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II. Identification of Plant Pests

1. HOW PESTS CAUSE DAMAGE

Animal pests damage plants mainly by feeding on their tissue or sucking their sap. Insects harmful to plants can thus be divided into two main groups: *biting* insects and *sucking* insects; the latter group includes also mites of the order Acarina. Plant parasitic nematodes form a third group. The degree of harmfulness of a pest is judged by which (particular) organs of the plant it attacks. Signs of direct injury done to certain parts of a plant may often help in the identification of a pest or of the order to which it belongs.

Damaged roots, for instance, indicate attack by termites, wireworms, grubs, or mole crickets; while feeding marks on shoots, buds or leaves may be caused by locusts, caterpillars, beetles or their larvae, or larvae of sawflies. Trunks, branches, twigs, or stems of many crops are mined by the larvae of beetles and caterpillars. Serpentine or blotch mines in leaves may be caused by caterpillars of moths or larvae of beetles or flies, according to the kind of plant injured. Leaves often also show various types of damage caused by beetles, locusts, or caterpillars. External or internal feeding injuries on buds and fruits are mainly caused by bugs, beetles, butterflies and flies in their larval stages.

Attacks of sucking insects, mites and nematodes may also produce characteristic injuries. Their sucking often causes distortion, dwarfing and gall formation; conditions which may finally lead to the death of the attacked part of the plant. Sucking insects also play an important rôle as vectors (carriers) of numerous virus diseases of plants.

An important point is the relationship of a pest to its host plant. Monophagous insects need one particular kind of host plant, while polyphagous insects attack a wide range of often unrelated plant species; they can therefore damage more than one type of crop.

Damage done depends largely on the state of development of a plant and on the seasonal occurrence of a pest. *Prodenia litura,* the cotton worm, for instance, starts its ravaging activity by eating

away the leaves of young cotton plants; later on, when buds and bolls are formed, it attacks and destroys these as well. Damage is sometimes done in such a way that the primary cause is concealed by secondary symptoms. Insects and nematodes, for instance, by injuring the roots of a plant, weaken it and reduce its resistance so that it is liable to be attacked by various surface pests. Plants growing under unfavourable conditions, and not thriving (even if only temporarily) are also more liable to attack.

Simultaneous attack on several parts of a plant by one or more insect species is also possible and leads to damage of various kinds. In cases of mixed infestation also it is necessary to detect the primary cause. In the first chapter of this book a simplified systematic table of the most important injurious insects is given which should make identification of a pest possible.

2. HOW PESTS ARE IDENTIFIED IN THE FIELD

Whereas the damage usually determines the species of a pest which has fed or is still feeding on a plant, the methods described below make it possible in many cases to identify a pest before its mass invasion and to take preventive steps. They should also make it possible to find adult stages of insects with complete metamorphosis and to determine the prevalence of certain pest populations; these being important data for the timing of control measures.

a) Isolating Attacked Plant Material

The best way to identify and determine a pest with complete metamorphosis in a field is to isolate the attacked plant material. Plant parts attacked by larvae, such as roots, stems, shoots, mined leaves, fruits and the like are enclosed in a cage or similar container covered with wire gauze and kept in a shady and moist place. When the larval development of the insects is complete, the adult stage, on which identification is based, can be seen. Thus, leaving the enclosed plant material where it was found in the insects' natural biotope, valuable data concerning possible times of emergence and flight of the majority of those insects can be ascertained. Parasites of the captured larvae, ichneumons, parasitic flies or other parasites are thus also isolated and can be observed. This method is also suitable for the study of various other biological data, such as feeding habits, duration of larval development and pupal rest. Its use is recommended to all agriculturists interested in biology and entomology.

b) Odour Attractants

Insects can be attracted by setting odour baits. Substances which give off long-range odours, attractive to insects, are placed in the field. Odour baits, however, act more selectively than optical baits (see below) and their use is therefore restricted. Since their attracting properties are affected by the weather, tests should be made for several days. It is left to the field worker to experiment with other formulae than those given below.

Bait	*Attractive for*
1. Fermented rice or banana beer 1:1 diluted in water	Noctuids Microlepidoptera Rose chafers Vinegar flies
2. Sour wine, white or red, with 2% sugar added	Noctuids Microlepidoptera
3. Orange juice, fermented with sugar (2%)	Noctuids Microlepidoptera Longicorn beetles Rose chafers Lamellicornia Various flies
4. Rice bran decoction (100 grammes bran boiled for 15 minutes in 1 litre water)	Noctuids Microlepidoptera
5. Ammonium stearate 2% in water	Fruit flies
6. Protein-hydrolysate 0.25-0.5% in water	Fruit flies
7. Yeast hydrolysate	Fruit flies
8. Sliced potatoes buried 10 cm deep in the ground	Wireworms Grubs Millipedes
9. Deciduous trees (chips of trunk or twigs)	Weevils Bark beetles Longicorn beetles
10. Rhyzomes of banana or lightly crushed midribs of banana leaves	Banana weevils
11. Pieces of sugar-cane shoots	Sugar cane weevils
12. Injured sisal boles	Sisal weevils
13. Freshly felled and cut-up trunks of coconut palms	Rhino beetles Palm weevils

Liquid baits 1-3 can be made considerably more attractive by adding a few drops (4-5 per litre) of amyl acetate or malic ether.

Even plain water with a few drops of amyl acetate attracts insects flying at dusk, especially Microlepidoptera.

Liquid baits 1-5 are most successful when they are placed in wide preserving jars or shallow dishes. These are exposed among the plants 1-2 metres above the ground so as to be protected from ants and other non-flying insects. Checking is done daily in the morning for catches of moths, flies and other winged insects. In order to check insect flight in tree plantations such as Citrus or Mango, preserving jars, fitted with wire hoops and long strings, are hung in the tops of trees. For daily checking of the catches swimming on the attractive fluid, the jars are lowered to the ground.

Baits 6 and 7 are used in the "spot-treatment method", where they are applied to the leaves (Citrus) together with insecticides in order to attract fruit flies to the latter.

Vegetable substances (8-13) are spread in shady places all over a field and if possible checking of attracted insects is done daily. Fresh vegetable baits are often most effective 24-48 hours after being laid out but tend to dry out and lose their attractiveness after a few days.

c) Optical Attractants

The fact that a great number of dusk- and night-flying insects, such as beetles, butterflies, moths, flies, Orthoptera, Hymenoptera and others are attracted by light makes the use of light traps possible. Experience has shown that a lamp with a high content of ultra violet rays is very effective.

Highest night catches are made with Hg- or so-called mercury-vapour lamps (150-250 Watt = about 3000 c.p.). Where no electric current is available, the well-known long-burning paraffin or petrol vapour lamps (Aladdin, Coleman) are a useful substitute. Night traps are easily and quickly set and require a minimum of material. A white sheet, about 2 metres square, is fixed vertically between two poles so as to form a reflecting screen. The lamp hangs freely from a support at about 50-70 cm from the sheet so that the light illuminates its centre (see Fig. 51). It is important that the illuminated surface of the sheet faces the habitat of the insects (field, crop, bush etc.). Insects settling on the sheet can easily be transferred to a killing bottle. Where no sheet is available, a whitewashed wall may be used instead. Good catches can only be expected on dark, moonless nights. Since each insect species has its own flight period and the time of its presence at night is restricted, night catches are limited to dusk and the following 3 to 4 hours. Records of the prevailing temperature and weather conditions during night catches are very useful for further biological observations.

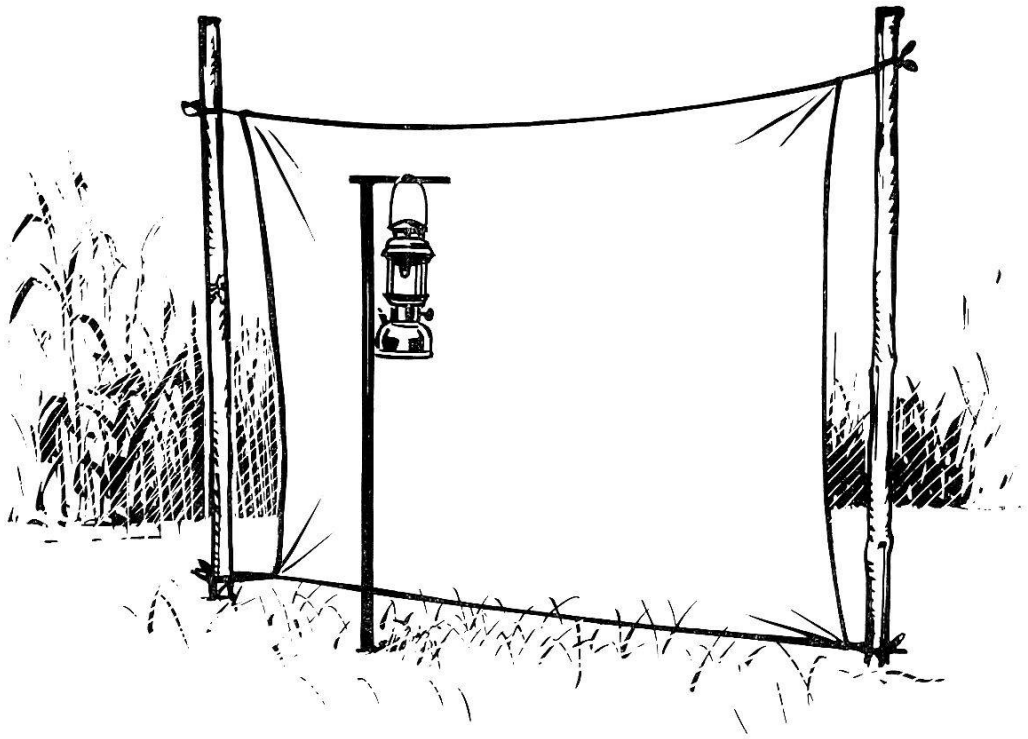

Fig. 51. Light trap with vapour lamp

Another very simple method of attracting insects by light, which can be used anywhere, consists of placing a pocket electric torch vertically, lamp upwards, on a suitable site. A glass dish containing alcohol or water is placed on the lens. The dish, thus in full light, acts as an automatic trap for many insects attracted by the light (see Fig. 52).

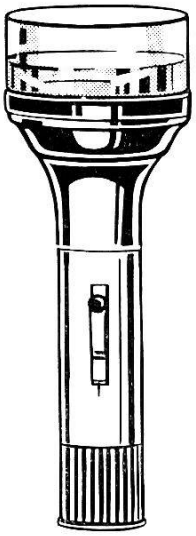

Fig. 52. Light trap with a pocket electric torch

Optical baits can also attract various insects in daytime. Basins, the insides of which are painted yellow or lined with yellow glossy paper, are placed where they can be seen easily, especially in low crops where they attract flying aphids as well as various species of beetles and flies. A disadvantage of these traps is that many insects

tend to fly away again after a short rest on the yellow surface and thus escape. This can be overcome by applying some insect glue to the surface (see page 481). These self-acting traps are checked at regular intervals and newly set up; the insects sticking to the glue are carefully removed and washed in alcohol or acetone for further examination.

d) Traps

For catching mole crickets, fairly tall preserving jars or food tins with wide apertures and vertical sides are most suitable. These are sunk in the ground so that the upper rim is level with the soil surface. To protect the traps from dirt or rain the mouth is covered with a slab of stone or a piece of wood forming a roof; the space under it has of course to be large enough to give the insects access to the trap. With 20-25 of these traps per hectare (10,000 sq. metres) the presence of mole crickets can be established.

The traps can be made considerably more effective by baiting them with some odour attractant. Decaying fruit, sliced potatoes or moistened bran attract earwigs, wood lice, millipedes and slugs.

e) Other Equipment for Capturing Insects

The simplest way of capturing insects is to use a butterfly net or a sweeping net. With the former, butterflies can be caught in flight, while the sweeping net is for more general use. A sweeping net is similar to a butterfly net, but the bag is made of strong cotton fabric instead of gauze and the frame is stronger (see Fig. 53a). The sweeping net is used in low crops. When swung several times over the plants with the mouth held sideways, insects are swept into the

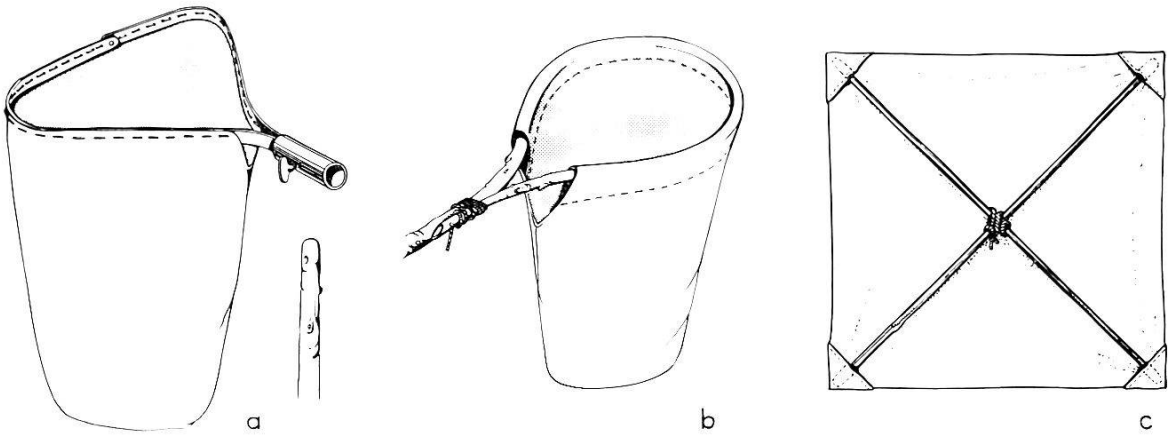

Fig. 53. Equipment for capturing insects
a = butterfly net or sweeping net; metal frame
b = butterfly net or sweeping net; frame made of a flexible stick
c = beating tray

bag, from where they can be removed for preserving. Best catches are made early in the morning and late at night when insects are less active.

Another way is with a so-called "beating tray". A square piece of material (e.g. canvas, linen etc.) 1 × 1 metre is spread out and two sticks forming a cross are pushed into pockets sewn in each corner. The screen (see Fig. 53c) is held under a plant which is then lightly stirred with a stick. Insects fall during the cool hours of the morning and evening and can easily be examined on the screen.

f) Insecticidal Sprays

To study the various insects which infest one particular plant species or crop the plants are sprayed with a fast-acting insecticide. Here also the insects' drowsiness during the cool hours of the morning is taken advantage of. A white paper or sheet is spread under the plant. Then the whole plant is rapidly and thoroughly sprayed with an emulsion of pyrethrum (0.1-0.2%). The knock-down effect of pyrethrum causes the insects to fall within a short time on to the white surface on which checking and examining can easily be done. This method is widely recommended; it also helps to establish the onset of infestation and density of insect populations.

3. EVIDENCE OF PLANT PARASITIC NEMATODES

The methods by which plant nematodes (except free living root nematodes) can be detected are relatively simple and require only little equipment. Exact determination of the parasite, however, requires thorough taxonomic knowledge and must be done by a specialist. The separated and preserved nematodes (for fixing etc. see pages 52 and 54 or the infested plant or earth samples are therefore best sent to an experimental station.

a) Leaf and Stem Nematodes

Method 1: Leaves and stems are plucked to pieces under water in a small glass dish or directly on a concave slide. Nematodes emerging from the tissue can be recognized by their undulating movements under the microscope or under a low-powered magnifying glass.

Method 2: Good and reliable results are obtained by the funnel method of Baermann. Finely chopped leaves and stems as well as earth samples are wrapped in a piece of gauze. The bag is put into a funnel filled with water and closed with a rubber tube and a pinch-cock (see Fig. 55).

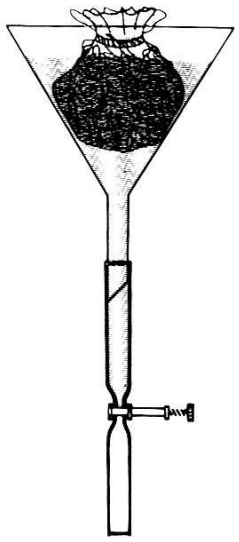

Fig. 54. Funnel method of Baermann

Nematodes contained in the plant tissue will soon appear in the water. By opening the pinch-cock the deposit accumulated at the bottom of the funnel is let into a glass dish where the nematodes can be seen. Since plant parasitic nematodes live mainly in healthy green tissue, only such plant material, separated from earth particles, should be used for investigations. Saprophylous, non-injurious species are frequently found in disintegrating or rotting plants.

Method 3: The presence of leaf or stem nematodes in plant tissue can also be shown by staining the material in the following way: Leaf or stem fragments are placed in a boiling solution of Naphtolblueblack/Geigy (0.02-0.05%), where they are left overnight and then rinsed in water. The nematodes appear dark blue under the microscope, whereas the plant tissue is slightly greenish or bluish.

b) Root Knot Nematodes

Method: Roots must be carefully dug out, washed and examined for knots. They are then placed in a solution of common salt (NaCl) or sugar (2.5%), and the knots are carefully opened with two pins. The white, pear-shaped females can then be released (see Fig. 48).

The releasing of the globular nematodes can be made easier by first making small cuts on the surface of the knots, and then placing them for a few hours in FFA fixative solution (see page 54).

c) Cyst-forming Nematodes

Method: On freshly and carefully dug out roots young cysts can be seen with the naked eye (see Fig. 49). In more advanced, that is, older foci the brown, globular cysts are found in earth samples. The inside of a preserving jar is lined with filter paper and filled with finely crumbled, well dried samples of earth (100-200 cc), taken 5-20 cm below the surface. About 750 cc of a solution of common salt 10-15% is then poured over the samples and stirred all the time. The cysts, being of lower specific gravity, rise to the surface and collect all along the jar wall, i.e. on the filter paper. The cysts, easily distinguishable from earth particles, can be detected by placing the filter paper under a magnifying glass (see Fig. 49). Samples should be taken at various places all over an infested field.

d) Free Living Root Nematodes

Nematodes which have penetrated into roots can be stained in the following ways so that they show under the microscope:

Method 1 of Goffart: The fine, possibly injured, roots are placed for 10-20 minutes in Lugol's solution (2 grammes potassium iodide in 5 cc water + 1 gramme iodine + 30 cc water). Then the roots are laid on a slide, dried with filter paper, and afterwards treated with glycerol or lactic acid. The nematodes show reddish-brown, while the plant tissue remains colourless.

Method 2: The fine roots are placed for several hours in a mixture of lactophenol and cottonblue (20 cc distilled water + 10 cc glycerol + 20 cc lactic acid + 20 grammes chemically pure crystallized phenol + 0.5 grammes cottonblue). Then they are quickly rinsed in water and immediately examined under the microscope in aqueous medium. If they are stained too dark, the colour can be reduced by placing the roots in lactophenol without cottonblue. Nematodes show blue, while the plant tissue remains colourless.

Method 3: Fine roots, suspected of nematode infestation, are opened by making a small cut with a needle. Then they are placed in boiling aqueous solution of Naphtolblueblack/Geigy (0.05-0.1%). After letting the staining solution come to the boil 2-3 times it is left to cool and act for 10-12 hours. Then the roots are rinsed in water and examined in aqueous medium under the microscope. The nematodes appear blue, while the plant tissue is greenish to colourless.

Free living root nematodes in earth samples can be detected by the funnel method of Baermann. However, from the various nematode populations contained in an earth sample, only a specialist can identify the plant parasitic species.

4. KILLING, PRESERVING AND PACKING INSECTS

It is important to choose a reliable and rapid killing method by which the characteristic points and features of the insects are not damaged. A proper killing method is also important for proper preservation.

a) Equipment for Killing Insects

1. *Cyanide bottle for butterflies and dragonflies*

The bottom of a 200-300 cc glass jar or celluloid container with a wide mouth is filled with 4-5 cm sawdust (Fig. 55 a,3), on which a layer of 2-5 mm powdered potassium cyanide (Fig. 55 a,2) is spread evenly. The poison is covered with 3-5 mm of a thick paste of plaster of Paris (Fig. 55 a,1).

After 24 hours a killing bottle prepared in this way is ready for use, and it remains effective for several months in tropical conditions. Hydrocyanic acid produced by reaction of carbonic acid from the atmosphere with potassium cyanide kills the insects in the bottle within a few seconds. Naturally these bottles must be clearly labelled and kept out of reach of unauthorised persons.

2. *Ethyl acetate killing bottle for beetles, locusts, crickets, cockroaches, bugs (large), ants, various Hymenoptera*

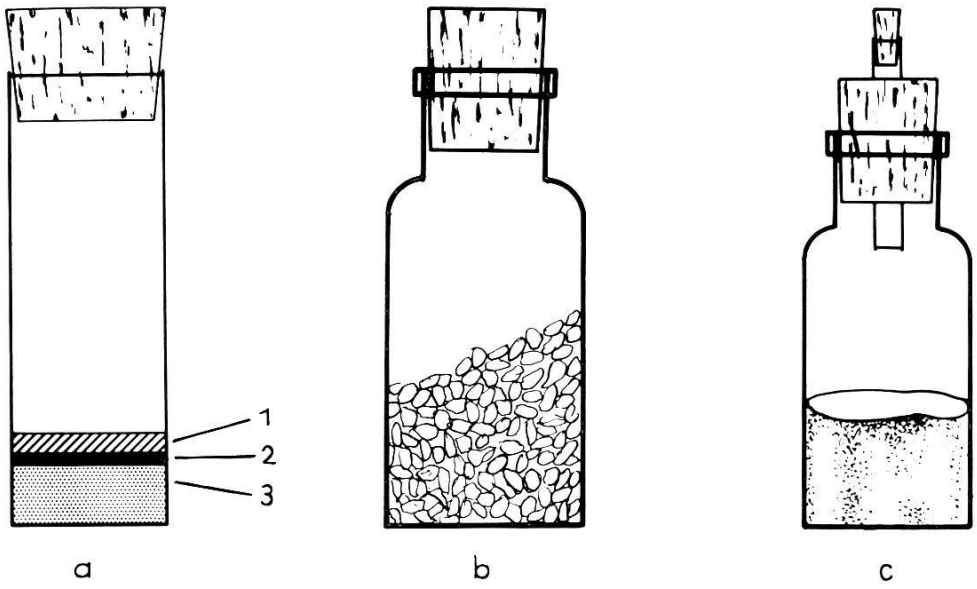

Fig. 55. Equipment for killing insects
a = cyanide bottle
b, c = ethyl acetate bottles

A wide-necked bottle with a well fitting cork or rubber stopper is filled ¾ full with fine cork chips or dry, boiled sawdust (free from resin) or small pieces of blotting paper (Fig. 55 b). This material is moistened with 1 cc ethyl acetate per 100 cc. The whole of the liquid must be absorbed, otherwise a surplus might damage the insects by sticking them together. Insects put into these bottles move downwards among the poisoned particles, where they soon die. In this way the insects can remain in the bottle for several weeks without becoming rigid.

3. *Ethyl acetate killing bottle for flies, midges, bugs (small), leafhoppers (small), Hymenoptera (small)*

This is the most suitable method for killing frail and delicate insects. Some cotton wool, 2-3 cm thick, is placed in a cork-stoppered jar and moistened with a few drops of ethyl acetate. A piece of blotting paper is laid over the cotton wool to prevent the tarsi and other prominent parts of the insects from being caught and torn away. If a glass tube, 10-15 mm in diameter and closed with a cork plug, is inserted in the stopper of the bottle, small insects can be transferred directly, without opening the bottle each time, and therefore without evaporation of the killing agent (Fig. 55 c).

A considerable prolongation of the effect of such killing bottles can be achieved by the following method: Dissolve small pieces of celluloid in ethyl acetate until a thick jelly is produced. Place 2-3 cm of the jelly in the jar, and cover the jelly with cotton wool and blotting paper as in Fig. 55 c.

Where the above mentioned chemicals are not available, other volatile substances acting as gassing poisons may be used, such as ethylether, chloroform, carbon disulphide and the like. A drawback is, however, that these chemicals make the specimens rigid immediately after death so that further manipulation of them is difficult.

4. *Killing agents for aphids, mealybugs, coccids, thrips, termites, caterpillars, pupae, insect eggs, mites, nematodes*

see preserving substances (pages 52 and 53).

b) Preserving

Most insects maintain their natural shape after death, because of their robust, chitinized skeletons which do not require special attention when being preserved. Insects need only be thoroughly dried in order to make them durable for a long time.

1. *Method for insects with a strongly chitinized body wall such as butterflies, beetles, locusts, crickets, cockroaches, flies, wasps*

To prevent decay, large insects, measuring several centimetres, must be slit open at the base of the abdomen and the inner organs drawn out and replaced by cotton wool. For easy manipulation the insects, when taken out of the killing bottle, are pinned on special entomological stainless pins. It is usual in entomological practice to pierce butterflies and wasps vertically through the centre of the thorax; midges, flies, and locusts through the right posterior portion of the thorax; beetles, bugs and cockroaches through the body near the anterior third of the right elytrum. After their extremities have been arranged as symmetrically and as close to the body as possible, the specimens can be stored in boxes, where they are dried and preserved. Special insect boxes can be bought from dealers, but empty cigar boxes may serve the same purpose when lined with cork, or "moll", or 2-3 layers of corrugated paper. With a few crystals of paradichlorobenzene or thymol the specimens can be protected from attacks of fungus or insect parasites. Time and place of capture are recorded on a label, fixed to the pin. Ants and other thickly chitinized insects smaller than 5 mm are best stuck to a strong card about double the size of the specimen. In this case the pin is pushed through the card immediately behind the posterior end of the insect and the specimen is then preserved in the same way as above.

In areas with relatively dry and hot climates, drying should not be particularly difficult. Specimens should dry within a few days if simply exposed indoors. In warm and moist areas, however, where the drying process is very slow, insects are liable to decay and disintegrate. In this case the specimens must be kept for 1-2 days (depending on their size) in a drying oven at a temperature of 40-50 °C. Where no such apparatus exists, the open storage box containing the insects is placed in a tin which is then heated over a spirit lamp. The lid of the tin is pierced with a few holes to let the steam out. A further improvised drying apparatus can be made by painting a perforated can black and exposing it to the sun. An air-tight container in which some common salt, silicogel or some other hygroscopic substance is placed, can serve the same purpose.

The danger of ants invading the insect material exposed outdoors or indoors can be overcome by placing the containers in a basin of water. The cans can also be surrounded by repellent substances such as phenol, lysol or vinegar.

2. *Method for soft, fragile insects, mites and nematodes*

The soft and frail nature of many small insects and their larvae makes it impossible to pin them. Such specimens are best preserved in liquid media. Most preserving media have, however, the disadvantage of bleaching, that is of extracting the insects' natural colours. The colours of the live insect should therefore be recorded and written with pencil or indelible ink and the label with this information placed in the liquid with the specimen.

Adult and larval stages of insects with incomplete metamorphosis

aphids	3-4 days in a mixture 1:1 of alcohol 96% and carbon tetrachloride, then in alcohol 80% or in a mixture 1:1 of alcohol 90% and lactic acid 60%
mealybugs	alcohol 70%
coccids	alcohol 70%
thrips	alcohol 60%
froghoppers (smaller than 3 mm)	alcohol 65%
leafhoppers (smaller than 3 mm)	alcohol 80%

Larval stages of insects with complete metamorphosis

fly maggots	* 2:1 alcohol 96% + formol 5% (formaldehyde)
caterpillars	ditto
larvae of beetles	ditto
larvae of wasps	ditto
larvae of midges	ditto

* Discoloration can be avoided by pouring hot preserving liquid over the specimens.

Small, delicate and hirsute insect larvae are killed in hot water and immediately placed in MacGregor solution*(Geigy/Herbig)* of the following formula:

100 cc formol 5% (formaldehyde)
2.5 cc glycerol
5 grammes borax
1000 cc distilled water

Insect eggs

soft-skinned eggs	a) 1:1 alcohol 90% + formol 4% or
	b) formol 4% + 5% glycerol or
	c) Cova Garcia medium *(Geigy/Herbig)* 3 grammes gum arabic pulv. 100 cc 1:10 formol + sodium chloride (0.8%) 2 cc glycerol
hard-skinned eggs	d) 1:1 alcohol 90% + formol 5%

Mites

spider mites and others	alcohol 80%

Nematodes

roots infested with nematodes	formol 4% (formaldehyde)
nematodes	alcohol 65%

3. *Methods for green plants (damage)*

Method 1 (after De Bolles Lee "The Microtomist's Vade-Mecum, 11th ed.):

90 cc alcohol 50%
5 cc formol
2.5 cc glycerol
10 grammes copper chloride
1.5 grammes uranium nitrate
2.5 cc acetic acid

The plants are first placed for 3-10 days, according to their size and thickness, in the solution, then preserved in formol 4%. Copper chloride should be reduced to 5 grammes for yellowish-green plants.

Method 2: Plant tissue is killed by placing the plants for 48 hours in a weak solution of copper sulphate. Then the plants are rinsed for 24 hours in water and preserved in a solution of

1000 cc water
40 cc glycerol
80 cc formol 4% (formaldehyde)

Method 3 (after Kaiserling): Plants are placed for 1-5 days in Kaiserling solution of the following formula:

170 grammes potassium acetate
90 grammes potassium nitrate
1600 cc formol (formaldehyde 38%)
8000 cc distilled water

Then the plants are laid in denatured alcohol 90% until their original colours are restored, after which they are immediately rinsed in water (plants are brittle!) and preserved in a solution of

1800 grammes potassium acetate
2000-4000 cc glycerol
10000 cc distilled water
20 cc phenol

c) Packing and Dispatching

Methods: For dispatching insects in insect boxes, the specimens are held by two additional pins arranged roof-like over the middle of the body or of the elytra. The pins must hold firm so that they remain in position during transport.

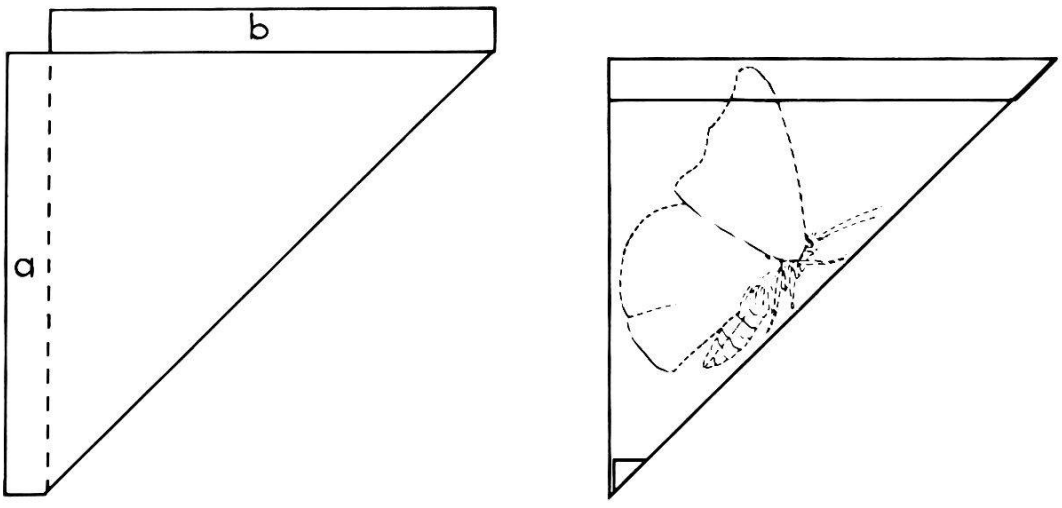

Fig. 56. Paper bag for packing and dispatching insects

For freshly killed and still soft insects, especially butterflies, bugs, beetles and locusts, the use of paper bags is recommended. A rectangular piece of paper is folded diagonally so that the parts overlapping extend about 1 cm each (see Fig. 56). When strip "a" has been folded under, the insect, wings upward, is put in the bag at "b" and then "b" is folded down.

The bag is tied up and labelled with records of time, place and date of capture. In this way even large numbers can be packed into a relatively small container. To avoid mould or attack by parasitic insects, paper bags or rolls should be impregnated with Merfen + 10% DDT as recommended by GEIGY and HERBIG (1955). The absorbent paper is laid for a short time in the solution and dried before being used for packing.

Large beetles, bugs, crickets and other specimens are rolled singly in paper before they harden, and the rolls are then placed in a box, well lined with wadding so that the specimens remain in position.

A further simple and handy packing material for unpinned insects of all sizes is a strong box with layers of cellulose. The bottom of a cigar box is lined with paper napkins or handkerchiefs and the insects, before hardening, are carefully transferred from the killing bottle to the box. Then a layer of cellulose is placed over them. In this way several layers of insects can be packed into one box. To keep them in place, the space between the top layer and the lid is filled with fluffed up cotton wool. A few crystals of thymol or paradichlorobenzene, spread between the layers, prevent mould and rotting. Insects should never touch the cotton wool, because their tarsi, claws or spiny processes might get caught in it and break when the insects are removed.

Ethyl acetate killing bottles, and also glass tubes containing insects in liquid fixative, can be mailed directly if they are carefully and suitably packed.

5. HOW TO MAKE SIMPLE MICROSCOPE SLIDE MOUNTS

Minute insects, mites and nematodes can often be identified and observed only when they are mounted on slides, so that examination of single organs or parts of the body, which are important for the identification of the specimens, is possible. Mounts also provide good material for reference and comparison. If no microscope is available, a strong magnifying glass can be used.

Material and equipment required:

glass slides 25 × 75 mm
glass coverslips 18 × 18 mm
lactic acid 60%
potassium- or sodium hydroxide 10%
carbon tetrachloride
alcohol absolute
xylene
acetone
nail varnish

FFA fixative: 100 cc alcohol 96%
30 cc formol 38% (formaldehyde)
5 cc acetic acid
200 cc distilled water

a) Mounting Media

*1. *Faure's medium after Puri*

8 grammes gum arabic
10 cc distilled water
70 cc chloral hydrate
5 cc glycerol
3 cc acetic acid

*2. *Berlese medium*

3 grammes gum arabic
5 cc distilled water
20 cc chloral hydrate
2 cc glycerol

Dissolve gum arabic in warm water, add chloral hydrate; when dissolved add glycerol and acetic acid, stirring well.

*3. *Polyvinyl-lactophenol medium after Heinze*

10 grammes polyvinyl alcohol
50 cc distilled water
20 cc chloral hydrate
10 cc glycerol
35 cc lactic acid
25 cc phenol 15%

Mix polyvinyl alcohol to a paste, with distilled water, continue to dilute it gradually on a water-bath. Add lactic acid, stirring all the time, then add glycerol and, after allowing to cool, pour in chloral hydrate previously dissolved in phenol. Filter through glass wool and if necessary thicken in drying oven or by just letting it stand. The advantage of this medium over Faure's medium is that it dries more quickly.

*4. *Methylcellulose-creosote medium*

Heat creosote on a water-bath and add powdered methyl- or ethylcellulose until the mixture has the desired viscosity.

5. *Canada balsam*

Specimens may be mounted in the media marked * without previous treatment.

b) Mounting for Quick Identification

1. *Soft or fragile insects such as aphids, mealybugs, coccids, thrips, other delicate insects, mites*

A. For bleaching, the specimens are placed in a few drops of lactic acid 50-60% (or potassium- or sodium-hydroxide 10%) between a slide and a coverslip. The slide is moved several times over a gas or paraffin lamp until all the air has escaped and steam is forming. Boiling lactic acid damages

delicate insects. Overheating must therefore be avoided and the specimens heated only until they become transparent. If stored horizontally such slide mounts may be kept for several weeks.

B. Live specimens or specimens kept in aqueous media are put into a few drops of Faure's medium on a slide and covered with a coverslip. Those kept in alcohol must be rinsed in distilled water before they are placed in a mounting medium, in order to prevent them from becoming opaque. After storing the slides horizontally for 2-3 days the mounting medium is set and the mount remains permanent. Crystallization of the mounting medium is prevented if some nail varnish is applied along the edges of the coverslip, overlapping by about 1 mm.

2. *Nematodes*

Nematodes are placed with a fine wood chip in a drop of water or a solution of formaldehyde 1% on a slide and covered with a coverslip, after which the slide is heated for a short time over a flame (50-60 °C).

c) Mounting for Permanent Preservation

Permanent mounts usually require preliminary treatment. By thorough bleaching and maceration the inner tissues are softened so that they can be removed. The state of an insect or its organs must be maintained as intact as possible so that the insects can be classified systematically. It should be possible to make permanent mounts of fresh specimens and of those preserved dry or in alcohol.

1. *Mounts of single organs, or parts of the body, or large insects (mouth parts etc.), or undissected small insects*

Soft internal tissue is removed by placing the insects for 1-3 days, according to their size, in potassium hydroxide 5-10% at room temperature. The softening process can be speeded up by boiling the specimens in the medium for 20-30 minutes. For pale specimens, however, the cold method is preferred, owing to the bleaching properties of hot potassium hydroxide. Both potassium- and sodium hydroxide tend to splash when boiling; great care must therefore be taken when these chemicals are handled (goggles!). Accidents can be avoided by putting a few small stones into the boiling fluid or by continually shaking the dish.

The chitinous structure is then rinsed in distilled water. Dissection and isolation of the organs to be mounted are done under water in a dissecting basin which consists of a glass dish filled ⅓ full with wax. Small insects, parts of insects, or insect organs are arranged on a slide in a small amount of preserving medium (*) of one of the above formulae; they are then covered with a coverslip and labelled. Fresh mounts must be stored horizontally until the preserving medium is completely set. A dab of nail varnish makes the mount air-tight, preventing evaporation, so that its durability is considerably increased.

Good mounts which keep for many years can be made with Canada balsam. The specimens, however, must be previously dehydrated, because Canada balsam is a resin dissolved in xylene. They are therefore placed

successively in alcohol 75%, 85% and 95% for 10-20 minutes each and in a mixture of xylene + alcohol absolute for 2-5 minutes and then in pure xylene before they can be mounted in Canada balsam.

2. *Mounts of aphids, mealybugs, coccids, minute larval stages, mites*

Perfect permanent mounts can be obtained with the Dosse method, by which the specimens are stained so that all the details show clearly. To fix the specimens and dissolve grease and colours they are placed for 24 hours in a 1:1 mixture of carbon tetrachloride and alcohol 95%, then in pure lactic acid to which a solution of Diphenyldeepblack/Geigy or Directdeepblack/Bayer is added (20 drops of 0.5% alcoholic stain solvent to 20 cc lactic acid). After this they are boiled for 2-3 hours and then rinsed thoroughly several times in fresh water and mounted in Faure's medium in the usual way.

A modified, simpler method consists of fixing the specimens and dissolving their colours and grease by placing them in 1:1 carbon tetrachloride/alcohol for 3 days. The specimens are next rinsed, first in alcohol 50% and then in distilled water. They are then boiled for 15-20 minutes in lactic acid and staining medium (see above), and finally rinsed again thoroughly with distilled water. They are then ready for mounting in Faure's medium.

3. *Nematodes*

Nematodes are transferred with a wood chip direct into Faure's or Berlese's medium on a slide. Squeezing of hypertrophic or globular specimens can be avoided by placing a few fine glass chips under the corners of the coverslip.

4. *Nematodes*

Nematodes are laid for 1-2 hours in FFA-fixative or alcohol 80%, then rinsed several times in distilled water and mounted in a mixture of methylcellulose + creosote.

5. *Mounts of various parts of insects, such as legs, hairs, bristles, wings, scales (interference colours), spores of fungi*

The dried specimens are laid in the centre of a slide and covered with a coverslip, the edges of which are dabbed with a quick-drying glue. The sealing glue should not be too thin, otherwise the coverslip squeezes it over too large an area.