

Chapter 15

Sampling insects: general techniques, strategies and remarks

by

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Abstract

Sampling insects requires knowledge of their biology, preferred habitats and activity patterns. An overview is given of the most frequently applied collecting and recording techniques and the insect taxa that they gather in largest numbers. Sampling strategies can be deduced for each of the included taxonomic groups. Following techniques are described and recommendations and restrictions are given for them: 1. Active collecting: pooter, portable suction devices, sweepnet, visual observation; 2. Passive collecting: coloured pan traps, emergence traps, sticky traps and suction traps. For light traps, Malaise traps and pitfall traps we refer to other chapters.

Keywords: Sampling strategies, coloured pan traps, suction traps, emergence traps, sticky traps

1. Introduction

It is virtually impossible to attempt at collecting all species of one particular taxonomic group with only one sampling technique. And it is considered very unlikely to collect all of them even with several methods. This is not only due to the specific life histories of the different species, and their numbers in the field, but also to features of the recording methods used. Preferably, at least two or three collecting techniques, and visual observations in the field are mandatory to get a representative idea of the present species richness. In a canopy sampling campaign for weevils (Coleoptera: Curculionidae), the three methods applied (fogging, sticky traps, light traps) each yielded a very large number of species, but proved strongly complementary in terms of collected species (Missa, 2000) (Fig. 1).

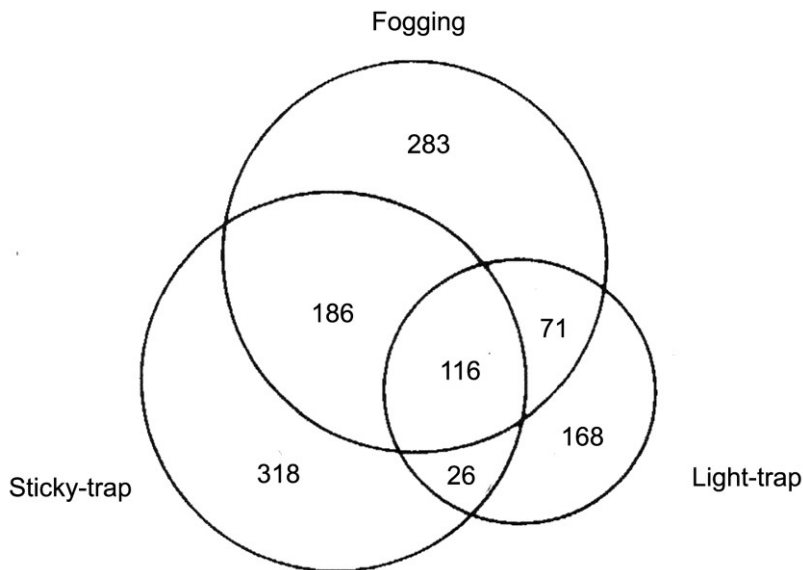


Fig. 1. Weevil species richness (Coleoptera: Curculionidae) as established by three collecting techniques in lowland rainforest in Papua New Guinea (Missa, unpublished data).

Sampling insects requires knowledge of their biology, preferred habitats and activity patterns. Like most invertebrates, many insects show oscillating population densities with cycles from 3 up to 10 years (Hunter & Price, 1998) (Fig. 2). In low density years, species' populations are difficult to measure and might give the impression that the habitat represents suboptimal conditions. In temperate and tropical climates, insects show a specific annual activity pattern, often referred to as phenology (Tauber & Tauber, 1981). In temperate regions these patterns are triggered by photoperiod in combination with temperature and humidity (van Asch & Visser, 2007), which renders species being most active during spring, summer, autumn, and even winter. Some species even have several generations per year disjunct in time. Apart from monsoon conditions it

remains unclear what exactly triggers phenology in the tropics, certainly around the equator, where photoperiod and temperature are subequal throughout the year. In tropical forests the fruiting of trees may be one of the triggers.

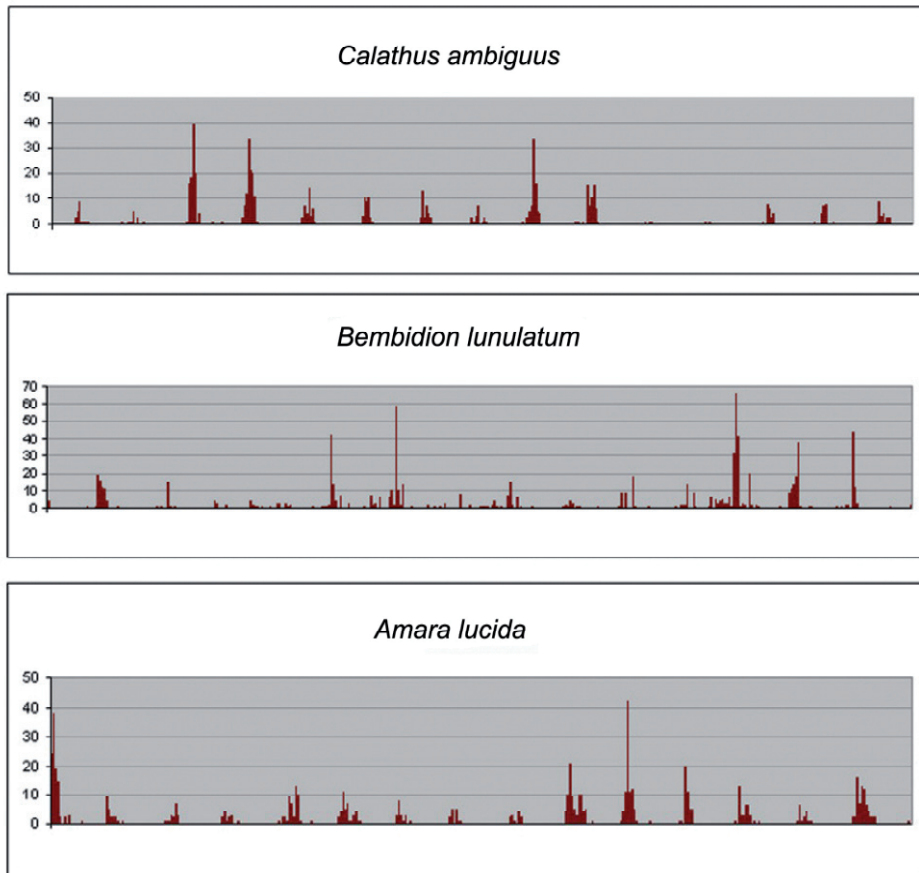


Fig. 2. Cycles of annual variations in population density of three ground beetle species (Coleoptera: Carabidae) resulting from pitfall traps over 15 years: the periodicity of population peaks varies between 5 years in *Calathus ambiguus*, and 10 years in *Amara lucida*. In low density years, populations are hard to establish (Desender, unpublished data).

An All Taxa Biodiversity Inventory (ATBI) *sensu stricto* is an illusion as well. It is not feasible to record all species at one particular site, even when sampling continuously, year-round and using different techniques. But the strategic employment of a particular combination of trapping techniques might yield a sufficiently representative portion of the species richness. Each collecting device has been constructed to gather particular taxa as efficient as possible, using species' features as mobility and attraction: e.g. Malaise traps collect a very diverse fauna of mainly diurnal flying insects; pitfall traps focus primarily on soil-

dwelling invertebrates, whereas coloured pan traps and light traps attract flying insects during the day and night respectively (Missa *et al.*, 2009).

Before initiating a sampling campaign, the goal of the action should be very clear. Also, aspects as coverage and intensity of the sampling in time and space, practical issues, treatment of material before preparation and logistics, and the handling of possible by-catches or residue samples should be taken into account prior to the start of the campaign.

It is very important to choose the collecting method and devices according to preservational aspects. Many taxa are to be dry-mounted by pinning or gluing onto paper cards as a standard preservation method. Collecting devices using fluid fixation agents prevent satisfying results in many cases (as for all Lepidoptera, pilose and coated specimens), and require ultimate liquid specimen preservation, also dependent on fixation agent, collecting periods, temperature, etc. In these cases passive collecting devices can be used without fixation fluids, but have to be serviced in short intervals. So fixation and preservation fluids must be selected according to the final purpose of the gathered specimens (e.g. DNA extraction requires 100% ethanol). See chapter 18 by Krogmann & Holstein.

Traps have been designed for each stratum, from the soil surface level (to collect soil-dwelling and weak flyers), over the near-soil stratum (most of the flying insects in herb and lower canopy levels) up to the upper canopy. The canopy can hold an unprecedented biodiversity as shown by Erwin (1982) who observed that about 2/3 of the arthropods of a dry tropical forest occur in the canopy. The present chapter deals only with the near-soil stratum. Collecting strategies and techniques for soil-stratum and canopy invertebrates are treated in chapters 9 and 8, respectively.

A clear difference should be made between discontinuous or occasional, and continuous sampling techniques, and both have their advantages and shortcomings. If practically possible, continuous sampling with traps is recommended because of the relatively low service time (especially as compared to the time needed to collect the same species richness actively), and the fact that traps remain in operation regardless of weather conditions.

Trapping devices can also be separated into attraction and interception traps. Attraction traps employ the phenomenon of attraction of the species by the trap, generated by agents such as light, colour, odour and others. Interceptions traps, on the contrary, form an obstruction on the path of organisms and lead them to a collecting device. A number of traps combine both sampling methodologies.

A third way to divide sampling activities is based on the involvement of the collector himself during the collecting activity and in this frame, active and passive collecting are distinguished. The former approach implies the direct and active involvement of the collector who effectively moves (around) in search for the focal taxa. Active sampling encompasses visual observation, sweep netting and the use of pooters and related recipients. Passive collecting, on the other hand, is based on the movement of the focal taxa towards the trapping device. This methodology includes all kinds of continuous traps such as Malaise traps, pan and pitfall traps, fixed suction traps, sticky traps, light traps and emergence

traps. All of these collecting techniques are presented below, except for Malaise, light and pitfall traps, which are dealt with in chapters 17, 16 and 9, respectively.

Collecting / recording techniques relevant for ATBIs of insects

Table 1 presents an overview of the most frequently applied collecting / recording techniques and the insect taxa that they gather in largest numbers. From this table, recommended sampling strategies can be deduced for each of the included taxonomic groups. Hereunder, the different techniques are described, and recommendations and restrictions are given.

Collecting techniques (see Text)	Active collecting					Passive collecting					
	2.1 pooter	2.2 portable suction devices	2.3 sweepnet	2.4 visual observations <i>fogging</i>	3.1 coloured pan traps #	3.2 emergence traps	3.3 light traps	3.4 Malaise traps	3.5 sticky trap	3.6 suction traps	<i>pitfall traps</i>
Collembola	<input type="checkbox"/>	<input checked="" type="checkbox"/>		<input type="checkbox"/>				<input type="checkbox"/>	<input type="checkbox"/>		<input checked="" type="checkbox"/>
Thysanura				<input type="checkbox"/>				<input type="checkbox"/>	<input type="checkbox"/>		<input checked="" type="checkbox"/>
Ephemeroptera			<input checked="" type="checkbox"/>				<input type="checkbox"/>	<input type="checkbox"/>			
Plecoptera			<input checked="" type="checkbox"/>				<input type="checkbox"/>	<input checked="" type="checkbox"/>			
Blattodea			<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>
Isoptera				<input type="checkbox"/>			<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>			
Orthoptera			<input checked="" type="checkbox"/>	<input type="checkbox"/>			<input type="checkbox"/>	<input type="checkbox"/>			
- Tettigonoidea			<input type="checkbox"/>	<input checked="" type="checkbox"/>			<input type="checkbox"/>	<input type="checkbox"/>			
- Acridoidea			<input type="checkbox"/>	<input checked="" type="checkbox"/>							<input type="checkbox"/>
- Tetrigidae			<input type="checkbox"/>	<input type="checkbox"/>			<input type="checkbox"/>	<input checked="" type="checkbox"/>			<input type="checkbox"/>
Embioptera				<input type="checkbox"/>			<input type="checkbox"/>	<input checked="" type="checkbox"/>			
Psocoptera			<input type="checkbox"/>	<input type="checkbox"/>				<input checked="" type="checkbox"/>			
Hemiptera			<input type="checkbox"/>					<input checked="" type="checkbox"/>			
- Cicadomorpha			<input type="checkbox"/>	<input type="checkbox"/>			<input checked="" type="checkbox"/>	<input type="checkbox"/>			
Thysanoptera			<input checked="" type="checkbox"/>	<input type="checkbox"/>				<input type="checkbox"/>			
Neuroptera			<input type="checkbox"/>	<input type="checkbox"/>			<input type="checkbox"/>	<input checked="" type="checkbox"/>			
Coleoptera											
- xylobionts (e.g. Cerambycidae, Scolytidae)			<input type="checkbox"/>	<input checked="" type="checkbox"/>		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		
- ground-dwelling beetles (e.g. Carabidae)							<input type="checkbox"/>				<input checked="" type="checkbox"/>
- phytophagous beetles (e.g. Chrysomelidae)			<input checked="" type="checkbox"/>	<input type="checkbox"/>			<input type="checkbox"/>				
- aquatic beetles (e.g. Dytiscidae)			<input checked="" type="checkbox"/>				<input type="checkbox"/>				

Strepsiptera									■
Diptera		■		■ W, Y	□				■
- apterous/brachyterous flies	□				□				■
- Dolichopodidae		■		■ W, Y	□			■	□
- remaining Empidoidea		■		■ W, Y				■	
- Phoridae				■				■	□
- Syrphidae		■		□				■	
- Stratiomyidae				□				■	
- Tabanidae								■	
Mecoptera		■			□	□	□		
Lepidoptera									
- Rhopalocera		■		□ ^W				□	
- Heterocera								■	■
Hymenoptera		■		□				■	
- Apoidea	□			■ ^Y				■	
- Cynipoidea	□	□			□			■	
- Parasitoids	□	□	□	□ ^{W, Y}	□	□		■	
- Formicidae	□	□	□	□ ^{W, Y}		□	□	■	□
- Ichneumonoidea	□		□	□ ^{W, Y}				■	
- Pompilidae	□		□	□ ^{W, Y}		○		■	
- Symphyta	□		□	□ ^{W, Y}				■	
- Vespoidea	□		□	□ ^{W, Y}		○		■	

W: white; Y: yellow pan traps

Table 1. Overview of techniques used to collect insect orders and some selected superfamilies and families. Only taxonomic groups for which at least one technique can be assigned as recommended are included. Explanation of covered collecting techniques follows the structure of the chapter; techniques not treated here are indicated in italics. Most recommended techniques are indicated as ■ (if two or more techniques are in this category, they are considered as equally recommended); useful, supplementary techniques indicated as □. If no techniques are indicated for a certain order, recommended techniques for the underlying families differ greatly.

2. Active collecting

2.1. The pooter

A pooter consists of a collecting jar closed by a cork or polymer stop with two flexible tubes inserted into it, a mouthpiece tube to aspire and a collecting tube to suck up the insect. At the inner end (in the collecting jar), the mouthpiece tube is covered by a fine gauze (Fig. 3) to avoid insects from entering the collector's mouth. Small insects are collected by positioning the collecting tube on top of the insect and abruptly sucking it up into the collecting jar. In between collecting actions, the outer end of the collecting tube must be covered or blocked by a stop to avoid the insects from escaping. Finally, the insects can be transferred to a killing jar or preservative by gently removing the stop. This method is widely used

to collect insects from all kind of surfaces (rocks, fences, tree trunks, etc.), from crevices and even from sweep net samples. This method is particularly interesting to gather insects that tend to stick to these substrates, and thus cannot easily be collected with a sweep net.

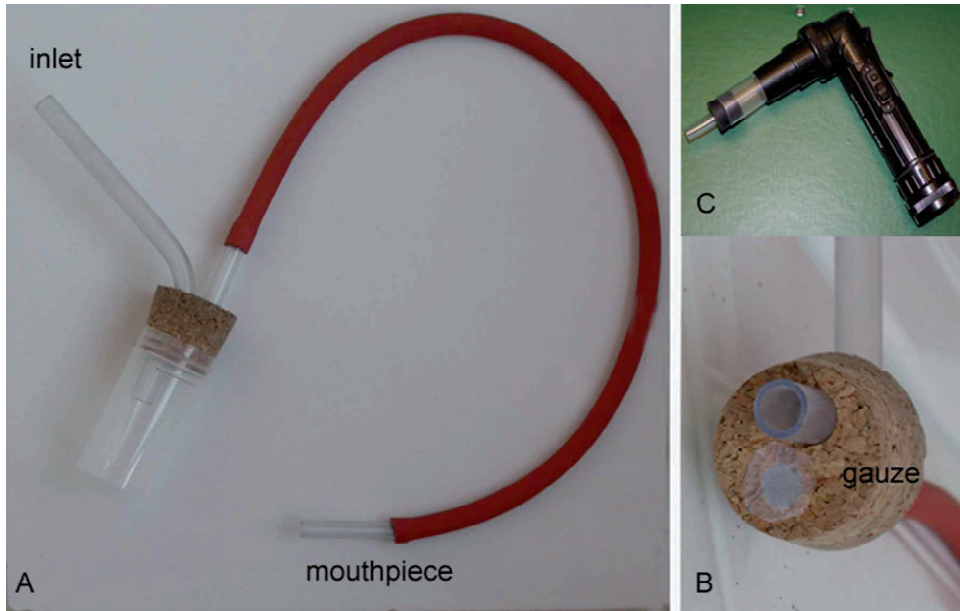


Fig. 3. A. Mouth-pooter; B. Gauze at the inner end of the mouthpiece tube prevents insects from being inhaled; C. Electric pooter (Photos A & B by Patrick Grootaert; C on <http://svalbardinsects.net/index.php?id=64>).

Recommendations:

- Use a distinctive mouthpiece tube to avoid confusion with the collecting tube;
- Glass collecting jars are prone to get broken, so transparent plastic vials are safer. However, be aware that some types of polymer corrode when in contact with a killing agent;
- Transfer the collected insects regularly to the killing jar so that the pooter jar does not become too crowded. By putting a piece of paper tissue in the pooter jar, the time interval between collecting actions can be increased and collected insects do not get too damaged during the trip;
- To kill the collected insects, a piece of paper tissue with some volatile killing agent can be deposited into the pooter jar prior to their transfer into a larger killing jar. Take care that the killing agent is entirely evaporated before the pooter is used again.

Restrictions:

- While aspirating, enormous amounts of germs (fungi, bacteria, viruses, mites and their eggs, springtails, etc.) can be inhaled which might cause damage to the respiratory system. It is also highly recommended when collecting insects from excremental surfaces to use a rubber bellow on the mouthpiece tube instead of an electric pooter;
- Ants and certain beetles emit noxious products when disturbed, and in these cases an electric pooter is recommended.

In the case of tree-trunk dwelling flies, an alternative and safer method consists of a transparent vial (a recipient with a diameter of 3 cm and a depth of 7 cm is very practical) that is rinsed with some alcohol solution. This leaves a thin wet layer on the inside of the vial in which flies and other flying insects get entangled while flying up when the vial is quickly put on top of them. In this way, a surprisingly high number of specimens can be collected during one collecting action before being transferred to an alcohol solution. This method is superior to all others for collecting *Medetera* spp. (Diptera: Dolichopodidae) and other arboreal trunk-dwelling long-legged flies. This method is well suitable for specimens that are ultimately wet preserved, but only to some extent to collect dry preserved insects.

2.2. Portable suction devices

D-VAC is a portable aspirator activated by a gasoline engine and carried on the back of a person. The advantage of D-VAC vacuum sampling as compared to other sampling techniques is the more complete extraction of tiny invertebrate species, and immature forms of even larger species from the environment. Due to the pressure built up by conventional nets while sweeping, insects of low body mass simply do not enter them as they are caught in the overflow of air pressure built up as the net is sent through the air. By applying suction to the collecting bag, this inertia of air at the entrance of the net is overcome and tiny forms are collected more readily. Using a similar motion as is done while swinging an insect net, the D-VAC is also suitable to sample more heavy insects like caterpillars, beetles, etc. For fragile insects like many flies, sweep netting is preferred over suction trapping, although the latter method might be applied successfully to capture cryptic species that occur in dense vegetations, within tussocks and in e.g. rot-holes of trees.

2.3. The sweep net

Sweep nets come in all shapes and sizes, each designed for a particular insect group (Stubbs & Chandler, 1978). Both the net shape and sweeping technique affect the yield as commented upon by Chalcidoidea (Hymenoptera) specialists (Anonymous, 2004). While employing a sweep net, the collector not necessarily targets a specific specimen, but sometimes carries out a random sampling of the fauna present in the vegetation or on the soil surface. The species diversity in

sweep net samples often resembles that of Malaise trap yields (Guevara & Aviles, 2009).

The sweep net is by far the most widely used device to collect insects, and has been the most important one for the past centuries. Its success can be explained by its practical use and the fact that it can be employed in almost every possible habitat, except for densely vegetated or inaccessible sites (reed marshes, mangroves, etc.) and thorny vegetations. Moreover, it is ideal for short-term large scale inventories as the gathering of the separate samples is not time-consuming and several sites can be visited during the same day. Also, it does not require the collector to return to the same site more than once to collect the yields.

When using a big-sized net selected insects can be gathered with a pooter. This holds true for small specimens only and is not feasible for *e.g.* Lepidoptera and medium-sized to large arthropods. If the entire content is to be conserved, the yield is gathered in the tip of the net by sweeping the net a few times and closing it manually. If the specimens must be stored dry, the tip can be put in a jar with a knockdown agent like ethyl acetate to kill the specimens. Subsequently, the sample can be exposed on a white sheet for immediate sorting. The collector should make sure that the specimens are dead (caterpillars and beetles might be harder to kill in this way). If the specimens are stored wet, then the tip of the net with the yield can easily be emptied in a collecting jar with an alcohol solution.

Beating vegetation with a strong sweep net or with a stick and subsequently collecting the fallen insects on a sheet or in an umbrella is an alternative way to collect arthropods like spiders, beetles, bugs and caterpillars. However it is not highly recommended to maltreat vegetation in a nature reserve, especially in the presence of park guards.

Recommendations:

- Use a net with the right mesh size; dipterists require a finer mesh size than *e.g.* butterfly or dragonfly collectors. Sweeping nets for sweeping through thorny vegetation must be made of a stronger fabric (*e.g.* linen), at least around the clamp to avoid ruptures;
- Transfer the sample to a collecting jar after a limited number of sweeps, depending on the size of the sample (this requires some experience). Samples collected during a long sweeping session tend to contain a high ratio of damaged specimens;
- Sweep gently (over) the vegetation; insects will fly up, end up in the net and will not be damaged, nor will the vegetation. If sweeping too severely, leaves and branches will end up in the net, damaging the specimens;
- Use an eversible stick which makes the collecting radius substantial larger;
- Take care when manipulating the sample (in the tip of the net) and watch out for stinging insects, especially when you are allergic;

- Join an experienced entomologist on one of his trips; you will learn more and much faster than studying manuals. Every entomologist has his personal technique that affects the yields.

Restrictions:

- Sweep netting of vegetation cannot be done when vegetation is humid or highly thorny. Fragile insects will be severely damaged which renders them useless for identification. As insect activity only starts when the temperature is sufficiently high, collecting with sweep nets becomes only efficient when the collecting sites are exposed to the sun. In practice, collecting starts best not before 8:00 a.m., especially in strongly wooded habitats, and lasts until the late afternoon (when the weather is dry). Poorly vegetated sites like beaches, especially in the tropics, are best avoided at noon when insects escape from the soaring temperatures and hide in the soil or on the soil surface within dense vegetations.

2.4. Visual observation

Visual observation is a technique that should not be underestimated. Moreover, it is the innate feeling of most entomologists nowadays that they spend too little time in the field to learn about the whereabouts of their animals of interest. Instead, sampling is mostly done as efficient as possible, using all kinds of trapping devices which can yield very large amounts of species and specimens but only rarely uncover aspects of their life history (see further). Observing insects in their natural habitat yields information on their behaviour, commotion and preferred (micro)habitats. *E.g.* many long-legged fly species (Diptera: Dolichopodidae) in the tropics demonstrate very specific habitat affinities and are sometimes entirely confined to *e.g.* springs, waterfalls, rapids and even splash zones of rocks amid rivers.

Well-sized specimens can be collected by hand or with a jar or vial, respectively. In this way, non-flying arthropods from substrates and from under rocks, stones or bark are usually collected.

During visual observation, specimens can be photographed and pictures and related information can be stored using PDAs (personal digital apparatus), which have rather recently been developed. Recording using only visual observation is only suitable for taxonomic groups that are easily recognized in the field. In all other cases, it is strongly recommended to collect voucher specimens for confirmation of their identity in the laboratory.

3. Passive collecting

3.1. Coloured pan traps

Next to sweep nets and Malaise traps (see chapter 17), the most frequently employed technique to collect flying insects is undoubtedly pan traps. These traps were initially used in pest species sampling, but more recently have

become part of the standard biodiversity assessment instruments mainly applied by North American entomologists, but only few European researchers (Baillot & Tréhen, 1974; Pollet & Grootaert, 1987, 1991, 1996). In contrast to Malaise traps and sweep nets that are manufactured exclusively for the collecting purpose, any kind of device that holds a certain amount of (preserving) liquid and that features a colour attractive to the focal taxon is suitable as pan trap. The material can range from garbage bags, vinyl sheets, plastic food trays to aluminium roasting pans, but the most practical are definitely round plastic bowls that are weather-proof (the colour should not change over time). The specific type to be used largely depends on sampling site attributes (*e.g.* accessibility, distance to the collector's residence). Nearby sites can be sampled with large and heavy pan traps (see Pollet & Grootaert, 1987, 1991), but most recommendable in all situations are light-weight and easily stackable types such as 12 oz plastic partyware bowls (see <http://www.partypro.com>). These bowls that come in 41 different colours have a flat rim of 2.3 cm, an inner diameter of 15.4 cm and a depth of 3.7 cm. They were recently employed successfully during an expedition in Ecuador (Pollet, unpubl. data) (Fig. 4A, B). Unfortunately, these devices do not seem to be found easily in Europe.

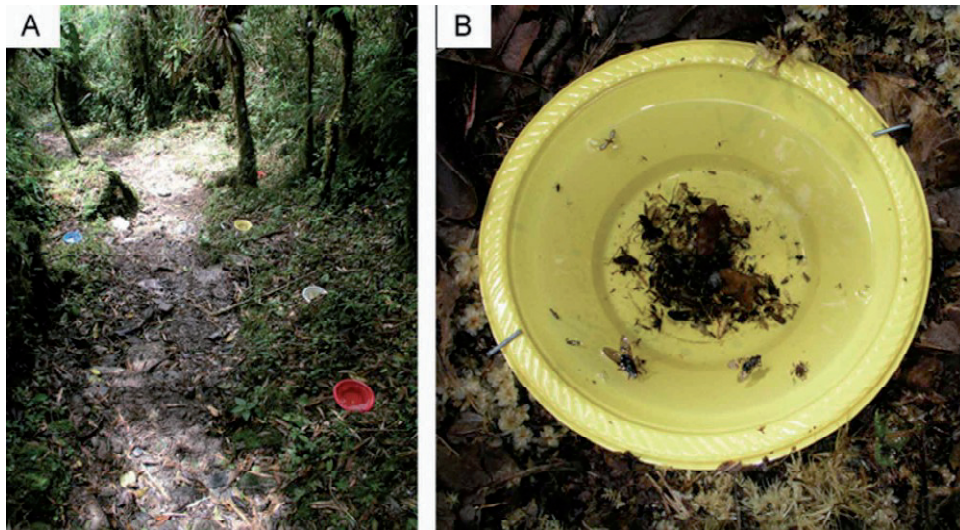


Fig. 4. A. Different coloured pan traps along a trail in a forest in Ecuador; B. detail of the insects trapped in a pan trap. (Photos by Marc Pollet).

One of the most significant advantages of the use of pan traps is their versatility: not only can the size and shape be varied infinitely but also the trap colour and its installation can be adapted greatly in order to optimise the sampling process (see Pollet & Grootaert, 1994). Traps with a bright yellow colour (often referred to as Moericke's traps) are by far the most widely used and attract a broad spectrum of low-flying insects, in particular Hymenoptera and predacious flies. Also white pan traps repeatedly proved to be excellent devices to collect certain fly families *e.g.* Syrphidae and Dolichopodidae: one trap type with a diameter and depth of approx. 9 cm yielded on average 116 and 248 dolichopodid specimens during one season in reedmarsh (Pollet, 1992) and marshland sites, respectively

(Pollet, 2001). These sampling campaigns gathered a total of 73 and 68 species using 77 and 54 traps, respectively. Moreover, a comparative study by Pollet & Grootaert (1994) involving white, yellow, and bluish green pan traps revealed that white and yellow traps collected a comparable number of species; the higher number of specimens yielded by yellow traps was explained by only one very abundant species. Most dolichopodid species thus appear to be most attracted by yellow and white and less by other colours as blue and red. This, however, does not hold true for arboreal dolichopodid species (e.g. *Medetera* spp., *Neurigona* spp., *Sciapus* spp.) that are collected in highest numbers in blue, and soil-dwelling species (e.g. *Campsicnemus* spp.) that are most numerous in red (and blue) pan traps (Pollet & Grootaert, 1987). Actually, thus far *Australachalcus melanotrichus* Pollet & Stark, a species that breeds exclusively in rot-holes of trees, has only been gathered by blue or bluish green traps in multicolour pan trap campaigns (Pollet, unpubl. data). Also other dipteran families with larvae that breed in plant tissue such as leaf miners (Chloropidae) and fruit flies (Tephritidae) are most attracted by blue pan traps.

The installation height also has a substantial impact on the yields. In general, pan traps sunk into the soil are most productive, both in terms of species and specimens (Pollet & Grootaert, 1987, 1991). Again, some species like the xerophilous *Chrysotus gramineus* (Fallen, 1823) and arboreal species are collected more abundantly in traps at 60 cm height (Pollet & Grootaert, 1987), or traps level with vegetation height (Pollet, 2001). As a result, blue or bluish green traps installed at a certain height are best employed if the research focuses on arboreal species communities. If a short-term assessment of the overall species diversity is the main aim, yellow or white pan traps are preferably used. And in case of faunas with a largely unknown ecology, a combination of yellow, white, red and blue coloured traps can be strongly recommended (as the distribution of species of the differently coloured traps holds information on their ecology).

Pan traps thus can be used in every terrestrial and semi-aquatic habitat but are most commonly installed at soil surface level. Traps that are installed on the soil only yield a fraction of the soil-dwelling fauna of e.g. carabid beetles and spiders, which are abundantly trapped in pan traps dug into the soil with their rim at soil surface level. In either case, they should be fixed to the soil by metal pins or any other device that prevents displacement. Pan traps can be put simply on the soil in habitats with a well developed herb layer, or sites that are subject to regular but mild flooding. In drier habitats traps are better sunk into the soil and are preferably deeper to prevent them from drying out.

Pan traps are usually filled for $\frac{3}{4}$ with water. A sufficient amount of detergent must always be added as a surfactant to break the surface tension. Depending on the servicing periodicity, salt can be added as a preservative. If traps are emptied daily or every two days, salt is not necessary, but it becomes absolutely essential with longer servicing intervals. A possible alternative that allows even longer sampling intervals is formalin solution. With a 5% solution as preservative, traps can remain in operation for at least 7 days, and for a fortnight with a 10% solution. Precipitation (rainfall) should be taken into account, especially in the tropics, which can cause a very quick and strong dilution. Deeper traps (over 5 cm) might reduce this effect, but are no guarantee for a good preservation of the

trapped specimens in the rainy season. To avoid the loss of (floating) specimens due to heavy rainfall, minute holes just below the upper rim of the pan trap work well as drainage. Further on, especially in forests and wooded habitats in general, falling leaves or branches might cover the traps largely to entirely, blocking any insect to be trapped. This can be prevented by constructing a framework of thin branches or metal wire covering the trap. As this can be rather time-consuming, it is more practical to service the traps at sampling intervals of at most 5 to 7 days.

The servicing process starts with removing large objects such as leaves, twigs, and vertebrates that might obstruct the collection of the trapped invertebrates and accelerate their decomposition. The remaining contents are subsequently scooped out with a fine mesh aquarium net while collecting the preservative liquid (in a supplementary trap) for reuse (after addition of some fresh solution if necessary). In order to recover the entire content, the net might need to be dragged several times gently near the bottom in one direction. The content of one trap can be kept separately or be pooled with the contents of other traps, depending on the specific objective of the sampling campaign. If the preservative liquid is significantly coloured (mostly by leaves), fresh solution should be used. The contents are transferred to collecting jars or (better) self-sealing plastic bags (*i.e.* whirl-pack type) and properly labelled. Preferably a 90% ethanol solution is added as preservative.

The pan trap technique holds a number of advantages as compared to Malaise traps (Pollet, 1988): (i) they are less striking in the field and as such less subject to damage or removal; (ii) yields are usually fair but not as massive as those of Malaise traps which allows processing in proper time; (iii) consequently, per sampling site a number of traps (Fig. 4A) can be installed to gather information on the heterogeneity of the fauna without jeopardizing the processing of the samples; and (iv) information on the ecology can be gathered using traps of different colours. Nevertheless, it is strongly recommended to employ both techniques in combination as they are largely complementary: a preliminary analysis of samples from Braulio Carillo National Park (Costa Rica) revealed that both trap types collected an identical number of species (26), but shared only 30% or 12 of the total number of species collected ($n = 40$) (Pollet, 2002). Actually, comparing the yields of both trap types also provides information on the flying activity and frequency of the trapped species.

3.2. Emergence traps

Emergence traps are based on the phenomenon that most insects move up towards the light after emerging. These traps very often reveal species that are rarely collected with other trapping techniques. This was recently illustrated by a field experiment (Fig. 5) along the Belgian coast (see further) that yielded 16 species of Diptera. Two of the species proved new to the Belgian fauna which was surprising as the same beach habitats have been sampled intensively for the past 30 years (Grootaert *et al.*, in litt.). Moreover, this kind of collecting method also gathers information on larval development time and food preference.



Fig. 5. Collecting insects on the beach with baited emergence traps. A. Freshly cut seaweed is put on top of a vial that is filled with sand and B. dug into the beach for two weeks; C. Subsequently the vials are transferred to the laboratory and D. a cover and collecting jar filled with 70 or 90% alcohol is attached. Emerging insects are collected weekly during a period of two months. (Photos by Wouter Dekoninck & Patrick Grootaert).

Several types of emergence traps are currently available. Some are installed for some period of time in the field, where emerging insects are gathered. Other types (see above) are baited to attract insects that deposit eggs into the intentionally provided substrate, and are returned to the lab for the larvae to accomplish their development and the adults to emerge.

Emergence traps in the field

A first type of emergence trap usually consists of a large pyramidal structure made of black fabric (nylon or other tissue) with a collecting jar on top (Fig. 6). Commercial wasp traps can be used as collecting jar and filled with alcohol. It is still unclear to what extent the climatic conditions within this trap are affected and what fraction of the present fauna eventually ends up in the collecting jar (Glen, 1976).

To collect xylobiont arthropods in the field on standing dead wood, an emergence trap can be attached to, or even constructed around the tree (Fig. 7).



Fig. 6. Emergence trap in the field. The collecting jar is a plastic commercial wasp trap filled with 70% alcohol. (Photo by Wouter Dekoninck).



Fig. 7. Emergence traps fixed around a dead tree to collect emerging xylobiont insects. (Photo by Kris Vandekerkhove).

Emergence traps in the laboratory (see also Berlese and Winkler samples, chapter 9)

Adult insects, especially Diptera, that are not easily collected with the usual sampling techniques are sometimes obtained by gathering soil, litter, dung, mushrooms, decaying fruits, wood or debris in the field, and transferring it to the laboratory for (adult) insects to emerge. Soil samples should remain undisturbed. Dead branches can be placed in large containers and can even be left for months or years as the developmental time of some xylobiont species last

several years. Xylobiont (beetle) species generally emerge in spring (April until June in northern temperate regions) and in this period, traps should be checked regularly.

- In some cases, insects are attracted by bait in order to deposit eggs. The substrates holding the eggs and larvae are subsequently transferred to the lab for the adult insects to emerge. This methodology was recently applied along the Belgian coast: jars filled with sterile beach sand were baited with freshly cut seaweed, and left in the field for about two weeks. It was assumed that fly species inhabiting the littoral zone would be attracted by the bait and deposit their eggs in the plant material. Minute holes in the bottom of the jars were provided for drainage to prevent the developing larvae from drowning. After two weeks, the jars with the soil and plant bait were brought into the lab where they were covered with a lid and a collecting jar was attached.
- A similar method is often used to collect parasitic species (mainly wasps and flies), by actively collecting the hosts in the field and rearing them in the lab. This approach enabled Dan Janzen to build an accurate idea of the tachinid parasite fauna (Diptera: Tachinidae) of caterpillars in the Santa Rosa National Park (Costa Rica) (Smith *et al.*, 2006; see also Stireman *et al.*, 2009).
- In each type of emergence trap, special attention should be drawn to the orientation and position of the collecting jar. As many emerging adult insects tend to be attracted by light, the jar opening is preferably on top of the trap and has a colour that is substantially lighter than the rest of the trap (Fig. 8). The collecting jar is best filled with an alcohol solution.

Recommendations:

- This method allows the collector to gather information on generation time and diet of the investigated species;
- Emergence traps in the laboratory are preferably held at room temperature (approximately 18-20°C);
- The humidity of the samples in the laboratory should be checked regularly. Samples that are too humid will cause mould and will stimulate mites to develop. An appropriate aeration is recommended in this case. Samples that dry too fast will cause a stop in the development of the insects or their death. If laboratory temperatures might be rather high (e.g. in summer), keeping the samples moist might be useful.



Fig. 8. Collecting jar of an emergence trap made of plumbing tubes. No glue is needed to fix the separate parts except for the mesh. (Photos by Filip De Block).

3.3. Light traps

Light traps are operated at night and are most effective from sunset till after midnight with clouded skies. Especially drizzly weather conditions are very productive, both in terms of species and specimens. This technique is generally applied for the collection of moths, scarabaeid beetles (Coleoptera, Scarabaeidae), and some Hemiptera and Hymenoptera. This trapping method is dealt with in chapter 16.

3.4. Malaise traps

Next to the sweep net, Malaise traps are the most widely employed insect collecting devices since the 70's. They work unselective and often yield high

insect diversities with huge amounts of specimens. Sufficient time should be reserved for timely processing of these large samples. This collecting method is dealt with in detail in chapter 17.

3.5. Sticky traps

Sticky traps constitute of coloured sheets covered with a thin layer of weather-proof glue. They are made of waxed cardboard, glass, wood, plastic cups, plastic sheets or trap boards, empty milk cartons, red apple spheres or any other surfaces. The sheet's colour represents the attractive agent and depending on the applied colours, particular insect groups will be trapped. Glue types that are applied to this kind of traps are transparent. Attractants can be applied in combination with the glue to lure flying or crawling insects. Tanglefoot Tangle-Trap insect trap coating is often used as adhesive and remains sticky during the entire collecting period (Fig. 9).



Fig. 9. Sticky traps: glue-covered white wooden boards are pulled up 20-30 m high in the canopy of rain forest in Papua New Guinea in order to observe dispersal of weevils (Coleoptera: Curculionidae) between trees. (Photo by Patrick Grootaert).

Recommendations:

- Unlike other traps, sticky traps can operate in inaccessible places such as the upper canopy (including tree trunks), and on top of water surfaces;
- Due to their versatility, sticky traps of different sizes and colours can be produced depending on the specific collecting purpose, similar to pan traps (see 3.1).

Restrictions:

- Insects collected with sticky traps are very hard to detach without causing damage or the loss of body parts. The technique is therefore mainly used for the collection of large insects such as beetles and wasps. The glue is usually dissolved with kerosene, which is highly inflammable;
- Another type of sticky trap consists of a transparent plastic sheet with glue on both sides and attached to tree trunks. This technique should not be employed in areas with rich and endangered arboreal lizard or amphibian faunas.

3.6. Suction traps

Different kinds of suction traps are currently available: traps of the Rothampsted type are high towers that suck in air at a height of at least 10 m, and are mainly used for the monitoring of pest species like aphids (Hemiptera: Aphididae) or gnats (Diptera: Ceratopogonidae). As such, they do not seem particularly fit for ATBI purposes.

Suction traps can also be combined with attractants. The BG-Sentinel (diameter: 36 cm / 14 inches; height: 40 cm / 1.3 feet) is a simple suction trap (Fig. 11) originally designed to collect mosquitoes. Due to its white coloured packing, however, it also proved to be attractive to a large number of pollinators (Grootaert & Dekoninck, in litt.). The trap is essentially a collapsible pop-up container with a white gauze cover, and an inlet at the top. Air is sucked into the trap through a black catch pipe at the top by an electrical fan, drawing approaching mosquitoes and other insects into a collecting bag. The air then exits the trap through the white gauze, generating ascending currents (Fig 11, red arrows). These are similar to convection currents produced by a human host, both in its direction, its geometrical structure, and due to the addition of artificial human skin odours (BG-Lure), also in its chemical composition (BioGents, 2007). Insects are gathered in the collecting bag and dried. The nylon collecting bag can be placed in a cooler and later on transferred to a deep freezer. Alternatively, dried insects can be sorted and pinned immediately or transferred to an alcohol solution. Specimens collected in this way prove suitable for DNA sequencing, even when collected after one week of sampling, which is a major advantage.

Recommendations (for the BG-Sentinel trap):

- A roof should be provided in (expectedly) rainy weather to cover the trap;
- Samples are best removed every two days to prevent damage to the dried insects by large live insects; this can be combined with replacing of the batteries.

Restrictions:

- While using a suction trap to investigate vegetation or the litter or soil layer, plant material and debris is collected which cause damage to the collected invertebrates;
- The working capacity of the batteries of the BG-Sentinel type is two days.



Fig. 10. The BG-Sentinel suction trap was originally designed to collect mosquitoes. The arrows indicate the convection stream with yellow arrows corresponding with the air that is sucked in, and red arrows showing the air stream carrying the odours of the lure out of the trap. Due to its white colour many pollinators are collected. (Photo by Wouter Dekoninck).

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