed the pheromones that had fared well in the laboratory yet failed in the field. Often they discovered that the addition of one or two more components to these single compounds improved field test results tremendously.

Amazingly the missing component sometimes had the same assortment of atoms joined to give the same chemical structure, but the shape was its mirror image. In other cases the mirror image of the pheromone had an opposite effect. In Japanese beetles, for example, contamination of its sex pheromone with just 1 percent of its mirror image compound dramatically diminishes its attractiveness. Researchers also discovered that for many insects, if the pheromone components are not combined in the proper proportions, the mixture loses its attractiveness—or attracts a different species.

New Technology

Throughout the 1960s and 1970s technical improvements dramatically quickened the pace and productivity of pheromone research. Among the improvements was the use of three techniques known as gas chromatography, mass spectrometry, and nuclear magnetic resonance. These techniques were used in combination with the electroantennogram (EAG). Gas chromatography is a technique for separating components in a vapor based on how quickly they travel through a column containing an absorbent material. Mass and nuclear magnetic resonance spectrometers are used to identify chemical compositions.

In 1970, several groups of researchers were working on identifying the pheromone of the codling moth, an apple orchard pest. Despite a massive effort to analyze the contents of around half a million glands from female moths, the pheromone remained elusive. Then in 1971, Wendell Roelofs and his colleagues at Cornell University made the identification by taking a novel shortcut. First, using gas chromatography, they separated extracts from the glands into fractions. They then tested each fraction with the EAG to determine which fraction contained the pheromone. At this point researchers would

1966

Chemist Robert Silverstein and entomologist David Wood demonstrate that all three components of the bark beetle's pheromone blend are required to attract the beetles—a phenomenon known as synergism.

1970s

British biologist John Kennedy develops the wind tunnel assay.

1971

Wendell Roelofs uses EAG as an analytical tool to identify the codling moth pheromone.

1980

Pheromones are used in more than a million traps to capture more than four billion beetles, curbing an epidemic of bark beetles in the forests of Norway and Sweden.

1967

Entomologist Harry Shorey shows that pheromones can be used to disrupt the mating of cabbage looper moths in the field.

1970s

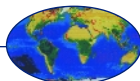
Farmers begin to use pheromones for monitoring insect pests in order to reduce insecticide use.

1978

First pheromone is registered in the United States for commercial use in mating disruption—against the pink bollworm on cotton.

1990s

Pheromones used for mating disruption effectively help curb insect damage in stone-pitted fruit orchards, and tomato, rice, cotton, and grape fields.



typically use spectral analysis to isolate the pheromone in the fraction, a slow and labor-intensive process. Roelofs and his colleagues sped up this step by testing a library of all possible mono-unsaturated compounds related to known pheromones. They used the EAG of the codling moth antenna to test each of the chemicals in the library. As they got closer to the actual structure the EAG response increased, peaking in response to two compounds, each containing a different double bond. This information led to the correct prediction that the pheromone compound contained both double bonds in one compound. When the Cornell team announced its identification of the codling moth pheromone, the news was met with disbelief. Only after the results were confirmed using conventional methods did the new approach gain acceptance.

All these techniques, and others, were used in various combinations. Gas chromatography was linked to mass spectrometry so researchers could both separate and identify the pheromone components in their mixtures. By coupling gas chromatography to the EAG, researchers could detect which components in their insect preparations prompted an electrical response. And the development of capillary gas chromatography allowed researchers to separate compounds that could not be resolved by previous methods.

Along with the physical tests researchers now needed new behavioral assays to determine which chemicals were actually part of the pheromone signal. In the 1930s, English zoologist John Kennedy had developed a special wind tunnel to study how insects orient and move upwind. By the 1970s, Kennedy became curious as to how insects track a sex pheromone back to its source. He used his wind tunnel—a clear plastic tube in which an odor is released at one end and blown through the tunnel by a fan. He knew from previous work on the yellow fever mosquito that flying insects use visual cues for guidance as they follow the trail of an attractant. Kennedy therefore equipped the tunnel with a moving patterned floor to simulate the changing territory beneath the insects' flight path. He found that moths use the same visual information when tracking pheromones.

Since the 1970s, Kennedy's wind tunnel and similar devices have proven invaluable to researchers trying to test candidate pheromones. If an insect is stimulated to fly upwind in the tunnel toward the chemical scent, then researchers usually conclude that the scent is indeed a pheromone. The wind tunnel also allows researchers to test various mixtures of



In the laboratory, entomologists prepare a flight tunnel experiment. Insects fly upwind when stimulated by the scent of a pheromone and use the moving spots on the floor as visual cues. (Photo courtesy of Scott Bauer/Agriculture Research Service Photo Unit)

chemicals at different release rates to find the optimum lure for field traps.

Thanks to a combination of all of these techniques the quantities of insects that researchers need to pinpoint new pheromones has dropped dramatically.

A Chemical Language

The exquisite specificity of insects' chemical language is not surprising, considering that it is often the only means insects have for finding each other. Researchers have now broken the code for the pheromone communication of more than 1,600 insects. In so doing they have found that pheromones serve many more purposes than simply attracting mates.

For example, queen bees emit a pheromone that affects the development of worker bees, and ants use



pheromones to recruit nestmates to a food source (which explains trails of ants at a picnic or in a kitchen). When laying their eggs, some flies, moths, and beetles use certain pheromones to repel insects of the same and competing species, thereby protecting their progeny from competition for resources. Other insects, such as aphids, give off alarm pheromones that urge neighboring aphids to flee from nearby predators. Honey bees use alarm pheromones to recruit nestmates to sting and pursue intruders. Some male moths use aphrodisiac pheromones to entice females to mate with them.

For the most part, insects' responses are automatic rather than resulting from the analysis of many sensory inputs. For many insects, love truly is blind. Male moths can often be seen trying to mate with plastic tubes containing the sex pheromones for their species, and one scientist who worked with the gypsy moth sex pheromone reported that he persistently drew male gypsy moths to him after inadvertently absorbing the substance on his skin and clothing.

Pheromones can be highly effective at low doses and great distances. Detection of just 30 pheromone molecules can prompt a response in cockroaches. In less than five days a single caged female pine sawfly attracted more than 11,000 males from the field. From a pest management standpoint, pheromones are a critical key to manipulating insect behaviors.

Pheromone Birth Control

In 1960, chemist Morton Beroza of the U.S. Department of Agriculture suggested using sex pheromones to jam the insect long-distance mating communication system. He reasoned that if an agricultural area is blanketed with many sources emitting the sex pheromones of a pest species, some or even most males of the species would follow the false trails. Instead of being happily united with appropriate females and producing a new generation of insects, the males would die as confused bachelors.

In 1967, entomologist Harry Shorey at the University of California, Riverside, followed Beroza's lead and was the first to show that pheromones could be used to disrupt the mating of an insect—in this case cabbage looper moths in the field. Precisely how the pheromones do this job is not known. Researchers speculate that the high loads of

pheromone not only confuse male insects, but also camouflage a female's pheromone emission and cause some males to tune out all sources of the pheromone.

Mating disruption has been a boon for farmers whose crops were plagued by insects that had become immune to broad-spectrum insecticides. In Mexico—where nearly half the tomatoes consumed in the United States are grown—the pinworm once regularly destroyed more than three-quarters of a year's crop. Then growers began broadcasting the pinworm's sex pheromone throughout tomato fields by means of plastic tubes attached to stakes or tomato stems or foliage.

Results were dramatic. According to one study only about 4 percent of the females were able to mate under these conditions. In neighboring untreated fields, by contrast, 50 percent of the female pinworms mated. Moreover, only about 30 percent of a year's growth of tomatoes was lost to pinworm damage in crops treated with mating disruption and other integrated pest management (IPM) measures, such as the use of an insecticide produced by bacteria. Neighboring fields treated with conventional insecticides lost as much as 80 percent of the tomato crop. The IPM approach was also less costly than the conventional approach. With such convincing findings, most growers of tomatoes in Mexico have adopted IPM programs using mating disruption for pinworm control.

Mating disruption has been beneficial for other crops as well. A pilot program to control the codling moth in apple and pear orchards in Oregon, Washington, and California reduced pesticide use by 80 percent and caused damage by this insect to be lower than in conventionally treated orchards. The pilot program's success boosted the use of codling moth mating disruption in Washington apple orchards from 1,000 acres in 1991 to more than 100,000 acres in 2000—about half the apple acreage in the state. Farmers are also using mating disruption extensively to control cotton pests in Egypt and the United States, rice pests in Spain, peach and nectarine pests in Australia and North America, and grape pests in Europe.

An Alluring Trap

Pheromones also are used as the bait in traps for pests. Such a mass trapping of bark beetles was credited with saving Norwegian and Swedish forests from a devastating epidemic of the beetles in 1980.



Researchers also are experimenting with using pheromones as lures for devices designed to spread disease in targeted insect populations or to render the insects sterile; in these cases, the temporarily trapped insects are released to affect the wild population.

Farmers now use pheromones to help determine when to spray various agricultural crops with pesticides. The timing can be crucial; to be effective against the codling moth, for example, the orchards must be sprayed during a critical period after the caterpillars have hatched but before they burrow into the fruit. Researchers have developed sticky codling moth traps baited with the moth's pheromone. Farmers check the traps in their orchards each day to detect when the number of moths peaks. After a certain number of sufficiently warm days, during which time the eggs are hatching, farmers spray the orchards with insecticide so that most of it lands on newly emerging caterpillars.

Some farmers rely on pheromone monitoring traps to spray their crops only when pests approach damaging levels, a practice that reduces insecticide levels in the environment. Pheromone monitoring has proven particularly valuable in setting off early alarms to indicate that certain voracious pests have invaded new territory and require pest control measures. When an area of boll weevil infestation is detected, for example, farmers

can destroy the weevil using insecticides, plant destruction, or pheromone traps. These efforts have been immensely successful at eradicating the cotton boll weevil from much of the southeastern cotton belt.

An added benefit of limiting insecticide spraying only to infested areas is that many beneficial insects are saved from destruction. The beneficial insects effectively control many cotton pests, such as bollworm and aphids. Once the boll weevil has been selectively eradicated from an area, farmers can generally reduce pesticide use by 40 to 90 percent.

Pheromone monitoring traps also have helped stem the spread of gypsy moths, which destroy the leaves of many different kinds of trees. Each year the U.S. Department of Agriculture deploys up to 350,000 pheromone-based monitoring traps to track the gypsy moth. Detection triggers spraying with a bacterial insecticide while population levels are still low enough to be controlled. Such efforts have stopped the invasion of the destructive caterpillars into Vancouver, British Columbia, and the San Francisco Bay area, among others.

Opportunities Ahead

Future research should help expand the successful use of pheromones in pest management. Researchers continue to study how insects produce pheromones, how they trigger a response, and what influences that response. For example, researchers are beginning to uncover the hormones that trigger pheromone production as well as the binding proteins that bring the pheromones to their receptors. Investigators also are discovering the neurological pathways the pheromones stimulate in a responding insect, and the enzymes the insects use to break down the pheromone so as to shut off its signaling. This fascinating basic research should lead to the design of new molecules that affect an insect's response to pheromones, as well as to better ways to use pheromones or other compounds to manage insect pests.

Researchers are working to improve pheromone dispensers in the field so that the chemicals are longer acting, less costly, more potent, and easier to release. In addition, basic research on insect ecology and population dynamics is being applied by agricultural scientists in assessing how many pheromone traps are needed and their most effective distribution.

Jean-Henri Fabre would probably be surprised to find how his observations of the great peacock moth



A pheromone trap is placed in an apple orchard to attract codling moths. (Photo courtesy of Scott Bauer/Agricultural Research Service Photo Unit)



have led to an effective means for controlling crop pests. Had it not been for his keen observations of insect behavior, and for the determination of Butenandt and a host of successors to identify the substances that prompt such behavior, many farmers would still be reaping very little of what they sow. And many fruit crops would be unable to pass the low limit for insecticide residues recently required for their sale in this country.

Indeed, as insects have become increasingly resistant to conventional insecticides—and as the American public has become increasingly wary of the adverse effects of insecticides—farmers of all kinds of crops might have been unable to control the insects that threaten their livelihoods. Fortunately the development of benign pheromone-based alternatives for pest management has given farmers effective new options in their endless battle with the bugs. As we have seen here, many of the scientific breakthroughs that led to these options stemmed from fundamental research, much of it publicly funded, conducted by curious people who merely wanted to understand how nature works.

“Insect Pheromones: Chemicals of Communication” was written by science writer Margie Patlak with the assistance of Drs. Thomas Baker, May Berenbaum, Ring Cardé, Thomas Eisner, Jerrold Meinwald, Wendell Roelofs, and David Wood for Beyond Discovery®: The Path from Research to Human Benefit, a project of the National Academy of Sciences. The project’s Web site is accessible at <www.BeyondDiscovery.org>, from which the full text of all articles in the series can be obtained.

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