PHEROMONES By Tim Braun

The term “pheromones” denotes chemicals emitted by living organisms to send messages to individuals of the same species.

Communication

Animals communicate in several ways and the human animal has gone to great lengths in ways to communicate. If you are using a computer to take this course you are using just one of many methods of communicating. Even computers are set up to communicate with one another.

Just recently in the August 14, 2010 issue of Time there was an article on how apes are taught to communicate with people. In this case a form of communication by the ape was done on laminated sheets with hundreds of colorful symbols that represented all the words that they’d been taught since they were small baby apes. In this particular case the name of the ape was Kanzi who used the finger pointing and arm movements to communicate with the people that worked with him. He had a vocabulary of 384 words using this icon symbol method.

I had a dog that could follow my finger pointing signals when he couldn’t find something. I would point to the object until he located it. Hunting dogs communicate with their human hunter about where the hunted bird is located in the brush of the field by pointing with their entire body.

Some species of flies use their huge eyes looking for females from a perch where they can easily see other insects. If a flying insect looks like it may be a female of the same species the male takes off and they meet. If they are of the same species and they are in a mating stage of life, they mate.

Butterflies are a good example of male and female attracting the opposite sex. Their colorful wings tell the courting butterfly what their species is for mating. Fireflies are the some of the best visual senders of messages. The female firefly flashes her codes to describe her species, sex and that she is ready to mate. Then the right male firefly will flash back the fact that he is of the same species and ready to mate. These flashing messages go on and on until the two fireflies find and mate one another.

There is also the use of noise that is music to the right ear that receives it. The male cricket makes most of the noise or music by rubbing their forewings together giving off that loud, raspy sound, but once he gets a female next to him the sound he makes is a softer courtship sound. I’ve tried hearing that softer sound but I guess I don’t hear as well as his female mate.

The cicadas’ songs are another form of communication between those insects that are ready to mate. The so called periodical cicada males come together forming large masses. There are usually about three different species in the huge mass of cicadas and they are all singing together. The female instantly knows which male cicada is her species and who is right for her in spite of the loud noisy chaos that exists.

The mole crickets that live in the ground build their entrance tunnels so that they are amplifiers to increase the volume of the sound coming from the rubbing together of their forewings. The death watch beetle, that feeds on old wood, bangs his head against the roof of his tunnel that he has bored into the wood. This sound coming out of the tunnel tells the female death watch beetle what the males species is and that he’s ready to mate. Other male insects just tap on anything that will give off a particular noise to communicate that a female of their species will know that he‘s ready to mate.

Chemical Communication

According to Wikipedia, the free encyclopedia, Semiochemical is defined as a chemical substance or mixture that carries a message or a form of chemical communication. These chemicals act as messengers within or between species. The word semiochemical comes from the word semeon which means “signal” in Greek. The term semiochemical is used in the field of chemical ecology and it includes the words: allomones, kairomones, attractants, repellents and pheromones.

Plants even though they don‘t move about or fly under their own power do communicate with other plants and even with insects and microbes. Researchers have been looking at this form of communication for several years. There isn’t any sounds or visual communication, but there is some chemical communication.

Plants communicate with the use of an allomone. Allomones are released by one species and affects the behavior of a member of another species to the benefit of the originator. Allomones produced and left in the soil by disked Sudan grass grown in the Yuma summer months will affect lettuce seedlings when the lettuce seeds are planted before the chemical, allomone, has broken down in the soil. The lettuce seedlings tend to gain yellow tinged leaves and they have the appearance of miss-applied pre plant fertilizer applications or residual herbicide damage. I have had several complaints from my application customers who mistake these allomone caused damages on herbicide and fertilizer pre plant applications. In most cases the lettuce outgrows the damage.

The chemicals, allomones, are selective. Black walnut trees will allow lima bean plants to exist nearby, but they put out an allelopathic chemical that will kill the tomato, eggplant and pepper plants growing near the tree.

Theophrastus, a Greek botanist, gave a written account in 300 B. C. reporting how chickpea exhausts the soil of nutrients and kills surrounding weeds. Research at Kyoto University in Japan showed that when lima beans are attacked by spider mites the beans released chemicals that lowered their flavor that the mites are attracted to. This same released chemical when released to the air in the field that landed on other lima bean plants caused those lima bean plants to release chemicals that lowered their flavor for the feeding mites. This is a case of one species, lima bean plants, releasing a chemical to cause harm to another species, namely an insect in the animal kingdom.

At the University of California at Davis, California, my old alma mater, researchers “Karban and Shiojiri, trimmed leaves on some sagebrush plants to copy the damage done by leaf feeding insects like grasshoppers and caterpillars. The damaged sagebrush produced chemicals that the insects found repulsive. The leaf feeding insects were repulsed by the chemical emitted by the plant when the plant had leaf feeding damage done to it. The chemical that the sagebrush emitted also caused the neighboring tobacco plants that hadn’t been damaged to activate enzymes that made their leaves repulsive in taste to the insect pests. In the tests the researchers found that the tobacco plants next to the clipped sagebrush had 60% less insect damage then tobacco plants next to sagebrush that hadn’t received the clipping damage to their leaves.

Kairomones are chemicals or chemical mixtures the are given off by a plant that causes a response of another species that is good for the one receiving the chemical. This is just the opposite reaction produced by the allomone that is bad for the one receiving the chemical. An example would be a chemical or mixture of chemicals given off that allows the receiver of the chemical to identify the giver of the chemical as something good to feed on or something that should be avoided because it could hurt the feeder.

Some trees like pines have pine needles that that emit chemicals that guide beetles to the tree. These chemicals will even notify the insect about the nutrition value of the tree whether the tree is still worth boring into or has lost most of its nutritional value. These emitted chemicals help the insect do damage to the species that emitted these chemicals. These chemicals are referred to as kairomones.

Kairomones are still in existence and that is a reason to know that they are in some cases good for the living thing that produces these chemicals. Otherwise the living plant or insect would not still be in existence in this world. Evolution doesn’t allow death to the emitters of good things to others or death to all the emitters. Karomones exist to inform their emitter of something that can be eaten. In the evolution of things the consumption of the thing getting eaten cleans up the forest of old trees that can cause diseases and cause fires to the forests. This result is good for the forest and that could be why some good comes out of what is considered bad for the poor old tree.

Attractant chemicals given off by one species causes the others species to desire making contact with the attracting species. The repellants given off by one species causes the other species to stay away from the reppellant emitting species.

History of Pheromones As Pest Control Aids

Communication between insects has been going on for millions of years, but we in the pest control industry have just started using this fact in the last half century. In the 1870’s a French priest, Jean-Henri Fabre, discovered the activity of insect pheromones when he observed some male peacock moths flitting through the open windows of his laboratory to land on a mesh cage that a female peacock moth inhabited. Fabre moved the female’s cage around the lab, hiding the female from the males, but the males always found her. Fabre carried out several experiments trying to find out how the male butterflies were able to find the female. After a great deal of research Fabre concluded that the female must be giving off some type of odor that was attracting only her species of male moths.

About that same time in New York, an entomologist, Joseph A Lintner , decided that he had the same thing happening when he placed a female spicebush silk moth on the office window sill. Several male spice bush silk moths crowded the window sill. Lintner decided that the female was emitting some sort of chemical that was drawing the same species of male moths and decided to get chemists involved. He hoped to create a new way of controlling insect pests. But in that day and age chemistry was not advanced enough to produce these chemicals.

In the 1930's in Czechoslovakia female deciduous fruit pests were used as bait to trap males in an attempt to monitor the population of the pest in orchards. Knowing a pest was present he then applied pesticides to the orchard. Later on at the Kaiser Wilhelm Institute for Biochemistry in Germany Adolph Butenandt decided to discover what chemical substance these moths emitted to attract male moths of their species. Butenandt was already famous for discovering the human sex hormones: esterone, testosterone, and progesterone. Like Lintner he hoped, that this would open up a new field of chemical pest control. For the next 30 years that included World War ll, Hitler's reign over Germany and the long recovery of Europe, and the world, Butenandt kept working on his new way to control insect pests.

At first Butenandt cut the tips of the abdomens of silkworm moths and ground them up. He used certain known chemical methods to isolate the chemicals from the ground-up mass of material. Then he tested these various chemicals on male silkworm moth males. Because in captivity the male silkworm moths were not able to fly they did flutter their wings when they were tempted with the chemicals that he isolated from the slurry that was produced from the abdomens of the female silkworm moths. These silkworm male moths reacted the same way when female silkworm moths were brought near them. This was the proof he needed to announce to the research organizations that he had isolated a mating material substance. This substance that Butenandt produced over the long thirty years he named Bombycid. Because silkworm moths have the Latin name of Bombyx.

Butenandt's final results happened in 1959 and at that time another German biochemist, Peter Karlson along with a Swiss entomologist, Martin Luscher came up with the term "pheromone". This is a Greek word for "carrier of excitement". Pheromone is now used to describe the chemical substance that animals emit that causes other animals of the <u>same species</u> to react in a certain way similar to the fluttering of wings that the silkworm male moth displayed. These scientists, Karlson and Luscher were experimenting with chemicals that termites emit to maintain the termite's unique caste system within each colony.

Other scientists were inspired by Butenandt's success and they continued to research and locate pheromones produced by other animals especially their affect on the physiological development of an insect. In 1961 at the Rothamstead Experimental Station in London Colin G. Butler tested the gland secretions of the honey bee mandibles to see if the secretions inhibited the worker bees from constructing special queen bee rearing chambers. It was already known that queen bees emitted a material that stopped worker bees from rearing other queens. It was also known that other than a few male drones for breeding purposes the bee hive population mainly consists of female bees. Butler was able to identify a specific pheromone that was produced by the queen bee that prevented the rearing of other queen bees but also a pheromone that stopped the other worker female bees from developing ovaries. Queen bees use this pheromone until old age and disease prevents them from using this method of bee hive control.

The need for pest control methods was greater than the use of pheromones for the study of beneficial insects like the silkworm moth and honey bees. Researchers were able to identify pheromones used as attractants for the pest that included: black carpet beetle, California 5-spined engraver beetle, the sestern pine beetle, cabbage looper moth and a leaf-cutting ant by using behavioral assays.

But scientists discovered that this method was a slow and limited research tool. They needed the availability of a more general test. In 1953 Peter Karlson had asked Biologist Dietrich Schneider to use Schneider's expertise in electrophysiology to develop an electrical means of detecting pheromones. During this period of time researchers had suspected that most of the moths were using their large antennae to detect pheromone molecules in the air around them.

Schneider decided to use these antennae as “sniffers” of pheromones. He felt that they would respond to the pheromone chemical with a tiny burst of electricity which is a usual response of nerve cells when they are stimulated. He removed antenna from a male silkworm moth. Kept it fresh with a bath of salt water. Put it between two electrodes that would sense any electrical activity. Then he gave the antenna a shot of air that had been swept over a material that he knew contained the pheromone, Bombycid. There was a peak of electrical activity in the antenna which was a reaction to the material containing the pheromone. Schneider called the pheromone prompted response of the antenna of the insect an “electroantennogram“. This work was reported by Dietrich Schneider in 1957.

The research at that time, late 1960’s, still was not producing any useable pheromones for insect pest control. The synthetic pheromones that were produced worked in the lab, but failed to do the job when tried in the field. The crude form of pheromones produced from female bodies didn’t attract male moths when isolated as a method of attracting males because they supposedly didn’t hold up long enough.

In the 1960s two researchers joined together in identifying a pheromone that causes both male and female bark beetles to aggregate in massive populations in specific pine trees. The two scientists were Robert Silverstein from the Stanford Research Institute in California and entomologist, David Wood at the University of California at Berkeley. As the beetles tunneled through the bark of the pine trees a pheromone was identified that attracted larger and larger masses of the beetles until they conquered one of the tree’s main defense, the resin that is created by the tree coming from the wounds of the tree. This defensive resin is a “repellant” given off by the invaded tree.

Because he thought the pheromone causing the mass aggregation must be in the sawdust from the borings done by the bark beetles mixed with their fecal matter. Wood sent about ten pounds of this mixture, or frass, to Silverstein in his lab for a chemical analysis of the contents.

To determine which component of the mixture contained the pheromone Wood and Silverstein observed which one of the components would attract the beetles when they were exposed to it. They placed each component in a position and when the beetles walked upwind toward the component they knew the chemical component attracted the beetles. When they broke down and fractionated the attractive component to three main chemical compounds they found that each independent compound made no effect on the beetles. When they mixed two of the chemical compound fractions that they had and did the same test in the lab the beetles were attracted to the mix.

When Silverstein and Wood tested the same combined two compounds in a field study the same combination of the two compounds failed to attract the intended bark beetles, but it did attract another different beetle named *lps latidens*. Then when they mixed the three isolated chemical compounds and ran the same test in the field the three compound mixture as a lure attracted the same amount of the intended bark beetles as when using live bark beetles as a lure. However this mixture of the three compounds did not attract the *lps latidens* species of beetle.

Other pheromone researchers got the same results when going back to the lab chemical compounds that failed in field tests. This technique of using all the different fractions of the chemical compounds required more time, but they found that adding and mixing pheromone chemical compounds gave improved results in the field tests that had previously failed in field tests, but were successful in lab tests. During the 1970’s the production of pheromone chemical compounds took a complete new turn in the intricate development of the compounds that were needed. Just adding a few extra components to the compound improved the field results.

In some cases the added component had the same chemical structure but the shape ended up being a *mirror* image and produced different or opposite results. In Japanese beetles a contamination of just 1% of the pheromone with a contamination of its mirror image ended up diminishing the pheromone’s needed results. (The New World Dictionary states that a mirror image is an image or a view of someone or something as seen in a mirror with the right side as though it were the left.)

Mirror images remind me of the term “Dead Ringer”. At one time morticians found that certain doctors were pronouncing some of the corpses that they received from these doctors as dead. But when some of these corpses were later investigated the morticians found that the people pronounced dead weren’t dead after all. They had tried to claw their way out of the closed coffins. The morticians started tying a string attached to a bell to the wrist of these supposedly dead people that they received from these doctors. If the bell started ringing they knew that they didn't have a dead person. They had a "dead ringer". The person looked and had the same structure, but they didn't act the same. This was true of the mirror imaged pheromones. They have to be field tested even though they look the same, but the pheromone may be a mirror image.

Mixing the ingredients of the compounds have to be done in the precise amounts. Any mistake in the mixing of the pheromone compounds will not result in an attraction to the target insect and this error may result in the attraction of an entirely different species.

The pheromone products in the 1960's and 1970's were increased at a more rapid pace with the new methods of testing and production that became available for pheromone researchers. The use of gas chromatography, nuclear magnetic resonance and mass spectrometry were used to separate components in vapor based on how fast they travel through columns that have absorbent materials that collect and then identify the chemical compositions. The gas chromatograph <u>separated</u> the components and the nuclear magnetic resonance and mass spectrometry were used to <u>identify</u> each of the chemical components.

A large group of pheromone research scientists were involved with identifying the codling moth pheromone. This was in the year of 1970. The codling moth is a pest of apple orchards. The particular pheromone of this insect pest was very hard to analyze and produce as a chemical compound that could be useful. There was a great deal of work put into this research. An analysis of the contents of nearly 500,000 glands of female codling moths were analyzed without producing a pheromone.

Then in 1971 a researcher by the name of Wendell Roelofs with his fellow reseachers at Cornell University used a different approach and identified the pheromone of the codling moth. They isolated extracts from the glands into different factions with gas chromatography as a first step. Then they tested each fraction using Schneider's electroantennogram method to find out which fraction held the pheromone. After doing this other researchers would use spectral analysis to isolate the pheromone that was in the fraction.

This was a slow process that required excess labor. Wendell Roelofs and his fellow researchers saved time and labor by testing a library of all possible mono-unsaturated compounds that are related to the known pheromones. They used the electroantennogram of codling moth antenna to test each of the library compounds. As they approached the actual structure of the codling moth pheromone the ellectroantennogram response increased by peaking in response to two library compounds. Each of these compounds contained a double bond. This information led to the prediction that the codling moth pheromone contained both double bonds in one compound. When this identification of the codling moth pheromone was announced by Wendell Roelofs and his Cornell University team many did not believe them. These results were not accepted until the use of the conventional, slow and labor intensive tests were run thereby confirming the same results. This new method of pheromone research was then accepted in 1971.

These tests were considered to be physical tests. The techniques were used in combinations. Gas chromatography used with mass spectrometry so that researchers could separate and identify the pheromone components in the mixtures. They could use the gas chromatography with the ellectroantennogram. This allowed the researchers to see which component in their insect preparations would cause an electrical response. The development of capillary gas chromatography gave the researchers a tool to separate compounds that they previously couldn't have done before these tools became available.

In the 1930's an English zoologist named John Kennedy was studying the insect behavioral activities. He developed a wind tunnel to observe how insects orient and move up wind. 40 years later in 1970 Kennedy during the active research going on with pheromones, decided to use his wind tunnel to find out how insects track pheromones to their source.

Kennedy's wind tunnel was a clear plastic tube in which an odor is released in one end and blown by a fan through the tunnel. He already knew from previous studies that the yellow fever mosquito used its eyesight to orient its movements when flying through the air when following the trial of an attractant. He set up his tunnel with a moving floor to simulate the change in location of the flying insect's flight path as it moved through the tube. Kennedy found that moths use the same visual information of their surroundings when following pheromones.

Researchers being what they are have made tremendous strides in the knowledge of chemical communication. The advancements made by scientists who learned how to handle very tiny amounts of chemicals plus the increase in electronics and computers has produced a record of 3 moth pheromones by 1965, 20 pheromones by 1970, over 40 pheromones by 1975 and more than 100 insect pheromones by 1978. We now have in this day and age a number greater than 1000 pheromones available. These known pheromones that have been isolated include other species besides the insect pheromones. Known pheromones for algae, spiders, fishes, crustaceans, amphibians, reptiles and mammals are now available

Human pheromones have been developed. Dr. Winnifred Cutler was one of many researchers to realize that there was a male pheromone that helps produce reproductive health in women. As I was reading the February 2010 issue of the magazine, Smithsonian, I found an add in the gift guide section for pheromones with the ATHENA PHEROMONES label. The add has a picture of Dr. Cutler as the creator of the pheromone and a co-discoverer of human pheromones in the year 1986.

Biosynthesis Of Pheromones

Enzymes that are used by insects to produce pheromones are found in mature insects and are controlled by a neurohormone. Once the process of biosynthesis of pheromone production starts the process occurs in the pheromone glands.

How the insect produces the pheromones is referred to as 'biosynthesis' and it is a very involved process. The chemical is basically produced from the food consumed by the insect. In some cases the food consumed by the insect has a great deal to do with the chemical make-up of the pheromone. If the insect is putting out a pheromone to draw other insects to the food that the insects is feeding on the pheromone will change as the food loses its taste value as the food ages.

There is some evidence that the insects internal bacteria and other body microorganisms break down some of the food to chemicals that go into the manufacture of the pheromones. Researchers have used bactericides to kill the microorganisms in insect bodies to see if the pheromone production of these treated insects could still produce pheromones without the help of their internal microorganisms. The insects treated with these bactericides in most cases continued to produce pheromones without the treatment. Also many of the insects needed the internal microorganisms to thrive. Not to make pheromones. Killing the microorganisms in many cases caused the death of the insect.

Fatty acids are parts of the food that the insect has digested or broken down into building material for the construction of pheromone molecules. Insects utilize hormone messengers to regulate their manufacture of pheromone. We do the same or similar processes in our bodies but we are not aware of the chemical processes. So even if the insect produces the pheromone or for that matter if we as humans produce certain chemicals in our bodies that we use in growth or digestion, we are not conscience of the ongoing processes. Various insects do these chemical processes, but they do not mentally initiate the manufacturing process . They don't say "I'm going to do this". Their individual species gene controlled enzymes initiate the activity and the chemical building of the pheromone is carried out by the system in the body of the insect. The building system is a program laid out by the genes of the specific insect.

When the biosynthesis of a pheromone using a fatty acid the fatty acid is stripped down to its skeleton shape. Then parts are added. The parts make up the building or the molecule. In this case the finished building or molecule would be a pheromone.

Because insect pheromones are used as a major form of communication between species the pheromones are required to be specific to the species. Only insects of the same species have the mechanism to identify what the message produced by other insects of their species means to them. The chemical causes a definite reaction in their system. This specificity is caused in two ways: The structure of the molecules is specific for the species and the blending of the molecules is specific. The right structured molecule and the unique blend of molecules that make up the pheromone are required in order to send the exact message to an exact mate that can interpret it.

In many cases the chemical may already exist in the food that the insect has eaten. Then the chemical can be used by the insect in its pheromone. This reduces the energy needed to build a new complete pheromone molecule. Most of the completed and used pheromones consist of more than one chemical molecule.

The insects food plays a very crucial part in its manufacturing of pheromones. One of the insects where the manufacturing of commercial synthetic pheromones is almost impossible is the Mediterranean fruit fly. This is because of the wide number of foods that the fruit fly feeds on. Researchers have isolated a food host range of more than 70 compounds. This increases the cost of making the numbers of pheromone chemicals to a tremendously expensive amount.

The pheromone molecule is made up of chain type structures. The short chain molecules are more volatile and affects the evaporation of the pheromone to the atmosphere. The longer chain molecules are less volatile and are more persistent. The less volatile would be used for laying trails. Alarm pheromones are smaller molecules and must be volatile to spread rapidly. Alarm pheromones are not as specific as the sex pheromones. Sex pheromones are specific and volatile. Aggregation pheromones are longer lasting. All of the pheromones produced by insects break down in time. Depending on the pheromone the breakdown can be very quick.

This biosynthesis or manufacturing of the pheromone takes place in the insect’s glandular cells. These glands are located in several parts of the insect’s body located in the epidermal or outer surface of the insect’s head, thorax and abdomen. In the younger insects the glands are located in the base of the epidermal cells. As the insect matures the glands enlarge toward the cuticle or outer cover of the insects body. In most cases sex pheromones are only present in insects who are old enough to mate. The age and development of the testes and ovaries as well as the food consumed by the insect determines the timing of the release of the pheromone.

Because these pheromone glands contain genomes that regulate what type of pheromone produced and genomes in the receiving insect regulate what type of pheromone it accepts there is some genetic research going on to determine how much the pheromone biosynthesis or manufacturing of pheromones is involved in species selection and change. This could effect the immunity of insects to certain control methods especially when it comes to sex pheromones where breeding is the final result.

After the manufacturing the pheromone is held in folds or wrinkles of the cuticle. Usually sex pheromones are not stored and they are used right after they have been synthesized. The stored pheromones would include the marking pheromones used by ants.

The cuticle or exoskeleton of and insect cover the surface of the insect. It is made up of a material called chitin and is composed of chitin filaments imbedded in a protein material that is nonliving unlike the living cells of the epidermis which the cuticle covers and protects. The cuticle is molted periodically and discarded. It can be very hard in some places and very soft and flexible in other areas covering the insect’s body. The glands producing insect pheromones are located in several parts of the body including: the legs, head, and the abdomen.

Release of Pheromones From The Insect’s Body.

Temperature has an effect on the calling and release of pheromones. As mild temperatures set in the amount of calling and release of pheromones increase. Cooler temperatures are more inducive for insects as a time of pheromone release and a period of calling. Moderate temperatures and warm days are not as good for pheromone release as cool night time temperatures and this timing is also conducive to insect mating activity.

The pheromones are released from the glands that have cells in clusters forming tubes to the outside of the body in some cases. The cell clusters that are referred to as secretory cells channel the pheromones to the outer air. In some cases the pheromone is slowly released as a liquid to objects, like stones or leaf stems. In the case of ants the liquid is rubbed on the surface of the area that the ant is traveling on. It could be a limb, leaf, soil or counter top in a kitchen. Bees have mandibular glands for pheromones and bumblebees release their pheromones by biting objects that the spread their pheromones onto. Some of the male moths use their wings to emit their pheromones when in contact with the female moths of their species.

Pheromones can be released in a variety of ways. The variation of release of pheromones by insects include: in single liquids or in blends as streams, droplets, gases or fine films. Some beetles mix their aggregation pheromones with their fecal matter. The pheromones used for mating can be released as gases that can be carried by the wind for long distances. There are instances where male silk worm moths have traveled distances of 30 miles following a sex pheromone trail in the sky.

Receiving Pheromones

Pheromone reception by insects is done by cells that are surrounded by cuticle tissue that is called sensilla. This sensilla tissue is made up of four chemical sensitive body parts which include hairs, pegs, plates and pits. They are located on various parts of the insects body: antennae, mouthparts, ovipositor (egg laying part of the insect's body) and cerci (appendages at the rear end of the insect's abdomen or stomach).

Out of these body parts of the insect used to receive pheromones the antennae is the organ that is most used by the insects. It is said that a single antennae may have over 8,000 classes of sensilla and this huge variation in classes of sensilla allow the insects to respond to a huge range of chemical stimuli that are present in the environment where the insect exists. The fact that the insect has two identical antennae positioned atop the insects head could increase the sensitivity and efficiency of the system that receives these pheromones. This placement on the head of the receiving insect more than doubles the reception of the pheromone molecules.

Pursuing Pheromones

As mentioned earlier in my history notes in 1970 on an English researcher. John Kennedy, with his wind tunnels observed the actions of the insect that is pursuing the wind carried pheromones. The wind tunnels used for this pheromone research isn't just a tunnel with wind blowing through it. In his tests with mosquitoes John Kennedy found that the mosquito used visual differences of its surroundings to orient their direction in relation to the wind direction. He set his tunnel up with a moving floor that had a broken pattern on it thus giving the researched insects something to visually see and guide itself in the wind.

Capturing Pheromones

As insects take in the right pheromone while in flight, they are stimulated by the pheromone molecules that enter their antennae. They get an actual electric stimulation of their body when their antennae takes in the pheromone. This stimulation encourages the insect to fly in the direction where they receive the pheromone molecules that trigger the insects nerve telling the insect that it is on the right track..

Insects intercept the pheromones from the air with their antennae. The greater the surface available for making contact with pheromones the more pheromones they can detect. The antenna of an insect isn't only used to catch pheromones. Some insects use their antennae to catch prey or hold females for mating. Usually the male insect has the larger and more adaptable antennae when compared to the female. The male is required to catch the pheromones of females who emit sex pheromones. The (*Nezara viridula*) southern green stinkbug male emits its own sex pheromone to females.

Pheromones are not the only chemicals captured by the insect antennae. Female mosquitoes use their antenna to catch carbon dioxide molecules thus giving them a method of locating a host for their blood meal. As animals put out carbon dioxide as they breathe the female mosquito picks up the carbon dioxide molecules with her antenna and follows the trail to the source of her blood meal. A female mosquitoes needs a blood meal in her diet to produce eggs.

Insect antennae vary in their ability to catch chemical molecules, but the pheromone molecule is made to do the job by itself. The antenna has to catch only one pheromone molecule out of the mass of pheromone molecules emitted by the male. The molecule after making contact with the antennae hairs enters pores in the hairs. Then the pheromone molecule travels to a gel made up of proteins that surrounds the cell nerve ends in the antennae. Antennae pheromone-binding proteins in the gel only attach to specific pheromone molecules of the same species which makes them ideal for IPM pest control programs. Predator insects are not involved because of the difference in their pheromones when compared to the target insect pest specie.

Pheromone Reactions In The Insect

The captured pheromone molecule and the binding protein react with the neuron cell sending the message to the male insect's brain telling him he's on the right course. During this reaction the pheromone molecule and binding protein are broken down. This cleans out the antennae allowing another pheromone molecule to enter the antennae pore. The male flies through several specific female pheromone bursts constantly changing course to get to the female.

If the binding protein was blocked with an enzyme the male couldn't clean out his antenna pore to catch new pheromones from the female. According to research report in the UC Davis News & Information dated August 2004 this binding protein in the antennae can be blocked with a chemical enzyme they have discovered. The use of this new chemical that blocks the binding protein could reduce mating and pest populations.

If the insect while flying upwind into the cloud of pheromones loses the stimulation the insect either stops and waits for another cloud of that same pheromone or it starts flying cross-wind back and forth endeavoring to pick up the cloud of stimulating pheromones. Kennedy found that the insect while following this trail has to use its visual equipment to know what direction it is going in and how far it has flown while trying to stay in the right direction to intercept the wind carried pheromones. Aircraft pilots use their eyes to check their flight instruments. There are ground stations that put out information telling pilots what their direction is in relation to where they want to fly. Some automobiles are equipped with satellite instruments and we are orally told where we are and what turns to make. We then use our eyes and ears to find our way to wherever we have a desire to go. Our nerves are stimulated putting our muscles to work in using the instruments that we have to get there.

After Pheromones Effects On Insect’s System

Research work done on (*Nezara viridula*) southern green stinkbugs that have found the vicinity of their prospective mate from the intake of pheromones starts using other forms of communication methods to finalize their mating process. If the two are on a plant they begin using vibrations. Then the use of calling or court ship songs that are specific to their species. Male and female use both vibrations and songs when they get close enough for these two forms of communication to work. Researchers who tested southern stinkbugs with natural and artificial signals found that female calling increased the release of male pheromones. The researchers were able to collect the male pheromone with fibers for solid phase micro extraction.

In this same research on the southern green stinkbugs it was found that when rival males in the vicinity put out mating songs the pheromone release of the males remained stable. The output of songs by rival males caused a reduction in emissions of pheromones by the courting males. According to the researchers this ability to control their pheromone emissions could reduce the amount of energy output by the male insects allowing them to save vital energy.

Sex Pheromones

Most of the insect sex pheromone are produced by the females. The male emitted sex pheromones often attract both sexes and are referred to as aggregation pheromones and sex pheromones. In the 1930’s live females of deciduous fruit crops were used as bait to trap male fruit pests to monitor the infestation of the crop. Now orchard growers use the synthetic sex pheromones that have been developed.

Male sex pheromones are usually found in Coleoptera insects or beetles. Female sex pheromones are dominant in the Lepidoptera insects or butter flies.

Some research on the sex pheromones is pointing out the possibility that the pheromone differences within a species led to some of the evolution changes that has occurred in insects. The female pheromone if changed slightly due to the gene make up of her glands that produce the pheromones that she emits and a male with sensilla cells in his antennae that due to his gene makeup will accept her pheromone then a genetic change will occur in their offspring. Usually the male of insect species carries the gene changes to the young that is produced by the sex act producing an egg that grows into a genetically different insect. In this case the male and female could both cause a change in the species. Genetic changes in insects is the cause of the immunity to our pest control endeavors. If what the researchers are finding in the work they are doing with genetic changes due to gene types in pheromone production we need to know how to spot it and curtail it. This would be especially important when using growth regulators where we work on species differences in insect growth stages.

Alarm Pheromones

The insects that use alarm pheromones include: aphids, ants, bees, termites and wasps. Mites (spiders) also use alarm pheromones. The alarm pheromones are emitted from the mandibles, anal glands or stinging part of the insects. The alarmed insects emit alarm pheromones “alarmed aphids flee”, “alarmed bees attack”. The pheromone causes the insect to disperse or be aggressive toward the source of the emitted pheromone. Large amounts of the pheromone are released and in the case of attack, the spray often reaches the attackers and is used to identify them as attackers. Physical reactions include raised heads or antennae, running, movement to the source, biting and stinging.

The alarm pheromones are very volatile and contain small chained molecules. They are synthesized from food that the insect consumes. Ants have a variety of alarm and attack pheromones as have the bees.

Wasps have less specific alarm pheromones and in some wasp species the wasp will not attack, but will hide when encountering alarm pheromones. The only alarm pheromone used in pest control is on the Russian wheat aphid. When released commercially for pest control it causes the aphid to pull its feeding tube from the plant and leave.

If you've observed persons working with active honey bees you have probably seen them use smoke making equipment. Smoke is used by beekeepers to mask the bees' alarm pheromones that signal bees to attack. Honey bees are a so called social insect like ants and termites where pheromones are vital in coordinating the activities of the colony. On the body of a bee there are 15 known glands that produce pheromones. The forager bees have their alarm emitter located next to the stinger shaft where they secrete a pheromone to alarm other bees to attack.

Aphids can and do defend themselves to a certain extent. Aphids will kick at an attacker and try to discourage any attack. The aphid will pull up their mouthparts from the plant they are feeding from and walk away and this includes jumping off of the plant. Some species of aphids can also spray out a waxy solution on their attacker.

During these defensive actions the aphid will emit a alarm pheromone alerting the surrounding aphids to the attack. Some species of plants have been found to put out the same pheromone to drive aphids off of their leaves. The aphid alarm pheromone is emitted in short bursts by the aphids whereas the plant pheromone is constant; therefore the aphids are not usually alarmed by the plant pheromone. Researchers have tried using plant pheromones to mimic aphid alarm pheromones to protect crops, but to date without success.

Primer and Releaser Pheromones

The queen bee emits several pheromones: to mate, to prevent worker bees from developing sex organs, to swarm, to identify eggs as hers and to initiate maintenance of the hive as needed. As the queen bee grows older the strength of her pheromones weakens until she dies or is replaced by another queen bee with younger active genes and enzyme systems.

Bees can do more work and increase the amount of pollination and honey production when primer pheromones are applied. Primer pheromones are produced by mainly social insects   Research on the effects of honeybee brood pheromone at Texas A&M results showed that brood pheromone collected from the surfaces of the larvae when applied onto honey bee colonies increased the overall growth of the colony when compared to untreated honey bee colonies. Pheromones increased brood nest bees able to rear larvae. This resulted in an increase in the number of younger forager bees that were carrying more pollen into the hive per trip than the forager bees in the untreated hives.

Very sophisticated pheromones have been developed in the social insects to do more than those pheromones that communicate mating, alarm, aggregation and trail making. These other pheromones are called releaser and primer pheromones by some researchers. The releaser pheromones stimulate the insects to do activities needed by their insect community to exist. These activities include social activities including foraging, nesting, food dispersal, storage, tending of fungi growth for food, husbandry of other insects that produce food for the colony and care of the young, eggs, and the queen,

The primer pheromones change the colony insects into different body characteristics needed for defense (warrior ants), large mouth parts to store foods and other body changes like the growth of wings for long distant migration or mating flights or the reduction of ovaries on females other than the queen to control egg production. These pheromone bodily changes make the changed insect into a useful instrument for the survival of the insect colony.

Aggregation Pheromones

Pheromones that attract large numbers of a species of insects to a feeding site are referred to as aggregation pheromones. The male produced pheromones attract female and male insects of the same species. These male produced pheromones attract female and are used as sex pheromones and mating occurs as well as mass feeding.

Once the insects get to the feeding material and start eating the pheromone that is produced includes the chemicals that are taken from the plant that the insects are feeding on. This blending of the plant chemicals in the digestive system of the insect produces pheromones referred to as aggregation pheromones.

Bark beetles that feast on forest trees are in many cases repulsed by the odors that contain chemicals that the forest trees put out. There are theories that say the bark beetles use a form of natural selection. This method of finding the right tree to feed on includes landing on several trees until the right tree is found. Then the beetle emits aggregation pheromones that attract the other beetles. The beetle has a very short life to live and finding the right tree by a general selection method would cut into the life of the beetles. Therefore the ability to know from the odor or chemical that the right tree has been located is vital to the beetles life span.

Once the feeding of the aggregated beetle feeding has started several changes in the pheromone chemical can take place. If the tree is rotting the chemical in the frass (the mixture of the food from the tree and the insect's pheromone) will change. This change will determine whether the arriving insects find the pheromone releases favorable to them or not favorable to them and they may be warned off to another feeding site.

The feeding beetles also produce an anti aggregation pheromone when the feeding site has become saturated by feeding beetles thus reducing the amount of food for all the beetles. The synthesis of this anti aggregation pheromone has been somewhat successful in reducing the damage to forests where the synthetic pheromone has been released.

Researchers testing pheromone aggregation systems have increased the food supply thus reducing the ratio of the number of feeding male insects to the amount of food. This led to an increase in the amount of pheromones attracting more insects to the feeding site. Once the amount of food was reduced the amount of aggregation pheromone was reduced in the area. This effect was stronger on males then on female insects.

Pheromone Use With Degree-Days Information

Plants and animals including insects require heat to grow from one stage of life to another. The method of measuring the amount of heat that occurs during a given period of time for development of insect and plant growth has been developed by researchers in agriculture. This quantity of heat is calculated as units and is referred to as heat units or degree-days. A period of 24 hours is the length of a degree day. The researchers know what temperature is too low for the particular organism to grow or develop. They also know what temperature is too high for growth. The degrees or heat units are either in Fahrenheit or Celsius. The low temperature for growth of the particular insect is subtracted from the high temperature for this insect to obtain the needed heat units.

The phenology database given by the UC IPM uses both scales,. Fahrenheit or Celsius. (This database is available at http.//www.ipm.ucdavis.edu/WEATHER/decongests.htm#ipmpagetop.
The farm advisor's office in the Arizona counties have heat unit data for Arizona areas.

When using the degree-days with pheromone applications the use of an individual insect biofix date is first determined. The biofix date is based on either the crop (date planted or bloom date) or insect (the first trap catch). This biofix gives a date when to start counting degree days. When the number of degree days or heat units equals the amount the insect needs to reach a certain stage of life, the pest pheromones in their traps of lures can be set out for capture and count. When the count of trapped insects is reached control measures can be started. Without knowing about the insect infestation costly missed applications may occur. The heat unit data is also used for predicting when to put on plant growth regulators as well as when to put out pheromones.

Pheromones Traps

There are several types of traps available from most of the pesticide companies. Traps are built with the fact that insects have a tendency to fly upward or search for sites that are protected. The wing type trap is a very popular trap used in monitoring. The cone or funnel type trap catches higher amounts of insects. There are other types of traps: sticky card, water pan traps, bags (the pheromone emits through the walls of the bags), laminated plastic flake (the pheromone is emitted through walls and edges of the flakes) and even plastic cups with oil in the bottom that kill the insect.

Disruption methods use pheromone saturated flakes, hollow fibers and other point sources that can be scattered throughout the field. Another type of pheromone trap is the rope type trap that is used in apple orchards. The trap trees have four ropes per tree. Usually a sticky type bottom on the trap is used to catch the insect for identification purposes. In some cases a toxic material is used rather than the sticky surface to make identification easier.

Pheromone Trap Placement

The placement of the traps in the location to be monitored will depend on the area, the insect, height monitored and the prevailing winds. In New Mexico trap placement for fruit orchards have a density of at least 3 traps per orchard. The traps should be hung from the trees on the northeast corner 1 to 2 feet inside the tree canopy. Traps should be 4 to 8 feet from the ground. The overall density of pheromone traps on various orchard acreages include: one trap per 5 acres in a 20 acre orchard; one trap per 10 acres in a 20 to 80 acre orchard; one trap to 20 acres in orchards that are over 80 acres in size.

The pheromone traps are more useful when placed in the field at the plant canopy level. In corn the pheromone traps where more useful when placed in the field at ear level. In food warehouses and processing facilities pheromone traps are placed in 50 ft. grids used for monitoring stored product insect infestations. In warehouses the traps can be placed near the fire extinguishers as a site for the traps. When food destroying moths appear in the grid traps more traps are set in that area to pinpoint the infestation. Not placing traps near exit doors and windows avoids catching insects from outdoors. The Indian meal moths and Mediterranean flour moths are two of the pests controlled by this method of monitoring with pheromone traps.

Mating Disruption

Mating disruption is an insect control method that uses several sources of pheromone released in an area to lead male insects away from the female. The theory is that the male expends all of his time and energy following the trails of released synthetic pheromone and doesn't mate. The pheromone is released from sources including flakes, fibers, whey, starch, soy proteins, paraffin wax and other materials. One of the better pheromone sources in mating disruption is paraffin wax because of initial entrapment and a constant release rate.

In 1960 Morton Beroza of the United States Department of Agriculture suggested using sex pheromones to jam the insect mating system by flooding the field or entire area with many sources of a sex pheromone of a particular pest species. His goal was to encourage the males to follow several false trails and eventually die without mating. This would hopefully reduce the number of offspring and reduce the damage that the insect could inflect.

Following Beroza's work Harry Shorey was the first to show that pheromones could be used to disrupt the mating of insects. He used the cabbage looper moths. Researchers think that the great amount of pheromone material in the crop field confuses the male moths and in some cases camouflages the female pheromones to the extent that the males just ignores the large amount of pheromones and pass up the need to mate.

Pinworm control using pheromone mating disruption techniques in the Culiacan area of Mexico where nearly 75% of the crop was destroyed by pinworms. When traps were used the fields ended up with only 30 percent of the tomato crop lost to pinworm damage. The growers planted stakes with glass tubes containing female pinworm moths synthetic pheromone throughout the tomato fields. Then they sprayed the field with bacillus Thuringeinsis *bacillus* or Bts. Bts do not kill adult moths. Bts kill worms or larvae.

According to the reports from the Mexican tomato field only four percent of the females were able to mate. 50 % of the females in the non pheromone treated fields mated. Neighboring fields of Tomatoes that didn't receive the pheromone treatment lost 80% of the crop due to pin worm damage. The cost of using pheromones followed with the BT treatments was less costly than the usual pesticide control methods. Most of the tomato growers with pin worm problems now use the pheromone method of pest control in combinations with the newer uses of pest control.

Monitoring Pest Infestations With The Use Of Pheromones

Citrus scale control in California is monitored by citrus growers with the use of pheromone cards. The timing of the flights of the male scale is estimated by the use of degree-days. The cards put out during the first flight in May; second flight in June-July; and fourth flight, Sept-Oct. When 1,000 scales are found during the fourth flight and fruit is infested at harvest, treatment is set up for the next season. Citrus growers try to keep the California red scale population at 10 scale per fruit when harvesting.

If the citrus grove has been treated with the predator, *aphytis,* the pheromone counts are not reliable because these predators control male scales that the count for control is based on. Aphytis predators parasitize female scale and when this occurs the male population will be increased giving low population of female scales and a too high count of male scale. The use of the insect growth regulators will be unreliable because the growth regulators primarily control male scale giving a count that underestimates the scale population.

During the growing season monitoring by checking pheromone traps every week in five to six orchards that have had scale infestations will improve the timing of insecticide sprays to control the scale.

Monitoring Cotton Boll Weevil In Arizona

After the declaration of the eradication of the cotton boll weevil in Arizona in 1991 a monitoring program was set up in 1992. The monitoring program established by the Boll Weevil Technical Advisory Panel in the fall months of 1991 set up weighted monitoring activities with emphasis on certain areas: An example: (One to 50 miles north of the Mexico border using one trap per 20 acres of cotton.) In 1991 seventy seven weevils were trapped. It wasn't until 1996 that one weevil was trapped. The trapped weevil was found in a trap that was located between the interstate freeway 10 and the main railroad line, and it was decided that the weevil was a hitchhiker from some form of transport vehicle that was passing through.

The boll weevil *(Anthonomus grandis)* is an insect that infects cotton crops. It is a beetle that is colored a grayish brown and is smaller than 0.24 inches. The boll weevil has a complete life cycle that lasts about three weeks during the summer. Adults emerge and enter cotton fields in March through July. The adult weevil feeds on the cotton petioles until cotton squares are ¼ inch in diameter. Then they lay their eggs on the squares. The female boll weevil lays close to 200 eggs in 10 to 12 days. One egg per square is usually laid. The egg laying of the weevil adult female causes a wound to the exterior of the flower bud. This wound causes a protusion or bump. The infested cotton squares flare, turn yellow and drop to the ground. The eggs hatch as larvae that feed within the squares and eventually after ten days pupate. The adult emerge in 5 to 7 days. The boll weeevil can have eight to ten generations during a cotton season.

This insect had been known to infest cotton in the West Indies and Central America before 1863 when it did enough crop damage in Mexico that the cultivation of cotton was stopped at that period of time. It crossed the border into the United states near Brownsville, Texas around 1892. In 1903 the damage caused by boll weevil was so severe that in 1903 the Texas legislature offered a $50,000 cash reward for a way to control the boll weevil. By 1922 boll weevils were a serious cotton crop insect from Texas through Oklahoma to the Atlantic Ocean. By 1981 the insect was a cotton growing problem in California, northwestern Mexico and Arizona.

DDT along with other chlorinated hydrocarbons were used on the boll weevil until the weevil became resistant to the pesticide. Organophosphates were used without any sign of resistance even though some boll weevils in Central America have shown some resistance.

In 1966 researchers were able to isolate a pheromone that the male boll weevil produced. By 1972 improved synthesis of the heavily used pheromone, Grandlure ocurred. This pheromone attracted both male and female beetles being a sex pheromone and an aggregation for both male and females.

Some of the pheromone lures available by brand name include: TUA-OPTIMA lure, Femilure, Qlure-CC dispenser, Rhylure 700, Prolure.
Some of the pheromones: Qlure-AGS (turnip moth), Qlure-AGI (black cutworm)

The use of pheromone traps for monitoring outbreaks of insects is carried on by federal, state, county and district agencies throughout the world. Individual growers especially in tree and vine crops use pheromone trapping techniques to monitor insect infestations before and after application. Most of the pesticide dealers have pheromone traps and chemicals available.

The use of trade names in this course is solely for the purpose of providing specific information. It is not a guarantee or warranty of the products named, and does not signify that they are approved to the exclusion of others of suitable composition. Use pesticides safely. Read and follow directions on the manufacturer's label.

Acknowledgements
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Bts By Tim Braun

Chemical reactors used to manufacture insect pesticides aren't used when BT pesticides are produced. The Bt Pesticides are produced by bacteria in a process called fermentation similar to making cheese. Instead of making carbon formed molecules certain bacteria are fed nutrient mixes that allow them to produce toxic proteins and spores that kill specific pests as disease infestations do.

The rod-shaped bacteria lives in the soil and other natural surroundings. Bacillus *thuringiensis* bacteria grow at body temperature and during the stage of its life when it is producing spores it also produces a diamond-shaped crystal that is toxic to certain insect larvae. It is now used as a pesticide in several countries.

Bacteria history

Christian Gottfried Ehrenberg a German naturalist, zoologist and scientist was first to use the name, *bacterium*, in 1838, but before that Antonie van Leeuwenhoek, was believed to be the first person to observe bacteria in 1676. Antonie van Leeuwenhoek using a single-lens microscope that he had designed observed bacteria and he named them "animalcules" in a series of letters that he sent to the Royal Society.
van Leeuwenhoek, a linen merchant by trade, was not a trained scientist , but he was very intelligent, self educated and he was from a well-to-do family with plenty of free time on his hands to amuse himself with science. He made powerful lenses that he used to amuse himself. Antonie van Leeuwenhoek examined saliva, cork, leaves, seminal fluid, urine, and scrapings from his teeth. The research that he carried out he kept to himself. He made very good notes of his work though and even made drawing of what he observed. He did submit letters to the Royal Society and was elected a Fellow of the Royal Society of London.

In 1859 it was still believed that the growth process of fermentation was caused by spontaneous generation from non-living matter. This theory of spontaneous generation was thought up in ancient Greece by Aristotle. When the microorganisms were first brought to the scientific world through the use of microscopic lens a great discussion came about over the origin of life. The arguments were both pro and con. One researcher, Van Helmont, was quoted on how to produce mice. "You put wheat grains with cheese in a soiled linen leaving it in a receptacle for a time in an attic. Mice will then appear." There were many who gave examples to prove that spontaneous generation of living things was true.

This argument on spontaneous generation verse the existence of microorganisms went on for several years. Several experiments where the microorganisms were destroyed by some form of sterilization were carried out to prove that the microorganisms actually existed. The fact that Leeuwenhoek's animalcules were seen moving about gave proof that animal and vegetation matter if left to stand for a period of time contained a force that produced the moving animalcules.

One scientist, Louis Joblot boiled water containing hay. Then he split the fluid into two containers. He covered one and left the other container uncovered. The one that was covered didn't grow animalcules while the uncovered container did. This should have proven that the animalcules came from the air, but another scientist carried out the same experiment and both the covered and uncovered containers were found to have animalcules. At that time the presence of bacterial spores that can live even through some forms of sterilization weren't discovered yet. In this case hatched spores were seen by the observers.

In 1859 Louis Pasteur, a French chemist and microbiologist, was able to demonstrate that a fermentation process is caused by the growth of contaminating microorganisms. He proved this primarily in defense of the wine industry of France at that time. The wine that tasted fine in France turned sour when shipped to other countries. Pasteur after much study found that by heating the wine and beer made in France and then sealing it to keep the animalcules from getting to it allowed the French wines to taste just the same as before they were shipped. He maintained that as long as the wine or beer was not allowed to be contaminated by the equipment or the dust in the air the wine and beer would not spoil. Wine had to be heated for some time at a temperature of 50 to 60 degrees centigrade and beer at 55 to 60 degrees centigrade to kill the animocules. This process was called Pasteurization and it is still used today. An example is the use of pasteurized milk heated at 62 degrees.

In 1867 an English surgeon, Lord Lister, realized that wounds become infected by bacteria that floated on dust particles, on instruments and on the skin of surgeons and other personnel in the hospitals. He used an apparatus that gave off a continuous mist of carbolic acid solution in the areas used for surgery. Lister also used antiseptic dressing to prevent the entrance of bacteria to the surgical wounds. This hampered the activities of the surgeons but the incidence of infections dropped considerably. He was influenced to carry out these activities by the work of Pasteur.

The methods used by microbiologists were still very crude and advances in their field of microbiology were very slow. The main reason for this was the tremendous mixture of different kinds of microorganisms that were present in their cultures that the scientists had to work with. Pure cultures of microorganisms were impossible to attain and this made progress impossible until a German scientist with the name of Robert Koch happened to study the specific disease, anthrax. Koch was practicing medicine as a county heath officer. In his official capacity he was investigating the anthrax bacteria which is a disease of man and other animals.

The researchers viewed the microorganisms that were in fluids. The microorganisms where constantly moving about either on their own with the use of their flagella and tails or through the molecule movement in liquid which is referred to as "Brownian Movement". This movement and the fact that they couldn't see through some of the solid microorganisms made it very difficult to study them with any type of accuracy.

Koch who also took photographs decided to spread the solution in order to get rid of the liquid and let the microorganisms dry out. This worked real well with the anthrax bacteria and the bacteria didn't shrivel or dry out when the liquid evaporated.

The next problem that microbiologists encountered was the fact that microorganisms had almost transparent bodies. This made it very difficult to observe the structure of their bodies and for photographing it was almost impossible.

People involved in research made a practice of trading information on how to overcome these problems that occurred. One German friend of Koch named, Weigert, who worked with him became aware of the use of staining the microorganisms.

This method of observing microorganisms was first used by Cohn and others. Cohn and his associates were involved in the study of tissues. They were using natural dyes that highlighted their tissues when stained with the dyes under the microscope.

At that period of time, 1875, a chemist by the name of Paul Ehrlich developed coal-tar solutions. It was in 1910 that this chemist Paul Ehrlich, developed the first antibiotic. Ehrlich received the Nobel prize in 1908 for his work on immunology. He based his work on the identification of bacteria changing stains to detect and identify the bacteria that causes syphilis. Then he used these observations to use compounds, called antibiotics, that selectively killed the syphilis bacteria.

The use of these stains, primarily Ehrlich's methyl-violet (modified), are still used today. In 1884 a scholar by the name of Gram used the methyl-violet as a stain, but he added iodine which made the methyl-violet stain stick or fasten to the bacterial bodies. The use of alcohol could not remove it from the bodies of most bacteria, but when he applied it to some other bodies he found that as soon as alcohol was applied the stain was removed, and the bacteria became transparent. These bacteria that lost their stain when washed with the alcohol could be stained again with other dyes.

This staining became used in the naming of bacteria. Bacteria that can be stained with the methyl-violet stain are called "Gram Positive" and the bacteria that lose their stain are referred to as "Gram Negative". Bacillus *thuringeinsis* is classified and named Gram Positive Bacteria.

Gram staining, culturing bacteria with different bacteria food sources and other biochemical methods of identifying the types of microorganisms are still used today, but newer methods of identification are being developed and used at a steady pace. The use of molecular techniques and DNA technology have aided the researchers in speeding up the identification process and increase the number of cures for several diseases. Because I have had several cancer invasions of my body I am excited every time they come up with another weapon to fight diseases. I'm hoping that they can improve on some of my cancer treatments like Chemo and target the specific type of cancer to reduce the very damaging side effects of the Chemo treatments that they use today.

The Three Domain System

In 1990 Carl Woese using the sequences of the microbe genes introduced a biological classification system called the "three domain system". This system divides creatures with living cells into three domains. The cellular life form domains are named: archaea, bacteria and eukaryote. He divided each domain into several different kingdoms.

The Archaea Domaine

The archaea domain is considered to be some of the oldest species of organisms living on earth today and possibly in other parts of the world. Many of these one celled organisms are able to live in very harsh environments thriving in acid and very hot temperatures. They can thrive in salt and use hydrogen gas and carbon dioxide for energy sources. Archaea have also been found to live in a broad range of places like oceans, soils and swamps. They reproduce asexually, dividing or splitting into two identical bodies or growing buds that break off and grow into identical offspring. Archaea do not form spores. Because of their durability they are thought to have been some of the earth's first inhabitants when the earth was a very hard place to exist. Some data place the Archaea on this earth as far back as 2.7 billion years.

A small number of biologists argue that the Archaea and the Eukaryota (animals, plants and fungi) emerged from a group of bacteria. The archaea unlike the Eukaryota do not have membrane-bound nucleus or any other membrane-bound organs. They were originally thought to be a group of bacteria, but they are now classified as a separate domain.

The two domains, archaea and bacteria, are alike in size and shape as one celled living organisms. The differences are evident when their genes and their molecular changes are examined with the newer identification methods. Some of the archaea exist in the guts of humans and vegetable feeding animals helping to digests foods. They are used to produce gas from sewage at sewage treatment plants. In some ways the archaea are closer to the Eukaryota (animals, plants and fungi) than the bacteria.

Eukaryota Domaine

The Domain of Eukaryota (animals, plants and fungi) has more than a single cell and their cells contain a membrane-bound nucleus and membrane-bound organs. Plants like the pine trees and other eukaryota produce spores instead of seeds as one of their forms of reproduction. Most plants produce seeds. Animals produce eggs.

Bacteria Domaine

The third Domain is the Bacteria which like the Arkaea are one celled, without a nucleus and do not have membrane-bound organs. Bacteria do produce spores. There are three species of Bacillus bacteria: Bacillus *anthracis*, Bacillus *cereus* and Bacillus *thuringiensis.* Bacillus *thuringeiensis* when under magnification is distinguished from Bacillus *anthracis* and Bacillus *cereus* by producing intracellular crystals (CRY proteins) when a spore is formed. Bacillus *anthracis* and Bacillus *cereus* have a sporangia for the spore but they do not have the intercellular crystal (CRY proteins) that the Bacillus *thuringiensis* bacteria contains.

In the human body the cells present can be classified as: human, fungi, bacteria, and archaea. Of these the bacterial cells make up ten times as many cells as the human cells. Most of these bacteria are harmless because of the immune system that is present in the human body. This immune system consists of human biological structures and processes that identify and kill tumor cells and diseases. These protective items evolved over several years in the human body and are constantly changing as the diseases and tumors change or evolve. Some of the bacteria that are harmful include tuberculosis, cholera, syphilis, anthrax and leprosy.

Bacterial cells are about one tenth the size of human cells. These bacteria cells are small, but some are large enough to see with the naked eye. These bacterial cells exist in several shapes. Some are like balls others are rod like and some are worm shaped and coil up. The spherical (ball) shaped bacteria are called *cocci.* Bacillus thurengeinsis are rod shaped.

The shape of the bacteria is determined by the cell wall and the cytoskeleton. The cell wall of the bacteria gives support and protection. Plant and fungi cell walls are made of cellulose and chitin while the walls of bacteria are made of sugars and amino acids. The bacteria cell wall is mesh-like thus filtering the liquid that enters the bacteria’s body. The filtered cell wall of the bacteria prevents over-expansion when water enters the bacteria’s body.

The cell wall of Bacillus *thuringiensis* is thick and classified as Gram positive. (The cell wall can be stained with the Gram staining method.) Gram positive cell walls can contain 40 layers of the polymer, peptidoglycan. The Gram positive cell wall is outside the cell membrane.

Bacillus *thuringiensis* has a small section of space between the cell wall and the inside plasma membrane of the cell. This space is necessary for protection.

The Gram negative bacteria are thinner than the Gram positive bacteria and do not stain with the Gram stain like the Gram positive bacteria.

Gram negative bacteria cell walls are much weaker than the Gram positive cell walls with only a few layers of the polymer. The Gram negative cell wall has another membrane around the outside of its cell wall. Bacteria surround their cells with a slime layer or capsule that is used as another form of protection. The capsule is formed by secretions of the bacteria.

The cytoskeleton in the liquid inside of the bacteria is made up of proteins and acts as a skeleton giving the bacteria structure and strength. The cytoskeleton have fine filaments that cause forces for movement by stretching at one end and shrinking at the other end.

Although the body or cell of the bacteria does not have its internal organs contained in the body with individual membranes like the Eukaryotas, the cell does confine these organs to certain locations in the cell’s body with the use of interlocking protein shells.

I will use the terms “genome” ,“chromosome”. “genes” and “DNA“. The genome contains chromosomes and chromosomes contain genes and genes contain DNA.

Bacteria have only one chromosome. Plants and animals have several chromosomes.

Bacteria do not have a membrane covered nucleus like plant and animals. They have an irregular shaped body inside their cell called the nucleoid. This nucleoid is in the cytoplasm and contains a single circular genome which contains one chromosome that has deoxyribonucleic acid (DNA) that contains the genes with the genetic information for the development and functioning of all the bacteria’s activities.

These activities include the break down of food to make the cell’s living parts. DNA makes ribonucleic acid (RNA). RNA encodes genetic information that directs the building of proteins from amino acids that come from the breakdown of foods that the bacteria eats.

This same activity structure takes place in the cells of all living organisms.

(DNA makes RNA, RNA makes protein, and proteins make us. By FRANCIS CRICK)

The bacterial nucleoid is made up of 60% DNA with some proteins and a small amount of RNA. The proteins aid in compaction or bending of the DNA structure into the nucleoid mass. There are different kinds of RNA that are made by the DNA.

(rRNAs) make ribosomes. (mRNAa) carry messages.

The ribosome starts manufacturing proteins using the RNA as a blueprint for the type of protein that it manufacturers. The material used for manufacturing is gained from the amino acids that are available from the food that the bacteria eats and then breaks down to make the cell body parts and organs. Some of the ribosomes move out to the cell wall where the proteins that are manufactured are used on the wall or even passed through the wall to the outer area. Ribosomes make proteins that will be used for growth of the bacteria and eventual splitting of the bacteria creating another bacteria.

The proteins produced include: enzymes that are used to make new molecules and these enzymes catalyze or turn on all the chemical processes going on in the bacteria. These enzymes are used to make ; structural components that give the organs in the bacteria bodies their shape and help them move about; hormones that transmit signals throughout the body of the bacteria; and antibodies that identify foreign molecules and transport molecules that carry oxygen.

Another part of the cell that is found unattached in the cytoplasm is the bacterial plasmids. Plasmids are found in the three major domains: Archea, Bacteria and plants and aren‘t found in animals. Plasmids are molecules that come from the genome. The plasmids contain individual genes and they can break away from the genome and reconnect with the genome of the cell or another cell‘s genome.

Plasmids can pass through the cell wall of the bacteria and enter other bacteria or cells of plants that are compatible to them. When gene engineers want to transfer genes they often take the gene they want on isolated plasmids instead of the main genome.

Some of the gram-positive bacteria which includes Bacillus *thrurengeinsis* form spores. Other living organisms form spores for reproduction. Fern plants form spores instead of seeds. Fern spores and other plant spores are a form of sexual reproduction, but these spore forming bacteria form the endospores.

They are called endo spores because they are formed inside the bacteria cell. They are basically used as dormant structures. The endospores are formed when living conditions become harsh and damaging to the bacteria’s existence. This is somewhat similar to bears hibernating in bad winter weather.

The endospores have a center of cytoplasm with a genome containing DNA. RNA, ribosomes and proteins surrounded with a cortex layer. This cortex layer is protected with a coated wall that is rigid and impermeable. The spore can stay dormant until surrounding conditions improve. Some bacteria endospores can exist for millions of years and still become living bacteria when conditions are suitable. Most of the bacteria domain cannot change to the endospore stage but *Bacillus* can.

Because of the hard material that coats the endospore the gram stain will not stain the coat of the endospore. A special stain called the Moeller stain is used. The Gram stain shows up colourless while the Moeller stain will stain the endospore red while the rest of the bacteria cell is shown as blue.

When conditions for bacterial growth are bad enough the bacteria produces a spore. The development of the endospore begins with the splitting of the genome, the bacterial RNA, some proteins that are present and ribosomes. After this splitting a spore wall is formed around the material that has split off. This new walled area with identical parts becomes the endospore inside of the bacteria‘s cell.

The bacteria’s plasma membrane and wall still covers both the old bacterial nucloid and the endospore. The plasma membrane pinches together between the two, then the two, the bacteria cell with it’s parts and the newly formed endospore with its very strong wall separate.

Because the bacteria cannot survive in the conditions that caused the formation of the endospore, the bacteria cell degenerates and breaks down into the surrounding material leaving its protected endospore to survive until the environment changes to a climate that the bacteria can exist and multiply in.

When conditions improve the spore loses its hard protective coat or wall and becomes a bacteria once again. With an abundance of food the bacteria begins growing and dividing into more bacteria.

These bacteria spores can exist for long periods of time. Two people at the Cal Poly University, Raul Cano and Monica Borucki, recovered and re-animated bacterial spores from the digestive tracts of bees that had been entombed in amber for an estimated 25 to 40 million years.

`Bacterial spores can cause several very serious diseases. These diseases include: botulism, gangrene, tetanus, food poisoning and anthrax.

Bacterial Reproduction

Bacteria get their energy from various sources. Some bacteria use sunlight; some use inorganic compounds; and some use organic compounds like bacillus *thurengeinsis.* Bacteria were originally classified by what they ate. Now in modern times their genetic make up or DNA is used to classify them.

Bacteria reproduce asexually. When the bacteria consumes food and reaches a certain size they split or divide into two bacteria that are alike. The splitting of bacteria into two cells that are alike is referred to as binary fission. The binary fission process starts as the genome splits. The two identical bacteria cells that are formed both survive and go on to divide again as long as the conditions are compatible for bacterial growth. Under very good growing conditions bacteria can double in population in a period of 9.8 minutes. As nutrients start to be deleted by consumption the growth in the population is reduced and spore formation begins. Spores are bacteria cells that are dormant until conditions improve.

The DNA is made up of a double strand of molecules. After the strands separate during binary fusion a copy is formed of each of the two strands. Each copy then has an existing strand joined to a new formed strand. This makes two identical double strands. After the new formation of DNA is formed each formation moves to opposite ends of the cell. When they reach the cell plasma membrane they cling to the membrane. This contact with the cell plasma membrane between the DNA and its group of ribosomes and proteins triggers the growth of the bacteria's cell wall and plasma membrane creating a double sized body.

Once the bacteria begins doubling its size, proteins in the center of the bacterial body start to grow around the middle of the bacterial cell forming a ring at the division site of the cell wall. These proteins bring the wall and membrane together in a seam. This ring of proteins causes a division of the enlarged bacteria forming two identical daughter cells or two separate but identical bacteria. The DNA, RNA, ribosomes and associated proteins in each of the two new bacteria are released from the cell plasma membrane and move in the cytoplasm back to the center of the bacterial body.

Physical Or Chemical Damage to DNA And RNS

The asexual reproduction in bacteria produces identical offspring and does not produce bacteria that can prevent an outbreak of a new disease that can destroy all of the identical bacteria. Asexual reproduction does not produce offspring that have a different make up in their bodies that can resist the disease, but the genome that contains the genes that bacteria inherit can be changed by several sources of damage that can break the DNA or RNA molecule and then have them join together in a different configuration. The change that results can produce a different combination of genes.

When a change in genes occurs due to damage to the genes the resulting next generation of bacteria that inherited the different genes may be weaker or stronger depending on the calamity that strikes them. In this group of genetically different bacteria, some of them will survive and some will die.

Bacteria and other living organism gene changes have been responsible for the survival of many species of bacteria in a new type of calamity. Within the genome which is the bacterial DNA, RNA, Ribosomes and proteins there are proteins that can repair damages caused by mutations that result from physical or chemical damage to the genes. When a problem is strong enough to cause permanent damage to the DNA the next time the bacteria multiplies the result will be different bacteria with different genes.

Conjugation

There are some bacteria that can transfer genetic material directly between their bodies without damage. Bacteria have plasmids. A plasmid is a DNA protein that is separate from the main genome's DNA. The plasmids are made when strands of DNA and RNA are peeled off of the main bacterial round shaped chromosome. The pieces of genetic materials are copies of the bacterial chromosome. These pieces of DNA and RNA are circular and contain DNA genes that can be transferred between bacteria.

This transfer of genetic material between bacteria is referred to as conjugation. The plasmid has a system that makes sure that the bacteria receiving the genetic material does not already have a similar element of genetic material. This transfer of genetic material can be good for the receiving bacteria. It will contain a material (genes) that allows the receiving bacteria to use things that are involved in its growth, development and reproduction. The two bacteria cells come in contact and the plasmid that contains the genes is transferred between the bacteria through their walls and membranes. Researchers using E. coli have timed the transfer and note that it takes about one hundred minutes.

Bts History

A Japanese bacteriologist, Dr. Ishiwatari, discovered a bacteria that caused a disease called *sotto* (sudden-collapse disease) in silkworm farms in 1901. In 1911 the bacteria was discovered to have insecticidal activity as a pesticide of the larva of flower moths in the province of Thuringia, Germany. Ernst Berliner isolated the bacteria. Ishiwatari had named the disease: *Bacillus sotto* back in 1901 but the name was ruled invalid. The name Bacillus *thuringiensis* (commonly called Bt) came from the name of this province (Thuringia, Germany). Ernst Berliner noted the existence of pesticide proteins crystals in Bt in 1915.

Bt was used as an insecticide in the 1920's and as commercial pesticides from Bacillus thuringiensis made from commercialized spore based formulations with the name, Sporine, in France during 1938. In the 1950s Bacillus *thuringiensis* was sold in the United States as an insecticide. Researchers discovered the parasporal crystal in 1956. The discovery of this protein crystal opened the door to a large amount of research to use Bts as pesticides for a wider range of pests. In 1961 Bacillus *thuringiensis* (Bt) was registered as pesticide in the United States by the EPA.

During the late 1970s there were only 13 known Bt subspecies. These Bt subspecies were toxic to lepidopteran larvae (caterpillars) but this low number of pesticides limited its use in the pest control industry. With the discovery and isolation of the BT protein crystal the toxicity to other insect pests was discovered. In 1977 a subspecies was found that was toxic to dipteran (flies and mosquitos). By 1983 another strain of BT was found to be toxic to coleoptera (beetles).

Today there are thousands of strains of BT. The uses and money available for the research of Bts have increased primarily because of the awareness of environmentalists who found out that some pesticides were harming the environment. The main reason for the success of Bts as a pest control material was the resistance of pests to the synthetic pesticides like parathion and DDT. Growers who wanted an organic pest control method without persistence in the environment were sure that BT is the answer.

To date over 150 known insects are susceptible to Bts pesticides. The use of Bts represent about 1% of the total insecticide market worldwide but this is changing to a higher percentage as the Bt toxin genes are genetically engineered into crop plants like cotton, corn and several other crops. The agricultural seed suppliers have been purchased or gone into partnerships with ag chemical companies to market Bt engineered seeds.

On February 23rd, 2007 the genome for the Bacillus *thuringgiensis* was sequenced. This sequencing gave scientists knowledge on how mutations can further produce different types of the bacteria that may grow stronger against agricultural pests.

One of the main causes for the decline in the global use of synthetic pesticides is the use of (IPM) Integrated Pest Management methods . These management methods use all the inputs in growing modern crops. These methods depend on combining the use of pesticides along with predator control, biologically engineered seed crops and intense pest monitoring with the use of pheromones.

Although hard pesticide use has declined the growth of predator friendly or biopesticides have gone from 672 million dollars in 2005 to one billion dollars in 2010. Biopesticides include Bts, insect growth regulators, Pheromones, predators, genetic engeneered Bts and others. The decrease in overall pesticides is due in part to the increase in regulations on harsh chemicals and the success of the use of integrated pest management.

The country of China is making great strides in isolating genes of the Bacillus *thuriengienisis*. Chinese researchers have isolated fifty new genes of Bacillus *thuringiensis.* The researchers are concentrating their efforts on the *cry8-type genes.* which are toxic to colopteran (beetles & weevils) pests and scarab (beetles) pest species.

The research into Bts has gotten a huge boost with the advances that have been made in molecular biology. The spores of Bt that exist naturally on the surfaces of living and recently dead things. They can be found on many plant surfaces. Some subspecies of Bts have been collected and raised from pine trees, broadleaf trees and vegetables. Bt can be found in some stored products and growing in the soil. Bt subspecies have been taken from dead or dying insects. These Bt bacteria found in natural growing conditions are isolated and transferred to commercial processing plants where they are allowed to ferment (grow) on solid media or under submerged fermentation conditions in large tanks.

Bt Production

During the commercial fermentation process Bt bacteria cells grow and produce insecticidal proteins and spores. Both the insecticidal proteins and the spores of Bt are toxic under the right conditions. The insecticidal proteins are in single crystal and crystal-complex form. The ICP (Insecticidal Crystal Proteins) are the main source of insect toxicity. The ICP is the primary active toxic ingredient in Bt. Some Bt is cultured to produce only ICP's which are toxic protein crystals, but most of the commercial Bt contains spores and Insecticidal Crystal Protein (ICP).

The ICP or (Insecticidal Crystal Protein) is just what it says it is: a protein. Proteins are nitrogen containing molecules. That together with water are the main ingredients of bacteria and other living organisms. They are formed into a tremendous variety of structures. Their size when compared to the other molecules in living organisms are very large. Proteins can reproduce themselves rapidly and exactly from simple units. This is why they maintain and enlarge the system that they are a part of. Proteins can be the stored product of the cell or they can be part of the metabolism of the cell. Proteins can also exist as crystals that can be dissolved in different solutions.

All proteins contain carbon. They contain from 50 to 54 percent carbon. Proteins have 16 to 18 percent nitrogen, about 7 percent hydrogen and 20 to 25 percent oxygen. Phosphate is also present in proteins as part of their structure. Plant proteins have less than 2 percent sulfur when it is present. Other elements like potassium are present in proteins in living organisms.

The main fertilizer elements that are added to growing agricultural crops include nitrogen, phosphate and potassium. The nitrogen is used for the structures or proteins; the phosphate transports the energy around the organism and the potassium activates or turns on the plants living system. Potassium is often called "the Key'.

Amino acids come from tissue that was formed by living organisms from their food sources. The amino acids are joined together either in long chains that are folded in a globular or fibrous form making the large protein molecules.

As the Bt cell forms its spore because of poor growing conditions, protein crystals are grown on the hard protective wall that covers the spore. These crystals are formed by the directions of a gene that is present in the genome of the Bt bacteria that is also in the Bt spore's DNA complex or genome. These insecticidal protein crystals (ICPs) can be isolated from the spore and sold separately or as a Bt spore and ICP mix as Bt pesticides.

How Bt Spores And Crystals Kill

When applying the Bt pesticides, the spores and crystals of Bt must be eaten by the pest larva before they can act as poisons. Bt is therefore referred to as a stomach poison. Bt crystals dissolve in response to intestinal conditions of susceptible insect larvae. One of the conditions is a high pH solution of about 9.6. The protein crystals dissolve in the stomach, attach to the stomach wall cells and cause holes to form. This paralyzes the cells in the gut, interfering with normal digestion and triggering the insect to stop feeding on host plants. Bt spores can then invade other insect tissue. Once the feeding conditions improve the spores lose their hard protective coat and become active Bt cells that multiply in the insect's blood, until the insect dies. Death can occur within a few hours to a few weeks after Bt application, depending on the insect species and the amount of Bt ingested.

During the growth of spores Bacillus *thuringiensis* subspecies produce Beta exotoxin proteins as well as the (Insecticidal Crystal Proteins) ICP. These Beta exotoxins are toxic to all forms of life including human life. Because of this threat of human toxicity the manufacturing process for Bt includes very strict monitoring to prevent beta exotoxin from contaminating the Bt. Pesticides.

Both the ICP and the spores in Bt cells are toxic to some insects. This toxic effect of the Bt spore is due to either the toxin in the protein crystals from the coating of the spore or the vegetative growth of consumed spores. The two of them, ICP and spores when used in combination as an insecticide increases their effectiveness as a poison to the target pest. Because both the toxic proteins (crystal proteins) and the spore is found inside the Bt bacteria cell they are referred to as an endospore and an endotoxin, but never exotoxins.

The Bt spore and crystal protein have a half life of a few hours to 10 days in agricultural conditions. Both the spores and the pesticide crystals break down in sunlight. When mixing and transporting Bt pesticide mixtures, plastic tanks that sunlight can penetrate should not be used.

By itself the Bt spore is able to survive for long periods of time due to its resistance to the stresses of heat, lack of moisture and food. Spores when surrounding conditions improve lose their thick hard coat and begin to split asexually growing vegetatively to become new Bt bacterial cells that can produce endospores and Insect Crystal Proteins (ICP) or endotoxins if conditions turn bad.

In the sedimentation or the mud of water the spores of Bt can last for at least 22 days. This would include medium flowing streams, ponds and other bodies of water where mosquito larvae can exist. Bt pesticide granules are spread over these water basin areas for mosquito control.

Bt insecticide formulations are either powders or liquids containing the ICP crystal and spores. Some formulations of Bt only contain ICP crystals without spores.

A protoxin is not toxic until it encounters the right condition that allows it to become toxic or poisonous. The Insecticidal Crystal Protein (ICP) in Bt is a protoxin. Bt is not effective as an insecticide unless it is eaten by the specific insect larva that has conditions in its gut where the Bt can become a toxic material that can kill the insect. .Bt is only effective against the worm life stage (larva) of the insect. Bt does not control adult insects.

The target insect must have mid gut fluids that have a high pH, above 9.5. The target insect must have specific enzymes in its mid gut that turn the ICP into a poisonous crystal. The target insect must also have specific receptors (hooks) located on the internal wall of its midgut for the specific toxic protein to attach to. This is similar to the lock and key method of security. Only the right key fit's the lock. These conditions for control make Bts a pesticide that is safe to other mammals and other living organisms..

Bt kills by going through the following procedure:

1. The susceptible insect larva eats the Bacillus thuringeinsis spore and ICPs (insecticidal crystal proteins).
2. If the insect is susceptible to the strain of Bacillu thuringeinsis its gut fluids will be at a high range of pH which is above 9.5. At this pH the insecticidal crystal proteins will dissolve and become active.
3. If the target insect has enzymes in its gut that will react with the Bacillus thuringeinsis insecticidal protein, the enzymes will turn the ICP into a toxic chemical that will kill the target pest. Now the ICP is an endo-toxin.
4. If the target insect has the right kind of receptors (hooks) in its gut that the Bt endo-toxin will cling to, then these receptors will hold the activated endo-toxin against the gut's wall.
5. The endo-toxin of the Bt will paralyze and destroy the cells of the insect's gut wall to form pores (holes). These pores allow the gut contents as well a the endo-toxin along with the Bt spores to enter the body cavity of the insect..
6. The low pH(acid) of the target insect's body cavity's fluid which includes the blood of the target pest will create an environment where the vegetative Bt spores will lose their thick, hard coating and become a mass of multiplying Bt bacterial cells. The abundance of food cause the Bt vegetative spores which have now become asexually multiplying Bacillus thuringeinsis cells in the body cavity causing septicemia or blood poisoning. Insects without the specific gut conditions are not killed by Bts. This is one of the Insect Pest Management qualities of Bts.
7. The specific insect will stop feeding after eating the Bts even though it does not die for 2 to 3 days. The insect that consumes BT stops feeding and its gut is paralyzed. Symptoms include vomiting and diarrhea. After death caused by Bts the insect's body becomes brown to black before decomposing.

During the 1960s in the San Jauquin Valley cotton growers would harvest the black dead cabbage loopers that they found on cotton plants and then break the bodies down and apply this dead worm mixture in water to cotton plants that were infested with worm pest populations. The growers that did this claimed that they were able to gain some control over the cabbage looper infestations that were skeletonzing their cotton fields.

The Bt bacteria from the spores that grow in the infected insect will not multiply to the extent that they infect other generations of the target pest. Bt will not survive for more than a few days in the dead insect. Bt applications must be repeated to be effective.

Bt pesticides are applied to the surfaces of plants for the control of lepidopteron (worms) and coleopteran (beetles). For the control of diptera (mosquitoes and black flies) Bt is applied to waters where the worm (larva) stage of growth occurs.

The target insect has to consume the Bacillus Thuringeinsis pesticide that has been sprayed on the plant. Good coverage is required. On vegetables the use of spray equipment with three nozzles arrangements per row will work. In some cases the use of five nozzles per row has been even more superior.

Monitoring the crop before application should be done to make sure the larval stage is targeted; therefore timing the application to spray larvae in the first to second instar is recommended. The use of pheromone traps to verify the presence of adults is a good monitoring practice to start looking for eggs. Then set up the spraying to co-inside with egg hatch. Alternating Bacillus thuringeinsis pesticide sprays with chemical pesticide sprays will reduce the development of insect resistance to Bacillus thuringeinsis pesticides.

Bt Pesticides

Bt pesticides have subspecies that target specific insects. The subspecies include: Bacillus *thuringeinsis* subspecies *kurstaki,* Bacillus *thuringeinsis* subspecies *aizaway,* Bacillus *thruringeinsis* subspecies *tenderloins*, Bacillus *thuringeinsis* subspecies *israelensis and* Bacillus *thuringeinsid Japonensis.*

The protein crystals (Cry) are classified by the insect that is susceptible to them. This is a partial list of some of the protein crystals that are available in Bt pesticide formulations.

Cry I Lepidoptera specific kills (worms like looper etc.)
Cry II Lepidoptera and Diptera specific kills (worms and mosquitoes)
Cry III Coleoptera specific kills (beetles)
Cry IV Diptera specific kills (moquitoes)
Cry V Coleoptera and Lepidoptera specific kills (beetles and mosquitoes)

I will use the terms “genome” ,“chromosome”. “genes” and “DNA“. The genome contains chromosomes and chromosomes contain genes and genes contain DNA.

In the cell of the Bt bacteria has only one chromosome where the DNA with all the genes of the bacteria are stored. Bacteria consist of only one cell. The gene has a specific location on the chromosome with other genes that decide a particular characteristic and guides the activity of an organism. Our human genomes store our genes in our cells. The genes decide whether we inherit blue eyes or black hair, whether we get diabetes or not, among other inherited characteristics.

The type of Crystal Insecticidal Protein (ICP) inherited by the Bt bacteria is decided by a gene on the chromosome of the bacteria. This is referred to as a Cry (crystal) gene. The CRY gene of a Bt strain causes the Bt cells that are produced with this CRY gene to produce this specific crystal protein on the wall of a forming bacterial spore in a bacterial cell. This particular crystal protein will then turn into an endotoxin (toxic crystal). The endotoxin crystal when eaten by the insect that could be either a looper, beetle or mosquito causes the insect that swallows it to die. This crystal protein is the Insecticidal Crystal Protein (ICP) which is the primary active pesticide ingredient in the Bt pesticides.

Some of the trade names for the Cry IV (mosquito) proteins are Skeetal, Vectobac, Gnatrol, Bactimos and Mosquito Attack. These Cry IV protiens are from the strain, B. *thuringiensis var israelensis* (Bti). They are used to control black fly that carry some of the tropical diseases. They are used to control other diptera insects including mosquitoes and fungus gnat larvae.

Strains of the Bti will not control larval stages of the so called “higher” flies such as the house fly, stable flies or blow flies. Bti is used to a large extent by abatement districts and vector control.

The Cry III protein include the Bt strains: B. *thuringiensis var san diego* (Bt sd), now called B. *thuringiensis var tenebrionis* (Bt te). They are used to control Colorado potato beetle and boll weevil. Another Bt strain used for scarabid beetles is the B. *thuringeinsis Japonensis* (Btj). The trade names for these Bt include

Trident, M-One, M-Trak, Foil and Novodor.

The Bt pesticides are available in several formulations; dry flowable, granule, water dispersible granules, technical powder dust, emulsifiable suspensions, wettable powder, oil flowable, bait granules and bait bran. Bt formulations can be mixed with other chemical pesticides.

Bt pesticides have been developed so that different Cry protein genes can be incorporated into one Bt pesticide product in order to have activity against more than one insect pest. The method of doing this is referred to as tranconugated strains of Bts. Raven is a Bt insecticide that was genetically engineered to kill Colorado potato beetle. Raven uses two different gut binding Cry III (beetle) proteins as well as Cry I (worm) proteins to kill caterpillars.

Advantages of Bt over other insecticides:

Bt insecticides usually require no re-entry period on the label.

The bodies of insects that have been killed by Bt do not pose the problem as being toxic to birds and other mammals that consume them.

Because by the way in which Bt becomes toxic to specific insects Bt is used on food crops.

Resistance by target insects have been very rare considering the length of time that Bt has been used as an insecticide.

There have been no reports of plant injury with the use of Bts.

Bt has <u>not</u> been found to be detrimental to the environment.

Bt can be used without the fear of causing an outbreak of other pests like mites.

There are disadvantages to the use of Bts.

Because Bt is so specific as an insecticide other pesticides need to be added to kill additional pests that are present.

Bt is a stomach pesticide and has to be applied so that the pests eats it.

Bt toxin breaks down in sunlight and has a short half life.

Bt isn’t effective against adult insects.

Genetic Engineering of Bts

Genetic engineers can excise Cry genes from Bt cell bacteria and place them on different Bt chromosome DNA of another Bt cell chromosome and produce several desired combinations of Bt insecticides.

Cry genes are located on circular ribbons of DNA that are independent of the Chromosomes called plasmids. These plasmids with individual Cry genes can replicate themselves on an independent basis. Increasing the number of them enormously. The DNA ribbons or plasmids with genes on them can go in and out of the DNA main ribbon or the genome of the cell becoming part of it.

These plasmids can also move in and out of the Bt bacteria cells and into other Bt bacterial cells. Plasmids with the genes exist in plant cells and bacteria cells. This movement in and out of different strains of Bt is referred to as a conjugation-like process. Some Bt cells have as many as 5 or 6 plasmids at one time. The plasmids with their genes from other bacteria can move into and become part of the main genome of any other Bt bacteria cell.

This process increases the number and variation of the individual strains of Bt Cry proteins which are eaten and activated by different insects. The insects’ resistance is reduced because the insect must be resistant to a larger number of Cry toxins. This transfer of genes that increases the number of different acting toxins for Bts occurs naturally. So when you hear about genetic engineers processing a CRY toxin gene into a Bt cell they are copying a natural process that has been taking place for centuries.

Crop plants can now internally contain the indo-toxin gene from Bt. This has been a pesticide application technique since 1996. Plants are genetically engineered to grow part of the active Cry protein in their tissues. This increases the areas of the plant that can be treated. The Bt pesticides which were only applied to the surface on the plant can now be growing as part of the plant’s internal areas. Many insects that bore into the plant and feed can now be controlled with Bts without an application. The Bt comes in the seed.

Genetic Engineering Is the human use of manipulation on an organism’s genetic material that doesn’t occur under natural conditions. It involves the insertion of a gene or genes taken from a plasmid or a genome with the desired genes into an existing DNA on a genome. Genetic engineering does not include animal breeding or plant breeding. The plants and animals that are produced with this method are referred to as genetically modified organism.

Bacteria were engineered genetically for the first time in 1973. Mice were genetically engineered in 1974. The engineered insulin producing bacteria were sold commercially in 1982. Genetically modified food has been sold since 1994.

The genetically engineered plants were field tested in France and the United States of America in 1986. In this procedure tobacco plants were engineered to be resistant to herbicides.

In 1995 potatoes engineered with Bacillus *thuringeinsis* cry genes were approved to be safe by the Environmental Protection Agency. This was the first approved crop to produce its own pesticides in the United States of America.

Genentech Company was the first engineering company founded by Herbert Boyer and Robert Swanson in 1976. They announced the production of genetically engineered human insulin in 1978. By 2009 eleven transgenic crops were grown in 25 countries. The largest growers of this group of countrys included: Brazil, Argentina, India, Canada, China, Paraguay, South Africa and The United States of America.

In 2010 scientists at the J. Craig Venter Institute created the first synthetic bacteria and named it Synthia. This bacteria is the world's first synthetic life form. They created the first bacterial genome and placed it in a cell that did not have DNA or a genome thus making a synthetic life form.

The process of putting a gene into the DNA of a living plant starts out by isolating the particular gene that is needed. Once the gene has been identified it is put into a bacterial plasmid. I described bacterial plasmids earlier. Plasmids are parts of the cells genome DNA which is the biological heredity information of the organism. We have a genome in each of our cells. These items decide the way we exist. In the plant and bacterial cells there are the plasmids that have parts of the genome DNA. The gene wanted for transfer is on one of these plasmids. Animals do not have plasmids.

With the gene that is needed there is an enzyme (promoter) that activates the making of a copy of the gene. Also there is an enzyme terminator that stops the making of a copy of the gene.

There is a third item necessary in this group. It is called a marker.

Markers: The gene of interest that is to be transferred is accompanied with a marker gene on the plasmid. Cells with marker genes are resistant to antibiotics that kill cells that do not have marker genes. Antibiotic Marker genes are added to plasmids along with a gene wanted by the engineer that is being transferred into plant cells. When an antibiotic is added to a solution of cells that have plasmids that have resistant gene markers along with the desired gene, the cell with them (markers) will survive the antibiotic and the other cells without the marker gene will die.

Example: The surviving cells with the wanted gene will multiply and we have the cells we want genetically engineered into a plant. The gene wanted in Bt cotton would be a cry gene from Bacillus thuriengensis that produces a poison inside the growing plant that will kill worms that destroy cotton bolls.

Once the genes are isolated then they are allowed to multiply. In the genetically engineering of genes the use of a genetic library that contains clones of this particular gene is now commercially available. The market for this type of product has grown to be a multimillion dollar business.

The synthesis of various DNA needed for genetic engineering can now be done by several companies. The gene may already be on plasmids and once they are available the plasmids are ready to be transferred through the cell wall and into the cell cytoplasm where the plasmid may join up with the target DNA chromosome. The particular gene markers, promoters and terminators for the particular gene are also commercially available.

Gene Transfer From One Bacteria To Another Bacteria

The plasmid with its cry gene on board that will make the plant that it enters produce the Bt proteins that are the insecticidal protein pesticide is now ready to be transferred to and into a agricultural plant like cotton.

Scientists have a names for gene transfer from one organism to another organism: Horizontal Gene Transfer or Lateral Gene Transfer. This is the process whereby an organism receives genetic material from another organism when the receiving organism has not been produced by the producing or parent organism either by asexual or sexual birth or reproduction. Whereas the Vertical Gene Transfer term refers to the transfer of genes from parent organisms or ancestors to offspring organisms by asexual or sexual reproduction.

Natural Gene Transfer

Some bacteria are naturally able to take in DNA. These bacteria able to do this (naturally) amount to only one percent or all the bacteria. The use of heat shocks or electric shocks can loosen up the cell membrane so that DNA molecules can enter the other cells. There is a problem because of the damage caused by the shocks and the transfer is not as efficient as the microinjection and the Agrobacterial transfers.

Needle Injection

The microinjection process of DNA transfer uses a very fine needle that penetrates the single cell. This is done under a microscope. This method can be used on plants. The benefit is that a person knows what cells have received the DNA because only one cell is treated at a time. The DNA molecule in the cell can receive the injection directly and not wait until a plasmid with the gene has entered the cell’s Main DNA. The draw back is the speed of the operation. It is much slower than natural transfer of DNA.

Biolistic Injection

Another method of DNA transfer into cells is the biolistics particle delivery of the DNA into a cell. This method of DNA injection uses a gun to fire a bullet made of tungsten which was coated with plasmid DNA into a callus of cells. A callus is a cell culture in a gel media of nutrients including nitrogen, phosphorus and potassium with water. The cells are usually vegetable. The callus is in a petri dish that sits at the bottom of a pipe.

The plant cells are messed up from the blast, but the cells that are not obliterated have some of the tungsten coated DNA inside them. The DNA migrates to the cell chromosome and provides the new gene for making proteins. The cells are then treated with plant hormones, such as gibberellins, and auxins causing the cells to divide and grow into tissue cells or entire plants. The new plant that survived and received the transferred DNA may develop into a genetic engineered plant. If the DNA came from a Bacillus *thurengeinsis* bacteria the new gene would help control insect pests in an agricultural crop.

The gene gun technique is used when the cell wall of the intended is not able to be invaded by the other methods of DNA transfer. The original gene gun has been modified by replacing the tungsten with gold and silver. Both materials are more expensive, but tungsten can be toxic to cells. Gold and silver cost more and the use is limited, but it is still in use on plant cells and on animals cells .

Agrobacterium Transfer

Another method of genetic engineering is the use of a Gram-negative bacteria genus called Agrobacterium with an ability to horizontally transfer DNA or genes from its cell to plant cells by using a natural horizontal gene transfer method.

Taxonomy classifiers have reclassified many of the Agrobacterium species as Rhizobium species because of their properties and behaviors which include causing galls or tumors on plants. Many of the galls are detrimental to the plants, but some have beneficial effects for the plant.

The bacteria genus, Rhizobium, is a Gram-negative soil bacteria that colonizes plant cells within plant root nodules similar to galls and tumour-like growths. The bacteria are able to fix nitrogen gas from the atmosphere and convert the nitrogen to ammonia then provide it to the plants. The plant in turn provides the bacteria with organic compounds that it makes with photosynthesis. Plants like alfalfa and soy beans get most of their nitrogen needs from Rhizobium bacteria species.

The bacteria genus, Agrobacterium has an ability to transfer DNA or genes on plasmids from itself into cells of living plants. This transfer ability has become a basic tool by Genetic engineers that want to move useful genes into plants thereby improving the plant.

Scientists Marc Van Montagu and Josef Schell at the Ghent in Belgium discovered this natural method of transferring genes between Agrobacterium and plants. Nicholas Davidson from Glasgow originally first discovered the bacteria.

Normal *Agrobacterium tumefaciens* is the species of bacteria that forms tumors in the plant by using its ability (having nitrogen fixing features from its Rhizobium association). Agrobaterium tumefaciens bacteria moves in the soil to a plant root or shoot where the bacterial cell makes contact with the plant’s cells. The plasmids in the bacteria move freely into the plant cells where they enter the plant cell genome. The plasmid is carrying DNA that can change nitrogen from the atmosphere into ammonium.

The ammonia joins with the plant's produced carbon foods that the plant makes from it photosynthesis and produces a different food in these new plant cells. The plant can't use this different food but the new cells can thrive and multiply on this food. Then these new plant cells grown with the Agrobcterium tumefaciens transferred genes multiply into a tumor or gall. The gall formation gradually takes over the infested plant and the plant dies.

In 1983 researchers headed by Dr May-Dell Chilton were able to remove the gene in the Agrobacterium tumefaciens transfer plasmid that caused the build up of toxic tumors or galls in the plant. Since then the doctored transfer plasmids of Agrobacterium tumefaciens that do not carry tumor forming genes have been used to transfer other genes that could carry what the researchers wanted to inject into plants cells that wouldn't form deadly tumors that kill the plant but would produce results that the researchers wanted.

Once the selected gene or genes is on the plasmid the plasmid is transformed into Agrobacterium cell that does not have plasmids. The Agrobacterium will then naturally insert the selected genes into the targeted plant cells. To hasten the transfer the plant cells can be treated with enzymes that breaks down cell walls. Plant cell walls are made of polysaccharides that have cellulose and chitin. Both materials are not soluble in water, but when treated with certain enzymes they start to break down. This allows the transfer of the gene carrier plasmid to penetrate the cell wall. After the plasmid enters the plant cell the plasmid joins with the genome and the genes become a part of the DNA of the plant cell.

The plant cells are in leaf discs which are parts of plant leafs from the plant that is to be engineered. These plant discs have their cells weakened by the enzymes in a solution. The plant cells in the discs are incubated with the Agrobacterium using a plant tissue culture method that produces plants that are alike or cloned in a fairly rapid period of time. The growing plant tissues are treated with antibiotic and the plants that survive because the gene marker that they have with the crygenes will survive to become Bt cotton.

There are several Bt crops that have been engineered with the Agrobacterium method. These include: Bt cotton, Bt corn, Bt soybeans, Bt sugar beet, alfalfa, wheat, rapeseed (canola), creeping bent grass and rice. The benefits have been worth the period of time that is required to produce these engineered Bt crops. Producing Bt crops requires the extra years needed to allow the engineered plant to germinate and produce seeds.

There is a drawback to this plant tissue genetic engineering. The Bt toxin is exposed to a great many insects increasing the chances for the treated insects developing resistance to the Bt strain.

To reduce this build-up of insect resistance to the bio-engineered Bt, farmers are planting part of their acreage with the same species of the crop but without the engineered Bt toxin. In this way the toxin resistant insects will not have the advantage over susceptible insects in breeding. This will extend the period before total resistant species of insects will dominate.

Some Good Research Results

One important study that was carried out by a group of research scientists and reported in the journal, *Science*, says that Bt corn genetically engineered to control corn borers was providing pest control to farmers who weren't using the Bt corn but were growing in the area where Bt farmers were using Bt corn. The researchers estimate that in Iowa, Minnesota, Nebraska and Wisconsin Bt corn growers between 1996 to 2009 received benefits of nearly seven billion dollars and the non Bt corn growers received benefits of four billion dollars during the same period.

The same researchers estimated that the corn borer populations on non Bt corn farmers fields adjacent to the Bt corn planted fields had reductions of corn borer populations of 28 to 73 percent. Growers that farm non Bt crops like potato, green bean and other crops in the area that aren't protected by Bt engineering benefit from the neighbors who use Bt corn. Wound sites caused by corn borers allow harmful molds to enter the corn but on the Bt corn areas the reduction of the corn borer populations reduced the mold infections.

The team of researchers gathered 14 years of information. This team or researchers was led by William Hutchison of the University of Minnesota and included Rick Hellmich a United States Department of Agriculture entomologist at the Corn Insects and Crop Genetics Research Unit operated at Ames, Iowa, by the Agricultural Research Service.

The use of trade names in this course is solely for the purpose of providing specific information. It is not a guarantee or warranty of the products named, and does not signify that they are approved to the exclusion of others of suitable composition. Use pesticides safely. Read and follow directions on the manufacturer's label.

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IGRs By Tim Braun

Chemicals have been used over the years for the purpose of controlling insect pests. These pest control chemicals were called broad-spectrum pesticides. They included dust based chemicals containing calcium arsenate in the 1920's. DDT was developed and used during the second world war for insect control. In many situations of pest control the insects rapidly became immune to the chemicals. Organophosphates were used and in some situations like the cotton boll weevil programs the organophosphates were used for a long period of time without an immunity problem.

For a number of years the use of nerve pesticides or neurotoxins were and are still used safely and effectively in the pest control industry. Neurotoxins kill rapidly and residues are usually gone in a short period of time. The organophosphates like Parathion are still a tool for killing insect pests. I partnered with another fellow with a crop dusting operation for several years and when the pilots were putting on organophosphates I remember checking the pupils of their eyes in the dark with a flashlight when they were applying organophosphates at night. We also checked their blood on a regular basis as well as the ground crews who worked with them.

Other means of pest control have included the following: 1. Destroying the crop residues where pests could infect the following crop. 2. Growing crops when and where pests were less abundant. 3. Physically pulling from the crop plants infected fruit. 4. Covering the fruit with cloth or paper to prevent pest damage. These and other methods of crop protection are physical activities to prevent pests from harming the crop rather than applying chemical pesticides.

During the second half of the 20th century pest control researchers began studying the biology of the pest to find other ways of controlling insects. Reasons for a search for other methods of control included the fact that pests were becoming resistant to the chemical toxicity and the residues of the chemical pesticides were presenting an environmental problem in some cases.

Chemicals are basically used in a number of ways for pest control: chemicals that interfere with the pests nervous system; (neurotoxins) and chlorinated hydrocarbons; stomach poisons; chemicals that modify the pests behavior (Pheromones); and chemicals that regulate the pests growth (Insect Growth Regulators or IGR's).

The IGR researchers worked on the development of the chemical hormones that the insect pest produced to exist in this world hoping that this knowledge would result in an insect control method. They first had to study the chemical biology of the target pest. Researchers were hoping to find chemicals that were specific to each individual pest that regulated phases in the insect's life history. The chemicals they researched were hormones.

IGRs Development History (Paper Towel Discovery)

In 1956 the juvenile hormone (JH) was isolated from a crude extract taken from the male Cecropia moths, Hyalophora *cecropis* (L.). Topical application of this hormone were made to this species and it prevented the insect from being able to carry on metaphorphosis thus the insect did not multiply.

In 1965 researchers at Harvard noticed that cultures of the linden bug, *pyrrhocoris apterus L.* that had come from Czechoslovakia had low hatch rates of eggs and that instead of adults many larvae were formed. The researchers found that this abnormality was caused by a material in the paper towels that were in the rearing jars. The towels were Scott brand towels brand # 150. The active material in these towels was found to be juvabione and came from the balsam fir trees. The tree producing this chemical was from the Abis balsam fir tree. This tree is one of the main pulp trees used in the United States paper industry. It's used to make newspaper, magazines and other paper needs. Juvabione is a methyl ester of domatuic acid which was found to be a juvenile hormone copy or mimic of the hemipteran family, Pyrrhocoridae. The discovery of this specific material encouraged industrial interests to use JH as a tool in developing the insecticides referred to as IGRs

The first insect juvenile hormone's chemical structure, JHI (Junior Hormone I) was explained through clarification in 1967. Schmialek was the first to explain for clarification in 1961 the junior hormone active isoprenoids farnesol and farnesal in feces of Tenebrio molitor. As early as 1965 Bowers, Thompson and Uebel synthesized the IGR compound Junior Hormone III. They were able to recognize the hormones biological activity and suggested that it had the same characteristics of a hormone. About this time the federal government as well as the chemical industry produced a great many chemical analogs of juvenile hormones that could be used in pest control.

Insect Life History

The insect life history (life cycle) occurs during the time needed between the egg to another egg. In some cases the life cycle can take a year or in the case of the 17 year locust, it can take 17 years. Fruit fly life cycles are quick and fruit flies can have as many as 25 generations in a year. The length of an insect life cycle depends on the insect and the weather. Completions of life cycles are slower in the north and faster in the south due to temperature.

Insect species survive changing conditions with a growth method of body changes called metamorphosis. An insect life history from egg to egg is one generation. Insects change in form, structure or function as they develop from the egg or embryonic state to the adult state. This is possible through the insect's complete change from a non egg laying, wingless, and voracious feeding body (the larvae or nymph) to a flying, egg laying body (the adult).

Some insect adults, butterflies, can live on sugar sources like flower nectar. Their larva, caterpillars, need vegetative or fiber sources of food. In the life history of some insects the insect can become sheltered in eggs or as pupa to survive long harsh winter conditions. Other insects like aphids have life stages where two stages (nymph and adult) physically look alike and they only feed on plant juices. The adult aphids can grow wings when needed to change location or mate and aphids have an egg laying stage to survive the winter months.

The life history of an insect that includes physical body development in different stages is called metamorphosis. The difference between the two divisions of insects is referred to as those insects that have a complete life cycle with a pupa stage of growth and those insects that have an incomplete life cycle that does not contain a pupa stage of growth.

Holometabolous Insects

An insect with a complete metamorphosis (known as; Holometabolous) goes through four life cycle stages: "egg - larva - pupa - adult". It starts out as an egg, then develops into a larva, a pupa and finally develops into an adult. (Some authors refer to the larva stage as the nymph stage or the larva stage.). Holometabolous insects go through a larva stage during their life cycle. During this larva life stage the insect does not look like the adult stage of its life cycle. The holometabolous insect has wings that develop inside their bodies and do not appear until after the pupa life cycle of the insect.

Hemimetabolous Insects

The insects that have an incomplete metamorphosis (known as: Hemimetabolous) go through three life stages of development: “egg - nymph - adult. The adult insect that develops through incomplete metamorphosis is similar in appearance to the young nymphs except that it is usually larger and it can mate under the right circumstances. Hemimetabolous insects do not go through a pupa stage. Some hemimetabolous insects have wings. When these incomplete life stage insects have wings their wings develop on the outside of their bodies In most cases the adult insects going through an incomplete metamorphosis have wings or the capability to grow wings if conditions require wings. The nymphs usually have wing pads on the outside of their bodies that are visible but can‘t be used for flying. Aphids are an example of the hemimetabolous form of incomplete metamorphosis.

There are some incomplete or hemimetabolous insects that spend all their nymph stage in water because they are aquatic. These nymphs do not look quite like their adult forms who are land insects. They are still classified as hemimetabolous insects because they do not have a pupa stage and their wings are outside of their bodies. The Damsel and Dragon flies, Mayflies and Stoneflies are in this group of insects.

INSECT STAGES OF GROWTH

EGG STAGE

The egg is one of the periods for an insect to over winter in a semi protected state in its life history. The pupa stage is another protected state or period in its life history. Some insects like the loopers lay eggs with protective shells that have enough nourishment in them to take care of the young insects’ needs until they hatch from the egg. The insect that enters its new life after they hatch from an egg with a shell do not receive nourishment from the egg laying female once the shell is formed around the embryo. (Cockroaches carry egg cases in their bodies until the eggs with shells are ready to hatch. Then they lay the entire case.

One of the pest species of insects is the Lepidoptera. This includes cabbage loopers. They lay their eggs and are called oviparous or egg-layers. Their eggs have a yolk that is surrounded by a material called cytoplasm. The yolk is used by the embryo as a source of food until the embryo has grown enough to escape from the egg and find other food sources. There is a protein material layer that encloses the cytoplasm and yolk. This is referred to as an envelope. This is inside the chorion which is a protien membrane that protects the egg from external forces. In some Lepidoptera species there is a waxy layer that is inside the chorion. This waxy layer protects the contents of the egg from drying out. The respiration of the embryo is aided by the presence of a layer of air-pores in the solid material of the chorion.

There is a small depression in the top of the egg with a small central cavity. When the egg is in the female this cavity is where the sperm of the male can enter after the mating process. The cavities are either globular, conical of cylindrical. The eggs of this species, Lepidoptera, are usually round and small. The colors of the eggs can be white, pale green, bluish-green or brown.

The butterfly and moth eggs are spherical, conical, cylindrical, lenticular or lens shaped. They can be barrel shaped and pancake shape. These eggs can be turban or cheese shaped. They can be angled or depressed on both ends, ridge or ornamented, spotted or blemished. Eggs are laid in clusters or singly. Usually eggs are laid near a food source. The eggs laid for over wintering are usually larger in in order to provide food for the embryo. Eggs laid on woody vegetation are often larger that eggs laid on vegetative surfaces. It’s been noted that older females lay smaller eggs than those that have been laid by the younger females. The new born larvae of many species of the Lepidoptera eat the chorion. The egg shell has an area of weakness around the cap of the egg shell.

The white fly females lay their oval eggs in a singular, haphazard manner. They will also lay their eggs in spirals or circles on the underside of the plant’s leaves. The eggs of the white fly have a peg-like pedicel that the female can push the egg to insert this pedicel into the leaf after she makes a slit with her ovipositor into the surface of the leaf. The female may lay eggs directly into the stomata openings of the leaf. A glue like substance is deposited at the base of the pedicel on the leaf to cement the egg in place. The egg can draw water into itself to prevent desiccation of the egg before hatching.

There are some insects that do not produce a shell around their young embryos and these insects provide nourishment for the embryo in the egg sac until the young insect is able to survive on its own. Then the adult gives birth to a living young insect.

Aphids do both. They give birth to live nymphs and in other cases they lay shell protected eggs. Ants will protect aphids in order that the aphids provide the honey dew material that ants use as a source of food. Ants have also been known to carry aphid eggs to the ant nests where the eggs are cleaned to protect the eggs from fungus. Researchers have found instances where certain insects like the desert locusts uses egg protectants and cements to protect their eggs. Female stick insects lay their eggs singly or by dropping them on the soil to prevent predators from feeding on a cluster of her eggs.

Some plants like the pine trees produce a defense against the insects that lay their eggs on their leaves. The plant reduces the vigor or growth of their leaves that insects have laid their eggs on. The reduction of growth vigor in the plant’s leaf lowers the ability of the leaf to fight fungus and other diseases that are constantly trying to infect the leaf’s surface. These growing pathological microorganisms diseases then attack the insect eggs that the insect pest has laid on the leaf surface.

The predaceous insect known as the lacewing lays its eggs on a post that the female builds from a thick fluid that she produces and allows to harden before she deposits her individual egg on top of it. The post is high enough from the leaf surface that it prevents other hatched lace wing larva from attacking the lace wing eggs and destroying its brothers and sisters.

THE LARVA AND NYMPH STAGE:

LARVA (The word LARVA in Latin means *Ghost*) Insects that go through a complete metamorphosis have a young stage called the larva. They have common names: Diptera or flies, mosquitos (maggots) ; Lepidoptera (caterpillars, cabbage loopers, corn ear worms) Coleoptera beetles (grubs). The larval stage of insects in most cases does not look like the final stage or adult stage.

During the growth stage of the larvae and nymph the young insects have outer skins that have to be shed once the insect has filled them with the growth of their bodies. They then grow a new larger skin inside the old skin that they break out of and shed. The new skin expands until the insect grows enough to fill it. Nymphs and larvae both grow into a series of larger stages.

The skin shedding process is called molting. Depending on the insect, molting can occur 3 to 15 times. Each stage of molting is referred to as an instar. The 1st instar is the growth between the egg hatch and the first molt. The 2nd instar is between the 1st molt and the 2nd molt. . etc. The nymphs molt until they become adults while the larvae molt until they become pupa. Incomplete metamorphosis nymphs do not have a pupa stage in their life cycle. Complete metamorphosis larvae have a pupa stage in their life history.

Molting is triggered by steroid hormones that stimulate the making of chitin and protein in the epidermal or skin cells of the larva. This chitin and protein mixture gives support to the insect’s body as an exterior skeleton. The skeleton of the larvae is soft enough to be flexible depending on the insect as in the case of mosquito wrigglers and fly maggots. The glands that produce these hormones and cause molting to occur, wither away and become inactive as the nymph reaches the adult stage or as the larva begins to pupate.

Large differences exist between insect adults and larva. An example of this vast difference occurs with the predaceous insect called the lace wing. Lace wing adults are beautiful insects with large clear wings grown from a clean, green body. The larva is similar to a very small alligator with a large voracious appetite for white flies and other crop pests. The larval stage in most insect pests is considered to be the most damaging to growing and stored crops. The larvae eat a great deal of food because they need a great deal of energy to go through their next stage of life history which is their metamorphosis.

The larval stage in many cases lasts longer than the adult, egg and pupa stages and in other insect species this stage of life can be shorter. Some species of cicadas live for a long 17 years in their larval stage of life. The life stages of the larva vary in the number of growth stages. Many insect larvae are without legs, because they have no use for legs. Mosquito larvae live in water as well as many flying species and haven’t a need for legs. They swim by wriggling their bodies and are referred to as "wrigglers". Social insect larva that are fed by their colony members like honeybees and ants do not need legs. Parasitic insect larvae whose egg stage are laid inside other insects are legless.

Lepidoptera Larva

The Lepidoptera caterpillar larvae have chewing mouth parts and several of them feed on growing plants. The larval body of the Lepidoptera insect has three sections: the head, thorax and abdomen. The larvae have soft bodies that are tube shaped. The body contains hair-like projections. The body has 13 segments. Ten of the segments are located on the abdomen. Three segments are on the thorax.

The larva has 3 pair of true legs and up to 5 pairs of prolegs. These prolegs of the Lepidoptera larvae are not segmented like the real legs, but they do have hooks on the ends of their legs. When scouting leafy vegetables for caterpillars the trail of scar damage from there proleg hooks is sometimes visible. The Lepidoptera, leaf minor, has smaller thoracic legs than the larva of loopers.

One item of identification of the Lepidoptera larva is the presence of an upside downward line shaped like an inverted or upside down Y on the front of its head's face. The head has a mouth at the bottom of the inverted Y. The mouth has an upper lip or labrum that has a notch in it where plant leaves are held for chewing. Below the labrum are the mandibles that are used for chewing. On either side of the larva's head are located six stemmatas or eyes. Below the eyes, stemmatas, are the two larva antenna. On the labrum of the Lepidoptera larva are silk glands which are modified saliva glands. These glands are used to produce silk for cocoons and shelters. Often the leaf area around the larva has fluffy or white material present.

The Lepidoptera larvae usually go through 5 instars or growth stages. The skin or cuticle that first covers the larva is very loose and flexible. As the Lepidoptera larva grows the cuticle expands to a certain point then it hardens and the larva has to break through this hardened cuticle before starting to grow another soft pliable cuticle. The old hardened cuticles are abandoned. Each one of these cuticle growths are called ecdysis. This is an important part of this hour's lesson because we will discuss IGRs that are referred to as (Ecdysone Inhibitors). During the last instar or ecdysis the hardened cuticle splits and forms a small ball at the rear end of the pupa.

Coleoptera Larva

The Coleoptera beetle larvae are referred to as grubs, wireworms and click beetles. The larvae are called the feeding stage and these insect larvae live up to the feeding name causing huge feeding damage to crops. The predators of the Coleoptera larva include the famous lady bugs larva. The Coleoptera larva pests include the boll weevil, alfalfa weevil and Colorado potato beetle which are crop destroying feeders.

The Coleoptera larvae have chewing mouthparts and some have segmented legs and feed on plants but unlike the Lepidoptera larvae they lack prolegs with hooks. They usually have a darkened head and several spiracles along the side of their bodies. Some Coleoptera larva that have flat bodies and are able to move about rapidly and are called ground and rove beetle larvae. There are Coleoptera beetle larvae that have dark head capsules and tiny feet that look like hardened worms. The Coleoptera beetle larvae go through several instars. The number of instars varies by species.

Diptera (Flies) Larva

The Diptera larvae do not have true legs. The head of this larva looks like the rest of the body and is hard to make out. In some diptera larvae the head and its features like the mouth and antennae are withdrawn into the thorax which is undistinguished from the abdomen and therefore the larvae look like they have no head or thorax. The division to the thorax and the abdomen are almost non existant. The larvae of the diptera are called maggots. These diptera maggots are without legs or limbs, but in some cases they will have prolegs. In most species the eyes and antennae are not present and in some species. The abdomen of the diptera maggot has no appendages or thorns or any other things sticking out of either side of their body.

The lack of mobility features on the diptera larvae bodies is because these body appendages and parts are not needed in the environment where the larvae exists. These larvae hatch from eggs that are laid in rotting oganic matter or in liquids that have sufficient liquid type foods. I have a dog and I constantly have to pick up the fecal matter that the dog excretes. I know that diptera house flies lay their eggs while the fecal matter is fresh and soft. If I water the lawn with dry dog fecal matter on the lawn the water will soften the fecal matter attracting house flies to an ideal place for laying their eggs that rapidly hatch. In our neighborhood fly populations come from dog fecal matter. Nobody has any livestock other than the dogs we keep.

The fly larvae are similar to the legless beetle and have mouth hooks used to loosen tight organic food particles. The social insect larvae like termites and bees do not require chewing mouth parts because they are fed liquid materials that do not require chewing. Because some diptera larvae exist in water their ability to obtain oxygen in a somewhat airless environment is noted. They have tracheae and other external body spiracles that they use to obtain their oxygen needs for respiration. Some Diptera larvae get their oxygen through their skin from the water without the use of spiracles.

Mosquito larvae go to the surface of the water and use a siphon tube projected through the water film to suck in their oxygen needs. The anopheles species lay parallel with the surface of the water to obtain their oxygen because they do not have siphon tubes. Mosquito pest control methods include spraying the water surface with pesticide oils that prevent mosquito larvae from obtaining their oxygen needs.

INSECT NYMPH STAGE

The young stage of the insects that have an incomplete metamorphosis is referred to as the nymph stage of growth. Unlike the insect larvae the nymphs in most cases are similar to their adults. Some nymphs that grow to become adults with wings have wing pads on their bodies that will become wings when they gain adulthood. Because the insect nymphs are hemimetabolous, their wings are attached <u>out</u>side their bodies. Nymphs, like the larvae, do not mate because only adults can mate.

Aphids Nymph Stage,

Aphids that encounter diseases, climate, starvation etc.. can survive if they have the right set of genes. The greater the mix of genes the greater the chances of survival for aphids with the right gene mix. Just the fact that the sexual mating of male and female aphids can produce an egg that will survive the tough winter increases the chances of the aphids' species survival. The sexual union produces an egg and a mix of genes both vital to the survival of the aphid species.

As the weather warms up and the food supply increases aphids begin to multiply rapidly. Aphids born after conditions improve are born by asexual reproduction. Asexual reproduction doesn't require male aphids or a sexual union. When times are good the need for a fertilized egg that has a protective shell to withstand the winter months or other harsh conditions is not required. During good conditions the female aphid produces an egg cell in her ovary and it starts dividing producing growth within her body. (No male sperm is needed.) The dividing cells produce various body parts: legs, head, antennae, abdomen ect...A female embryo is formed that becomes a nymph. This female nymph is born live and immediately begins feeding.

After the female nymph goes through four molts or instars she becomes an adult and she can give birth to more aphid nymphs both male and female without having sex. The newborn aphid nymphs can be aphid adults producing aphid nymphs in just 7 to 8 days.

These female adults that hatched as nymphs from the shell protected fertilized egg can produce daughters by asexual reproduction called "parthenogenesis". The first born female nymphs that are born this way, asexually by parthenogenesis, from adult females reared from fertilized eggs, are called "parthenogens". These first asexually born daughters of sexually produced aphid mothers are parthenogens but they are not "clones". Clones are genetically identical to the mother. The first offspring birth through asexual parthenogenesis is a process that creates new individuals from varied genetic material that was contained in the egg cell from the sexually produced mother. The aphid daughters asexually produced offspring will be parthenogens with no twins and with varied genes just like her sexually produced mother.

Now. The offspring nymphs of these asexually produced parthenogens will be clones identical to their adult mothers. Inside the body of the parthenogen aphid female, unfertilized egg cells are released from the ovaries and start developing into aphid embryos and the egg cells inside those embryos start developing embryos. A female aphid nymph may be carrying her daughter's embryos and her grand-daughter's embryos that are developing in her daughter.

Within 8 days after being born the aphid nymph carrying the embryos may grow through 4 instars or molts and become an adult stem mother that gives birth. Then her aphid nymph daughter after 8 days gives birth and on and on. This telescoping asexual birth of aphids is why populations can explode in an alfalfa field. Asexual birth requires less time and energy then sexual birth, but remember that the aphid nymph has to go through the instars growth cycle to become an adult. Then she can give birth. Only adult insects give birth and also only an adult insect can mate. These asexually produced parthenogen aphids adults are referred to as stem mothers.

Termite Nymphs

The termite nymphs hatch from eggs. The termite nymphs go through five instars or molts before they become adults. The nymphs are dependent on adult termites for their food which consists of wood that has been eaten by the adult termites then regurgitated for the nymphs. The termite nymphs are very mobile and move about the nest for food and changes in their environment. As the nest changes from hot to cool the nymphs move to the more compatible areas of the nest. They move from cold nesting areas to mild nesting areas as the worker termites dig deeper (cool areas) or move closer to the surface of the soil (warmer areas). These nymphs even though they resemble the adult termites are unable to feed themselves by consuming wood like their termite adult tenders who have bacteria in their guts that breakdown the consumed wood. The digested products become food and nourishment that can then be consumed and used for growth.

White Fly Nymphs

The silver leaf whitefly (Bemisia argentifolii) is one of the species of the whitefly that has built up the highest resistance to pest control methods. This is one of the whitefly species that IGRs have been used on with some success. Unlike the termite nymphs whitefly nymphs have to feed themselves from the moment they hatch from their eggs. During the first of their instars the whitefly moves from the egg they hatched from. White fly nymphs are called *crawlers.*

The whitefly nymphs have piercing sucking mouth parts. While in the first of the four instars of the silver leaf whitefly nymph, the nymph moves about the plant leaf until it locates a spot where it inserts its piercing mouthpart into the leaf tissue. This may take several hours and the distance traveled can be as much as 30 millimeters.

The host plant leaf is penetrated by the nymph to the plant’s phloem cells where the sugar laced plant juices are located. Part of the sugary material is sucked into and used in the nymph’s anterior midgut. The excess liquid is passed on to the nymph’s hindgut and excreted out the anus. This excreted liquid attracts fungi and ends up coating the lower plant parts with black sooty mold.

During their second, third and fourth star instars the whitefly nymphs remain in one spot and do not move about. The whitefly nymph during their first instars are referred to as crawlers. They have six legs that they use to move about the leaf finding a place to inject their piercing sucking mouth parts. The size of these nymphs is so small the use of magnifying lens are necessary to make out their legs. Once the crawler finds this feeding spot it pulls its legs in under its body and becomes stationary through the next three instars.

One way of monitoring whitefly populations is to check certain leaves for counts. Adults, eggs and first instar nymphs are found on young leaves. Second and third instars nymphs are found on middle aged leaves and fourth instars nymph which includes their so-called pupal cases can be found on middle aged and older leaves.

The 3rd instar of the white fly nymph is usually large enough for the naked eye to see. There are two yellow spots on the third instar. On the fourth instars of the whitefly nymphs the appearance of the red eyes of the just appeared adult whitefly is visible on the so-called pupa case which is the old body covering of the fourth instar whitefly nymph. Whiteflys have an incomplete life history that does not include a pupa stage. The red eye white fly is the adult and can have sex and lay eggs.

Citrus Red Scale Nymphs

Another insect nymph that is referred to as *crawlers* is the citrus red scale. Red scale is a serious pest in California citrus. This scale insect is a treated pest in central Arizona. The Yuma citrus area has a public program that monitors and controls red scale insects and is considered citrus scale free.

The nymph stage of the red scale born from the adult female scale that produces one hundred to two hundred nymphs that are referred to as crawlers the same as the whitefly nymphs which are also referred to as crawlers. The crawlers have six legs which they use to move about the plant. Their movement is aided by the wind and other objects that brush up against the crawlers and move them about. In this way the crawlers are able to infest large areas of the crop (citrus).

The eyeless female nymph scale hunts for the ideal feeding spot on a leaf, stem or fruit as a crawler. When she finds the feeding spot she inserts her piercing sucking mouth piece into the spot and terminates any further movement. During this first instar the nymph secretes a material that covers her body forming a white cap. As the cap forms a circular ridge is made that forms a nipple on the top of the cover. The liquid that is forming the cover flows onto the plant surface and becomes a whitish gray.

The feeding tube is pulled out of the plant when an instar or molt of the scale nymph's skin occurs. The scale nymph is sealed off with the cover it has formed from its first molt or cast off skin and the growth of its body which has a yellow cast to it. This takes about four days.

During the second instar the scale nymph reinserts the feeding tube and begins feeding. An orange ring is formed in the nymph's cover from the remains of the first molt's cast off skin. Counting the number orange rings around the scale nymphs dome is a method used to determine the stages of the scale. A skirt is formed around the scale nymph's cover. This gray colored skirt is a secretion of wax and protein produced by the scale nymph during its second instar. The cover or dome is sealed to the scale nymphs body and cannot be separated.

The female scale nymph goes through another molt of its skin. Not of its cover or dome. As it sheds its skin it again pulls out its feeding tube and when the skin is shed it replaces its feeding tube and resumes feeding. The second instar takes about 6 days and the orange ring formed from the second skin that was cast is visible.

The third instar stage has a more circular shape to it. The bottom edge of the third instar scale nymph's body protrudes from the females body to the outside of the gray colored skirt around the body. The winged males are able to locate this appendage and mate with the female. After the female has mated with the male the appendage which is referred to as a pygidium withdraws under the dome to prevent mating with other males. After mating the female scale is no longer a nymph. She is now an adult. Females that do not mate continue to grow but do not become mature adults.

The male scale nymph looks about the same as the female scale nymph during the first instar. They have six legs and move about as crawlers. As the male scale nymph goes into the second instar of its life changes to its body are different from the female scale nymph. The male gets a longer body whereas the female's body was rounded. The male scale nymph develops eyes. The female doesn't.

The male scale nymph has a brown pigmentation at the posterior of it's body referred to as a pygitium. The pygitium becomes v shaped. After five days the second instar male scale nymph goes through another molt. This change brings about the squaring of the pointed rear appendage or pygitium and it loses its brown pigmentation. This pygitium later becomes the male scale nymph's genitalia. A day and a half later the male scale nymph goes through another molt and now has a pointed genitalia. During the next three days the male scale nymph grows to be an sexually active adult with prominent wings, antennae, head, thorax and abdomen. The male adult can and does fly looking for female scales with the use of its antennae that takes in pheromones that the female emits.

Pupa Stage

The adult body is formed during the next stage which is the pupa stage for insects with a complete metamorphosis. Complete metamorphosis occurs when the insect's larvae finishes their last instar and starts forming a covering to enter their third life history stage as a pupa. Pupae like the caterpillar's are formed in a chrysalis which is in the open and attached to leaf surfaces. A chrysalis is bright colored and sculpted into an attractive looking object which provides protection by camouflage. The last larva skin of the larva is either discarded while making the pupa skin or the pupa skin remains inside this last larval skin.

Moths can make another covering of their pupa called a cocoon which is made of wooden fragments and leaves woven with silk strands or just straight silk excreted by the last larva. Pupae are located above or below ground or hang from branches or twigs. Because the pupa is almost defenseless it is either hidden, camouflaged as part of its surroundings or protected by strong protective pupa skins which include the cocoon covering.

The major physical changes of the insects that have a complete metamorphosis occurs in the pupa stage. As the last stage or instar of the larva ages, the same juices put out by the larval insect to dissolve the food it consumes starts working on dissolving the larva's own body. This dissolving of the insect's body with its own digestive juices is referred to as histolysis.

There are certain parts of the insects body that do not dissolve. The insect has carried embryonic stem cells formed in the fetus and carried by the larvae through each instar to its pupal body. This group of stem cells in the pupa that are not digested are called histoblast. The embryonic stem cells divide and multiply to become a complete adult body.

In order to grow these adult body parts like the wings, legs and sex organs the dividing stem cells use the juices of the preceding larval body to form a new adult body. The pupa stage can take as little time as a couple of weeks or it can go into a resting period of an entire season. When the right temperature and moisture conditions occur a fully grown adult insect emerges.

Lepidopter Pupa Stage

The butterfly pupa is called a chrysalis. Unlike other Lepidoptera the butterfly has a number of shapes and an abundance of colors. The caterpillar during its last instar as a larva attaches itself to a branch or other object that is projecting so that it can hang with the help of a small wad of silk. The chrysalis is formed by the caterpillar while hanging attached to a silk pad by a hook-like process called a cremater. Some butterflies produce a chrysalis that is held upright with the use of a silk girdle that supports the hanging chrysalis. Other butterflies hang with their head pointed down.

Moths on the other hand make a dull colored cocoon from spun silk. The cocoon is used to protect the pupa while it goes through metamorphosis. Other moth's simply make a cell in a small tunnel under the ground for protection of the pupa.

During the pupa stage the adult parts of the body: the wings, antennae, eyes, head on the thorax, and the six legs are visible. In most cases the Lepidoptera pupa is stationary, but in some cases the pupa can move or wiggle some of their body parts. There are Lepidoptera pupa that can move back and forth in the tunnels with the use of spines on their back. Some Lepidoptera pupa can make noises like rattling or clicking sounds that startles would be predators.

In some of the Lepidoptera insects adult males mate with the female pupa that are about to emerge. Other female Lepidoptera in order to avoid mating cap their reproductive organs or put out anti-pheromones that discourage any male Lepidoptera that may approach the pupa.

Coleoptera or Beetle Pupa

Once the coleoptera pupa goes into its resting stage, it stops feeding and its body tissues begin dissolving. Once the old tissues become liquid new tissues are formed becoming the body parts of the adult beetle. The pupa is not tightly covered by a chrysalis of the butterfly or a cocoon of the moth. The coleoptera pupa is covered with a thick chitinous layer that provides protection from dehydration.

During the pupa stage for the beetle the body parts that are formed include: compound eyes, wings, antennae and sex organs. Most of the species of the coleoptera's larva are built to be eating machines with teeth and a large fleshy body to handle the digested food whereas the adult form of the beetle that replaces the larva is more adapted to sexual propagation of the species. There are exceptions however with some species of beetles where the adult is a big eater and many of them are responsible for crop damage as severe as the larva stage of beetle.

Diptera Pupa

'Tumblers' is the name given for mosquito pupae. Once the mosquito larva reaches its fourth instar it inters the pupa stage. It stays in the water another one to four days and because it is lighter than water it floats on the surface. The pupa takes in oxygen through two breathing tubes. If something startles the pupa it will dive with a jerking movement "tumbling" under the water's surface. Then it floats back to the water's surface. Like other pupa insects it doesn't eat while going through the pupa life stage.

The mosquito pupa is covered inside a pupa case that it has made before going into the pupa stage. While in the pupa case it goes through metamorphosis using fluids from the breakdown of its larva body to grow antennae, wings, legs, and the rest of its adult body. The adult mosquito split's the pupa case and steps out onto the surface of the water where it stands until its body has time to harden. This is one of the reasons that mosquito adults lay their eggs on calm waters that allow the new adults to stand on the water while the adult body dries out. Then it is able to fly away.

House Fly Pupa

The house fly eggs are laid in manure, or garbage that can supply liquid food for the maggots or larva that feed and grow through four instars. At the end of the fourth instar the pupa stage begins. The pupa case is formed around the larva and the insect grows into an adult house fly. The pupa case is made of the last larva skin which can be yellow, red, brown or black as the pupa grows inside the case.

The larvae is long, greasy and cream colored. The house fly pupa is shorter than the larva with a blunt rounded body. The change to an adult with wings, legs, antennae and body parts takes from two to six days in warm weather and seventeen to twenty seven days during cool weather. The adult breaks out of the larva skin by swelling and shrinking and using a part on the front of its head as a jack hammer to break out of the pupa case.

The Adult Insect

IGRs are not a pest control product for adults, but without the adult insect there is no egg, nymph, larva or pupa. Some of the Insect Growth Regulator labels state that when exposed to IGRs some female adult insect pests are unable to reproduce. The IGR brand name, Talus, states that "Talus" also suppresses egg-laying and causes egg sterility in treated adults through secondary hormonal activity." another IGR product states that "Pedestal" also reduces adult female fertility." Therefore this course will include information on adult insect pests.

The final stage of the insect is an adult that is capable of mating and producing the first stage of the next generation which is the fertile or infertile egg. Both types of adult insects, those with complete metamorphosis and those with incomplete metamorphosis, can reproduce and grow functional wings if the species requires wings. The adult cuticle of most insects harden and are darker than the larval and nymph cuticle which is flexible and usually clear. The adult external skeleton with its hard cuticle cover prevents water loss and water entry. It provides protection from heat, cold and its enemies.

Insect adults have antennae or feelers on their heads and their muscular thorax or chest supports their wings. They have six legs and an abdomen. An adult insect uses its wings to rise up to levels in the air where prevailing winds carry them for long distances. Some adult insects like the mayfly last as adults for one day while the adult honey bee queens live 4 to 5 years. The adult mound building termite queens can last as adults for more than 50 years.

The Lepidoptera Adult

Lepidoptera adults, at least most of them, have mouthparts used for piercing and sucking liquids. There are some with no mouth parts that do not feed. Others who had ancestors with chewing mouth parts but through genetic changes that included not eating during adulthood lost their mouth parts.

The sucking mouthpart of the adult Lepidoptera is made up of two tubes connected together with hooks called a proboscis. The Lepidoptera adult gains the suction from contraction and expansion of a sac in the head with muscles that control it. The proboscis is kept curled under the head unless the insect is feeding. Some adult Lepidoptera feed on flower nectars and others feed on rotting fruit juices or fermenting tree sap with a proboscis that have tiny holes in the sides and a closed end to strain out the solid parts. Some Lepidoptera adults have a proboscis that allows them to consume pollen.

The adult Lepidoptera butterfly has two compound eyes. Butterflies have different colored eyes. They can be brown, golden brown of red. The antennae surface is covered with scales and hairs that are used primary for intercepting pheromones.

The thorax, the middle part of the insect body, has three divided segments on the Lepidoptera adult: the pro thorax is the front segment of the thorax with forelegs on it; the forewings and the middle pair of legs are located on the meta thorax or middle section of the thorax; and the hind wings and hind legs are located on the mesa thorax or hind section of the thorax. The three pair of legs are used for support, tasting and smelling. The organs used for these activities are visible on their legs. All three pair of the legs of the Lepidoptera adult are covered with scales

The two pair of wings held together with fibers are thickened with hollow ribs. They are covered with a membrane above and below. The membranes are covered with scales that contain hairs on their jagged ends. The adult Lepidoptera operate their wings with muscular movement, contraction and expansion of their thorax. Wings are not only used to fly. Adult Lepidoptera also use their wings to defend themselves. Wings are used as a means of camouflage when they blend in with the immediate surroundings or give a menacing appearance to their enemies. The wings are varied in their design and size.

The adult Lepidoptera have veins that run through the membranes used for vital liquid transfer. The Lepidoptera has what is called an open circulatory system through which the fluid that contains water, fats, sugars, proteins, hormones and etc.. flows. In insects this system does not carry oxygen as systems do in other animals. Insects and other lower animals receive their oxygen needs directly from their body surfaces. This fluid does contain nutrients. This liquid transfer system aids in the growth of the insect acting like a hydraulic system allowing the insect to expand its segments before they harden. There are also nerves running through the wings that activate muscles to control motion.

The Lepidoptera are covered with scales. This is where the name, Lepidoptera, comes from. The Greek words, lepis, or, lepidos (fish scale). Scales are found on the Lepidoptera adult thorax, head and abdomen. There are even scales found on parts of the genitalia. The scale are easily removed from the adult Lepidoptera body without harming the insect. The scales give the adult Lepidoptera its color. This color is dependent on the direction that the light passes through the scale. These colors of the adult Lepidoptera is used as camouflage. The colors of predator edible butterflies and moths are changed by Lepidoptera to inedible colors in order to fool and escape the predators.

The abdomen of the adult Lepidoptera has nine segments. The role of the Lepidoptera adult is mainly courtship and mating. The abdomen’s end segments are the genitalia used for the mating process. The genitalia of the Lepidoptera insects are made to fit the specific female genitalia like a key in lock. The differences in genitalia are specific for each species.

When mating occurs the male sperm is in a capsule that enters the female. The sperm capsule inside the female releases the sperm which swims to a receptacle in the female and stays their until egg laying takes place. Then the sperm is released into the vagina for fertilization of the eggs produced by the female. The sperm can be held in the female receptacle for hours, days or months after mating depending on when the female lays her eggs.

Coleoptera Beetle and Weevil Adults

The coleoptera adult is distinct in the fact that it is armored. Most of the coleoptera species have wings. Their fore wings which are called elytra are not used for flight but are used to protect the hind part of the body. They are raised to allow the second pair of wings to be used for flying. The elytra are fused together to defend beetles that do not fly. An example of this type of non-flying beetle would be the ground beetles.

Both the adult and larva beetles consume food unlike many of the Lepidoptera insects therefore the adult mouthparts are similar to the larva‘s mouth parts. Their tooth parts, called mandibles, are like large pincers that move horizontally to grasp, crunch and cut food or in many cases their enemies.

With the large number of beetle and weevil species that exist the number of variations in the coleoptera adults is enormous. The eyes of the coleoptera are compound and in some cases the eyes are split. This is true of the water beetles to view the areas above and below the water line.

Their antennae are used to smell as well as physically feel their surroundings. Some use their antennae for defense and mating. Their antennae may be clubbed or short and some beetles have long antennae.

The adult coleoptera have legs attached to the thorax or mid section as well as their wings. They have six legs like other insects and the legs may be used for other activities besides walking. The water beetles have legs adapted to help them swim. Some species have legs that are widened to aid them in digging. Other coleoptera have long hind legs for leaping. Flea beetles are and example of beetles that leap.

The oxygen used by the adults is taken in by tracheal tubes and pumped through the their interior by pumping movements of their body in the fine tubes that exist throughout their bodies. They have an open system of liquid circulation instead of the heart blood pumping system that is found in the higher animals.

The main activity of the adult coleoptera is mating and laying their eggs. The adult stage of the coleoptera can last for hours, days, months or in some cases for years.

Diptera Adults (Flies and Mosquitoes)

A main feature of the fly that identifies it from other adult insects is the presence on its body of two wings. (Diptera, meaning "two wings"). The body of the diptera adult is built for flying and is short and streamlined. Even though the diptera have two wings they also have knobs or halteres that aid in the diptera adults balance during flight. They can hover in mid air and turn in very little space.

The diptera adults eyes that in most cases are compound which means that the eye consists of thousands of individual photo receptors. They can detect fast movement which comes in handy when doing intricate, speedy flight movements. The surface of the compound eye is rounded giving sight in different directions.

The diptera adult mouth parts are used for liquid uptake and do not include chewing mouth parts. The mouthparts of the diptera adult are either piercing and sucking or lapping and sucking. The mosquito female with its piercing sucking mouth parts takes in the blood meal that it needs. Male mosquitoes feed on fruit and liquid sugars of the flower nectars. Females will feed on fruit and liquid sugars, but before or after mating she needs the blood meal to provide protein for her eggs. She can store the male sperm in her body and uses it to fertilize her eggs right before she lays them. The female mosquito looks for a place to lay the fertilized eggs that will provide food for the embryo's growth inside the egg.

This location that she lays her fertilized eggs in is usually water, but in some cases she will lay her eggs in an area that will receive water after she lays the egg. The eggs can survive a long period of time before water is available but must receive water in the future. Without water the hatching larvae will perish. These egg nests could include empty cans, tires, bird baths, etc.. In these cases where water is coming the egg goes into a diapause or dormant stage and will hatch when water levels, temperature and oxygen levels are ideal to allow the egg's dormant stage to come to an end and the eggs can hatch.

The female also makes sure that when there is water the surface of the water will remain calm enough that after metamorphosis the emerging adult mosquito can stand on the water while it castes off its pupa case, stretches its new body and flies away. There is no more growth for the adult once it goes through the metamorphosis stage.

The Incomplete Life Cycle Adult Stage

The main difference between nymphs and adults for those insects that do not have a pupa stage and do not go through metamorphosis is the presence of wings and mating. There are exceptions to having wings and giving birth. Many of the species of hemimetabolous (*insects that do not go through metamorphosis*) do not need wings and therefore do not grow them. Some insects like the aphids do mate as adults, but they can and do produce offspring without mating through asexual reproduction.

The Great Things Happening With Insect Growth Regulators

When I first started in the pest control business back in 1958 after graduating from the University of California at Davis their was a great deal of interest in combining the use of insect predators with pesticides to control the damage done by pests in producing agricultural crops. The book by Rachel Carson, "Silent Spring" came out during that time and some people were pushing to do away with pesticides. People like myself who worked with and just started using pesticides needed to find a way to keep using these products.

I was working in Bakersfield, California when a research group at the University of California at Riverside, California led by Dr. VM Sterm came up with a pest control program that combined several sources of information to control pest in a specific crop field. This system, Intergrated Control, (IC) later became the Intergrated Pest Management (IPM) method of pest control used in agriculture today. Dr Sterm's group of entomologists first formed their concept of combining field checking to know whether a crop's damaging infestation existed and whether chemical pesticides were required and what chemical pesticide should be used that would kill the damaging pest without entirely eliminating the predator population.

In 1958 when I first checked fields in Bakersfield, California the insecticide that I used most frequently was Systox a systemic material that controlled several pests including the Atlantic red mite that was toxic to cotton. The Bakersfield growers had a serious outbreak of spotted alfalfa aphid that was very damaging. The organophosphates including parathion, malathion and TEPP (tetraethyl polyphosphate) stopped the ruin of the California alfalfa industry, but after a few years of control the spotted alfalfa aphid was coming right back in greater numbers with a building immunity to the organophosphates.

Stern and his group ran several field tests in the alfalfa and found that fields treated with the systemic organophosphate, *Systox*, left many predators that hatched after treatment and that the spotted aphid populations were reduced by these predators and constant applications weren‘t needed. The Systox material went into the plant without leaving a residue that killed the predators but it did destroy leaf sucking spotted alfalfa aphids. The predators that either entered the field after the pesticide application or hatched from eggs laid before the application killed spotted alfalfa aphid that entered the field.

The entomologists stated that the chemical (Systox) and the predators (biological) were an Integrated Control combination that works. The Integrated Pest Management, IPM, is a common term used by many people in the agricultural industry today. The use of pheromones are now used to check or monitor fields for pesticide use. IGRs that kill specific insect pests and leave predators alone are part of the IPM program used by growers and their pest control advisors.

In the State of Arizona and the lower deserts of California the use of Insect growth regulators were added to the IPM program to control the whitefly under a section 18 in 1996. The industry had been using one of the first measures of IPM, field monitoring, before adding the pesticides. Treatment was made when a count of five adult white flies were found per leaf when 30 leaves were examined in a field. (15 each at two spots in the field). This plan of treating was used by the cotton growers using parathyroid pesticides that were very good at controlling the whitefly.

The spray plan worked well, but because the biological part (working with predators) of Stern’s plan wasn’t used, the program started breaking down. The broad spectrum chemical, pyrethroids, killed the predators and the white flies came back very strongly. By 1995 a new strategy was needed and the new chemicals called IGRs that were being used in Israel greenhouses were tried out.

The IGRs were Knack and Applaud. They were easy on predators in cotton. The EPA issued a section 18 emergency exemption in 1996 in response to the emergency need due to the 1995 outbreak. The use of these two IGRs, the use of BT cotton seed and the use of a late season application of parathyroid have lowered the pesticide application costs in Southern California and Arizona cotton crops. IPM methods are doing the job and new pesticides materials and procedures are still coming into our industry.

IGRs cause death to the insect in three ways:

1. (Chitin Synthesis Inhibitors) Inhibiting the production of chitin by the insect. Chitin is a primary material used to build the insect’s external skin after molting It is the insect’s skeleton holding the body together. Internally chitin supports the lining of the stomach, the respiratory systems, reproductive ducts and some of the gland ducts of the insect’s body. Chitin is also present in the cell walls of microorganisms such as fungi and protozoa. The first chitin inhibitor was introduced in 1993 and was used against the larvae of the Lepidoptera, common cutworm.

This chitin inhibitor was also effective against the Coleoptera and Diptera. Other chitin inhibitors were used against nymphs of plant hoppers, leafhoppers, white flies and scale insects. An orally administered chitin inhibitor was used against fleas. The flea chitin inhibitor effected the egg and larva development. The female fleas that bit animals treated with the chitin inhibitor produced undeveloped eggs. The flea larvae were undeveloped when feeding on flea dirt that was contaminated with blood from the treated fleas.

Chitin inhibitors prevent insects from forming a skeleton therefore the insect dies. Chitin inhibitors are quicker acting than the other types of IGRs. The timing of application of chitin inhibitors isn’t as critical as the other two types of IGRs. There have been problems with the chitin inhibitors with predators. They affect some predators and some fish.

2. (Juvenile Hormone Mimics) Chemicals that mimic or copy hormone activities and force both the nymphs and larvae to molt before their body is ready to molt. Juvenile Hormone Mimics produce deformed wings and reproductive parts resulting in adults that lay infertile eggs. The insect produces several hormones that are vital in the molting stage for nymphs and larvae alike. Without the proper mix of hormones present in the insect's system at a precise time of their growth, the insect will not become an adult. This will result in reducing the insect reproduction process causing a reduction in insect populations.

Because a narrow range of insects are affected by juvenile hormone, mimics of this type of IGR are less harmful to insect predators. The juvenile hormone mimics were easier to synthesize or make and they were more specific in their control activity then the actual insect juvenile hormones. The demand for safe pesticides for home use encouraged the use of the first junior hormone mimic. It was called Methoprene it was incorporated into dog and cat collars for control of fleas.

3. (Ecdysone Inhibitors) Ecdysone is a hormone produced by the insect that causes the insect nymph, larvae and pupa to keep molting. IGRs that inhibit the insects production of this hormone causes the insect to stop feeding and the insect never becomes a pupa or an adult. The insect slowly dies. To increase the efficiency of Ecdysone the addition of horticultural oils are recommended.

IGRs should be used in a pest control program where spraying starts early. If a high population of insects is present a fast acting pesticides like the pyrethroids should be used. Then spray a week later with the IGRs. By using all three kinds of IGRs in a program a wider variety of the pests will be controlled. This rotation will also help prevent resistance.

IGRs

Juvenile Hormone Mimics include: Knack (Pyriproxyfen), Esteem, Distance, Seize, Confirm, Intrepid and Precision

Chitin Synthesis Inhibitors include: Applaud (Buprofezin), Talus, Dimilin, Citation, Trigard, Pedestal and Adept

Ecdysone Inhibitors Include: Azatin, Ornazin, Aza-Direct

Insects controlled by IGRs include: Mealybugs, whitefly, scales, leafminers, aphids, thrips and caterpillars.

The use of trade names in this course is solely for the purpose of providing specific information. It is not a guarantee or warranty of the products named, and does not signify that they are approved to the exclusion of others of suitable composition. Use pesticides safely. Read and follow directions on the manufacturer's label.

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