FORENSIC ENTOMOLOGY

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Preface

Forensic Entomology is an emerging discipline that has garnered considerable attention in recent years. It holds great promise in providing evidence that can illuminate many unsolved murders or plots. This rare challenge of our century is a new branch of science.

The book, comprising three sections, offers comprehensive information on the history of forensic entomology, its applications, and standard protocols. Additionally, the book includes a list of insect species that can be utilized in forensic entomology cases, categorized by order and family. This comprehensive guide is expected to broaden the knowledge of both undergraduate and graduate students.

SECTION 1

CHAPTER 1

FORENSIC ENTOMOLOGY: MEANING, HISTORY, SCIENCE, AND APPLICATIONS

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Meaning of Forensic Entomology

Forensic entomology is a science that utilizes insects as evidence in courts. This science involves not only entomologists but also those who provide information about the proper uses of chemicals or pesticides, those who control fly populations in animal waste, and those who can ascertain insect damage to structures and the contamination of food by insects. Cases typically referred to in civil courts that require evidence from forensic entomology are quite common. Hence, this study seeks a precise understanding of forensic entomology which is concerned with the use of insects during the investigation of questionable deaths or other legal cases. Forensic entomologists are the main experts who are expected to present an investigative report or testimony in a law court by using their knowledge of entomology.

Basics of Forensic Entomology

Forensic entomology is simply defined as the study of insects in forensic science. It is the use of the knowledge of insect biology to legally investigate cases of questionable deaths of humans and animals. In some cases, other arthropods are incorporated into the examination to achieve a similar goal. Terrestrial and aquatic insects have been studied with a view to understanding forensic entomology. Hence, forensic entomology is not restricted to land because it also examines insects in water (Anderson, 1995; Keiper and Casamatta, 2001; Hobischak and Anderson, 2002). In practice, terrestrial insects have received wider attention than aquatic insects. Similarly, corpses recovered in outdoor scenes have received broader forensic attention than indoor scenes. However, the examination of the corpses found indoors is not limited to insect infestations yet they constitute the biggest part of it. Recently, forensic entomology has evolved into entomotoxicology, which is a novel approach in forensic toxicology that alternatively carries out the analysis of toxins on insects that have fed on decomposing cadavers.

For a better interpretation of crime scenes, the forensic entomological investigation of mysterious deaths critically evaluates the micro-environmental variables at crime scenes to elucidate how the variations affect the presence, development, and diversity of insects to estimate when the death occurred.

Insects are known from ancient times to colonize both invertebrates and vertebrate carrions. Casual observations of these allogenic communities reveal a diverse group of insect species. Thus, when the taxonomy of insects became a core discipline in biosciences, the identity of every organism based on morphological attributes helped specific insects associated with vertebrate carriers to be identified. However, in relation to forensic science, insects, especially fly larvae, were largely ignored during autopsy as they were regarded as disgusting elements of decay (Catts and Goff, 1992). It is noteworthy that immediately after death, insects will be attracted to the body often within minutes or hours depending on the location of the dead body and the season of the year unless their access to it is intentionally blocked. Interestingly, insects constitute numerically the largest invertebrate fauna on earth, and hence they are more diversified than other invertebrates. This diversity makes insects available in every ecosystem. The groups of insects that are useful in forensic investigations include the true flies in the following families: Calliphoridae, Muscidae, Sarcophagidae, Beetles, Cleridae, Dermestidae, Histeridae, Staphylinidae, etc. The immature stages of flies are one of the true carrion feeders. In some beetles, both the immature ones and the adults will eat the body. The groups that consume the body are referred to necrophagous insects (Smith, 1986). Other families of insects found on corpses or cadavers are predators or parasites of necrophagous insects.

Historical Perspectives of Forensic Entomology

The book " The Washing Away of the Wrongs: Forensic Medicine in the Thirteenth-century China" written by Sung T'zu in 1247 and translated into English by B.E. Mc Knight in 1981 is one of the books of reference on the origin and application of knowledge of insects in a murder case. It was reported in the book that a man was murdered in a rice field after being inflicted with multiple injuries. It was suspected that the injuries were caused by a sharp metal object. The investigator of the murder asked the suspects (rice farmers) to assemble their sickles on the ground. One of the sickles attracted blowflies, probably because of the traces of blood tissues on the sickle. The owner of the sickle was interrogated and he confessed to the killing of the man. Research findings have authoritatively established or at least clarified the understanding of insect metamorphosis, especially that of flies, which disproves the theory of spontaneous generation. The knowledge of the insect life cycle is the core of forensic entomology which remains effective in law court. To exemplify, Bergeret (1855 as cited in Amendt *et al*., 2004) reported that the French courtroom witnessed the first application of forensic entomology in 1850. In that report, it was written that the mummified child was found behind a chimney during the house renovation. The insects found on that mummified corpse were admitted as evidence that the current occupants were not the culprits and they were acquitted. During that time under review, forensic examiners did not have clear knowledge of insect biology but rather their perceptions were based on casual observation. Yovanovich and Megin were recognized as the first forensic examiners because they evaluated insect succession on corpses, properly establishing the science of forensic entomology (Amendt *et al.*, 2004). Dead animals, including human corpses, begin to deteriorate minutes after death due to the changes in the physiological circulation which have come to a halt, thus leading to putrefaction. Insects especially flies are accepted as the evidence of this deterioration since they are the first to detect the change and arrive on the carrions. In addition, beetles arrive on the carrions a few days after death to feed on the soft and dried tissues which aid to skeletonize the carrions (Abajue *et al.*, 2013). Smith (1986) categorized these insects into four carrion ecological communities:

1. Necrophagous insects- these are insects that feed on the carrions.
2. Predators and parasites of necrophagous insects- these are insects that solely feed on other insects or arthropods on the carrion.
3. Omnivorous insects- these include insects such as wasps, ants, and some beetles feeding on both the carriers and their colonizers.
4. Adventive species- these are insects such as springtails and spiders that use the corpse as an extension of their environment.

Forensic entomology utilizes insects in the first two groups. They are mainly insect species from Coleoptera and Diptera Orders. Their succession on carrions/corpses can be distinguished into different phases over the various stages of decomposition despite being controversial. About taxonomic composition and facts, African carrion communities comprised at least three hundred species of arthropods in about forty taxonomic families, primarily of insects. These figures can be compared with the lists from the Holarctics (Payne 1965; Smith, 1986) and the Neotropics (Carvalho and Mello-Patiu, 2008; Almeida and Mise, 2009), which were reviewed by Villet (2011).

There are some challenges in the study of insects associated with carrions in Africa, especially in Nigeria. One of them is the proper identification of insects at the species level based on the morphological features of the adults. While very few of the identification keys can be meticulously found, the keys to the larval stages may not be found though meticulous work. The challenge is a heavy liability that poses an enormous task on forensic entomology as it does right now and makes few carrion researchers rear the larvae to the adult stage. However, this procedure may be hampered as the larvae may die during the rearing process. On the contrary, developed countries in the United States and the United Kingdom have developed molecular methods of identification, enabling the identification of all stages including eggs, thereby linking (with ease) the immature stages to adult identity. This calls for molecular advancement in places where crude methods of identification are still very much operational.

The Science of Forensic Entomology and Its Applications

The goal of forensic entomology is primarily to determine elapsed time since death. Thus, in the last twenty years in developed countries, forensic entomology has become more and more common in police investigations. Most cases that involve a forensic entomologist are the ones that have stayed up to 72 hours and beyond because insect evidence is often the most accurate and sometimes the only method to determine the elapsed time since death after the first three days.

There are two main approaches to using insects to determine elapsed time since death:

- Using maggot age and development.
- Using successional waves of insects.

Any approach used is determined by the conditions surrounding each case. In general, the first approach is used when the death occurred less than a week before discovery. The second approach is used when a corpse is between a week to a month, a year, or more. The first approach is considered by using maggot age and development which can give a date of death accurate to a day or less, or a range of days, and is used in the first few weeks after death. Maggots are immature stages of dipteran flies. The flies used in this method are those that arrive first on the corpse/cadaver. They are mainly the Calliphorids (blowflies) that arrive on a corpse immediately after death. They lay their eggs on the orifices of the corpse/cadaver or in a wound if present. The development of the eggs into adults follows a predictable cycle. The eggs laid on a corpse/cadaver by flies hatch into the first larval instars after a specified period. The larvae will begin to feed on the corpse/cadaver until they molt into second larval instars. They will continue to feed on it and molt into third larval instars. The third larval instars will feed on the corpse/cadaver for a while and stop feeding. They will begin to wander away from the corpse/cadaver into the clothes or the soil or nearby debris for pupation. The non-feeding wandering maggots are called the pre-pupae. At this stage, they lose their outer skin but remain inside the skin. The outer skin hardens into a hard protective outer shell, or pupal case, which safeguards the insect as it transforms into an adult. The freshly formed pupa is usually pale in color but darkens to a deep brown after a few hours. After a few days, an adult fly will emerge from the pupa and the cycle will begin again. When adults emerge, it leaves behind empty pupal cases as evidence of successful development and emergence. Each of these developmental stages (egg, larva, and pupa) takes a set of recognized times. This identified time frame is based on the availability of food resources and the temperature. However, food availability is not usually a limiting factor in the case of a human corpse. Note that insects are "cold-blooded" animals, and therefore their development is extremely temperature dependent. Hence, their metabolic rates are increased with the increased temperature resulting in a faster rate of development. Consequently, the duration of development is reduced linearly with the increased temperature and vice versa.

When analyzing the carrion community, the oldest stage of insect (larva) on the corpse and the temperature of the region in which the body was discovered will lead to a day or a range of days on which the first fly laid its eggs on the corpse. This will in turn lead to a day, or a range of days during which the death occurred. For instance, if the oldest insect (larva) is estimated to be 5 days old, then the decedent is estimated to have been dead for at least 5 days.

This method can be used until the first adults from the first batch of eggs begin to emerge. Beyond this period, it is not possible to determine which generation is present. Therefore, after the first batch of blowfly generation has been completed, the time of death is determined using the second approach that is centered on the use of insect succession. The second approach is centered on the fact that a human body, or any kind of carrion, supports a very fast-changing ecosystem going from fresh decay stage to dry decay of bones in a matter of weeks or months depending on the geographical region. During the decomposition stages, the remains go through rapid physical, biological, and chemical changes. The different stages of decomposition are attractive to different insect species. Some species of flies are often the first witnesses to a crime. They usually arrive within less than the first 24 hours of death if the season is suitable and the cadaver is not intentionally covered to prevent flies from accessing the body. The first groups of flies are the calliphorids (blowflies) or the muscids (houseflies), especially in a closed-door scene. Other insect species are not interested in the corpse when the corpse is fresh due to their nutritional and reproductive requirements but are only attracted to the corpse later. Probably due to protein fermentation of the body's tissues other insect faunas especially the beetles, begin to arrive successively. Some of the insects are not directly attracted to the body but arrive as predators to feed on the other insects on the carrion body. Many insect species observed at each stage of decomposition as well as another group of insects overlap with the ones adjacent to them somewhat. Therefore, with the knowledge of the regional entomofauna and times of cadaveric colonization, the insect assemblage associated with cadavers can be analyzed to determine a window of the time when the death occurred.

This method is used when the decedent has been dead for a few days to a year or several years in some cases, with the estimated window of time that extends as the time passing since death increases. It can also be used to indicate the season of death, a dry season, a rainy season, etc. Thus, knowledge of insect succession is required for this method to be successful.

1. ESTIMATES OF TIME OF DEATH

In medicine, a medical pathologist can reasonably estimate the time of death of a cadaver when the body is not beyond three days. The pathologist's approach is based on the historical use of features by studying the body condition such as the fall in body temperature and biological parameters such as lividity, rigor mortis, and post-mortem cooling. Others include changes in the chemical constituents of the body, autolysis, and putrefaction. However, beyond approximately three days, all the known pathological information will remain invalid to estimate accurately the time of death of the individual. This time estimate is referred to as the post-mortem interval (PMI). Estimation of time of death (PMI) is very crucial in forensic investigations; hence, an alternative for this challenge is sought. Thus, forensic entomologists present a means of the possible post-mortem interval based upon the life cycle stages of specific fly species recovered from the corpse/cadaver, or the succession of insect fauna present on the body. This entomological estimate can be valid over weeks or years. The indicator of this estimate is based on the knowledge that the beginning of the post-mortem interval is considered to coincide with the time when a fly first laid its eggs on the body and its end to be the discovery of the body based on the recognition of life stage of the oldest colonizing species infesting the cadaver. The time taken to reach this stage concerning the particular stage of decomposition gives an accurate measure of the estimated length of time the person or the animal has remained dead. This may be the best estimate that is alternatively available.

Stages of Decomposition of a Corpse/Cadaver

The stages of decomposition of a corpse/cadaver vary based on the zoogeographical differences of the ecosystem and other exogenous parameters. However, there are three identified processes in corpse/cadaver decomposition. These are autolysis, putrefaction, and digenesis.

In autolysis, enzymes such as lipases, proteases, and carbohydrates begin to digest the cells of the body. This causes the cells to naturally break down. This process is rapid within the organs such as the brain and liver (Vass, 2001). The cells that have broken down become nutrients that serve as a food source for bacteria.

In putrefaction, tissues are broken down by bacteria. This process leads to the emission of gases such as hydrogen sulfide, sulphur dioxide, carbon dioxide, methane, ammonia, and hydrogen. In addition, anaerobic fermentation occurs when the volatile fatty acids, propionic and butyric acid, are formed. When the cadaver body undergoes decaying, protein sources are broken down into fatty acids by bacteria. Fatty acids and other compounds such as skatole, putrescine, and cadaverine are significant members of these decomposition products (Vass, 2001). However, these volatile compounds may be absent on buried bodies (Vass *et al*., 2004).

In digenesis, skeletal bones decompose after the soft tissues are removed. Skeletal materials composed of organic and inorganic remains are further broken down by environmental conditions and are finally reduced to components of the soil. However, skeletal decomposition is temperature-dependent. ***Note that the three decomposition process is synonymous with every corpse/cadaver irrespective of its location on land or in water.***

On land, a corpse/cadaver can be allocated to one of the five identified post-mortem conditions which can be linked to different waves of insects or arthropod colonization. However, no obvious distinction is observed from one stage of decay to the next. Moreover, no stage of decay has a fixed duration; each stage can be associated with a specific assemblage of insects. The sequence of this insect colonization on a corpse/cadaver remains universally constant at the family level among locales. However, at the species level, the colonization of a body is influenced by environmental and location-specific factors depending on the zoogeography of the region (Early and Goff, 1986). Hence, post-mortem changes are broadly recognized in these stages:

- **Fresh stage:** This stage commences from the moment of death to the first sign of bloating of the body. The first specimens to arrive on the corpse/cadaver are the calliphorids (blowflies). However, the species may vary concerning zoogeographical locations and seasons.
- **Bloated stage:** This second stage is the continual breaking down of the body due to putrefaction and perhaps it is the easiest stage to recognize. Anaerobic bacteria cause the nutrients to metabolize and, as a result, generate gases causing the corpse/cadaver to bloat. The initial evidence is the abdominal swelling that precedes the whole body's becoming stretched like an air-balloon. This stage of decay attracts more blowflies probably as a response to the odor of the breakdown gases. Beetles such as Histerids and Staphylinides may be attracted to the body at this stage because of fly eggs and maggots that serve as ready meals for beetles. As they are predators, they can alter the interpretation of the range and life stages of the existing insects on the corpse as they predate on the maggots or remove pupae (Smith, 1986).
- **Active/Wet decay stage:** This third stage is recognized as an inflated and ruptured body because the skin of the body breaks up and starts to slough from the body. The sloughing allows the decomposition gases to escape, and thus the distended body begins to subside gradually while putrefaction goes on. At the peak of putrefaction, there occurs fermentation, and butyric and caseic acids are produced (Gennard, 2007).

After that, a period of advanced putrefaction appears involving ammoniacal fermentation of the body to which different species of insects are attracted. These insects include clerids, dermestids, histerids, staphylinids, and maggots of calliphorids, ulidiids, and stratiomyids (Abajue, 2016).

- **Post/Dry decay stage:** This fourth stage may be the remnants of the previous stages which include the skin, cartilage, and bones as well as the gut contents. This stage of decomposition may be omitted during the rainy season in eastern Nigeria (Abajue, 2016). The evidence of this stage is the conspicuous number of beetles and their juvenile stages. Blowfly maggots are as well drastically reduced leaving behind their pupae while ulidiid and stratiomyid maggots become preponderance beneath the cadaver body (Plates 1, 2, 3, 4).

Plate 1. A fresh pig cadaver exposed to insect infestation.

Plate 2. A Bloated pig cadaver infested with fly eggs and larvae.

Plate 3. Active decomposing pig cadaver infested with fly larvae and beetles.

Plate 4. Dry decomposing pig cadaver infested with beetles and their larvae.

Plate 5. A skeletonizing pig cadaver infested with beetles and their larvae.

Skeletonization

This stage is recognized by the absence of body tissues except for the hairs and the bones. Insects found at this stage are mostly keratophagous beetles (clerids and dermestids). Decomposition is finalized at this stage as further breakdown is best described in terms of the decay of individual components of the body such as the bones of the feet, skull, vertebral column, and ribs.

2. EVIDENCE OF PHYSICAL ABUSE

One major contribution of forensic entomology is to provide the estimated time of a questionable death. However, forensic entomology has a role to play in the investigation of other crimes in which the victim is alive because insects can also provide evidence in cases of neglect or abuse. Blowflies, for instance, are attracted to odors emanating from ammonia due to urine or fecal contamination. They tend to infest a baby who is deprived of regular diaper/nappy changes or an incontinent old individual who has not been assisted to maintain sufficient hygiene (Gennard, 2007). Flies, if allowed access, may lay eggs in the clothing or on the skin. If the eggs are not discovered, they will hatch into maggots that start to feed on the flesh or in wounds, ulcers, or any of the orifices of the body. This is referred to as myiasis. With time the flesh will be eaten away and the region may be further infected by bacteria as well as invaded by other insects.

Similarly, insect attacks can also happen to animals such as rabbits, pigs, dogs, and sheep. These animals can be victims of a fly strike because of urine or fecal material attached to the furs, fleece, or hind quarters through neglect or poor caging and living conditions as well as ill-health reflected by scouring. Such cases are considered to be instances of physical abuse since victims are unable to remove the eggs or maggots themselves. The effects can be serious and require attention from a veterinarian, otherwise, they may lead to the death of the animal or require its euthanasia. Therefore, caution has to be taken in making assumptions about the existence of physical abuse or assault before death.

3. ENTOMOTOXICOLOGY

Entomotoxicology is a new area of entomology whereby insect evidence on a corpse is analyzed to determine whether drugs or toxins were used before death. The insects that feed on a corpse/cadaver are a good potential reservoir of undigested flesh from the corpse as the tissue from the corpse can retain drugs for some time in parts or in a whole that had been consumed by the deceased before death and which may even be the cause of death. Thus, drugs may be recovered if present by analyzing the insects that have fed on the corpse.

This branch of forensic investigation is very necessary when the decomposing body is difficult to toxicologically examine due to a lack of appropriate sources such as tissues, blood, or urine. At this stage, immature insects such as maggots and pupae of flies may serve as an alternative material (Nolte *et al*., 1992; Goff and Lord, 1994; 2001; Introna *et al*., 2001 and Campobasso *et al*., 2001). No information up till now has, however, stated clearly the role of drugs or poisons in insect immaturity (Gennard, 2007). In addition, how immature insects bio-accumulate or eliminate drugs or poisons is not yet understood (Gautam *et al*., 2013). However, numerous empirical and case studies have reported that certain drugs of abuse have the potential to increase the rate of development of insects feeding on a poisoned corpse or cadaver (Goff *et al*., 1989; 1991; O'Brien and Turner, 2004). Similarly, some poisons in the form of pesticides may decrease the rate of development of cadaveric insects (Bourel *et al*., 1999; Gunatilake and Goff, 1989). Therefore, overestimation or underestimation is possible if such variability is not considered when determining the PMI of a corpse. Therefore, it is necessary to evaluate the life style of the victim and pay attention to the antemortem mood swings of the victim.

4. INSECTS AND DRUG TRAFFICKING

Forensic entomology in a broad sense is not restricted only to the questionable deaths of humans but includes inquiry into poaching activities in game reserves as well as tracing the origin of plant materials legally prohibited. It has been reported that two separate cannabis seizures along with eight Asian species of beetles, as well as wasps and ants in New Zealand, were clear evidence of the role of insects in drug trafficking.

The beetles were identified as belonging to the families of Bruchidae, Carabidae, and Tenebrionidae. Considering the geographical distribution of the insects and the level of overlap of their distributions, entomologists concluded that the cannabis came from the Tenasserim region, between the Andaman Sea and Thailand. In affirmation, one of the two suspects confessed on the basis of this evidence (Crosby *et al.* 1986). This kind of forensic information is considered to be useful to customs, excise officers, and drug law enforcement agencies.

5. INSECTS IN FOOD INDUSTRY

Stored product insects may be eaten along with food or seen on commercial foods. In many instances, this is unacceptable to the consumer and can be taken as food contamination or poisoning. Stored product insects such as saw-toothed grain beetle may be found in cereal packages. Others include wireworms which may be sold along with freshly cut lettuce or it may be processed into lettuce and tomato sandwiches. In developing countries where fish and meat processing is done locally, insects are attracted to infest them because they are left in the open place to dry. Hence, fish and meat bought from the open market are not always free from beetle and fly larvae. Undoubtedly, these insects may potentially cause illness when they are eaten alongside fish or meat. Hence, forensic entomologists do entertain questions to submit their professional views in civil cases relating to the food industry where synanthropic insects are found to contaminate food. However, this does not make insect diet (entomophagy) an unhealthy practice because insect species are consumed globally. For instance, *Lethocerus indicus* Le Peletier & Serville, a giant water bug is a delicacy in South-eastern Asia while chocolate-covered bees and canned fried grasshoppers are sold in the UK and North America. Presently, Thailand is known for the mass production of crickets sold in tins for food (De Foliart, 1988). In Africa, wide varieties of insects at different stages of development are eaten extensively to augment the protein requirements of children and lactating mothers.

CHAPTER 2

FORENSIC INSECTS: CHARACTERISTICS AND ECOLOGY

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Overview

Identifying the identity of insects that are of forensic importance is one of the first crucial steps in ascertaining the role of the species infesting the corpse/cadaver in addition to their habitats and environmental requirements. This endeavor requires knowledge of insect structure as well as an understanding of various parts of insects in general. Scientific keys to these identification requirements have been written by insect taxonomists. Paying attention to the keys will help entomologists easily identify the insects morphologically. Moreover, newer and better methods of identification now exist in the form of DNA analysis which even takes care of the immature stages as an advantage over the former. However, the two methods are still within the scope of entomology and are acceptable and valid in a law court.

The knowledge of college biology still refreshes our memory that insects are arthropods with six legs (three pairs of legs). The whole body structure of insects is divided into three sections; the head, the thorax, and the abdomen. Each of the sections has three dimensions. The top part view is referred to as the dorsum; the underneath is the sternum while the sides are referred to as pleura (pleuron-singular). The head section is a capsule that contains the mouthparts and the sensory organs such as the eyes and antennae (the feelers). The thorax section is segmented into three parts. The first segment proximal to the head is called the prothorax which bears one pair of legs. Each of the legs has five sections starting at the point nearest to the body called the coxa which is followed by the trochanter, the femur, the tibia, and the tarsus in that order. The tarsus ends up with tough tiny and spiny-like structures called claws. The second segment of the thoracic body is referred to as the mesothorax which bears another pair of legs. It also bears a membranous pair of wings at least in a particular stage of its life history. The last segment of the thoracic body is referred to as the metathorax which also bears the last pair of the insect legs in addition to the second pair of wings (which is replaced with organs called halters in dipteran flies).

These characteristic features such as the antennae in various forms and shapes, the legs, and the membranous wings aligned with serially arranged veins are taxonomic features in insect identification. Precise and accurate identification is such a vital step in forensic entomology that the help of expert taxonomists should be consulted in cases of doubt about the identity of a particular insect species. Hence, it is recommended that forensic researchers intending to use entomological evidence as a career or profession in a forensic investigation should familiarize themselves with cadaveric insects, especially the Calliphorids (blowflies). Moreover, special attention should be given to the identification of insect larval stages of blowflies because their diagnostic features are not readily covered by literature and should be reared by adults. This is achieved by engaging in good observation and careful documentation which will help to produce the collection of entomological data as a standard routine in forensic inquiry. The more cautious the investigation is, the more accurate and precise the method will become.

Insects (hexapods) by systemic identification are in the Phylum Arthropoda. The class is divided into several groups called orders. Each order is further divided into several families. Each family also contains some genera and each genus may have more than one species.

Diptera

The order Diptera contains true flies such as blowflies, houseflies, fleshflies, horseflies, and craneflies. Others include midges and mosquitoes. Adult flies generally have just one pair of wings. The second pair is modified as organs for balancing called halteres. Flies such as blowflies, houseflies, and fleshflies are synanthropic and, certainly, the most important insects associated with corpses/cadavers or carrions. The larval stages are generally called maggots. Fly larvae have distinct heads but with no defined thoracic legs except the abdominal prolegs. It is advisable to consult the bibliography in the Diptera catalogue for each Zoogeographical region. This, however, limits entomological studies in developing countries.

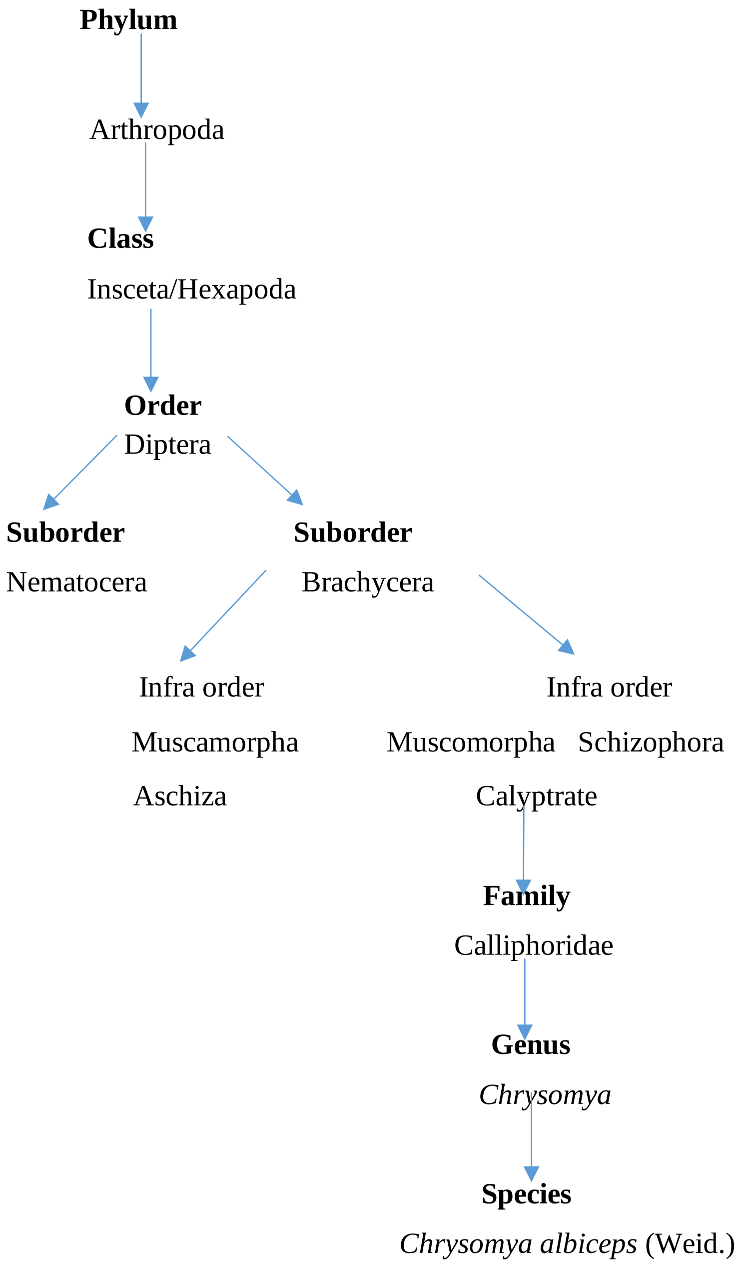


Figure 1. Schematic Classification of *Chrysomya albiceps* Weid. (blowfly)

Calliphoridae

This family constitutes the blowflies that are of greatest importance in forensic entomology for determining the time of death (PMI) of human and animal cadavers. The family consists of over a thousand described species and is well-represented in all zoogeographical regions. A large number of blowfly species develop in decaying organic remains including cadavers. Thus, bionomics of blow flies has discovered different species including species of myiasis importance in the Old World (Zumpt, 1965). Moreover, the taxonomy of blowflies is not easy as many species of forensic importance are continuing to get attention more than the already reported species in the literature. Thus, for accurate data, forensic entomologists should study in detail the cadaveric flies in their zoogeographical region with references contained in the appropriate Diptera catalog.

Important genera in this family include the *Calliphora*, *Chrysomya,* and *Lucilia* with different species restricted to different geographical regions and as well influenced by seasons and environmental habitats. One of the most common species in eastern Nigeria is *Chrysomya albiceps* Weidmann with a characteristic large metallic blue or green fly. It has a yellow or white thoracic spiracle. It has dark bands across its abdomen. Its legs are dark. The larvae of *Chrysomya* species are hard to distinguish in appearance except for employing mitochondria DNA analysis.

Muscidae

Muscid flies such as houseflies, face-flies, stable-flies, sweat-flies, and others have worldwide distribution. Several species of muscid flies are of medical importance due to their relationship with man and his dwellings. They feed on excrement, garbage, compost, sewage, carrion, etc., and may become mechanical transmitters of diseases when they settle on foodstuffs intended for human consumption. Because of their synanthropic habit, some muscid flies are likely to be involved in medico-legal cases, particularly in domestic situations. *Musca domestica* Linnaeus, for instance, has followed humans around the world. The adult is characteristically distinguished from other house flies by possessing a sharply angled outer portion of the fourth long-wing vein. The fly is mouse-grey in color with an average of 6-7 mm in size (Smith, 1986). It is recognized by four narrow black stripes along its thorax. *M. domestica* L. found in Nigeria is distinguished by a characteristic yellow abdomen.

Sarcophagidae

Sarcophagid flies are a large family of diverse distribution. The members are commonly referred to as flesh flies. The adults are generally large (4-16 mm long). The body is silvery-grey and has black strips at the thorax and a tessellate or spotted abdomen with strong bristles and reddish eyes (Smith, 1986). Flesh flies are found to associate with carrions. They prefer cadavers exposed to the sunlight. *Sarcophaga inzi* Curran, for instance, was found to visit pig carrion within three minutes of exposure in an open fallow land (Abajue *et al.,* 2013 and 2014). Females are viviparous because they deposit active first- instar larvae. *Sarcophaga* species in the tropics are very similar in appearance and difficult to identify both as larvae and adults. Larvae should be reared into adults before identification. Many of the species develop in excrement, carrion, or other decomposing remains. *S. inzi* Curran may come before the forensic entomologist (personal observation). Rearing the larvae associated with carrions through adults (Abajue, *et al.,* 2017) is very essential because the diagnostic feature described by Smith (1986) for *Sarcophaga haemorrhoidalis* (Fallen) is morphologically similar to that of *S. inzi* (Curran).

Stratiomyidae

Stratiomyids are fairly large flies measuring about 5-15 mm in length. They are referred to as soldier flies because of the ‘armament’ of spines on the scutellum of some species. Their colors range from brilliant metallic green and purple to black and yellow. Their bodies are generally flat with discal cells in their wings. The larval stages are found in rotten woods, under bark and in dung, lavatories, and compost heaps and will probably feed on any decaying remains of animal or vegetable origin. Stratiomyid larvae are distinguished by their roughened appearance, like shark skin. They have flattened mouthparts moving vertically parallel to each other like a pair of hooks (Smith, 1986). One of the species generally found to associate with carrion decomposition in Nigeria is *Hermatia illucens* Linnaeus (Ekanem and Dike, 2010; Ekrakene and Iloba, 2011; Abajue *et al.,* 2013 and 2014).

Bionomics of the carrion community is continually dominated by the assemblage of flies mainly during the active decompositions as primary necrophages. The flies usually recovered from carrions but not forensically discussed for being used to estimate the time of death include species in the following families: Coleopidae, Drosophilidae, Dryomyzidae, Ephydridae, Fanniidae, Heleomyzidae, Milichiidae, Phoridae, Piophilidae, Psychodidae, Sepsidae, Sphaeroceridae, Syrphidae, Trichoceridae, etc. The forensic importance of these and other families not covered in this book is that their recovery of the corpse will give clues about the location of the crime scene as well as the season.

Coleoptera

Coleoptera Order constitutes the group of insects generally called beetles and weevils. Beetles are distinguished by the possession of hardened fore-wings that forms elytra or wing cases which usually cover the abdomen, thus giving them an armor-plated appearance. Most beetles can fly but they are usually found on the ground, in the soil, and under the bark of trees and leaf litter among organic debris.

Many beetles have been reported to be associated with carrions. In literature, however, many of them are predators while few of them are true necrophages. Both the adults and larvae are found on carrions but their feeding habits may differ. Nevertheless, beetle species have received more forensic discussion than fly species only because of the fact that the greater mobility of flies facilitates them to reach a carrion/cadaver before the beetles. In addition, the faster rate of larval growth of flies enables them to complete their development before the carrion resource is exhausted. This competition suggests why carrion beetles have behaviourally evolved to succeed the flies at a later drier stage (Crowson, 1981). While larval predating beetles such as histerids, staphylinids, etc. regularly exist on carrions during the wet decay stages, core keratophagous beetles (clerids and dermestids) also arrive on carrions during wet decay stages (Abajue *et al*., 2013; 2015), yet they tend to avoid the wet tissues.

Cleridae

Clerids are small beetles with hairs, especially on the thorax. These beetles are one of the stored product pests of hides and skin especially on dried fish in eastern Nigeria (personal observation). They are mainly bluish-green. Some species are distinguished by having a red color on the thorax and the anterior part of the abdomen. Both the adults and larvae are probably predaceous on maggots. They constitute the major carrion/cadaveric fauna during the drier stages of decay. The most frequently submitted clerid in forensic entomology is the genus *Necrobia* (*Necrobia ruficolis* Fab. and *Necrobia rufipes* Deg.).

Dermestidae

Dermestids are moderately small-sized beetles densely covered with short hairs or scales which are often conspicuously grey. Both the adults and larvae specifically feed on dried fish, hides, skins, furs, etc. The larvae are characteristically distinguished by their brownish urticating hairs. Species of forensic value as frequently reported are the genus *Dermestes* spp.

Histeridae

Members of this family usually have moderately short elytra that leave the last two abdominal segments visible. They are most squat in shape. Their colors range from bluish-green to black. These beetles have been predictably found on carrions during the bloating stages of pig carrions in eastern Nigeria. Their presence on carrions synchronizes at least the first instars of blowfly larvae which they frequently predate on. Species in the genus *Hister* are abundant all through the year in Eastern Nigeria.

Staphylinidae

Staphylinids are moderately tiny beetles with very short elytra. Many of the species associated with carrion are distinguished by the yellow color of the thorax. They are usually not noticed and observed unless carefully searched for because of their fast disappearance into holes and debris underneath and beside carrions when a slight disturbance is felt on the body. Both adults and larvae are active maggot predators. Two forensic important genera; *Philonthus and Staphylinus* are usually reported in forensic entomology.

Other beetles that have been reported in forensic literature include these families: Carabidae, Geotrupidae, Hydrophilidae, Leiodidae, Nitidulidae, Ptinidae, Rhizophagidae, Scarabaeidae, Silphidae, Tenebrionidae, Trogidae, etc. The assemblage of these beetles has roles to play as indicators of the carrion/cadaver's environment where they are discovered.

The Life Cycles of Insects Associated with Dead Human and Animal

The life cycle is the predicted metamorphosis that is exhibited in the development of animals, it gives the general overview of the life forms of an individual, whether oviparous, viviparous, ovoviviparous, or some other forms of development.

Flies and beetles are among the insects that have life cycles that show four stages of metamorphosis. It means that each stage of the life cycle is unique and different from the other stage. The life cycles of these insects start when the adult females lay eggs which develop into another stage called larvae. Then, the larvae will undergo developmental changes to pupae and adult stages. The estimated duration of each stage and summation of the stages from egg laying to adulthood in both flies and beetles associated with vertebrate decompositions have been documented (Reiter, 1984; Greenberg, 1991; Anderson, 2000; and Grassberger and Reiter 2002). The estimation of these stages of insects on cadavers is the primary hypothetical tool used by forensic entomologists to estimate when the animal died.

Flies associated with vertebrate cadavers tend to lay eggs in batches. Egg clumps are laid in the parts of the corpse that offer protection, moisture, and food (Gennard, 2007). It was suggested that *Calliphora vicina* may lay 2000-3000 eggs in their lifetime (Hinton, 1981). Eggs of blowflies which are the most reported egg samples recovered from vertebrate cadavers are usually very shiny and white with sizes ranging from around 0.9 mm to over 1.50 mm long and 0.3-0.4 mm wide (Rognes, 1991). The emergence of the first instar larvae from the eggs called eclosion is a term also used to describe any form of hatching. However, this may not be observed in the flesh flies (Sarcophagidae) which do not lay eggs on the carrion but deposit first instar larvae. This type of development has been observed in some *C. vicina* flies in which fertilization takes place without a suitable oviposition site available (Erzinçlioğlu, 1996). The blowfly larvae have been reported to have twelve segments. The blunt posterior end has two brown circular areas on the final segment called the posterior spiracles (Gennard, 2007). The larvae have three larval stages or instars and each stage is distinguishable. The specific life stage of each larva can be identified by the number of slits present in each posterior spiracle. There is one slit in the first instar, while two slits in the second instar and three slits in the third instar are present (Ekanem and Usua, 2000). There is normally a difference in the size of larvae in the three larval stages of blowflies. Nevertheless, size is not an entirely reliable measure of the age of the larvae because it differs according to the amount and quality of the food available on the flesh considered to be an ample repository of food for insects (Gennard, 2007).

Larvae in the late third instar tend to stop feeding and become migratory, searching for a drier place for pupation. This is the final developmental stage of metamorphosis into the adult stage. This is called the larval post-feeding stage. Usually, the post-feeding larva attempts to bury itself in soil or some other dark locations. They may be found by searching in the first 2-3cm depth of soil at outdoor crime scenes. The pupa is simply the transition stage between larva and adult. It is found inside a barrel-shaped puparium which is the hardened and darkened skin of the final larval instar. The pupal case changes color over time becoming an oval object resembling an uncut cigar in a color between reddish-brown and dark mahogany brown or black (Gennard, 2007). Some attempts have been made to relate the color development of the pupae to post-mortem interval but, to date, the method has not shown great accuracy for the period after the first 24 hours (Greenberg, 1991). At the end of the life cycle, the fly pushes the cap (operculum) out of the pupal case using the ptilinum, a blood-inflated region on the head, and the adult emerges (Gennard, 2007). The immature stages of blowflies and other flies associated with carrion decomposition are poorly documented in comparison to adults.

Beetles as well, show complete metamorphosis in their development. Before reaching adulthood from the egg stage, they pass through three to five larval stages depending on species, and a pupal stage. Beetle eggs tend to be oval, spherical, or spheroid in shape usually considered very similar, irrespective of family, and they are mostly found buried in a specially constructed chamber or in the soil while they are pupating (Gennard, 2007).

The length of the life cycles of beetles varies depending on the family and species of the beetle. While it takes days in Staphylinids, the Carabids take up to one year to complete one life cycle to adulthood. In some species, the number of instars in the larval stage is not fixed as it is dependent on environmental conditions. Hinton (1945) reported as many as nine instars in dermestids while Smith (1986) noted that there is only one generation of beetles per year (cited in Gennard, 2007). The pupal duration of dermestid species can range between 2 weeks and 2 months and these species tend to overwinter in the pupa chamber due to unfavorable weather conditions or till it becomes late winter (Smith, 1986). The information available about beetles' life cycles is less detailed than that about flies.

Ecology of Insects of Forensic Importance

Micro-environmental conditions are ecological variables that define a micro-habitat in terms of vegetation. Soil types as well as the meteorological conditions of the area will clearly have a major impact on the types and diversity of insect species present as well as their seasonal availability. Insects that are attracted to vertebrate carrions are influenced by these micro-environmental variables. These variables are of vital importance in interpreting a crime scene and estimating the length of time a body has been dead.

Insects may be present or absent on carrion depending on the micro-environmental conditions of the area. Seasonality affects the daily activity patterns of flies which also differ from one geographical location to another. Greenberg (1990) reported that calliphorid flies do not fly in the rain. Hence, seasonal factors such as cold and rainy weather may inhibit or even prevent fly activity and thus delay oviposition (Erzinçlioğlu, 1996). Female insects choose to lay eggs in places on a body that provide sufficient food for the new generation along with protection, moisture, and a consistent microclimate needed for larval development. As a carcass, a human corpse, for instance, decomposes, it offers a food source large enough to support colonies of several different fly species (Gennard, 2007). Archer and Elgar (2003) reported that preferred colonization sites on a carcass changed from the orifices to skin folds after the carcass was exposed to outdoor conditions for 24 hours. These sites include locations such as those between the legs or under the ear pinnae. They concluded that the migration of fly larvae, for instance, to a more favored site was in response to food depletion.

Blowflies usually show peaks of oviposition activity in the early afternoon (Baurmgartner and Greenberg, 1987; Greenberg, 1990). This shows that light intensity and over-lapping temperature as well as relative humidity influence oviposition in flies. In laboratory experiments, the developmental times of calliphorids were increased under a cyclical temperature regime compared to the developmental times at a constant temperature (Byrd and Allen, 2001). Variation in the duration of the life cycle occurred at higher temperatures (35 - 45°C) where adults failed to emerge and when cultures were kept at a constant temperature of 40°C or 10°C. This observation indicates that the development of *Phormia regina* tends to get inhibited when the monthly temperatures reach an average below 10°C.

Chrysomya albiceps is commonly the initial colonizer of a corpse in Afro-tropical regions and oriental regions from India to China, Central South America, and Southern Europe (Hall and Smith, 1993). It was also recorded as one of the two most encountered species in forensic cases in South Africa (Mostovski and Mansell, 2004) where it is recognized as a spring and summer species. This species was considered to fulfill the initial colonizing role played by *Calliphora* and *Lucilia* spp. in the temperate zones (Smith, 1986). *Chrysomya* species have been recorded in Northern France, Austria, and central Europe (Erzinçlioğlu, 2000; Grassberger *et al*., 2003). The level of interaction of these species with other species rather than the influence of higher temperatures alone is suggested by Grassberger *et al*. (2003) as an important factor in determining the changes in its distribution.

The carrion community presents many instant changes over three-time scales: circadian duration, annual duration, and the duration of decomposition. Being ectothermic, most carrion animals are less active in colder conditions such as at night and in winter. The range of mechanisms affecting community dynamics over the life span of a carrion source is more complex (Villet, 2011). The occurrence of circadian cycles in adult blowflies and flesh flies is important in forensic contexts because it determines the hours when eggs can be laid, and this enables the estimation of post-mortem intervals (PMI). If an animal dies early in the evening, flies will probably not lay eggs on it until the next day, and an estimation of the PMI based on the development of the immature flies will need to take this disparity into account (Villet, 2011). Nocturnal oviposition has never been reported from outdoor sites in temperate climates, but there are several reports from tropical Asia where nocturnal temperatures are higher (Singh and Bharti, 2001, 2008; Villet, 2011). Circadian rhythms also affect the timing of other activities of invertebrates such as the migration of mature maggots from carrion and the eclosion of adults. Thus, Villet *et al.* (2010) suggested that the circadian impact on the accuracy of such PMI estimates by norming the timing of these developmental landmarks to particular times of the day produces a variable bias and precision that limits the accuracy of such estimates. Similarly, seasonal cycles determine whether a species breeds on carrion at all. The core membership of the carrion community is therefore more strongly affected by a seasonal variation than by the time of the day (Villet, 2011). As earlier stated, temperature truly regulates the seasonal occurrence of many carrion arthropods, producing changes in which species represent their association with one another. In South Africa, *Dermestes peruvianus* is more common in winter and *D. maculatus* in summer. *Calliphora croceipalpis* is characteristically active in winter, *Chrysomya chloropyga* in spring, and *C.*

putoria in later summer. In the case of the flies, these differences in seasonal occurrence are reflected in their thermo physiological tolerances and their geographical distributions (Richards *et al*., 2009a, 2009b) (Villet, 2011).

In a nutshell, the decomposition of vertebrate carrion is generally influenced by environmental conditions. This has however affected the variations in the arrival of some insect families in different geographical regions. For instance, dermestids known to inhabit dry carcasses have been reported sooner on carrions. Van Laerhoven and Anderson (1996; 1999) in North America recorded dermestids 21 days after death when the body was reported to be in early advanced decay. Oliva (2011) in Argentina found them as early colonizers between 10 and 30 days after death. However, reports from Nigeria have reported them on day 4 (Abajue *et al*., 2013) and day 10 respectively (Ekanem and Dike, 2010; Ekrakene and Iloba, 2011). Ideally, this suggests that data about insect succession on carrion for a particular region where death occurred should be used.

Applied Forensic Entomology

The best way to respond to a call to visit a crime scene is to get a ready-made carrying container or bag having all the requirements for collecting insect specimens and other entomological valuables. This is to avoid unnecessary delay in responding to a call. If a forensic entomologist is asked to attend a crime scene by the police, he/she needs to collect reasonable entomological samples so that they can be available when they are legally requested by a fellow forensic entomologist to make his/her valuation for the defense. This is very important to be well-equipped to answer questions that must emanate from the reports when presented.

Collection of Insects at Crime Scene

The collection of insects on a decomposing body at the crime scene is the first and most crucial step in the journey to provide entomological evidence to forensic investigators. Therefore, insects collection equipment that is needed includes plastic or poly-carbonate screw-top sampling jars for both preserved specimens and live cultures, forceps, stepping plates to preserve the scene from contamination, a killing jar containing ethyl acetate, labels, indelible markers with fine points, fine forceps, artists’ paint brushes, an entomological net, killing agents for larvae such as boiling water, and insect preservative. Several preservatives could be used, including 70-80%

alcohol, KAAD, and Kahle's solution. Each of these preservatives has its benefits (Gennard, 2007).

Bear in mind that live maggots must be recovered from the site; therefore, it is vital to bring some food for them. Pig's liver or ground beef is the most suitable food. However, research indicates that larvae show varying growth on different body parts. Ground mackerel fish was substituted for pig's liver and ground beef in Abajue (2012). Any food chosen at a particular time due to proximity and availability should ideally be at room temperature, not frozen or chilled when the maggots are placed upon it. When returning, the cultures should be kept at a low temperature; at least below the base temperature of the maggots is ideal. A mobile refrigerator for the car or van or a cool box with artificial ice blocks would be ideal. A thermometer should be included in the container to ensure that the temperature during transport is known. A carrying box or packaging for the specimens should be included. The sample jars of preserved and live maggots from each site on the body should be retained together in pairs. If samples are collected by a crime scene officer instead of a forensic entomologist, it is better to package and seal the samples to prevent risking their validity. The storage packages can be cardboard boxes. The packages require pin holes and the lids of the culture jars need pin holes too or a porous covering that is firmly attached to the top of the container. Maggots are very artistic in escaping from containers by pushing through a top if it is not tightly secured. When they escape, evidence is lost and thus all efforts become useless. In addition, polythene bags are used to package maggots at a crime scene. Pin holes are made through the bag to avoid a build-up of carbon dioxide, whilst preventing the larvae from escaping.

The maggots should be immersed for at least 30 seconds in boiling water to kill them from each site of colonization on the body and fix their maximum length. Boiling water can be brought to a crime scene in a portable thermos flask. Alternatively, prepare on-site hot water using a small camping stove and kettle (don't forget to go with your matches or gas lighter in this regard).

Other prerequisites include studying the general habitat of the crime scene. Observe whether the body is wrapped or covered and how. If the body is indoors, check whether the windows are open or closed. Ascertain the slope of the ground if the crime scene or the body is outdoors. Study the nature of the vegetation if any and give a general description of the scene. Take photographs of the scene and record the scene temperature along with the degree of light shed at the scene. Thus, a thermometer should be included in your entomological equipment case. The thermometer should be calibrated so that it reads accurately and does not give readings that must be corrected. Use a digital probe thermometer for safety reasons. However, an alcohol thermometer is preferred to a mercury thermometer. If possible, go with a weather recorder to the crime scene in order to record temperature, light intensity, humidity, wind direction, and speed, which can all be recorded over a specific time. Use a portable thermo hygrometer to record at least the ambient temperature and relative humidity of the scene.

Strategy for sampling entomological specimens at crime scenes

Eggs

Once a crime scene is sealed for investigation and necessary permissions are obtained, the body is thus searched in an orderly sequence. The head is first examined followed by a thorough search of the trunk and later on moving onto the legs and the toes. Any wounds are precisely noted. Once the top side is checked, the body should be turned over and the underneath should be examined. Clothing should be quickly examined on-site, especially the pockets, sleeves, and clothing folds. A more thorough search can as well be extended at the mortuary when the clothes have been stripped from the body. Fly eggs, for instance, are laid in batches and can be mistaken because everything is from yellowish-white mold to sawdust or an encrusting of salt on the body whereas beetle eggs are laid individually and therefore they may be easily missed at the crime scene.

Fly eggs are laid in dark, moist orifices of a body (ears, nose, eyes, mouth, anus, or genitalia). Other areas include folds of skin, the back of the ears, the inside of the joint folds, or the parts of the clothing that have absorbed body fluid exudates. Therefore, every part of the body must be thoroughly examined. It is also necessary to attend the post-mortem examination to check further for insects. This is necessary if the body is fully clothed or has been wrapped in some stuff. The clumps of eggs should be collected individually from the body and carefully placed in a container without food. The humidity in the container must be maintained by using a damp paper towel carefully placed on the container to prevent the eggs from getting dry. Every sample should be well tagged with crime scene details such as crime scene no., name of the officer in charge, name of the collector, date, item no., and location and description of the scene. These details should be written with indelible ink but not ballpoint ink. The label should be duplicated. One duplicate should be placed on the body of the container while the other one (non-adhesive version) inside the container. This practice of double placement of information inside and outside the container is to limit the chances of losing the information and ending up with a sample of an unknown source. To get the paper label into a container, roll the paper round a pencil and deposit the roll through the neck of the container and it will unroll itself. The label data must be recorded in the scene log book.

Larvae

Larvae will be found as the body is explored for eggs. They are most likely to be in the orifices and wounds which were inflicted on the body. The larvae should be sampled from each site in batches or sets of 15-30 per jar so that no additional heat or ammonia is produced during delivery. More than one sampling jar per infestation site may be required. The first larval instar is the smallest and most vulnerable of the three larval stages and the larvae, if collected at this stage, can easily die. It is, therefore, necessary to protect them from drying out when collecting and culturing the larvae from a corpse at a crime scene.

Boiling or hot water in a portable thermos flask is poured into a container such as a styrene cup or a collecting jar to a depth of 4 cm depending on the volume of the jar and the number of the larvae collected. Larvae which are to be preserved from the specific site are then added to the collecting jar. They are allowed in the hot water for at least 20-30 seconds before the contents of the jar are poured through a small sieve and collected in a large container. Large bottled water container of any choice is ideal to serve such a purpose. The contents of the container when full can be poured into an excavated pit away from the crime scene.

Larvae are known to mass together when they reach the late second and third instar stages. These are referred to as maggot masses which are capable of raising the temperature above air temperature and the additional heat can affect the rate of larval development. If a larval mass is observed, it should be photographed and the mass temperature should be recorded in reference to the location being sampled. The temperature of every maggot mass ought to be recorded at each site on the body. This is necessary because it can be elucidative in the calculation of the thermal history of the crime scene.

Pupae

Fly pupa is usually seen at some distance away from the body. These are the third instar post-feeding larvae that have left the carrion resource and can be found in soil about 3-5 cm below the soil surface. They can also be found in pockets, under carpets, in leaf litter, or in any corners and crevices which are available in buildings. If the pupae are found on the body, it is then assumed that either there are obstructions that may have caused some restrictions to larval movement, or a particular species of insect is indicated. Pupae change color from white to dark brown over a specific period, hence, all pupae of whatever color should be collected.

A well-planned search strategy should be employed to do the collection. The ideal is to thoroughly search every grid of a meter apart over a 40 square meter area surrounding the decomposing body especially if it is not in a house. This is a gentle and time-consuming activity in which the soil should be sampled at the intercepts of the grid, using a hand trowel to a depth of about 8-10 cm. The soil may need to be sieved over a tray or white cardboard sheet, or it can be hand-searched. As earlier specified, the pupae recovered are placed in a container with a moist paper towel and properly labeled. The pupae do not require feeding but should be taken back to the laboratory for identification. They should be cultured in laboratory conditions until their emergence to ascertain the identification of their species. The pupal cases should also be retained as added evidence. Pupae that fail to hatch provide examples of preserved specimens from the crime scene.

Sampling adult flying insects at the crime scene

Flying insects found at the crime scene should be collected first with a sweep net, before collecting other specimens probably crawling on or around the body. This is because they can only be captured with a net as they tend to disappear when the scene is disturbed. The net is flicked from behind the insect and lifted up or simply moved up in a sweeping motion with an instant wrist swing so that the net opening could be held up to keep the insects inside it. At this point, the bag can be grasped with the other hand and the insect in the net base can be restricted so that a container can be placed over it. A firm shake usually retains the insect in the bottom of the tube for sufficient time to put a lid on top.

These insects can either be retained in individual killing jars, or they can be kept until death in a single killing jar as a collection of flying insects from the crime scene. Later, they can be transferred to individual specimen jars. Because insects are mobile, they can be the representative of the crime scene as a whole. In all cases, correct labeling and recording are essential.

If the crime scene is a car, for instance, important evidence can be obtained by collecting any insects which have been trapped in a radiator grill, inside the bonnet, or on the windscreen of the vehicle. This may provide trails or details of the movement of the body. The temperatures in the car may be imperative as the inner side of the vehicle is possible to get fairly hot, which may affect the speed of the insect development, as flying insects have gained access and lay eggs.

Sampling adult crawling insects at the crime scene

Insects such as beetles are visible on the surface of the body. They can be collected by hand-picking and placed individually in a labeled container. This is a functional precaution because beetles may be carnivores and may eat other specimens, thereby destroying your evidence. In an indoor crime scene, it is beneficial to check the corners and crevices of the room for crawling insects as this provides additional information about predators and the conditions in which the body has been found. Leaf litter or ground cover, in an outdoor scene, can also be collected at regular intervals and the contents can be sieved. Pitfall traps can also serve for catching crawling insects near the body in an outdoor crime scene. Another method of collecting crawling insects is the use of Tullgren funnels which can be used to recover the soil organisms living under the decomposing body. Several samples of soil (around 5-8 g each) are ideal. Each is placed in a Tullgren funnel and a light is positioned above the soil sample. As the soil dries out, the organisms are compelled down into the container below containing 70% alcohol. These specimens can later be identified to give a profile of the specimens below the body and elsewhere at the crime scene.

Collecting meteorological data at the crime scene

It is very important to determine the temperature at which the insects were growing on the dead body before it was discovered. The estimates of time since death depend on the figures gathered at the crime scene and those obtained subsequently from other sources.

The body temperature of the dead body should be determined by placing a thermometer on the body's surface. The ambient temperature should be taken at a height of about 1.1 meters or 4 feet (chest height). This offers a measure of air temperature at a comparable height to those taken at the meteorological station. Caution should be taken to avoid holding the actual thermometer, and therefore a protector or a rubber band should be wound around the end. Do not expose the thermometer to direct sunlight, because this may increase the temperature and gives a false reading. Take ambient temperature under a shade. In addition, the temperature directly beneath the body and the soil temperature should be taken successively. It is wiser to use a soil thermometer to take the temperature of the soil so that there is little chance of the thermometer breaking as it is forced into the ground.

Rearing Entomological Specimens Recovered from the Crime Scene

The larvae of flies and beetles recovered from the crime scene should be reared to the adult stage in laboratory conditions. This should be undertaken by the forensic entomologist so that the identity of the species of insect can be ascertained. Rearing the specimens from the egg stage to the adult, under conditions that simulate the crime scene allows an accurate post-mortem interval to be estimated (Abajue *et al.*, 2017).

As each life stage is reached in the laboratory, samples developing from those recovered from each location on the body at the crime scene should be shipped in boiling water and preserved in Kahle's solution. Data relating to details of temperature and time to reach this stage should also be recorded both in a laboratory note book and on the sample pots so that the pots collected at the crime scene can be related to this information. The record of the data, along with the specimens, may be requested by the court, or be used in court to illustrate your methodology (Gennard, 2007).

Insects recovered from a dead body at a crime scene for rearing must be kept in conditions that enable them to grow successfully. Although all of the insects feed on dead bodies, it is not appropriate from both the health and safety standpoints or in terms of human tissue retention laws, to utilize flesh from a corpse to feed insect specimens in the laboratory. A supply of food like a pig's liver will provide a better alternative to be used at the crime scene to provide food for the samples of living larvae collected from each site on the body. The pig's liver should also be the food used for the rest of the rearing period.

Taking entomological evidence to the laboratory

The conditions under which the insects are kept are particularly important. Insect specimens must always be kept in proper conditions at the laboratory so that they will be stored without damage or reared through their life stages. The larvae recovered from each of the locations on the body should be separated from each other and stored in separate containers.

The likely influence of the temperatures when the specimens are being transported to the laboratory should always be taken into account. If the insects have been collected by a crime scene investigator, they should be grouped according to their life stages. The larvae especially that of flies collected from each of the locations on the dead body should be separately taken to the laboratory from the crime scene at a temperature below the base temperature for those insects expected to be found at the crime scene and handed over to the forensic entomologist as fast as possible. The temperature may need to be determined for local conditions. Myskowiak and Doums (2002) stated that a temperature as low as the normal refrigeration temperature of 4°C may cause alteration in the duration of life stages and the time taken to reach the adult stage. They stated that 10 days of refrigeration before the rearing conditions led to an alteration of 9-56 hours from the duration of 15.5 days counted under normal circumstances where the larvae of *Protophormia terraenovae* were reared at 24°C. Hence, transit temperatures should be taken into account, mainly if there is any deviation from the expected life cycle in the laboratory.

Conditions for fly-rearing in the laboratory

When larvae are recovered from the crime scene in foil packages of meat, each package containing about 30 larvae will be placed in containers such as polystyrene cups. These containers can be stored in controlled and dark environments, such as a cabinet or a room, until the larvae reach the post-feeding stage. A pierced lid is placed on the top of each container to reduce drying out; one per sample from each location, for each type of larvae collected. The pierced lid will aid some gas exchange and stop the build-up of ammonia as the larvae grow. These containers should be maintained at a relative humidity between 60 and 65 % or in baths of water at a suitable temperature so that the microclimate within the containers can prevent the eggs or initial larval instars from drying out. Introna et al. (1989) revealed that larvae of flies reared in growth cabinets under conditions reminiscent of the wild do not statistically go through alterations in the durations of their normal life cycle. In this instance, they used Lucilia sericata for the trials. As a precaution against loss and intermixing of cultures, each container can be placed in a second container such as an aquarium. Each container should be specifically labeled with the date, case, collector, and item number.

For certainty, some adult flies should be kept in large cages to let them mate and to obtain another batch of eggs for development through to the stage they were first recovered at the crime scene. The cages should be around 40 x 30 x 40 cm with a mesh cover to allow light to enter and air to circulate while retaining the culture. If the cage is too small, the insects' wings will become battered and the flight will be affected, hence mating may or will not take place successfully. Access to the cage is necessary to replace the food through a sleeve opening in the front panel of the cage. The adults are provided with a supply of water continually either through a screw-top jar with a wick emanating the water inside it from the top, a Petri dish with water and stones, or a sponge impregnated with water all of which prevents the flies from drowning while drinking water.

The nutritional requirement of the adults is a 50:50 mix of sugar and water to retain the culture (if sugar is used alone without water, the insects may potentially become reduced in size. However, artificial diets have been successfully used to rear blowfly larvae). Meat or liver is also placed in the cage both as nutrients for ovary development in the females and as a place where the eggs are laid. The meat can be grounded or can be palm-sized pieces of liver. While pig liver has been used most successfully, ox or sheep liver can also be used but care should be taken to be consistent with the type and source of meat used to avoid introducing unnecessary variables. Kaneshrajah and Turner (2004) reported that there was a reduction in the rate of larval growth of *C. vicina* on the liver, in contrast to the growth observed when heart, lungs, kidney, or brain tissues were given as nutrients. However, the majority of researchers successfully use ad libitum liver as the food source for carrion feeders from forensic sites without causing any impact on the duration of life cycle stages. Hermes (1928), however, proved that an effect on the sex ratio of *Lucilia sericata* was caused by the amount of food available to the larvae, so *ad libitum* feeding is imperative. The food should be placed in a foil case, or a tub with a partial lid so that the area of access at the top can be reduced. This restriction stimulates flies to lay eggs and lets the food retain its moisture for a longer period. Care should be taken to keep the relative humidity above 50% and ideally, 65 % to prevent the eggs from drying out.

Flies should be reared at the most suitable temperature, either at the one measured at the crime scene or at the one that allows for swift development. This can be better achieved with a controlled environment cabinet although a room with a temperature with a limited and recorded fluctuation can also be used. Preliminary research indicates a suitable temperature for this end. Information about the expected duration of the life stages comes from several sources including Kamal (1958) who studied the life cycles of 13 fly species at 26.7°C and 50% relative humidity. Anderson (2000), Byrd and Castner (2001), Higley and Haskell (2001), Greenberg and Kunich (2002), and Donovan *et al*. (2006) are among the others reporting a suitable temperature. The amount of exposure to daylight and dark during a day length which has been successfully used to avoid influencing the life cycle was calculated as 16 hours of daylight and 8 hours of dark (16L:8D; Vaz Nunes and Saunders, 1989). Nevertheless, the most suitable day length to use is the average day length for the season in which the specimens were recovered from the crime scene so that the conditions in the environment before the recovery of the body are mirrored.

Conditions necessary for successful rearing of flies to the adult stage

Whenever the fly larvae have reached the third instar stage, they must be transferred into conditions that ensure that the post-feeding larvae can migrate successfully, while averting loss of the evidence. The ideal is an aquarium with vermiculite, sand, or sawdust in the bottom, kept in a controlled environment cabinet at the same temperature as that found at the crime scene. This offers the larvae a suitable room for burying themselves to pupate. Therefore, placing the third instar larvae in an aquarium tank or alternative container at this stage of their life cycle if the aquarium has not previously been used as a means of secondary containment. The existence of some extra space is important in that it can affect the degree of success in the completion of the life cycle. Byrd and Castner (2001) noted that the time spent by the larvae in a particular stage could be extended and the PMI estimation will be inaccurate as a result if natural pupation is altered or prevented.

Rearing beetles in the laboratory

Rearing beetles such as Cleridae, Histeridae, Silphidae, and Staphylinidae requires keeping them in transparent plastic containers, glass jars, vials, pots, or buckets. They may need to be kept individually to prevent one beetle from eating another. The containers should have a layer of moist peat, soil, or sawdust in the bottom, depending upon the family of beetles, and places in which insects can hide, such as half-plant pots.

Silphid beetles need a temperature of about 20°C and a daylight regime of 16:8 (L:D) (Eggert *et al*., 1998). They should be kept either individually or in a maximum group size of six beetles of the same sex. Pairs can be placed for breeding in a container including a small carcass such as a defrosted mouse. A large piece of beef, pork, or chicken can aslo offer enough food (Byrd and Castner, 2001). If one is keeping *Nicrophorus* beetles, a piece of meat left in a container placed on the top of peat will be buried and eggs will be laid in the soil around that piece of meat (Kramer Wilson, 1999). Such eggs can be recovered and retained on moist filter paper at 20°C until they hatch. Eggs of silphid species such as *Nicrophorus vespilloides* need an average 56 hours to hatch at 20°C (Müller and Eggert, 1990; in Eggert *et.al*., 1998).

Water in a Petri-dish with damp cotton wool or a vial containing water with a wick of paper or cotton wool pushed through the lid should be supplied in the tank for both adult beetles and larvae. The vial is sunk into the peat to let the beetles easily have access to it. The larval culture, each in individual containers, can be supplied with a carcass with a hole in it so that a larva can penetrate it. The cultures can be maintained under a regime of total darkness in an incubator or controlled environment cabinet.

Other beetles like the dermestids, do not require a whole carcass to successfully complete their life cycle and can be reared adequately on dried meat or an artificial diet. Dermestids breed optimally at a marginally higher temperature than 20°C and are ideally kept at 25°C and 80% relative humidity (Coombs, 1978). They can also be kept as cultures in aquaria or glass jars. Dermestids need wood sawdust, or some solid medium such as polystyrene or cork where they can burrow to pupate. This should be covered with several layers of paper to simulate conditions in the body. A supply of water must be provided, either as a piece of folded and dampened paper or as a container of water with a wick. Keep the water away from the food to prevent fungal growth. Black paper, in the form of a ‘concertina’, provides an egg-laying site from which it is quite simple to see and recover eggs.

Nutritive requirements of insects reared in the laboratory

Nutritional requirements of both flies and beetles are germane in that they have specific nutrient requirements to be able to complete their life cycles. Flies need carbohydrates as an energy source, water, and protein. Protein is specifically needed by the females for the development of the ovarioles and egg production. They as well require several vitamins and minerals.

Beetles associated with carcasses require a diet that integrates the important nutrients. It is suggested that they should be provided with dead mealworms as food every 3-4 days (Eggert *et al*., 1998). This is the proper food for ground beetles like carabids which can also be fed on ant eggs such as those used for feeding fish, or maggots and pupae. This inclusion of insects in the diet is particularly appropriate for the Cleridae and Silphidae. Others such as Histeridae and Nitidulidae can also be fed in the same way.

Conversely, larvae and adults of skin beetles (Dermestidae) - *Dermestes maculatus* or *Dermestes lardarius* can be fed on dried dog food or fish food. They prefer pelleted food rather than flakes but can consume both. Such food should be checked every 2 or 3 days to ensure that it is sufficiently consumed and of good quality. The food should be available in excess so that the dermestid life cycle is not influenced (Gennard, 2007).

To be sure that dermestids can breed successfully, it may be necessary to provide them with meat on an intermittent basis for the adequate supply of all of the required nutrients for reproduction. To simulate the conditions at the crime scene, using meat such as pork that has dried out may be a more proper food source than artificial foods.

Preserving and mounting insect specimens

This activity equips an entomologist with the skills that would be required for presenting entomological evidence in court. One will need to mount both adult fly and beetle to provide evidence of the identification features for the species.

Mounting insects is imperative because it offers examples of insects of different species which can be used for illustrative purposes in the court as well as providing a voucher collection of specimens regarding a particular geographical location. This will allow an entomologist to ascertain whether there are any unusual species present and thus predict the possible movement of a cadaver.

The adult insects at the crime scene may have been killed by placing them in a killing jar containing ethyl acetate or any other killing agent. A new jar should be used for each location on the corpse at which the insects were collected. Those from the general crime scene may all be added to the one jar.

Note: **Ethyl acetate does not cause the insects to become rigid when they die; this means they are easy to position for pinning**.

Gennard (2007) outlined the following steps in mounting insects:

1. Each large insect, like a blowfly, is removed from the killing jar with the help of fine forceps or a fine paint brush. This is necessary to position it on a cork or polystyrene tile, or insect mounting block. If a mounting block is to be used, choose one large enough on which to sit the thorax so that the insect's wings and legs can be stretched out.
2. Using a pair of entomological forceps, a pin should be carefully forced through the insect exoskeleton. The pin should be in the size of No. 1 and ideally of a non-rusting type.
    - For beetles, the pin is placed through the right elytron, as near as possible to the top margin of the wing case.
    - Large fly specimens should have the pin placed in the middle of their thorax (mesothorax region), exactly between the wings. Smaller flies may be glued to a card.
3. The legs of both flies and beetles should be positioned in a way that enables stretching them away from the body. For example, the first pair of legs of a beetle should be positioned forward, the middle pair of legs should be positioned at a 90° angle to the body and the third pair of legs should be positioned away from the body at a 45° angle to the body. They may need to be held in that position onto the cork or polystyrene, with their wings and legs retained by

small strips of paper with their ends pinned down. Alternatively, the fly or beetle can be positioned as naturally as possible so that the femur is held in this position and the legs hang down at an angle of 90°. The specimens are left to dry for several days until they are firmly in position. Smaller flies and beetles are less easy to position. They are mounted on a triangular point by gluing. The triangle is usually 1 cm in length and made of white card. Clear nail varnish or Solvate wallpaper paste can both provide a cheap and accessible source of glue. The side of the fly or beetle is glued onto the point of the triangle.

The procedure for pinning the specimen and positioning the labels made of white cards is the same. This is irrespective of whether the insect is mounted onto a pin directly, or the triangle plus insect is mounted at the mid-point along its base. To explain how to do this, the pinning and labeling of a fly are described as follows:

4. A pinning block is used to position the fly about 1 cm from the head of the pin. Below this, two labels of an appropriate size for the information and the fly are positioned parallel.

5. First, the pin point is pushed into the fly so that it pierces the thorax. The fly is then moved up the pin into position, by locating the pin point into the hole in the highest step of the pinning block and pushing.

6. Next, the fly is removed from the hole in the top step and the first label is placed on the second highest step. The fly plus pin is pushed into the center of the end of the label so that the information can be read. The label should contain the following information:

- Name of the collector.
- Place of collection (crime scene), including where on the body.
- Item number and case number.
- Date collected.

7. The label is pierced by pushing the pin through the label, using entomological forceps and resistance from the block. Once the pin has emerged through the card label, it is slid up the pin to the correct position. This is achieved by pushing the pin down through

the second hole on the pinning block. It is in a position when the pin hits the bottom of the block.

8. A second label indicates the species name of the insect, when it is identified, and who did the identification. This label is put in the proper position using the same sequence of movements but using the third hole in the lowest step of the pinning block. Both labels are held parallel to each other and written in indelible ink or printed using a laser printer. By using this sequence for mounting insects, the danger of losing information about the case number and location is lessened. The insect can, after all, be re-identified in the unlikely case of loss of the second label on the pinned specimen.

Estimating the Post-Mortem Interval of a Corpse

After collecting and identifying the insects from the body, the next stage is to relate the information to the temperature at the crime scene. Temperature data including the period since the person was last seen alive are usually obtained from the local meteo¬rological station. These data are 'corrected' using a correction factor calculated from the meteorological office data and half-hourly temperature readings which have been recorded at the crime scene for 3-5 days after the body was discovered. These corrected data helps to estimate the temperatures at the crime scene before the corpse was found. Entomologists, using this information, can determine the length of time the flies took to grow from an egg to the developmental stage recovered from the body. By inference, this is the best estimate of the post-mortem interval (PMI) that is available.

These estimations of time since death are centered on the speed of insect growth. Insects are 'cold-blooded' animals; hence, their growth is influenced by temperature. Below a temperature threshold, development stops; above a specific temperature threshold, the rate of growth also slows down. Between these two points, however, the rate of growth of the juvenile insect is considered to have a linear relationship with ambient temperature.

Upper threshold temperatures are not often experienced in investigating most crime scenes, so this factor is not always important. However, if temperatures do remain at or near the maximum for an extended period of time, this will affect the accuracy of the PMI estimate, as the growth of the insect will be slower than expected. Equally, at a very low temperature, development may not take place at all. It is pertinent to note that such temperature fluctuations have not been observed in the tropics (personal communication).

The temperature threshold below which growth and development will not take place is referred to as the base temperature. This will vary from species to species and with geographic locations. Thus, Davies and Ratcliffe (1994) demonstrated a threshold of 3.5°C for C. vicina in the north of England while Marchenko (2001), working in Russia, recorded a base temperature of 2°C for the same species. Donovan et al. (2006) reported that the base temperature of C. vicina grown in London, at temperatures between 4°C and 30°C, was 1°C. Therefore, Oliveira-Costa and de Mello-Patiu (2004) opined that calculations which do not consider or use inappropriate base (lower threshold) temperature will overestimate the accumulated physiological energy budget - termed accumulated degree hours (ADH) or accumulated degree days (ADD). Thus, it is likely that a forensic entomologist may give a false post-mortem interval. Such species adaptation has to be ascertained at crime scenes and the base temperature for common fly species in such locality ought to be predetermined.

How to get the base temperature

The precise base temperature for a particular species is worked out in the laboratory from the insect's growth rate at set experimental temperatures. The calculation is based on the premise that the cooler the temperature, the more slowly the insect develops. The base temperature is calculated by plotting temperature against 1 divided by the total days to develop, i.e. the time between the larvae initially emerging from the egg and the emergence of the adult, using a range of temperatures. If the line of the graph is extended down to the x-axis, the point where it meets the x-axis (abscissa) can be read off. This is the base temperature for that particular species. This graphical method of determining the base temperature is called the linear approximation estimation method. This base temperature estimation has not been worked out in the tropics hence, the steps outlined by Gennard (2007), are thus recommended for reference.

Using the length of larvae to estimate post-mortem interval

If a corpse is discovered indoors, or in a controlled environment where the temperature is not fluctuating, the relationship between temperature and growth can be used in another way. In such conditions, the length of the oldest larva killed by immersing in boiling or hot water in a flask can vary by the time passing since the larva hatched. Graphs are produced, under controlled conditions in the laboratory, for the time since the hatching of the insect against the average minimum length. The time since hatch can then be read directly off the graph based on the length of the individual larvae collected from the crime scene. These graphs are called isomegalen diagrams and have been calculated for Lucilia sericata, Protophormia (= Phormia) terraenovae and C. vicina (Grassberger and Reiter, 2001, 2002; Reiter, 1984). Such graphs have been developed for Chrysomya species, Hermatia illucens, and Chrysomyza africana by Abajue (2016).

A second type of graph where life cycle stages from egg hatch to the time of emergence of the adult (eclosion) have been plotted against time can be used at specific temperatures. Each line indicates a change in the life cycle to the next stage. The areas between the lines relate to identical morphological stages. These are called isomorphen diagrams and they have been calculated for the same three species as contained in isomegalen graphs. A similar graph was obtained by Abajue et al. (2017) for different insect families associated with decomposing pig carrions in Okija, Anambra State Nigeria. Isomorphen diagrams are beneficial when post-feeding larvae and/or pupae are collected from a crime scene. From the stages, the post-mortem interval can be read directly off the graph, provided that the temperature has been the same.

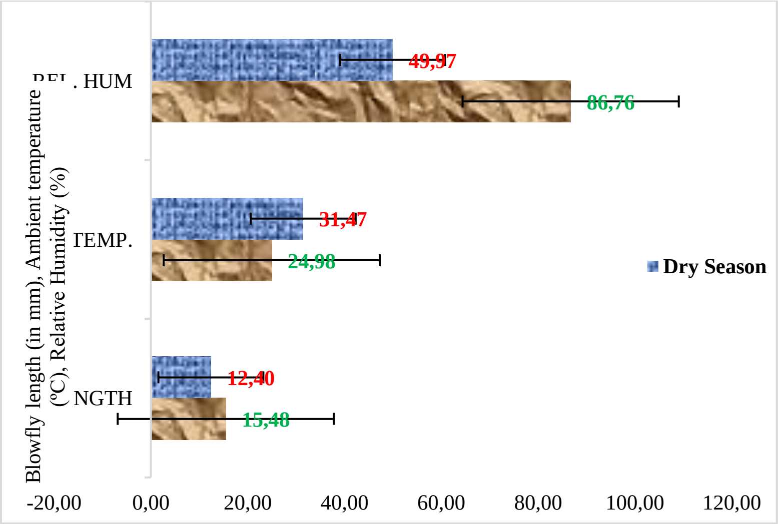


Figure 2. The mean length of blowfly larvae, ambient temperature, and relative humidity at Awka, Nigeria during a rainy and dry season (2014 & 2015), respectively Source: (Abajue, 2016)

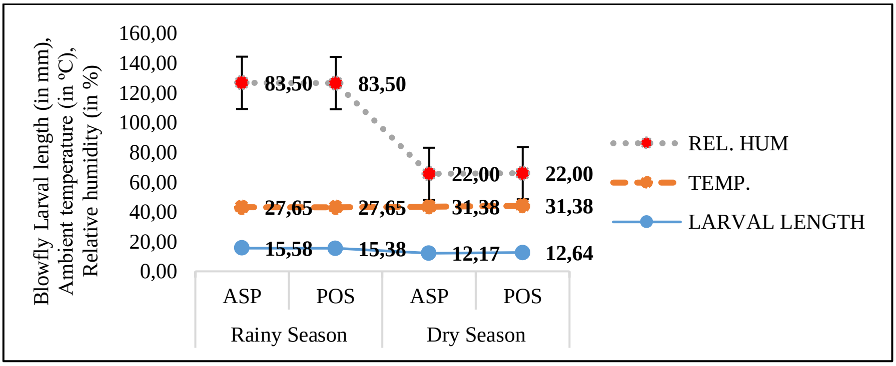


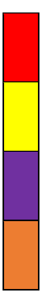
Figure 3. Larval length of blowflies collected from asphyxiated and poisoned pig carrions during a rainy season and a dry season, respectively, under ambient temperature and relative humidity in Awka, Nigeria.

Note: Larvae were collected from day 3 to day 7 as of the death; ASP = asphyxiated pig carrion; POS = poisoned pig carrion; TEMP =ambient temperature; REL. HUM = relative humidity.**Source:** (Abajue, 2016)

Using succession to calculate post-mortem interval

Examining post-mortem intervals for a period that has elapsed to at least more than one month may mean that there is a large group of flies, beetles, and other insects present on the body. The insects can be used for calculating the PMI using another method. This method entails that every insect is first identified at least at a family level. After that, an attempt is made to relate this allogenicity of decomposition fauna to the succession of insects which regularly colonize a corpse at that site. Ascertaining which insects are present and which are absent locally and in what season helps the entomologist estimate the post-mortem interval of the corpse (Abajue et al., 2013; Abajue and Ewuim, 2016).

Key



Fresh

Bloated

Active decay

Dry decay

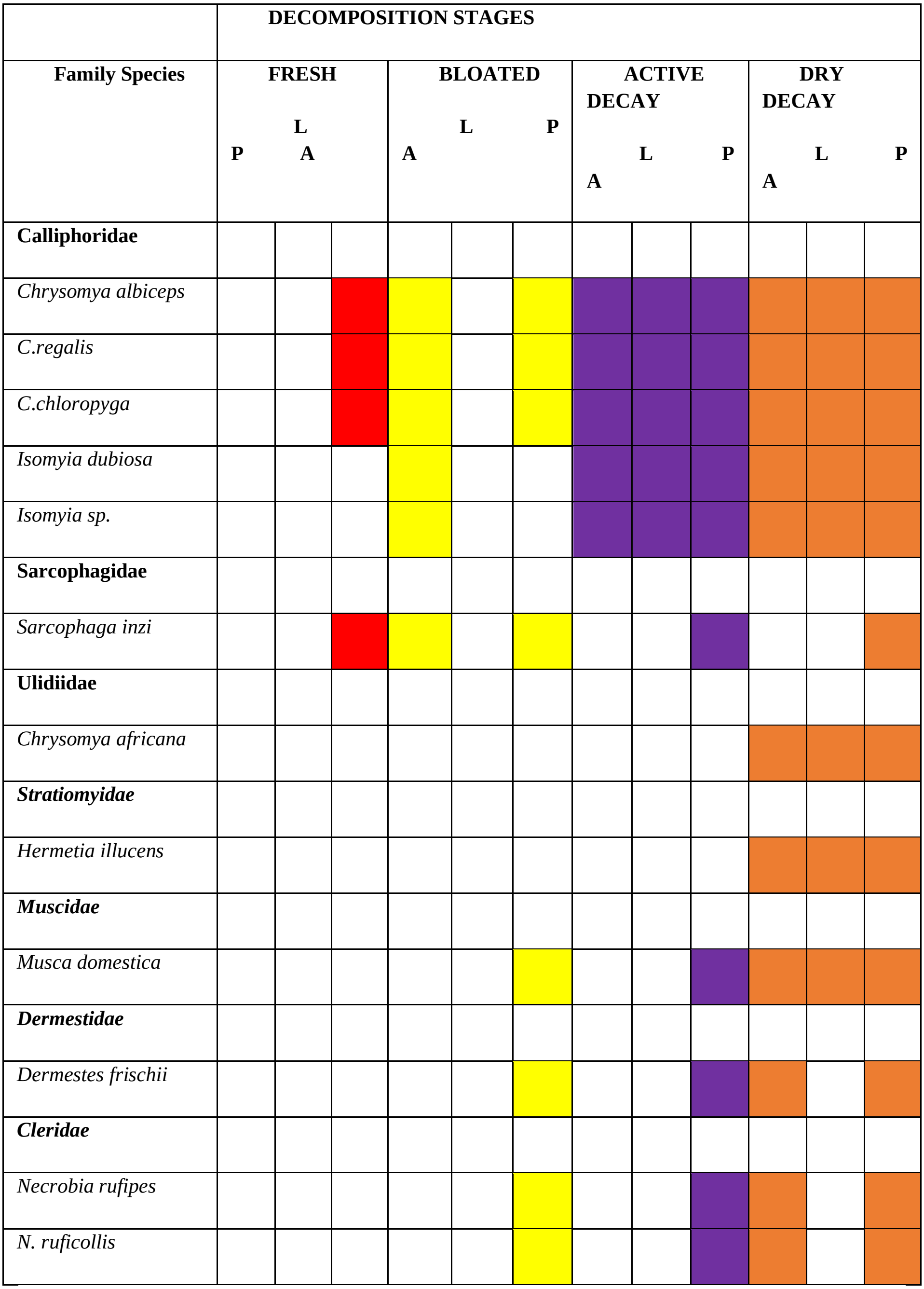


Figure 4. Insects succession on a pig cadaver in Okija, Nigeria (Abajue and Ewuim, 2016)

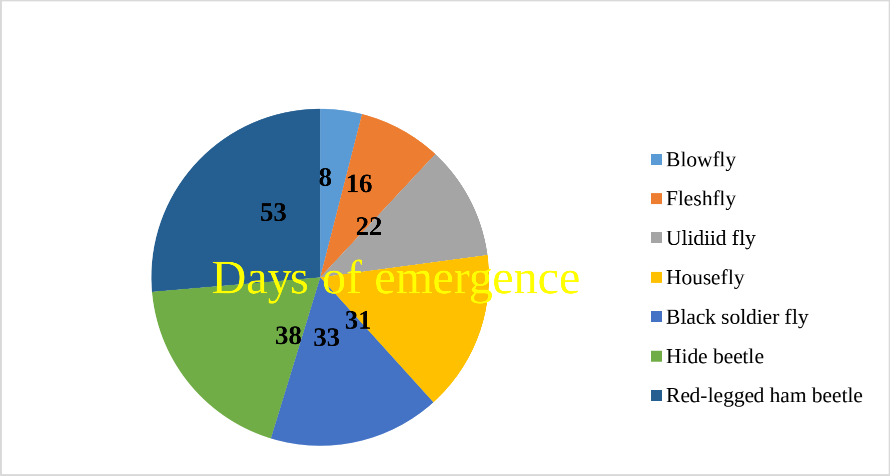


Figure 5. Days of the emergence of insects collected as larvae on pig carrions in Okija, Nigeria, Source: (Abajue and Ewuim, 2016)
Source: (Abajue and Ewuim, 2016)

Movement of the corpse

A specific group of insects existing on a corpse is also a good pointer to whether or not the body has been moved. The existence of an unanticipated species which is more typical of a different habitat or geographic region indicates that the body may have been moved. This in turn depends on the entomologist's knowledge of the local entomofauna which has been documented, peer- reviewed, and possibly published. Hence, such local content can provide a basis for one's conclusion which will be acceptable in a law court.

Predating insects feeding on necrophagous insects

As long as a body remains undiscovered, the possibility for the insects such as wasps and ants to consume those insects feeding directly on the body increases. This obliteration of evidence can cause an interpretation difficulty relating to time since death. Ants, for instance, may carry away the insect eggs and this may reduce the population of the next generation of colonizers. Similarly, beetles such as Carabids and Staphylinids may feed on the adults and larvae which are present on the body. Sometimes, voracious feeding occurs at night; hence, one will be less aware of their presence. Moreover, others will feed on the younger life stages or predate adults during daylight. In such cases, there will be an alteration in the sequence of the succession of insects, and some species which would be expected to be present, may not appear. This clue about predation can be important when interpreting the data especially if the individual has been dead for a period greater than a couple of days.

CHAPTER 3

SPECIALISED TOPICS IN FORENSIC ENTOMOLOGY

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INTRODUCTION

It is interesting to emphasize that the core duty of a forensic entomologist in crime investigation concerning mysterious death is to provide an estimate of the time when the descendent died. He is never a witness for or against the suspect or the victim but he is required to provide unbiased reports related to the time of death using insects recovered from the body. In fulfilling such an obligation, he needs to submit detailed reports that are consonant with the requirements of the court. Therefore, the report has to follow a reasonable sequence so that a non-expert can understand its content. In many related acts, the statement may be read aloud in court unless it is directed that the report cannot be oral. In this case, it has to be written in very simple terms. With the agreement of the judge, the entomologists appointed as expert witnesses can be called to the court to explain the contents of their statements.

Each statement or report submitted by the entomologist should have several sections and start with a front sheet that specifies:

- Status of the report, thus stating whether the report is for evidence assessment or a Statement of Witness?
- To whom it is addressed.
- Who is writing the report or giving the statement hence, his full name is very necessary and because he is a forensic entomologist, his institution or employee address is also needed.
- The entomologist must sign all the pages of the report with dates.
- State whether he is a joint single expert or has been instructed as an expert witness by one party to the case or the other.
- He should list the names of people who instructed him to undertake the work, whether it is an individual, a solicitor, or the police force.
- He should write his professional details for emphasis to include his name, professional address, and contacts.
- Finally, he should insert the court reference number; this may not be necessary for many report writing.

The next page may include the "Contents" and after that comes a glossary page including the terms in alphabetical order. A glossary should be added because the entomological terms need to be defined for the non-experts to understand. It is better to embolden in the text the entomological words whose meaning can be found in the glossary, as this will help to speed up reading the report.

The main body of the forensic entomology report may be split into ten(10) sections to include: an introduction, the mandatory wording of the statement of truth, a summary of the background, instructions, a summary of conclusions that he has drawn, a description of what he sampled in response to the instructions, investigations at the locality or the crime scene, follow-up investigations (laboratory approach), follow-up investigations (meteorological approach) and experimental analysis of relevant entomological data.

Forensic entomology report as a statement of witness in a courtroom

The court may decide to invite the forensic entomologist to appear in person, in order to expand on the points in his “statement of witness”. He will first be asked for his full name and address and to state his qualifications. So he should be prepared to explain the meaning of the acronyms for the qualifications which he holds. The court may also want to be reassured and therefore may require whether he has been keeping up to date by regularly attending entomology and forensic entomology conferences, contributing to learned journals, and holding any membership of professional societies. Next, he may be required to clarify further any points in his report though he will be generally asked initially to summarize his findings and conclusions. For easy communication, a summary before details are required. Many forensic practitioners in the developed world also hold registration as expert witnesses through the Council for the Registration of Forensic Practitioners chin can be regarded as a measure of desire for professionalism.

The Role of Professional Associations for Forensic Entomologists

Profe There are many professional associations for forensic entomologists in the UK and US but they all have two goals. The first goal is to maintain a medium for discussing what professionalism entails as regards forensic entomology; in other words, to maintain decorum through the exchange of information and the development and maintenance of best practices in forensic entomology. The second role is to make sure that every practitioner adheres to the maintenance of standards in the practice of forensic entomology. Two major professional forensic entomology associations working seriously to move the development of stan¬dards forward are the American Board of Forensic Entomology and the European Association for Forensic Entomologyssional Organizations Related to Forensic Entomology in Nigeria

A forensic entomology organization is a society that ought to require its members to have a postgraduate qualification and appropriate experience in forensic entomology as well as crime scene management. It may require enthusiastic entomologists either registered in the *Entomological Society of Nigeria* or other organizations to come together to form the *Nigerian Association of Forensic Entomologists* or whichever name they choose to adopt. Thus, until now no registered professional organization is known to be accredited for or practicing forensic entomology in Nigeria. Therefore, those making research on forensic entomology in Nigeria are still within the ambient of *the Entomological Society of Nigeria.* Hence, such professional organization is a prerequisite in developing forensic entomology to a status that can easily be incorporated into the Nigerian legal system in investigating cases related to questionable deaths. Such organization should be mandated to develop the following goals:

- To develop a collective protocol in forensic entomology,
- To raise good ethical practice in insect collection and analysis about forensic entomology, and
- To provide a strong scientific basis so that forensic entomology can be an effective analytical tool.

In setting the above goals, the organization will inspire the recognition of forensic entomology as a formidable crime scene investigative tool, which has a role in assisting where necessary, in the investigation of crimes relating to questionable deaths.

Common protocols for forensic entomology

Presently, there is no mechanism for accrediting standard protocols for forensic entomology as it has not been utilized worldwide. However, there are protocols used by Catts and Haskell (1990). Similar protocols were highlighted by Byrd and Castner (2001). For now, these protocols form the general guide as a working practice and offer a case study form to be completed at the crime scene. Forensic Entomologists have continuously highlighted the need to develop a sampling protocol that can be generally accepted by forensic entomologists, and also by those crime scene investigators who collect insect specimens at the crime scene on their behalf.

Therefore, workshops and field visits can be explored by sampling dead pigs or other cadavers and subsequent identification of the insect species collected; thus, discussing their forensic importance. Such workshops and meetings can provide a basal platform whereby harmonized methods of collection, processing, and shipment could be assembled, thus leading to the exchange of methodologies for good working practice. The protocols when highlighted will be deliberated, verified, and progressively become accepted by the community of forensic entomologists.

The Knowledge and Biology of Stored-Product Insects in Forensic Entomology

“Stored-product insects” appears as one of the sub-fields of forensic entomology. The knowledge of a stored-product entomologist is highly needed in forensic entomology because he understands the biology of insects associated with foods in the stores. As a professional, he may be needed in litigation cases to interpret the effect of insect infestation or contamination of commercially distributed foods. Thus, stored-product entomologists generally deal with arthropod infestation or contamination of a wide range of commercial products. For instance, beetles or their parts found in candy bars or flies found in ketchup may lead to litigation action. Similarly, stored product insects may be found in drugs consignment imported into a country. In all these cases, when products are legally presented before the litigation office, the services of a stored-product entomologist require them to interpret the relationship between the insects and the stored products with the health implications of consuming such infected or contaminated food.

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SECTION II

ASPECTS OF STORAGE ENTOMOLOGY

CHAPTER 1

STORED PRODUCTS ARTHROPOD PESTS

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INTRODUCTION

A pest is a plant or animal that causes nuisance and epidemic disease associated with high mortality in humans, agriculture, and livestock production. Arthropods are regarded as the largest group in the animal kingdom that constitutes serious pest problems to man and its interests. They are characterized by jointed appendages, a segmented body, a central nervous system, the presence or absence of wings, an exoskeleton made of chitin, an open circulatory system, a brain that connects with ventral nerve cord, highly specialized or well-developed sensory organs, specialized digestive system, specialized respiratory system for different habitats, and a clear distinction of sexes. Insects and mites are the major arthropod pests that play a key role in the destruction of agricultural products on the field and in storage. Insects from the orders Coleoptera (beetles and weevils), Lepidoptera (moths), and Isoptera (Termite-causing damage to storage structures) are major threats to stored products (cereals and legume seeds) due to the degree of damage they cause. Mites belonging to Astigmata, Mesostigmata, and Prostigmata Orders have a great effect on the stored product environments (Lale, 2002). Apart from damaging stored produces, they also cause a reduction in their quality and also contaminate the products through their feces, odor, dead insect bodies, and other body parts (Odeyemi, 2005).

Stored products are commodities which are stored after the harvest. They include grains, leather, tuber, flour, chips, etc. In tropical and subtropical regions of the world, the storage of harvested farm products poses a great challenge for farmers and retailers of stored products. The arthropod infestation of stored products is a major problem because various stages of insect development cause a great threat to the nation’s economy and loss in value, and quality of grain commodities. Year in and year out, the insects are developing various ways to keep on surviving in the storehouses and warehouses where the products are stored for distribution and future purposes. It is then important for scientists, farmers, government, and other stakeholders to develop a way of dealing with this problem. Many nations spend a larger percentage of their agriculture budget on the control of the outbreak of pest infestation. Various methods have been developed ranging from synthetic to organic ones for controlling the arthropod pest.

Arthropod Pests and Classification

Arthropod pests have the potential to cause economic damage to crops, stored agricultural products, and other house hold items, and harm to human and animal health (Lale, 2002). They always compete with human interests in diverse ways. Insects are the major pest of stored products or commodities. However, it should be noted that some insects could play a dual role as in the case of termites which help the decaying of waste material on the farm (bio-degradation). In another case, they could damage materials and agricultural products stored at home. There are various criteria used in classifying pests. Arthropod pests based on different capabilities can be classified as follows:

A. Based on the ability or inability to initiate damage to a wholesome grain

Under this criterion, arthropod pests are classified into primary and secondary pests.

Primary Pests

These are pests that have the innate ability to initiate damage on a wholesome or undamaged grain. They possess a special ability to penetrate the grains (the intact coat) e.g. *Callosobruchus maculatus* (cowpea beetle), *Sitophilus zeamais* (maize weevil), and *Rhyzopertha dominica* (lesser grain borer) among others.

Secondary Pests

These are the group of pests unable to initiate damage to a wholesome or intact grain (seed coat). They feed on already damaged commodities by the primary pest or those that have been mechanically damaged during harvest processes and other activities. Examples include *Tribolium castaneum* (red rust flour beetle) and tropical warehouse moth.

B. Based on the degree of damage

The classification under this heading is conducted through the assessment of the level of damage that arthropod pests cause in varying degrees of losses. Hence, they are classified as major and minor pests.

Major Pests

These are the pests that cause substantial loss in stored products. They could either be a secondary or primary pest in nature. Amongst insect pests that cause damage to field crops, food grains, and stored grains, the most devastating ones are beetles (Coleoptera) and moths (Lepidoptera). Of these two, beetles are far more diversified and highly destructive types of stored grain insects in comparison to moths. Both grubs (larvae) and adult insects attack the stored food material but in moths, only the caterpillars are a harmful life stage that causes the damage. Some of the major stored grain pests are *Sitophilus oryzae* Linn. (Rice weevil), *Trogaderma granarium* (Khapra beetle), *Rhyzopertha dominica* (Fabr), *Tribolium castaneum* (Herbst) (Rust red flour beetle), *Sitotraga castaneum* (Herbst) (Rust red flour beetle), *Sitotraga cerealella* (Olive), and Grain and flour moth, *Bruchus chinensis* (Pulse beetle).

Minor Pest

These are arthropod pests which do not breed on stored grain but their presence in the stores is harmful because they generate filth and nuisance. They do not cause large damage to food grains but create noxious smell and debris. These insects are cockroaches, ants, crickets, silverfish, psocids, and termites. Few mites also cause an infestation in grain flour and other stored products.

It should be noted that the level of damage a storage pest is capable of inflicting is supported by environmental factors such as humidity, temperature, and light. The fecundity of stored product insect pests is high. Females of stored grain insects enormously breed and multiply the insect population in a short span of inside go downs in uncontrolled conditions. The most infectious stage is the adult and the larva stage because the adult female lays the egg either on or inside the grains. The adult and larva have a great effect on the economy of the farmer and reduce the quality of the grains (Ashamo, 2016).

Major Arthropods associated with Stored Products

The groups of arthropods associated with store product damage are insects and mites. Insects associated with stored products belong primarily to three insect orders: Coleoptera, which includes beetles; Lepidoptera, which includes moths; and Psocoptera which includes the psocids. The order

Acarina is the most remarkable one associated with the destruction of stored products among mites.

Coleoptera Order (Beetles and Weevils

This is the largest insect order in the class Insecta of which 400,000 species have been described (Hammond, 1992; Hunt et al., 2007). Beetles thrive in diverse habitats and are found almost everywhere in the world as they are capable of feeding on all sorts of plant and animal materials (Borrer et al., 1989). There are about seven families that are considered economically important. Bostrichidae, Bruchidae, Curculionidae, Dermestidae, Laemopholidae, Silvanidae, and Tenebrionidae families are the ones associated most with stored products as they cause serious economic damage. Specific examples of storage pest coleopterans include Saw Toothed (*Oryzaephilus* sp.), Flat Grain Beetles (*Cryptolestes* sp.), Lesser, Larger Grain borers (*Prostephanus* sp.), Red and Confused Flour Beetles (*Tribolium* sp.), Granary, Rice, and Maize weevils (*Sitophilus* spp.), and Cowpea beetle (*Callosobrochus maculatus*) among others (USDA, 2015). Damages to stored products are done by both the larvae and the adult life stages.

Lepidoptera Order (Moths

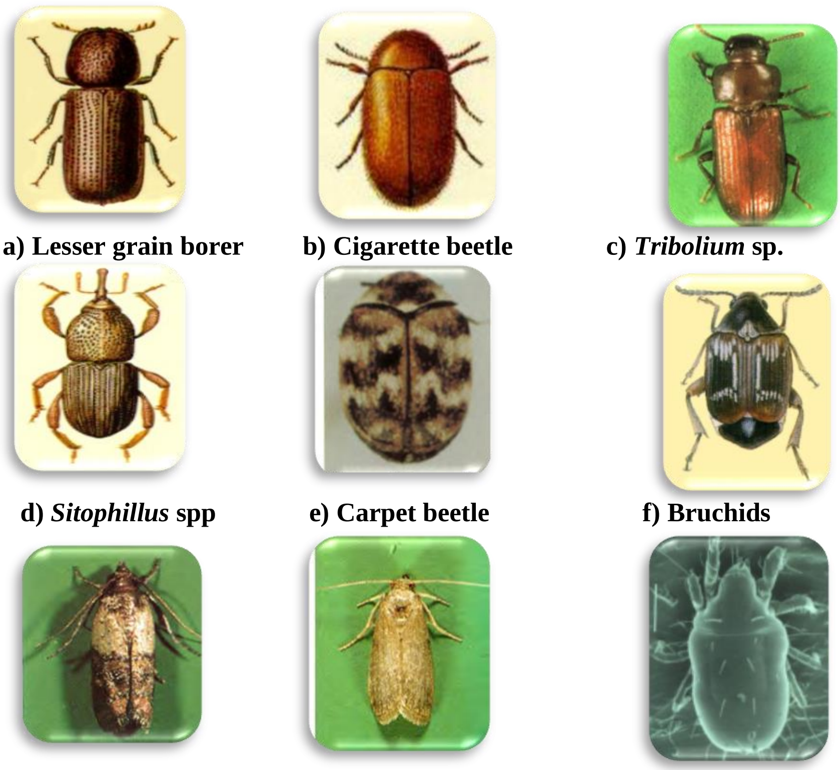
Gelechiidae, Oecophoridae, Pyralidae, and Tineidae families account for a vast number of Lepidoptera that have been reported in various stored products. However, only a few members of Pyralidae are associated with infesting agricultural products. Storage pest lepidopterans constitute a serious pest problem only in the larvae stages as the adult does not feed. This is a sharp contrast to beetles which have both the larvae and adult stages as seriously damaging pests. Angoumois grain moth (*Sitotroga cerealella*), Indian meal moth (*Plodia interpunctella*), Rice moth (*Corcyra cephalonica*), Meal moth (*Pyralis farinalis*), and etc. are some of them (USDA, 2015).

Psocoptera Order (Book lice, Bark lice)

Members of this order are mostly referred to as psocids. They are cosmopolitan, with a sizeable amount of species that are truly tropical while others are restricted to temperate regions. They are small, fragile insects capable of causing severe damage to stored grains. Most of them feed on fungi and lichens, or epiphytic algae, but a few eat dried animal material and stored flour and cereal products. Occasionally, when conditions are humid, psocids can be serious pests of stored foodstuffs and large populations can develop. Examples of psocids are *Liposcelis* sp. and *Trogium pulsatorium* L. (the Death Watch) (USDA, 2015).

Mites: Acarina Orde

This is another group of arthropods other than insects known to be storage pests. Important genera in this order include *Acarus*, *Blomia*, *Glycyphagus*, *Lepidoglyphus*, and *Tyrophagus* (Van Hage-Hamsten and Johansson 1992). Infestation of mites occurs when the products are in storage rather than being transferred from the field. An infestation by mites indicates a serious food safety problem because they are known to produce allergens (Van Hage-Hamsten and Johansson, 1992). Some of these allergens could present themselves as itching (e.g. grocer's itch) and diarrhea in humans. They could also transmit fungi that produces mycotoxin (Franzolin *et al*. 1999).



a) Lesser grain borer **b) Cigarette beetle** **c) *Tribolium* sp.**

d) *Sitophillus* spp **e) Carpet beetle** **f) Bruchids**

g) Flour Moth **h) Indian Meal Moth** **i) Mites**

Plate 1. Common insect pests of stored cereals and pulses (Source: GRDC, 2011; USDA, 2015)

Contaminants associated with storage pests

Contaminants associated with the occurrence of storage pests in stored commodities are broadly divided into 3 classes namely physical, biological (microbial), and chemical contaminants (Olsen, 1998). Physical contaminants include feces, body fragments, and exuviae of storage pests. Biological contaminants include pathogenic organisms such as viruses, bacteria, fungi, and protozoa carried to stored products by stored product pests. Humans or animals get infected by these pathogens by consuming the infected product. Chemical contaminants present themselves in the form of undesirable odors and smells, carcinogens, and allergens. These are secreted by storage pests during their destructive activities. A typical storage pest known for producing allergens is the mite.

CHAPTER 2

CONTROL OF STORED PRODUCT ARTHROPOD PESTS

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INTRODUCTION

There is an urgent need to control stored product arthropod pests because the injuries and damages they cause have cognate effects on ecology and equally on food security.

The following methods can be employed in the control of arthropod pests.

1. HYGIENE

A store that is well-aerated and in a hygienic condition can help reduce problems associated with pests. There is a need for an effective assessment of the efficacy of hygiene and structural treatment prior to the in-loading of grain. Good hygiene in the grain store or storage depot is important in maintaining grain and seed quality. The following are prescribed hygiene actions in and around the storage.

- Keep the storage area clean- this includes sweeping the floor, removing cobwebs, dust collection, and removal of any grain spills.
- Clean the storage room after products are emptied and the action may include spraying walls, crevices, and wooden pallets with effective insecticides before reusing them.
- Place rat traps and barriers in drying and storage areas.
- Inspect the storage room regularly: Inspection of the stored grains once a week for any sign of insect infestation is necessary. This should be carried out by an expert who is a trained pest control technician. The storage room or the seed stork may be sealed with tarpaulin and treated with fumigant. Proper hygiene also connotes building maintenance. Blocking of leakages and sealing of openings, pipes, holes, joints, and a leaky roof are necessary (Banks and Fields, 1995).

Physical Methods

This method has to do with maintaining atmospheric conditions to inhibit the reach of insect pests. Various physical methods can be employed in pest control.

2. TEMPERATURE REGULATION

Low-temperature storage

A low temperature of 0°C is also effective in controlling stored product insects (Ashamo and Odeyemi, 2001). The grains are stored under the ground, simply because the temperature below the ground is very low and it is not a favorable environment for the growth of insects. The favorable temperature for the growth of insects ranges between 25-35°C and a temperature between 15 and 25°C results in a slower development leading to a reduction in the number of eggs laid and an increase in lifespan. Reducing the temperature to 18.2°C extends the duration needed for completion of the generation to 15 weeks for *Sitophilus oryzae*. A female will lay 4 eggs in a week and produce 4 females in 10 weeks. Temperature also can be lowered by forcing cool air through grain bulk.

Low-temperature control/treatment

Huis van (1991) reported that eggs of stored product insect pests are more susceptible to cold temperature than insects in the other stages of the lifespan. Ashamo and Odeyemi (2001) reported that all stages of *Callosobruchus maculatus* were eliminated at 0°C for 30-32 days. This could be the reason for disinfecting grains needed for an experiment at temperatures between 0°C and 4°C for 72 hours. Despite its advantage, the cost of providing a durable cold environment is tough for farmers and retailers.

High-temperature storage

High temperatures of about 45°C will control stored product pests because they are always sensitive to higher temperatures (Zettler and Cuperus, 1990).

The use of high temperature

Eggs, larvae or nymphs, pupae, and adults of many insects can be killed by high temperatures.

Methods of generating high temperatures

a. Use of artificially generated heat

There are different ways of generating high temperatures in stored products:

1. Use of ovens of the domestic cooker

In the oven method, the oven compartment of the cooker is heated to a very high temperature and the batches of grains or smoked/dried fish to be treated are placed in a tray and left in the oven for a period of time sufficient enough for the pests on the surface and inside of the products to be killed. The temperature always up to one hour at the temperature of 57.3ºC will not affect the germination of the seed.

2. In pots heated over the open fire.

The infested food grains or seed is fitted in a pot over an open fire generated with fuel wood after which the grains or seed are admixed with hot sand and turned continually to make sure that all the parts of the grains have direct contact with hot sand. This is continued until the heat penetrates the grains to kill the insect pests present within them. This method is highly effective against Bruchids together but it can only be used in the protection of grains for family use. Unlike the oven method, the oven fire method cannot be regulated and may therefore not be suitable for grains that will be used as seeds.

Solar Disinfestations

This method uses the solar radiation generated by the sun as a means of driving insects, mites, and other arthropods from infested grain and could also kill eggs, larvae (or nymphs), pupae, and adults that may be living among the product or larvae pupae that may be inside the grains. This also reduces or inhibits oviposition in exposed females. This method is effective against all stages of stored product arthropod pests. This method is only visible and practicable in the African savanna where there is abundant sunshine virtually throughout the year, but particularly during the hot season when the open day temperature reaches 40 – 50ºC and relative humidity is low as 10-20%. The spreading pattern of the grain also affects the effectiveness of this method. It has been reported that grain infested with *C. maculatus* can be treated by exposing it to solar heat for 6 hours at the temperature of 47-51ºC. This method kills *C. maculatus* of all stages. This method is widely used by farmers in rural areas (Lale, 2002).

3. LOW GRAIN MOISTURE CONTENT

The moisture content of stored products affects the number of offspring, rate of development, longevity, and survivability of adult storage pests. Many larvae can absorb atmospheric water moisture directly at a relative humidity of 55% and above (Ashamo and Odeyemi, 2001). One of the methods in controlling stored product arthropod pests is to reduce the moisture content of the grain to a safe level. This level varies by the type of grains, for instance, Groundnuts 7%, Sorghum 12.5%, Wheat 13%, Maize 13%, Rice 13%, Paddy 14%, Pulses%, Millet 16% (Lale, 2002). The moisture content of the food affects the rate of growth, production of offspring, life span, and the total number that will successively become adults (although the moisture content also varies from one century to another).

4. HERMETIC STORAGE

This is an act of controlling the atmosphere of the grain environment. The oxygen in hermetic storage drops to zero and prevent the infestation of insect and mold. Hermetic storage fires a gaseous composition of the storage atmosphere through the respiration of cowpea seeds, microorganisms, and insects. This method eventually kills the insect through asphyxiation. If the grain is stored in a sealed container e.g. drum steel is filled with cowpea seed, the oxygen concentration will drop to zero within two weeks. Ofuya and Reichmull (1992) reported that 100% carbon dioxide or nitrogen atmosphere killed *C. maculatus* and *C. subinnotatus* of all stages in 5 days at 32°C. New and Rees (1988) reported that vacuum packaging under different pressures had varying degrees of success against *C. maculatus*.

5. IMPACT

Insects can die when moved with the grain from one place to another, especially from where they will be consumed or stored. Some moving methods like carrying in a screw gauge or a bucket elevation can cause considerable mortality to the insect(Fields and Muir, 1995) whereas pneumatic conveyors are very effective for controlling insects at the farm level (White et al., 1997) or unloading ships (Balor, 1991). Impact machines or entoleters have been used in flour mills since the 1940s to control insects in grains or flour (Reichmult, 2000). Insects or grains with internal infestations hit the pins and insects are killed on impact.

6. INERT DUST

These are materials that set the insects more sensitive to desiccation or dryness. The inert dust may be ash, clays, minerals, diatomaceous earth, and synthetic silica aerogels (Roeli, 2000). The inert dust damages the cuticle of insects and causes the insect to lose water and leading to the death of the insects by desiccation. Some of the limitations of diatomaceous earth include the followings:

i. It is very dusty for a worker to apply.
ii. It reduces the bulk density of grain (4-10%) (Korunic et al., 1998).
iii. It reduces the flow rate of grain.

7. CULTURAL CONTROL

This is an act of interrupting the environmental factor to lower the rate of pest influence on commodities. This is one of the simplest and cheapest methods of pest control. It includes keeping the storehouses clean and removing dirt, eggshell, and dead lawn. This also deals with the variation of the planting and harvesting time, the planting of pest-resistant varieties, seasonal manipulation, plant rotation, and harvesting method. Below are some cultural control methods for the stored products against insect pests.

- **Cleanliness of storehouse-**This is one of the simplest and cheapest methods of controlling insect pests. Removal of waste material and previously used sacks and bags reduces the rate of production of the insects by preventing the formation of habitat suitable for them.
- **Proper Storage-**Equipment used in storage such as processing machine, dryer, elevator, and storehouse vicinity must be properly cleaned. Store sanitation should be maintained. Broken-infested grains are removed and burnt before new grains are stored. Before the storage, the store should be fumigated properly and closed until the time of harvest comes.
- All cracks in the walls of the store and the roof should be filled well. Stores should be white washed or painted by repellant paint.
- Quarantine- All goods for storage should be properly checked before the export and import of food grains.

8. BIOLOGICAL CONTROL

Several biological agents are used for the suppression of stored grain insect population living organism or their products are used for the control of the stored product insect pests. Various parasitoids, predators, pathogens, and other living organisms are used as biological agents to control the stored products of arthropods. Equally, insect pathogen is also employed as biological control agents. The order hymenopteran is commonly used as a parasitoid to reduce stored-produced insect pests (Lacey, 2001). Some Hemipteran (bug), *Xylocoris flavipes* (Reuter), and several other bugs such as anthocorid bugs of the sub-family Lyctocorinae are more frequently used in the store and warehouses. This predator has a great potential to reduce both Coleopteran and Lepidoptera insect populations in stores and warehouses. Two common parasitoids placed in stored products are *Bracon hebetor* (Say, 1836) (Hymenoptera: Braconidae) and *Venturia canescens* (Graven horst, Ichneumonidae) and they are used to suppress *E. cautella* populations. Dermestid larvae are also reduced by *Laeluis pedatus* (Say) (Bethylidae). *Habrobracon hebetor* (Say) (Hymenoptera: Braconidae) was used to prevent the infestation of Indian meal moth (*Plodia interpunctella*) (Hubner). Egg parasitoid *Trichogramma* Dion Riley (Hymenoptera: Trichogrammatidae) and larval parasitoids alone or together were utilized for the control of cowpea weevil (*Grieshop* et al., 2006). Entomopathogenic *Beauveria bassiana* was also used to control cowpea weevil *C. chinesis* exoskeleton (Cortesero et al., 1997). In addition, *Bacilus thuringiensis* is another parasitoid used to control coleopteran pests of stored wheat (Abdel-Razek, 2002) while *Eupelmus vuilleti* Crawford (Hymenoptera: Eupelmidae) can control cowpea beetle (Cortesero et al., 1997). The parasitoid *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae) parasites and develops in late instars of five different stored product insects that typically complete their development inside seeds of grain or legume species or other dry commodities. Parasitoid adults are significantly larger and heavier when they develop on cowpea weevils irrespective of the parasitoid population. *Lariophagus distinguendus* Förster (Hymenoptera: Pteromalidae) is used as a biological control agent against the granary weevil *S. granarius* (L.) in grain stores. *Theocolax elegans* is used to reduce the population of *Rhyzopertha dominica* on wheat (Flinn and Hagstrum 2002). Parasitism decreased the larval host size and development time. Similarly, the larva of the next generation also has a reduced developmental period.

Microbial Control

This is an alternative to synthetic pesticides. It involves the use of microbial insecticides in the form of toxins or spores e.g. Bt toxins produced by *Bacillus thuringensis*. Many entomopathogens are also used for the control of stored grain pests. This method, most of the time, is used in combination with the botanical control method (Diaz-Gomez et al., 2000). For instance, four fungal species, *Beauveria bassina, Lecanicillum lecanii, Metarhizium* *anisopliae*, and *Paecilomyces farinosus* are used for controlling 68 Indian meal moth (Buda and Peciulyte, 2008). As another example, saw toothed grain beetle (Coleoptera: Silvaidae) can be controlled using the entomopathogenic fungi, *B. bassiana* (Throne and Lord 2004.).

Natural Plant Products

These are alternative methods to the use of synthetic insecticides. Synthetic insecticides are not ecologically friendly and it takes a long time for them to get removed from the environment after being applied. Equally, some insects have developed resistance against most of these synthetic insecticides (Zettler and Cuperus, 1990). Many plant products have been proven to have insecticidal properties in different stages of life against insects (Rahman and Talukder, 2006). Many plants' essential oil and chemical constituents have been reported for the ability to cause inhibition of some developmental activities of insect pests (Mollaei et al., 2011).

Beside the crude oil extract itself, the toxic effects of oil constituents like d-limonene and linalool have been demonstrated on the stored product insect pest (Weaver et al., 1991). The plant product has little or no effect on the environment and non-targeted organisms (Matthews 1993). For example, the essential oils of *Artemisia annua* (L) show toxic, repelled, and developmentally inhibitory activities against two species; (Herbst) and *C. maculatus* (L) Adult beetle of *T. castanum* were significantly and economically repelled by oils of *A. annua* of 1% concentration.

Plant essential oil

Apart from the plant extracts, essential oils have shown insecticide activities against stored products and insect pests (Palacios et al., 2009). Many of the oil have shown high oviposition and growth inhibitory activity (Tripathi et al., 2001) e.g. Volatile constituents, methyl salicylate from securidaca longapedunculata exhibited repellent and toxic properties for *S. zeamais* and *R. dominica* (Jayasekara *et al*., 2005). Also, volatile compound diallyl disulfide isolated from neem has shown potent toxicity and feeding deterrent activity against stored grain pests. The active ingredient from leaves of *Artimisia princeps* and seeds of *Cinnamomum camphora* (L.) has been shown to have repellent and insecticidal activity against *S. oryzae* and *Bruchus rugimanus* (Liu et al., 2006.). These plant products show enormous toxicity against several stored product pests and provide prolonged protection to the seeds which may be due to the high mortality of adult insects.

Plant volatile aldehydes are used as natural insecticides to control stored product beetle (Godfrey, 2004). Methyl allyl disulfide and diallyl trisulfide obtained from the essential oils of garlic are used to control stored products pest. Volatile constituent, di-n-propyl disulfide extracted from seeds of neem, *Azadiractita indica* is toxic when applied as a fumigant to *Tribolium castanaum* adult and larva and *S. oryzae* adult Di-n-propyl significantly reduces with moderate utilization the growth rate and dietary by moderately inhibiting food consumption in both insects. This component is a potent toxic, fumigant that also acts as a feeding deterrent to stored grain pests (Sabbour, 2003).

9. MECHANICAL METHOD

This has to do with a method such as handpicking, sorting, or separating by sieving materials so that larger ones than insects can be separated. This method is not effective for smaller insects such as *Oryzaephilus* spp. and there is currently a new approach in mechanical method which has to do with storage or packaging food stored in containers that insects cannot easily enter. The major disadvantage of this method is that it cannot protect the stored product once the insect pest finds its way to them after packaging.

10. REGULATORY METHOD

This is the process by which government provides several ways of screening, and restricting plant materials or products that are likely to carry diseases or pests into the country through importation. This method is also known as Phytosanitary method. The Food and Agriculture Organization of the United Nations (FAO) has formulated a document as regards to importation and exportation of plant materials to prevent the coming in of any that contains insect pests or diseases. As part of Phytosanitary measures, the requirements of the International Phytosanitary Certificate (IPC) must be followed in the importation of plant materials into the country. This method is not effective in Africa because of the degree of corruption in the national borders and this has caused the spread of disease in insect pests in Africa.

11. CHEMICAL CONTROL

The use of chemical insecticides has been regarded as an essential component in storage pest control. Synthetic insecticides, such as malathion, pirimiphos-methyl, chlorpyrifos-methyl, pyrethrum, deltamethrin methoprene, and fumigant phosphine (Bond, 1984; Snelson, 1987; Weaver and Petroff,

2005) are currently some of the major products used by many grain storage experts to reduce losses of stored grain to pests (Arthur, 1996). Its use is considered the only option when a pest population in stored grains reaches the economic threshold (White and Leesch 1996). Chemical control is sometimes the last tactic considered due to its negative impacts on human health and the environment.

Fumigation:

Stored grain pest infestation is controlled by various methods and fumigation is one of the most effective methods in which insect pests are exposed to a poisonous gaseous environment, produced by applying a synthetic chemical insecticide in the form of fumigant. This is applied for pest control in stores, warehouses, buildings, soil, seed, small bags, and stored products. The fume from the fumigants enters the insects' body through the spiracle and disperses into the trachea and tracheoles, and binds to the hemolymph materials (Brattsten et al., 1986). Fumigants such as ethyl formate, sulfuryl fluoride, carbonyl, and ethane dinitrile are employed in the control of termites, cockroaches, and mites. Ethylene dichloride and carbon tetrachloride mixture (3:1 ratio) are used for fumigation to kill the eggs, larvae, and adults of stored insect pests in general. It has no harmful effects on the grain even if they are intended to be used for seed purposes. Apart from this, other fumigants like Grain-O- Cide are a mixture of carbon bisulphide and carbon tetrachloride (1:4 ratio) that is used to fumigate warehouses. Phosphine inhibits the development of eggs in the stored product pest *Liposcelis bostrychophila* (Psocoptera: Liposcelididae) (Nayak et al., 2003) and also affects the hatching of coleopteran insect pests, *Cryptolestes ferrugineus.*

12. OZONATION

The use of ozone is another method for controlling arthropods of stored products. Ozone (O_3) is an allotrope of oxygen (O_2) that can be produced by UV-light and electrical discharges in the air. Ozone has various advantages over all other synthetic insecticides. Ozone produced by electrical discharge is the most common one with greater sustainability and cost affectivity. The half-life of ozone is short (20-50min) and it decomposes rapidly into diatomic oxygen, a nature substance in the atmosphere. Ozone can be easily generated at treatment sites using electricity and air and its safety makes it more advantageous than the conventional post-harvest pesticides. Ozone does not store up chemical substances and leaves no residue on the treated commodities. Equally with the short half-life, it can easily revert to natural oxygen (Law and Kiss 1991).

Ozone, a known sterilant, can be used as an insect-controlling agent in stored food products at less than 45 ppm. It is readily generated from oxygen in the atmosphere and environmentally safe when used as a fumigant. Furthermore, it is unstable and can quickly breakdown into molecular oxygen (Mason et al., 1998).

13. INSECT GROWTH REGULATORS (IGRs)

These are chemicals that are capable of mimicking the hormone that controls molting in insects and then interrupting the developmental processes of such insects. According to many studies reviewed by Oberlander et al. (1997), it shows that IGRs are a safe method for controlling the insect pest population of stored products. Most of the published research involved exposure of eggs, first instars, or adults on treated grains, in diet or vials with subsequent measurements of progeny production. El-Sayed, 1988 reported that some of the studies showed morphological effects and reduction in fecundity in adult stored product insects. In the test by Arthur (2001), the late-instar larva of *T. castaneum* (Herbst), the red flour beetle, and *Tribolium confusum* (du Val) were directly exposed to concrete treated with hydroprene. Larvae failed to molt to the pupal stage and adults were morphologically deformed and died quickly. Equally *T. castaneum* (Herbst) appeared to be more morphologically deformed and died quickly. *T. castaneum* was more susceptible to hydroprene than *T. confusum.*

Currently, there is a great demand for the generation of reduced-risk low toxicity chemicals and the generation of new formulations of IGRs, to replace older conventional products used in many agricultural firms including post- harvest protection in areas where food is stored. For example, the newly formulated hydophone was equivalent to the registered product Gentrol. *T. confusum* was less susceptible than *T. castaneum* and residual control of *T. confusum*, lasted 6-12 weeks (Arthur and Hoeremann, 2004). IGRs have been developed commercially and are being used to control insect pests in agriculture, forestry, public health, and stored products. IGRs affect the biology of treated insects, for example, both embryonic and post- embryonic development, reproduction, behavior, and mortality. Abnormal morphogenesis is the observed effect of IGRs' action on the insects. Many of them are more potent than current insecticides even against eggs. IGRs have the following advantages over other conventional chemically synthesized insecticides: it does not exhibit quick knock-down on insect or cause mortality, but the long-term exposure to these compounds largely stops population growth as a result of the effects mentioned in both the parents and progeny (Mondal and Parween, 2000).

14. BEHAVIORAL CONTROL USING INSECT PHEROMONES

Insect pheromones are used in controlling targeted insect populations. This is done either by applying female or male-specific pheromone substances. This method is mostly used in the surveillance and detection of infested stored grains. It is used to attract and lure a mass of insects for trapping. Pheromones are used in very small quantities in traps and they are positioned in a warehouse at a considerable distance. It always captures the targeted insects and causes a severe reduction in the targeted insect populations. Disruption of mating with pheromones provides a wider reduction in the insect population. For instance, pheromones of *Trogoderma* sp. and the black carpet beetle *Attagenus megatoma* (Fabricius) are used either singly or in combination with bait to catch these insect pests in large numbers. The primary component of *Trogoderma* pheromone 14-methyl 8-hexadecenal is now used to capture and kill large numbers of *Trogoderma granarium*. Equally, wheat germ oil combined with sex pheromone is used to attract and trap *Trogoderma* larvae. Furthermore, male lesser grain borer *Rhyzopertha dominica* (F), produces an aggregation pheromone that attracts both sexes. Also synthesized pheromones are used in baited traps which are found effective in monitoring populations (Kaakeh 2000).

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SECTION III

CHAPTER 1

CROP PRODUCTION AND ASSOCIATIONS WITH INSECT PESTS

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INTRODUCTION TO CROP PRODUCTION:

Crop production is a branch of agriculture that is primarily concerned with the growing of crops for use as food and fibre for man, feed for animals, and raw materials for industries. It encompasses the art, science, and business components of production. Consequently, it involves all aspects of crop improvements using conventional breeding methods as well as the use of molecular techniques to produce new varieties with increased quality and yield. A crop is an aggregation of plants or cultivated species of the same kind which are being grown or cultivated in one place on a large scale. After harvesting, crops may be consumed raw, processed through value addition to other products, and stored or traded by commerce for their values. They may be arable/ field or horticultural crops. This will be discussed in detail under the overview of Crop Classification. The processes of crop production and storage are inevitably challenged by insect pests.

Importance of Crop Production:

The United Nations Development Programme (UNDP) pursuing the seventeen Sustainable Development Goals (SDGs) of 2015 underscores the importance of crop production directly or indirectly. The first three, “No poverty”, “Zero hunger”, and “Good health/well-being” aim at the member nations’ accomplishments and attainments in and stances on crop production. The importance of crop production includes:

Food security:

Food security is the availability and accessibility of all people to sufficient, safe, nutritious food to maintain a healthy and active life. One major component of food being referred to here is crops. Crops also represent basic raw materials for other food. To attain food security, crop production for the ever-increasing populace is inevitable.

Nutrition security and hidden hunger:

Similar to food security, nutrition security defines a community’s access to essential nutrients. Nutrition security is essential for adequate nutrient supplies (vitamins, micro-minerals, macro-minerals, etc.), nutrition, phytochemicals, system balancing, balanced diet, disease prevention, etc. Hidden hunger is a nutritional deficiency caused by a lack of balance in nutrition despite the ‘filling’ of the food. Nutrition security forestalls hidden hunger, and it is highly dependent on crop production.

For a community to attain food and nutrition security and prevent hidden hunger, the following steps are crucial:

- Campaign on food/nutrition security and its benefits to households
- Awareness of the importance of different crops
- Awareness about hidden hunger, its causes, and its prevention
- Campaign about crop production and consumption of nutrient-dense food
- Government policy for supporting farmers to reduce the cost of production
- Distribution of improved seed/seedlings to households and farmers
- Extension of best practices to farmers by extension agents
- Demonstration of recipes to households
- Encouraging household farming
- Advertisements in strategic places such as hospitals and media houses about the benefits and consequences of vegetable consumption
- Adequate provision of storage facilities for post-harvest purposes.

Science of Crop Production Management:

Production of crops is incomplete without the management of the crops. In order words, the activities related to crop production are one half; the other and bigger half, which completes the process, is the management of crops that are being produced. There are five main phases of crop production that require management: Pre-planting, planting, post-planting, harvesting, and post-harvest stages. These management practices have to do with agronomic practices before, during, and after planting which eventually leads to maximum yield. Pre- planting and planting management are comprised of the following operations: i) site selection; ii) management of the climate, temperature, light, wind, frost, and rainfall; iii) soil- texture, structure, depth, drainage, ph, and nutrients; iv) topography- aspect and altitude; v) water availability- rain-fed and irrigation; and vi) accessibility and labor management.

With recent development, crop production management starts before planting with the choice of soil amendments which may either be organic or inorganic. Considering the SDG goals which are 3 - good health and well-being, 6-clean water and sanitation, 11- sustainable cities and communities, 12- responsible consumption and production, 13 – climate actions, 14 – life below water, and 15 – life on land, the organic production of crops is a better choice given its implications on health and the environment. Organic farming gives rise to sustainable agriculture. Sustainable agriculture is a philosophy based on human goals and their understanding of the long-term impact of their activities on the environment and other species. This philosophy guides the use of the latest scientific advances to develop integrated, resource-conserving, and equitable farming systems.

Organic growing is a production system that consciously rejects the use of fertilizers, pesticides/fungicides/nematicides, growth regulators, and any other crop additives which are synthetically formulated for crop production. These chemicals are intentionally avoided in order to circumvent species imbalance caused by the use of pesticides and quick-release fertilizers thereby producing crops with as little disturbance as possible to the balance of microscopic and larger organisms found both in the soil and in the above-soil zone. In organic crop production, therefore, maintenance and management of soil productivity and tilth, plant nutrient supplies, control of insects, other pests, and weeds largely depend on crop rotations, crop residues, mulches, animal and green manures, legumes, off farm organic wastes including spent mushroom substrates, mechanical cultivation, mineral bearing rocks, and biological means of pest control either with the use of natural enemies or phyto-control (the use of plants extracts with anti-microbial activities). The taste of organically grown crops is said to be better than conventionally produced crops. Furthermore, organic growing encourages greater resistance to pests and diseases because of the balanced nutrition of the crop.

Post-planting, harvesting, and post-planting management involve mainly crop protection practices to prevent all forms of crop failure. The key practice is the management of insect pests. Others include the management of weeds, nematodes, diseases, other pests, and also humans. This is further discussed in the subsequent chapters.

Types of Major Crops:

Major crops may be found in any of the following:

Arable, Field, or Horticultural Crops:

Arable crops: These are the crops that can be cultivated on arable lands. Arable lands are agricultural lands.

Field crops: These are crops other than fruits and vegetables that are cultivated commercially on a large scale for agricultural purposes.

Horticultural crops: These are garden crops (fruits, ornamentals, vegetables including mushrooms) that are cultivated intensively for food, medicinal purposes, and aesthetic gratifications.

Crops may either be permanent or temporary. They may also be field or horticultural crops. Permanent crops are crops that are planted once, stay on the land for several years, and need not be replanted after each annual harvest. Temporary crops, on the other hand, are those which do not occupy the land for so long after planting and are harvested annually, biennially, or even perennially. Perennial crops do not last as long as permanent crops. Examples of permanent crops are cocoa (*Theobroma cacao*), coffee (*Coffea arabica*), and rubber (*Hevea brasiliensis*). Examples of temporary crops are cabbage (*Brassica oleracea*), bitter leaf (*Vernonia amygdalina*), and peanut (*Arachis hypogeal*). Permanent crops also include flowering shrubs, fruit trees, nut trees, and vines, but exclude trees grown for wood or timber.

The Major Food Crops:

The major food crops are:

- Cereal or grain crops: Cereals are grasses grown for their edible seeds, the term cereal being applied either to the grain or to the plant itself. They include wheat (*Triticum aestivum*), rice (*Oryza sativa*), maize (*Zea mays*), and barley (*Hordeum vulgare*).
- Legumes for seed: Legumes or leguminous plants are grown for their seeds. They are important protein sources. They include peanuts (*Arachis hypogea*), beans (*Vigna sinensis),* and Bambara nuts (*Vigna subterranea*).
- Forage crops: Forage refers to vegetable matter, legumes, and grass used fresh or preserved for feeding animals. Forage crops include grasses such as Cogon grass (*Imperata cylindrica),* and Pigeon pea (*Cajanus cajan).*
- Fibre crops: These are plants that yield fibres. The fibres are useful for making sacks, twines, textiles, etc. Examples are Jute (*Corchorus olitorius*), Cotton (*Gossypium* spp.), Kenaf (*Hibiscus cannabinus*).

- Sugar crops: These are crops cultivated for their sweet parts from which sucrose is extracted and crystallized. Examples are sugar cane (*Saccharum officinarum*), beet root (*Beta vulgaris*)
- Oil crops: These are crops whose fruits or seeds yield useful oil. They are used as cooking fats and in industries as raw materials for the manufacture of soap, margarine, and pomade. Examples of oil crops include peanut (*Arachis hypogea*), soybeans (*Glycine max*), coconut (*Cocos nucifera*), and sesame (*Sesamum indicum*).
- Mushrooms: These are reproductive structures of edible fungi. They are eaten as vegetables. They are useful as food for humans, feed for animals, compost for plants, and also in restoring (bioremediation) polluted environments. They are special crops gradually attracting the interest of humans. Examples include oyster mushrooms (*Pleurotus pulmonarius*), Shiitake *(Lentinus edodes)*, and wood ear mushrooms *(Auricularia auricula)*.
- Vegetables: Vegetables are herbaceous plants some of which are eaten either cooked or raw as the principal part of the meal. Vegetables can also be defined as edible seeds or roots or stems or leaves or bulbs or tubers or non-sweet fruits of any of numerous herbaceous plants. They may be leafy, fruit, root, or tuber crops. Examples include okra (*Abelmoschus esculentus*), Irish potatoes (*Solanum tuberosum*), and Ugu (*Telfairia occidentalis*).

Overview of Crop Classification:

Crop may be classified according to the following parameters:

Classification according to geographical distribution: This classifies crops as tropical or temperate crops depending on where they are found.

Classification according to life cycle: This classifies crops according to their life span. There are three groups:

Annuals- This group refers to the crops which complete their life cycle in one season. Examples are maize and rice.

Biennials- This group consists of plants that require two seasons to attain full development. Examples include Cocoyam (*Xanthosoma sagittifolium*), and Cabbage (*Brassica oleracea*)

Perennials- This group refers to some plants that live for several years. They may produce seeds each year but they do not die with seed production. An example is the bitter leaf (*Vernonia amygdalina*).

Classification according to economic/practical use as described under major crops.

Classification according to special purpose:

Cover crops: Cover crops are those seeds to provide a cover for the soil.

Catch crops: Catch crop is a substitute crop planted too late instead of regular crops or after the regular crop has failed An example is Clover (*Trifolium* spp.).

Silage crops: These are the crops which are cultivated for silage. Example: Guinea corn (*Sorghum bicolor*).

Advances in Crop Production:

Crop production is dynamic. Recent development encourages growing crops in nutrient solutions or other mediums without the use of soil. This is referred to as a soilless culture system. It may be water culture (hydroponics) or medium culture (gravel, sand, or another medium). There is a growing interest in soilless culture and it can be practiced as backyard gardening or on a large scale for commercial purposes. A special type of medium culture is vertical farming or aeroponics which maximizes the vertical space for crop production. There are advantages and disadvantages of soilless culture, yet it yields better results than conventional crop growing.

Insect Pests Associations: An insect pest is an insect organism with characteristics that people see as damaging or unwanted since it harms agriculture by feeding on crops or parasitizing livestock. An insect can also be a pest when it causes damage to a wild ecosystem or carries germs. The term insect pest is used to refer specifically to harmful insects. Therefore, insect pests are insects that cause physical damage to man, animals, and crops and interfere with the production of crops affecting quality and/or yield.

Types of crop pests

Important pests of crops are grouped into the following classes:

- Insects

- Birds
- Rodents
- Monkeys
- Man
- Nematodes

The insects

The class Insecta, or insects, are the Arthropoda that have three pairs of legs, a segmented body divided into three regions (head, thorax, and abdomen), one pair of antennae, and usually wings. They have 3 pairs of legs and are sometimes called Hexapoda. Insects are the largest group in the animal kingdom which constitute 75% of all living animals. There are more than one million living species in the world and many more are yet to be discovered. They were the first flying creatures and still the only invertebrates that can fly. They are the most successful animals inhabiting every conceivable ecological condition from volcanoes to glaciers.

Although only 2% of the insect species are obnoxious to man, they are enough to cause heavy damage to crops, livestock, and the man himself, which makes them very important for human attention. Pest species are difficult to control, and cockroaches, flies, and mosquitoes are as abundant as ever despite about a century of constant efforts made for fighting against them. Insect bodies are extremely tough due to their exoskeleton and they possess enormous muscle power. They are endowed with the capacity to survive in the most inhospitable situations.

Insects help us by pollinating our food crops, decomposing organic matter, providing researchers with clues to a cancer cure, and even solving crimes. They can also harm us by spreading diseases and damaging plants and structures.

Insects are arthropods. All animals in the phylum Arthropoda have exoskeletons, segmented bodies, and at least three pairs of legs. Other classes that belong to the phylum Arthropoda include Arachnida (spiders), Chilopoda (centipedes), and Diplopoda (millipedes).

Hexapoda encompasses all of the insects on the earth. It is most often divided into 29 orders. These 29 orders use the physical characteristics of the insects to group similar insect families. Some insect taxonomists organize the insects differently, using evolutionary links instead of physical traits. To identify an insect, it makes more sense to use the system of 29 orders since the physical similarities and differences between observed insects can be seen.

Insect pest

Insects and humans co-habit the Earth and have developed complex relationships. Insect pests (less than 1% of all species) are those insects that feed on, compete for food with, or transmit diseases to humans and livestock. Ecosystems modified by human activities have provided opportunities for insects, and species that successfully adapt to the changing conditions are often pests.

Insect pests can have adverse and damaging impacts on agricultural production and market access, the natural environment, and our lifestyle. Insect pests may cause problems by damaging crops and food production, parasitizing livestock, or being a nuisance and health hazard to humans.

Crops and major pests

Crop	**Pests**
Beans	Cowpea weevils (*Callosobruchus maculates*)
Mushroom	Sciarids (*Lycoriella ingenua*) or fungus gnats, Phorids (*Megaselia halterata*), and Cecids (*Mycophila speyeri*)
Rice	Rice weevil (*Sitophilus oryzae)*, stem borers, army worms, and leaf rollers
Maize	Maize weevils (*Sitophilus zea)*, stem borers, and army worms
Yam	Yam beetles and rodents
Cocoa	Stem borers, root mealy bugs, aphids, black tea thrips, and scales
Groundnut	Leaf worm, aphids, boll worms, snails, and hoppers
Cotton	Cotton strainers and boll worms
Sorghum	Weevils, boll worms, aphids, and sorghum midge
Stored grains	Weevils

EFFECTS OR ECONOMIC IMPORTANCE OF INSECT PESTS IN CROP PRODUCTION

1. Insect pests destroy crops in the field through their biting, chewing, boring, sucking, and defoliation activities.
2. They cause a reduction in the viability of stored produce.

3. Site of injuries by insects may pre-dispose crops to disease attack.
4. They increase the cost of production by controlling them.
5. They render vegetables and fruits unattractive and unmarketable.
6. Some are carriers or vectors of diseases.
7. The profits of the farmers are reduced.
8. They reduce the quality of products either in the store or in the field.
9. They generally reduce the yield of crops.
10. They can also cause the total death of crop plants.

INSECT PESTS OF FIELD CROPS

Field crops are traditionally marketed to the wholesale or commodity market. Farmers must produce them in considerable volumes. Economic efficiencies and commodity marketing both contribute to profitability. For example, an economic unit for most grain crops is 1500-2000 acres. For animal farms in this area, acres are needed to spread manure and feed several animals. Most field crop producers farm a combination of owned and rented land.

Points to consider before growing field crops include:

1. Do you know what the **soil type** is on your farm? The choice of field crop and a field rotation should be aligned with the productivity of your soil.
2. Do you have any **access to a market or appropriate storage** for the crop?
3. Do you **have the savings for all the input purchases**, such as seed, fertilizer, manure and spreading, lime, pesticides, and fuel? These expenses will not return any income until the crop is harvested, stored, and sold.
4. Do you **have enough acres** to justify machinery purchases or custom (rental) work? If renting or hiring a custom operator, do you have access to the equipment? Some areas may not have enough machinery dealers or custom operators or there may be competition for the custom operator’s time.
5. If you are **considering organic field crop production**, have you checked your *soil organic matter* rate, and do you know *what weeds will need to be monitored* for and controlled during the transition

process to organic certification? Do you understand *what is required to be certified organic*? What is the *market*? Will you get an *organic premium* to help pay for additional costs to grow organic crops?

6. Have you **ever taken a soil test** to know what nutrients you need to grow the crop?
7. Do you have a **soil conservation plan** for the farm? More information about a conservation plan can be obtained by contacting your local USDA-NRCS (Natural Resource Conservation Service) office. The soil conservation plan has valuable information in it such as soil type and a suggested rotation.

The budget helps you determine the potential profitability of the crop to be grown.

INSECT PESTS OF AGRICULTURAL IMPORTANCE

RICE WEEVIL (*Sitophilus oryzae* L.)

Scientific Classification	
Kingdom	Animalia (Animals)
Phylum	Arthropoda (Arthropods)
Subphylum	Hexapoda (Hexapods)
Class	Insecta (Insects)
Order	Coleoptera (Beetles)
Family	Curculionidae (Snout and Bark Beetles)
Subfamily	Drtophtorinae
Genus	*Sitophilus* (Granary Weevils)
Species	*Sitophilus oryzae* (Rice Weevil)

Sitophilus oryzae

The **rice weevil** (*Sitophilus oryzae*) is a stored product pest that attacks several crops, especially the grains of cereals or cereal products including wheat, rice, maize, barley, occasionally peas, and raw processed cereals such as pasta.

Identification: The adult rice weevil is small (2.5 to 4 mm), dark brown with 4 distinct reddish to reddish yellow patches on the elytra. It is identical in external appearance to the maize weevil (*S. zeamais)* and only male genitalia provide a reliable diagnostic feature; therefore, dissection is required to distinguish between the species. Larvae are white, legless grubs that develop within the kernel. They thrive well in tropical or subtropical environments but can survive in temperate regions in protected situations.

Biology and life history: Adult rice weevils live about 4-5 months. Females lay 2-6 eggs per day and up to 300 over their lifetime. The female uses strong mandibles to chew a hole into a grain kernel after which she deposits a single egg within the hole, sealing it with secretions from her ovipositor. Females generally lay eggs within a kernel but they may lay multiple eggs per kernel and more than 1 larvae can develop within a single kernel. The larva develops within the grain, hollowing it out while feeding. It then pupates within the grain kernel and emerges 2–4 days after eclosion. Adults make a small, circular emergence hole, compared to large, oblong emergence hole made by the granary weevil, and can fly.

Male *S. orzyae* produce an aggregation pheromone ((4S,5R)-5-Hydroxy-4-methylheptan-3-one) to which males and females are drawn. A synthetic version is available which attracts rice weevils, maize weevils, and grain weevils. Females produce a pheromone that attracts only males.

Damages caused: Larvae develop in seeds or pieces of seeds or cereal products large enough to house larvae but will not develop in flour unless it has been compacted. Feeding contributes to heating and infested grain is often damp due to moisture added by the insects' respiration. Adults also feed on whole seeds or flour. They also create round holes in grains formed by exiting adults.

Control: Control of weevils involves locating and removing all potentially infected food sources. Rice weevils in all stages of development can be killed by freezing infected food below -17.7 °C (0°F) for three days or by heating it to 60 °C (140 °F) for 15 minutes.

MAIZE WEEVIL (*Sitophilus zeamais)*

Scientific classification	
Kingdom	Animalia (Animals)
Phylum	Arthropoda (Arthropods)
Subphylum	Hexapoda (Hexapods)
Class	Insecta (Insects)
Order	Coleoptera (Beetles)
Suborder	Polyphaga (Water, Rove, Leaf, and Snout Beetles)
Subfamily	Dryophthorinae
Genus	*Sitophilus* (Granary Weevils)
Species	*Sitophilus zeamais* (Maize weevil)

Sitophilus zeamais

The maize weevil (*Sitophilus zeamais)* is a common pest of grain crops in tropical regions worldwide. It is a cosmopolitan pest of stored products and one of the most important pests on stored maize in Africa. It can be found infesting a variety of grain and food materials. It attacks both standing crops and stored cereal products, however, it is most often found in corn, oats, barley, rye, and wheat. It cannot breed in finely processed grain but will readily breed in manufactured products such as macaroni, noodles, and milled cereals that have become caked from excessive moisture. It has even been known to attack fruits such as apples while in storage. Rice and Maize Weevils are widely distributed in tropical and sub-tropical areas and will be carried to temperate areas on imported commodities.

Identification: The maize weevil is a close relative of the rice weevil, and has a length of 2.5 mm to 4 mm. Its color varies from dull red-brown to nearly black, and it is usually marked on the back with four light reddish or yellowish spots. The maize weevil has fully developed wings beneath its wing covers (elytra) and can fly readily. It has a long, thin snout, and elbowed antennae. The thorax is densely pitted with somewhat irregularly shaped punctures, except for a smooth narrow strip extending down the middle of the dorsal (top) side. *S. zeamais* appears similar to the rice weevil (*S. oryzae*) but has more clearly marked spots on the wing covers and is slightly larger.

Biology and life cycle: The complete development time for the life cycle of this species averages 36 days. The female chews through the surface of the grain, creating a hole. She then deposits a small oval white egg and covers the hole with a waxy secretion that creates a plug as the ovipositor is removed. The plug quickly hardens and leaves a small raised area on the seed surface. This provides the only visible evidence that the kernel is infested.

Only one egg is laid inside each grain. The egg then hatches in a few days into a soft, white, legless, fleshy grub which feeds on the interior of the grain kernel. The larvae will pupate while inside and then chew a circular hole for exit, and finally emerge as an adult beetle. The development rate of the maize weevil is slightly slower than the rice weevil's development rate, yet its life cycle and habits are similar to those of the rice weevil. A single female may lay 300 to 400 eggs during her lifetime. Adults can live for 5 to 8 months. Breeding conditions require temperatures between 15 and 34 °C and 40% relative humidity. When the adults emerge, the females move to a high surface and release sex pheromones. Males are then attracted to this pheromone.

Damage Caused: These weevils are very destructive grain pests. They frequently cause almost the destruction of grain in elevators or bins where conditions are favorable and the grain is undisturbed for some length of time. Infested grain will usually be found heating at the surface, and it may be damp sometimes to such an extent that sprouting occurs. *S. zea mais* larvae feed on the interior of individual grains, often leaving only the hulls, flour-like grain dust, mixed with frass. Infested grains contain holes through which adults have emerged. A possible indication of infestation of the grain is that it floats up on the surface when placed in water. Ragged holes in individual grains, similar to damage caused by the rice weevil and granary weevil, may indicate infestation. In large stores of grain, an increase in temperature may be detected. Like many other stored grain pests, *Sitophilus* species tolerate or prefer low moisture levels in their food.

CHAPTER 2

PRINCIPLES AND PRACTICES OF PEST CONTROL

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PRINCIPLES AND PRACTICES OF PEST CONTROL

Crop protection is the science and practice of managing plant diseases, pests (both vertebrate and invertebrate), and weeds that damage crops and forestry. The crop plants may be damaged by insects, birds, rodents, bacteria, etc. The principles and practices of sustainable crop protection include the use of cultural practices, biological control, pesticides, and host resistance including plant breeding to control pests (insects, nematodes). The methods of control vary considerably from one pest to another depending on the kind of pest, the host, and the interaction of the two. However, a combination of two or more of these control methods can be employed in order to achieve more favorable results.

METHODS OF PEST CONTROL

Pests of crops can be prevented or controlled through the following methods:

Preventive method

Natural control

Physical control

Cultural control

Biological control

Chemical control

Preventive method: Insect pest situations can be prevented through crop husbandry practices that discourage their establishment or timely intervention that will render their presence in the field or store non-significant. Some of these practices include:

Regular monitoring of insect pests' presence and population increase to know when control measures are necessary to avoid economic loss.

Reduction of the rate of bush burning as this practice destroys predators, parasites, and parasitoids of insect pests.

Planting of certified insect-free seeds and other planting materials.

Avoidance of movement of soils from insect pest endemic zones/areas to other zones that are insect pest free.

Avoidance of alternating crops of common insect pest species in a crop rotation program.

Careful timing of planting seasons to avoid peak periods of insect pests that can lead to total crop loss.

Avoidance of indiscriminate planting of any crop at all seasons as the off-season fields will act as a sanctuary for the insect pest to multiply and infest the crop of the next season.

Quarantine and inspection laws which simply refer to the enactment of laws to prevent the introduction of foreign pests into a country through proper inspection of plant materials at points of entry.

Natural control method: This is the control method that does not depend on human manipulation of the environment or insect pests. The activities are natural and are responsible for the control of more than 70% of the insect pest population in our environment. These natural agents are:

Climatic factors such as rainfall, temperature, wind, thunder, storm, and humidity.

Physical barriers like mountains, valleys, rivers, oceans, and deserts.

Organisms such as predacious and parasitic animals, insects, birds, fishes, and reptiles.

Other environmental stresses like starvation, lack of mates, and destruction of habitats.

Micro-organisms such as fungi, bacteria, and viruses that attack insect pests.

Physical control: This involves the physical removal of pests by:

Handpicking of insects and larvae

Shooting rodents with gun

Mechanical barriers such as fencing around the farm with wire nets, ditches, traps, screens, and sticky bands to prevent pests from reaching their host crops.

Temperature treatments such as low temperature which is achieved through refrigeration or the use of high temperatures obtained via techniques such as super heating by fire or electric heating.

Radiation through the use of light traps such as back light which attracts insects into traps that destroy them.

Ionizing radiation which is a process employed to kill insect pests, especially stored insect pests, through ionization.

Cultural control: This method involves the use of farm practices to prevent or control pests, especially those in the field. This entails the manipulation and/or adjustment of crop production techniques aimed at eliminating the pests from the crops or from the area in which the plants are growing (eradication), increasing the resistance of the host to the pest, or creating conditions unfavorable to the pests to prevent and reduce the establishment of insect pests in the field/store. Examples of cultural control include:

Proper storage of planting materials in insect-proof environments.

Crop rotation keeps down the level of insect pests by starving them to death in a plot when a host crop is followed by a non-host crop in the rotation.

Farm sanitation involves regular weeding and removal of plant debris that could be a source of breeding insect pests.

Tillage and other cultivation practices which expose the eggs, larvae, and pupa of insects to desiccation thereby reducing the population by the next cropping season.

Proper timing of planting and harvesting of the target crops to check insect pest infestation and multiplication in the field.

Use of resistant varieties of such crops to prevent establishment of the insect pests.

Destruction of crop residues by either burning or burying in order to destroy those developmental stages of insect pests that could have completed their life cycle in such residues.

Rouging which is the removal of crops with undesirable characteristics, or which may pose as hosts of certain insect pests from the field.

Improvements of growing conditions of crops and creating conditions unfavorable to the pests.

Bush burning which kills insect pests in the vegetation and soil especially the larvae and pupa of non-soil insects and all stages of development of soil insects.

Biological control: This involves the introduction of the natural enemies of pests to control or keep the pest population under control. These natural enemies may either be antagonistic to the pests or parasitize the pest themselves. The biological agents used in this method include predators, parasites, parasitoids, and pathogens. Such enemies eat up or feed on these pests thereby reducing the population of the pests.

Biological control is also achieved by the encouragement of natural biological control agents through ecological manipulations or by the mass rearing and release of any of these agents. Some micro-organisms such as bacteria and fungi may also be used as biological control agents. This method has been successful with Bacillus thuringiensis on caterpillars, beetles, mosquitoes, and flies. The use of pathogens is reliable because the pathogens are specific in their attack on the insect pest species and often do not have any side effects on the environment.

Chemical control: Chemical control involves the use of chemicals to control pests of crop plants. All chemicals used in the control of pests are called Pesticides. These chemicals which are in the form of powder, liquid, granules, and tablets are used on pests through various methods like spraying or dusting seeds or plants to check pests. They do not act against all pests indiscriminately, rather a few pesticides are known for each of the major groups of pests as follows:

Insecticides: chemicals to control insects

Rodenticides: chemicals to control rodents

Avicides: chemicals to control birds

Nematicides: chemicals to control nematodes

PESTICIDE FORMULATION AND CALIBRATION OF EQUIPMENT

Pesticides are poisons that selectively kill pests when used at recommended doses. They are used to control, repel, attract, or kill pests, for example, insects and weeds that are considered a nuisance or threat to crops. Pesticides are most often, but not always, poisons; thus, farmers must be careful not to overuse chemical pesticides as they could cause harmful side-effects to non-target organisms, especially people, including the users (farmers), who can easily come in contact with the chemicals during use, and consumers when residues of pesticide are left behind on crops that are then eaten.

Some pesticides occur naturally while others are manufactured or synthesized. Some pesticides are unstable compounds, therefore they present storage problems. As most pesticides are insoluble in liquid media such as water and oil concentrates, the manufacturer prepares these chemicals in stable forms which can easily be diluted in water or mineral oils to meet the strength of solution required for field use. This state of the pesticide is termed formulation. Pesticides usually contain a stabilizer to prevent chemical degradation.

The real pesticide formulation content is known as an active ingredient or active material. Most formulations contain only a single pesticide e.g. attack 2.5 EC, but some formulations contain double or two different pesticides e.g. crack down-lambdacy habithium, and dimethoate. The non-active part or non-killing part of a formulation is referred to as inert materials or inactive ingredients.

FORMULATION

Pesticide formulation is the process of transforming a pesticidal chemical into a product that can be applied through a practical method to permit its effective, safe, and economic use. The formulation may be either liquid, solid, or gas.

LIQUID FORMULATION

These are those formulations that can be applied as sprays. Some pesticides are soluble in water and are applied as solutions e.g. sodium, potassium, and amine salts while others are ordinarily insoluble in water.

Liquid formulations include:

Emulsifiable Concentrates (E.C): This type of liquid concentrate has an important component known as an emulsifier which is a surface active agent that is partly hydrophilic and lipophilic. They are the most convenient and therefore most popular forms in which most pesticides are used. The stability of emulsions is affected by the hardness and pH of the water used when mixing for spraying and also the conditions under which the concentrate is stored. High temperatures can adversely affect the formulation. Examples of E.C. includes Action 40, Chlorpyriphos, and Demiforce.

Oil Concentrates (O.C): These are liquid formulations containing high concentrations of active ingredients. They are generally used after dilution to a practical or convenient low concentration with an inexpensive hydrocarbon solvent e.g. Xylene, Fuel, Oil, or Diesel.

SOLID FORMULATION

Wettable Powder (W.P): These formulations sometimes called dispensable or sprayable powders are formulations of pesticides fully divided into particles with surface active agents that enable the obtained powders to be mixed with water to form a stable homogenous suspension. Wettable powder frequently contains 50% active ingredient but some contain a higher concentration. Wettable powder formulations are not compatible with most other types of formulations.

Aqueous Concentrates (water-soluble concentrates): These are concentrates of pesticidal chemicals dissolved in water. The most common type is the salt of herbicidal acids e.g. 2, 4, D-Amine. Because herbicidal acid is the normal active ingredient, concentrate is generally expressed in terms of kilogram of acid equivalent per liter. Other examples are Azodrin 60%wsc, Bladex wsc, etc.

Invert Emulsion (I.E): An invert emulsion is very viscous in water emulsion that has changed to water (H2O) in oil emulsion. The phase of mixing the water in the oil occurs at the nozzle of the sprayer. This formulation is very difficult to apply and is currently applied aerially with specially designed aircraft.

Ultra Low Volume Formulation (ULV): In some areas of the world where valuable crops are produced, water (H2O) for spraying is a problem for farmers. To solve this problem of lack of water, some pesticides are applied virtually as a technical material without dilution. This is applied as small spray droplets to achieve effective coverage. This type of formulation requires solvents that are sufficiently non-volatile for a spray deposit to remain liquid for days or weeks rather than minutes or seconds.

GAS FORMULATIONS

Fog Formulations: Pesticides such as pirimiphos methyl (actellic 25EC, actellic 50EC) which has a fumigant effect are ideally applied as an aerosol spray of fog provided that the appropriate concentration is retained for a sufficient time.

Smoke: In this type of formulation the pesticide is mixed with an oxidant and a combustible material that generates a large amount of hot gas. A special form of gas is the mosquito coil. The coils are made from an extruded ribbon of wood dust, starch, and various other additives and coloring matter, often green, together with natural pyrethrin or allethrin.

All solid formulations except wettable powder are applied on the field in dry form as dust, granules, or pellets. The dust bases or concentrates and wettable powder are intended for further dilution to field strength before final application as a spray, using low-cost diluents such as clay and talc for dust bases and water for wettable powders. The major formulation types are dust bases (concentrates, wettable powder, dust granules, and pellets).

Dust Bases or Dust Concentrates: These are free-flowing powders containing high concentrations of active ingredients. Pesticide fertilizer mixtures are often made by mixing the dust concentrates with dry fertilizer, a sticker being added in the case of granular fertilizer to prevent segregation of the fine particles of the pesticide base. Dust is the very finely-ground powder of dry pesticide, usually formulated to field strength ranging from 1-10% active ingredient, according to the potency of the pesticide and the rate of application. Dust must be free-flowing so that it can be accurately monitored in application equipment. The particle size of dust may vary, but it is usually below 74mm. The advantage of dust is the inherent billowing around the foliage and covering the underside of the leaves and plants e.g. Vetox 5.

Granules: The granular pesticides are distinguished according to mesh size which for granules lies between 4 to 80 mesh. The concentration of active ingredients in granular pesticides varies from as low as 1% to as high as 42% depending upon the properties of the active ingredients, characteristics of the carrier, the potency of the pesticide, and the desired rate of application of the finished product e.g. Furadan 3G, 5G, 10G.

Pellets: Pesticide pellets are dry pesticide formulations in which the particle size is larger than granules i.e. greater than 4 mesh. There are no fixed maximum sizes, but diameters may be as large as 0.6cm in practice and the possibility of 1.3cm exists.

Poison Baits: These are special formulations designed preferentially to lure and poison certain types of insects and rodents near or in their natural environment. Sometimes, they are used to intercept the immigration of insects such as locusts into grain fields. Other usages include applying rodenticide baits around tree trunks in orchards to prevent rat attacks and adding them to pellets or food for municipal and residential rat control and the control of rats and mice in farm buildings and grain storage areas e.g. Klerat, stoprat, and power kill.

INSECTICIDES

These are chemicals used in controlling insect pests. Insecticides are generally poisonous to man and animals and therefore must be handled with precaution and only by trained personnel. Chemical insecticides must be applied with the appropriate equipment and as recommended by the manufacturer and should be used only at population levels at which serious damage could be done (Economic threshold level), and the use of specific insecticides will spare the lives of beneficial insects (natural enemies of the insect pests) which are constantly working to regulate the population of the pests in both the natural and agricultural ecosystems. Chemical insecticides can only be used as a last resort only after all other control measures have failed because most of these chemicals have side effects that may make them undesirable.

The need for chemical control

Despite all the drawbacks and side effects that arise from the use of chemical insecticides to control pests, there are still many reasons that can persuade farmers to still choose to use them:

The biggest advantage of insecticides is they are readily available and much easier to use compared to alternative methods such as biological control and other similar methods which can take a long while to plan and often have no immediate effect on pests.

When insect pests must be controlled over large areas of land, insecticides prove to be very cost-effective and practical considering less human labor is needed to maintain the pesticide process. The general effectiveness of the program and its economic benefits are increased greatly still when insecticides are used in a way that reduces the likelihood of the pests becoming resistant to the chemicals used to fight them. If all the correct precautions are used, including using no more than the recommended level, then chemical control of pests can be performed effectively.

The rapid increase in human population and the need to produce large quantities of food all year round call for the use of fast and effective insect pest control methods. Chemical insecticides are generally fast-acting, and this limits the damage done to crops.

Side effects of chemical insecticide

The development of resistance by the insect pest species eventually renders the insecticides ineffective and that leads to the resurgence of these pests.

The residual effects of these insecticides on the soil render the soil unfit for crop growth.

The pollution of water through run-offs kills fish and other aquatic organisms.

The concentration of insecticides through food chains.

The storage of insecticides in fat tissues.

The promotion of secondary pests to primary pest positions is a result of the unconscious elimination of their natural enemies through the regular application of chemical insecticides.

The unconscious distribution of poisonous food products by harvesting treated crops earlier than normal.

The general creation of more pest situations by unbalancing the equilibrium of the ecosystem through the elimination of the food source for non-pests by using insecticides, resulting in the survival of pests.

CLASSIFICATION OF CHEMICAL INSECTICIDES

Insecticides are broadly classified into 6 categories:

Organo-chlorines: These are persistent insecticides, and they are no more in common use because of their persistence in plants, animals, and the environment, e.g. DDT and Lindane (gamma-BHC).

Organo-phosphates: These are not persistent but have high mammalian toxicity. They are currently in high demand because of their high reliability e.g. Malathion, Parathion, etc.

Carbamates: These insecticides are very effective but have a very short half-life which demands very frequent application to achieve an expected result e.g. Carbaryl furadan (3G, 5G, 10G).

Pyrethrins: These are manufactured from plants and they are very useful in insect control in food industries. The pyrethrins are very effective but their effects are short-lived.

Pyrethroids: These are chemically formulated pyrethrins used just as pyrethrins with similar qualities.

Pheromones: These are sex hormones that attract insect pests into traps or poison that destroy them.

INTEGRATED PEST MANAGEMENT

The ineffectiveness of individual insect pest control methods has led to the articulation of an insect pest control program commonly referred to as the Integrated Pest Management program. This involves the combined use of all compatible control methods including chemical control at the appropriate time with a proper understanding of the biology of the insect pest, with the result of no adverse effect on the ecosystem.

The success of this method depends on a well-planned control strategy. It is important that a good knowledge of the pest, ecosystem, and population trends is obtained before insecticides are applied and such insecticides must be highly specific for the target insect. The integrated method is the best control method, but its level of sophistication demands hard work and patience on the part of the agriculturist. Notwithstanding this, farmers and other agricultural practitioners are advised to imbibe this concept which aims at maintaining a balanced environment for both plants and animals.

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