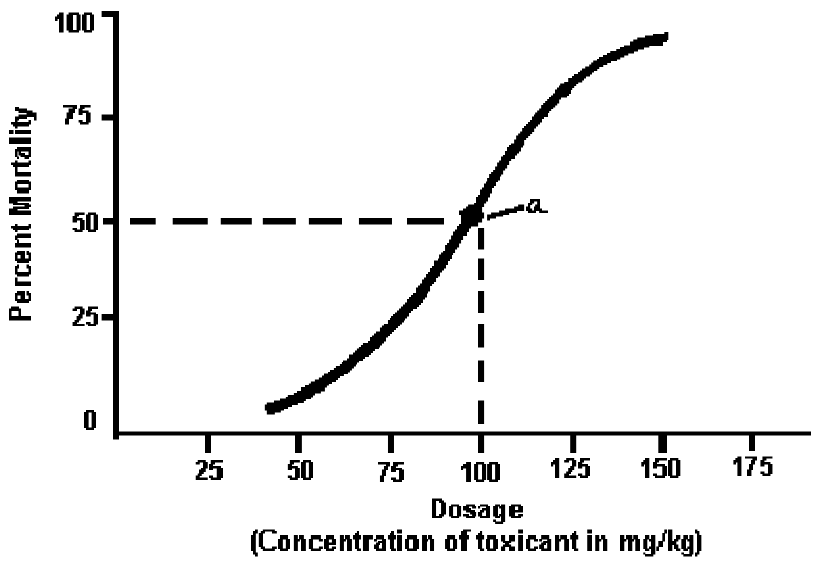
Toxicity

When we say that conventional insecticides are toxic, everyone understands that this means they can kill you. But it's not quite that simple. Common table salt is also toxic. It can be used as a pesticide. Yet salt is an essential component of all living organisms; we would die without it. In fact, it turns out that most chemical substances are toxic if their concentration (dosage) is high enough. As toxicologists often say, **"The dose makes the poison."**

The graph below illustrates this principle:



This is the general form of a **dose response curve**: low mortality at low concentrations of the toxicant increasing to high mortality at higher doses. The mid-point of a dose-response curve (point "a") is known as the median lethal dosage (usually abbreviated LD_{50}). It represents the level of toxicant at which mortality would occur in 50% of the subject population. In the above example, LD_{50} = 100 mg/kg.

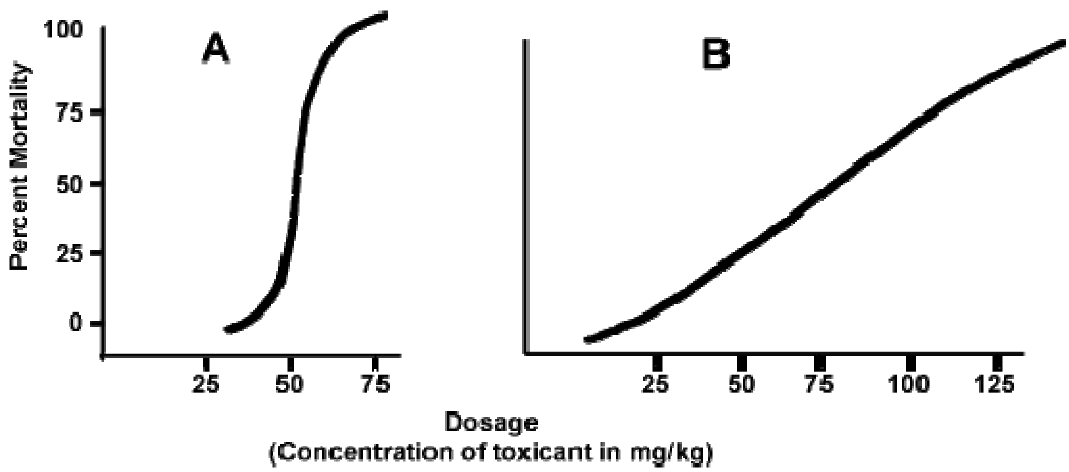
Different species often respond very differently to the same dosage of a toxicant. Some pesticides, pyrethrum is a good example, have a very low LD_{50} for humans and other mammals. These chemicals are relatively safe to use (low hazard) because the rate we use to kill pests is far below the dosage that would injure humans. Other insecticides (e. g. parathion and aldicarb) are far more hazardous because their application rate is much closer to their lethal rate for humans.

The LD_{50} value also depends largely on how an organism is exposed to a toxicant, whether it is ingested, inhaled, or absorbed through the skin, and whether exposure occurs over a short period of time (acute exposure) or over weeks or months (chronic exposure). For this reason, toxicity data always includes information about how the toxicant was administered: acute oral, acute dermal, chronic oral, or chronic dermal.

Which compound is **LEAST** toxic to a human applicator (based on acute oral LD_{50}):

Name of insecticide	Mammalian Toxicity
Sevin	300 mg/kg
Guthion	13 mg/kg
Kryocide	35 mg/kg
Ambush	2000 mg/kg

The slope of a dose-response curve gives a good indication of how a target population will respond to a toxicant. When all members of the population react in a similar way, their dose-response curve is quite steep (Figure 2A). The population is said to be **homogeneous**. On the other hand, if some members of the population are much more sensitive than others (the population is **heterogeneous**), then the dose-response curve is flatter, like Figure 2B.



Based on what you know about the process of natural selection, which of these populations would have the greatest potential for developing **resistance** to this pesticide? Explain your reasoning.

Insect Communication

Communication may be defined as any exchange of information between individuals. For members of the Human species, it is an essential part of all social interactions. We communicate through speech, written language, sign language, body language, Braille, Morse code, and many other cultural and technological inventions. There is even some evidence that we use body odor in communication. Our brains are uniquely adapted for symbolic communication, but most of our "language" skills are acquired through learning.

Insects also have many ways to communicate but, unlike humans, their "language" is almost entirely innate. Each individual is born with a distinctive "vocabulary" that is shared only with other members of its own species. Learning plays little or no role in the ability to produce these signals or to understand them.

An act of communication is not always overt or obvious. No physical entity passes from one individual to another, so it is not always possible to know when exchange of information occurs. The situation is analogous to an alien from another planet who comes to Earth and observes human behavior. Without knowing our language and customs, the alien would be unlikely to recognize a black arm band, a coy wink, or a "yellow ribbon 'round the old oak tree" as forms of human communication. We are in much the same predicament when we study insect communication. The only way to distinguish communicative behavior from non-communicative behavior is by looking for evidence of a change in the behavior (or sometimes, physiology) of another individual.

For experimental purposes, ethologists (scientists who study animal behavior) often define communication as:

An action or condition on the part of one organism that alters the behavior of another organism in an adaptive way.

Thus, an insect may send a communication signal by doing something (e.g. make a noise, release a chemical, or flash a light) or the signal may simply be an inherent part of the insect's physical makeup (e.g. wing pattern, body color, or surface chemistry). In either case, the signal must elicit some behavioral change in order for a human observer to recognize its existence.

Why do insects communicate?

Some form of intraspecific communication is a prerequisite for any behavior that involves the participation or cooperation of two or more individuals. Intentionally or not, insects may also communicate with members of other species (interspecific communication). The adaptive value of these communication signals may include:

1. Recognition of kin or nestmates
2. Locating or identifying a member of the opposite sex
3. Facilitation of courtship and mating
4. Giving directions for location of food or other resources
5. Regulating spatial distribution of individuals -- aggregation or dispersal; establishing and maintaining a territory
6. Warning of danger; setting off an alarm
7. Advertising one's presence or location
8. Expressing threat or submission (agonistic behaviors)
9. Deception / mimicry

How do insects communicate?

Like all other animals, insects use their five senses to acquire information about their environment; any of these sensory modalities may serve as a pathway for the exchange of information. Taste and touch are both contact senses, therefore, exchange of information can occur only when two individuals are touching one another. Vision, olfaction (smell), and hearing are remote senses -- information signals may propogate through the air (or water) over considerable distances.

Each signal modality has unique advantages and disadvantages. Follow the links below to find examples of each signal system and learn more about insect communication.

Chemical Communication

It is probably safe to say that insects rely more heavily on chemical signals than on any other form of communication. These signals, often called **semiochemicals** or **infochemicals**, serve as a form of "language" that helps to mediate interactions between organisms. Insects may be highly sensitive to low concentrations of these chemicals -- in some cases, a few molecules may be enough to elicit a response.

Pros and Cons of Chemical Communication
Advantages: • Not limited by environmental barriers • Effective over distances and around corners • Effective either day or night • Longer lasting than visual or auditory signals • Metabolically "inexpensive" because only small quantities are needed   ***Disadvantages:*** • Low information content (presence/absence) • Not effective in an upwind direction

Semiochemicals can be divided into two groups based on who "sends" a message and who "receives" it:

1. **Pheromones** are chemical signals that carry information from one individual to another member of the same species. These include sex attractants, trail marking compounds, alarm substances, and many other intraspecific messages.
2. **Allelochemicals** are signals that travel from one animal to some member of a different species. These include defensive signals such as repellents, compounds used to locate suitable host plants, and a vast array of other substances that regulate interspecific behaviors.

Allelochemicals can be further subdivided into three groups based on who "benefits" from the meassage:

1. **Allomones** benefit the sender -- such as a repellent, or defensive compound (*e. g.* cyanide) that deters predation.
2. **Kairomones** benefit the receiver -- such as an odor that a parasite uses to find its host.
3. **Synomones** benefit both sender and receiver -- such as plant volatiles that attract insect pollinators.

Insects use their sense of taste or smell to detect the presence of semiochemicals. Specialized receptors may be located anywhere on the body, but are especially common on the feet, antennae, palps, and ovipositor (see Chemoreception). The sense of smell (olfaction) is used for remote chemoreception -- detecting semiochemicals with low molecular weight that are volatile enough to become airborne. The sense of taste (gustation) is used for contact chemoreception -- detecting molecules that adhere to a substrate or to the outside of an insect's body.

Which type of signal is involved (pheromone, allomone, kairomone, or synomone)?

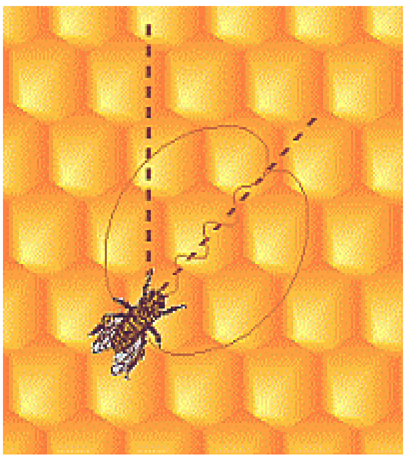
1. A parasitic fly, *Euclytia flavahe*, is attracted to the odor of its host.
2. Ants are able to recognize their nestmates by the taste and smell of hydrocarbons (lipids) found on the surface of the cuticle.
3. Caterpillars that feed on plants sometimes unwittingly induce those plants to release a defensive compound that attracts parasitoids of the caterpillars.
4. Caterpillars of *Lycaena arion* produce a chemical that attracts ants and elicits care-giving behavior. When carried into the ant's nest and placed among the brood, these caterpillars will eat the ant larvae.

Tactile Communication

"Keep in touch!" For you, it's probably just a metaphor, but for some insects it's really a channel of communication. Since many insects have poor vision and sound perception, physical contact provides an important avenue of communication. In blister beetles (family Meloidae), courtship begins with a series of antennal taps by the male on each side of the female's body. She signals her receptivity by lifting her wing covers (elytra) and allowing him to climb on her back. But to complete his quest, the male must continue tapping, alternating from side to side at just the right frequency until the female is stimulated to extend her genitalia and begin mating.


Tandem running in ants

Antennal tapping is also an essential component of communication in both ants and termites. It's not clear exactly what information may be exchanged, but it certainly involves nestmate recognition and leads to exchange of food through trophallaxis. In some cases, instantaneous feedback allows the sender to monitor the receiver's response and alter the signal if necessary. Antennal tapping on the hind legs is used during **tandem running** in both ants and termites. This is a "follow-the-leader" behavior in which the tapping informs the leader that she has not lost her disciple. If tapping stops, the leader instinctively turns around and searches in ever-widening circles until she re-establishes contact with the follower.



The "dance" language of honeybees is largely a tactile communication system, performed in total darkness on the vertical surface of the honeycomb. A "round dance" signals to nestmates the presence of a nectar source in close proximity to the hive (usually less than 80 feet). It consists of a series of circular runs with more or less frequent changes in direction. The greater the frequency of direction changes, the better the quality of the nectar source. The "waggle dance" is used for longer distances. It involves a figure eight pattern with a series of abdominal waggles on a straight run after each half-circle turn (see figure at left). Distance is indicated by the duration of the straight run and the frequency of the waggles. Direction is indicated by the angle of the straight run (relative to vertical) and corresponds to the horizontal angle between the sun and the direction of the food source.

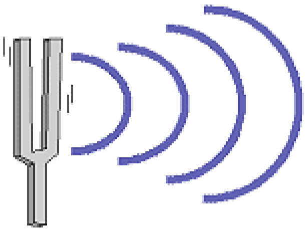
More about
DANCE LANGUAGE

Tactile cues generated by ripples on the water surface allow whirligig beetles (family Gyrinidae) to constantly monitor the location of dozens of other nearby whirligigs. Thanks to this tactile communication system, the whirligigs can swim rapidly in circles, avoid bumping into other members of their own species, and still detect the presence of nearby predators or prey.

Pros and Cons of Tactile Communication
Advantages: • Instantaneous feedback • localized area • Individual recipient • Effective in the dark (*e. g.* caves, wood galleries) **Disadvantages:** • Not effective over distance • Organisms must stay in direct contact • Message must be repeated to each recipient • Vibration signals can be intercepted by predators

Certain treehoppers (order Hemiptera: family Membracidae) produce vibrations in the tissue of their host plant that can be felt by all other treehoppers on the same plant. The signal travels throughout the plant in much the same way that banging on water pipes in your apartment creates noise throughout the whole building. The signals apparently work as an alarm system, and in some species, they may be used by nymphs to elicit protective maternal behavior. Substrate vibrations can be a particularly effective communication system for small insects who cannot generate an acoustic signal loud enough to be heard more than few inches away.

Acoustic Communication



Sound is produced when a physical object vibrates rapidly, disturbs nearby air molecules (or other surrounding medium), and generates compression waves that travel in all directions away from the source. We perceive these waves as sound when they collide with our ear drum and cause a mechanical disturbance that is detected by sensory neurons in our inner ear. Both living and non-living objects produce sound, but only animals use it as a means of communication.

Sound serves as a very effective communication modality. It can be made to vary in **frequency** (high pitch vs. low pitch), **amplitude** (loudness), and **periodicity** (the temporal pattern of freqency and amplitude). Together, these three variables can create an extremely wide and complex range of signals -- from an insect's mating call to human speech and vocal music. Since sound waves move rapidly through air (about 331 m/sec), acoustic signals can be quickly started, stopped, or modified to send a time-sensitive message.

Pros and Cons of Acoustic Communication

Advantages:

- Not limited by environmental barriers
- Effective over distances and around corners
- Highly variable, fast change -- high information content

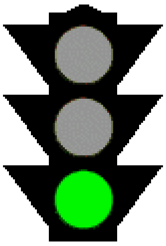
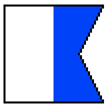
Disadvantages:

- May reveal location of sender to a potential predator
- Less effective in "noisy" environments (e.g. seashore)
- May be metabolically "expensive" to produce
- Attenuation -- intensity falls rapidly with distance from source (cube-root function)

At best, the human ear is able to detect sound frequencies only within the range of about 20-20,000 hertz (vibrations per second). But some insects (as well as other animals like bats and dolphins) produce and detect sounds that are well above this frequency range. Some grasshoppers and moths, for example, produce ultrasonic sounds as high as 80,000 hertz. Entomologists study these high-pitch sounds by using an audio transducer, an electronic device that converts inaudible high frequencies to lower audible frequencies.

Most insects detect sound with a **tympanic mambrane** in the abdomen (e.g. grasshoppers and moths) or in the tibiae of the front legs (e.g. crickets and katydids). Mosquitoes have antennal hairs that resonate to certain frequencies of sound. But sound vibrations can also travel through solid objects, and some insects (e.g. some species of ants, bees, termites, and treehoppers) can sense **substrate vibrations** with mechanoreceptors (chordotonal organs) in their legs. Since these signals are "felt" rather than "heard", they are usually regarded as a form of tactile communication.

Visual Communication

Many insects communicate with visual signals. The color patterns and other markings on the wings of butterflies and moths facilitate species recognition in much the same way colored uniforms reveal the players' affiliations on a football field. Some insects use bright colors, eyespots, or other distinctive patterns to scare away predators, to advertise their ability to sting, or to mimic the appearance of another unpalatable species. Other insects use dance-like body movements to attract a mate or to communicate with nestmates. Most of these signals are effective only as long as they are visible in daylight. But a few insects (fireflies, for example) can generate their own light and use visual signals that can be seen at night.

Pros and Cons of Visual Communication

Advantages:

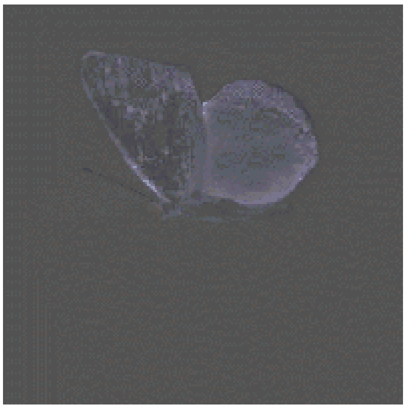
- Effective over long distances
- Can be used while moving
- Fast -- speed of light
- Effective in all directions (independent of wind)
- Passive signals require no expenditure of energy

Disadvantages:

- Requires a clear line of sight
- Visual signals may be intercepted by predators
- Only effective in daylight (in fireflies, only at night)
- Active signals may be metabolically "expensive" to produce

Passive signals, such as eyespots and color patterns can serve as a form of "free advertising". The colorful wings of a butterfly, for example, are a "billboard" publicizing its species identity. Individual insects incur little or no metabolic cost for displaying these messages because they are an integral part of the integument. It may be prudent to hide these signals from a potential predator, so some insects have a way to conceal their message when necessary. The red admiral butterfly, for example, has bright, distinctive markings on the upper wing surface and drab, protective coloration on the underside.

Active signals, like body movements and light flashes, are more costly to produce, but they can be withheld from use at inappropriate times. They may also have a higher information content because signal frequency, duration, or periodicity may convey additional meaning. In fireflies, for example, pulses of light are used in a courtship dialogue between a male (usually flying) and a female (usually perched in the vegetation). Each species has a unique flash pattern and response time. Males of *Photinus pyralis* emit a single "J"-shape flash during a rising flight movement. A female responds with a single flash after a two second interval. In *Photinus consumilis*, males emit a series of 3-5 short flashes and the females respond with a double flash. Roles are reversed in some tropical species where the females fly and the males signal from perches in the vegetation.

- 
- Ultraviolet reflection from a male alfalfa butterfly in simulated flight

Unlike humans, many insects have the ability to see ultraviolet light. Not surprisingly, some species communicate using wavelengths in this part of the spectrum. Female cabbage butterflies, for example, have ultraviolet-reflecting scales on the dorsal wing surface. When they fly, each downstroke of the wing creates a brief "flash" of UV that males apparently recognize as the flight signature of a potential mate. A "flashing female" may attract several males who engage in aerial courship displays. In alfalfa butterflies, only males have the UV-reflective scales. They flutter in front of the females to create a flickering courtship display. Missing scales reduce the wings' reflectivity -- a sign of aging that impairs a male's ability to seduce a mate.

ENTOMOLOGY 401/801. INSECT PHYSIOLOGY

Dr. David W. Stanley

CHAPTER THIRTEEN. INSECT IMMUNOLOGY: HUMORAL IMMUNITY

One question entomologists and university administrators might wonder about now and again would be along the lines of "What are some of the emerging areas of insect physiology that are likely to have great impact on how entomology might be carried out in the future? Several topics come to mind, including understanding the roles of peptide hormones, the significance of biogenic amines, more detailed appreciation of sensory physiology, and intracellular signal transduction mechanisms.

However, above all of these topics, and this is certainly not to be taken as an exhaustive list, the study of insect immunology may well develop into one of the most active and most important areas of insect physiology.

INSECT DISEASES

The idea that insects can have diseases goes well back to antiquity. The idea predates the science of microbiology by centuries. The earliest records come from descriptions of a pest of honey bees by Aristotle. In the infancy of microbiology, Pasteur showed that two diseases of silkworms were caused by microbes. As you might expect, much of Pasteur's work was motivated by economic concern: the silk industry suffered considerable losses caused by diseased silkworms.

In more recent times insect pathogens have used microbes as agents of biological control of insects. Although many microorganisms have been studied as actually or potentially useful control agents, none have so far received as much attention as *Bacillus thuringeinsis*. The potential of various microbial forms, including bacteria, fungi and protistans, as insect control agents has driven an impressive body of work on insect microbiology and pathology.

The study of insect immunity went along with much of the very early work on the general question immunology. There was a period of considerable activity in the 1920's. A second surge of work on insect immunity started in the late 1950's, when it was discovered that a certain immunity could be induced in some insects. This induced immunity can be compared to a sort of standing, or constitutive, immunity. The most recent period of research on insect immunity began in 1980 with the first isolation and purification of an induced antibacterial factor. Modern work on insect immunity is particularly satisfactory because it is one of the areas of insect biology where molecular biological tools have yielded a rich harvest of information that would not otherwise have been generated.

It is appropriate to define immunity. Immunity is "resistance to or protection against a specified disease". This definition draws attention to the phenomonology, and it is independent of specific immunological mechanisms. The distinction is important because the contemporary wisdom in some circles holds that insects do not have an immune system because they do not produce antibodies of the mammalian sort.

THE MAJOR IMMUNITY MECHANISMS

There are two major areas of insect immunity, cell-free immunity and hemocytic immunity. These are sometimes called humoral immunity and cellular immunity, respectively. These two immunological responses are complimentary, and both may be seen in response to the same infection. Please note that immunity in insects and in other invertebrates differs from the immune systems of mammals in fundamental ways. First, insects produce no lymphocytes, of which there are many sub-types including eosinophils and neutrophils, no T-cells and no helper cells. So the cellular immunity of insects is not to be compared to the cellular immunity of mammals, although some people have tried to make that sort of comparison. Second, insects do not biosynthesize immunoglobulins, such as gamma-globulin. The important point here is that the immunity of insects lacks the molecular specifity of antibodies from mammals.

INDUCED ANTIBACTERIAL PROTEINS

Wounding or injection of certain bacteria some insects induces the biosynthesis of antibacterial proteins called cecropins. These proteins were first isolated from the hemolymph of *Hyalophora cecropia,* from whence the term cecropin was coined. Molecules with similar structure have since been isolated from the waxmoth *Galleria,* the tobacco horn worm *Manduca sexta,* several species of Diptera and from a beetle. Not all insects appear to produce cecropins, because a cricket, a cockroach and a locuts do not produce them. The blood-sucking bug *Rhodnius* produces antibacterial proteins that differ from cecropins. *H. cecropia* produces cecropins A, B and D as well as several minor cecropins, C, E, and F, which differ from the major cecropin by only a single amino acid substitution. All of these proteins are about 4000 in molecular weight, each with 35 to 37 amino acids. There is a very high level of homology among these proteins, all of which feature long hydrophobic reaches in the C-terminal portions of the molecule. The very close homology among these proteins is consistent with the idea that they have arisen through gene duplication.

The cecropins are not active against many, but not all, bacteria. Cecropins A and B are active against a large number of gram-positive and gram-negative species, including some that are occasional insect pathogens and some that are obligate insect pathogens and others that are non-pathogenic species. None of the cecropins are active against *B. theringeinsis*.

ANTIBACTERIAL ACTIONS

Cecropins lyse bacterial cell membranes; they also inhibit proline uptake and cause leaky membranes. These actions at the level of procaryotic membranes are consistent with the structures of cecropins. They are all cylindrical, amphipathic molecules with long hydrophobic regions on one end. In effect, these protein act like detergents. Synthesis of proteins that are analoges to cecropins show that activity against a broad spectrum of bacteria species is lost when amino acid substitutions that disrupt the alpha-helix structure are made.

The genes that code for cecropins have been cloned. These molecular biological approaches give insight into the biosynthesis of the cecropins. The gene for cecropin B, for example, codes for 26 amino acids in the N-terminus of the protein that are removed during post-transcriptional processing to produce the mature cecropin B. These are removed in 3 steps, first 22 residues are removed in one cleavage, which produces a pro-cecropin. The pro-cecropin is further processed by two sequential cleavages that yield the final product.

ATTACINS

A second set of antibacterial proteins are called attacins. These also have been isolated from *H. cecropia* and three other moth species. Attacins are considerably larger than cecropins, made up with over 180 amino acids. The antibacterial activity of these proteins covers a rather narrow spectrum compared to the cecropins, with high activity only against a few species of bacteria in the alimentary canal. Their site of action is at the outer bacterial membrane. These proteins appear to work in synergism with the cecropins and with lysozyme so that the three types of immune proteins can work together.

LYSOZYME

Peptidoglycan is a large, bag-shaped macromolecule that encases bacterial membranes. It is universally present in bacteria. Minor variations of peptidoglycan occur in among different bacterial species. Lysozyme is an enzyme that hydrolyzes the glycosidic bonds of peptidoglycan, and thereby causes bacterial cell lysis. This enzyme constitutively occurs in low levels in hemolymph of most insects. Lysozyme is rapidly induced immediately upon bacterial infection.

STILL OTHER ANTIBACTERIAL PROTEINS

Antibacterial proteins widely occur in vertebrate and invertebrate animals. It is not too surprizing, then, to learn that representatives of various insect orders produce different antibactieral proteins. The attacins and cecropins are known mostly from lepidopterans, although they have been detected in other insects and even in intestine of pigs.

Honeybees produce another family of such proteins that are known as apidaecins. These are small peptides, molecular weight about 2100. Apidaecins do not appear to disturb bacterial membranes, and it is not yet clear how they do work. It is speculated that these are bacteriostatic, rather than bacteriocidal, proteins. Royalisin is another antibacterial peptide is found in the royal jelly of honeybees. This is also an amphipathic protein, and its mode of action may be similar to cecropins. It is a small molecule, molecular weight estimated at 5523.

Dipterans also produce induced antibacterial proteins. Two peptides from *Phormia* are called defensin and diptericin. Another dipteran peptide is a male-specific antibacterial product from *Drosophila melanogaster*. This peptide is known as andropin, and it thought to protect seminal fluid and the male reproductive tract against microbial infections.

One of the most exciting findings in insect immunology is the discovery of an insect immune protein called hemolin. This protein belongs to the immunoglobulin superfamily, that is, it shares sequence homologies to mammalian immunoglobulins. It is thought that this protein is one of the first proteins to appear in the hemolymph of the giant silkmoth *H. cecropia*. This protein binds to the surface of bacteria, and it is likely that the binding may be the first step in the insect immune response.

BACTERIAL RESISTANCE TO INSECT IMMUNITY

With respect to evolutionary ecology, our discussion of insect immunity offers a sort of physiological perspective on host-parasite relationships. Within the context of these relationships, we can imagine strong evolutionary pressure on the microbes to develop mechanisms that circumvent immunity reactions with hosts. Two such mechanisms are known in some of the bacteria that invade insects. These are called passive resistence and active resistence. Please note that resistence in this context refers to the parasite side of host-parasite relationships

Passive resistence is related to the structures of some bacterial envelopes. These envelopes happen to be resistent to antibacterial activity of immune proteins. In these cases the resistence is a feature of outer membranes, rather than something bacteria might actively produce. There appear to be mutants of insect-pathogenic bacteria that have lost this sort of passive protection and are comcominantly less virulent.

Active resistence refers to active biochemical breakdown of immunity, rather than a sort of side-stepping as seen in passive resistence. B. theringeinsis, for example, produces an immune inhibitor called InA that works by proteolytic breakdown of cecropins and attacins. A beautifully complex relationship occurs in a nematode with a symbiotic bacterium appropriately named *Xenorhabdus nematophilus*. This bacterium is sensitive to two cecropins, A and B. However, the nematode produces an immune inhibitor enzyme that digests cecropins and attacins, thereby rendering them without activity.

One co-evolutionary response to these bacterial proteases that inactivate the insect anti-bacterial proteins may be another protein produced by some insects that inhibit the bacterial proteases. A molecule known as alpha2-macroglobulin has been identified in the hemolymph of some arthropods, but not yet from insects. Alpha2-macroglobulins are protease inhibitors, well known from mammals. These inhibitors do not work by competitively inhibiting the proteases, which is the most general mechanism of inhibiting proteases. Instead, these molecules form "cages" around protease enzymes. The cages inhibit protease activity by screening larger molecules out. Smaller peptides are not screened out, so they can get into the active sites of the proteases, where they can be hydrolyzed. This forms a sort of selective inhibition of proteases. It is speculated that the alpha2-macroglobulins are a regular feature of arthropod hemolymph. They are thought to clear protease activity from hemolymph by collecting the proteases, then delivering them to hemocytes where they participate in a endocytotic protease clearance pathway that is similar to protease clearance pathways known in mammals.

THE BIOLOGICAL SIGNIFICANCE OF IMMUNITY

There are over two hundred species of bacteria that live in one or another association with insects. These can be categorized with repect to their pathogenicity:

1. harmless forms that are not pathogenic

2. occasional pathogens

3. obligate pathogens

Induced insect immune systems do not have antibacterial activity against all, or even, most pathogenic species. *B. theringensis* is a good example of a pathogenic species that is not troubled by immune systems. On the other hand, as far as can be known, insect immune sytems are effective against all non-pathogenic systems. What sense can we make of this? One suggestion is that insect immunity has more to do with regulating population dynamics of the non-pathogenic bacteria that naturally occur in insect bodies. My speculation is that many pathogenic bacterial species have secondarily lost protective and resistence mechanisms such that they have become labile to the induced proteins.

OTHER HUMORAL IMMUNITY FACTORS

We most often regard phenyloxidases in terms of sclerotization, or tanning, of cuticle. These enzymes are found within cuticle and in hemolymph of insects. In hemolymph phenyloxidases are involved in melanin synthesis by way of quinones. Phenyloxidases are very active enzymes, and the intermediates and products of phenyloxidase reactions can be harmful to animals. These enzymes are nearly always found in a pro-phenyloxidase form. Phenyloxidases are activated in response to bacterial infections, and melanin, a tanned insoluble material is deposited around clumps of bacteria. A similar reaction occurs at the site of wounding in insect integument.

Activation of phenyloxidase has been studied in a few insects. This usually involves a signal from the surface of bacterial walls, such as the putative beta-1,3-glucan receptor. Conformational changes in this receptor activate a serine protease called prophenyloxidase activating enzyme. The activating enzyme removes a 5-kD peptide from prophenyloxidase, which yields phenyloxidase. These sort of cascade reactions are common biochemical activating mechanisms. Another important reaction cascade is the blood-clotting reaction in mammals.

Lectins are agglutinating molecules that are well known from plants and many classes of animals. Lectins have been purified from the hemolymph of many insect species, including a meat fly, a cricket and a grasshopper. A possible function of some lectins may be agglutination of microbes, but the this has been clearly shown in only a single case.

ENTOMOLOGY 401/801. INSECT PHYSIOLOGY

Dr. David W. Stanley

CHAPTER FOURTEEN. INSECT IMMUNOLOGY: HEMOCYTIC IMMUNITY

As we mentioned last meeting, insects defend themselves from challenges by microorganisms with a combination of humoral and cellular responses. The distinction between these two response categories is rather artificial for a couple of reasons. First, hemocytes can release some of the protein factors that follow from challenges. Hemocytes produce what we regard as humoral responses. Second, although infections elicit a complex of multifaceted responses, the responses are coordinated and integrated into a single theme: host defense. Nevertheless, it is very convenient to treat hemocytic and humoral immunity as separate topics. One point of separation is that hemocytic responses are rapid. They occur almost immediately an insect is invaded by microorganisms. Humoral responses are much slower, they are usually not seen until some hours after infection. These longer-term responses may be more important in sustaining the hemocytic host defense reactions initiated earlier in the infection cycle.

Circulating cells are regularly found in the hemolymph of insects. These cells are known as hemocytes. Hemocytes are involved in conferring a cellular, or hemocytic, immunity to insects. These cells are formed in haemopoietic organs that are variously well developed in different insect groups. In the cricket *Gryllus* haemopoietic organs are well developed, distinct organs found in the second and third abdominal segments along either side of the heart. In other insects haemopoietic organs are amorphous clumps of cells irregularly distributed along the dorsal vessel.

You might enjoy a quote on the classification of insect hemocytes: "Classification of insect hemocytes is an issue that stimulates an instantaneous loss of good manners and an induction of bad temper at any conference on insect physiology or immunity" . {Lackie, A.M., 1988, In: Advances in Insect Phyisology, Ed. by P.D. Evans and W.B. Wigglesworth, Academic Press, London} Given detectable levels of disagreement on the subject, a list of some hemocytes and their function follows:

Prohemocytes (PRs) are thought to be the stem cells from which all hemocytes arise.

Plasmatocytes (PLs) are larger cells involved in major immunological actions. PLs are capable of phagocytosis, a kind of entocytosis of smaller foreigh cells. Phagocytosis usually follows low doses of invading yeast and bacteria. PLs are also involved in encapsulation of clumps of invading microbes. There are typically more PLs than other types of hemocytes.

Granulocytes (GRs) are characterized by the presences of many intracellular granules. Electron microscope studies have revealed differences among the granules in these cells. There are structureless and structured granules. The biochemistry of the different types of granules is not yet understood. GRs are major players in encapsulation reactions.

Other hemocytes include spherulocytes, oenocytoids and adipohemocytes. These cells are regarded as "nonimmunocompetent" by some authors, although there is considerable ground to disagree with this idea. Gupta prefers to use the term "immunocytes" for GRs and PLs and "hemocytes" for all the other types. He uses these terms to stress the idea that GRs and PLs make up most of the immune responses. Spherulocytes may be involved in silk formation, melanization and secretion of some hemolymph proteins. They may also be important in hemolymph clotting responses to wounds. Oenocytoids may contribute to the final stages of cuticle formation. Other authors suggested that these cells may recognize "non-self".

DEFENSE MECHANISMS

Again, hemocytic defense responses occur immediately insects are invaded by microorganisms. GRs and PLs are responsible for a number of cellular immune reactions. They are able to phagocytize small biologic particles such as bacteria and yeast. Phagocytization refers to the process of cellular internalization of foreign invaders. Phagocytosis is not the same as killing the microbial cells. Microbes are sécondarily killed after phagoccytosis.

GRs and oenocytoids are attracted to sites of wounds, where they play major roles in coagulating hemolymph to stop bleedings. We know virtually nothing about the signalling process that is responsible for moving cells to wound sites.

Another defense mechanism is nodule formation. Nodules are aggregates of hemocytes that entrap invading microbes. This is a particularly effective means of clearing the hemolymph, and it is considered to be of greater importance than phagocytosis in clearing large doses of bacteria. There appears to be a sequence of trapping by GRs, followed by aggregation by PLs. The precise sequence of events will remain a point of controversy. In addition to bacteria, viral polyhedrons, fungal spores and protozoans are taken up in nodules.

When organisms or clumps of organisms are too big to be phagocytized or to form nodules, they may be encapsulated. Encapsulation is the sequesterization of these organisms or clumps with multilayered aggregates of hemocytes. This also involves release of coagulum, in a response similar to wound-repair. The coagulum probably aides in forming layers of cells. Encapsulation was first observed by the Russian zoologist Elie Metchnikoff in 1892. He placed rose thorns into a starfish larva. The next day he saw that the thorns were completely surrounded by tiny cells. Metchnikoff called these cells phagocytes from the Greek words for eating and cells. Metchnikoff eventually moved from the University of Odessa to the Pasteur Institute, and later shared the Nobel Prize for his studies of cellular defenses in mammals and invertebrates.

We are not sure of the killing mechanisms that hemocytes elaborate. Some of the suggested mechanisms include peroxidation, melanization, and lysozymes. By comparison to our scant knowledge of invertebrate immunity, very much is understood about the killing mechanisms of mammalian host-defense cells. There are two sorts of mechanisms. One sort does not depend on oxygen. These include protease, esterase, acid-phosphatase and lysoszyme mechanisms. Another sort does depend on oxygen, including formation of singlet oxygen and superoxide anions, which are very reactive and very toxic species. Work on killing mechanisms of insect hemocytes will produce important and very novel insights

Insect Defenses

Compare your response time to the speed of a cockroach's escape reflex. Click **"start"** to begin and then click **"stomp"** as soon as a cockroach appears in the white window.

Can you beat 50 milliseconds?

DHTML script courtesy of Dynamic Drive

For many insects, a quick escape by running or flying is the primary mode of defense. A cockroach, for example, has mechanoreceptive hairs (setae) on the cerci that are sensitive enough to detect the change in air pressure that precedes a fast moving object (like your foot). Nerve impulses from these receptors travel through giant neurons to thoracic ganglia at speeds up to 3 meters per second, triggering an evasive response by the legs in less than 50 milliseconds. House flies have a similar reaction time when you try to swat them. They leap into the air and begin flapping their wings 30-50 milliseconds after sensing a threat.

Tiger moths (family Arctiidae) can detect ultrasonic echolocation by bats. At low intensity, they fly away from the bat, but if the bat's call increases to a certain threshold they quickly drop from the air in an evasive, looping dive. Other alarm reactions may be less dramatic, but just as effective: Madagascar cockroaches hiss when disturbed; cuckoo wasps curl up into hard, rigid balls; tortoise beetles have strong adhesive pads on their tarsi and hold themselves tight and flat against a leaf or stem. Other insects simply "play dead" (**thanatosis**) -- they release their grip on the substrate and fall to the ground where they are hard to find as long as they remain motionless.

An insect's hard exoskeleton may serve as an effective defense against some predators and parasites. Large weevils are notorious for their hard bodies - as you may discover for yourself the first time you bend an insect pin trying to push it through the thorax. Most diving beetles are hard, slick, and streamlined; even if you can catch them, they will often squirm out of your grip.

Spines, bristles, and hairs may be effective mechanical deterrents against predators and parasites. A mouthful of hair can be an unpleasant experience for a predator and parasitic flies or wasps may have a hard time getting close enough to the insect's body to lay their eggs. Some caterpillars incorporate body hairs into the silk of their cocoon as an additional defense against predation.

Some insects have a "fracture line" in each appendage (often between the trochanter and the femur) that allows a leg to break off easily if it is caught in the grasp of a predator. This phenomenon, called **autotomy**, is most common in crane flies, walkingsticks, grasshoppers, and other long-legged insects. In most cases, sacrificing a limb in this manner creates only a minor disability. In fact, walkingsticks (especially young nymphs) may regenerate all or part of a missing appendage over the course of several molts.

Chemical Defenses

Many insects are equipped to wage chemical warfare against their enemies. In some cases, they manufacture their own toxic or distasteful compounds. In other cases, the chemicals are acquired from host plants and sequestered in the hemolymph or body tissues. When threatened or disturbed, the noxious compounds may be released onto the surface of the body as a glandular ooze, into the air as a repellent volatile, or aimed as a spray directly at the offending target.

Defensive chemicals typically work in one of four ways:

1. **Repellency** -- a foul smell or a bad taste is often enough to discourage a potential predator. Stink bugs, for example, have specialized exocrine glands located in the thorax or abdomen that produce foul-smelling hydrocarbons. These chemicals accumulate in a small reservoir adjacent to the gland and are released onto the

body surface only as needed. The larvae of certain swallowtail butterflies have eversible glands, called **osmeteria**, located just behind the head. When a caterpillar is disturbed, it rears up, everts the osmeteria to release a repellent volatile, and waves its body back and forth to ward off intruders.

2. **Induce cleaning** -- irritant compounds often induce cleaning behavior by a predator, giving the prey time to escape. Some blister beetles (family Meloidae) produce cantharidin, a strong irritant and blistering agent that circulates in their hemolymph. Droplets of this blood ooze from the beetle's leg joints when it is disturbed or threatened -- an adaptation known as **reflex bleeding**. Irritant sprays are produced by some termites, cockroaches, earwigs, stick insects, and beetles. The notorious bombardier beetles store chemical precursors for an explosive reaction mixture in specialized glands. When threatened, these precursors are mixed together to produce a forceful discharge of boiling hot benzoquinone and water vapor (steam).
3. **Adhesion** -- sticky compounds that harden like glue to incapacitate an attacker. Several species of cockroach guard their backsides with a slimy anal secretion that quickly cripples any worker ants that launch an attack. Similarly, members of the soldier caste in nasute termites have nozzle-like heads equipped with a defensive gland that can shoot a cocktail of defensive chemicals at intruders. The compounds, which are both irritating and immobilizing, have been shown to be highly effective against ants, spiders, centipedes, and other predatory arthropods.

4. **Cause pain or discomfort** -- Saddleback caterpillars, larvae of the io moth, and various other Lepidopteran larvae have hollow body hairs that contain a painful irritant. Simply brushing against these **urticating hairs** will cause them to break and release their contents onto your skin. The consequence is an intense burning sensation that may last for several hours. Many ants, bees, and wasps (the aculeate Hymenoptera) deliver **venom** to their enemies by means of a formidable **stinger** (modified ovipositor). The venom is a complex mixture of proteins and amino acids that not only induces intense pain but may also trigger an allergic reaction in the victim.

Protective Coloration

Biologists recognize that there is usually an underlying rationale for the great diversity of shapes and colors found in the insect world. We may not know why a particular species has parallel ridges on the pronotum or black spots on the wings, but we can be reasonably certain that this shape or color has contributed in some way, however small, to the overall fitness of the species. It is obvious that at least some of the colors and patterns serve a defensive function by offering a degree of protection from predators and parasites. These patterns, collectively known as **protective coloration**, fall into four broad categories:

1. **Crypsis**

Photo Gallery of Insect Camouflage

Insects that blend in with their surroundings often manage to escape detection by predators and parasites. This tactic, called **cryptic coloration**, involves not only matching the colors of the background but also disrupting the outline of the body, eliminating reflective highlights from smooth body surfaces, and avoiding sudden movements that might betray location. Obviously, this tactic loses much of its effectiveness if an insect moves from one type of habitat to another. Well-camouflaged insects usually stay close to home or make only short trips and return quickly to the shelter of their protective cover. Many ground-dwelling grasshoppers and katydids, for example, have colors of mottled gray and brown that help them "disappear" against a background of dried leaves or gravel. On the other hand, closely related species that live in foliage are usually a shade of green that matches the surrounding leaves. The larvae of some lacewings improve their camouflage by attaching bits of moss or lichen from their environment onto the dorsal side of their body.

Insect Camouflage

Camo Moth

Brimstone Butterfly

Crescent Moth

Flower Mantid

Geometer Moth

Grasshopper

Katydid

Lichen moth

Notodontid Moth

2. **Mimesis**

Photo Gallery of Mimesis

Some insects "hide in plain sight" by resembling other objects in the environment. A thorn could really be a treehopper; a small twig might be a walkingstick, an assassin bug, or the caterpillar of a geometrid moth; and sometimes a dead leaf turns out to be a katydid, a moth, or even a butterfly. This "mimicry" of natural objects is often known as **mimesis**. It goes far beyond imitation of plant parts:

- Some swallowtail larvae resemble bird droppings, others have false eyespots on the thorax that create a convincing imitation of a snake's head.
- The likeness of a caterpillar can be found on the outer edge of many lepidopteran wings, perhaps serving to fool predatory birds that may peck at the wing margin instead of the butterfly's body.
- Many butterflies and moths have eyespots on the wings that emulate the face of an owl or some other large animal.
- Slug caterpillars and hag moth larvae look like hair balls or small furry mammals.

Mimesis

Butterfly Larva

Dead Leaf Butterfly

Leaf Insect

Mourningcloak Butterfly

Polyphemus Moth

Puss Moth Larva

Spanworm

Swallowtail Larva

Treehoppers

3 .Warning Colors

Photo Gallery of Warning Colors

Insects that have an active means of defense (like a sting or a repellent spray) frequently display bright colors or contrasting patterns that tend to attract attention. These visually conspicuous insects illustrate **aposematic coloration**, a term derived from the Greek words *apo-* (from a distance) and *sema* (a sign or signal) -- meaning "a signal from afar". A predator quickly learns to associate the distinctive coloration with an "unpleasant" outcome, and one such encounter is usually enough to insure avoidance of that prey in the future. A few individuals will die as sacrifices, but for the species as a whole, it pays to advertise!

Warning Colors

Bumble Bee

Honeybee

Hornworm

Milkweed Bug

Monarch Butterfly

Palm Beetle

Spanish Festoon

Velvet Ant

Yellow Jacket

4. Mimicry

Photo Gallery of [Batesian Mimicry]

If a distinctive visual appearance is sufficient to protect an unpalatable insect from predation, then it stands to reason that other insects might also avoid predation by adopting a similar appearance. This ploy, essentially a form of "false advertising", was first recognized and described by Henry W. Bates in 1861. Today, it is commonly known as **Batesian mimicry**. Viceroy butterflies (mostly palatable to birds) are largely protected from predation because they resemble monarch butterflies (very distasteful). Many species of bee flies, flower flies, robber flies, and clear-winged moths are similarly protected because they mimic the appearance (and often the behavior) of stinging bees and wasps. Batesian mimicry is usually a successful strategy as long as the model and mimic are found in the same location, the mimic's population size is smaller than that of the model, and predators associate the model's appearance with an unpleasant effect.

Batesian Mimicry

Viceroy Butterfly

Flower Fly

Sesiid Moth

Robber Fly

Redspotted Purple Butterfly

Conopid Fly

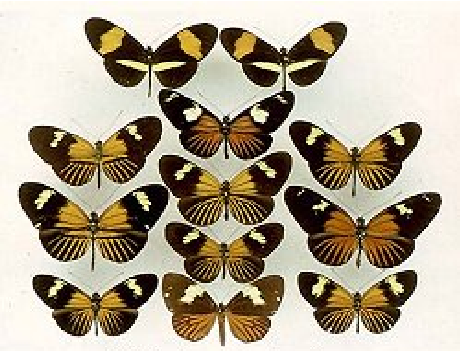
Flower Fly

Largid Bug

Stilt–legged Fly

In 1879, Fritz Müller recognized that two or more distasteful species often share the same aposematic color patterns. Many species of wasps, for example, have alternating bands of black and yellow on the abdomen. This defensive tactic, commonly known as **Müllerian mimicry**, benefits all members of the group because it spreads the liability for "educating the predator" over more than one species. In fact, as the number of species in a Müllerian complex increases, there is a greater selective advantage for each individual species.

Mimicry has been carried to extremes in some tropical Lepidoptera where both related and unrelated species resemble each other in size, shape, color, and wing pattern. Collectively, these butterflies (and sometimes moths) form **mimicry rings** that may include both palatable and unpalatable species. In South America, for example, longwing butterflies (Family Nymphalidae) form a mimicry ring that includes at least twelve different species (including one moth).


Butterfly mimicy ring

Although natural selection favors individuals in a population with the best camouflage or mimicry, it also favors the predator or parasite with the best prey-finding acumen. As a result of these competing interests, coevolution between predator and prey populations inevitably leads to an ongoing escalation of offensive and defensive measures -- a scenario that Leigh Van Valen of Chicago University describes as an evolutionary "arms race".

In order to survive in the arms race, both predator and prey must constantly evolve in response to the other's changes. Failure to "keep up" concedes a competitive advantage to the opponent and may lead to extinction.

The idea that perpetual change is necessary just to maintain the status quo has been coined the **Red Queen's Hypothesis**. This name refers to a scene from the stories of Alice in Wonderland by Lewis Carroll. In *Through the Looking Glass*, Alice meets a chess piece, the Red Queen. After running hard to follow the Queen, Alice discovers that she has not moved from where she started. Asked about this paradox, the Red Queen replies, "Here, you see, it takes all the running you can do to keep in the same place."

Pests of Medical Importance

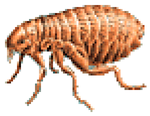
It is impossible to measure the full impact of insects and other arthropods on human health and welfare. These organisms have the capacity to inflict injury, disease, discomfort, or distress. They can be a direct cause of illness, pain, and suffering through bites and stings, infested wounds, or allergic reactions. They feed on blood or body tissues and they may transmit deadly pathogens or parasites. Economic losses associated with these pests are borne not only by the affected individuals and their families, but also by human society in general. Losses include not only the direct costs of medicine and health care, but also indirect costs resulting from stress, absenteeism, and reduced productivity. These are costs that are not easy to measure in dollars and cents.

Biting Insects

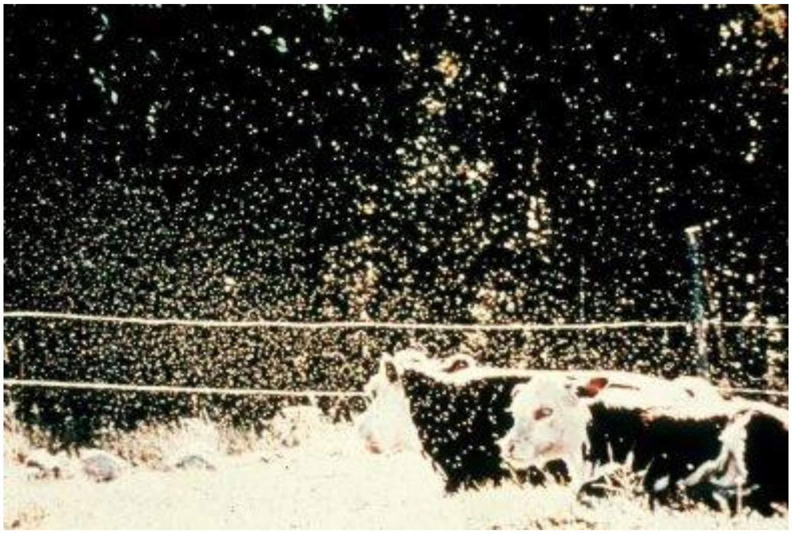
Most mandibulate insects are not strong enough to pierce human skin with their mouthparts. Their bite is usually little more than a pinch, serving primarily as a defensive behavior (e.g. ground beetles and ants). Even fire ants (*Solenopsis* spp.), whose painful "bite" is well known, lack the ability to penetrate skin. In fact, their "bite" is actually caused by a sting at the other end of the body!

All of the arthropods that can pierce human skin have mouthparts that are especially adapted for piercing, cutting, or burrowing. These include:

- Diptera (mosquitoes, black flies, horse flies, deer flies, stable flies, sand flies, and various biting midges)
- Hemiptera, (bed bugs, assassin bugs, water bugs)
- Thysanoptera (thrips)
- Phthiraptera (sucking lice)
- Siphonaptera (fleas)
- the class Arachnida (spiders, mites, and ticks).



Most of these arthropods are **hematophagous** (they feed on blood). Their mouthparts are designed either to cut the skin and induce bleeding (horse flies and stable flies) or to pierce far enough under the skin to reach capillary blood (mosquitoes, bugs, fleas, etc.). Salivary enzymes and other compounds, such as anti-coagulants, anesthetics, and vasodilators also may be injected by the mouthparts during feeding. The localized reaction to an insect bite (pain, swelling, redness, etc.) is usually a physiological (inflammatory) response to these injected compounds.



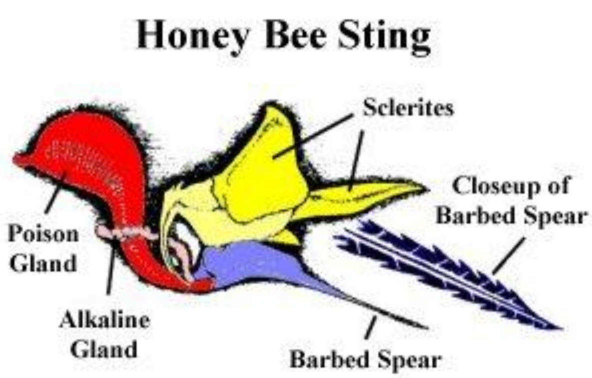
Although the amount of blood taken by each insect may be quite small, the cumulative effects of blood feeding by large populations of hematophagous insects can be life-threatening. In the summertime, small herds of reindeer living above the arctic circle have died from desanguination (massive loss of blood) caused by hoards of mosquitoes and black flies.

Some arachnids (e.g. spiders) and most water bugs (Notonectidae, Belostomatidae, and Gerridae, for example) bite only defensively. Their strong, piercing mouthparts can inject toxins or digestive enzymes that may cause pain and localized swelling. In North America, the black widow spider (*Latrodectus mactans*), the brown recluse spider (*Loxosceles reclusa*), and two species of scorpions (*Centruroides sculpturatus* and *C. gertschi*) are the only arthropods with a venom that is toxic enough to cause serious illness or death.

Itch mites, mange mites, and harvest mites (chiggers or redbugs), burrow into the skin of humans and other animals where they feed on body fluids and tissues. These infestations (variously known as acariasis, mange, or scabies) cause redness, itching, and flaking of the skin. The itch mite, *Sarcoptes scabiei hominis*, causes a severe, itching rash in humans; other subspecies of the same mite infest dogs, swine, cattle, and sheep. Infestations of various mites can become a severe economic problem in commercial poultry and swine production. The northern fowl mite, *Ornithonyssus sylviarum*, is a fast breeding pest that lives around the neck, vent, and tail of chickens. Under ideal conditions, this species completes its life cycle in less than seven days. Population growth is explosive, and often difficult to control.

Thrips have rasping/sucking mouthparts capable of abrading the skin and causing irritation. These insects are primarily herbivores, but they may be quite annoying to people who work near infested plants. Occasional reports of predatory thrips that suck blood are unsubstantiated.

Stinging Insects



Ants, wasps, bees, and scorpions are the only arthropods that have a true stinger. Some predatory and parasitic insects sting to kill or immobilize their prey, whereas most other species sting only as a defensive behavior to kill or drive away potential predators. In all of the stinging (aculeate) hymenoptera, the stinger is modified from structures of the ovipositor. The shaft of the stinger is hollow, occasionally barbed, and connected internally to a venom gland that produces a complex mixture of compounds that may destroy cells (hemolytic and proteolytic enzymes), increase blood flow (hemorrhagic enzymes), break down intercellular connective tissue (hyaluronidase), and cause neurotoxic or other pharmacologic effects on nerve cells.

Arthropod venom is rapid-acting and frequently associated with considerable pain. Most people experience an intense local reaction that subsides after several hours and heals within a few days. But for other people (estimates range from 2-5% of the U.S. population) a single sting may elicit **anaphylactic shock**, a life-threatening allergic reaction caused by hypersensitivity to insect protein or any other venom component. Anaphylaxis is characterized by rapid loss of blood pressure, fainting, and respiratory difficulty. If the patient does not receive prompt medical attention, unconsciousness and death may occur within minutes. Emergency treatment of anaphylactic shock usually consists of deep subcutaneous or intramuscular injections of a cardiac stimulant (such as norepinephrine) in an effort to counteract the physiological reaction, increase heart rate, and stabilize blood pressure. Emergency bee sting kits, containing auto-injectable norepinephrine, are available by prescription to patients with known sensitivity to insect stings. In the United States, about 20 deaths per year are attributed to stinging insects -- in nearly every case death results from anaphylactic shock, not the sting itself.

The larvae of certain flannel moths (Megalopygidae) and slug caterpillars (Limacodidae) do not have a stinger, but they also inflict a sharp, stinging pain upon contact. These insects have specialized **urticating hairs** that inject a painful chemical when touched. The sensation, like that of a stinging nettle, is an intense localized pain that gradually fades after several hours. Saddleback caterpillars (*Sibine stimulea*) are probably the most conspicuous of the stinging caterpillars. They feed on a variety of trees and shrubs, including cherry, plum, elm, and poplar.

Irritants and Allergens

Skin and eye irritation, respiratory inflammation, and various types of chronic allergies may also be caused by insects and related arthropods. An allergic reaction can be induced by nearly any component of an insect's body, but hairs, scales, exuviae, and fecal products are the most common culprits. Allergies to household dust, for example, can often be traced to dander from fleas (Siphonaptera), cockroaches (Blattodea), or house dust mites (*Dermatophagoides* spp.). Larvae of the browntail moth (*Euproctis chrysorrhoea*) have hairs that cause an irritating rash on the skin of many people.

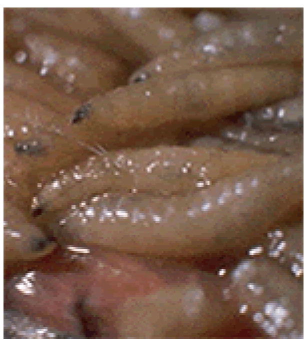
Human exposure to sensitizing antigens usually occurs in one of four ways:

- inhalation of airborne particles
- ingestion with foods
- dermal contact, or
- unintentional injection (as by rubbing the eyes)

Immunological responses of the human body vary, but sneezing, watery eyes, a runny nose, or skin rashes are common manifestations. Chronic asthma, allergic rhinitis, and eczema are frequently aggravated by exposure to arthropod antigens.

The prognosis for treatment of "arboallergies" is not very promising. One option, immunotherapy, involves successive exposures to increasing concentrations of the offending allergen in an effort to desensitize the immune system. This approach may provide some relief for some patients, but the only "sure cure" is complete avoidance of exposure to the allergens.

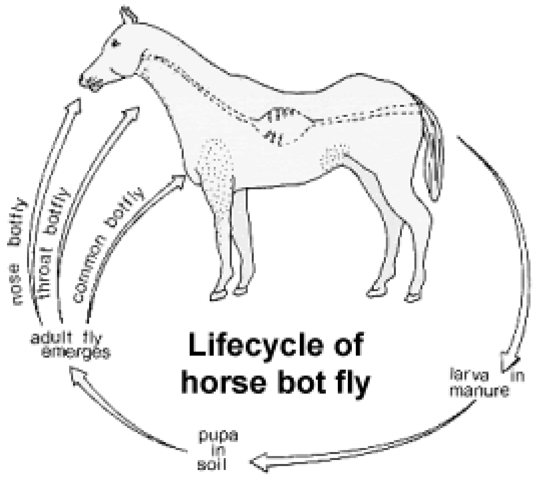
Myiasis



Of all the arthropods, only the larvae of certain flies (Diptera) are adapted to invade and consume the tissues of a vertebrate host. An infestation by any of these flies is known as **myiasis**. In North America, this type of parasitism is most common among domestic animals (particularly sheep, cattle, and horses), but it can also be a problem among the poor and elderly of the human population where it is usually associated with neglect and unsanitary conditions.

Some of these flies breed in carrion or manure; others live in spoiled food. Eggs or larvae may be ingested and survive in the vertebrate's intestinal tract, or larvae may crawl into the bowel through the anus. The screwworm fly (*Cochliomyia hominivorax*) lays its eggs in open, festering wounds. Larvae feed on these injured tissues and prevent healing. Screwworms are endemic to Mexico and the southwestern United States where they are a major pest of sheep and cattle. A sterile-male release program (see Chapt. 19) has eradicated screwworm populations from Texas and New Mexico, but ranchers in southern Mexico still suffer heavy losses from these flies.

The family Oestridae includes warble flies, cattle grubs, and bot flies whose larvae also parasitize domestic animals. Cattle grubs (*Hypoderma bovis* and *H. lineatum*) lay their eggs on the legs of cattle. After hatching, larvae burrow through the skin and migrate upward, first to the digestive system and eventually to the back where they produce swellings known as "warbles". Full grown larvae cut their way out of the warble leaving a hole that decreases the hide's value when it is tanned for leather. The sheep bot fly (*Oestrus ovis*) produces live young and deposits them in the nostrils of sheep. These larvae migrate into the sinus cavities where they complete development. Large fly populations can weaken or kill the sheep.



Larvae of flies in the family Gastrophilidae (the horse bot flies) are internal parasites of horses and mules. Most species lay their eggs around the animal's nose or mouth. After hatching, the larvae burrow or crawl into the horse's mouth and eventually move down the digestive tract, attaching themselves to the walls of the stomach, duodenum, or rectum. The horse bot fly (*Gasterophilus intestinalis*) lays its eggs on the legs, flanks, or shoulders of the horse. These eggs hatch immediately when they are licked and ingested by the horse. The presence of bots (fly larvae) in the animal's digestive system often causes malnutrition, ulceration of the digestive tract, and even complete blockage of the intestine. Mature larvae leave their vertebrate host by passing out as excrement. They pupate in the soil and emerge as adults several weeks later.

Transmission of Diseases

Infectious agents that cause illness or disease in other living organisms are known as **pathogens**. These agents may include a wide variety of microorganisms (e.g. mycoplasmas, bacteria, protozoa, spirochetes, and rickettsias) as well as fungi, helminths (roundworms and flatworms), and viruses. A **host** is any living organism that is infected by a pathogen, regardless of whether or not symptoms of the disease are present. Some hosts die quickly, others may kill or inactivate the pathogen, and still others may retain the pathogen in a condition of readiness to infect other hosts. Whenever a host serves as a source of new infections for other hosts (of the same or different species) it is known as a **reservoir**. Since pathogens do not have legs or wings, they are often "carried" from one host to another by **vectors**. Insects and related arthropods are among the most important vectors of pathogens. The term **zoonosis** (or zoonotic disease) refers to any infectious disease that is transmitted (by a vector) from an animal reservoir to a human being.

Although epidemics of arthropod-borne disease have been well-documented throughout human history, it was not until the late 1800's that insects and related arthropods were convincingly linked to the spread of human disease. In 1878, Patrick Manson first demonstrated that mosquitoes can transmit filariasis, a disease of the blood and lymph caused by roundworms (nematodes). His research was followed in rapid succession by evidence that *Anopheles* spp. mosquitoes carry malaria (Ronald Ross, 1897), fleas spread bubonic plague (P. L. Simond, 1898), and mosquitoes, particularly *Aedes aegypti*, transmit yellow fever (the U. S. Army Yellow Fever Commission headed by Walter Reed, James Carroll, Jesse Lazear, and A. J. Agramonte, 1900).

Today, we know of over 200 human diseases that can be spread by insects and related arthropods.

Despite the efforts of modern medicine, spread of arthropod-borne disease is still one of the most serious concerns facing public health officials and the medical community in general. The World Health Organization (WHO) estimates that as many as 4 million people die each year from the consequences of arthropod-borne disease. Obviously, the problem is most severe in underdeveloped countries where access to good medical care is limited. But even here in the United States, encephalitis (mosquito-borne), Lyme disease (tick-borne), and Rocky Mountain Spotted Fever (tick-borne) are still regarded as epidemic-scale problems. The Communicable Disease Control Center (CDC) in Atlanta, Georgia estimates that eight out of every ten Americans will be infected by an arthropod-borne disease sometime during their lives. Pathogens coexist in elaborate ecological relationships with their hosts and vectors. Without a vector, the pathogen could not infect new hosts, and without new hosts, the pathogen would eventually become extinct. The term **biocenosis** (pl. biocenoses) can be used to denote an ecological group that includes a pathogen and all of its hosts and vectors.

Some pathogens may have multiple hosts, reservoirs, or vectors. For example, *Pasturella tularensis*, the tularemia pathogen, can be transmitted to humans by deer flies, ticks, fleas, or body lice from reservoirs in rodents as well as other humans. Other pathogens, like the *Plasmodium* species that cause malaria, have a much narrower biocenosis -- they survive only as long as they remain within their human hosts and mosquito vectors. Finally, there are pathogens (e.g. the causal agents of cholera and amoebic dysentery) that can survive for long periods outside the bodies of living hosts. These infectious agents are often spread to humans by flies or other insects that visit sewage and garbage.

In some cases, pathogens may simply adhere to a vector's feet or mouthparts, catching a quick ride to a new host. This is known as **mechanical transmission**. Most of the pathogens that are transmitted mechanically are able to survive short-term exposure to the atmosphere and sunlight. Many types of **arboviruses** (short for **ar**thropod-**bo**rne **viruses**) are spread by mechanical transmission on the mouthparts of mosquitoes.

In contrast, **biological transmission** occurs when the pathogen survives for a time inside the vector's body, and is later spread to another host. Some of these pathogens relocate within the body of the vector, traveling from the gut to the salivary glands, for example. Others, such as the rickettsia of rocky mountain spotted fever, remain dormant in the vector's body and only become activated after feeding commences. Removing a tick within 1-2 hours of attachment usually will ensure too little time for activation and transmission of its pathogens.

Some pathogens can reproduce in the vector, and a few (the plasmodium of malaria, for example) must complete a part of their life cycle inside the vector's body.

Newly hatched insects are usually pathogen-free. After feeding on an infected host, the vector may be able to infect new hosts immediately (in the case of mechanical transmission) or there may be a waiting period (latency) of days to weeks (in the case of biological transmission) while the pathogen migrates or reproduces inside the vector's body. Once infected, a vector may retain the pathogen for the rest of its life (**persistent infection**) or may eventually eliminate or inactivate the pathogen (**non-persistent infection**).

Although vectors typically acquire a pathogen only by feeding on an infected host, there are a few species that pass infection through the egg stage from generation to generation. Such **transovarial transmission** occurs most commonly in ticks.

Arthropod-borne diseases have shaped the course of human history. Ever since medieval times, epidemics of bubonic plague have swept through the civilized world. It is estimated that nearly one-fourth of the population of Europe (20-25 million people) died of plague (The Black Death) during the 14th century. Edgar Allan Poe described the gruesome death of plague victims in his story *The Masque of the Red Death*:

"No pestilence had ever been so fatal or so hideous. Blood was its Avatar and its seal -- the redness and the horror of blood. There were sharp pains, and sudden dizziness, and then profuse bleeding at the pores, with dissolution. The scarlet stains upon the body and especially upon the face of the victim, were the pest ban which shut him out from the aid and from the sympathy of his fellow-men."

Malaria and typhus, both arthropod-borne pathogens, played major roles in the outcome of several wars. In 1815, during Napoleon's final campaign, his army suffered 105,000 war casualties but lost 219,000 fighting men to typhus. After their rout at Waterloo, one disappointed Frenchman complained that they had been defeated by "General Famine, General Winter, and General Typhus".

Forty years later, in 1856, the Russian army, weakened by tularemia after marching through fly-infested swamps in Turkey, was soundly defeated by the allied forces of Europe to end the Crimean War. 1.2 million cases of malaria were treated during the American Civil War (1861-1865) and 8,000 soldiers died. More than 100,000 troops were sidelined with malaria during World War II, yet the Allied army was in much better health than the Japanese largely due to widespread use of a "new" insecticide (DDT) to control lice and mosquitoes. Many historians believe that, even without the atomic bomb, Japan's army would not have survived much longer against the onslaught of typhus and malaria.

Demographics and civilization have also been shaped by arthropod-borne disease. Deteriorating socio-economic conditions during the Russian Revolution (1917) prompted Lenin to declare, **"Either socialism will defeat the louse, or the louse will defeat socialism."** Even today much of equatorial Africa is uninhabitable because of endemic sleeping sickness vectored by the tsetse fly. The World Health Organization estimates that more than 500 million people throughout the world currently suffer from the debilitating effects of arthropod-borne disease.

Insect Vectors of Human Pathogens

Hemiptera -- True Bugs

Reduviidae

Assassin bugs (or kissing bugs) in the genera *Triatoma* and *Rhodnius* transmit a protozoan pathogen (*Trypanosoma cruzi*) that causes **Chagas disease** in South and Central America.

Phthiraptera -- Lice

Pediculidae

Human lice (*Pediculus humanus* and *P. capitus*) spread *Borellia recurrentis*, a spirochaete pathogen that causes **epidemic relapsing fever**. They also carry the rickettsial pathogens that cause **epidemic typhus** (*Rickettsia prowazeki*) and **trench fever** (*R. quintana*).

Diptera -- Flies

Simuliidae

Black flies spread *Onchocerca volvulus*, a parasitic roundworm. **Onchocerciasis**, the disease caused by infestion of these worms, may cause blindness in peoples of Africa, Mexico, and Central and South America.

Psychodidae

Sand flies in the genus *Phlebotomus* are vectors of a bacterium (*Bartonella bacilliformis*) that causes **Carrion's disease** (oroyo fever) in South America. In parts of Asia and North Africa, they spread a viral agent that causes **sand fly fever** (pappataci fever) as well as protozoan pathogens (*Leishmania* spp.) that cause **Leishmaniasis**.

Ceratopogonidae

Punkies are the vectors of parasitic roundworms in several genera, including *Acanthocheilonema, Dipetalonema, Mansonella*, and *Onchocerca*.

Culicidae

Mosquitoes in the genus *Anopheles* are the principle vectors of **malaria**, a disease caused by protozoa in the genus *Plasmodium.* *Aedes aegypti* is the main vector of the viruses that cause **yellow fever** and **dengue**. Other viruses, the causal agents of various types of **encephalitis**, are also carried by *Aedes* spp. mosquitoes. *Wuchereria bancrofti* and *Brugia malayi*, parasitic roundworms that cause **filariasis**, are usually spread by mosquitoes in the genera *Culex, Mansonia*, and *Anopheles*.

Tabanidae

Horse flies and deer flies may transmit the bacterial pathogens of **tularemia**(*Pasteurella tularensis*) and **anthrax**(*Bacillus anthracis*), as well as a parasitic roundworm (*Loa loa*) that causes **loiasis** in tropical Africa.

Chloropidae

Eye gnats in the genus *Hippelates* can carry the spirochaete pathogen that causes **yaws**(*Treponema pertenue*), and may also spread **conjunctivitis** (pinkeye).

Muscidae
Calliphoridae
Sarcophagidae

House flies (family Muscidae), blow flies (family Calliphoridae), and flesh flies (family Sarcophagidae) often live among filth and garbage. They can carry the pathogens for **dysentary** (*Shigella dysentariae*), **typhoid fever**(*Eberthella typhosa*), and **cholera** (*Vibrio comma*) on their feet and mouthparts. They have also been suspected as vectors of the viral agent that causes **poliomyelitis**.

Glossidae Tsetse flies in the genus *Glossina*transmit the protozoan pathogens that cause **African sleeping sickness** (*Trypanosoma gambiense*and *T. rhodesiense*).

Siphonaptera -- Fleas

Pulicidae Rat fleas, especially *Xenopsylla cheopis* (the Oriental rat flea), are the principle vectors of *Pasturella pestis*, the bacterial pathogen of **bubonic plague**. Fleas can also transmit **murine typhus** caused by *Rickettsia mooseri*.

Pesticide Resistance

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Photograph by Scott Bauer.

Reasons for Pesticide Failures

- The pest was not identified correctly and the wrong pesticide was used
- An incorrect dosage of pesticide was used or the pesticide was applied in an improper manner
- The pesticide was not applied at the appropriate time (i.e., pest was not in the area at the time of treatment or was in a life stage not susceptible to the pesticide)
- Pests re-infested the area following the pesticide application

Terrapin scales on blueberry.
Photographs by John Meyer.

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Reasons for Pesticide Failures

- Pest Resurgence: the natural enemies (i.e., predators and parasites) of the pest as well as the pest are eliminated by a pesticide application, the natural enemy populations can take longer to rebound than the pest population, therefore pest populations increase rapidly as the pesticide residues decrease
- Secondary Pests: certain pests that usually do not occur at significant levels can reach damaging levels after a pesticide application because their natural enemies are eliminated by the pesticide
- Pesticide Resistance: after repeated applications, pests become resistant to one or more pesticides

Pesticide Resistance

- Humans have been using pesticides for thousands of years to control pests
- In the last 50 years, synthetic pesticides have been used widely to combat pests of agriculture and public health
- Early success with synthetic pesticides (such as DDT) suggested that the war on pests had been won by humans

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Pesticide Resistance

- However, very soon the effectiveness of synthetic pesticides began to decline
- Increased use of these pesticides resulted in an even more rapid decline in effectiveness
- Pests had become resistant to synthetic pesticides

Jack Bacheler

Bill Tarpenning

Classic Examples of Pesticide Resistance

- Resistance of mosquitoes to DDT and other insecticides
- Resistance of corn earworms to multiple insecticide classes

P. Koehler, University of Florida

USDA/ARS

Magnitude of Pesticide Resistance

- Over 500 species of insects
- Over 270 species of weeds
- Over 150 plant pathogens
- About 6 species of rodents

Dale Monks

Mike Stringham

Jim Baker

How Do Pests Become Resistance?

- Pest population is exposed to pesticide
- Some members of the pest population survive because of a genetic predisposition to be resistant to pesticide
- Surviving members of the pest population pass along the genetic resistant to their offspring

Brian Nault

Bill Tarpenning

Factors Contributing to Pest Resistance

- Rapid reproduction rate of pests (i.e., insects that have many generations per year)
- Repeated applications of the same or similar pesticides
- Use of “broad-spectrum” pesticides (i.e., insecticides that kill all insects, including beneficial insects)
- Pesticides used as the sole means of pest control

Jim Baker

Jack Bacheler

Multiple Pesticide Resistance

- Multiple pesticide resistance (“cross-resistance”) is the resistance of pests to more than one pesticide or pesticides in more than one chemical class (e.g., DDT and pyrethroid insecticides)

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Combating Pesticide Resistance

- Use an integrated approach to pest management not overly dependent on pesticides (e.g., cultural practices, biological controls, pest monitoring)
- Use pesticides judiciously (i.e., only when needed and at the lowest rate necessary to achieve control)
- Alternate the use of pesticides in different chemical classes with differing modes of action against the pest

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