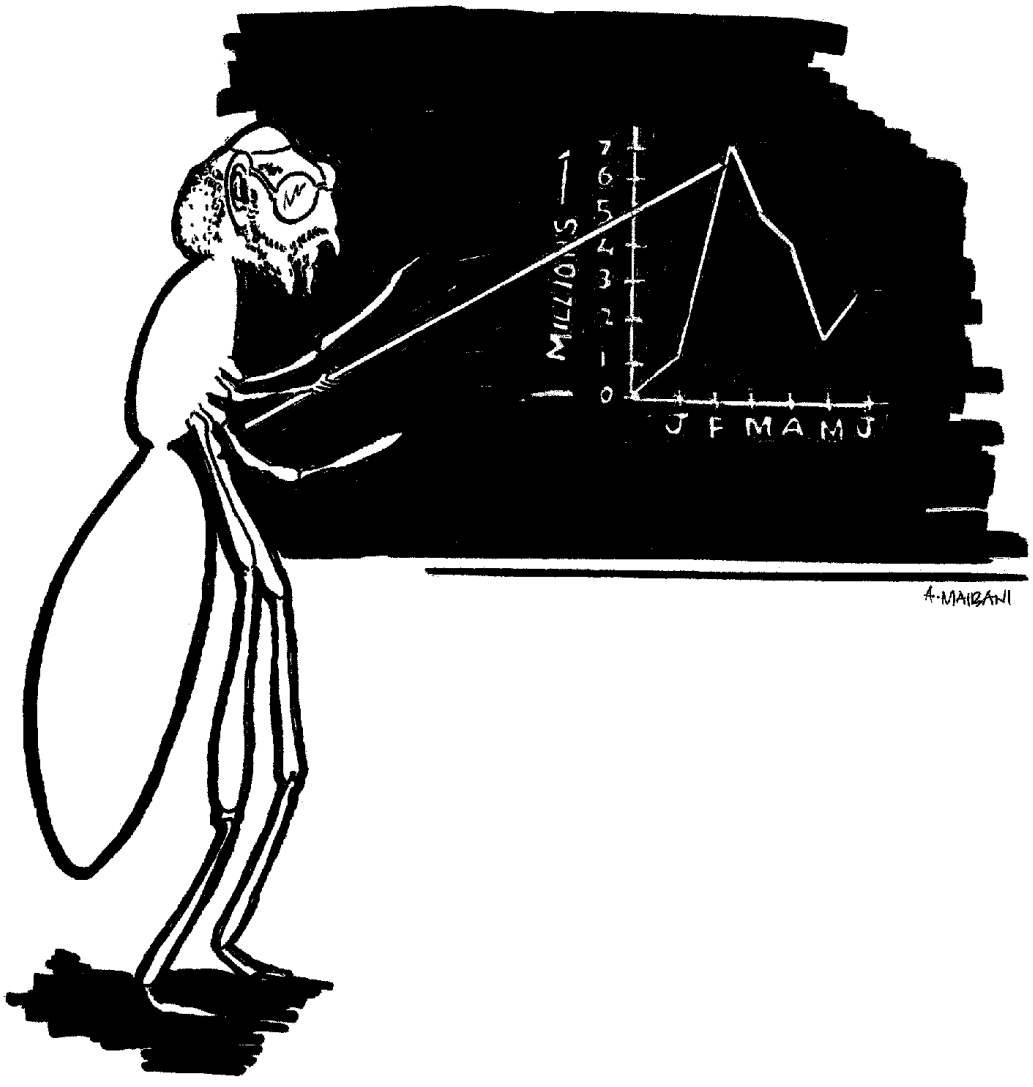
Chapter 7

Assessment of Insect Populations



This chapter introduces some commonly used sampling and monitoring strategies and outlines basic methods for the collection and preservation of insects.

Biologists, foresters and agriculturalists are interested in the assessment of insect populations for several reasons:

A **biological survey** is carried out to collect specimens for taxonomic studies, to study the population biology of an insect species and to find out what kind of insects occur in a particular area. Biological surveys become more and more important since, based on the results of the survey, management strategies are developed and applied for the conservation of biological diversity in a certain area. Moths seem to be a convenient, quick and reliable tool for the assessment of biological diversity. Furthermore, the findings of a survey can be used for the preparation of environmental plans and environmental impact assessments in general. The insect fauna is studied prior to the implementation of a proposed project, for instance a mining or logging project. Regular monitoring during the course of the project might indicate toxic contaminants or other impacts on the environment, if particular insects are no longer present in the area. Aquatic insects are usually good indicators of the quality of aquatic habitats.

A **detection survey** is usually carried out by applied scientists, foresters and agriculturalists in order to find the insects causing damage to particular crops. Moreover, it is important to assess insect pest populations on a regular basis so that outbreaks can be predicted at an early stage and suitable curative measures be taken as a remedy. Further surveys are carried out after the application of an insecticide for instance in order to estimate its success and effectiveness. The latter case is referred to as **pest control evaluation**.

7.1 Monitoring Strategies

A monitoring strategy depends on the objective of the proposed assessment. Basically there are the following two different sampling methods:

- **Parameter-estimating sampling** or **census** or **total counts** gives an **accurate** estimate of a population and provide information about the population size, the absolute density, the composition, etc. Accurate in this context means true, meeting the actual number of individuals, even though the result is characterised by a large standard deviation. For instance the result of a population estimation of a known 500 animals might be 492 ± 221.
- **Decision-making sampling** or **monitoring** or **sample count** allows **precise** estimates and is suitable to detect variations in the number of individuals during the course of time. In this case precise means repeatable, with a low standard deviation. The actual number of individuals is of no relevance as long the estimated mean is well below a certain threshold value. The result of an estimation of a known 500 individuals could be 316 ± 13.

Agriculturalists and foresters are usually interested in whether there is an increase or decrease in the population size of a particular pest, so that the situation can be classified rapidly for decision making, eg. to apply insecticide or not. For that purpose instant and precise results, as they are provided by decision-making sampling, are desired. A method having a large error however, is also tolerable, as long as the mean is far away from a given threshold level. Eg. it is acceptable if the threshold level is 500 individuals per unit, the mean is 100 and the error is ± 200.

Ideally the result of any monitoring method should be **accurate** and **precise**, however usually the experimental design neglects one feature. Therefore, the kind of result for the purpose of the planned assessment must be considered. The choice of the method will also depend on how much money can be spent on the intended assessment. The costs of a survey depend on the size of the area, the survey design, the number of visits and the time spent at each visit, the distance to the survey area, the amount of data, the time for data analysis, the required manpower and equipment, etc.

7.1.1 Sampling Techniques

Total counts of insect populations are in most cases labour intensive and time consuming and as a result too expensive and thus not feasible and appropriate. Therefore, the area has to be divided into sample plots. The assessment of a certain number of plots allows a more or less precise estimate of the insect population, provided that the sample plots are representative of the range of insect abundance in an area. The number of sample plots has a direct influence on the precision of the estimate, and of course, on the costs of the monitoring exercise. The sample plot design can be a systematic, random or stratified random set-up, eg. along a line or belt transect. **Stratification** in this context means that the samples are taken from different **strata** of the host plant, eg. from the stem, from the leaves and from the roots. Furthermore, the plots should not established too close to the boarder of the area, because the **ecotone**, the area where two different habitats join, usually shows higher diversity due to the edge effect. If plots are established there, they will be no longer representative for the area and the results will be biased. In some cases the establishment of control plots is required. The spatial distribution (**chapter 4.7.3**) is also of great importance in this context. The frequency of the assessments depends on the life cycle of a particular pest. If the insect has a short life cycle of a few days, the survey has to be carried out more frequently than for a species that requires a month for its development from the egg to the adult.

7.1.2 Absolute Methods

Absolute methods yield estimates in density per unit such as locusts per land area, eggs per leaf or pupae per tree. Every insect per unit has to be counted. Most reliable data can be gathered from insect counts in square plots or standardised rings of 0.1 m² area that are placed along a transect line. The results of absolute methods can be directly compared with the results of previous assessments or assessments at different locations, however the price of this advantage is literally very high not only in terms of the required time and labour. Suitable absolute methods are leaf-counts, manual collection, the use of suction devices, rotary nets, emergence traps and Berlese extractors. An interesting absolute method for the assessment of the population density is the **capture-recapture-method** (Peterson-Lincoln-index or proportionality method). Insects are captured randomly and marked, for instance with coloured nail polish. Of course, marking should not handicap the insect. After marking the animals are released. Care must be taken to ensure, that the animals intermingle with the

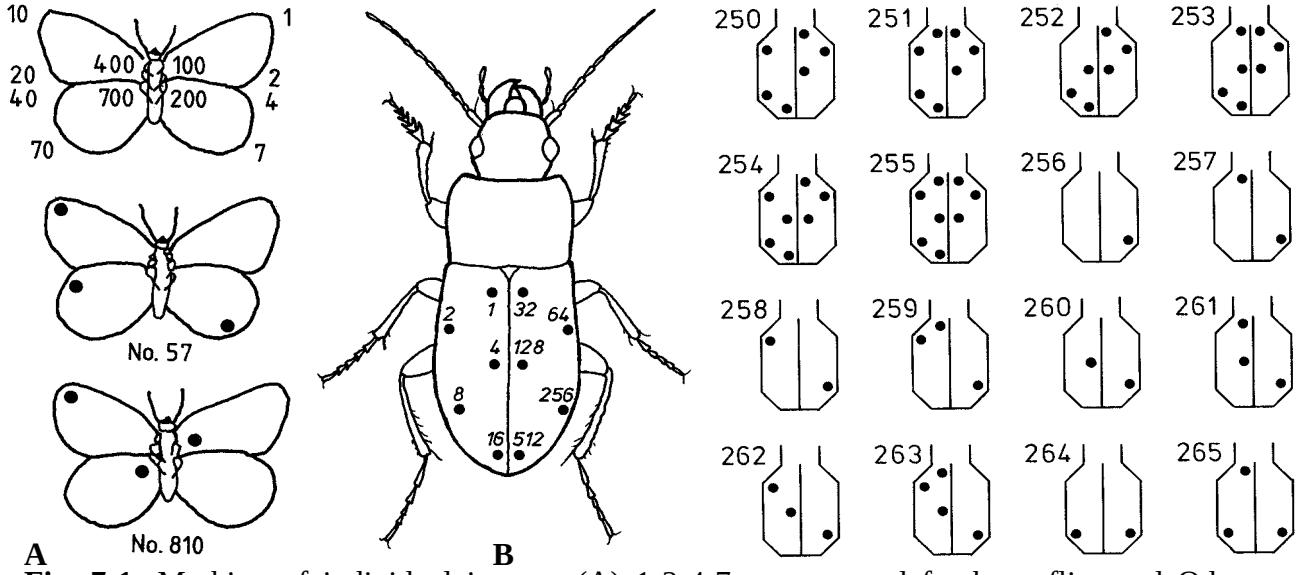


Fig. 7-1: Marking of individual insects: (A) 1-2-4-7 system used for butterflies and Odonata; (B) binary system for beetles; see text for further explanations (reproduced from Mühlenberg, M., 1993)

rest of the population before recapturing is carried out. An estimate of the population density can be calculated from the ratio between the number of marked and released insects, the recaptured insects and the marked recaptured insects, according to:

$$\text{Population Density} = \frac{n_1 \times n_2}{n_3}, \text{ where}$$

$\mathbf{n_1}$ = number of individuals marked and released
$\mathbf{n_2}$ = total number of individuals recaptured
$\mathbf{n_3}$ = number of marked individuals recaptured

For capturing suitable traps, nets, etc. can be used. At least 20 to 50 % of the individuals of the entire population have to be marked in order to gain reliable results. A disadvantage of this method is that migration of marked animals might influence the result. The method is subject to a relatively high error, unless the procedure is carried out repeatedly. Scientists of the Division of Entomology at CSIRO for instance tried to assess the number of individuals of termite colonies by the use of the capture-recapture-method. Wood stained with a microscopic dye was offered to the termites and the number of termites feeding upon this bait was recorded. These animals eventually became coloured since the dye in the wood stained particular tissues of the termites. After some time, termites were recaptured to work out their abundance. However the method failed, apparently due to the fact that the food-gathering animals feed other individuals of the colony, which then also become coloured, thus increasing the number of marked termites.
Individuals of a population can be marked individually, which is required for particular studies. **Fig. 7-1** shows several schemes that allow the marking of large numbers of insects. The 1-2-4-7 system suitable for butterflies, dragon- and damselflies can discriminate 1554 individuals using a simple marking scheme. The figures represented by 12 possible marking positions simply have to be added, for instance 10 + 40 + 7 = 57. Another method used for beetles allows the marking of 2^{10} - 1 = 1023 individuals. The number of an individual is calculated by addition of the binary codes represented by the ten marking positions.

7.1 3 Relative Methods

Relative methods do not relate to any defined unit and count a more or less consistent if unknown proportion of the population. Since the area, the volume or generally the unit in which the count was made is unknown, it is difficult to compare the results with those of previous counts or counts in other areas. Relative methods are less labour and time intensive, amass large amounts of data and are therefore commonly used by entomologists. Most catching and trapping methods are relative methods such as visual searches, fixed time collection, sweep-net catch, shaking and beating, vacuum traps, Malaise traps, window pane traps, sticky traps, pitfall traps and traps using attractants like pheromones.

7.1.4 Direct and Indirect Assessment

Direct assessment aims at the assessment of a pest population causing particular damage. This is, however, in many cases not possible because the insects might be hidden in the plant, like termites, or the pest may be living in the soil and emerges only during night. Therefore, the assessment is often indirect and actually does not count the number of insects, but their representatives such as frass, the number of cocoons, exuviae, egg shells, etc. A simple but interesting assessment method has been developed to estimate the presence of termites in soil: untreated wooden pickets are driven into the soil of the assessment area and left for several weeks. The degree of decomposition of the up-rooted pickets can then be graded as shown in **fig. 7-2**.

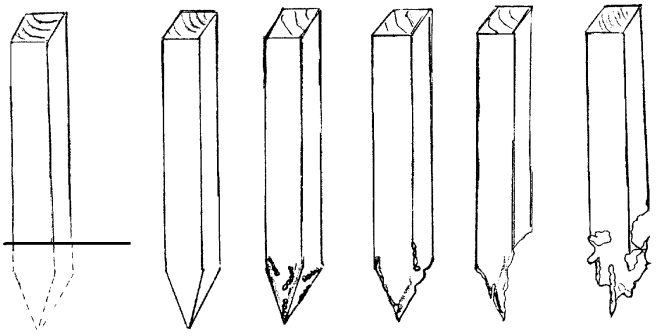


Fig. 7-2: Indirect assessment of the presence of termites in soil; see text for further explanations (modified after Denfop, B., 1988)

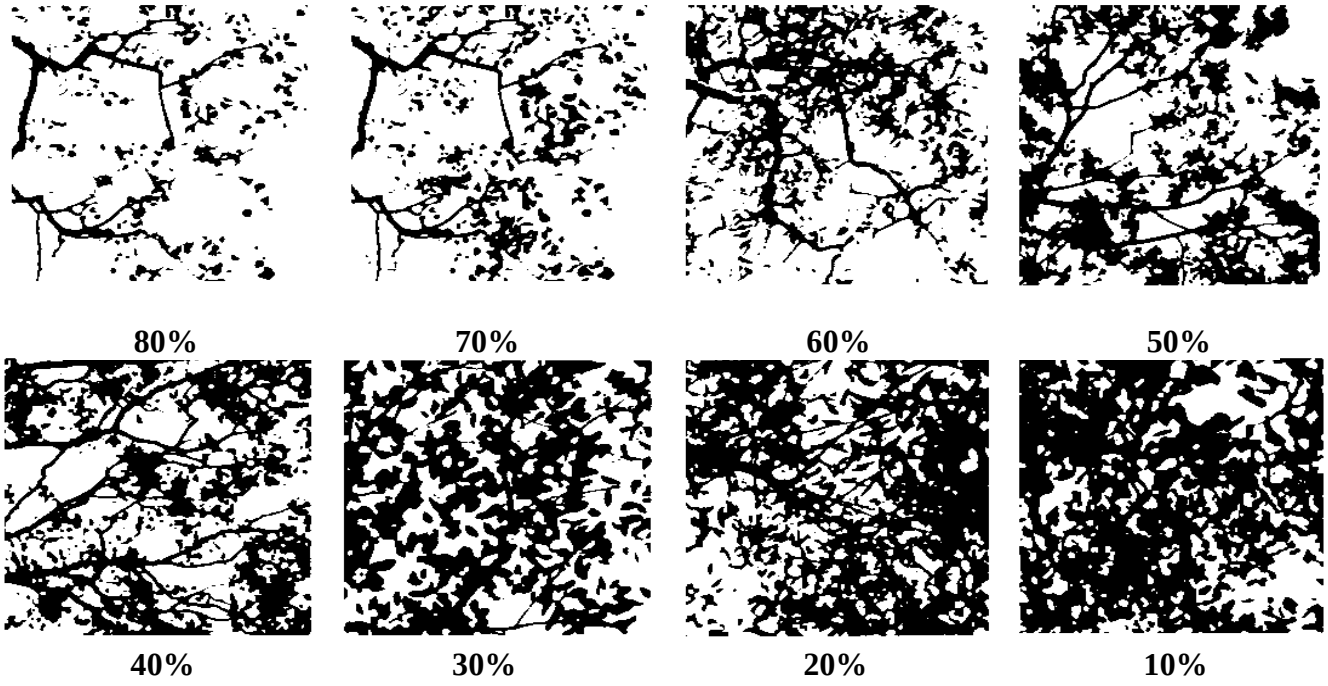


Fig. 7-3: Damage assessment as percent defoliation of the host tree (source unknown)

7.1.5 Damage Assessment

An agriculturalist's or forester's major concern is the actual loss of a crop rather than the number of insects causing the damage. The damage to a crop is another indicator of the number of pest insects present on the particular crop. Therefore, the damage or **yield loss assessment** is an indirect method for the estimation of the insect pest abundance. Pest management strategies are often based on the results of damage assessments, for instance if the damage exceeds a certain threshold value, the application of an insecticide would be justified. The calculation of the economic loss caused by a pest is further outlined in **chapter 8.9**. Damage assessment, however, is not only of interest for decision making in pest management but also at the level of governmental policy decision making. The production of food or cash crops for domestic and export markets is of great importance for the economy of a country. Therefore, research, pest control and extension officers on a higher level depend very much on the results of damage assessments in order to forecast insect outbreaks and to apply control measures at the right time.

The choice of the damage assessment method generally depends on the crop and the damage that is caused by a pest. In agriculture, a **plant growth analysis** can be carried out when the relationships between insect infestation, damage and yield loss are not obvious and simple. The comparison for instance, of plant dry weights and the leaf dimensions of infested and healthy plants during different times of the plant's life cycle can provide a good estimation of the photosynthetic production of a plant as an indicator of the degree of infestation. In forestry, plant growth analysis is less common. Other possible methods are:

- the degree of defoliation of a host tree is a useful measure of the damage and is commonly indicated in percent according to **fig. 7-3**
- the use of satellite images (**plate 11 K**) and aerial photos allows an effective damage assessment of large scale plantations. Changes in the light absorption of diseased host plants, usually in the lower energetic end of the visible light or in the infrared (IR) can be visualised by the help of false colour films
- the damage caused by termites can be assessed by tapping the base of an infested tree. A hollow sound is audible where the trunk has internal termite galleries. This method is usually more suitable for hardwoods than for softwoods

- the damage caused by wood boring beetles can be assessed by counting the number of boreholes per area, eg. 10 cm x 10 cm, or by measuring the height from the base of the tree up to where bore holes occur
- radiography is a sophisticated, therefore less appropriate, but interesting method of visualising the damage caused by wood and bark boring insects inside the trunk of a tree. An X-ray photo of diseased bark is shown in **fig. 7-4**.

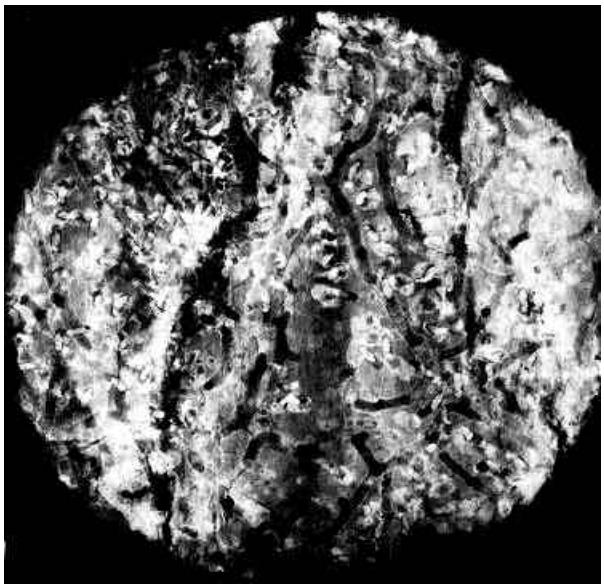


Fig. 7-4: Radiograph of bark infested by bark beetles; late larval instars are visible in the galleries (reprod. from Coulson, R.N. & Witter, J.A., 1984)

7.2 Collection and Trapping Methods

There is a large variety of methods available for catching and trapping insects, each being suitable for a particular assessment method and group of insects. Traps usually immobilise insects and are either active (with bait) or passive (unbaited). Baited traps attract insects actively to a more or less specific bait. Suitable baits are food, water, carrion and faeces to attract flies, or the very smelly Limburger cheese or CO_2 suitable for mosquitoes. Other attractants are light for nocturnal insects, particular colours for a large range of diurnal insects, pheromones, like sex pheromones to attract males, etc. Unbaited traps do not have any attractant effect on the insect and are therefore unbiased. The time and the location are important for successfully collecting and trapping insects. Even though this seems to be trivial, some basic information on the biology of the insect is required to decide where and when to trap an insect most successfully. Traps have to be placed at a location where the insect can be encountered sooner or later. The time of the day or night, the phase of the moon, the weather and the season have to be taken into consideration as well.

Some common trapping devices and collection methods are:

- **manual collection** is definitely the method of choice for many purposes. Beetles, most moths, all sorts of larvae can be easily collected by the use of hands, without any further devices. Sometimes the use of forceps is recommended, eg. for the collection of caterpillars with stinging hairs. Beetles and moths are sometimes easier to catch if a container like an empty jar is placed over or below the insect. In the latter case the collector can make use of a beetle's or moth's behaviour: these insects usually drop down when disturbed and with a bit of experience right into the sample glass or collecting jar
- during **visual searches** all individuals of the species to be assessed are counted or collected from the leaves of the host plant, from the stem, from the litter below the plant, from under rocks and fallen logs, etc.

Important: to avoid habitat destruction, rocks and bark, etc. have to be put back after the assessment

- **fixed time collection** is a relative method that uses visual searches carried out for a certain time. For example, the pests on a particular host plant are counted for ten minutes
- **nets** are the ultimate devices for catching flying insects like butterflies, that are difficult to catch by any other means. Once the insect is trapped the net is flipped over its rim so that the insect cannot escape
- nets can also be used as '**sweeping nets**' shown in **fig. 7-6 A**, a relative method for the assessment of ground-dwelling insects and insects associated with low vegetation. The

method is suitable to monitor changes in the abundance of insect populations if a certain number of 'sweeps' at a particular angle, for instance 90° are done along a transect line and if the assessment is carried out regularly for a longer period of time. A standardised net size, a constant number of sweeps and the same sweeping technique have to be used in order to produce reliable data

• **light traps** are used to catch nocturnal insects like moths, beetles and many more. Those insects are actually attracted to light because they become completely confused by an artificial source of light. The only light that nocturnal insects usually experience is the moon which does not markedly change its position relative to the insect. This is different with an artificial light source that changes its position in relation to an approaching insect. The insect tries to correct the change of position and as a result it approaches the source of light spiral-like, as shown in **fig. 7-5**. The insects attracted to the reflecting screen can be easily caught. A simple way to set up a light trap is to use a white bed sheet and a kerosene pressure lamp, eg. a Coleman lamp (**fig. 7-6 D**). Black light fluorescent tubes (**fig. 7-6 C**) or Mercury vapour lamps (160 or 250 Watt, **photo on page V**) are more effective, since these lamps emit light that moths readily respond to. Most moths are sensitive to wavelengths of 383, 460, 500 and 620 nm, which is ultraviolet, blue, green and orange light, respectively. Care should be taken when using a Mercury vapour lamp since it emits ultraviolet light which is harmful to the eyes. Avoid looking directly at the lamp and consider the use of sunglasses. Light trapping does not work during full moon due to the reduced activity of the moths. The time of the night also has an effect on the result of the method. For instance, particular moths such as **Sphingidae** are active in the very early morning hours and are rarely encountered before midnight

• **Malaise traps** (**fig. 7-6 G**) and **quick traps** are tent-like or soccer goal-like devices that are commonly used for the relative assessment of agricultural pests. The traps are open on one side allowing access for the insect. Many

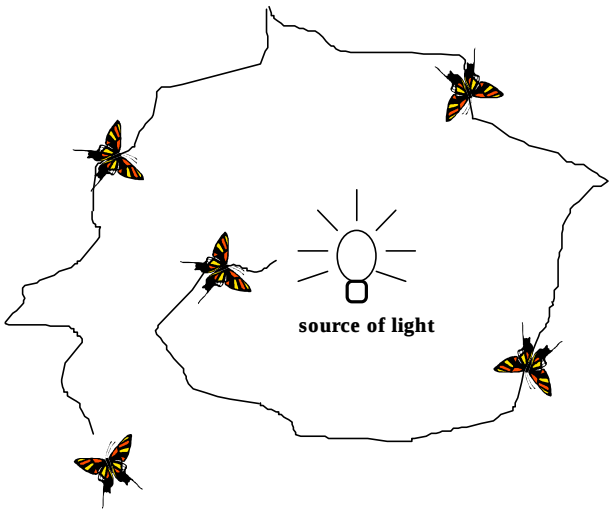


Fig. 7-5: Attraction of a nocturnal insect to an artificial source of light (graphic Schneider, M.F.)

insects crawl upwards when encountering an obstacle. This behaviour is used in Malaise and quick traps: a trapped insect crawls up into the dome of the trap where it falls into a collection vial that contains a suitable preservative. These traps can also be equipped with attractants or baits. Fast moving insects such as Diptera and Hymenoptera get mostly caught in this kind of trap

• **vacuum** or **suction traps** and **aspirators** are suitable for the relative assessment of ground dwellers and of insects on low vegetation. Suction devices suck insects into a net from which the trapped animals can no longer escape. The current of air is either produced by a hand-held vacuum cleaner-like device (**fig. 7-6 E**) or simply by the lungs of the collector (**fig. 7-6 P**)

• **Berlese** (**Tullgren or Beauman**) **funnels** shown in **figs. 7-6 F** and **K** are used for the absolute assessment of minute soil dwelling insects and other arthropods. A sample is punched out of the soil using a corer shown in **fig. 7-6 B**. The soil sample is immediately transferred into the extraction apparatus and exposed to the light and heat produced by a light bulb. As a result, the soil sample slowly dries from the top downward, forcing the animals deeper into the soil until they eventually fall through the mash base into a container with a preservative such as picric acid. The drying process has to be carried out very slowly, the extraction procedure takes up to two weeks


A

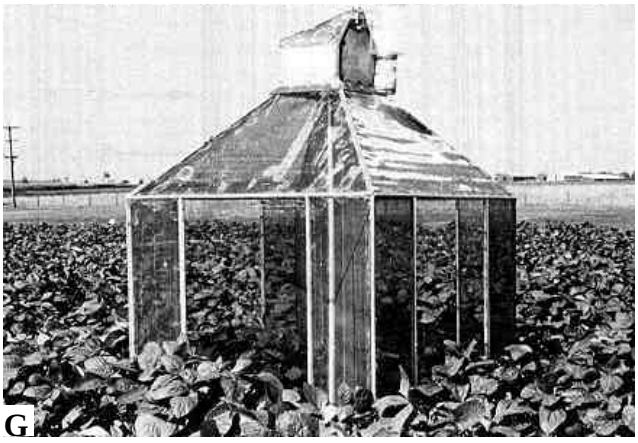

B


C


D


E


F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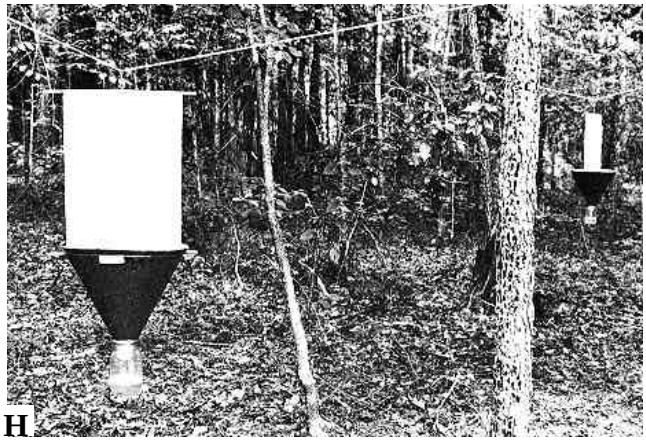
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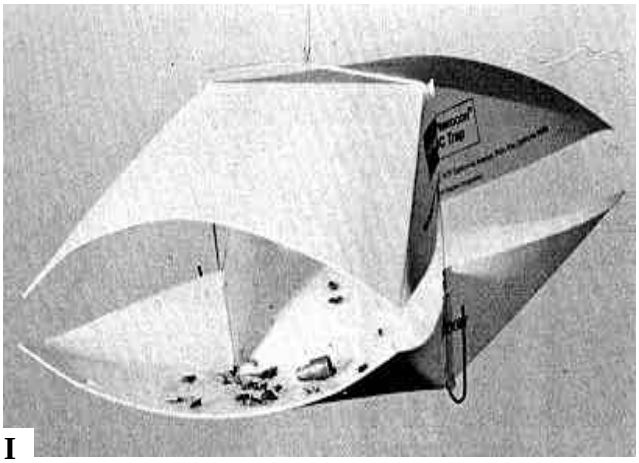
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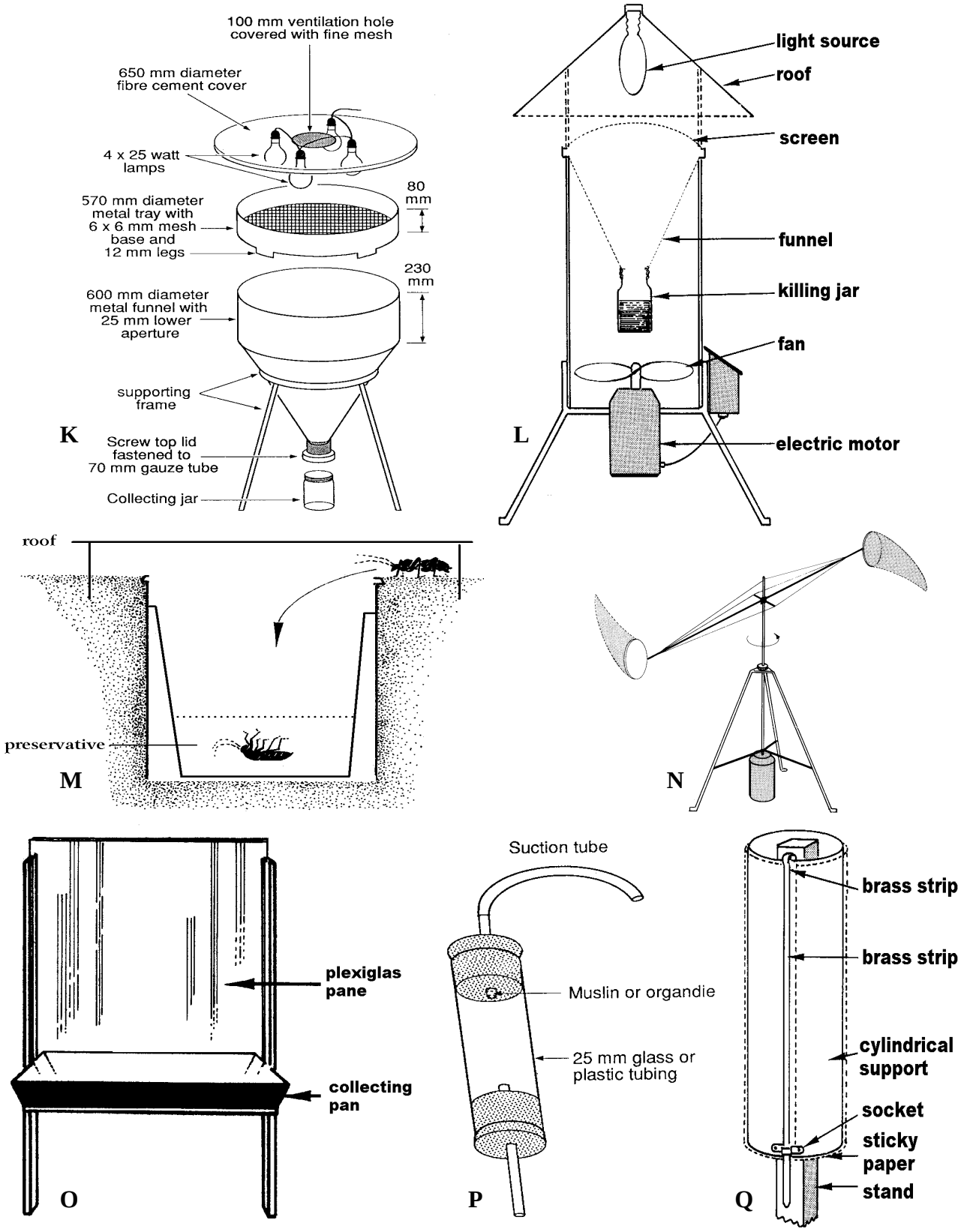

Fig. 7-6: Trapping and Catching Methods: (A[‡‡]) sweeping net method carried out by the author for the assessment of immature plague locusts; (B[‡]) soil corer; (C) stationary fluorescent light trap; (D[§§]) light trap using a kerosene pressure lamp; (E[‡]) motor driven suction trap; (F) Berlese funnel; (G) Malaise trap; (H[‡], I) pheromone traps; (J[§§]) sticky trap; (K[§]) Berlese funnel; (L) combined light and suction trap; (M) pitfall trap; (N) rotary net; (O) windowpane trap; (P[§]) aspirator; (Q) cylindrical sticky trap (reproduced from Metcalf, R.L. & Luckmann, W.H., 1975; Upton, M.S., 1991[§]; Coulson, R.N. & Witter, J.A., 1984[‡]; photos Holtmann, M.[‡‡]; Schneider, M.F. [§§])

- **Barber traps** or **pitfall traps** are used as a relative method for the assessment of ground dwelling insects, mostly beetles or other arthropods, that walk on the ground and accidentally fall into the pit (**fig. 7-6 M**). Pitfall traps can also be lured with attractants or baits. Corers are convenient for punching the pit. In tropical countries, a roof and a drain should be provided in order to prevent the specimens being washed away by rain. Usually 3% aqueous **picric acid** is contained in the trap as preservative, in which the trapped insect drowns. Picric acid has neither an attractant nor a repellent effect on insects. Caution should be taken when using picric acid as a preservative. Solid picric acid is highly explosive. The aqueous solution was used as a dye at the beginning of this century, and irreversibly stains clothes, skin, etc.

- **windowpane traps** (**fig. 7-6 O**) consist of a transparent plexiglas screen mounted vertically above a trough containing a suitable preservative. When an insect hits the invisible screen it drops down into the pan containing preservative and drowns. This method is also suitable for determining the flight direction of insects

- **combined light and suction traps** attract nocturnal insects to the source of light. When an insect is close enough, it is sucked into the trap by a current of air. The trap is equipped with a funnel with smooth and steep walls (**fig. 7-6 L**), from which a trapped insect slips into a killing jar. A disadvantage of this kind of trap is that larger specimens like moths are easily damaged in the trap

- **emergence traps** are used for the absolute assessment of insect larvae and pupae hidden in soil or litter. The cage-like device is placed over the respective site and left there to trap the adults emerging from the soil

- **shaking and beating** is widely used by agriculturalists as a relative assessment method. The method is suitable for catching insects associated with lower vegetation like smaller trees and shrubs. A beating tray, canvas or piece of cloth is held or placed below the plant to be assessed. Then the stem of the plant is beaten or vigorously shaken so that insects fall on to the beating tray from where they can be collected. Fast moving insects however might easily escape from the tray or canvas, therefore an aspirator can be very helpful

- **rotary nets** are devices used for the absolute assessment of insect populations. The devices consist of two or more revolving nets (**fig. 7-6 N**) in which flying insects get caught. The method yields good results independent of the wind speed

- **canopy fogging** is commonly used for biological surveys of the rain forest canopy. A cartridge containing a suitable insecticide is shot into the crown of a tree. The killed insects fall down on to a huge canvas sheet that is placed beneath the tree

- **sticky traps** and **greasy traps** are used for the relative assessment of diurnal insects. The square or cylindrical trap (**figs. 7-6 J** and **Q**) is set up either vertically or horizontally, suspended from a tree or mounted on a pole. The device is coated with a very sticky adhesive or grease suitable for immobilising insects. Insects are attracted to the trap either by its bright colour like yellow or white or by an attractant like a sex pheromone. The advantage of a greasy trap is that the immobilised insect can be removed from the trap in one piece. The grease stuck to the specimen can be washed off with chloroform

- **pheromone traps** (**figs. 7-6 H** and **I**) are used for the relative assessment of insect populations. Such devices are common, for instance in forestry for trapping bark beetles. The attracted insects either fall into a container with preservative or are immobilised on adhesive, if the trap is used in combination with a sticky trap. Pheromone traps containing sex attractants usually attract only one particular sex, eg. the males. The traps are very simple, cheap and effective, but have the disadvantage that they can be used only for one particular insect species because pheromones are species-specific.

A modified device releases high concentrations of a sex pheromone that superimposes the lower concentration of the pheromone released by females of a certain insect. Thus the males become confused and are no longer able to find

their female counterparts. Devices of this kind are in fact chemical control methods and are not really suitable for the assessment of insect populations.

7.3 Collection and Preservation of Insect Specimens for Identification

Insects are collected by naturalists and scientists for research purposes, or by local collectors for income generation, or by people who collect insects as a hobby. Applied entomologists, agriculturalists and foresters usually collect pest insect specimens that occur on a particular crop to study the pest's biology or for the identification of the insect.

Insect specimens have to be preserved so that they can be stored and will last for some time. If the preservation of the specimens is done thoroughly and if the specimens are properly stored, they can last for many years, even in a tropical country. However, insect specimens are likely to be attacked by ants, rot and mould and deteriorate very quickly, if the procedure of preservation is done in a negligent way.

When there is a problem related to insect attack the insects causing the damage have to be located, they have to be caught, preserved and posted to an entomologist, who can give a piece of advice. These steps are followed by agriculturalists and foresters when collecting and preserving insect pests for identification:

1. **locate the insects that are responsible for the damage**
2. **collect at least six specimens**
3. **label the specimens and provide additional information**
4. **preserve the specimens**
5. **mail the specimens to an entomologist**

In most cases the collection and preservation of insects does not require any expensive and sophisticated materials. Many items of daily life such as wrappers and other packing materials can be used instead of the materials used by professionals. Professional equipment is usually not obtainable in PNG and has to be ordered from overseas, eg. from **Australian Entomological Supplies** (see **Addresses**).

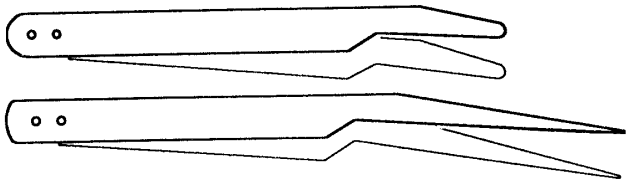

Fig. 7-7: Blunt and sharp feather light entomological forceps for handling delicate specimens

Required Materials and Equipment:

- entomological pins of different sizes
- insect net
- specimen tubes; alternatively empty peanut butter jars or similar containers with an air-tight screw-type lid can be used
- setting-boards that can be made from timber, styrofoam or card board
- sharp blade or knife, pair of pointed scissors
- methylated spirit (70% ethanol) as preservative for wet specimens
- a pair of feather light entomological forceps to handle delicate specimens (**fig. 7-7**)
- cotton wool
- insect paper; old newspaper can be used instead to fold specimen envelopes; cellophane from wrappers of cigarette boxes, tea, etc. can be used as setting paper; plastic foil however, is not suitable
- paper for labels
- soft pencil
- killing jar; can be made according to **fig. 7-8**
- killing agent preferably ethyl acetate or chloroform; ethanol is less suitable
- disposable hypodermic syringe (1 to 2 ml) with a thin needle (0.5 mm ∅);

Never take used syringes from hospitals since they might be contaminated with Hepatitis B or HIV!

7.3.1 Collection of Insect Specimens

Insects are collected by using a suitable collection or trapping method, according to **chapter 7.2**. In most cases the manual collection of specimens is the most appropriate and suitable method. Each part of the plant has to be thoroughly searched for insects. A pair of entomological forceps should be used for small or soft insects and for caterpillars to avoid

squeezing of delicate specimens and to prevent any hazards like being stung or bitten. Only good quality specimens should be collected. Specimens for instance with broken wings should be released.

Be careful, some insects might bite, sting or cause skin irritations with their hairs!

At least six specimens have to be collected. **This is important because some specimens might become damaged during transport and handling. Furthermore, specimens of the same species rarely look the same due to variability**. The individuals of a species always differ in little details, as in humans, where some individuals are tall and others are short, some are slim and others are obese or some have curled hair, whereas others have straight hair.

7.3.2 Rearing of Immature Insects

In most cases holometabolous insects can only be identified correctly from the adult stage. Therefore, immature stages like caterpillars and pupae have to be reared and fed with their respective food plant in a suitable enclosure until they eventually develop into the adult, which can then be used for the identification. This is particularly important if the kind of insect occurs for the first time on the host plant. When rearing insects a certain number of the reared larvae or pupae always fall prey to parasitization. This seems to be very discouraging, but precious information on the parasite - a potential biological control agent - can be gathered at the same time. The parasite will then have to be reared, too, so that it can be identified and studied in more detail.

7.3.3 Killing Methods

Insects are usually killed right after they have been collected or trapped. This is particularly important for butterflies and moths, that vigorously move their wings in order to escape and thus are likely to become damaged. **Killing should be done in such a way that the insect suffers as little stress and pain as possible.** Suitable killing methods are:

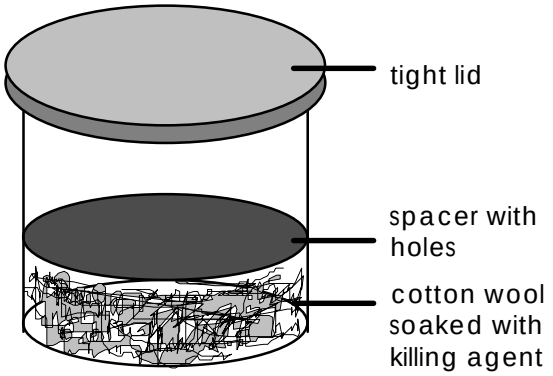

Fig. 7-8: Killing jar (graphic Schneider, M.F.)

Killing Jar:
Most insects can be killed by exposing them to the fumes of a poisonous killing agent in a killing jar (**fig. 7-8**). Commonly used killing agents are volatile organic solvents like ethyl acetate, ether or chloroform. Ethanol is less suitable, because it takes a long time for an insect to die. Some entomologists use the poisonous sodium cyanide. The specimens have to be left in the killing jar for a few minutes. Larger specimens should be exposed to the poison for five to ten minutes, so that they will not recover once removed from the killing jar. Contact of the insect with the killing agent should be avoided.

Injection of Killing Agent:
Larger specimens of moths, butterflies, grasshoppers, beetles, etc. can be easily and instantly killed by the injection of a small amount of killing agent into their thorax and abdomen. Usually 0.1 to 0.2 ml of ethyl acetate or chloroform are sufficient to kill the specimen. The use of a small hypodermic syringe with a thin needle is recommended. The specimen is held firmly and the killing agent is injected from the ventral side.

Caution: Use the syringe carefully to avoid any health hazards!

Deep Freezer:
A good killing method for almost any kind of insect is to simply freeze the specimen to death in a deep freezer at -20 °C. The cold-blooded insect slowly falls asleep as the temperature decreases and after one to two hours it definitely will not recover any more.

Squeezing of Thorax:
A common killing method for butterflies is to squeeze the thorax firmly for several minutes. This method does not require any materials, and the specimen remains in a perfect condition, if crushing of the thorax is avoided.

7.3.4 Labelling of Specimens

Specimens are labelled as soon as they are caught. The date, location, catching/trapping methods and name of the collector are stated on the label. Additional information should be provided since this might be helpful for the entomologist to identify the insect. Important might be the host species, its age and height, the number of affected trees, the percentage of damage, the number of eggs, larvae, pupae and adults viewed per host plant, the presence of predators like wasps, birds, etc. The label has to be written with pencil on white paper. Most ball point pens, felt tip pens, etc. are not suitable, because the ink fades away during the course of time or blurs if the label is put in alcohol. For wet specimens the label is put together with the specimen into the preservative. A label stuck to the outside of a specimen tube might come off. For specimens supposed to be set at a later time, the required information is written on the storage envelope (**fig. 7-9**) containing the specimen. Later, when the specimen is set, a new label is written and pinned together with the specimen. In the latter case, the size of the label should not exceed 10 x 15 mm. Several labels can be attached to the pin of a specimen if required.

Note: a specimen without a label is lacking important information and is thus worthless.

7.3.5 Wet Preservation of Specimens

Wet preservation is a very easy and quick method of preservation and requires far less time than dry preservation. Live specimens can be simply killed and preserved at the same time by putting them into spirit together with the label. As easy as this, spirit preservation is finished and the specimen can be stored away.

Dry preservation is more common but more difficult than wet preservation. However, soft specimens like termites and grubs or caterpillars cannot be dried - at least not that easily - due to shrinking. They have to be preserved in alcohol. In general, wet preservation is possible for all insects except moths and butterflies. Especially minute insects that are too small or too difficult for pinning are preserved in spirit. The most commonly used preservative is 70% methylated ethanol. Only spirit that contains 70% ethanol or more is an effective preservative. Poisonous methanol is added by the manufacturer so that the spirit is no longer suitable for human consumption. Each year there are a number of casualties caused by the consumption of methylated spirit, which might be fatal or at least cause blindness.

Warning: methylated spirit is not to be drunk!

For the wet preservation of particular groups of arthropods special preservatives might be required. Most of the formulations contain small percentages of formalin, chloroform, glacial acetic acid, glycerol and other organic solvents that are added to ethanol. Soft specimens usually shrink a little bit and become stiff after being exposed to alcohol. However, wet specimens are not as brittle as dried ones and are therefore more suitable for microscopic studies. The spirit has to be replaced after about one month, because the preservative will be diluted by the water contained in the specimens thus decreasing the preserving properties. The spirit level in the specimen containers should be checked from time to time and more spirit should be added if necessary.

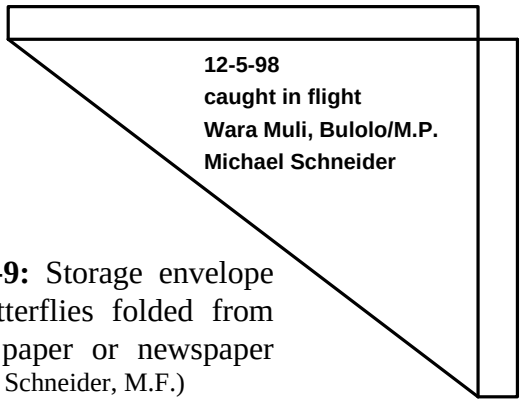

Fig. 7-9: Storage envelope for butterflies folded from insect paper or newspaper (graphic Schneider, M.F.)

7.3.6 Dry Preservation of Specimens

The specimens should be pinned and set soon after the collection, as long as the specimens are still flexible. The specimens can also be dried and temporarily stored, eg. in triangular envelopes made from glassine insect paper or newspaper (**fig. 7-9**) and relaxed later. The whole procedure is summarised in **fig. 7-11**.

Pinning, pointing and carding:
The specimens have to be pinned and set prior to drying. Pinning of the specimens is done according to **fig. 7-10**. For instance beetles are pinned through the right side of the wing cover. The pin is driven perpendicularly through specimens of most insect groups, but obliquely through wasps. Different types and sizes of pins with and without head are available and should be used according to the size of the specimen. It is important to use only high quality, stainless steel entomological pins. Some pins suitable for most purposes are:

entomological pin	**dimensions** (length x ∅)		
macropins, size 1	38 mm	x	0.40 mm
macropins, size 4	38 mm	x	0.55 mm
micropins, size B3	15 mm	x	0.0076”
micropins, size B3	15 mm	x	0.0100”

Minute insects may be glued onto an elongate or triangular-shaped piece of cardboard (**carding**), that is then pinned on a macropin. Another possibility is to use micropins for minute specimens. With the help of forceps, the specimen is pinned under a stereo microscope. Then the specimen is pinned onto the pointed side of a piece of triangular-shaped cardboard or PE-foam (**pointing**). This piece of cardboard or Styrofoam is then pinned together with the label on a macropin.

Setting and spreading:
Particular insects are set after being pinned but prior to drying. The wings of butterflies and moths, larger flies, wasps, etc. are spread and opened, so that the upperside of the hindwings

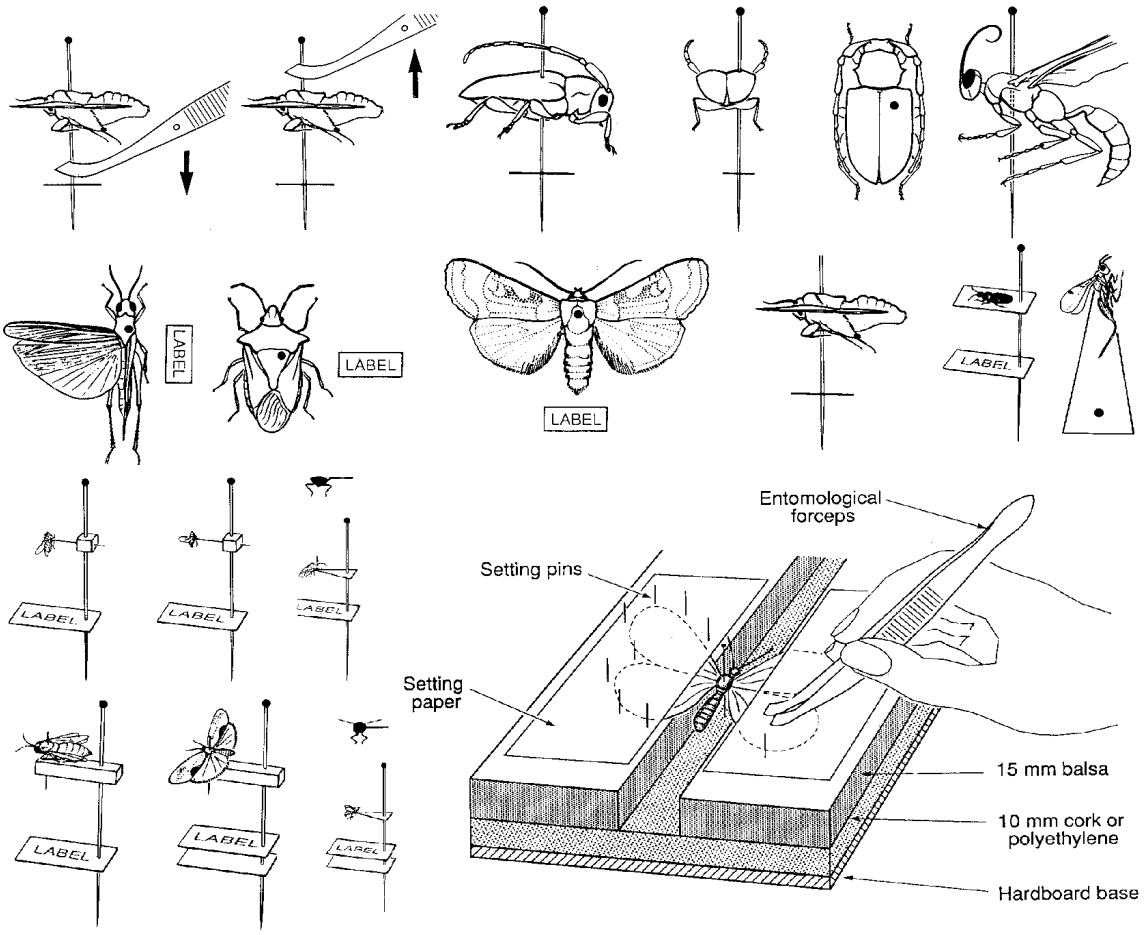

Fig. 7-10: Handling, Pinning, Pointing, Carding and Setting of Specimens (reprod. from Upton, M. S., 1991)

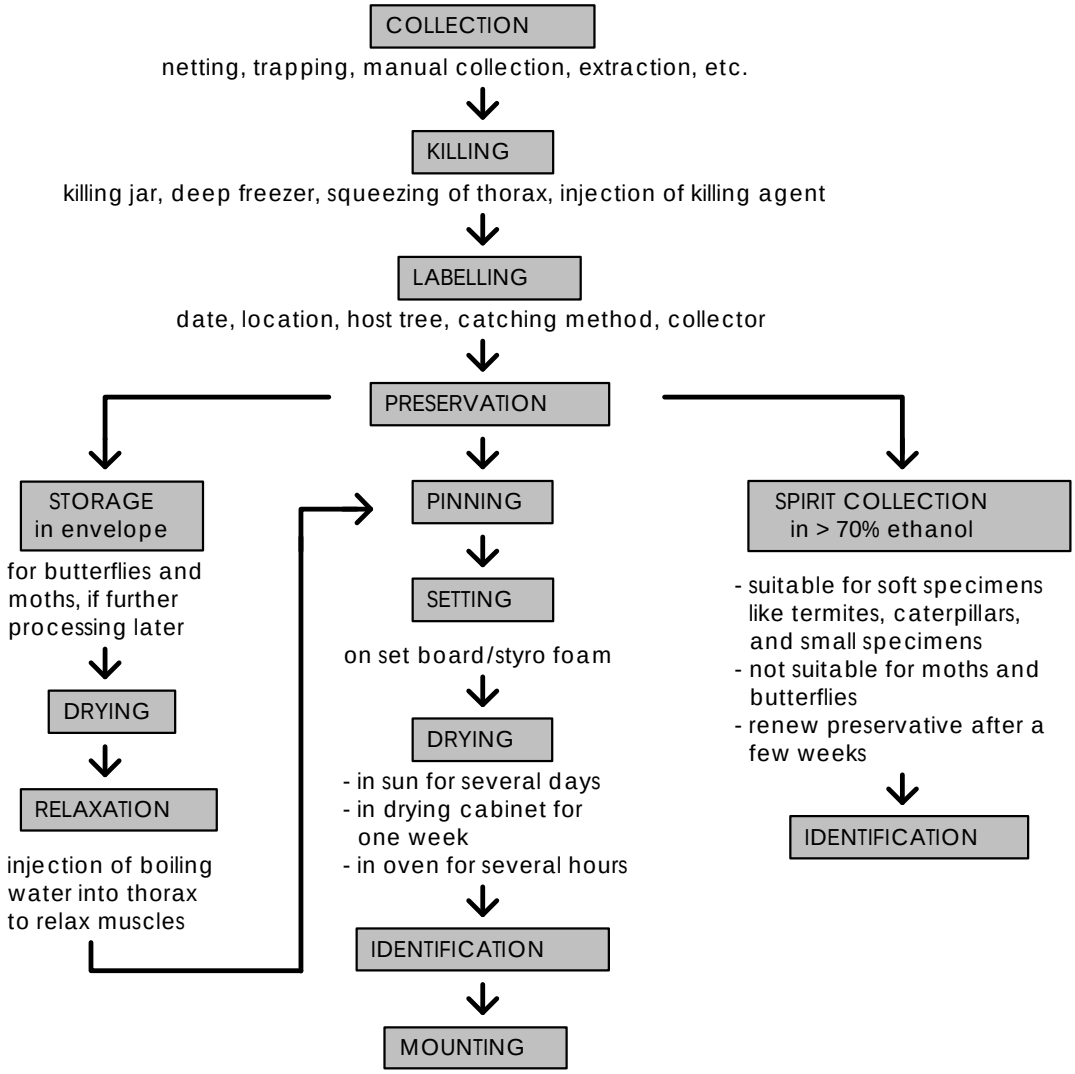

Fig. 7-11: Preservation of Insect Specimens - A Summary (graphic Schneider, M.F.)

becomes visible. The left pair of wings of grasshoppers, praying mantids, stick insects, etc. can be set, whereas the wings of the other side are left folded. Therefore, the specimen is placed and pinned into the groove of a setting board. Forceps should be used to open up the wings or to set the legs. As per convention, the wings are set in a way that the hind margin of the forewings forms a right angle with the median line of the insect's body. A suitably sized piece of insect paper or cellophane is placed over the opened wings and pinned onto the setting board. Setting boards can also be made from styrofoam or balsa. The pins should not be driven through the wings. Finally the label is attached next to the specimen. The antennae and the legs of larger beetles, grasshoppers, spiders, etc. are set, too.

Removal of Guts:
The guts of larger, soft specimens like spiders or stick insects have to be removed in order to avoid rot or shrinking. To operate on the guts, the abdomen of the specimen is cut open from the ventral side. The intestines, fat body, etc., are removed with forceps and the cavity is then stuffed with cotton wool or soft tissue paper. Once the specimen is in its original shape, the edges of the cut are joined nicely and the specimen can then be set and dried. Sewing of the cut is not necessary.

Drying:
Insect specimens have to be preserved by drying in order to avoid attack by ants, rot or mould. Drying is possible for all insects except soft specimens. The specimens can be dried

either in the sun or above/underneath a light bulb for several days or in an oven at a low temperature (< 50° C) for several hours. Sun-drying is commonly done by local insect collectors or during biological surveys, when the specimens are to be set at a later time, eg. upon returning to the lab after several weeks. During the process of drying the specimens have to be protected from ants, eg. beyond a barrier that is inaccessible to ants. Sufficient protection can be gained by keeping the specimens on a small table-like rack that can be made from cardboard and sticks. Each leg of the rack is placed in a small container with mineral oil (engine oil) or water plus a little bit of detergent. Such barriers usually cannot be crossed by ants.

Note: Dried insect specimens are very brittle and delicate and have to be handled with extreme care. Pinned specimens should be held only at the pin, preferably by the use of forceps.

Relaxing of Dried Specimens:
Dried specimens have to be relaxed prior to setting to avoid breaking off parts of the brittle specimen. For relaxing moths and butterflies, boiling water is injected into the thorax to relax the flight muscles so that the wings can be spread without breaking. Beetles and other tougher specimens can be dipped into boiling water for a few seconds, then their legs and antennae can be set.

7.3.7 Transport of Insect Specimens

The transport of insect specimens requires suitable packing so that the specimens are not damaged. As long as the specimens are fresh and still flexible, they can be stored between layers of cotton wool in a cardboard box as shown in **fig. 7-12**. Once the specimens are dried and brittle, they have to be packed very carefully. If a professional postal box is unavailable, a layer of styrofoam, cork, etc. is firmly fixed to the bottom of a suitable-sized cardboard box. The pinned specimens should be driven firmly into the base, so that the specimens cannot fall off during transport. Larger specimens like Lepidoptera should be hemmed in with several pins inserted into the cork obliquely in order to prevent vertical as well as rotary movement. Moth balls or plastic containers, etc. should not be included in the box containing the pinned specimen but should be packed separately to avoid damage of the specimens. Wet specimens should be restricted to the bottom of their container by a plug of filter or tissue paper so that they will not break as a result of turbulent movement of the fluid. The insect box should be surrounded by a layer of wood wool or other packing material, not less than 5 cm thick and the whole should be enclosed in a strong cardboard box. Specimens should be sent by 'Air Mail' rather than by 'Surface Mail'.

Fig. 7-12: Freshly killed specimens are safely transported between layers of non-absorbent cotton wool (photo Schneider, M.F.)

7.3.8 Storage of Dried Insect Specimens

Specimens should be kept in almost air-tight storage or display boxes made from plywood or metal. The bottom of the storage box should be equipped with a 7 mm thick cork or poly-ethylene foam (plastazote) layer, in which the pins of the specimens are driven. Lining paper can be pasted on to the bottom of the storage box. A preservative should be applied to the specimens to avoid damage by mould and pests

like booklice (**Psocoptera**) and the tiny museum beetle *Anthrenus* (**Dermestidae**). Suitable preservatives are naphthalene flakes (moth balls) against insect pests and chlorocresol against mould. Damage caused by insect pests can be recognised by fine dust below the specimen. In this case the box should be fumigated with aluminium phosphide (Fumitoxin ®), chloroform or paradichlorobenzene and sealed for several days.

Warning: aluminium phosphide and paradichlorobenzene are highly toxic and might be fatal when inhaled and therefore should be handled with the greatest care.

Preservatives like moth balls have to be secured so that the specimens are not damaged if the box is moved during handling. Even in a tropical country, insect specimens can last for many years, if they are maintained and looked after properly. Ideally, the specimens are stored in almost air-tight insect cabinets in an air-conditioned, dehumidified room of about 20° C and less than 40% relative humidity. The colours of some insects like grasshoppers and praying mantids will fade or even disappear during the process of drying. However, the coloration of most butterflies, moths and beetles persists for years.

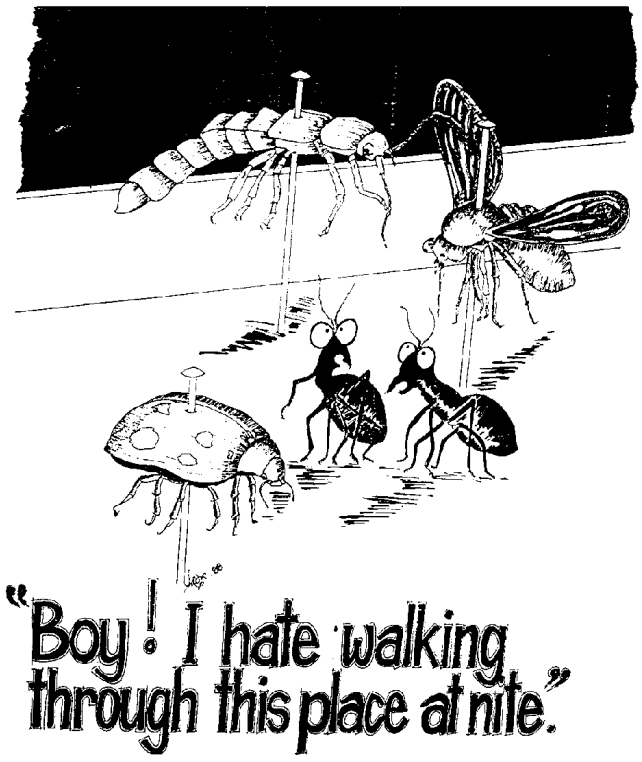

Fig. 7-13: Identification of specimens in the field lab by staff of FRI (photo Schneider, M.F.)

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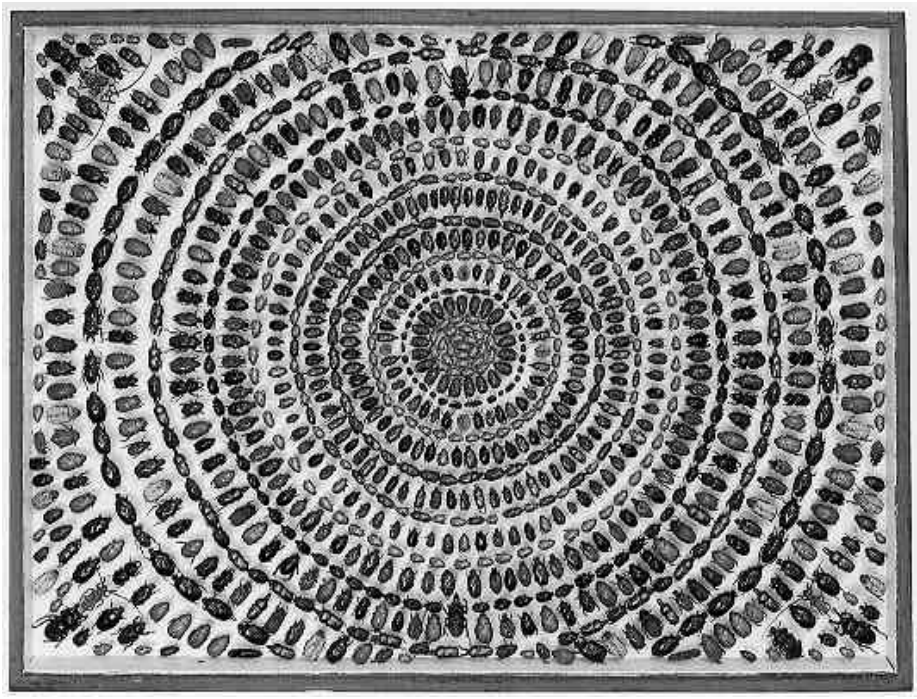

Fig. 7-14: 'The Grand Parade' containing many species of Christmas beetles, flower chafers and stag beetles (reproduced from Monteith, G., 1991)