Chapter 17

Flight interception traps for arthropods

by

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Abstract

An overview is given of flight interception traps for arthropods since the discovery of the principle by R. Malaise. New and rare designs are described and suggestions for improvement and low cost improvisation are made. The effectiveness of the traps is discussed. An overview of killing agents and preservatives and their effects on specimens is given. Good and bad practices are listed and safety is discussed. Finally methods for preparing Hymenoptera and Diptera from alcohol are described.

Keywords: positive phototropism, Malaise trap, Schacht trap, window pane trap, placement of traps, Townes design, new designs, effectiveness, preparing Hymenoptera and Diptera, killing agents and preservatives, safety, ethics

1. General introduction

The aim of this chapter is to give an updated overview of the available flight interception traps, outlining their use, advantages and disadvantages, to facilitate the choice of the appropriate designs and to improve the efficiency and quality of the collecting of arthropods. The operation of interception traps is based on the interception of arthropods (in most cases insects) in the air by means of a vertical or oblique barrier. The subsequent reaction is positive if the intercepted insects are attracted by sunlight to fly or walk to the top of the trap ("positive phototropism"). If the insects try to hide by walking down or allowing themselves to fall down the reaction is negative ("negative phototropism"). Defined in this way Malaise traps are a kind of flight interception trap and the latter name should be applied to traps using both positive and negative phototropism. Flight interception traps can be used in any habitat where insects occur, but will be most efficient if corridors ("flyways") are present to be blocked by the trap. Their applicability is equal in temperate and tropical habitats, but abnormally low temperatures will lower trapping efficiency.

Collecting a large number of specimens from groups of no interest to the collector poses a potential ethical problem. Therefore, it is recommended that the unused portions are stored in central depositories (*e.g.*, national museums of natural history) at low temperature and in darkness. There the material can be made available to other specialists, who may extract the specimens of interest to their study. The problem of catching protected or flagship taxa is very rarely encountered, but in these cases either an extra mesh before the entrance of the collector could be used or the trap could be placed just outside the area where these taxa occur. Hardly anything has been published on the impact of flight interception traps on the local populations of insects. It has been assumed that at most about 20% of the Hymenoptera entering the trap is finally caught in the collector (late H.K. Townes, pers. comm.); as far as the authors are aware no estimates have been made for other traps. Experiments to ascertain the effects of trapping on insect populations would need careful design, and the results would be expected to be highly site and organism dependent. In publications the design of the trap (including the measurements of the sampling surface), the way it was used and the position of the trap related to the sun and vegetation should be stated.

It should be strongly borne in mind that many of the fluids used as preservatives are highly toxic to vertebrate animals that will frequently try to drink them, and this risk to wildlife as well as to domestic animals needs always to be minimised.

Placement of traps

According to Darling & Packer (1988) the effectiveness of a trap depends first of all on its placement within the micro-habitat, second on its design and last on the mesh-size. According to Matthews & Matthews (1983) the design is the most important, followed by its correct placement in the flyways of insects. Obviously, an effective placement is extremely important; poor placement may lower the

catches by more than 50% in the same micro-habitat (van Achterberg, unpublished data). Relatively small changes result in large differences in collection efficiency (Matthews & Matthews, 1983). In general the trap should be either blocking a corridor (*e.g.* a path in the forest) or placed perpendicular to a barrier (*e.g.* border of a forest, with the collecting head directed to the border and the sun). Malaise (1937) was already very aware of the importance of placement: "The chief difficulty in using this trap is to find a suitable place. A trap put up in an open field would doubtless catch insects too, but the number of insects passing that special spot is a restricted one compared with a place where they are for some reason or other concentrated. Such concentrations are not uncommon; the insects are, *e.g.,* more numerous along the border of a wood or field than in the middle of it. Most, if not all, flying insects have an instinctive fear of being blown away by the wind, and are therefore always trying to keep against it, thereby taking advantage of depressions and other irregularities of the earths surface, that will furnish them shelter or help them in advancing against the current. Stronger insects are not so dependent on shelter, but have nevertheless a special liking for streamlets, ravines, shores, wood-fringes, forest-roads, clearings, etc. where they patrol back and forth. Weak fliers very often prefer such openings to the dense wood. Such places are as a rule very good for traps, which must be expanded at right angles to the main direction, and preferably with the entrance away from the prevailing wind, so that insects working their way against the current may enter the trap". The collecting head or collector should always be in the sun, especially in the morning when most of the flight activity takes place. Protection from interference is first by finding secluded but still promising places; easiest on private property without free access. Sometimes this is impossible and protection is needed by *e.g.* barbed wire and attaching an information sheet for the public.

For an overview of preservatives, killing agents, frequency of change, quality of specimens, problems, precautions and treatment of the material, see Table 1. Ethanol may also act as an attractant for some groups (*e.g.*, insects associated with rotting organic tissue and their parasitoids). To avoid this 80% isopropanol may be used (Wilkening *et al.*, 1981), though unlike ethanol this will not preserve DNA and the condition of the specimens is only fair. A solution of 2.5% formalin should not be used; it is dangerous for the user, the specimens are irreversibly hardened and rendered useless for molecular studies. Cyanide (KCN or NaCN) is also dangerous and may cause extreme reddening of specimens.

Table 1 (next page). Overview of killing agents and preservatives and their effects on specimens.

Note: 96% Ethanol includes denatured ethanol B, and 70% ethanol includes suitably diluted IMS (= industrial methylated spirits). Dichlorvos (*e.g.*, Vapona strips) = 2.2-dichlorovinyl dimethyl phosphate; cyanide is KCN or NaCN encapsulated within plaster of Paris. Specimens killed by ethyl acetate vapour and air-dried specimens yield very degraded DNA (Dillon *et al*., 1996)

Killing agent/preservative	Availability	Maximum change over time	DNA preservation	Specimen condition	Problems / precautions	Minimum further treatment before	Optimal further treatment before
96% Ethanol	--	1 month	++	- (brittle)	higher evaporation and fire risk	rinse in acetone or 96% alcohol	AXA or CPD method
80% Ethanol or 80% Isopropanol	--	3 weeks	+	+ (fair)	id.	id.	id.
70% Ethanol	+	2 weeks	±	++ (good) (transfer to ethanol 80 or 96%)	swelling/softening if alcohol becomes too diluted	id.	id.
Ethylene glycol/ propylene glycol (+ alcohol) (antifreeze)	++	2 weeks	--	++	swelling/softening if glycol becomes too diluted	re-store in 80-96% ethanol/ rinse in acetone	id. (first re-store in 80-96% ethanol)
Satured salt solution (NaCl)	++	1 week	--	-- (poor)	damage by rinsing	id.	id.
Water + detergent ("soapy water")	++	2 days	--	--- (very poor)	disintegration of specimens	id.	id.
Dry with killing agent (cyanide or dichlorvos)	+	1 day	-	- (brittle + dirty)	high toxicity of agent to humans; add absorbent tissue	relax and clean before mounting	relax and clean before mounting
Dry without killing agent	++	few hours	-	- (brittle + dirty)	mutual physical damage (beetles!); add absorbent tissue	id.	id.

On average traps can be emptied daily (dry collecting), once per week (wet preservation, high season, tropics) or up to once per month (low season). This depends on the preservative used, the number of insects collected per day and the supposed use of the material. If 70% alcohol is used, the material will still be useful for molecular studies if the material is collected every week but it should be separated immediately and transferred to 80% to 96% alcohol. The material should be kept as cool and dark as possible; if the collecting bottle is subhyaline it may be covered by aluminium foil. In general the catch is first cleaned from large butterflies, moths and beetles (check for small insects clinging to them!), followed by pouring off the old preservative and replacing it by 70% or 80% alcohol. A fine sieve could be used to avoid losing minute specimens when the old preservative is poured off. A set of sieves of different mesh size can be used to sort the catch in several fractions, but this requires a lot of fluid and may cause damage to specimens. The sorting can be done by the unaided eye, with a head-lens or in small batches under a binocular microscope. The latter is the best option, but also the most time-consuming.

Safety

Fieldwork has its normal dangers for the researcher: in the tropics the chance of getting insect-borne diseases such as malaria and dengue can be lowered by using bed nets and prophylactic medicines against malaria. Impregnated bed nets are useful but may cause an allergic reaction. Legs and arms should be covered after 5 PM to lower the chance of contact by infected mosquitoes. Leeches are a nuisance but with the use of DEET on the shoes and eventually on leech-socks the problem is limited. The bleeding of the bites can be limited by using small pieces of tissue and the bites should be disinfected after bleeding has stopped. Both in temperate and tropical climates it is important to be aware of poisonous snakes. In case of allergic reaction to stings from aculeate Hymenoptera (*e.g.*, hornets and yellow jackets) an antidote should be taken in the field.

Preservatives used in the traps should be covered with a mesh or fine wire netting if there is a risk of its being drunk by mammals and birds; this is normally only a problem when there is an open reservoir below a flight interception trap. Some chemicals used in the traps, such as cyanide, dichlorvos and deltamethrin, are poisonous or can cause allergic reactions in humans and should be treated with care or avoided. During the processing of the material contact with xylene should be avoided and a fume-hood has to be used; if used outside the laboratory it should be done in a well ventilated room *e.g.*, by opening window(s) or in the open air.

In summary, a top 10 of the "does" and "don'ts" is given in Table 2.

Does	Don'ts
1. good position to block flyways or flight corridor	1. trap in shadow, *e.g.* collector of trap tight to a tree
2. good position for the collector: in the sun between 10 AM and 4 PM	2. leaving trap catches in sunlight after fetching
3. good position at border of habitat(s)	3. trap in habitat with a lot of butterflies (or use coarse mesh at the entrance of the collector)
4. perpendicular to a border when no flyway or flight corridor can be detected	4. bottle of collector filled up completely without free space above preservative
5. back of trap should be straight to guide the insects directly to the collector	5. placement near ant nest
6. monitor fabric near entrance of collector for holes and spider webs	6. use of 96% alcohol when the material has to be transported before sorting
7. clean inside of collector before use	7. trap well visible near places with many human visitors
8. inform local people about the traps and arrange protection with a fence of barbed wire or of chicken-wire netting	8. large traps in low vegetation because of unnecessary long distance to collector
9. reduce amount of alcohol or other preservative before transporting the catches	9. collector made of non-transparent material
10. refresh the alcohol or other preservative the same day after acquiring the catch	10. use of formalin

Table 2. Top 10 of good and of bad practices.

2. Traps with collector at top of trap, using positive phototropism

2.1. Introduction

The operation of the trap is based on the interception of the path of insects by means of a fabric or acrylic vertical or oblique barrier and subsequent positive phototropism. The intercepted insects are attracted by the sunlight to fly or walk to the top of the trap where the collector is situated. In principle, all flying insects are collected but groups with strong positive phototropism, such as most day-active Hymenoptera, Diptera and Lepidoptera, will be most abundant (Fig. 1A, B). Wingless insects and small flying insects may walk up the barrier ("diaphragm") in Malaise traps and the roof in Schacht traps to the collector, but the sampling is much less efficient than for actively flying insects. If small parasitoid Hymenoptera (mainly Platygastroidea, Chalcidoidea and Diapriidae) need to be collected, fine meshed material (mesh size 0.3-0.5 mm) should be used for construction. In most other cases a medium-sized (1.0-1.5 mm) mesh will be sufficient and may be more effective because of less interrupted air movement. The intercepted insects fly or walk to the collector, where they fall into a jar or bottle with a preservative.

2.2. Traps with a central diaphragm

2.2.1. Original unilateral and bilateral Malaise traps

Malaise traps are among the most important instruments for collecting day-flying (and to some degree also night-flying) species of Hymenoptera and Diptera. Other groups are also collected, but in general less efficiently (Figs 1 & 2). The trap is named after the Swedish Hymenopterist, insect and art collector Dr. René Edmond Malaise (1892-1978), who had the first versions made in Burma in 1934. He discovered the principle when he was camping in Sweden because of an opening in his tent where a considerable number of insects were gathered (Malaise, 1937). He proposed three types: a unilateral trap with lateral collector, a bilateral type with a lateral collector and one with a central collector. Even at that time he suggested the use of a framework to hang a bilateral trap in the canopy.

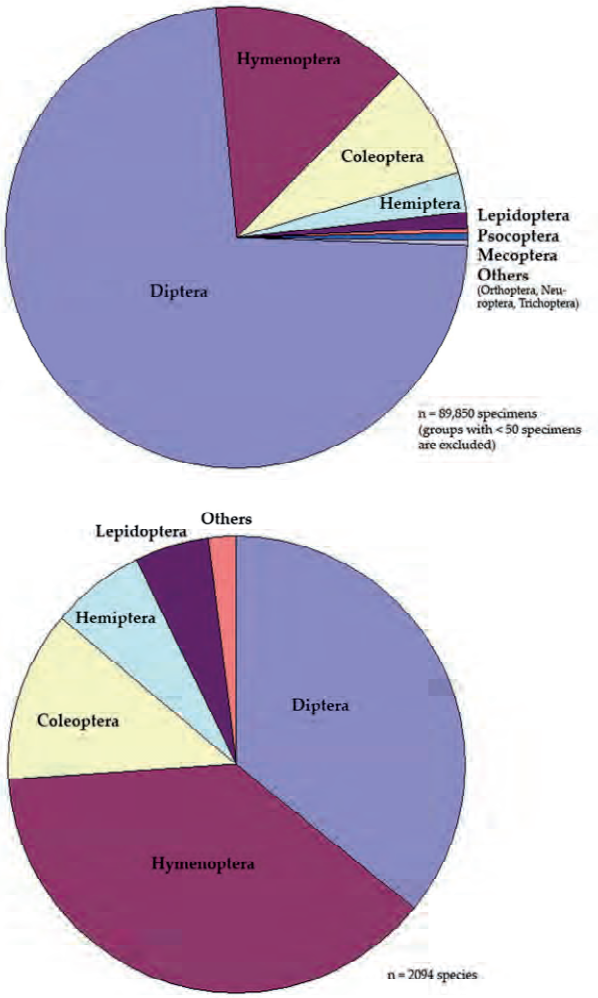


Fig. 1. Pie-diagrams of catches by a Malaise trap (Townes design) during 7 months (17.iii.-28. x.1990) in "De Brand", near Tilburg (the Netherlands; data from van Zuijlen *et al*., 1996).

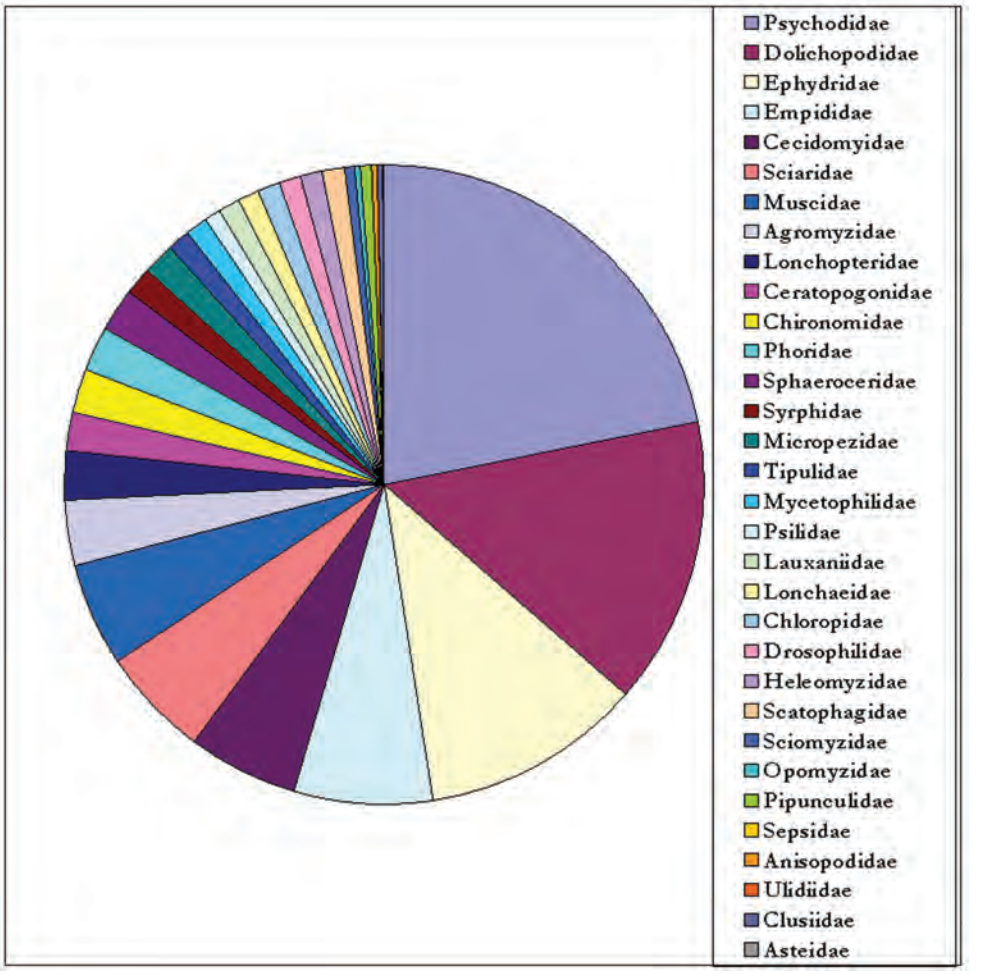


Fig. 2. Pie-diagram of Diptera catches by a Malaise trap in a very humid tropical biotope near a polluted river in SE Asia (P. Grootaert, unpublished).

The bilateral type with a lateral collector (Fig. 3) was used for the Townes design, but with the length of the diaphragm twice the depth of the lateral opening; the latter modification was also suggested by Malaise (1937).

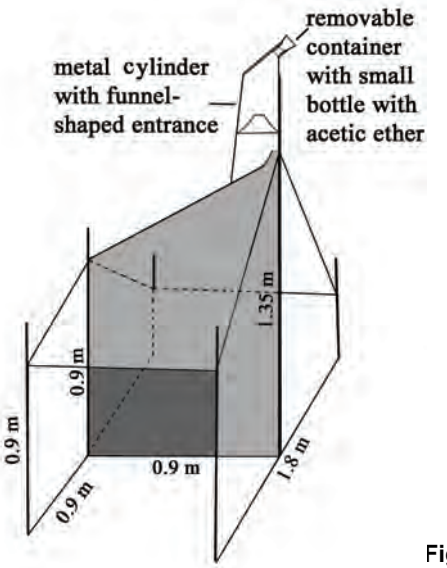


Fig. 3. Original design of bilateral Malaise trap.

2.2.2. Townes' redesign of the bilateral Malaise trap

A major break-through was the simplified design of Dr. Henry Keith Townes, Jr. (1913-1990) which he published in 1962. Townes type Malaise traps (Townes, 1962, 1972; Fig. 4) are the most commonly used design; they have a handy format and low weight, are open at two sides, with a diaphragm of about 1.6 m in the middle as barrier and with one lateral collector with a bottle at the summit. Either black with a white roof or completely black; the efficiency of having the trap white, black or bicoloured is a matter of continuing debate. The first author did not notice negative differences when using all-white traps compared with all-black traps; for some groups like sawflies and Syrphidae the catches seemed even higher than normal when completely white traps were used. A white object may better attract insects normally attracted to plants because it reflects all colours including yellow and green. The bilateral Townes design is vastly superior in collecting as compared with the "Cornell type" (Matthews & Matthews, 1983). The latter is a quadrilateral design with a central collector comparable to the SLAM (= Sea Land and Air Malaise trap) design (see Fig. 13).

Fig. 4. Townes design Malaise trap. (Photo by C. van Achterberg).

The collecting head or collector deserves special attention; the commercially available designs have a horizontal entrance and are degraded by UV light and/or are comparatively complicated and expensive. Hutcheson (1991) proposed a cheap, but not durable, alternative consisting of two polycarbonate bottles glued and taped together with the trap directly connected to the upper bottle. The first author designed in 1979 (Figs 5/6) a simple and durable collector with a 45º angled entrance made of PVC sewage pipe, at the top closed with a circular Perspex cutting and with an opening made opposite to the entrance and covered with a piece of Perspex (van Achterberg, 2009). It is almost indestructible, cheap and not degraded by UV light; the type recently made together with students at the Zhejiang University at Hangzhou is even cheaper to manufacture by using plastic drinks bottles (Fig. 7).

Fig. 5. Large grey PVC collector for Malaise trap (75 mm/45 degrees, 3.2 mm + insert) with 1 l bottle. (Photo by C. van Achterberg).

Fig. 6. Small grey PVC collector for Malaise trap (50 mm/45 degrees, 3.2 mm) with 0.2 l bottle. (Photo by C. van Achterberg).

Fig. 7. White UPVC collector for Malaise trap (Hangzhou type) (75 mm/45 degrees, 3.2 mm + insert) with 1 l bottle. (Photo by C. van Achterberg).

A half-height copy of the Townes design has been used successfully by the first author in relatively windy sites, when the vegetation is low and/or the trap needs to be inconspicuous to avoid theft. The half-height copies catch far fewer butterflies than the normal size and also have a smaller (two thirds the usual diameter) PVC collecting head, as designed by the first author in 1979 (Fig. 6). Large numbers of specimens may be collected and, if properly placed for several weeks or months in the right season, it collects a good sample of the fast and slow flying taxa present. Depending on the size of the trap, but normally from near-ground up to 0.8 m height, there is good sampling of the area.

Townes type traps can be used in nearly every habitat, even if no corridor for placement is available, *e.g.*, boreal tundra. Light-weight designs can be suspended in the canopy. The most commercially sold version of the Townes design has on average a total sampling surface (= sum of surface of both openings) of 3 m^2 (Matthews & Matthews, 1983), resulting in a sampling surface of 1.92 m^2 per m length of diaphragm. The designs are generally fairly weather resistant except under winter conditions with heavy (melting) snow loads on the roof of the trap. The traps are fairly portable and one person can set up a trap, but for large numbers of traps two people will perform much better.

Disadvantages are the cost (€ 100-400 per trap, depending on the design, place of manufacture and quality of the material), the visibility of the trap (they are fairly large objects difficult to hide from monkeys, humans, cattle, etc.), the time

needed to find promising places (preferably a corridor) and the total weight (normally including liquid preservative) if more than a few traps are used. Some of these disadvantages could be diminished by using thick thermo-sealed transparent Nylar film; not polyethylene plastic film, because that would deteriorate too fast in sunlight (Marston, 1965). The collector is made of a simple bag-shaped wire frame, covered with a bag and a second bag with alcohol is taped to it. Another approach is to use an insect bed net as a unilateral trap and add a plastic bag with some alcohol as collector at the top (Butler, 1965).

2.2.3. Malaise traps with two collectors

Gressitt & Gressitt (1962) published a greatly enlarged design; actually two Malaise traps joined at their rear parts, with two summits, each with a collector and a bottle. It results in a large trap (Fig. 8A) with the opening about 2.3 times longer than in the common Townes design: 6 m long in the commercially sold version (www.johnwhock.com). The trap has an opening at one side of 4.5 x 1.3 m, thus for both sides a total of 11.7 m^2 sampling surface, resulting in 2.6 m^2 sampling surface per m length of diaphragm. The migration trap is a modified Gressitt design: insects are separately collected per side to allow determination of the flight direction (Gressitt & Gressitt, 1962; Fig. 8B). The Gressitt design is frequently used for mosquito research. The design is made more complicated by having two collectors, and its large height (about 3 m) will negatively influence the catch of weakly flying and minute Hymenoptera. A recently developed smaller version (Fig. 8B) is lower and easier to place and a version with four collectors is being developed to determine four flight directions.

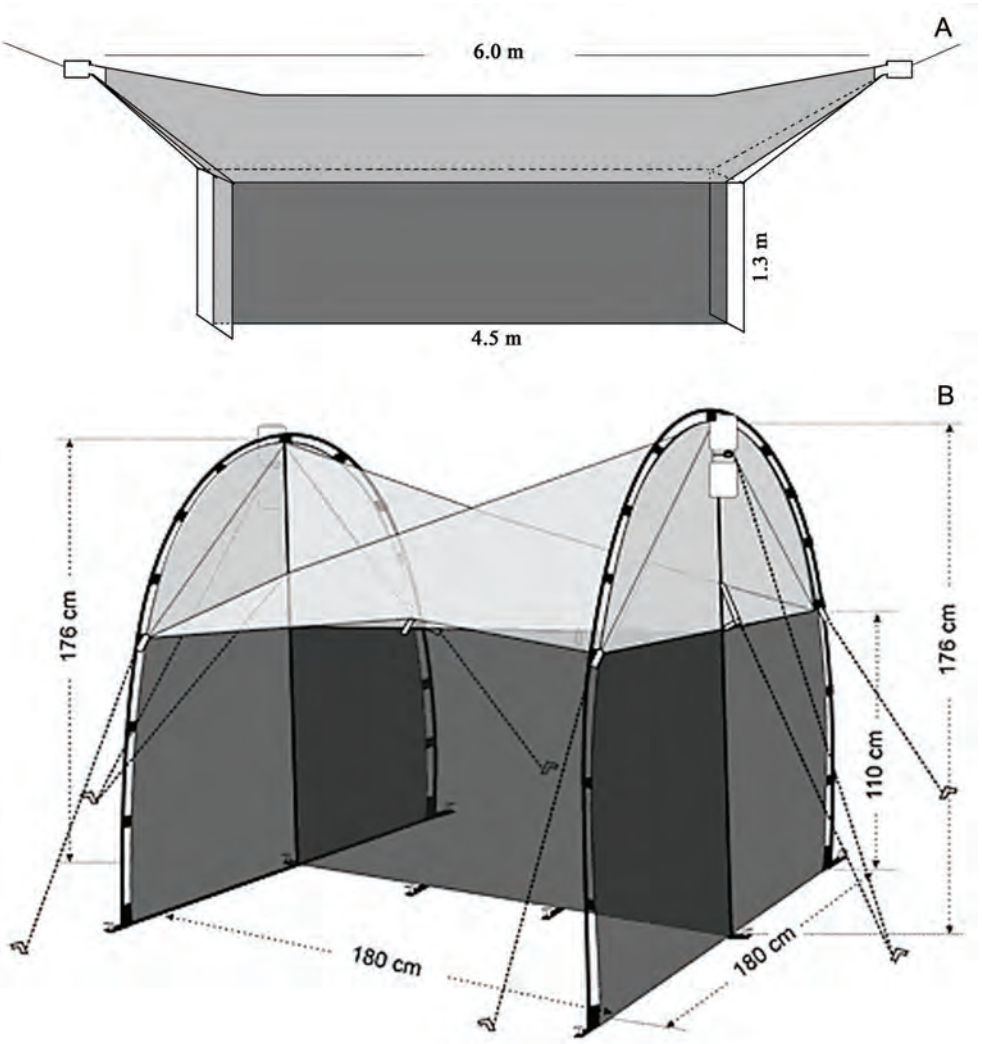


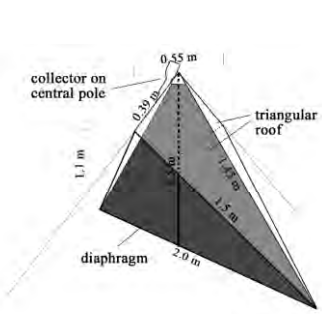
Fig. 8. A. Gressitt design of the Malaise trap; B. Scheme of small Gressitt design ("ez-migration trap") (from: http://bugdorm.megaview.com).

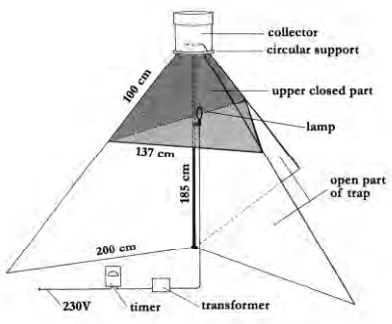
2.2.4. Malaise trap with triangular opening and a central collector

Three versions have been developed: the quadrilateral design (Cornell trap: Matthews & Matthews, 1983), the trilateral design combined with a light trap (Dufour, 1980; Fig. 9) and, recently, a light-weight bilateral design (Figs 10-12) by Mr. J. de Rond (Lelystad). The new bilateral design was aimed at collecting small parasitoid Hymenoptera (especially Bethylidae) in low open vegetation. The sampling surface is 1.1 m^2 per m length of the diaphragm, less than that of the Townes design, but the new design has a simpler construction, has a lower weight and should sample small walking parasitoids better. The first results are promising and the design is probably fairly weather-resistant. Its efficiency might

be improved by having the roof 30 cm wide at ground level (instead of a few centimetres in the prototype).

Fig. 9. Scheme of trilateral design combined with light trap (after Dufour, 1980).





Figs. 10-12. Bilateral Malaise trap with triangular opening and a central collector. Photos and sketch of design supplied by its designer, J. de Rond (Lelystad).

2.2.5. Freestanding, floating or hanging polyester fabric quadrilateral traps with a central collector

For use at a water surface or in the canopy the special SLAM (= Sea Land and Air Malaise) design has been developed (Fig. 13). It is freestanding (no supporting rods) and is easily erected by one person. It may be combined with a bottom collector(s) to become a hybrid between Type I and II flight interception traps (Fig. 14). The design with one bottom collector (Fig.15) is suitable for sampling different heights from ground level to the top of the canopy by attaching several free hanging traps to each other.

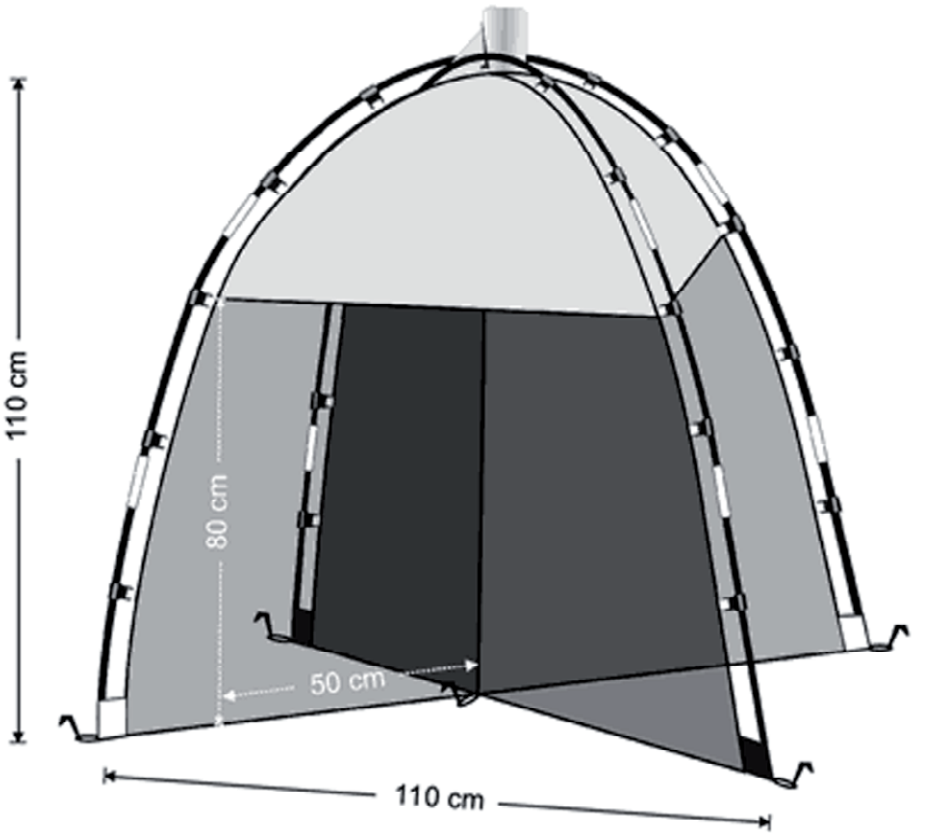


Fig. 13. Scheme of quadrilateral SLAM design (from: http://bugdorm.megaview.com).

Fig. 14. Hybrid SLAM design with collecting trays (from: http://bugdorm.megaview.com).

Fig. 15. SLAM design with a bottom collector (from: http://bugdorm.megaview.com).

2.2.6. Epsilon tsetse fly unilateral trap

This is a triangular fabric trap that attracts flies because it is contrastingly coloured. The oldest design was a box-type trap for collecting eye gnats and blow flies (Parman, 1931). It is easy to place and to remove for sampling tsetse fly populations (for details see www.nri.org and Fig. 16).

Fig. 16. Epsilon tsetse fly trap (from: www.nri.org).

2.2.7. Bilateral freestanding trap with rounded roof

Recently, a modified Malaise trap was developed with a rounded roof, no supporting rods and with a screen to prevent butterflies and large moths from entering the collector (Fig. 17). It is easily erected by one person and may be more weather-resistant than the Townes design. The sampling surface ratio of this design is 2.0 m^2 per m length of diaphragm, thus slightly improving the Townes design by about 5%. The incomplete diaphragm may have a negative influence on the efficiency of the trap, especially for larger arthropods. (for details see www.//http.bugdorm.megaview.com).

Fig. 17. Malaise trap with rounded roof design. (Photo by C. van Achterberg).

2.2.8. Redesigned bilateral Malaise trap

The sampling surface of the most frequently used type of Malaise trap, the Townes design (see above), is comparatively low. To enlarge the sampling surface (and probably its efficiency) the first author (van Achterberg, submitted) proposed an improved design to considerably enlarge the sampling surface without losing all the advantages and the simplicity of the Townes design. The redesign is based on four approaches. First is to direct the rear corners of the roof upwards (they are down in the Townes design), second to place the transverse sections more outwards (Figs 18 & 19), third to use a somewhat longer and higher diaphragm and finally to use the improved collector (see under Townes type).

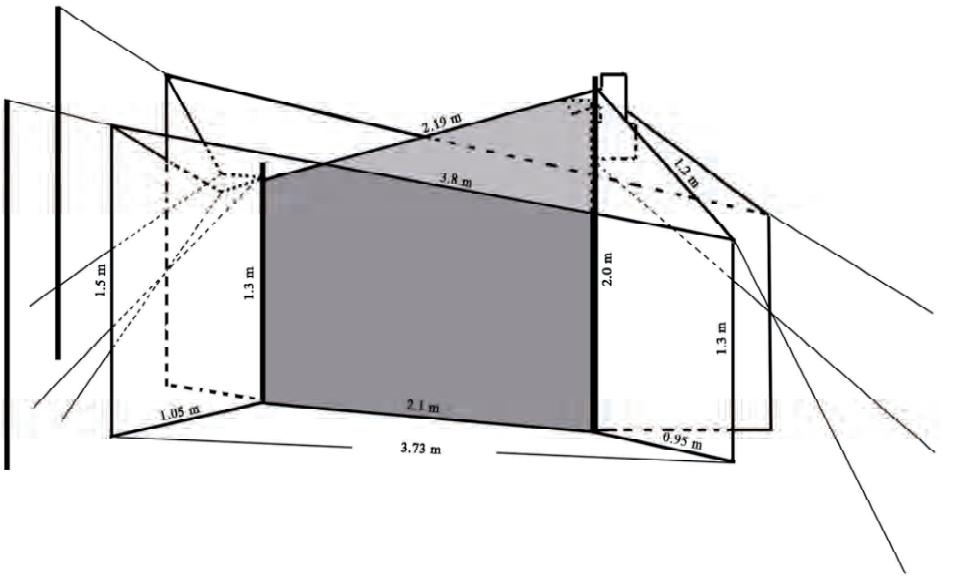


Fig. 18. Scheme of the redesigned Malaise trap.

Fig. 19. The first version of the redesigned Malaise trap. (Photo by C. van Achterberg).

The new design has a sampling surface ratio of 2.73 m^2 per m length of diaphragm, thus improving the Townes design by 42%. The ratio is similar to that of the Gressitt trap but the latter is twice as high and, therefore, less efficient if the height is taken into account. In addition, the Gressitt trap has two collecting heads and is heavier. The first impression of the catches by the new model is that the amount of specimens of some groups is about doubled, but the improvement differs per family. The trap has not been used for long enough to give comparative data yet. The new model will be commercially available in the near future; please contact the first author.

2.2.9. Freestanding quadrilateral Perspex trap with a central collector

Mr H.J. Vlug (Scherpenzeel) designed a small freestanding trap of two PMMA (= PolyMethylMethAcrylate, Plexiglas or Perspex) plates, triangular at the top, one indented at the base, the other at the top, and connected perpendicularly. On top of the plates there is a polyester fabric roof with a small central collector. This small trap is useful for collecting in low vegetation, but it is comparatively heavy and the construction of the collector is rather complicated.

2.3. Traps without a central diaphragm

2.3.1. Schacht trap

The Schacht trap (Schacht, 1988) was designed by Mr. Wolfgang Schacht (research associate at the Diptera section of the Zoologische Staatssammlung München). The trap is based on the idea that insects hitting an oblique surface will walk up the surface and, in the case of the trap, to the collecting bottle (Fig. 20). It is a rather new and little known trap, originally designed for collecting Diptera, but the Schacht trap may be recommended also for collecting Hymenoptera in addition to the use of Malaise traps. Although it is less effective, considering its size and the number of insects collected, it better collects small insects that tend to walk all the way up to the top, probably also because it works partly as an emergence trap. There is no diaphragm because it would deter insects; up to 80% of Hymenoptera flying into a Malaise trap may escape according to the late Dr. Townes (pers. comm.). The first results show that the Schacht trap is an excellent trap to sample a large area as a kind of emergence trap and it attracts (because it is a large white object) and intercepts a large variety of Diptera and Hymenoptera.

Fig. 20. Schacht trap (5 m long version). (Photo by C. van Achterberg).

2.3.2. Cheesecloth flight trap

This is a cage trap designed by Mr H.B. Leech (1955) for collecting Diptera and parasitoid Hymenoptera in large numbers. The trap has an equal-sided frame of 1.8 m covered with cheesecloth and with a door on the lower part of one side. The opening should be facing north or east; it traps insects in a way similar to, for instance, a garage with an open door facing north and a closed window at the other end. Herting (1969) used the same principle, but with dark textile for the sides and roof with a large opening at the back. The transparent front is against the wind; the trap needs to be checked several times per day and the numbers are rather low.

2.3.3. Manning trap

About thirty years ago the Manning trap was developed for collecting horse flies (Tabanidae). The dark (preferably black) central ball hangs free from an open box with a transparent cover with a central collector at the top (Fig. 21). The ball is warmed by the sun and is moved by the wind, mimicking a target for the flies. After discovering the lack of a suitable host they fly off to the sun and are intercepted by the upper part of the trap. Recently, the "LOER-2007" or "dazenval" (Dutch for horse fly trap) was designed by Mr. F. van Dungen (Heesch) for the same purpose. It has a massive black ball to attract the flies and is half covered by a white fabric hood; the flies are intercepted by the hood and

die in the central collector from heat on sunny days (Fig. 22). For collecting 200-400 horse flies per sunny day the ball should be far from ground level (the total trap height is about 3 m) and the trap should be placed near woodland edges and in the sun.

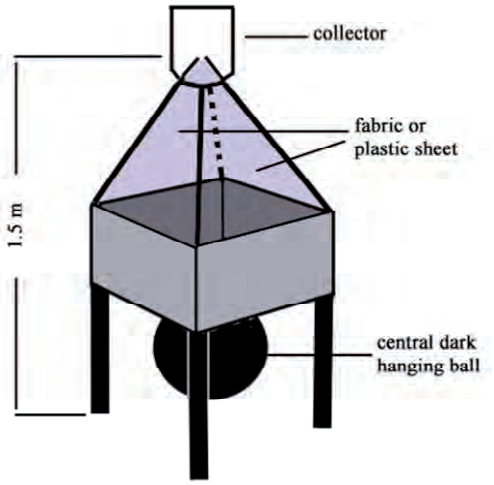


Fig. 21. Manning trap for collecting horse flies.

Fig. 22. Ball and hood (LOER-2007) trap for collecting horse flies. Left collector with dead flies (From: www.dazenval.nl).

3. Traps with collector at the bottom of the trap, using negative phototropism

3.1. Introduction

Many insects associated with the bottom layer of a micro-habitat fly just above ground level and fall to the ground when they collide with a vertical object. Flight interception traps with a bottom collector make use of this behaviour to trap insects, especially Coleoptera (Fig. 23). They are most effective at trapping relatively heavy, slow-flying insects such as beetles, cockroaches and crickets, groups that are hardly or not collected in Malaise traps.

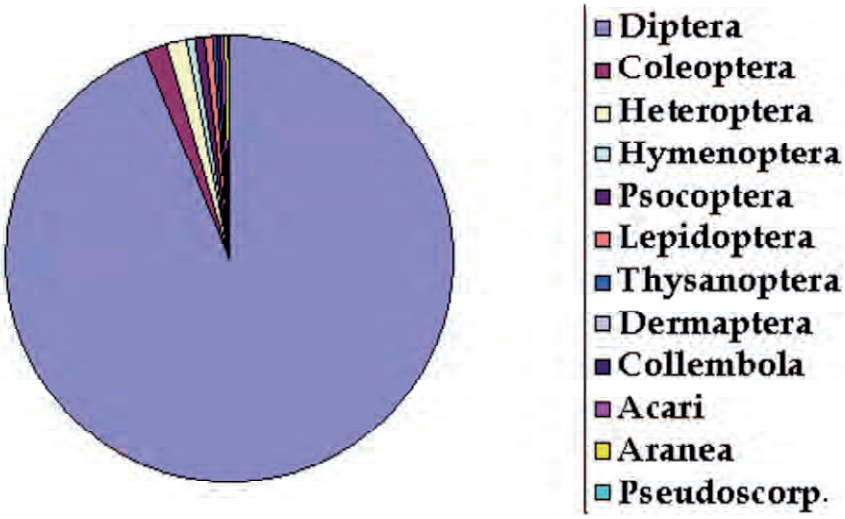


Fig. 23. Pie-diagram of catches by a window-pane interception trap in the temperate climate zone.

3.2. Transparent bilateral flight interception traps

A vertical screen of glass ("window flight trap" of Chapman & Kinghorn, 1955), Perspex or transparent plastic, such as PVC cling film, stretched between two stakes and a trough (or row of *e.g.*, ice cream containers) with preservative fluid (*e.g.*, water with propylene glycol and detergent) is arranged below its bottom edge (Figs 24-31). This is sometimes called a "window trap", but this name is applied to all kinds of unrelated traps, and therefore, the name "windowpane trap" is preferred for the framed types with glass, plastic or Perspex. A cover may be placed on top of the trap to avoid flooding by rain (Figs 25 & 26) and small holes may be made near the rim of the reservoir to allow overflow from rainfall without loss of trapped material. Nijholt & Chapman (1968) proposed a trap without fluid to collect living insects. The conical trough under the screen is open below and connected to a cylinder. The cylinder has a clear plastic bag or a removable glass jar at the end for collecting the live insects. Chapman & Kinghorn (1955) suggested the combination with a light source and the use of

transparent plastic screen without a frame. The presence or absence of a frame did not significantly influence the avoidance of the trap by Colorado beetles (Bouteau, 2000), though other tests have not been reported. A modified design has been used for sampling the forest canopy (Hill & Cermak, 1997; Fig. 26). If using collecting fluid is a problem the vertical screen can be made sticky and the insects adhere to the screen (sticky flight interception traps).

Fig. 24. Perspex bilateral window-pane interception trap. (Photo by P.S. van Wielink).

Fig. 25. Perspex bilateral window-pane interception trap with a plastic roof. (Photo by P.S. van Wielink).

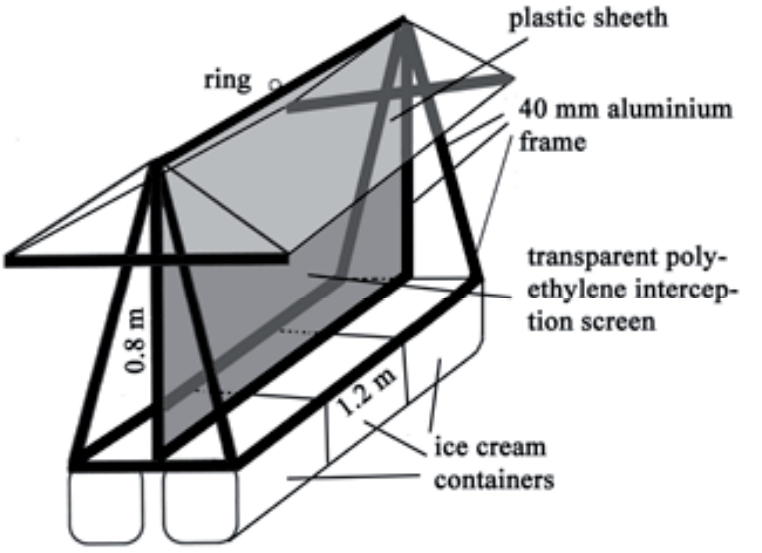


Fig. 26. Canopy flight interception trap with a polyethylene screen (after Hill & Cermak, 1997).

3.3. Transparent quadrilateral flight interception traps

Perspex window-pane interception traps with four collecting sides (= quadrilateral) are easier to place because of the 360 degrees collecting angle. This might either stand on four rods over an open reservoir with fluid (Fig. 27), or be constructed with an integral collector under it (Wilkening *et al.*, 1981). The latter version can be hung over a stack of wood or in a tree (Fig. 28) or combined with an upper collector (Wilkening trap; Fig. 29). Hines & Keikkenen (1977) and Furnes (1981) used a non-transparent cylinder for interception, *e.g.* one made of 33 cm diameter aluminium pizza plate.

Fig. 27. Perspex quadrilateral window-pane interception trap. (Photo by P. Grootaert).

Fig. 28. Suspended Perspex quadrilateral window-pane interception trap with a collecting bottle at the bottom. (Photo by B. Mériguet).

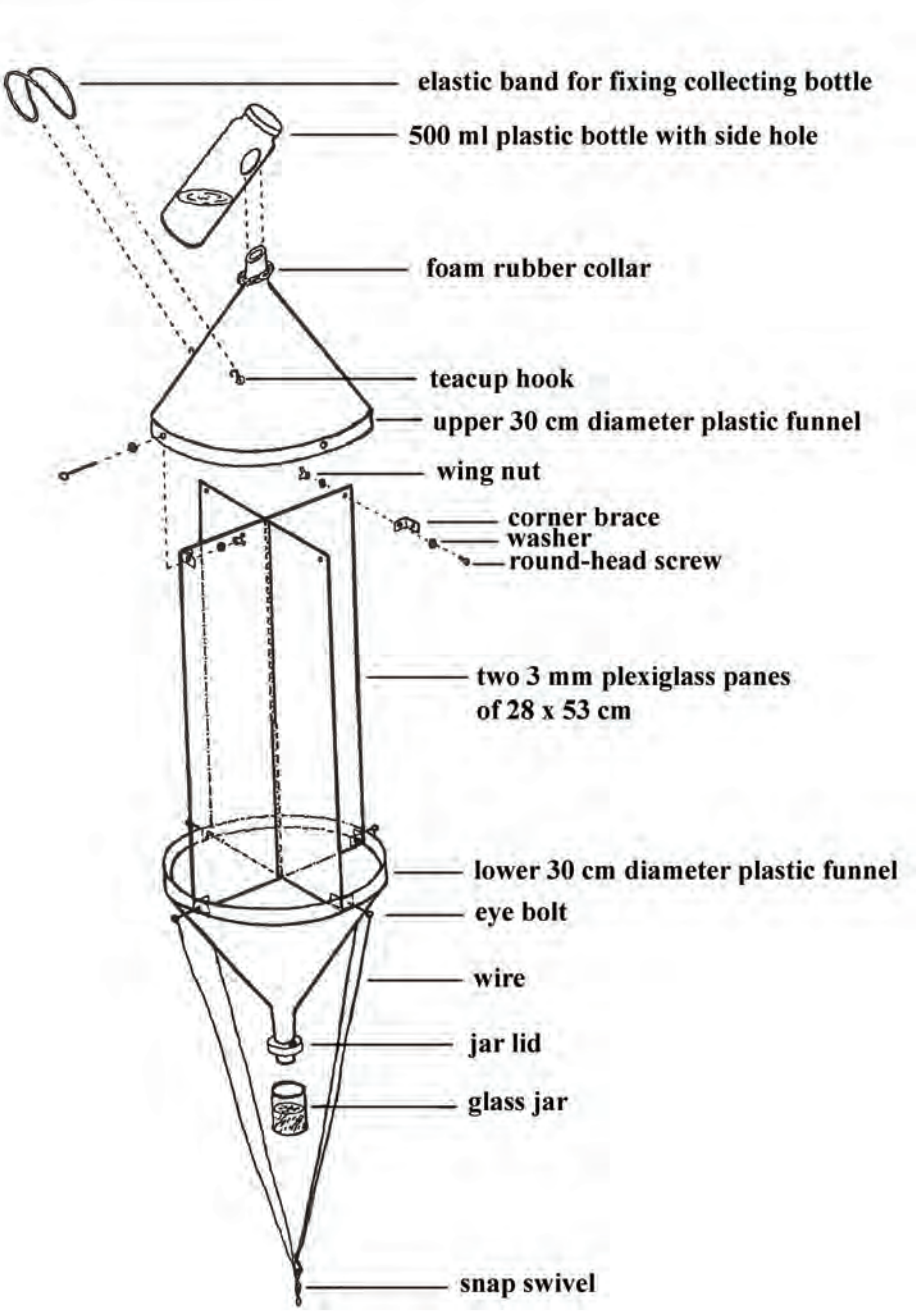


Fig. 29. Scheme of Wilkening trap (after Wilkening *et al.*, 1981).

3.4. Fabric screen interception traps

A vertical screen ("diaphragm") of fabric is stretched between two poles (Fig. 30). Trays, yellow pan traps or a plastic trough filled with water, a preservative and some detergent or with an antifreeze-alcohol solution are placed under the screen (Fig. 31). The disadvantages are the necessity of a flat horizontal area without protruding roots, stones, etc., the habit of some beetles to cling to the fabric with their claws and walk away, the need for transport of sufficient quantities of fluids, the risk of flooding by showers, the drinking of the fluids by vertebrate animals and the necessity to collect the captured insects at comparatively short intervals. Placing a plastic cover on top of the trap may avoid flooding by rain and using a bitter additive could avoid the drinking of the collecting fluid by animals. The EPPS biting fly trap (http://www.horselineproducts.com; Fig. 32) is designed for collecting flies, especially biting flies, near farms by providing a large, contrasting surface area and two semi-transparent areas (the deflectors). Many biting flies are attracted to large objects of contrasting colour (mimicking potential hosts like cattle, deer, and horses) and tend to circle around the host. Flies probably see the deflectors as open spaces, try to fly through, hit the deflectors, fall into the soapy water of the trays below and drown.

Fig. 30. Fabric interception trap (with separate trays). (From: http://www.inbio.ac.cr/papers/manual_coleoptera).

Fig. 31. Fabric interception trap with several small trays in a large tray. (From: http://mississippientomologicalmuseum.org.msstate.edu).

Fig. 32. EPPS fly trap using soapy water. (From: http://www.horselineproducts.com).

3.5. Trays below the diaphragm of a Malaise trap and use of insecticides

Yellow tray(s) with water, propylene glycol and a bit of detergent or a saturated salt solution are placed below the diaphragm of a Malaise trap (Figs 33 & 34; Robert, 1992). Insects (especially beetles) that bounce off will fall down into the trays with preservative. Masner & Goulet (1981) proposed the application of insecticide (pyrethroid: deltamethrin) to the diaphragm of the trap to make the collecting of small insects (especially Hymenoptera) more efficient. Altogether these measures will about double the collecting by a Malaise trap according to Campos *et al.* (2000). The disadvantages are the same as for fabric screen interception traps, but the results are much better.

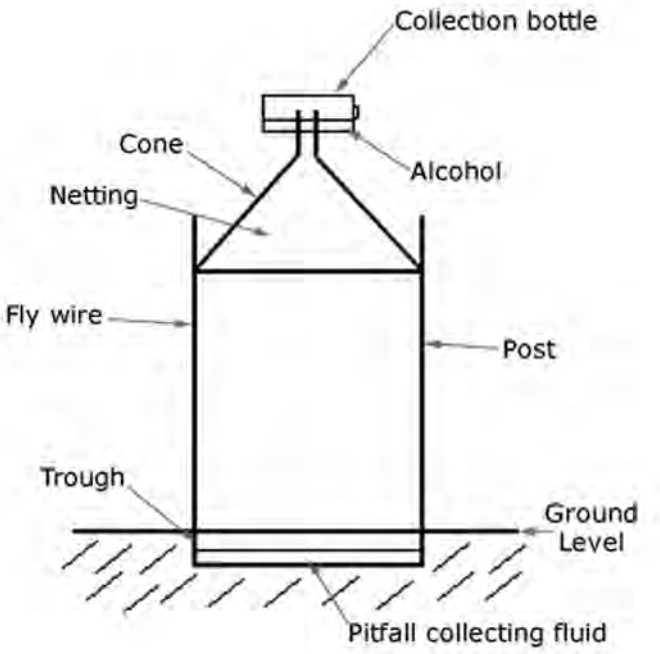


Fig. 33. Hybrid unilateral trap with rear diaphragm. (From: http://www.ento.csiro.au/education).

Fig. 34. Hybrid trap with the central diaphragm sprayed with insecticide and with a large yellow reservoir below it. T = top collector; B = bottom collector (From: Campos *et al.*, 2000)

4. Direct collecting

4.1. Suspended plastic bottles

A low-cost trap can be made from an array of 4 transparent, 2-liter polycarbonate beverage bottles suspended by their caps in a 2 x 2 array centred on the underside of a 20 x 30 cm piece of 1.3 cm thick exterior grade plywood. The plywood platform rests on four 2.5 m long metal rods; this conformation stabilizes it in windy conditions and protects it from rain (Fig. 35). The bottles each have a 17 cm wide and 13 cm high strip in its side removed to allow the entry of arthropods. When viewed from the side, the area of the opening in each bottle is 10.5 x 13 cm. The intact bottom of each bottle serves as a reservoir for about 200 ml of collecting fluid (Carrel, 2002). The preliminary results are similar to a glass or Perspex windowpane trap (*e.g.*, Dobony & Edwards' (2001) Perspex trap). The results might be improved for Hymenoptera and some other groups by painting the part of the bottle opposite to the opening yellow or white and the trap could be protected by wrapping chicken-wire netting around it.

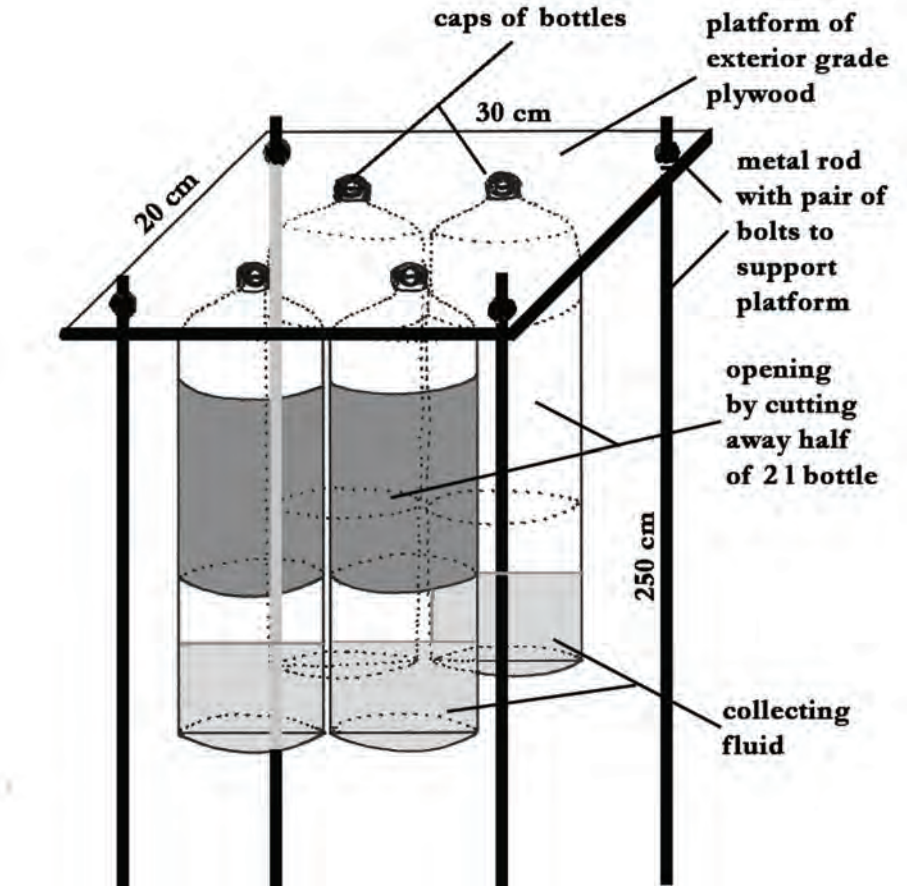


Fig. 35. Scheme of the plywood platform with four suspended transparent polycarbonate bottles.

4.2. Suspended sticky traps

These are usually yellow or blue plastic (polyethylene) or cardboard panels of 20 x 40 cm with a rain-resistant wet-sticky type of glue (*e.g.* Tangle) applied to both sides. The glue may be baited with pheromone to promote the collection of a certain group. The traps may be transparent, white or coloured: yellow for whiteflies, aphids, moths, leafhoppers and leaf mining Diptera and light blue for thrips. Also other groups will be collected by interception. The traps are widely available because they are used as part of integrated pest management programs in horticulture, being a non-toxic way to control and monitor insects. The glue does not dry out and the traps will last until the surface area is completely covered with insects (but they are of course prone to dust). Several traps are often suspended among vegetation, including the canopy. Recovering valuable specimens is problematic; the glue has to be resolved by warm kerosene, the specimens need extensive cleaning before preparation and fragile specimens will often be damaged. Although the low price of the traps and their easy use is a potential advantage, they are not usually a good method for specimen collection.

5. Acknowledgements

The first author thanks Prof. Dr. Xuexin Chen, Dr. Jiangli Tan and Mr. Shujun Wei (Hangzhou) for their help in assembling the new collector, Mr. Jeroen de Rond (Lelystad) for contributing illustrations and data on his recently developed trap, Mr. Paul van Wielink (Tilburg) for supplying illustrations of the windowpane traps and Mr. Theo Peeters (Tilburg) for providing information about the "dazenval", Mrs Josephine Cardale (Canberra) for kindly supplying details of CPD.

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7. Appendix : Preparation of Hymenoptera and Diptera from alcohol

Most groups of unprepared Hymenoptera are usually stored in 70% alcohol. This is a safe method, but there are some hazards; dilution of alcohol (of whatever strength) in which specimens are stored should be avoided, otherwise a precipitate may form on the specimens. The specimens should be transferred to fresh 70% alcohol after being collected. Be sure that it is 70% or higher! Lower percentages often cause precipitation of dissolved fats, etc. and spoil the specimens. Never put vials containing specimens in alcohol in sunlight (UV-radiation, temperature!) and store samples in alcohol as cool as possible; to put them in the freezer is no problem. Dried out alcohol samples should not be discarded (van Cleave & Ross, 1947); with a 0.25-0.50% aqueous solution of a commercial grade of trisodium phosphate specimens are restored in a few hours (at 35° C in about one hour)!

The preparation of insects stored for a considerable time in 70% alcohol can be done well by three methods:

1. The most elaborate and most costly method is critical point drying (CPD; Gordh & Hall, 1979). The specimens are transferred to a small "basket" (a small numbered mesh container), which restricts the method only to small specimens. The results for *e.g.*, Eulophidae (Hymenoptera) are much better than air drying as the heads do not collapse. Freeze-drying is a similar method.

2. The Alcohol/Xylene-Amyl acetate-method (AXA); a less expensive and less time-consuming method than critical point or freeze-drying and the results are usually comparable. It is also suitable for large Hymenoptera and large quantities can be treated at once. It is based on the alcohol-ethyl acetate method used for the preparation of Syrphidae in the Canadian National Collection of Insects at Ottawa (Vockeroth, 1966). The ethyl acetate was replaced by amyl acetate by the late Dr. W.R.M. Mason (working at the same institute) for the preparation of Braconidae from 70% alcohol. The first author successfully used the modified version explained below during over 30 years for Braconidae and other Hymenoptera in the collection of the National Museum of Natural History (Naturalis) at Leiden.

The alcohol is poured off (carefully, to avoid loosing specimens) and the vial is filled with a mixture of 40% xylene and 60% alcohol made out of a concentration of 96% alcohol. After 1-3 days this mixture is poured off again and replaced by amyl acetate; do not use any kind of (plastic) vials that are susceptible to amyl acetate and avoid inhalation of the chemicals or contact with the skin. The insects can be prepared after 1 day (or longer) in the amyl acetate. With forceps the specimens are taken from the fluid and with the wings stretched out laid on any kind of slowly absorbing paper (*e.g.*, 180-250 grivorite paper). If the wings are not well stretched out, the procedure should be repeated or a drop of fluid is added with the tip of the forceps. After about 15 minutes the specimens are ready to be pinned or glued. Pinning should be done not later than 25 minutes after taking out of the amyl acetate to avoid losing legs or its head during pinning. An alternative is to put a limited number of specimens in a thin layer of amyl acetate and let it evaporate.

3. Heat-assisted air-drying from acetone (Trumen, 1968; Walpole *et al.*, 1988) is an easy and fast method for specimens preserved in alcohol for less than one year. The specimens may be removed from the 70% alcohol and kept for a few hours in water, followed by a few hours in acetone. If the specimens are cleaned before by rinsing them in 70 or 80% alcohol the results are generally slightly less than of the AXA method or CPD. However, according to Ware & Cross (1989) and van Noort (1995) the results are the same for some groups of Chalcidoidea. The direct slow drying of the alcohol (Noyes, 1982) gives much worse results, especially the wing venation is often less visible because of distortion of the wings. The latter method lowers considerably the quality of the material of relatively weakly sclerotised, delicate or small specimens (like Braconidae, Chalcidoidea and Diptera) and should be avoided unless the specimen is collected within a few hours. However, for many relatively robust and large Ichneumonidae, rinsing in 96% alcohol and drying onto absorbent tissue (which will often enable the wings to dry flat) can be the most practical way to achieve fairly good and consistent results. Some specialists advocate the use of HMDS (hexamethyldisilazane) for insects (*e.g.*, Heraty & Hawks, 1998), but the chemical is expensive (about € 900 per kg plus shipping costs), and in some trials with Braconidae and Chalcidoidea the results were less good than those obtained with the CPD, AXA or acetone methods. In addition, HMDS has an unpleasant smell, is highly flammable and has a strong corrosive effect on eyes and to a lesser degree on skin and mucous membranes.

8. Glossary

AXA method: the use of xylene and amyl acetate to prepare material from alcohol.

Bilateral trap: trap with two open sides or 180º collecting angle.

Central collector: collecting device situated at centre of the trap.

Cornell type Malaise trap: small quadrilateral Malaise trap.

CPD: critical point drying method.

DEET: an insect repellent: N,N-diethyl-meta-toluamide.

HMDS: hexamethyldisilazane.

Lateral collector: collecting device situated at one of the sides of the trap.

PMMA: Perspex or polymethylmethacrylate.

PVC: polyvinylchloride or polychlooretheen (PCE).

Quadrilateral trap: trap with four open sides or 360º collecting angle.

SLAM: Sea Land & Air Malaise trap design.

Unilateral trap: trap with one open side or 90º collecting angle.

UPVC: unplasticised polyvinylchloride.

Chapter 18

Preserving and Specimen Handling: Insects and other Invertebrates

by

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Abstract

Up-to-date field techniques for preserving and handling invertebrate specimens with special emphasis on insects are summarized. Different preservation techniques for specimens sampled for molecular and morphological analyses as well as for natural history collections are presented and hints for scientific labelling of specimens in the field are given. Fluid fixation of molluscs, annelids, nematodes, and plathelmints are briefly discussed. Fluid or dry preservation of insects and other arthropods depends on the purpose of the field work and on the taxonomic groups. The most commonly used killing agents, fixation fluids and sample containers are discussed. Direct pinning and card mounting of insects in the field is explained and hints for transport of specimens are given. The recipes of frequently used reagents and solutions are listed in an appendix.

Keywords: specimen sampling, killing, labelling, transport, fluid preservation, dry preservation, direct pinning, card mounting

1. Introduction

This chapter will discuss the steps following immediately after the collecting event. The first consideration is what is the purpose of the fieldwork, *e.g.,* what is supposed to be done with the collected invertebrate specimens. If the specimens shall not be kept alive for experimental studies or for rearing larvae to adults (this will not be dealt with in this chapter) the first logical step is the killing or in some cases the narcotisation of the specimens. If traps like Malaise traps, flight interception traps, yellow pan traps or pit fall traps have been used this step can be skipped in most cases as the specimens are normally killed by a fluid with which the collecting containers in the traps were filled. When the specimens have been collected alive it must be considered if the specimens will be used as a whole, *i.e.* as voucher specimens in natural history museums or if they shall wholly or partly be used for molecular or morphological investigation.

2. Specimen sampling and labelling

2.1. Specimen sampling for molecular analyses

Molecular analyses, such as DNA sequencing, require particular considerations that preserve DNA and are only briefly considered here. Usually the best option to preserve the DNA for long time storage is to transfer the specimens to high percentage ethanol (95-99%) which serves as both, as killing and fixation agent. A second option is to keep the specimens alive during transport and have them fresh frozen in the laboratory. This allows storing suitable DNA for decades. The biggest enemy of DNA is humidity so long time storage in low percentage ethanol (70%) should be avoided as well as leaving the specimens in moist atmosphere. Specimens preserved in ethanol should be put in dark and cool conditions as soon as possible and not left out in daylight during fieldwork (or in the laboratory!). This applies to all invertebrate specimens sampled in ethanol whether they are to be used for DNA studies or not. If only low percentage ethanol is available it may a better option to kill the specimens with a killing agent, let them dry quickly and store them (or just selected body parts of them) in the freezer and/or high percentage ethanol in the laboratory. Even if the specimens are stored in dry collections they usually allow extracting suitable DNA for ten or more years (in some cases even hundreds of years) but it seems that the success rate decreases significantly over time.

2.2. Specimen sampling for morphological analyses

If specimens shall be used for morphological investigation (*e.g.*, anatomical dissections, thin-sectioning) it is generally most appropriate to kill and store them directly in a fixation fluid. The fluid normally depends on the taxonomic group and/or the morphological analyses. Specimens that will be used for histological work can be preserved in a number of different fluids that often contain formalin. Fluids like Kahle's or Bouin's solution (Appendix 1) are the best choice for insect larvae as they fix tissues. Kahle's solution also prevents larvae from discoloration while Bouin's solution may change the colour of larvae to light yellow. Before formalin is used it should be considered that it contains

formaldehyde which cross-links proteins and makes tissue samples unusable for DNA extraction.

2.3. Specimen sampling for natural history collections

In the majority of cases specimens are collected to be stored in natural history collections for documentation and research. Even though this does not preclude that parts of the specimens may still be used in future molecular or morphological studies, the primary purpose of the fieldwork is to yield specimens that should be preserved and stored as a whole. The question that arises is if the specimens are to be stored in a dry or a wet collection (*i.e.* usually an ethanol collection). In most cases specimens that are to be stored in an ethanol collection will already be killed and fixated in ethanol directly in the field (70-80% is the standard ethanol concentration). It can be suitable to add a small amount of glycerol to the ethanol, which makes the specimens less stiff. Also glycerol does not evaporate which can be an advantage when containers do not close hermetically. However, use of glycerol should be avoided for small winged insects, such as Micro-Hymenoptera as it complicates the subsequent dry-mounting of these specimens. Glycerol softens the wings in small insect specimens excessively, so that they will not stay flat when specimens are air or critical point dried and card-mounted. Only for small winged insects that need to be slide-mounted (*e.g.* Thysanoptera), glycerol-ethanol solutions are a good option. Ethanol vials should be completely filled which makes specimens less prone to damage during transport. Even small air bubbles that slosh around in the vials can cause damage to very fragile specimens, so special care should be taken to minimize these risks in the field. It should be considered that glass vials that are completely filled with ethanol may crack or even explode in the hold of an aeroplane. With plastic vials these problems can be overcome but it is still useful to seal the screw-cap of the vial with stripes of Parafilm® as it may become loose or undone during transit. An authorization is needed to transport ethanol in an aeroplane and therefore dry storage of specimens (see 3.3.4) during transit is more advisable. Specimens that are to be deposited in a dry collection are normally killed by a gaseous killing agent and stored dry before they are further processed (*e.g.,* pinned and mounted). Keeping and storing specimens dry in the field usually requires more care from the collector as specimens are more fragile and prone to damage compared to specimens preserved and transported in a fluid fixation agent. This is even more severe when specimens are completely dried, which can occur within a few hours on a hot and dry collecting day. Especially dry insects are very delicate and care must be taken to prevent specimens from loosing legs, heads or antennae during transport. Many collectors therefore transport the specimens in a moist atmosphere, which can be a plastic box that is laid out with wet tissue. A few drops of thymol-camphor solution (Appendix 1) or a few crumbs of crystalline thymol should be added to the tissue to prevent the specimens from moulding. If smaller specimens numbers are collected it may also be appropriate to pin the specimens directly after collecting (*e.g.,* in the field or immediately after in the hotel or field station) which secures specimens and facilitates subsequent preparations. Special transport boxes can be obtained from entomological suppliers. Also in these dry boxes it is appropriate to add thymol as larger specimens that cannot dry fast may get mouldy.

2.4. Labelling

Even experienced biologists tend to inappropriately label specimens so this step needs special attention as biological specimens loose their significance for research and documentation if they are not or insufficiently labelled. Labelling should be done in the field, directly after collecting the specimens or after emptying the traps. It can be convenient to prepare the labels in advance and already print parts of the information (*e.g.*, parts of the locality data, name of collector) beforehand and just add the specific data (*e.g.*, date and altitude) in the field. External labelling of tubes or transport boxes can be useful but does not replace a proper labelling of the individual specimens or samples inside the respective container. The most widespread mistake during fieldwork is to just add numbers to the specimens and to list the collection data on separate sheets. Even though the collector has strong intentions to properly label his samples "some when" after the fieldwork there is always a high risk that this will never happen and that the collected specimens will loose their scientific value.

It is crucial that specimens are labelled with all necessary collection data:

- **Locality** (Country, Province, nearest City, Region)
- **Name of project** (if available)
- **GPS data** (if available)
- **Altitude**
- **Collecting method**
- **Date of collection**
- **Name of collector**

Further data (*e.g.,* habitat type, host plant, weather, and temperature) should also be added on (an) additional label(s). The golden rules (Table 1) should be followed to minimize the risks of mixing samples or loosing locality information.

Labels for dry specimens should be written with water proof pens or pencils. Labels for ethanol vials should preferably be written with ethanol-proof ink, *e.g.*, Micron archival ink pens (SAKURA corp.) or alternatively with a pencil. Laser printed labels will not last in ethanol and should not be used. Wet preserved samples should be generally labelled on tight paper which is not negatively affected by the fixation agent. Handwritten labels can later be replaced by proper type-written labels in the laboratory but care needs to be taken that spelling mistakes are avoided. Long-term storage and viability of ink on collection labels is a big challenge for curators of natural history collections and cannot be addressed in this field manual. However, every collector should make sure (prior to collecting) that long term storage of his natural history specimens and the necessary curatorial care can be guaranteed by the respective institute.

Rule 1	Always label the specimens and add the collection information to the specimens immediately (*i.e.*, directly in the field).
Rule 2	Preferably every specimen gets an individual label, but if this cannot be achieved due to high specimen numbers at least every sample gets an individual label.
Rule 3	A sample contains only specimens which have identical collecting data and which are clearly separated in an individual container from the other samples.
Rule 4	If specimens are pre-sorted into smaller samples every sample needs to get a proper label.
Rule 5	Labels are always placed inside the vials, labelling the vials just from the outside is insufficient.
Rule 6	Numbering of samples does not replace locality labels and may later result in confusion and loss of information.

Table 1. The six golden rules for labelling scientific specimens in the field.

3. Invertebrate taxa

The right treatment of collected invertebrate specimens is not only dependent on the purpose of the collecting (see 2.1-2.3) but also on the invertebrate taxon and its life history stage. Soft bodied invertebrates generally require fixation as they suffer from shrinkage if air-dried while hard bodied, sclerotized invertebrates can often be air-dried and may even be damaged if put in ethanol. However, there are many exemptions from this rule and many taxa require a special treatment which made it necessary to devote a separate chapter to the different terrestrial and limnic invertebrates that can be the subject of fieldwork.

3.1. Molluscs (Mollusca)

If the soft parts of the animals shall be preserved as well as the shell (if present), it is necessary to narcotize the specimens prior to the killing. This ensures that the organisms are expanded and fully display their characteristic features. For this, terrestrial gastropods are best placed in a jar of water. The animals will die in a relaxed position (outside the shell if it is present) within 1 or 2 days (Sturm *et al.*, 2006). Afterwards the specimens should be transferred into a preservative, which can be 80% ethanol, a mixture of ethanol (80%), water (15%) and glycerol (5%), or formalin. Several different preservation methods have been described for molluscs (see Piechocki & Händel, 1996; Sturm *et al.*, 2006) but not all of them are practicable for field trips.

Molluscs, which are anticipated to be included in DNA studies, should be transferred immediately after collecting into 95-99% ethanol.

3.2. Round worms (Nematoda), flat worms (Plathelminthes), and segmented worms (Annelida)

Nematodes are usually killed and preserved in the laboratory after they have been extracted from plant or animal tissue or from soil samples. Due to their small body size, nematodes are always handled in fluid medium under a dissection microscope. General techniques for handling, killing and preserving nematodes are summarized in Kleynhans (1999).

Flat worms are a diverse group of organisms from which only the Turbellaria contain non-parasitic groups. The parasitic groups are not included here but their preservation is discussed in Piechocki & Händel (1996). Aquatic and terrestrial Turbellaria are best preserved in FAA (Appendix 1). Alternatively, formalin (5%) can be used as a suitable fixation agent. The problems of specimen contracting can be overcome by a variety of techniques which are discussed in Knudsen (1972) and Piechocki & Händel (1996). Some of these techniques require the use of mercuric chlorides which we would not recommend (especially not in the field) due to its toxic nature. Final storage of the flatworms should be in formalin (5%) or in ethanol (70-80%).

From the segmented worms (Annelida), only free living earthworms (Oligochaeta: Lumbricidae) and leeches (Hirudinea) are dealt with here. Oligochaete worms shall not be placed immediately in ethanol (unless they are to be used for molecular study) as they shrink. The specimens are washed in a shallow dish and killed in a weak formalin solution (1-2%). It is important to slew the specimens with forceps constantly in the formalin solution which limits the number of specimens that can be dealt with to about five specimens per treatment. After the oligochaete worms got immobilized they are stretched outside the solution. The specimens are then placed on blotting paper and permanently wetted with formalin solution. Alternatively, specimens can be covered by cellulose which has been imbued with formalin. After the specimens hardened (after 30-40 minutes) they need to be transferred into glass vials, which should be long enough to house the specimens. The vials can be either filled with formalin solution (5%) or ethanol (70-80%). Leaches are narcotized in 5-15% ethanol until they do not show any reactions anymore. This may take ½ to six hours depending on the size and physiological condition of the specimen. Fixation occurs in formalin solution (1:4), ethanol (70%) or formol-alcohol (Appendix 1).

3.3. Arthropods (Arthropoda)

Arthropods are the most diverse group of terrestrial organisms and their overall abundance and diversity makes them an important target group for fieldwork. Soft bodied arthropods are best transferred by spring steel forceps which allows safe handling without damaging the specimens (Fig. 1). Small, hard bodied arthropods are best handled or divided into smaller samples with the help of an aspirator (Fig. 1). In the following we will discuss different preservation methods for arthropods, which are best practicable in the field.

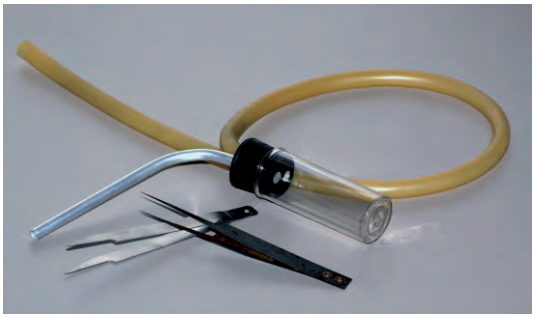

Fig. 1. Handling arthropods in the field. Delicate, soft bodied specimens can be handled with spring steel forceps. Minute, hard bodied specimens are best transferred by an aspirator which can be obtained from entomological suppliers. (Photo by authors).

3.3.1. Fluid preservation of arthropods

There can be a difference between the collecting and fixation fluids which are used for fieldwork and fluids which are used for permanent preservation in natural history collections. Here we only discuss those methods which are used during fieldwork, *i.e*. which concern the collecting and short-term storage during transport. In most cases specimens are directly killed and preserved in the same fixation agent but sometimes the killing agent can differ from the fixation agent. For example, it can be more suitable to collect (and kill) arthropods in water or salt water (*e.g.*, in a pan trap) as ethanol or other fixation agents may act as repellent or attractant thus artificially altering the species composition and diversity of the samples. However the time specimens are kept in non-fixation agents must be held to a minimum as specimens will start decaying within 1-2 days (depending on the temperature).

Standard fixation fluids for arthropods are:

- 70 -80% ethanol (higher alcohol concentration should only be used when specimens are to be included in molecular investigations). This is by far the most common fixation agent and suitable for the vast majority of arthropods.

- 2% acetic acid (like the concentration of vinegar), also feasible for permanent preservation. It is normally used for well sclerotized taxa like Coleoptera, Heteroptera or ants, which shall be dry- mounted in the laboratory. The specimens stay soft and elastic and normally do not need to be relaxed prior to dry-mounting. However, acetic acid is not feasible for most Arachnida and Crustacea because the specimens become too soft and Crustacea will even loose their integumental calcium deposits.

- Acetic acid-glycerol-alcohol solution (AGA) is suitable for small wingless arthropods such as mites (Acari) and for small winged forms like thrips (Thysanoptera). AGA is not suitable for winged forms that are intended to be dry-mounted.

- Lactic alcohol is suitable for aphids (Aphidoidea) and scale insects (Coccoidea).

- Saturated picric acid solution (odourless, only used as fixation liquid, sample has to be transferred into ethanol afterwards). A negative side effect is that specimens fade into yellow according to the luminous yellow colour of the picric acid.

The standard preservation fluid for short and long term storage of arthropods is 70 -80% ethanol. There are a number of different vials available from which those with a screw top should be preferred. Glass vials (Fig. 2) are commonly used but during field work they always bear the risk of being broken, which may lead the specimens to be lost and the collector to be injured by scattering glass pieces. The best option for field work is to use transparent plastic vials with screw tops (Fig. 3). Alternative preservation fluids which may depend on the taxonomic group or the purpose of the field work can be found in Appendix 1. In general, formalin is not recommended for collecting and preserving arthropods. Specimens become very rigid which complicates the handling. In some cases

this effect may be desirable, as for soft-shelled specimens or larval instars or for specimens that are intended to be included in anatomical dissections.

Fig. 2. Handling Glass vials are not a good choice for storing specimens during field work as they are heavier and less safe than plastic vials. (Photo by authors).

Fig. 3. Plastic vials are safer than glass vials during field work and also less heavy. A screw top with a ring gasket tightly closes the vials and prevents evaporation of the ethanol. The transparency of the vials allows the collector to check the content and labels without the need to re-open the vials. (Photo by authors).

3.3.2. Dry-mounting of insects after fluid fixation

The used fluid can be of great importance if the collector's intention is to dry-mount specimens after fixation in a preservation fluid. Acetic acid (2%) is recommended for well sclerotised taxa like beetles (Coleoptera), bugs (Heteroptera), and ants (Formicidae). Specimens in ethanol mostly become rigid and handling and mounting is complicated. Better results are only accessible via more elaborate methods like chemical treatment, heat impact or critical point drying. Inapplicable for dry-mounting after fluid fixation are Lepidoptera, Diptera, as well as pilose and coated Hymenoptera. Micro-Hymenoptera as well as any other small and delicate insect specimens should only be mounted after critical point drying. In these cases it is necessary to transfer them along an ethanol series in the laboratory, in which the ethanol concentration is gradually increased from 70-80% via 90% and 95% to absolute ethanol. Also for some insect larvae and small arachnids it can be more advisable to mount them after critical point drying instead of storing them permanently in ethanol.

3.3.3. Standard methods for dry preservation and mounting of insects

The standard method for dry preservation of well sclerotised insects is the use of specific insect pins. All characters of the specimen should be readily visible by mounting it in a characteristic manner like spreading wings and limbs. There are some different setting and mounting recommendations according to the taxonomic group to be mounted.

Dry-mounting and pinning is recommended or even necessary for the following groups:

- Lepidoptera
- Coleoptera
- Hymenoptera
- Diptera (partim: most Brachycera, single Nematocera groups)
- Heteroptera
- Saltatoria and other "Orthoptera"
- Odonata (imagines and exuviae)
- Neuropterida (partim)

All other taxonomic insect groups as well as insect larvae, Arachnida, Myriapoda, and Crustacea are best killed and preserved in 70 -80% ethanol.

There are 3 established alternatives of pinning (with some modifications in special cases) which are determined by the specimens' dimensions:

- Direct pinning of the specimen with commercially available insect pins of different size for well dimensioned objects.

- Direct pinning of the specimen with commercially available minuten pins of different sizes for small specimens (standard method for Micro-Lepidoptera). Minuten pins are to be pinned into small double mount strips, which are again pinned with common insect pins (see Schauff, undated, for more information).

- Gluing the specimens onto small paper cards of different size and shape. This method is also referred to as card-mounting.

Direct pinning of specimens should be done with an insect pin that fits to the specimen's size. The standard size for insect pins is 1 or 2 which fits for most Lepidoptera, large Hymenoptera and many Coleoptera. Larger specimens should be pinned with size 3, 4 or 5, while for smaller specimens, pins with size 0, 00, or even 000 are available. However, it should be noted that pins with size 0 or smaller are difficult to handle. Pinning through the labels or through the paper layer of insect boxes should be done with great care as the thin pins are easily twisted. Therefore, it may be more appropriate to use minute pins or glue for very small specimens. Direct pinning of insects should be done in a way that the pin is in a right angle to the body (Fig. 4). The insect specimens should rest about 1/3 of the pin length away from the top. This gives enough space to handle the specimens, *i.e*. to grip the top of the needle by the thumb and the index finger without damaging the specimens with the fingertips or fingernails. The specimens should not rest further away from the top of the needle as the bottom space is needed for collection and determination labels. The pin is usually inserted through the mesothorax but the exact insertion point depends on the insect group (Figs 5A-D). Bugs (Heteroptera) are pinned submedially through the scutellum (Fig. 5A). In Hymenoptera and Diptera the insertion point is slightly removed laterally from the median axis (Fig. 5B). This allows median sculpture or bristle patterns to remain intact and visible medially and also on one side. Beetles (Coleoptera) are pinned through the right elytron (Fig. 5C). In butterflies and moths (Lepidoptera) the insertion point is in the middle of the mesothorax (Fig. 5D).

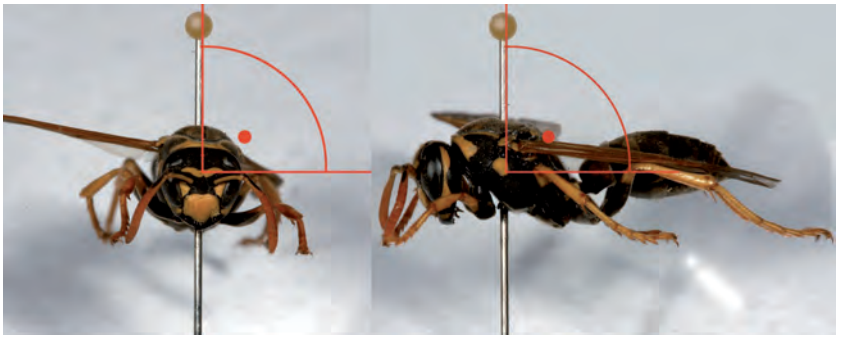

Fig. 4. While pinning an insect specimen care must be taken that the needle is in a right angle to the body of the insect. This needs to be checked in frontal and lateral view. (Photo by authors).

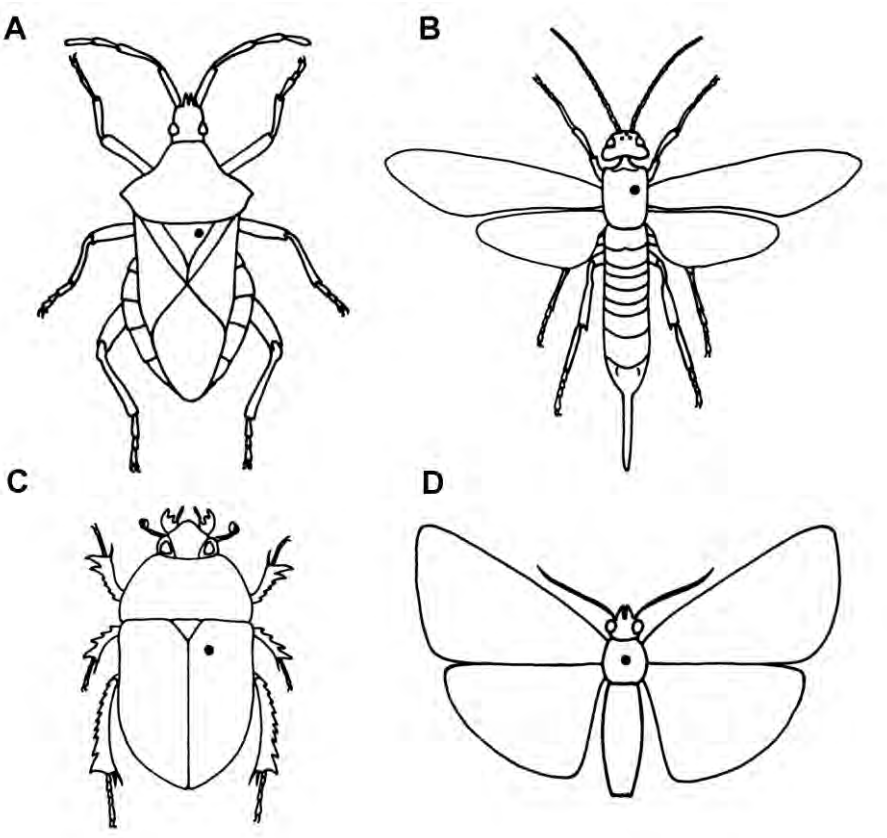

Fig. 5. Insertion of the insect pin, as exemplified in the orders of A. Heteroptera; B. Hymenoptera; C. Coleoptera; and D. Lepidoptera (after Abraham, 1991). See text for more details.

Very small insects (body length below 3 mm) should never be directly pinned on minutens as specimens will always be damaged or lost over time. Card-mounting is the method of choice for small beetles, bugs and Micro-Hymenoptera. Beetles and bugs are usually glued on rectangular cards (Fig. 6). Small Hymenoptera can either be glued on rectangular cards or on the tip of card points, which are small triangles of stiff paper (Fig. 7) (Noyes, 1982, 2009). The latter method has the advantage that the specimen can also be observed in ventral view. The paper cards with the mounted insects are pinned with common insect pins of larger size (sizes 3 to 5). Pins of that size can easily be inserted through the paper cards. The glue should be water-soluble or ethanol soluble so that specimens can easily be removed from the card in case they need to be re-mounted without being damaged. Noyes (2009) recommends glues, which were made from animal products. The best option is to use shellac, a resin produced by lac bugs (Coccoidea). It is important to use shellac (or any other glue) in the right solution, *i.e*. the glue should not be too thin (the specimen will sink in the glue) or too thick (the specimen will not attach tightly enough). Shellac can easily be brought to the right viscosity by adding drops of ethanol or by letting part of the ethanol evaporate from the glass tube in which

the resin is deposited. Shellac is commonly used in North America but less widespread among European entomologists. Shellac resin can be obtained from entomological suppliers in the United States. However, even if we would recommend shellac over other glues, there are also a few drawbacks of shellac which are best summarized in Noyes (2009). Seccotine (fish glue) is a water-soluble glue and a good alternative to shellac. Noyes (pers. comm.) recommends the use of shellac for card points and seccotine for card rectangles. For long and slender insect groups, *e.g.* ichneumonid wasps, it can be an alternative to glue them on to the side of an insect pin with shellac.

Fig. 6. Card mounting is the method of choice for small sized insects. Small beetles are usually mounted and glued on rectangular paper cards, which are available in various sizes from entomological suppliers. (Photo by authors).

Fig. 7. Card points are small triangles of stiff paper that allow specimens to be observed from all sites if specimens are glued laterally to the tip of the triangle. This is a suitable method for mounting Micro-Hymenoptera. (Photo by authors).

Accepted killing methods for arthropods to be dry-mounted are:

- Jar, containing absorbent paper saturated with ethyl acetate (Fig. 8B). This method is suitable for most insects apart from Lepidoptera. Avoid too wet content of the jar because of possible agglutination of small, pilose, or coated specimens.
- Potassium cyanide inside a killing jar (Fig. 8D). This is best method for Lepidoptera, except for some resistant groups like Zygaenidae moths. It is also feasible for most other taxonomic groups. Safety regulations are essential to avoid intoxication! It is the responsibility of the collector to make sure that the killing jars are always kept under supervision and do not get into the hands of others!
- Ammonium chloride, injected via syringe (Fig. 8C). This can be used for larger butterflies and moths effecting rapid killing and for softening rigour mortis.
- Freezing.

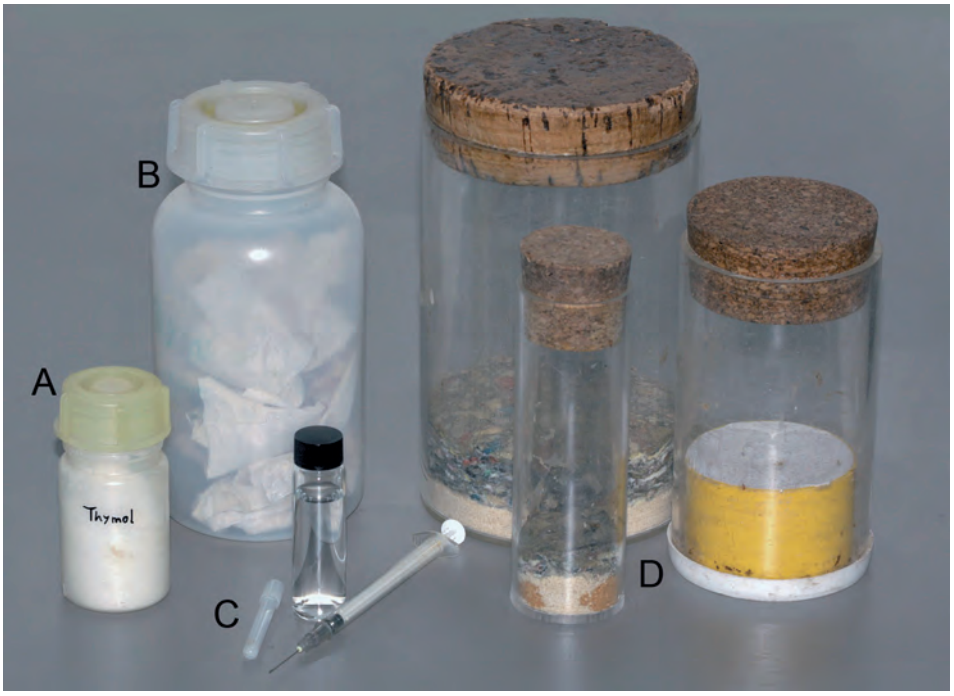

Fig. 8. Devices against moulding and for killing specimens. A. Thymol prevents specimens from fungal damage and can be applied crystalline or in a solution; B. Ethyl acetate is used for most insects apart from Lepidoptera; C. Ammonium chloride is used for larger Lepidoptera and injected by a syringe; D. Killing jars containing potassium cyanide can be purchased from entomological suppliers in various sizes. Specimens are usually killed in smaller jars and transferred into a large jar after they are narcotized. Layers of tissue between the specimens prevent them from mechanical damage during fieldwork. (Photo by authors).

3.3.4. Preservation in the field and transport

If time availability and the amount of samples permit, it is good practice to mount or prepare for dry preservation.

Even if this is not possible, the right preparations and transportation conditions are essential for best quality of set specimens. When pinning and mounting sheetings and/or setting boards, transport and store boxes are available they should be used already in the field. Protected and dry storage is important; especially intruding ants could be fatal. In many cases setting in the field is not possible. In these cases, the following recommendations should be noted to facilitate later setting and mounting:

- Use cardboard tubes of different diameter for transport (Fig. 9). Both sides of a tube are to be closed with a cotton plug. The freshly killed sample is placed directly inside the tube. It can be transferred into a soften chamber afterwards for preparation of setting and mounting. Check adequate labelling! This method is feasible for strongly sclerotized specimens (*e.g.*, beetles) which need to be stored during fieldwork before they can be dry-mounted in the laboratory. However, scaled, pilose, and coated specimens could be rubbed off during transport. Cardboard tubes are preferred over glass or plastic ones as they are lightweight, fracture-proof and absorb moisture. The tubes are to be stored inside of feasible sealed transport boxes containing crumbs of thymol (Fig. 8A) against moulding.

- Use butterfly envelopes of different size, made of vellum (Figs 10, 11). This is the best method to transport or even store dry unset Macro-Lepidoptera, but also feasible for other winged insect orders like Odonata and Neuroptera. It is important to “close” the specimens inside of the envelope with the wings folded upwards. This protects the more important upper sides of the wings (as identification characters) against rubbing and facilitates later setting and spreading. If this is not possible in case of *rigor mortis*, the specimens have to be injected by syringe with ammonium chloride to soften *rigor*. Placing more than one specimen into one envelope should be avoided as they may damage each other during transport. Every single envelope has to be labelled individually with the full locality data! The envelopes are to be stored inside of sealed transport boxes containing crumbs of thymol against moulding (Fig. 11). For softening the specimens the whole envelope has to be transferred into the soften chamber without removing its content.

- Use small plastic boxes laid out with layers of cellulose wadding. Freshly killed insect specimens can be placed between the layers and will be protected during transport. Thymol should be added against fungal damage.

- Use transport boxes with plastazote foam pinning bottoms (Fig. 12). Insects can be pinned without setting and plunged into the box in a space-saving manner. For later setting, mounting or spreading, they can be softened easily inside a soften chamber. This is feasible for all well sized specimens, which are to be pinned and set. Adding labels to every single specimen is essential! Fragile Micro-Lepidoptera that cannot be transported on setting boards (this is the preferred option) should be pinned directly onto plastazote in small transport boxes (Upton, 1991). Spreading the wings with

minuten pins can easily be done in the field and the roughness of the plastazote will hold the wings in place (Fig. 12). This method does not replace proper spreading on a setting board but will greatly facilitate this as the wings are already partially spread.

Fig. 9. Cardboard tubes are ideal for hard bodied insects, such as beetles. Specimens are ideally protected during transport and less prone to moulding as the tubes absorb moisture. (Photo by authors).

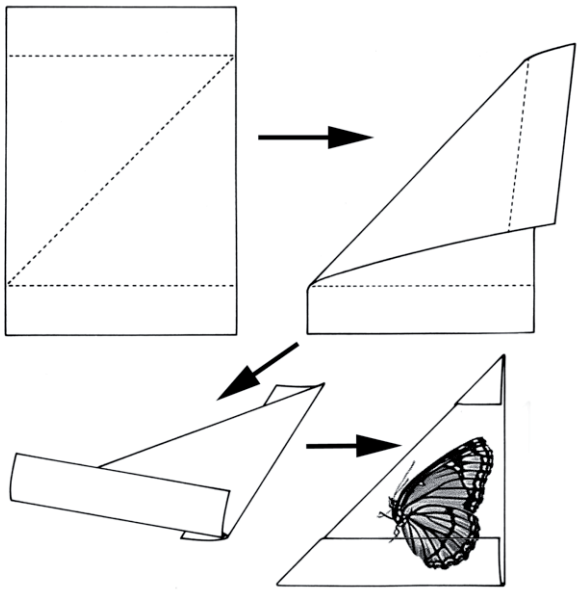

Fig. 10. Envelopes for storing insect groups, such as Lepidoptera can be easily folded from rectangular paper (after Abraham, 1991).

Fig. 11. Vellum envelopes are a simple option for storing Lepidoptera specimens as they do not need to be hand-folded but can be readily purchased in various sizes from philately purchasers. The envelopes are best stored in tightly lidded boxes which can be laid out with wet cotton. The moist atmosphere keeps the specimens relaxed before mounting. Thymol must be added to prevent moulding. (Photo by authors).

Fig. 12. Transport boxes with plastazote foam pinning bottoms are ideally suited to transport pinned insects in the field. Fragile Micro-Lepidoptera that cannot be transported on setting boards should be pinned directly with minutens onto the plastazote. The wings should be spread and the roughness of the plastazote will hold them in place. This greatly facilitates later spreading on a setting board. (Photo by authors).

4. Acknowledgements

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6. Appendix I: Reagents and solutions suitable for field work

Acetic acid-glycerol-alcohol solution (AGA) (fixation of small arthropods and thrips)
1 part of glacial acetic acid
6 parts of ethanol (95%)
4 parts of H_20
1 part of glycerol

Alcoholic thymol-camphor solution (prevention of mould)
100 ml ethanol (96%)
5 g camphor (crystalline)
10 g thymol (crystalline)

Bouin's solution (fixation of insect larvae for histological work)
70 parts of picric acid solution
25 parts of formalin
5 parts of glacial acetic acid

Formal-acetic-alcohol (FAA) (fixation of flatworms and other animals)
10 parts of formaldehyde solution (saturated) (= 100% formalin solution)
50 parts of ethanol (95%)
2 parts of acetic acid
40 parts of H_20

Formalin
Refers to a saturated solution of formaldehyde. Formaldehyde comes in a saturated solution of 39-40% which equals a 100% formalin solution. That means that *e.g.* a 10% percent formalin solution can be obtained by adding 1 part of formaldehyde (saturated) to 9 parts of water.

Formol-alcohol (fixation of some annelids)
1 part formol
2 parts ethanol (80%)

Kahle's solution (= Pampel's fluid) (general fixation of insect larvae)
30 ml ethanol (95%)
10 ml formalin (35-40%)
2 ml glacial acetic acid
60 ml H_20

Lactic alcohol (for aphids and scale insects)
2 parts of ethanol (95%)
1 part of lactic acid (75%)