

# FOREST INSECT STUDIES: METHODS AND TECHNIQUES

## KEY CONSIDERATIONS FOR STANDARDISATION

An overview of the reflections of the  
“Entomological Forest Inventories” working group  
(Inv.Ent.For.)

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## *EDITORIAL NOTE*

This publication is invaluable: it is invaluable because it is comprehensive. Such an assertion may surprise where the insect world is concerned. Naturally, it does not deal with every species found in the forest environment. Indeed, several volumes would not be enough for that task. Its originality and usefulness is to be found in the range of themes it addresses and the manner in which they are tackled.

This publication insists on the necessity to think at one and the same time in terms of the sampling plan and the capture method (or methods) in relation to the defined objectives. Both the advantages and drawbacks of these methods are described because human and financial contingencies mean that choices have to be made. These choices have a major influence on the conclusions that could be drawn from the data gathered.

A good description of the sampling and capture technique will also allow the data to be pooled as effectively as possible and comparisons to be made, and these in turn will permit judgements to be made as to the appropriateness of a given action on the environment. The present publication is thus intended not only for naturalists and scientists but also for land managers. It acts as a bridge between these different actors ensuring that together they fully understand the study options.

The originality of this document also resides in the fact that it brings together mutually complementary authors therefore offering the reader the best and most up to date knowledge available, invariably placing the information provided in the context of defined objectives and expected results. The authors have worked together over a long period, comparing and contrasting their personal experience. They deserve our warmest thanks.

At a time when the challenges of biodiversity conservation are calling for ever more specialised expertise, the building of databases to provide input for analyses and scenarios is a necessary precondition. This publication is a major contribution to that effort. The system of information on the natural environment and landscape driven by the Ministry responsible for ecology and the French National Museum of Natural History will thus benefit from a reference work for forest entomological inventories.

Once it has been widely diffused, this document will allow entomological inventories to be developed and contribute towards helping improve our knowledge of biological diversity and its conservation.

Jacques Trouvilliez  
Director, Natural Heritage Department  
National Museum of Natural History



## FOREWORD

The Inv.Ent.For. working group was set up in 2001 following numerous requests regarding entomological inventories from forest managers (the French national forestry service [*Office National des Forêts*], French nature reserves, [*Réserves Naturelles de France*], the regional nature parks, [*Parcs Naturels Régionaux*] and the regional conservation agencies for natural areas, [*Conservatoires Régionaux d'Espaces Naturels*]).

The group is of an informal nature and its members fall into two main categories:

1) Entomologists with forestry experience from a diverse number of different bodies:

- The French forest health department, *Département de la Santé des Forêts*,
- The French national agricultural research institute (INRA) in Bordeaux, Orléans, Montpellier and Versailles,
- The French institute for scientific and technological research for the environment (CE-MAGREF) in Nogent/Vernisson,
- The French National Museum of Natural History, *Muséum National d'Histoire Naturelle*, in Paris,
- The Troyes Museum of Natural History,
- French and Belgian universities: Grenoble, Orléans, Toulouse (Purpan engineering school) and Gembloux,
- The Luxembourg Museum of Natural History,
- The French national forestry service, *Office National des Forêts* (its internal entomologist network)
- The bureau for insects and their environment, *Office Pour les Insectes et leur Environnement*,
- A number of regional entomological associations,
- Consultancies (independent professional entomologists).

2) Managers looking for inventory methods, or inventories as such, and institutional partners:

- *Office National des Forêts*,
- *Réserves Naturelles de France* (forestry network)
- French regional nature parks,
- French Ministry of Agriculture and Fisheries,
- French Ministry for Ecology, Energy, Sustainable Development and the Sea,
- Regional local government authorities.

The working group had the following objectives:

1. To establish a set of technical specifications for inventories laying down a minimum inventory framework. The latter must detail the groups of insects to be inventoried in accordance with defined inventory objectives, along with the sampling methods and minimum inventory duration.
2. To list the resources for determination.
3. To draw up an ethical framework for the use of inventory data.
4. To make recommendations for forestry management that protects entomofauna.

The present document essentially addresses the first of these objectives, aiming **to offer a minimum standardised technical framework to those responsible for the management of forest areas and to entomologists conducting inventories at the request of managers**. As a consequence, this publication can in no way be seen as a detailed entomological manual containing an exhaustive list of all known inventory methods for the various insect groups making up forest entomofauna.

The production of this document has been made possible by financial contributions from the *Office National des Forêts*, Cemagref, the *Office Pour les Insectes et leur Environnement* and *Réserves Naturelles de France*.





## SUMMARY

Due to their enormous diversity, crucial ecological role and, in some cases, their use as bio-indicators, insects have been increasingly taken into account in the management and conservation of natural areas over the last decade or so.

However, the study of insects is currently suffering from a lack of professional resources (professional entomologists, training) and insufficient background knowledge among land managers, despite their strong interest in this vast group.

Ranging from rough inventories to the examination of the effects of a given management approach (comparative studies) including monitoring, any approach to entomological diversity will involve specific sampling methods and techniques.

Despite the fact that forest managers have long conducted entomological studies, sampling protocols and study groups often differ from one site to the next. Such virtually independent approaches between managers lead to difficulties in comparing the results from different sites.

In 2001, at the request of land management bodies, a working group entitled, 'Inv.Ent.For.', was set up to reflect upon how consideration could be given to entomological fauna within forest areas. This group, composed of professional entomologists (researchers, research managers in consultancies and not profit associations, etc.) and management bodies for natural areas (e.g. ONF, *Réserves Naturelles de France*), aimed to define a minimum technical and standardised framework for the insect groups to be targeted in entomological studies and the methods for their sampling. These proposals are detailed in the five chapters of this report.

Chapter 1 contains conceptual and practical information on conducting inventories and sampling. It sets out the imperative stages any scientific study must go through and the importance of a small number of broad principles to be followed.

Chapter 2 provides a detailed list of techniques for insect sampling in terrestrial (not only forest) ecosystems as well as aquatic environments. It goes on to detail, with accompanying practical advice, four methods proposed by the working group for use in temperate forests: pitfall traps, window flight traps, Malaise traps and light traps.

Chapter 3 describes a small number of insect groups that can be captured for tropical forest inventories, along with the specific techniques required.

Given the impossibility of encompassing the huge diversity of forest insects, the members of Inv.Ent.For. have defined five insect groups that merit systematic attention in the forest context: ground beetles, saproxylic beetles, hoverflies, butterflies and moths, and red ants. Each of these is discussed in turn in Chapter 4, addressing the benefits to be gained from studying them, and the methods whereby they can be sampled in accordance with the objectives defined by forest managers.

The final chapter contains some practical advice on the management of those insect samples that have been collected, from packaging after sampling, to the use of the data and including the mounting of individual insects.



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## INTRODUCTION

(Louis-Michel Nageleisen)

Over the last decade or so a growing need for entomological inventories has emerged with:

- The setting up of forest reserves (nature reserves, managed and strict biological reserves, etc.) and the obligation to create management plans that include entomofauna;
- Development of the Natura 2000 network;
- Revision of the ZNIEFF inventory (*Zones Naturelles d'Intérêt Écologique, Faunistique et Floristique* / Natural Zones of Ecological, Animal and Plant Interest);
- Demand for sustainable development indicators;
- Ignorance of the status of most forest species....

In order to carry out inventories, managers of forest areas approach various bodies, but in most cases these are regional non profit associations due to the lack of resources in the professional domain. Indeed, we can only deplore the gradual disappearance of professional entomologists working in universities and other such institutions.

After more than ten years of inventorying, we can clearly state that forest managers are generally profoundly ignorant of the insect world. One consequence is that the demands made for inventories are often unrealistic, especially for example in terms of the timeframes imposed for the delivery of results. Moreover, contrary to other forest related domains (felling, forestry practice and management planning, etc.), managers do not usually lay down any technical specifications (defining objectives, methods, etc.) due to their limited knowledge of entomofauna.

Organisations, official bodies or quite simply individual entomologists charged with conducting inventories carry them out conscientiously, often with great entomological skill that is in many cases acknowledged at national or even international level. However, the usefulness of the results is sometimes debatable due to these entomologists' lack of knowledge of the forest environment and inadequate formulation of the work commissioned by the forest management body. Such inventories cannot in most cases be compared with each other due to the lack of standardisation of the methods.

At a time when human, financial and other resources are more limited than ever, this absence of standardisation leads to results that are disparate and cannot be compared or merged to cover wider areas (regional or national) whereas the need for standardised data is making itself felt more than ever before, in order to improve our knowledge of the status of insects at regional and national levels.

There is therefore a need to put in place a dialogue between entomologists and forest managers with a view to better understanding, firstly, of the specific characteristics of entomofauna on the part of forest managers and, secondly, of the constraints on forest management on the part of entomologists.

The benefits of taking insects into account when managing a natural ecosystem such as a forest hardly need demonstration. This is because insects make up a dominant part (greater than 80%) of the biodiversity of forest fauna. They are actors in the functioning of ecosystems and they are involved at every level of trophic networks. They may be primary consumers (phytophage insects), secondary or tertiary consumers (predators, super-predators, parasites, and hyperparasites). Saprophages (saproxylrophages, necrophages, coprophages, and detritivores) are key actors in the matter cycle (organic material, minerals, etc.). The presence or absence of certain species, or rather of certain corteges, makes it possible to verify whether an ecosystem is functioning properly or not. In this way, insects can be good indicators of ecosystem quality and management impacts on the forest habitat. From the smallest among them to the biggest, from the most insignificant to the most spectacular (or 'pretty'), they are a heritage that we can no longer afford to ignore.

But however necessary and relevant the study of forest insects may be, it is complicated by numerous constraints linked to the biological characteristics of insects.

The first constraint is the number of insect species (over 10,000 in French forests). It is not possible to inventory or to monitor everything. This means that it is necessary to limit efforts to certain key groups on the basis of the desired objectives. Insect populations are subject to fluctuations in space and time that are usually poorly understood, and this must entail the use of robust sampling plans that take such fluctuations into account.

Lastly, unlike botanical or ornithological studies, where determination is done largely in the field during an inventory without 'collecting' specimens, insects are not identifiable, with some rare exceptions, other than at high levels of magnification (20x-100x) which in turn entails the gathering of specimens which then, after completion of the field activity phase, need to be sorted, cleaned and mounted in a dry collection for subsequent determination. In this last phase, a current major constraint is the lack of specialists for every group or family of insects, as well as systematic documentation of recent date. Some difficult-to-access groups (for species determination) cannot be included in standardised entomological studies across the entire national territory.

For forest managers, the relevant issues may relate to the impact of management on entomofauna, risks to stands due to conservatory management (e.g. the link between dead trees and pests) or the presence of insects of particular importance (e.g. protected species, 'heritage' species). The studies 'commissioned' from entomologists are in such cases only the initial phase in the process of expanding knowledge. It is a phase that is obligatorily followed by a decision stage that takes into account some of these factors in managing the forest environment. It leads on to the establishment of directives in management plans that are subject to official regulations. This series of phases means that studies often need to be completed very rapidly. Lastly, one of the major constraints on managers is the budget allocated to studies which may be demanding in time and effort before significant results can be obtained.

It is against this backdrop, taking into account, firstly, managers' imperatives and expectations and, secondly, entomofauna-related constraints and limitations, that the Inv.Ent.For. working group carried out its tasks, bringing together managers and entomologists. The ultimate goal is to **offer** those responsible for the management of forest areas and to entomologists engaged in inventories at the request of such managers, **a minimum technical framework that is standardised**. Such a framework should make it possible to obtain results that are useful at local level for improved knowledge and for management, that are comparable with similar studies carried out elsewhere and which can be scaled up to contribute to an overview of entomofauna status at regional and national levels.

## **CHAPTER 1**

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### ***DESIGNING AN INVENTORY:***

***How should a sampling plan be defined?***

**(Emmanuelle Dauffy-Richard, Philippe Bonneil and Christophe Bouget)**





When carrying out an entomological inventory, we are engaged in sampling without always being aware of the fact. Unfortunately, if we are unaware of the principles underlying sampling, and therefore of the limitations on our inventories, this can lead us to make incorrect interpretations or prevent us from reaching conclusions.

How then should we proceed in order to avoid such pitfalls? The crucial stage is the correct definition of the inventory's objectives prior to commencing the study, as the sampling strategy will depend on them. These objectives must be aligned with the biology of the groups to be studied, must be realistic in light of the resources available and reflect a consensus shared by all those involved in the study, to ensure that inventory data are not used subsequently for purposes for which the data has not been correctly obtained.

If the objective is properly pinned down this will help later at each stage in building the sampling plan, and especially in correctly defining (i) the situations between which we wish to compare entomofauna and (ii) the sampling unit and method that flow from them. Standardisation of methods, repeating, controlling and balancing sampling units between the situations we are seeking to compare will enable bias to be limited and assure the accuracy of the results.

## **I – INVENTORY OR SAMPLING?**

An inventory of the insects on part of a defined area involves drawing up the most exhaustive list possible of all the species present and seeking, if applicable, indications of how abundant they are, their biology and their ecology, the impacts of one or more natural or anthropogenic factors, etc.

However, an entomological inventory is a sample before it is anything else because it is impossible to succeed over any large area in conducting an exhaustive census of mobile and highly diverse organisms such as insects (Conroy, 1996).

We need to bear in mind that a sample is a subset representative of a larger set of entities that it is sought to represent (known as the '**target statistical population**'; e.g. species of hoverfly on a given site, populations of a species in a given type of habitat, ant colonies at various forest management stages, comparing young with old stands in a plantation of maritime pine, etc.; cf. Part IV of the present chapter.). The sample must provide the most representative snapshot possible of the study target at a given moment and in a given place in relation to a precisely defined question.

## **II – A SAMPLE OF WHAT?**

It is possible to sample a **population** if the focus is on just one species (e.g. a pest, a heritage species) in order to determine its demographic parameters (numbers, fertility, mortality), its intra-species diversity (genetics), its ecological requirements (habitat, food, etc.) or its distribution (mapping, movements of individuals).

Examples: The monitoring and distribution of the pine processionary (*Thaumetopoea pityocampa*, Lepidoptera Thaumetopoeidae); study of the status of violet click beetle populations (*Limoniscus violaceus*, Coleoptera Elateridae).

All species in the same group present at a given time on the same site, (*i.e.* a **community**) can also be sampled, with a list of species being drawn up with indications or not of their abundance (relative or absolute). It is possible in this way to determine interspecies diversity, species distribution (map or atlas) and their ecological requirements, making comparisons between sites, biotopes, approaches to forest management, etc.

Example: Studies of the effect of windfall clearings on ground beetles.

## **III – SAMPLING FOR WHAT PURPOSE?**

In order to know 'what' to sample and 'how' to sample it, what we need to know above all is why we are sampling it and how we want to use the information gathered subsequently.

Indeed the objective defined for the inventory will greatly influence the way in which the sample is acquired (what should be sampled, where, when and how) and, looking at it from the opposite direction, the sampling thus chosen will limit the scope of the results (cf. Figure 1, Table 3 and Insert 1).

Hence the need to define, very precisely, very early on and in conjunction with the organisation commissioning the work, the key objectives for the inventory, doing so in the form of a question to be answered or an expected result. If these objectives are then translated as soon as possible into a sampling strategy that is concrete and appropriate for the questions to be answered, this will allow the inventory objectives to be revised later if they turn out not to be achievable given the material circumstances of the study (timeframe, human resources, or simply the existence, in reality, of different practical expressions of the factor whose effect is to be assessed, and so on).

Below are some examples of objectives and their associated sampling constraints (cf. Gosselin and Gosselin, 2004):

**Information on the presence of species in a given place at a given time (faunistic approaches)**

This may involve drawing up as exhaustive a list as possible of the species present in a given place and at a given time (e.g. assessments of the status of reserves, heritage overviews, production of an atlas) or simply to carry out an active search for certain heritage species for the classification of sites subject to a particular status (required for ZNIEFF<sup>1</sup> species, or Annexes II and IV of Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora).

Although such an inventory is no more than the first stage in obtaining knowledge (Debinski and Humphrey, 1997), it is nevertheless necessary because information is patchy for many sites. For example, only 21% of Strict Biological Reserves (*Réserves Biologiques Intégrales*) and 16% of Managed Biological Reserves (*Réserves Biologiques Dirigées*) benefit from levels of knowledge considered ‘fairly good’ or ‘good’ where dragonflies and damselflies or butterflies and moths are concerned.

Sampling conditions (cf. Insert 3):

These objectives entail a need **to maximise the exhaustive and representative nature of the sampling** applied to the site, including all environments coexisting there, and with regard to the species that are actually present. This therefore leads to (i) a diversification of sampling approaches in order to detect species with the widest possible variety of habits, (ii) allocation of a high level of sampling effort in order to contact a maximum number of species (including those that are rare) and cover the whole of the relevant area (cf. Part VI of this chapter), (iii) in addition to using all pre-existing data sources (naturalists’ observations, collections, bibliographies, and so on).

However, the reverse side of the coin is that lists derived from such approaches are usually difficult to compare (between sites or different points in time), unless sampling is totally exhaustive (which is impossible to achieve) or unless certain sampling biases can be corrected after the fact, this being either very problematic (e.g. Dufrêne and Desender, 2006, with a view to building a distribution atlas or red list) or quite impossible. This is so because the more diversified the sampling methods and conditions and the greater the effort devoted to local sampling, the more difficult it will be to reproduce the protocol identically in the various different circumstances to be compared (cf. Figure 1, Table 1).

Despite this, such inventories are often reused as the baseline for the monitoring of a site’s entomofauna over time, or to build a species distribution atlas, or even for ex post assessments of environmental effects, and so on. All such subsequent uses necessarily entail comparisons between sites or between points in time.

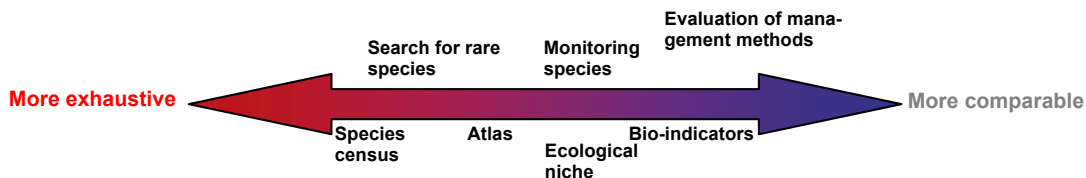


Figure 1: Priority can be given either to the exhaustiveness or to the comparability of sampling according to the inventory objective.

<sup>1</sup> *Zones Naturelles d’Intérêt Ecologique, Faunistic et Floristique* / Natural Zones of Ecological, Animal and Plant Interest.

**Comparison of entomofauna in widely different situations (comparative approaches)**

This amounts to comparing the presence (or abundance, etc.) of species between sites, over time, between habitats or between forestry management situations, in order to detect, for example, differences in species composition between communities (presence/absence), changes in the abundance of one or more species, or variations in the demographic parameters of different populations of a given species, etc.

Examples:

- Monitoring over time of a population or a community (with reference to a baseline state): STERF (*Suivi Temporel des Rhopalocères de France* / Temporal monitoring of butterflies in France), OPJ (*Observatoire des Papillons des Jardins* / Garden butterfly observatory).
- Evaluation of environmental effects on entomofauna; seeking to identify bio-indicator species:
  - The reaction of saproxylic beetles to storms or clearcutting (disturbance of natural or anthropogenic origin);
  - Ant species characteristic of deciduous as compared with coniferous forests (bio-indicators);
  - Appearance/disappearance of species of Lepidoptera as a function of altitude (differentiation of communities along an external gradient).

Sampling conditions:

If we are to be able to compare different situations, the sampling needs to be:

- Either totally exhaustive (impossible!),
- Or standardised, reapplying the same sampling methods, parameters and effort in the situations to be compared (sites, dates, approaches to forestry management, etc.). In this latter case, **rather than maximising exhaustiveness the need is to equalise the degree of exhaustiveness between the situations for comparison.**

However, other parameters contribute to sampling comparability. The stringency of the requirement will vary according to the degree of reliability desired in the results (cf. Insert 1, Table 1 and Table 3).

**Insert 1: The degree to which sampling is constrained depends on the approach adopted (cf. Richard, 2004).**

Standardisation of collection methods and correct representation of the targeted entomological group (the statistical population) are necessary conditions to permit comparison between inventories and delivery of the initial elements of an answer to exploratory questions (e.g.: atlas, monitoring heritage species, characterisation of the ecological requirements of a species, and so on).

However, satisfaction of these two conditions is not always sufficient for robust results. This is because there is a risk that chance trends may appear due simply to random or other hidden factors (Anderson *et al.*, 2001). It will therefore be possible to offer hypotheses at the conclusion of the study (*a posteriori*), but it will not really be possible to test them.

To go further, those hypotheses must then be verified (or contradicted) by a later, more tightly focused study (the **confirmatory approach**), using a sampling plan built in order to simulate a field experiment (a mensurative experiment, Hurlbert, 1984; Krebs, 1999). For example, in order to know whether the biodiversity of ground beetles differs according to whether forestry management is based on regular or irregular stands, we need to stratify the sample according to the factor 'forestry management mode', making sure at the same time that the principles of repetition, control and balance of the sample units are adhered to (cf. insert 4). These precautions will allow the core hypothesis of the study to be tested, as formulated according to previous research (bibliography) or derived from one's own experience as a naturalist. E.g. 'H1- Irregular stands should provide a habitat for more forest species than regular stands'.

Nevertheless, despite its rigorousness, this, like the previous approach, is still **descriptive**. If we are to be able to apply the results generally, it is necessary to repeat the mensurative experiment in different contexts or endeavour to understand the process underlying these patterns of correlation.

The latter case entails a need to look closely at the mechanisms for the phenomena observed in nature, which amounts to **an effort to demonstrate the existence of a causal relationship between two phenomena** (E.g. 'Does clearcutting cause ground beetle species to disappear? And if so, after how many years?'). However, only tools such as modelling and genuine experiments (**manipulative** experiments, Hurlbert, 1984; Krebs, 1999, cf. insert 6) can help explain natural phenomena.

Table 1: Sampling conditions to be met as a function of the desired objective. The symbol ‘+’ means ‘condition to be met’: symbols from ‘+’ to ‘++++’ (read vertically) indicate that a condition is increasingly essential to attainment of the desired objective (read horizontally). NB: ‘\*’ also includes conditions of representativeness, precision, robustness and comparability.

			Required sampling characteristics						Type of approach			
			exhaustive	representative	Precise, robust	comparable	Mensurative*	Manipulative*	Faunistic	comparative exploratory	comparative confirmatory	Experimental mechanistic
Local species census	++++	++	+	+	+							
Search for rare species	+++	++	+	+								
Atlas, mapping	++	+++	++	++	+							
Monitoring over time	+	+++	+++	+++	+							
Species autoecology	+	+++	+++	+++	+							
Detection of an expected difference due to the effect of a factor (descriptive pattern)	+	++	++++	++++	++++							
Search for the cause of an observed difference (causal process)	+	++	++++	++++	++++						++++	

## IV – STAGES IN THE SAMPLING PROCESS

The approach usually adopted is the following (cf. Part VI of this chapter for more details on the concepts listed below):

### 1. Defining the question to be answered:

This is often a question raised by the forest management body when facing a problem linked to a specific context involving a particular goal.

Example: “Will a change from coppice with standards to high forest have an impact on entomological diversity?” The problem: conservation of biodiversity. The context: a change from coppice with standards to high forest. The goal: To reconcile the conservation of biodiversity with wood production.

### 2. Based on the literature (scientific, historical, observation of the natural environment) and the questioning of experts, translate this question, identifying:

- The group to be studied (according to its ecological role, its diversity, the ease with which it can be studied, the availability of time and experts for determination, etc.) and the sampling method;
- The entomological and observational studies already carried out on the site;
- The hypotheses to be tested (disturbance, species succession, and so on);
- The explanatory variables (the year in the case of monitoring over time, forestry treatment types for assessment of management, etc.) and covariables (other influences: site class, land area, tree species, etc.);
- The response variables: direct measurement of the community (species presence/absence or abundance), useful for gaining information on species composition, and more synthetic descriptors (species richness, equitability indices, similarity, etc.) for the whole of the community and for each ecological group;
- The analytical methods.

### 3. Identification of constraints and resources:

- Natural constraints (broken terrain, underrepresented habitat, habitat subject to flooding, etc.);
- Resources: technical resources (pre-existing distribution maps, identification keys, reference collection, etc.), human (time, staffing and available skills) and financial (number of traps, etc.);
- Mathematical constraints (complex analytical methods, costly software, etc.).

### 4. Designing the sampling plan:

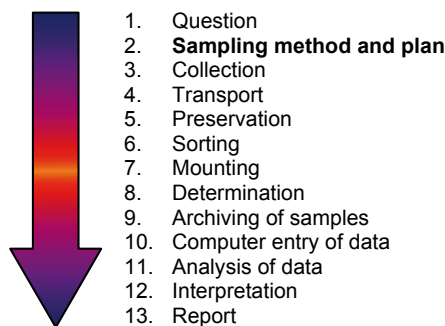
- Use as a basis as detailed a map as possible of the ecological units (CORINE biotope typology, map of forest stands, stand types, forest modification works, etc.);
- Depending on the objectives, define the scale of the sampling unit (types of forest stand, micro-habitats, forested area) and the target statistical population;
- Choice of the most suitable type of sampling plan: random, systematic, or stratified depending on the explanatory variables (cf. Insert 2, Insert 3 and Insert 4);
- Repetition of the sampling units as permitted by available resources, taking care to ensure that they remain independent in time and space (cf. Insert 5);
- Replication to be evenly balanced along the gradients (or between the factors) to be studied;
- Control of interference variables, potential sources of bias and factors conducive to confusion (e.g. stand-related, year of collection, etc.);
- Adjustment of the sampling method (type and number of traps per sampling unit, duration of the experiment [number of years, number of seasons, duration of seasons], etc.) and its standardisation across all sampling units;
- Selection and visible indication of sampling points in the field, either following random selection within limits predetermined on the basis of predefined constraints, or by surveying the site in accordance with documented criteria.

**5. Execution of sampling and building the data:**

- Collection (installation and periodic emptying of traps), transportation, conservation;
- Measurement of variables and explanatory covariables in the field (measured characteristics of trees and stands, etc.) or in the laboratory (aerial photos);
- Sorting, mounting, determination, archiving of samples;
- Input of data on fauna and the environment.

**6. Data analysis (cf. Gosselin and Gosselin, 2004) and drafting of the report:**

- Quantification of biodiversity on the basis of the species/trap records table (cf. response variables);
- Once the suitability of the statistical methods for the category of data has been checked, the descriptors should be compared between forestry treatments or the response variables correlated with the explanatory gradients: graphs, univariate or multivariate statistical analyses, and so on;
- Interpretation of the results and drafting of the report for the body commissioning the study.



**Figure 2: The place of sampling in the overall context of an entomological study.**

**V – CHOICE OF SAMPLING METHOD**

(cf. Chapter 2)

The selected group will of course be the primary criterion in choosing a sampling method. Following this, a number of other criteria are relevant:

- Effectiveness (the representativeness of the sample obtained in relation to reality);
- Selectivity with regard to the group to be studied;
- The possibility of using the method in comparisons (standardisation, repeatability);
- Feasibility (cost, availability, time needed for implementation, etc.).

The chosen method must be capable of maximising the **effectiveness** of insect capture, i.e. of providing an image that is as close as possible to reality. The capture device must for this reason limit as far as possible the avoidance or escape of individual specimens (e.g. fast kill, limited openings). For traps incorporating lures, it will be necessary to verify the radius of attraction for species, because if this is too great there is a risk that mobile species will be captured after wandering in from environments other than the one being sampled.

In addition to the type of sampling method chosen (size and characteristics of trap, collection fluid, etc., cf. Chapter 2), the effectiveness of the method is also related to the number of measurements made within a single sampling unit (e.g. how many traps should be placed on the study plot?). In order to be able later to estimate the detectability of a species and identify possible sources of bias (cf. Bonneil, 2005; Dauffy-Richard and Archaux, 2007), it is useful to repeat measurements micro-locally, resources permitting (e.g. at least two traps per study plot). This may also be useful to forestall risks of trap destruction or disturbance. Care should however be taken to avoid systematic consideration of such measurement micro-repetitions as genuine sampling repetitions (cf. Part VI-(3) of the present chapter and Insert 5).



If possible, the chosen method should maximise the capture of species in the target group and minimise capture of non-target groups (**selectivity**), due to ethical concerns and in order to reduce the time devoted to sorting.

The method **must lend itself to standardisation** in order to allow comparisons between sites, between several sampling campaigns, over time, and so on. To achieve this, the detection of all species in the group must not only be satisfactory but above all equivalent between the types of environment being compared, and if possible between species. If the method tends to detect some species more readily, it is preferable to avoid summing species abundance figures and to work on the basis of relative species abundance. This condition for comparability entails the standardisation not only of the device (type of trap) but also the protocol (installing and emptying traps) with a view to minimising bias due to disturbance when installing the traps, installer-related effects, and so on.

And lastly, the method must be **useable**, which in turn means that the constraints represented by equipment cost, ease of implementation or trap installation, availability from suppliers or the possibilities for building it, the workforce available, and so on, are all considerations to be taken into account in choosing the method to be used.

## VI – WHICH SAMPLING PLAN? PRINCIPLES TO FOLLOW

Unfortunately, there is no single recipe for a sampling plan to suit every situation. Everything depends on the inventory objectives (cf. Table 3), and the particular features of the data to be acquired (degree of variability in insect populations, potential confusion effects, etc.). General recommendations can be derived from two broad types of supplementary statistical tools (Frontier, 1983; Goupy, 1988; Ims and Yoccoz, 1997; Jayaraman, 1999; Krebs, 1999; Ancelle, 2002):

- Sampling techniques, which are aimed at **the best possible description of existing reality**, i.e. a description that is **representative** and **accurate**, estimating the mean and the variability of a descriptor for a given statistical population based on a sample of that population (cf. Insert 2),
- Planned experiments, which are aimed at **testing the effects of predefined forestry treatments on a response variable**, comparing the values for that variable between randomised manipulative treatments (cf. Insert 6).

### Insert 2: How can representativeness be assured?

**Random selection** (e.g. Figure 3.a) of a large number of sampling units (**repetitions**) will ensure that the sample is representative of the target statistical population (e.g. insect communities on the site): an image that is reduced in size but nevertheless faithful, *i.e.* free of bias (Ancelle, 2002).

However, in the field of ecology, **systematic sampling**, which involves selecting sampling units that are regular in space and/or in time (e.g. the grid in Figure 3.b), is frequently preferred to random sampling, especially for mapping or monitoring purposes, because it is more practical and less costly in the field when aiming at good coverage of the study area. Nevertheless, it will be necessary to verify the conditions for its validity (cf. Greenwood, p 79; Ims and Yoccoz, 1997, p 66; Krebs, 1999, p 291-293).

In order to take account of the effect of a fundamental factor (e.g. the effect of regeneration cutting), **stratified random sampling** involves subdividing a heterogeneous population into sub-populations (or strata) that are more homogeneous, mutually exclusive and collectively exhaustive. Within each stratum the sampling units are selected at random and independently of each other. According to the objective, the number of sampling units may be identical from one stratum to the next or proportional to the size of the stratum, or its scarcity (Frontier, 1983, p 92-108; Legendre, 2007).

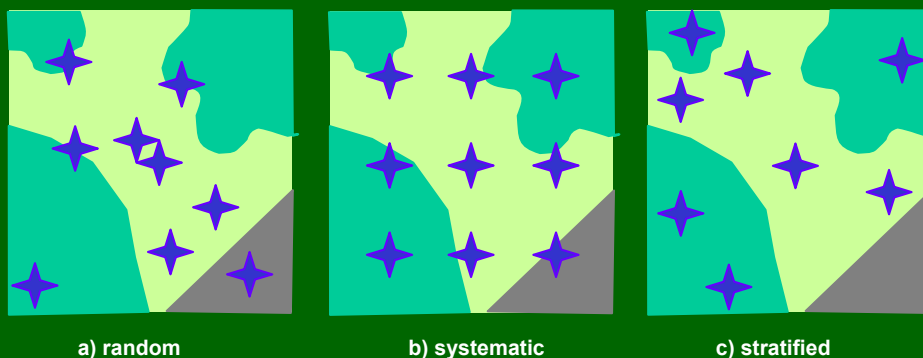


Figure 3: Illustration of three types of sampling plan applied to the same part of an overall area: (a) random sampling (observations at coordinates selected at random within a forest); (b) systematic sampling (sampling grid with one point every 100m); (c) stratified sampling according to soil cover (observations distributed at random in young forestry stages and respectively in older stages).

Whatever the objectives, a single approach and a few broad principles must be adhered to in designing the sampling plan (cf. Part IV of the present chapter):

**(1) Identification of the scale and type of sampling plan best suited to the question to be answered**

This entails a need to determine exactly what one wishes to sample ('What?') in order to determine the best way to sample it:

- ❖ The **explanatory variables** and the scale of the **sampling unit** which derives from them (Table 2):
  - **Explanatory variables** are quantitative (gradients, e.g. altitude) or qualitative (factors, e.g. cutting) effects that it are desired to evaluate with regard to the response variable (species richness, species abundance, etc.). The choice of the domain in which the explanatory variable is examined (number and values of different expressions of the factor, range of variation for a gradient) will have a knock-on effect on the ability to detect the targeted effect (magnitude and accuracy).
  - The **sampling unit** usually corresponds to a site on which the explanatory variables (environment) and responses (insect communities) are to be measured. It is crucial to define the spatial and temporal scales for this unit in order to apply the replication principle at the correct level (cf. Table 2, Part VI-(3) of the present chapter and Insert 5). To achieve this, two questions must be answered: What effect is being sampled? and: To what does the explanatory variable apply? This is so because these scales must be chosen to ensure that it is possible to take into consideration a value that is meaningful with regard to the explanatory variables (intra-unit homogeneity), and to vary their different expressions from one unit to the next (inter-unit heterogeneity).
- ❖ The **target statistical population**, the whole set of situations of interest (cf. Part I of this chapter), from which the sampling units will be drawn, and which will then represent the validity range of the study's results (Conroy, 1996, p 127).

**Table 2: The scales to be considered will vary according to the question to be answered.**

Effect on ...	of ...	→ Repeat for...	for a conclusion on ...				
<b>Response variables</b>	<b>Explanatory variables</b>	<b>Sampling units</b>	<b>Target statistical population</b>	- species richness of the community	cessation of forest harvesting	forests containing a reserve	a forested or biogeographical region
				- species abundance	thinning cycle	forest stands with varied thinning dates	an acidiline oak-hornbeam forest
				- life history features	windthrow	micro-habitats associated or not with windthrow	a land area presenting different storm impacts
				- total size of a population (capture - marking - recapture)	season	'trap x period' combinations	all relevant traps on a given site in a given year
				- number of contact points (radio tracking)	felling	felled and un-felled forest stands	all forest stands included in survey
					habitat type	individuals fitted with transmitter collars	species preferences between available habitat types

- ❖ The **type of sampling plan** (cf. Insert 2):

When the main focus is on the effect of certain explanatory variables (e.g. felling), the sampling plan should be **stratified** to reflect them, with random selection, repetition and balancing of sampling units within each of the **forestry treatments** (e.g. before vs. after felling), *i.e.* the combination of different expressions of the various explanatory factors being studied (cf. Insert 4). Complete **cross-correlation** of the explanatory variables (orthogonality) is imperative if it is to be possible to measure their respective effects independently of each other. A sampling plan stratified on the basis of forestry treatments



also provides the best possible simulation of a field experiment (**mensurative experiment**, cf. Inserts 4 and 6).

In other cases, a straightforward **random or systematic** sampling plan can be used, with care being taken to cover the whole of the target statistical population (cf. Insert 3). The granularity of the sampling will then depend on the compromise to be found between the extensiveness of the inventory site and the desired sampling effort.

**Insert 3: How can an overview of the status of the fauna in a reserve be built up?  
Example of a sampling plan for a species census.**

The objective here is to ensure that the observations are as **exhaustive, mutually complementary and representative** as possible in order to arrive at as complete a census of the species as possible in a given area, while at the same time retaining the possibility of **later comparison** of part of the resulting species list with other sites or to monitor the same site over time. The most effective approach is to explore the widest possible variety of habitats, seasons and times of day, combining more than one mutually complementary collection method (trapping, sight surveys, beating, etc.) while at the same time ensuring that certain of these methods are standardised (e.g. trapping).

Similarly, the recommended sampling strategy is a combined one: covering the whole of the area using random or systematic **sampling** (also including the more 'ordinary' parts of the study zone, cf. Insert 2), and supplementing this with more in-depth exploration of certain potentially species-rich micro-habitats (Sutherland, 1996).

Meeting all these conditions necessarily involves an initial definition of the target statistical population: extent of the site, environmental diversity and the diversity of the insects it is wished to include in the inventory, and so on.

**(2) Repetition of observations across a sufficient number of sampling units**

The purpose of replication is to take into account the natural **variability** of the phenomena studied by taking a diversity of possible cases into account. Replication therefore improves the **representative character** of the sampling in relation to the target statistical population. It is an essential condition to be met in order to avoid observing events that are due purely to chance.

The term 'repetition' (or 'replication') refers to sampling units that correspond to the same treatment or to graduated values of a quantitative explanatory variable (environment, space or time). For example, several areas subject to cutting for seeding purposes will constitute the same number of repetitions for 'cutting' treatment; in order to replicate the 'nature reserve' forestry treatment, it will be necessary to process several forested areas (*i.e.* separate reserves); plantations of differing ages count as repetitions for the quantitative variable 'plantation age'; stands of oak more or less rich in Scots pine will constitute repetitions when studying the effect of different degrees of mixing in the case of the quantitative variable 'percentage land area occupied by pine'. (cf. also Insert 4 and Table 2).

Repetition can enable the uncertainty surrounding a result to be calculated (e.g. variance, standard deviation, confidence interval around a mean). Replication will also increase the chances of detecting the effects being studied (**analytical power**) by improving the **precision** of their estimators; e.g. the higher the number of replications, the narrower the confidence interval around the mean.

This is so because if an effect is to be detected, the observed differences between treatments (e.g. cutting *versus* mature) must be greater than those observed within the same treatment (e.g. variability within areas subject to cutting). What is looked for therefore is **inter-treatment variability that is greater than intra-treatment variability** (Debinski and Humphrey, 1997). As a consequence, the greater the natural variability within the same treatment (noise), the more intra-treatment repetitions will be needed to highlight a difference between treatments. As a general rule, in order to double estimation precision (*i.e.* in order to reduce the width of its confidence interval by half) it will be necessary to multiply the number of repetitions by a factor of four (cf. Greenwood, 1996, p 74, 81-104; Ims and Yoccoz, 1997).

In more concrete terms, although the number of repetitions required for a given sampling plan also depends on the number of explanatory variables, and on the form, magnitude and degree of variability of the expected effects (cf. Krebs, 1999, p 229-260 for the basic principles of power studies), the usual recommendation is to provide for **at least 10 repetitions per treatment type** (in order to take account of possible interactions between factors) and **between 10 and 30 repetitions for each of the quantitative environmental variables**.

Naturally, a compromise needs to be found between the number of repetitions, which must be sufficient, and the effort this represents in terms of human and financial cost and the time taken to deliver the results. Where resources are limited, it may sometimes be preferable to look at a single explanatory variable with a satisfactory number of repetitions rather than covering numerous gradients, but with too few repetitions to reach any conclusion.

However, in order to gain the benefits of replication, the repetitions must be allocated without bias within the sampling plan. This entails a need to begin by defining the right level of replication, i.e. the scale of the sampling unit (cf. Part VI-(3) of the present chapter and Insert 5), and to ensure that confusion effects can be limited (cf. Parts VI-(4) and VI-(5) of this chapter).

**Insert 4: How can the effect of a given mode of forestry treatment on ground beetle biodiversity be studied?  
Example of a mensurative experiment**

In order to find out whether ground beetle communities differ between regular and irregular stands (from cluster to cluster), an effort is made to estimate the effect of the forestry management technique (explanatory variable 1) on Carabid species richness for each ecological group (response variables), but it may be surmised that this effect will also depend on forestry management stage (interaction with explanatory variable 2). In light of the available knowledge, one of the underlying hypotheses would be: *“In the case of regeneration stages, regular stands will be richer in species associated with open habitats than irregular stands, whereas the contrary will be true of older stages”*.

The right sampling plan will be one that is stratified with regard to the two most important explanatory factors, and entirely cross-correlated and balanced between the six treatments resulting from combinations of their different versions:

- Forestry management technique, a factor with two facets (regular vs. irregular),
- The stage reached, a factor with three facets (regeneration-clearing / intermediate / mature).

Ten repetitions per treatment, evenly balanced over the whole of the sampling plan, will be required for an examination of the interaction between the two factors (*“The effect of the forestry technique will depend on the growth stage reached”*). One highly problematic case would be an absence of any repetition simultaneously for both mature regular stands and irregular stands (i.e. the diagonally opposite boxes are both empty): in the sample, the irregular stand would be more ‘mature’ than the regular stand. The two factors ‘treatment type and ‘growth stage’ would then merge and it would not be possible to separate out their respective effects.

		Forestry management stage		
		Regeneration - clearing	intermediate	mature
Type of forestry treatment	regular	10 stands	10 stands	10 stands
	irregular	10 stands	10 stands	10 stands

The sampling unit to be replicated is a forest stand that is homogeneous not only in forestry management terms (treatment, stage reached), but also ecologically (covariables). This is the case because other variables will influence the ground beetle communities. The values of the potential disturbance variables will be fixed *a priori* in order to avoid certain sources of bias, by limiting sampling to a single forested area (historical and biogeographical bias), to acidiphile oak and hornbeam stands (stand and dendrological bias), and to stands more than 100m from the edge of the forested area (edge-effect). Seasonality can be controlled by repeating observations at the same periods for all six treatments. It will then be possible to measure other covariables *ex post* (e.g. soil cover around traps) to be included in the analyses.

And lastly, within these predefined sampling envelopes (statistical population), the coordinates of ten observation points per treatment are selected at random, under the imposed constraint that stands subject to the same treatment must be at least 300m apart in order to limit spatial autocorrelation. However, if all maps required to build the sampling envelopes are not available, stands can be surveyed in the field until at least 60 sampling points meeting the predetermined criteria have been found.

**(3) Allocation of site distribution independently in space and time**

In order to avoid the autocorrelation of sites located too close to one another, the repeated sampling units must be mutually **independent** (cf. Insert 5).

One of the first conditions to be met if such dependence is to be limited is to define the right sampling units for repetition (cf. Table 2, Insert 5). This is so because several seasonal observations at the same trap, or even several traps in the same stand, do not count as genuine repetitions for the comparison of different types of stand. Similarly, when using capture-marking-recapture (CMR) procedures in order to estimate population density, marked individuals do not count as genuine repetitions for the evaluation of the effect of felling on species density. It would be preferable to repeat the CMR scheme on different plots, both cut and uncut. Other examples: in order to define the habitat preferendum of a

species using radio-tracking, it will be necessary to fit several individuals with transmitters because multiple observations of the position of the same individual over time do not constitute independent repetitions for the achievement of the defined objective. Such observations provide information only on the single individual tracked.

#### **(4) Balancing numbers of replications between forestry treatments**

There should be a similar number of repetitions for all treatments, since otherwise those with greater representation will exert more influence on the results, due to the enhanced precision of their estimators.

In addition, what must be avoided at all costs in the sampling table is a situation in which diagonally opposite boxes are under-represented or empty in relation to the others (correlated explanatory variables), as it will then be impossible to separate out the respective effects of the two explanatory variables (**confusion of effects**, cf. Part VI-(5) of the present chapter and insert 4; and Ims and Yoccoz, 1997, p 98-100).

#### **Insert 5: What should be replicated? Beware of pseudo-repetition!**

At each level in a sampling plan (trap, study plot or site, forest block, whole forest, etc.), repetition of sample points will provide information on and improve the precision of the estimations of the response variable at that level. However, the most important need is to **replicate the sampling units at the level that relates to the question to be answered**, i.e. according to the explanatory variables of the study.

In order to assess the effect of regeneration cutting on nocturnal Lepidoptera (Bonneil, 2005), the priority will be to replicate forest stands that are at the same forestry management stage, rather than the traps set up on each study plot. This is because a simple comparison between a mature area and an area that has been felled will be insufficient to test the effect of cutting even if a hundred traps were to be installed in each of the two areas. This is the case because such intra-area traps are not sufficiently independent of each other in terms of the 'cutting' factor to be considered as genuine repetitions. They are merely **pseudo-repetitions** (Hurlbert, 1984) because they are too closely linked geographically, ecologically and from the standpoint of their management history to count as more than one distinct situation representing the same management stage.

Likewise, seasonal observations are not themselves genuine repetitions because they are associated in time and the phenological differences between species mean that they provide supplementary information best added to cumulative data for the sampling campaign as a whole.

#### **(5) Controlling disturbance variables in order to limit confusion of effects and bias**

Disturbance variables are variables that are in danger of preventing proper highlighting of the effect studied, by influencing the response variables without being initially a target for the study. To ignore them in preparing the study will hinder or impede subsequent interpretation of the results, and it will not be possible to remedy the situation. This is the case because if in the sampling plan such disturbance variables vary along with the explanatory variables (correlation), their respective effects will be inseparable (**confusion of effects**), this will prevent any conclusion being reached on the effect targeted at the outset.

An example: in order to test the hypothesis for community succession over a forestry management cycle, it will be necessary to avoid a situation in which the youngest growth stages are on ground that is wetter than for the oldest stages (stand bias) or at lower altitudes (altitude bias) or on former farmland (historical bias), and so on. In the absence of such precautions, it will not be possible to separate the effect of growth stage from the effects of these various sources of bias.

In order to forestall this problem, the planned experimental approach sets out to control the sampling conditions ahead of the survey, in addition to randomising forestry treatments.

- Control of known (or suspected) disturbance variables requires:
  - **the setting of predetermined values for the disturbance variables** (sampling limited to certain types of soil, to even aged stands, etc.), which will limit the choice of sampling units and by the same token restrict the scope of the results (statistical popu-

lation) while on the other hand guaranteeing satisfactory statistical power (low intra-treatment variability);

- or **cross-correlating the disturbance variable with the other explanatory variables**, which in fact amounts to stratifying the sampling plan in relation to an extra variable, yielding results that are more easily generalised. However, this latter solution can increase the extent of the sampling substantially if it is desired to retain satisfactory power to detect the effects initially targeted (need for a greater number of repetitions to offset greater intra-treatment variability).
- In order to escape possible confusion with hidden disturbance effects (unknown sources of bias), and to reach an unambiguous conclusion on the effect initially targeted, it will also be necessary to **randomise**, *i.e.* allocate on a random basis the treatments relating to previously controlled experimental units (cf. Insert 6). However, since this stage assumes that it is possible to manipulate the explanatory variable, this type of approach is rarely used in the natural setting.

**Insert 6: How can an effect be proved? Example of a manipulative experiment “How many years before clear cutting will cause the disappearance of populations of the forest species *Leistus rufomarginatus* (Coleoptera, Carabidae)?”**

If a causal relationship is to be proved between an explanatory variable ('clearcutting') and a response variable ('abundance of *Leistus rufomarginatus*'), it will be necessary to eliminate all alternative explanations for the concurrence of these phenomena, *i.e.* all the disturbance effects that may potentially be confused with the initially targeted effect ('clearcutting'), whether known, simply suspected or hidden.

That in turn requires a full-blown planned experiment (a manipulative experiment) based on four core principles (Imb and Yoccoz, 1997; Jayaraman, 1999):

- Definition of the treatments to be applied: 'clearcutting' *versus* 'no cutting' (control);
- Repetition of the experimental units: at least ten forest stands for each treatment;
- Control of the experimental conditions: division of the forest areas into blocks that are uniform from the point of view of stand, species composition, and so on, and in which the experimental units will be located;
- Randomisation: application of the treatment on a random basis in the experimental units in a manner such that each of those units has the same probability of receiving the 'clearcutting' treatment or remaining 'uncut' as an experimental control.

Only the randomisation stage can forestall the risk of confusion with hidden effects by eliminating all systematic errors (bias). Repetition and local control seek to keep residual random error to a level that is as low as possible (accuracy).

In the chosen example, an experimental design of the type Before/After-Control/Impact (cf. Koivula, 2002) would involve carrying out, simultaneously in more than one mature forest area, the clearcutting of half the area (treatment), and leaving the other half untouched (control), and then monitoring the effect over time of this treatment on *L. rufomarginatus*. For each area (block), the clearcut part will be selected at random (randomisation). The impact of clearcutting would then be tested by monitoring changes in the abundance of *Leistus rufomarginatus* over time (the years preceding and following the clearcutting), by comparing the control and clearcut stands for all blocks. Such an experiment would need to be planned over a timescale long enough to hope to measure the targeted effect (10 to 20 years).

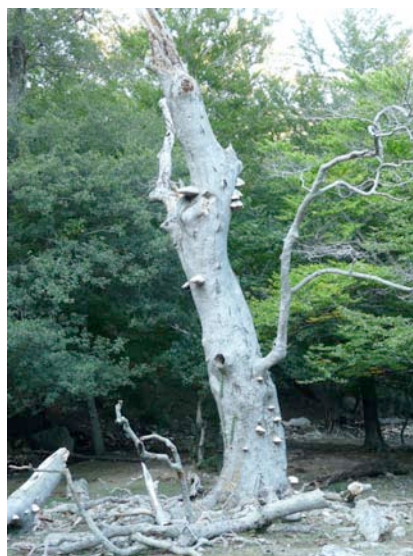
This type of sampling is equivalent to a manipulative experiment.

However, the manipulation and random allocation of forestry treatments, as well as the timescale required to realise the effects to be explored, all constitute constraints that are difficult to reconcile with entomological field studies. It is for this reason that instead of creating treatments by direct manipulation of the ecosystem, the effort is in fact focused on taking advantage of sharply differing situations found readymade in the field in order to simulate such treatments; e.g. a comparison of the entomofauna of mature blocks *versus* blocks that have been cut previously (a synchronic rather than diachronic study, or 'space-for-time substitution'). Therefore, this is in fact a mensurative experiment (or an observational study) for which the sampling plan is stratified according to pre-existing treatments instead of randomising the application of those treatments. As a consequence, we do not escape the hidden effects and the conclusions reached will be less robust (simple correlation). Nevertheless, if the three other conditions are met (stratification based on already existing treatments, control of disturbance variables and repetition of sampling units), a mensurative experiment can maximise the circumstantial evidence for the covariation relationship that is being tested (Ancelle, 2002).

## VII – CONCLUSIONS

**Rather than offering an off-the-peg solution (a ‘turnkey’ sampling plan applicable to every study) that unfortunately does not exist, what we are proposing here is a shortlist of the questions one needs to ask when preparing a study in order to take the project forward on the most robust basis possible. These questions are accompanied with indications of the answers for the main possible cases in Table 3.**

- What is the phenomenon to be highlighted?  
→ agree on an objective, framed as a question or questions and one or more hypotheses.
- Will elements be compared in this study?  
→ a comparative approach  
or will the results be compared with those of other studies only subsequently?  
→ a standardised faunistic approach.
- What is the effect to be assessed? (for a comparative approach)  
→ choose the explanatory variable(s)  
→ define the sampling unit to be repeated (being careful to avoid pseudo-replication)  
→ choose the most suitable type of sampling plan (stratified, random or systematic).
- Which aspect of the entomofauna is relevant?  
→ target the group with its own specific observation method, evaluate the constraints  
→ define the response variables.
- What are the desired spatial and temporal scales for the results?  
→ define the target statistical population  
→ define the land area and duration of the study.
- What sampling effort can be assumed to be available (i.e. resources, time)?  
→ maximise the number of replications: at least five per treatment (preferably  $\geq 10$ )  
→ adjust the number of effects studied and the target statistical population accordingly.
- What other effects might disturb the results?  
→ set fixed values for certain factors  
→ balance the other factors  
→ make sure the replications are mutually independent.



**Photo 1: Old dying beech acting as host to very large numbers of saproxylic insects (nature reserve in Massane forest).**

**Table 3: How should the sampling plan be designed to match the desired objective?**

Approach	Main objective		Key sampling conditions	Strategy	Limits on interpretation
Faunistic	Census of species on a site	Search for heritage species	Exhaustiveness Representativeness	Combining: - random (or systematic) sampling - observation methods that are as varied as possible	Exhaustiveness not attainable -> Without standardisation: results not comparable
Ecological Comparative Exploratory	Atlas/map	Species / space link	Representativeness Spatial comparability (Exhaustiveness)	- Random or systematic sampling - Definition of the extent of the zone to be studied, size of sampling unit, minimum distance between sampling units. - standardisation of methods	The trends observed allow hypotheses to be formulated only but not tested
	Monitoring over time	Species / time link	Spatial and temporal comparability	- Random or systematic sampling - Definition of the extent of the zone to be studied, minimum duration of monitoring, size of sampling unit, distance between sampling units and interannual frequency of observations. - Standardisation of methods	
	Ecological requirements	Species / environment link	Spatial and environmental comparability	Ditto, mapping and environmental measurements	
Ecological Comparative Confirmatory	Detection of an effect (correlation)	Testing a descriptive hypothesis (link)	Mensurative experiment - pre-existing treatments - repetitions - control	- Sampling stratified according to the explanatory variable, balanced and repeated for each treatment - Control of bias and confusion of effects - Beware of pseudo-replication - Standardisation of methods	- Detection is not proof - Generalise by repeating the experiment
Ecological Experimental Mechanistic	Proof of an effect (cause)	Testing an explanatory hypothesis (mechanism)	Manipulative experiment - manipulation of treatments - repetitions - randomisation - control	- Allocate treatments randomly to experimental units. - Beware of pseudo-replication - Standardisation of methods	The mechanism revealed will be more universal in its application but its consequences in reality will not always be observable.



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**CHAPTER 2**

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***INSECT SAMPLING METHODS***

**(Philippe Bonneil, Christophe Bouget, Hervé Brustel, and Anne Vallet)**



There are several publications that discuss the methods for sampling insects (Colas, 1974; Southwood, 1978; Basset, 1985; Schauff, 1986; Robert, 1991; Mora, 1994; Marshall *et al.*, 1994; New, 1998, among others). However, few are exhaustive or provide practical information for those responsible for managing natural areas. Most methods or categories of method are presented below. Following this, methods for sampling forest insects identified by the experts in the Inv.Ent.For working group are described in detail.

**I - METHOD CATEGORIES AND CLASSIFICATION**

(According to Brustel, 2004)

Entomological sampling methods can be characterised on the basis of a number of criteria that enable the method or methods to be used to be chosen in accordance with assigned objectives. They can be classified according to the nature of their action or the nature of their results (cf. following table).

**Table 4: Designations and characteristics of entomological collection methods (sources: Southwood, 1978; Marshall *et al.*, 1994; Fraval, 1997).**

Method	Characteristics	Examples
• Active ...	• Collection is carried out in the field by entomologists using a variety of tools.	• Daylight capture of Lepidoptera using butterfly nets.
• ... or passive	• Sampling is entrusted in the field to standalone traps left for varying periods between installation and collection.	• Capture of saproxylic beetles using window flight traps.
• Absolute ...	• All invertebrates present on a given area of land or in a volume are counted by a cumulative, continuous capture system.	• Census of invertebrates in a one square metre sample of soil extracted using a Berlese apparatus.
• ... semi-exhaustive	• The sample is correlated with an area of land or an estimated volume.	• Sweeping ('scything' motion) of the vegetation with a net.
• ... or relative	• The number of individuals cannot be related to such a unit and can be used for comparison only with another figure established under the same conditions.	• Hoverfly census using Malaise traps.
• One-off ...	• Each item of data (date stamped) is linked to an action to be reiterated.	• Trapping moths during a single night using automated light traps.
• ...or cumulative	• The data is generated over a period.	• Inclusion of all trapping nights during a given season.
• Based on unit of effort	• Collection is constrained by a timeframe, a given distance, a defined result, etc.	• Monitoring of Lepidoptera along a transect of predetermined length.
• ... or free-ranging	• Collection is carried out as the surveyor wishes.	• The vegetation is swept to sample Hemiptera.
• Exhaustive	• All individuals in the population are counted.	• A count of all individuals emerging from a piece of dead wood.
• ... or sample-based	• The individuals in one or more samples of the population are counted.	• A count of all Orthoptera in a square metre.
• Direct ...	• Individuals and/or species are counted.	• Number of individual larch needleworm collected in pheromone traps.
• ... or indirect	• The phenomena linked to the activity or presence of individuals and/or different species are counted or measured.	• Counting of colonies of red ants.
• Destructive ...	• The insects counted are killed or removed from the population.	• Sampling of floricolous insects using coloured traps.
• ... or non-destructive	• The insects are left undisturbed.	• Sampling Odonata by sight count and capture for identification and immediate release.

In the following pages of this chapter, we classify methods according to the environment involved in the sampling (terrestrial – surface and soil – aquatic) and according to the involvement of the surveyor (active or passive methods).

## II - A CATALOGUE OF ENTOMOLOGICAL SAMPLING METHODS

(Philippe Bonneil)

### *II.1 - Methods used in terrestrial environments*

#### *II.1.1 – Active methods*

(Cf. Table 5)

▪ **EXPLORATION OF HOST MATERIAL, MICRO-HABITATS AND SIGHT IDENTIFICATION**

The surveyor searches for targeted or potential micro-habitats and host material for the species of interest. They then determine the species either from a distance (e.g. Lepidoptera), or following capture, in the field or in the laboratory.

#### Equipment

All surveying and collection tools: net, mouth aspirator, pick, debarker, smoker, etc.

#### Sampled groups (according to habitats surveyed and target groups)

Lepidoptera Rhopalocera and Zygenidae, Hymenoptera, Odonata, floricolous Coleoptera, Orthoptera, Hemiptera, Neuroptera. Pollinators, phytophagous, floricolous, saproxylic and terricolous insects.

#### Advantages

Enables information to be obtained on species' micro-habitats (unless migratory). Can be selective. Enables captured specimens to be released alive.

#### Drawbacks

Low efficiency in terms of the ratio between time spent in collection and number of individuals collected. Can be very time-consuming. Major variation in effectiveness between surveyors. Requires good knowledge of the ecology of the target fauna. Capture bias towards the most visible and least mobile species. Small, cryptic and highly mobile species may be underestimated.

The following distinctions can be made according to the tools used and the micro-habitats sampled:

- ***Debarking***

Using a debarker, knife, wood chisel or pick, the operative can break open habitats in dead wood: fallen or standing dead trees at points that have reached various states of rot or different parts of the wood (bark, trunk, branches, stump), in addition to wood fungi. The resulting materials are collected in a plastic tray or a beating sheet for immediate examination. Individual insects can be captured using flexible forceps or a mouth aspirator. Alternatively, the debris can be screened using a Berlese apparatus for example (cf. Part II.2 of the present chapter).

- ***Brushing***

Using a brush attached to a long handle, a tree trunk can be brushed over a predetermined area and the invertebrates collected in a plastic tray, sheet or blanket.

- ***Beating***

Using a stick, the surveyor strikes or shakes energetically the branches of living or dead trees and bushes in order to cause the insects to fall on to a canvas stretched over a wooden framework or into a funnel.

- ***Sweeping***

Using a sweep net, the surveyor captures the insects by sweeping through the vegetation with a to-and-fro 'scything' movement.

- ***Sight identification and net collection***

Using a ‘butterfly’ net, the surveyor explores a homogeneous habitat and counts the number of species encountered, as determined at a distance by sight or after capturing them in the net, or possibly using a mouth aspirator.

- **OBSERVATION TRANSECTS**

The surveyor makes a visual count of the imagos found within a virtual cubic volume (5mx5mx5m) situated in front of them along a predefined route along which they travel at a constant speed (2kph). This transect is travelled regularly throughout the period of appearance of the species. If determination of the species requires it, individuals can be captured in a ‘butterfly’ net.

When monitoring Odonata, transects are parallel to the bank (in the case of a waterway) or perpendicular to it (in the case of a lake or pond).

Sampled groups

Used for monitoring diurnal Lepidoptera (Rhopalocera) and Odonata.

Advantages

Enables relative abundances to be determined for each species and the monitoring of changes in space and time (comparisons).

Drawbacks

Requires allocation of survey time throughout the whole period of species activity. Weather is a major constraint (need for sufficiently high temperatures and fairly cloud free skies). Surveys to be conducted at the times of day of maximum activity for individual insect species (usually the hottest hours).

- **INSECTICIDE FOGGING**

This method involves spraying an insecticide (Pyrethrin) on one or more plants, or over an entire tree, in order to collect all non-fixed invertebrates falling on to a sheet (placed on or above the ground) whose surface area can be predetermined.

Sampled groups

All non-fixed invertebrates on the host plant or their base material.

Advantages

Enables capture of the insects present on plants of large size.

Drawbacks

Costly and complicated to organise. Does not permit the capture of species fixed on the plant (e.g. sugarcane borers or subcortical species). Harmful to human beings and the environment (potential impact on fauna: birds, Chiroptera, etc.).

- **‘D-VAC’ ASPIRATORS**

An aspirator (of ‘D-Vac’ type or a garden aspirator with a net fitted to the intake) is positioned vertically on the ground and left to collect insects for a predetermined duration and over a predetermined area (intake diameter or aspiration area marked out on ground).

Sampled groups

Phytophage insects, pollinators, predators, etc. present in herbaceous vegetation (Hemiptera Auchenorrhyncha, Homoptera Aphididae, etc.).

Advantages

Enables absolute abundances to be estimated (number of species and individuals for a given ground area).

Drawbacks

Highly dependent on individual human performance and aspirator power. Ineffective if individual insects are highly mobile. Damages the most fragile species. Non-selective. Cumbersome to transport. Limited autonomy before refuelling is necessary. Really efficient only on dry herbaceous vegetation less than 15cm high not flattened by wind, rain or trampling (Southwood, 2000).

▪ EXTRACTION CYLINDERS AND CHAUVIN SELECTOR

This method involves covering the vegetation rapidly with a fixed-diameter cylinder and then aspirating or asphyxiating the trapped invertebrates. When used with an aspiration system, the extraction cylinder is considered by Southwood (2000) to be the most effective technique for collecting invertebrates in the herbaceous stratum.

The **Chauvin selector** (Chauvin, 1948 *in* Robert, 1991) is a variant of this, and enables part of the vegetation or plant to be sampled (sampling by stratum). It comprises a box in two articulated parts with cutting edges (or with a foam covering) whose rapid closure will trap both plant and associated invertebrates (with accompanying sampling of the plant material or not as the case may be).

Sampled groups

Phytophage insects, pollinators, predators, etc. present on herbaceous vegetation (Hemiptera Auchenorrhyncha, Homoptera Aphididae, etc.).

Advantages

Theoretically, it enables absolute abundances to be estimated (number of species and individuals for a given volume of vegetation). The Chauvin selector enables sampling to be done by stratum and vegetation height.

Drawbacks

Certain taxa (Aphididae larvae or adults, for example) will remain firmly fixed to the base plant and are inadequately sampled. The most mobile individuals will escape when the cylinder is applied (the cylinder must be placed in position during periods of least activity such as night-time). The Chauvin selector with cutting edges necessarily picks up vegetation, thereby causing destruction of the habitat.

▪ COLLECTION BAG FOR FOLIAGE, TWIGS AND BRANCHES

Twigs and branches are quickly trapped in a bag that is closed with a short length of cord. Invertebrates are sampled on site after treatment with insecticide or off site after the cutting and transportation of the twig followed by treatment with insecticide.

Advantages

Enables densities to be calculated.

Drawbacks

The most mobile insects will escape when the collection bag is put in place.

▪ QUADRAT SAMPLING, COLLECTION SQUARE AND BIOCENOMETER (Lamotte, 1969 *in* Mora, 1994)

This method involves the exhaustive sampling of all invertebrates present in a predetermined homogeneous surface area of vegetation using all available collection tools. The area is marked out using a net placed high enough to prevent the escape of individuals (collection square) or a completely closed volume in which the surveyor or surveyors do their work (biocenometer).

Orthoptera can be sampled using this method by laying out a marker framework on the ground (possibly topped by a net to prevent individuals escaping).

Advantages

Theoretically, it enables exhaustive collection over a given area.

Drawbacks

Very costly in time and manpower. Does not permit highly mobile individuals to be captured since they will escape at the approach and placing of the device (this is true of Orthoptera for example).

▪ **CAPTURE AFTER ATTRACTION BY LIGHT ONTO A SHEET**

(cf. also Part III.4 of the present chapter)

This method involves using light to attract certain insects to a stretched white sheet illuminated by a lamp emitting short-wavelength light (ultraviolet). The individuals attracted are determined on site or captured and placed in a jar with a lethal substance for later determination.

Sampled groups

Lepidoptera Heterocera, various Diptera, Coleoptera, Heteroptera, Trichoptera, etc.

Advantages

According to power and survey objectives: wide attraction radius. Enables insects to be captured alive.

Drawbacks

Requires the permanent presence of a person with knowledge of the groups surveyed. Requires in most cases an electrical generator driven by a noisy engine (disturbance to fauna, pollution). Possible presence of tourist species.

▪ **RECOGNITION OF ORTHOPTERA SPECIES BY SONG**

This is the recognition by the surveyor of the characteristic stridulation of each species of Orthoptera, as well as Homoptera Cicadoidea. A sound recorder can help, allowing song types to be analysed later using special software (Audacity® freeware). Relative positioning in space can be noted and numbers of individuals counted.

Advantages

Does not entail any risk escape of individuals.

Drawbacks

Requires a high degree of skill. Cost of equipment (sound recorder and computer).

▪ **'TRAWLING'**

Using a net trailed by a vehicle (car, bicycle, etc.), this method involves collecting flying insects ('aerial plankton') when travelling along predetermined routes.

Advantages

Sampling can be done over long distances with little effort.

Drawbacks

Sampling is possible only on roads or tracks suitable for vehicles. Polluting. No obvious link with habitats.

▪ **COUNTING ANT COLONIES (RED ANTS)**

(cf. Chapter 4, Part V)

This method involves counting and characterising ant hills holding colonies of red ants (genus *Formica*) along transects or within quadrats. Enables a census of species in the genus *Formica* to be carried out plus an assessment of the biological quality of the forest.

Advantages

Non-destructive method. Enables estimation of biological quality of the forest (degree of disturbance).

Drawbacks

Requires time in the field. Only permits the inventorying of red ant colonies in the forest environment.

Table 5: Characteristics and constraints of active sampling methods in terrestrial contexts.

(-: low; +: moderate; ++: high; +++: very high)

Method	Principle method	Required skill level	Time-intensiveness (inc. sorting and identification)	Equipment cost	Dependence on climate	Risk of damage to equipment	Selectivity vs. 'Capturability'	Standardisation
Exploration of host material and micro-habitats / sight identification	Relative	++	Variable	-	++	-	S variable C variable	-
Bark sieving	Semi-exhaustive	++	++	-	-	-	S variable C +	-
Brushing	Semi-exhaustive	++	++	-	-	-	S variable C +	-
Beating	Semi-exhaustive	++	Variable	-	++	-	S variable C +	-
Sweeping	Semi-exhaustive	++	Variable	-	++	-	S variable C +	-
Anthill count	Semi-exhaustive	-	++	-	-	-	S +++	++
Survey transect	Relative	++	Variable	-	+++	-	S variable C -	++
Insecticide fogging	Relative	++	++	++	++	-	S - C variable	-
D-Vac suction sampler	Absolute	-	++	++	-	-	S - C ++	++
Extraction cylinder	Absolute	+	++	++	+	-	S - C ++	+
Collection bag	Absolute	++	++	+	-	-	S - C ++	+
Quadrat sampling	Absolute	++	+++	+	++	-	S variable C +++	+
Sheet interception with light lure	Relatif	+	++	++	++	-	S variable	-
Song recognition (Orthoptera)	Relative	+++	++	- / +	++	-	S+++ C++	+
'Trawling'	Semi-exhaustive	-	++	++	++	-	S - C ++	+



### *II.1.2 – Passive methods*

(cf. Table 6)

#### ▪ **WINDOW FLIGHT TRAPS**

(cf. also Part III.2 of the present chapter)

A collection receptacle is placed under an interception surface comprising a single vane (bidirectional interception), or two crossed vanes (multidirectional interception) oriented vertically. The trap will intercept highly mobile flying insects whose flight is heavy and which allow themselves to fall on collision with an obstacle.

#### Sampled groups

Saproxyllic insects, Coleoptera, Hymenoptera, Diptera, Homoptera, Heteroptera.

#### Advantages

Captures a wide diversity of rare or cryptic species. Standardisation possible. Low survey cost. Easy to construct. Can be combined with other methods.

#### Drawbacks

Plant debris (leaves, branches, etc.) often obstructs the collection pan or funnel, allowing insects to escape. Possible presence of tourist species. Visible and vulnerable to vandalism.

#### ▪ **MALAISE TRAP**

(cf. also Part III.3 of the present chapter)

This is an interception trap comprising a stationary tent like structure in fine-meshed cloth, the sides of which are open, and include a vertical central baffle and conical roof fitted with a collection device (jar with preserving fluid) at the apex. Insects intercepted in flight by the tent will seek a way out by flying upwards towards the light and are then collected in the jar.

#### Sampled groups

Flying imagos of Diptera, Hymenoptera, Homoptera, some Coleoptera, Lepidoptera, etc.

#### Advantages

Captures a large number of species and individuals. Widely used and easy to standardise. Can be combined with other methods.

#### Drawbacks

High cost (approximately €150 to €200). Complicated to construct. Samples only part of the aerial fauna. Possible presence of tourist species. Visible and vulnerable to vandalism.



**Photo 2: A typical Malaise trap**

#### ▪ **STICKY TRAP**

A device comprising a sheet or plate covered with a sticky substance which retains insects landing on or hitting it. Variants are the mist (cf. Part II.1 of Chapter 3) and stationary nets (see below).

#### Sampled groups

Saproxyllic and pest species, etc. of Coleoptera, Diptera, Hymenoptera, Lepidoptera, etc.

Advantages

Simple, inexpensive method.

Drawbacks

The material collected is often in poor condition (dried out) and it is difficult to recover (broken or damaged specimens).

▪ **AERIAL ROTARY AND SUCTION TRAPS**

This is based on the interception of insects in flight using a device comprising one or more nets oriented perpendicularly to shaft and rotated horizontally by a motor (**aerial rotary trap**) or by a fixed electrical aspirator fitted with a conical canvas structure and collection container (**aerial suction trap**).

Sampled groups

Small aerial fauna: Homoptera Aphididae, Coleoptera Nitidulidae, Coleoptera Bostrychidae, Neuroptera Coniopterygidae, etc.

Advantages

Offers the possibility of calculating density by unit of time or collected air.

Drawbacks

Little used. Its effectiveness depends on its position in relation to the dominant winds. Problems of cumbersome horizontal sizing, energy autonomy, duration of operation and cost. Some insects may be able to avoid the trap, be attracted by its movement (Diptera Tabanidae) or may escape the net by crawling or flying.

▪ **STATIONARY NET**

A net stretched out across the axis of the dominant winds will capture insects carried along or deflected from their route by the wind, along with migratory insects (if the net is across the migration flow axis). One variant is the mist net (cf. Part II.1 of Chapter 3).

Sampled groups

Aerial plankton: Aphididae, Thysanoptera, micro-Hymenoptera, etc.; migratory insects: Diptera, Chironomidae, Lepidoptera, etc. (using the mist net: Coleoptera and Hemiptera of fairly substantial size).

Advantages

Low cost. Useful for studying migratory insects.

Drawbacks

Requires human presence.

▪ **PITFALL TRAP OR BARBER TRAP**

(cf. also Part III.1 of the present chapter)

A container buried flush with the soil will intercept mobile animals falling into it.

Sampled groups

Mobile epigeal invertebrates: Coleoptera Carabidae, Silphidae, Staphylinidae, Formicidae, Dermaptera, springtails (+ Araneids, Opilionides, Diplopodes, Chiliopodes, Isopodes).

Advantages

Cheap, simple to use, set up and check results without delay; the container will collect large numbers of epigeal arthropods. High efficiency in terms of the ratio between the number of individuals and species captured and the time required. In very widespread use.

Drawbacks

Choice of preserving fluid (attractiveness, toxicity, cost, etc.). Frequently damaged by wild boars. May overflow. Captures non-targeted species (micro-mammals, reptiles, terrestrial molluscs).

▪ **PHEROMONE TRAP**

The principle behind this trap is the response of male insects to the emission of a pheromone by a female prior to coupling. Individuals attracted by a synthetic pheromone or by an unfertilised female are captured using various devices (funnels, glue).

Sampled groups

Pests: especially Coleoptera and Lepidoptera.

Advantages

Selectivity (suited to the capture of a single insect species). Population monitoring.

Drawbacks

High cost of synthetic pheromones (€10 to €25 per recharge), weak link with local habitat (attraction effective at distances of several kilometres in some cases).

▪ **BAIT TRAPS**

The principle here is the attraction of insects responding to a food-related stimulus. The response to the stimuli depends on the species (selectivity) and often the sex of the insect: the results of this type of trap therefore yield a biased picture of the actual community.

• ***Bait trap at ground level***

A trap that is a combination of capture by interception (pitfall trap) and by attraction (decomposing organisms or meat, excrement).

Sampled groups

Coprophage and coprophile Coleoptera, Diptera (on excrement), Coleoptera Carabidae and necrophages, Diptera (on meat).

Advantages and drawbacks

See above comments on the pitfall trap.

• ***Suspended bait trap or 'beer trap'***

A trap that combines capture by a suspended container (a combination of container and funnel) and capture by attraction (fermented [wine, beer] and/or sugary substances [honey, fruit], honey solutions, ethanol, benzyl acetate, turpentine, alpha-Pinene, etc.). Where decomposing bait is used, it is advantageous to place several such traps at different periods to capture species attracted by different stages of decomposition.

Sampled groups

According to bait type: Diptera, Hymenoptera, Coleoptera Elateridae, Cerambycidae, Buprestidae, Cetoniidae, Lepidoptera Noctuides, etc.

Advantages

Simple and inexpensive. Standardisation. Very advantageous for capturing numerous species of saproxylic Coleoptera considered to be rare, preferably in warm regions (Mediterranean) than in areas that are cool and humid (mountains included).

Drawbacks

Wasps and other large Hymenoptera may damage the captured insects. The large quantities of noctuid moths and Vespidae captured in late season (August) may also damage the samples by putrefaction and soiling with scales, and they may also fill the trap to overflowing. More effective in the warmest regions.



**Photo 3: White bucket containing a wetting agent and a benzyl acetate lure for the capture of Saproxylic Coleoptera.**

▪ **REFUGE TRAP**

**Artificial substrates** are left in place for the time necessary to allow insects to take up residence and lay their eggs and then, after collection, are examined in the laboratory or left until the adult forms emerge. For example, freshly cut bundles of small twigs of varying diameters are hung up in the forest in spring and collected in autumn (xylophages that will appear in the spring of the following year or after two years).

Sampled groups

Saproxylic Coleoptera Staphylinidae, Clavicornes, Scydmaenidae, Pselaphidae; solitary Hymenoptera, etc.

Advantages

Inexpensive and strongly linked to the surrounding habitat.

Drawbacks

Attracts relatively few species. Method difficult to standardise (volume, size, condition of wood used).

▪ **COLOURED TRAP**

This type of trap is based on the visual attraction of colours (imitating those of flowers) for heliophilic and floricolous insects. The attracted insects fall into the trap which contains a wetting agent and preserving fluid.



Sampled groups (according to colour)

Diptera and Hymenoptera (yellow), saproxylic Coleoptera (white and blue).

Advantages

Simple and inexpensive. Strongly linked to local habitat (small radius of action). Captures a large number of cryptic species.

Drawbacks

Needs to be emptied and refilled regularly (evaporation of liquid, decomposition of the contents, overflows in wet weather). The use of a preservative may affect its attractiveness. May be damaged by livestock and wild fauna or by human action. Beware of flattening vegetation when putting these traps in position since this can affect insect capture. Birds may also eat the trapped insects. Possible presence of tourist species.

**Photo 4: Typical coloured trap: a yellow tray set up on its base.**

▪ **AUTOMATED LIGHT TRAP**

(cf. also Part III.4 of the present chapter)

This trap combines a lure based on light (a tube emitting UV light with automatic triggering by timer or photocell) and an interception device (window flight trap of multidirectional type).

Sampled groups

Flying insects attracted by light: various Lepidoptera Heterocera, Trichoptera, various Diptera, Coleoptera, Heteroptera, etc.

Advantages

Standardisation. Automatic trapping requires no human presence. Short radius of action (according to power): surveys fauna in the local habitat.



Drawbacks

Capture varies from night to night (depending on weather conditions): requires several successive nights of trapping. The batteries need to be recharged (energy storage insufficient for more than one complete night). Costly.

▪ **MICROTUBE ANT TRAP**

A microtube containing a sugary solution (diluted honey) (tube one-third full) and plugged (half way down the tube with a hydrophilic cotton plug allowing slow diffusion of the odour of the sugary solution) is buried in the soil in order to attract ants. The microtubes may be collected an hour or more after placing and closed for subsequent laboratory identification of the trapped ants.

Advantages

Selective for ants generally.

Drawbacks

Requires regular human supervision (includes no capture system).

▪ **EMERGENCE TRAP OR NET**

Enclosures cover or surround a substrate (herbaceous vegetation and soil, trunk, dead wood, fungi, etc.) already colonised by larvae. Capture is based on the positive phototropism of the insects which, after emerging, will move in the direction of an opening fitted with a collection receptacle. The substrate may be left on site or taken off site. The time required for emergence may be long (several years according to species).

Sampled groups

According to substrate: saproxylic Coleoptera, Diptera.

Advantages

Strongly linked to local habitat or micro-habitat. Where emergence is 'on site': no destruction of habitat.

Drawbacks

According to the micro-habitat surveyed: difficult to standardise (volume of dead wood, ground area, etc.). If the substrate is extracted for emergence 'off site': habitat destruction, transportation and storage difficulties.

▪ **COMPOSITE ENTOMOLOGICAL TRAP (PEC, Robert, 1992)**

This is a device originally designed for monitoring rather than inventories and combines aerial interception (window flight and Malaise trap) and ground trap (pitfall trap) plus an attraction trap (coloured trap).

Sampled groups

All fauna captured by window flight, pitfall, coloured and Malaise traps.

Advantages

Good capture capability for (flying or crawling) fauna in the surrounding area. Mutually complementary groups captured.

Can be suspended in trees.

Drawbacks

Device complicated and expensive. Time required for installation and sorting.



**Photo 5: A composite entomological trap.**

**Table 6: Characteristics and constraints of passive sampling methods in terrestrial contexts.**

(-: low; +: moderate; ++: high; +++: very high)

Method	Trap type	Principle method	Required skill level	Time-intensiveness (inc. sorting and identification)	Equipment cost	Dependence on climate	Risk of damage to equipment	Selectivity vs. 'Capturability'	Standardisation
Window-flight trap	Interception	Relative	+	++	- to ++	+	++	S- C++	+++
Malaise trap	Interception	Relative	+	++	++	++	++	S- C++	+++
Sticky trap	Interception	Relative	+	++	++	+	++	S- C+	+++
Aerial rotary and suction traps	Interception	Semi-exhaustive	+	++	++	-	++	S- C+	+++
Stationary net	Interception	Relative	+	++		++	++	S- C-	++
Pitfall trap	Interception	Relative	+	++	+	-	+	S-à+++ C++	+++
Pheromone trap	Attraction	Relative	-	-	++	++	++	S+++ C++	++
Ground bait trap	Attraction	Relative	+	++	+	-	+	S++ C++	+++
Suspended bait trap	Attraction	Relative	+	++	+	++	++	S++ C++	+++
Refuge trap	Attraction	Relative	+	++	-	-	+	S++ C+	+
Couloured trap	Attraction	Relative	+	++	+	+	++	S+ C++	+++
Automated light trap	Attraction	Relative	+	++	++	++	++	S+ C++	+++
Microtube trap	Attraction	Relative	-	+	-	++	+	S++ C+	+
Emergence trap	Interception	Absolute	+	++	+	-	+++ ("in situ")	S+ C+	+
Combined entomological trap (PEC)	Combined	Relative	++	Very high	++	++	++	S- C+++	+++

## ***II.2 - Methods for sampling litter and soil fauna***

(cf. Table 7)

Sampling is based in this case on the extraction of invertebrates from a portion of the soil and litter using manual, physical or chemical means. Manual methods are also effective in extracting invertebrates from earth in cavities and the material produced by breaking up old tree wood (cf. Part II.1.1 of the present chapter).

### ▪ **SIEVE EXTRACTION**

A sample of soil (of a predetermined volume) is sifted above a white sheet using a sieve (beginning initially with a 4mm square mesh, subsequently reducing to 0.5mm). The invertebrates are sorted on the sheet and collected using flexible forceps or a mouth aspirator and placed in a jar containing alcohol.

#### Advantages

Can be selective (if individuals from non-targeted species are returned along with the extracted soil).

#### Drawbacks

Very time-consuming. Dirty work. Examination in laboratory necessary in-order to identify very small species.

### ▪ **BERLÈSE-TULLGREN EXTRACTOR**

A portion of soil (litter plus a spade's depth of earth) is extracted and placed in an apparatus brightly illuminated from above (wide-mesh sieve above a funnel), obliging the arthropods to flee downwards into the collection jar containing preserving fluid (alcohol).

#### Advantages

Extraction not dependent on human intervention.

### ▪ **WINKLER-MOCZARSKI ECLECTOR**

A device not dissimilar to the Berlèse-Tullgren extractor and comprising a number of cloth bags filled with litter and suspended above a funnel (in cloth or plastic) fitted with the collection receptacle. Attracted by the light and/or fleeing desiccation, individuals move to the litter surface and fall into the funnel.

### ▪ **FLOTATION EXTRACTION**

This method involves separating out hypogeal macrofauna from soil components (mainly mineral particles) exploiting density differences in a suitable solution (magnesium sulphate, sodium chloride, heptane, sugary solution, colloidal silica polymer or "Ludox"). A portion of the soil is agitated in a basin containing the chosen solution. The floating invertebrates can be collected using a pipette, a fine brush or flexible forceps.

#### Advantages

Unlike the other methods, this will also pick up inactive insect stages. It therefore allows extraction following fairly lengthy storage of the substrate.

#### Drawbacks

Very time-consuming. Dirty work. Requires a container and water outdoors, or if indoors, a sink that will not get clogged.

**Table 7: Characteristics and constraints of methods for the extraction of invertebrates from samples of soil and litter.**

(-: low; +: moderate; ++: high; +++: very high)

Method	Trap type	Principle method	Required skill level	Time-intensiveness (inc. sorting and identification)	Equipment cost	Dependence on climate	Risk of damage to equipment	Selectivity vs. 'Capturability'	Standardisation
Sieving	Actif	Absolute	++	+++	-	+	-	S ++ C +++	+
Berlese-Tullgren Extractor	Passif	Absolute	-	+	++	-	-	S - C ++	+++
Winkler-Moczarski eclector	Passif	Absolute	-	+	++	-	-	S - C ++	+++
Flotation extraction	Actif	Absolute	++	+++	+	-	-	S + C ++	+



### ***II.3 - Methods used in aquatic environments***

(cf. Table 8)

#### ***II.3.1 – Active methods***

##### **▪ EXPLORATION OF HOST MATERIAL AND MICRO-HABITATS / SIGHT IDENTIFICATION**

This involves surveying the micro-habitats that are present (stones, root hairs, gravel and sand, sediment, deadwood, aquatic plants, areas under banks, etc.) and using suitable tools to capture on sight those insects present (hand net, Surber sampler, substrate extraction, etc.).

According to the survey tools and the habitats involved, the following can be identified:

##### **• *Capture by hand net***

The net is immersed in the water and will capture aquatic insects when agitated in the water with a to-and-fro ('figure of eight') movement. The contents of the net are emptied out on to a sheet and sorted.

##### Sampled groups

Trichoptera, Plecoptera, Ephemeroptera, Odonata, Diptera, Heteroptera and aquatic Coleoptera.

##### Advantages

Quick and easy sampling.

##### Drawbacks

Difficult to standardise, like all active methods (dependent on the equipment and its user).

##### **• *Surber sampler***

When sampling benthic invertebrates, the Surber sampler (base surface area 1/20<sup>th</sup> sq. m. with a 0.5mm mesh for establishing an IBGN<sup>2</sup>) is positioned across the bed of the river or stream. Pebbles and gravel located within the horizontal frame are agitated in order to 'wash' them at the entrance of the net: attached animals and larvae will in this way be drawn into it. The Surber sampler is usually employed for the establishment of IBGN indices (AFNOR, 2004) for estimation of water quality in rivers or streams.

##### Sampled groups

Benthic invertebrates among Plecoptera, Trichoptera, Ephemeroptera, aquatic Heteroptera, aquatic Coleoptera, aquatic Diptera, Odonata, Megaloptera, Neuroptera Plannipennes.

##### Advantages

Formalised and standardised method (AFNOR, 2004).

##### Drawbacks

Underestimates species that are firmly attached to pebbles, in addition to the heaviest species (Trichoptera and Plecoptera larvae).

##### **• *Sampling the substrate using a drag net, a box net or a grab bucket***

The basic principle is to collect a sample of the substrate for subsequent sorting to extract benthic invertebrates. Using a drag net, the bottom of the net is weighted down with a stone and thrown as far as possible over the water or towards the opposite bank; it is allowed to sink and is then dragged gently back using the rope. Using a box net, the net is pulled or pushed in order to collect the substrate surface. Using a grab bucket, a bucket with two jaws mounted on a boat can be used to pick up a defined volume of substrate.

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<sup>2</sup> *Indice Biologique Global Normalisé* / French standardised general biotic index.

These methods are little used for sampling aquatic insects.

A box nets is used with a 0.5mm mesh to establish an IBGN index in areas with slow or no current by dragging it over a distance of 50cm (AFNOR, 2004).

Sampled groups

Benthic invertebrates, including Coleoptera Dytiscidae, Hydrophilidae, etc.

• ***Detection of exuviae***

Surveys are conducted with minute inspection of those elements constituting the river or stream bank in order to detect Odonata exuviae.

Advantages

No habitat or population destruction.

Drawbacks

Exuviae remain present on site for only a short period: surveys necessary after periods of good weather (emergence of imagos and exuviae not carried away by rain). Requires considerable time to be spent in the field throughout the emergence period.

***II.3.2 – Passive methods***

▪ **ARTIFICIAL SUBSTRATE TRAP**

This trap is intended to capture macro-benthic larvae by attraction to and colonisation of a metal cage containing an artificial substrate (stones and thick rope) placed on the bottom of an area of water. When recovered, the substrates must be cleaned and the fauna sorted and preserved as soon as possible (6 hours).

Sampled groups

Ephemera, Plecoptera, Trichoptera, Odonata.

▪ **AQUATIC EMERGENCE NET**

Imagos are trapped in a net when flying off after emergence. The trap is made up of a four-sided sloped-roof frame holding a net with a collection receptacle at the apex. The whole assembly is placed on the water (with the base submerged) on supporting feet.

Sampled groups

Trichoptera, Plecoptera, Ephemeroptera, aquatic Diptera.

Drawbacks

May be damaged by wave action. Difficult to use on sites with wide variations in water level.

▪ **BAIT TRAP OR NET FOR COLEOPTERA HYDROCANTHARES**

Predator and carnivore Coleoptera hydrocanthares are trapped by a cylindrical net in fine-mesh cloth with two funnels at the ends and bait in the middle (meat), placed under the surface (with a buoy at the top and attachments at fixed points to allow recovery).

This trap needs to be checked frequently: after a few days the bait will be gone, increasing the risk of cannibalism). The risk of capturing amphibians and reptiles is not negligible.

▪ **AQUATIC LIGHT TRAP**

This trap is made up of a large transparent net under the water fitted with a watertight light (a neon tube generating little heat) powered by a car battery and operating at night.

Sampled groups

Aquatic insects: Hemiptera, Coleoptera, Odonata, Plecoptera, Trichoptera, Ephemeroptera.

**Table 8: Characteristics and constraints of methods for sampling in aquatic contexts.**

(-: low; +: moderate; ++: high; +++: very high)

Method	Principle method	Required skill level	Time-intensiveness (inc. sorting and identification)	Equipment cost	Dependence on climate	Risk of damage to equipment	Selectivity vs. 'Capturability'	Standardisation
Surveys of aquatic host materials and micro-habitats	Relative	++	++	-	-	-	S++ C++	-
Hand net	Relative	++	-	-	-	-	S++ C++	-
Surber sampler	Relative	+	+	-	-	-	S- C++	+++
Drag or box net	Semi-exhaustive	+	+	++	-	-	S- C++	+
Grab bucket	Absolute	+	+	++	-	-	S- C++	++
Detection of exuviae	Relative	+	++	-	++	-	S++ C+	-
Artificial aquatic substrate trap	Relative	+	+	+	-	++	S+ C++	++
Aquatic emergence net	Semi-exhaustive	+	+	+	+	++	S- C++	++
Trap for diving beetles	Relative	+	++	+	-	++	S+ C+	++
Aquatic light trap	Relative	+	++	++	-	++	S+ C++	++

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### III - METHODS PROPOSED BY THE INV.ENT.FOR. GROUP FOR TEMPERATE FORESTS

#### III.1 - Pitfall traps

(Christophe Bouget)

##### Basic principles, groups caught and sources of bias

When sampling mobile epigeal Arthropods, the most frequently used method is the pitfall trap or Barber trap (Barber, 1931): a jar buried flush with the ground surface (Photo 6) intercepts mobile fauna. Its popularity is based on its practical advantages; it is cheap, easy to use and can be set up and checked for capture rapidly. It will catch large numbers of epigeal Arthropods.



**Photo 6: A pitfall trap in place, complete with roof.**

A pitfall trap will capture circulating epigeal invertebrate fauna, including Coleoptera Carabidae, Silphidae, Staphylinidae, Araneids, Opilionides, Diplopodes, Chilopodes, Isopodes and Formicidae.

For very many sites and species, the pitfall trap is to be preferred to the alternatives: Berlese, manual capture or D-Vac aspiration systems (Spence and Niemelä, 1994).

Like all interception traps, what it in fact measures is invertebrate activity/density or activity/abundance, weighting the numbers captured in accordance with species activity. Activity/abundance is correlated to local population density around the trap (Baars, 1979).

##### **Insert 7: Factors affecting variation in trap effectiveness.**

The random character of interception by this trap (i.e. its 'neutrality') is nevertheless biased by various trap parameters (Bouget, 2001). Among the acknowledged sources of bias, the following can be pointed to:

- The influence of local cover, and especially vegetation structure, on activity and therefore on the effectiveness of capture (Greenslade, 1964), a hypothesis invalidated by Judas *et al.* (2002).
- The influence of openness of the environment on the functioning of a trap with non-neutral preserving fluid. The question was asked in studies comparing open and closed environments as to whether the effectiveness of the trap varies with openness. This question is far from trivial where traps are not neutral but attractive for the trapped organisms. The underlying hypothesis is that the intensity of the attraction will increase with the level of emission and diffusion of the lure, and this will increase with openness of the environment.
- Capture effectiveness is species-dependent, as is recalled by Sunderland *et al.* (1995), with the smallest and least active species being underrepresented in samples.

**Variants and accessories**

A number of pitfall trap parameters may vary, among them its shape, which determines the area of the opening and the internal volume, the possible presence of a roof and side barriers and the preserving fluid. The following table sums up the advantages and drawbacks of the various different arrangements.

**Table 9: Parameters and characteristics of pitfall traps.**

Parameters	Advantages	Drawbacks
<b>Shape:</b> - Cylindrical jar - Long, deep collection pans - L-shaped pans  - Linear array of jars with side barriers	- Simplicity of installation - Increased interception area - Increased interception area  - Increased interception area	- Installation logistics - Installation logistics  - Installation logistics
Increased size (diam., etc.)	Increased interception area	Increased debris obstruction, trapped micro-mammals
Material (glass or plastic)	Glass: smooth surface Plastic: lightweight, less fragile	Glass: breakable Plastic: can be scratched (creating rough patches that may assist the escape of certain insects). Reduction of escape risk with liquid teflon (flouon) coating on internal walls.
Continuity of flush edge	Increases numbers captured for small species	The material around the jar edge introduces a disturbing factor into the local environment
Preserving fluid	Accelerates killing and prevents escape, predation, cannibalism and deterioration of samples	Differential trap attraction / repulsion

Cylindrical trap receptacles are the most widely used (cups, halves of 1.5 litre plastic soft drink bottles, agrifood glass storage jars and the like).

The diameter is also important: Koivula *et al.* (2003) shows that richness and abundance are greater with a diameter of 90mm than with a diameter of 65mm (cf. preceding table).

Testing to improve such traps has been done but any addition of complexity needs to seek a compromise between increased effectiveness and greater logistical effort.

The following table lists the accessories sometimes added to a simple pitfall trap:

**Table 10: Accessories and characteristics associated with pitfall traps.**

Accessories	Advantages	Drawbacks	Comments
Roof	Prevents flooding by direct precipitation Prevents foliage and debris obstruction Prevents evaporation of fluid	May offer a visual indication for insects May create a local microclimate above the trap (e.g. condensation, greenhouse effect) May act as a solarium for ant colonies	In aluminium (light, but reflects light and modifies the microclimate), plastic or wood (little modification of environment)
Wide-mesh grid-type lid	Prevents amphibians and micro-mammals falling in	Facilitates escape by certain insects able to cling on before falling in	
Raised grid at bottom	Sorts out small and larger invertebrates and prevents cannibalism		
Funnel opening	Reduces escape by flying insects?	Facilitates escape by insects able to cling on?	
Side barriers	Increases probability of encounter with trap by 'guiding' insects	Logistics	

**Preserving fluids:**

The chosen preserving fluid must limit:

- attraction of micro-mammals (and necrophages later);
- attraction of game animals that can cause disturbance (especially wild boar);
- handling risks (contact toxicity) as well as harm to fauna and the environment;

- cost;
- rigidity of the trapped material;
- viscosity (to facilitate immersion of trapped insects);

but at the same time must also maximise:

- effectiveness (acceleration of killing and prevention of escape by flight after floating on the surface);
- preserving capacity (at least the fluid chosen must be suited to the frequency with which the trap will be checked for capture).

The following is a non-exhaustive list of products in regular use:

- Vinegar (acetic acid);
- Brine (10% NaCl) alone or mixed with brown ale or wine;
- 50% monoethylene glycol (MEG, vehicle antifreeze<sup>3</sup>);
- 50% monopropylene glycol, less harmful on contact than MEG;
- Formol, at 5-8 %, highly attractive for certain species and a repellent for others (but causes specimen rigidity and is highly toxic: it is a carcinogen);
- Picric acid;
- A mix of ethanol and glycerol;
- Caustic soda;
- A solution of water, copper sulphate (3%) and a wetting agent (non-attractive, a good preservative and non-toxic).

Field comparisons have been carried out for some preserving fluids: 8% formol, 50% monoethylene glycol salted and unsalted, 50% monopropylene glycol, or none at all. Formol (or formalin), whose use is subject to major constraints, delivers maximum abundance and richness for captured Carabidae (Bouget, 2001), with many species being attracted by this volatile substance. In the case of monoethylene glycol, abundance and richness are generally more limited than if formol is used, but certain species are attracted (Holopainen, 1990). A few species (*Carabus auratus*, *Metallina lampros*) are more abundant in traps with propylene-glycol than with ethylene-glycol, but most species seem to be as abundant in traps with propylene-glycol (Gosselin, *com.pers.*). Moreover, Koivula *et al.* (2003) have shown that the species richness of captures is greater with ethylene-glycol than with brine.

Viscosity, cost, preserving capacity, capture effectiveness, attractiveness for mammals (Marshall and Doty, 1990) and the 'neutrality' of monopropylene glycol appear to be equivalent to ethylene (Weeks and McIntyre, 1997). Furthermore, it is less toxic (Hall, 1991), and less harmful, or even harmless, on contact (Mochida and Gomyoda, 1987), although ingestion seems to be hazardous (Dorman and Haschek, 1991). It has come into use only recently, and is spreading in the world of entomology (Grove, 2000, Lemieux and Lindgren, 1999, Weeks and McIntyre, 1997, Bouget, 2004).

In order to enhance its preserving capacity, we can use a 50% solution of concentrated monopropylene with 10% added salt. A few drops of neutral, odourless detergent rich in surfactants are then added to reduce surface tension and facilitate the immersion of insects falling into the trap, especially the smaller species. A product without scent additives should be chosen (e.g. dishwasher rinse agent, Teepol or Mir).

Dry traps are used to capture certain insect species alive. These entail regular checking (at intervals of less than a week). While they allow avoidance of the possible repellent effects of a fluid whose neutrality is unknown, they do lead to other interactions, and notably:

- putrefaction and attraction of sapro- and necrophages, with certain Carabidae showing positive or negative reactions to these odours;
- inter-species predation and cannibalism.

### **Recommendations**

We use cylindrical 85mm diameter and 110mm deep polyethylene pots (internal volume 0.55 litre), buried flush with the soil and topped with a square 10cm by 10cm roof in translucent Plexiglas approximately 10cm above ground level. The pot is half-filled with preserving fluid. The plastic roof prevents flooding from direct precipitation and obstruction by leaves and debris.

<sup>3</sup> NB: commercial antifreeze is often diluted at 25%, which reduces its preservative capacity.



The traps are installed by digging a cylindrical hole with a pedological auger into which the pot is inserted flush with the soil surface; continuity of the flush transition between soil and pot edge is ensured with added soil. When installing the trap, the fact that the soil is turned over is a cause of disturbance and this may temporarily attract or repel Carabidae (presence of prey on surface, changes to soil cover, etc.). In order to reduce this initial disturbing effect (Digweed *et al.*, 1995), we separate installation and initial activation of the trap by an interval of ten or so days (leaving a lid on the trap).

Concerning the preserving fluid:

- if the fluid can be recovered after use, use a mix of 50% monopropylene glycol + 50% water + 10% salt by weight; intervals between checks on traps: up to 30 days;
- if the fluid cannot be recycled, use saturated brine: water + 10% salt; intervals between checks on traps: 7-15 days.

The reduced attractiveness of brine is sometimes put forward as an argument for passive sampling for quantitative surveys, but doubts will continue to exist on this issue until such time as an objective comparison is carried out between brine and antifreeze<sup>4</sup> (cf. Koivula *et al.*, 2003).

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<sup>4</sup> NB: disturbance to traps by wild boar is a thorny problem (the option of placing barbed wire around traps is currently under consideration).



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### **Equipment suppliers and sites**

- Trap pots in rigid polypropylene

Distributors of plastic containers for the agrifood industry.

E.g. cylindrical pots, diameter 95mm, height 114mm, volume 555ml, type UNIPAK 5012 (<http://www.pro-jet.fr>); available in cartons of 1,000, €38.21 per 100 not including VAT (2007 edition of the catalogue). These pots are cylindrical ice trays of half-litre capacity and can be recycled for entomological use.

- Collection fluids

Distributors of chemicals through DIY chain stores in the case of monopropylene glycol.

E.g. Brabant Chimie in northern France (<http://www.charbonneaux.com/brabant.htm>); Gaches Chimie in southern France (<http://www.gaches.com/contacts.html>).

**III.2 - Window flight traps**

**(Christophe Bouget and Hervé Brustel)**

**Basic principles, groups caught and sources of bias**

Window flight or collision traps are traps that intercept the flight of particularly mobile insects that are heavy in flight and show positive geotactism when impacting an obstacle: i.e. they allow themselves to fall (especially Coleoptera). The technique was developed by Chapman and Kinghorn (1955) and later by Peck and Davies (1980). The most commonly used devices consist in a collection receptacle is placed under an interception surface with a single vane (bidirectional interception), or two crossed vanes (multidirectional interception).

Although this method does not permit association of the species with their micro-habitats, it has been used by numerous authors for sampling saproxylic fauna (Barbalat, 1995; Okland, 1996; Martikainen *et al.*, 1999; Grove, 2000; Brustel, 2004b).

According to Similä (2002), single-vane window flight traps will catch 60% of flying coleopterological fauna and produce a representative image of the saproxylic fauna (Siitonen, 1994). In northern spruce forests, the proportion of saproxylic taxa among the Coleoptera caught in window flight traps is high: studies indicate between 42% and 67% of species and between 39% and 47% of individuals (Stokland, 1994; Martikainen *et al.*, 2000; Sippola *et al.*, 2002).

Other traps are used to catch circulating aerial (notably saproxylic) entomofauna: sticky sheets, coloured traps and chemical lure traps. Window flight traps do seem however to offer superior effectiveness: greater numbers of individuals and species are caught in each trap (Barbalat, 1995, Siitonen, 1994, Brustel, 2004b). Selectivity, defined as the percentage of Coleoptera in the total sample, is also maximised in window flight traps (Canaday, 1987), and especially in baited traps as shown in the following table.

**Table 11: The selectivity (i.e. % Coleoptera/total arthropods) of the various trapping techniques in different types of forest in south-western France and the Pyrenees (according to Valladares, 2000; Noblecourt, 2001; Brustel, 2004b).**

% Coleoptera	Douglas-Fir	Fir-beech	Pine woods	Pine – various deciduous	Holm oak forest
Low beer trap	1.6	3.9	7.6	11.9	3.9
High beer trap	4.1	4.2	6.1	6.1	4.3
Coloured trap	25	37.5	32.4	44.8	30.7
Window flight	41.8	57.1	40.6	75.7	40.8
WF + terpenes	49.4	19.5	68.4	84.1	/

This trapping method can be replicated and standardised more easily than emergence nets or manual debarking. Continuous trapping enables numerous species to be captured that are not easily caught on sight or by debarking, especially in the case of species whose activity is seasonal, brief or nocturnal.

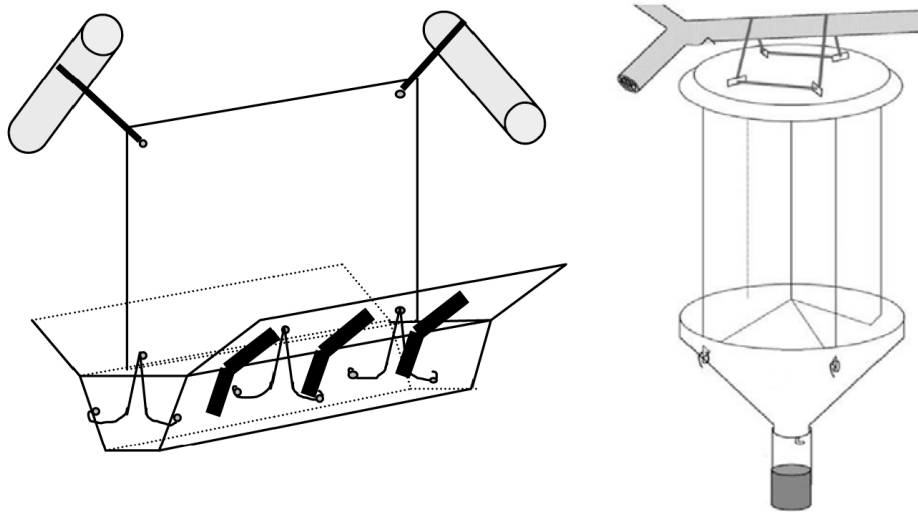
Like all interception traps, what is in fact measured is insect activity/density or activity/abundance. We hypothesise that activity/abundance is correlated to local population density around the trap (Baars, 1979). Due not only to the level of activity but also to reactions to the trap (weight, height and speed of flight: avoidance has been observed in the case of slow-flying species such as Cantharidae or conversely in that of rapid-flying, manoeuvrable species such as Buprestidae), the effectiveness of insect capture is species-dependent.

Randomness of interception (i.e. ‘neutrality’) is biased by various parameters of the device and its immediate environment (Bouget, 2001) such as forest stand density (concept of available flying space, and therefore ‘ease of frequentation’ and by the same token ‘ease of capture’), the proximity of certain resources or wind direction.

**Variants**

There are two window flight trap configurations (cf. Figure 4 and Insert 8):

- Single-vane, bidirectional, requiring two points of suspension of equivalent height and with a collection receptacle of ‘planter’ type; this is often narrow and sometimes fitted with inclined side panels for insect collection; this is because the kinetic energy of Coleoptera as they hit the window can cause them to rebound out of range of the narrow pan but they will then be deflected into it by the inclined panels.
- Multidirectional, with two crossed vanes perpendicular to each other positioned above a wide plastic funnel to which a collection bottle is attached; the total interception surface may be as great as a single-vane trap; these traps, which are less cumbersome, require a single point of suspension and can be hoisted up into the tree crown (Photo 7); the limited volume of the collection bottle reduces the use of preserving fluid compared with that necessary for the long collection receptacles of single-vane traps.



**Figure 4: Single-vane window flight trap (left) and a multidirectional Polytrap™ type (right).**



**Photo 7: Black multidirectional trap (Polytrap™ type).**

**Insert 8: Performance comparison between single-vane and multidirectional window flight traps.**

We compared in a methodological study the capture effectiveness of two traps each with an equivalent one square metre interception area but in different configurations: multidirectional, with two crossed vanes, and bidirectional, with a single vane. The configuration of a window flight trap (single-vane or crossed vanes) has a highly significant impact on the abundance and richness of the saproxylic Coleoptera caught in each trap: 2.5 times more individuals and species were collected in the single-vane traps (Bouget *et al.*, 2008a). 88% of local taxa were caught in the single-vane traps, whereas the cross-vane traps held only 46% of species at each site. On average, at a given site, nearly 54% of species were found only in the single-vane traps. Several families were notably more effectively caught with single-vane traps. (Latridiidae, Elateridae, Nitidulidae, Platypodinae, Scaptiidae, Mycetophagidae, Ciidae, Laemophloeidae, among others).

It should however be noted that the preserving fluid contained a lure. Indeed, for one square metre traps the volume ratio is in the region of 0.5 litre of fluid for the collection bottle of a cross-vane trap and 4 litres for the collector on a single-vane trap. This difference in volume of attraction fluid and evaporation area may have contributed to the differences in effectiveness.

The preserving fluid is saturated brine, monopropylene glycol (cf. Part III.1 of the present chapter), or ethanol, possibly diluted at 50%, plus an anionic detergent (to facilitate the immersion of the trapped insects).

In order to enhance the capture rate, a lure is sometimes added to the interception device:

- a fermentable or fermentative mix that preserves and attracts and which is based on beer, wine, sugar or ethanol diluted in water (Allemand and Aberlenc, 1991),
- or ethanol acting as a kairomone (cf. Insert 9),
- or a combination of ethanol and turpentine (terpene) in the case of coniferous stands.

**Insert 9: The attraction of alcohol as a kairomone**

Ethanol, a volatile compound released during the decomposition of the tissues of dead wood or the sap of damaged or dying trees, acts as a kairomone, a stress signal that will attract numerous Coleoptera associated with dead or ageing wood, enabling them to locate their host (Byers, 1992).

In ecology, a kairomone is a chemical produced by a living organism and released into the environment which triggers a behavioural response from another species and whose effect is positive for that receiving species.

The lure can be added to the preserving fluid in the collection receptacle or placed in a separate diffuser. If the preserving fluid in the collection receptacle is attractive (e.g. ethanol, fermentative mix), the large volume required in the pans of single-vane traps may lead to an increase in the attractiveness of such traps compared with cross-vane traps (cf. Insert 8).

Active devices (chemical or colour-based attraction, targeted positioning) entail a risk of interaction bias with the surrounding environment which will limit the validity of any comparisons (Insert 10). However, this bias may be deliberately exploited when searching for particular species in the context of a site inventory.

**Insert 10: Variations in the effectiveness of window flight traps baited with ethanol in sharply different forest environments.**

In a methodological study relating to the effects of use of an alcohol lure on the capture effectiveness of window flight traps, we observed that baited traps caught twice as many individuals and 40% more species than unbaited traps. The probability of detection is also enhanced in the traps with lures. Many species are more abundant in the samples caught by baited traps and no species was significantly less abundant in those traps (Bouget *et al.*, 2008b).

We then compared the effect of the lure in various forest environments in order to measure its influence in terms of the validity range of the comparison. This was because it might be thought that the use of a baited trap would bias comparisons between two types of environment with variable structures for example. We did in fact demonstrate that the difference between paired traps, whether or not they were baited, increases with the degree to which the forest environment is open (Bouget *et al.*, 2008b). It is likely that the emission levels and diffusion distance of the lure, and by the same token the intensity of the attractiveness of the baited trap, will increase with the openness of the surrounding area. Moreover, we have observed that in areas rich in fresh dead wood (after cutting for example), the high levels of ethanol emission interfere with the attraction of the trap (saturation effect, dilution), with the result that the intensity of the attraction diminishes, as does the difference between baited and unbaited traps.

Colour devices can also be added to window flight traps. A vertical black bar is sometimes added to the clear vanes of cross-vane traps in order to imitate the silhouette of a tree trunk (Photo 7), and this will attract certain xylophages (Chénier and Philogène, 1989; Zach, 1997) (cf. Lindgren-type traps for North American bark beetles).

In addition, a white or yellow funnel or collection pan can add the function of a coloured tray (cf. composite trap, Part II.1.2 of the present chapter).

The interception surface is limited by the fragility of the plastic and by wind stress: for this reason the vanes are sometimes replaced by very fine mesh net or stretched canvas whose dimensions can be much greater (Peck and Davies, 1980; Marshall *et al.*, 1994; Degallier and Arnaud, 1995), or by a more flexible, lighter plastic that can be folded for transportation (Meriguet, 2007).

For freely suspended traps, the standard total interception surface of one square metre is usual. Smaller window flight traps are used in direct association with a natural micro-habitat against a snag trunk or wood fungi providing a natural source of attraction (“trunk window trap”, Kaila, 1993). Muona (1998) considers that the latter method catches more rare species than free window traps. This is so because this technique orients the catch profile more towards guilds of stenoeic species (mycetophile, corticolous or cavicolous insects) depending on the chosen location.

### Recommendations

On a number of practical grounds (smaller volume of preserving fluid, greater robustness, reduced dimensions, quick set-up and easier transportation when broken down into separate parts) cross-vane window traps, and especially the standard Polytrap™ (Brustel, 2004a), are to be preferred to single-vane window traps.

The traps are hung on a natural support (e.g. a branch in the tree crown) at head height, a position that can be adjusted to suit the density of the sampled stratum and risks of disturbance by game animals.

Given the constraints described above (cf. Insert 10), we suggest:

- the use of a relatively ‘neutral’ preserving fluid for comparative studies (e.g. the propylene glycol/water/salt mix described in the section on “Pitfall Traps”);
- the addition of ethanol to the preserving fluid or its addition in a diffusion flask suspended from the trap in order to maximise catches forming part of an inventory programme.

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#### **Equipment suppliers**

Polytrap™ multidirectional window flight traps are marketed by the Purpan engineering school.

### III.3 - The Malaise trap

(Anne Vallet, Jean-Pierre Sarthou)

#### Basic principles, groups caught and sources of bias

The Malaise trap was developed by René Malaise in 1934 (Malaise, 1937). Malaise was a Swedish entomologist, explorer and art collector. He is famous above all for the invention of the insect trap discussed here. This technique is recommended for insects that are very manoeuvrable in flight and which present negative geotropism (i.e. a tendency to rise when faced with an obstacle). For further details on this trap the reader can refer to the bibliography of Steyskal (1981).

The Malaise trap (Photos 8) comprises a tent-like stationary structure in fine-mesh cloth whose sides are open and in which there is a central vertical baffle that guides the insects towards a conical roof fitted with a collection device containing preserving fluid. When an insect arrives at the central baffle, it will try to avoid the barrier by falling downwards or by flying upwards. Insects trying to escape by flying upwards are prevented from escaping by the roof and are directed towards the collecting receptacle, from which they cannot escape.



**Photos 8: Malaise trap.**

This is an effective trap that catches large numbers of a varied range of insects. The vast majority of insects caught are Diptera and Hymenoptera (Southwood, 1978), but it is also an effective method for catching Neuroptera and many other orders living in the herbaceous layer if the structure is set up on the ground. Coleoptera tend to allow themselves to fall when they hit an obstacle in flight and this technique is not recommended for them. However, the use of a single Malaise tent in Andorra over a period of 17 months yielded a catch of some 3,000 specimens of Coleoptera, with 41 families represented (Vazquez and Pujade, 1995). Interesting results for Coleoptera are claimed by Marshall *et al.* (1994), forest Coleoptera studies are entirely focused on this technique in New Zealand (Hutcheson and Jones, 1999; Hutcheson and Kimberley, 1999) and during a “treetop raft” expedition in Gabon, the technique led to new species of *Agriilus* (Buprestidae) being discovered (Curletti, 1999).

Malaise traps set up on the ground will catch insects flying up to a metre above ground level in or above the vegetation. They are the most effective devices for sampling arthropods at forest edges (corridor effect) but they can also be used in forests, bogs, meadows and ecosystems with sparse vegetation such as sand dunes, salt marshes and rocky areas. In wide open biotopes, the Malaise trap often acts as a focal point for swarms of Diptera, thus increasing the numbers captured.

The effectiveness of a Malaise trap is highly dependent on its shape, size and colour (Marshall *et al.*, 1994), sources of bias that can be forestalled by using standard traps (same shape and colour) available on the market.

The probability of the capture of an insect increases with the distance it travels. Malaise traps are among the most productive sampling devices in terms of species richness and numbers of specimens caught. This is probably their weakness, because the abundance of insects collected makes sorting

more time-intensive (approximately 10 hours for a Malaise trap that has been in position for a fortnight at the height of the season).

### Variants

There are several types of Malaise trap. The standard models are known as 'Marris House' traps (after their former manufacturer). In the United States the 'Townes' Malaise trap is in routine use (named after the inventor, Townes, 1962). They are larger than those used in Europe.

Malaise traps are interception traps that, theoretically, are not of attractive type. The presence of alcohol in the collection receptacle may become attractive for certain insects. In order to remedy this problem, it is possible to replace the alcohol with water to which salt has been added (preservative) and detergent (wetting agent). Alcohol or water plus detergent will kill the insects rapidly.

Malaise traps are available in black and in white, with the optional combination of both colours (a black barrier panel and a white roof, which reinforces the insects' tendency to seek escape in an upward direction).

The most widespread modification of the conventional Malaise trap is the addition of a collection receptacle along the central baffle, as is the case in Composite Entomological Traps (cf. Chapter II, II.1.2.) in order to catch insects that fall to earth after encountering the barrier. These receptacles may be identical to those used in pitfall traps, but they must be light grey in colour (non-attractive for the insects) and placed on the ground. This enables the unique capture of those insects that have been stopped by the Malaise trap central baffle. The material in the Malaise trap and that in the pitfall traps can then be analysed separately.

Basset (1985) reduced the dimensions of the trap in order to suspend it in the tree crown. In that study the taxa most often captured with the trap were Diptera Nematocera, Brachycera and Coleoptera, but in the case of the latter order, the results were very modest.

Malaise mini-tents fitted with a collection receptacle at the base and placed in the canopy were also tested by Barbalat (1995). The results for saproxylic Coleoptera were disappointing. However, they appear to be highly unpredictable if one is to believe the various results obtained with such trap configurations in south-western France and the Pyrenees (Noblecourt, *personal communication*). In France's Upper Savoy, fairly good results have been obtained with mutually complementary samples of the fauna being captured with Malaise traps and Polytrap™ window flight traps (Sarhou and Brustel, *personal communication*).

### Recommendations

We recommend the use of standard type Malaise traps (i.e. 'Marris House') available on the market from **B & S Entomological Services** in Ireland. The trap's purchase price includes the canvas, nylon suspension cords, anchor pegs for ground installation, a collection jar with a screw top, but does not include the poles. The package also contains a short explanation on how to assemble the trap.

The Malaise trap is held up by poles at each extremity. These poles are themselves held up by ropes attached to pegs driven into the ground. Aluminium pegs are the easiest to use but they can be broken by gusts of wind. Wooden pegs seem better suited. The collecting bottle is in white or translucent plastic and includes a side opening near the top which is at the apex of the trap and through which the insects fly. The bottle is approximately one-third full of 70° alcohol which will kill the insects rapidly without giving them time to damage themselves by attempting to escape. Where temperatures are high, it is advisable to fill the bottle a little higher than this to prevent drying out. Translucent non-denatured ethanol is to be preferred and yellow (denatured) ethanol sold on the open market should be avoided since it colours the insects.

Once folded down, these tents take up very little space and it is possible to carry several in the field. Assembly is also possible by a single individual after an initial helping hand. It is useful to have a hammer available to drive the pegs into the ground. The highest part of the trap should theoretically be positioned where there is most light. This is because the insects will prefer to try to escape by moving towards the light.

For comparative studies, the traps must be oriented identically (a compass should be used). Their placing in livestock pastures should be avoided (there is a high risk that they will be trampled) or if



this is done, a barrier should be added to stop animals coming too close. No damage due to wild fauna (roe deer, wild boar, etc.) has been reported.

For correct sampling in a habitat, trapping must cover the entire potential period of insect flight, which is usually between April and October in temperate regions. A period of trapping this long is necessary to sample taxa with differing phenologies.

Where the objective is a census of a particular ecosystem, sampling must be done with as many traps as there are habitats present.

Collection and recharging Malaise traps can be made easier by using extra collecting bottles filled with alcohol. All that needs to be done in this case is to unscrew the bottle already in position and to fit the new one. Do not forget to note the position of the trap and the date of collection on the bottle that has been removed. Transfer is made easier by the use of a small wash bottle to detach insects sticking to the sides. There is never any plant debris in the collecting bottles.

It is possible to leave captured insects in the bottle for several years before determination.

The useful life of this type of trap varies with use (3 to 5 years for continuous use during the growing season) because exposure to ultraviolet light will gradually make the cloth brittle.

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### Suppliers

Marris House Malaise Trap (€200 for the complete trap in 2009) from:  
*B & S Entomological Services* (Owners: Dr Brian Nelson and Dr Shirley Nelson)  
37, Derrycarne Road, Portadown, Co. Armagh,  
BT62 1PT, Northern Ireland, UK  
E-mail: enquiries@entomology.org.uk  
Tel: +44 (0)77 6738 6751 or +44 (0)28 3833 6922  
Fax: +44 (0)28 3833 6922

### *III.4 - Light traps*

(Philippe Bonneil)

#### **Basic principles, groups caught and sources of bias**

Light sources will attract a large number of insects active at twilight and during the night, especially when the light is in the ultraviolet range (wavelengths 10-400nm). This attractive property of light, which has been known since the Middle Ages, was initially used to combat pests before being employed in the study of insect fauna.

The use of urban light sources combined with active capture using nets yields interesting insect captures. Insects can also be attracted to a sheet or any white surface illuminated by a lamp (trapping with an illuminated sheet, cf. Figure 5, Photo 15 and Photo 16). Passive trap systems comprise an interception and collection arrangement added to the light source, which lights automatically (triggered by a photocell or a timer). The energy comes from a generator or an automobile-type battery.

The use of an illuminated sheet is particularly effective for census purposes. The individuals attracted are identified on site or captured and then placed in a collecting jar containing a lethal substance (ethyl acetate or potassium cyanide – to be handled with care) for later identification and counting.

Automated traps with a light bulb and an interception/collection system tend to be used for comparative studies and monitoring (standardisation) (Kato *et al.*, 1995; Summerville and Crist, 2002; Bonneil, 2005).

The trapping systems associated with this type of lure enable nocturnal Lepidoptera (Heterocera) to be captured in particular, along with various Diptera, Coleoptera, Hymenoptera, and so on. In the Mediterranean area, it is a very effective technique in periods of summer heat for catching certain Coleoptera Cerambycidae, Anobiidae, Alleculinae and Oedemeridae.

Like all relative sampling methods, the data obtained tend to reflect units of capture effort; they enable comparisons to be made between species distributions and assemblages, as well as species richness in time and space. However, it should be borne in mind that a number of factors can make biological interpretation difficult:

- Ongoing changes in population sizes;
- Changes in the number of individuals at a specific biological stage due to the phenology of the species (e.g. end of generation for adults);
- Changes in activity following a change in the environment;
- Differences in response between sexes or between species. This is because males seeking females for reproduction are captured in greater numbers and attraction behaviour varies between species, with some evidencing avoidance at greater or lesser proximity to the light source (Lamotte and Bourlière, 1969);
- Changes in trap effectiveness in certain circumstances.

This last point is particularly important: Muirhead-Thomson (1991), Southwood and Henderson (2000) review the various factors affecting trap effectiveness. In particular, these relate to climatic and lunar conditions whose effects on trapping have been studied for many years but which remain complex due to interaction between these factors (Williams, 1940):

- Increasing wind speed has a negative effect on insect capture;
- Trap effectiveness increases along with contrast against the surrounding environment and is dependent on lunar phase;
- High ambient temperature and relative humidity are conducive to capture;
- The effects of rain vary according to its intensity.

As a consequence, it is preferable to install traps only at new moon, on windless nights when the weather is relatively hot, and in the absence of driving rain.

#### **Variants**

For all these devices, the main variable is the power of the light source and its wavelength spectrum. The bulbs used are generally of mercury vapour or UV fluorescent tube types. The higher the power of the lamp, the greater the radius of attraction. For this reason it is necessary to adjust the power used to the area of the environment or site to be surveyed in order to avoid catching too many 'tourist' or

nomadic species. A 400W mercury vapour lamp for example will attract insects over a distance of approximately 6km on open land (Beaudoin, 1983)!

There are many different types of automatic light trap ranging from the simplest to the most complex, and some can be obtained from entomological equipment suppliers. It is also possible to build a system and adapt it for one's own purposes (bulb type, power source, type of interception, and so on).

A simple, effective trap for sampling Heterocera in comparative studies is the so-called 'Pennsylvania' light trap (Photo 9). A 15W tube is positioned vertically and surrounded by four transparent, perpendicular Plexiglas panels topped by a roof for protection from rain and under which a funnel and collection receptacle are placed. The tube is powered by a 12V car battery. Lighting can be manual or automatic (with a timer or a photocell allowing the operator to leave the site and run several traps simultaneously). The battery power must last for a sufficient period before recharging (especially if the trap is to operate all night).



**Photo 9: Automatically triggered light trap used in a comparative study of communities of nocturnal Lepidoptera (Bonneil, 2005).**

### Recommendations

For comparative studies we recommend the use of an automated light trap of 'Pennsylvania' type as described above.

The power of the 15W blacklight tube is sufficient to attract insects over a radius of approximately 25 metres and is well suited to sampling a forest plot. Energy autonomy is crucial: a 12V, 36A gel car battery (no leaks or liquids to spill) will power the trap for a whole night, or perhaps even two.

Trapping must be done around the new moon.

This type of trap yielded good results when used in the context of an ecological study in the French State-owned Montargis forest (Loiret) (Bonneil, 2005).

The price of these traps is fairly high (around €300 from the supplier – see below), not counting the cost of the batteries. It may be advisable to gather together the necessary components and to assemble such a trap oneself. It is possible to set up a common fund of equipment, which can then be used by several managers in more than one forest, but only in different years (if the stock is insufficient).

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### **Suppliers**

Automated 12V powered trap of 'Pennsylvania' type: system sold online on a German website (<http://www.bioform.de/>). Click on 'Entomologiebedarf' [entomological supplies], followed by 'Lichtfang' [light trapping] and then 'Leuchtfallen 12 V / 220 V classic' [classic 12V or 220V light trap].

## ***CHAPTER 3***

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# ***A BRIEF OVERVIEW OF SAMPLING METHODS AND OF INSECT GROUPS RELEVANT TO ENTOMOLOGICAL INVENTORIES IN TROPICAL FOREST ENVIRONMENTS.***

**(Julien Touroult and Pierre-Henri Dalens)**



As is also the case in temperate forests, the choice of a method for studying insects in tropical environments needs first to be matched with the desired objective. Most studies in tropical environments relate to the following:

- The effect on biodiversity caused by the degradation of 'primary' forest environments: fragmentation of forests, the effect of forest exploitation or conversion into plantations, etc.;
- Characterisation of the fauna on certain sites (e.g. reserves, national parks) by means of inventories of the species present;
- The search for species new to science, these often being discovered during site inventories.

In the first two of these cases, difficulties arise not from the inventory methods but from the choice of an appropriate taxonomic group, that is to say a group for which it is possible to meaningfully interpret the results: establishing whether a site is more or less species rich than another, whether it has species of special interest, whether a particular secondary forest is of interest with regard to biodiversity conservation, and so on. The need is to choose one or more collection methods to match the target taxonomic group and the type of study. The main methods used in Europe, and described in the preceding section, are also effective in tropical zones. In the pages that follow, we detail a small number of taxonomic groups and methods that are particularly appropriate or specific to tropical environments.

In tropical forests, the value of the seductive notion that certain groups of insects might be better bio-indicators than other groups, reflecting in synthetic fashion the biodiversity present or the response of the biotic community to disturbance, remains to be proved. Several studies, both in the seasonal tropical forest in Sulawesi (Schulze *et al.*, 2004) correlating the diversity of vertebrates and invertebrates, and in the equatorial forest of Cameroon (Lawton *et al.*, 1998) correlating diversity between groups of arthropods, show that such correlations are usually weak where response to disturbance is concerned. Responses often diverge in accordance with the diets of the different taxonomic groups, their capacity to disperse and their behaviour with respect to light levels.

## I - SOME TAXONOMIC GROUPS THAT MAY LEND THEMSELVES TO INVENTORIES AND ECOLOGICAL INTERPRETATION

### 1.1 - *Lepidoptera*

As is the case in temperate environments, there is a satisfactory level of knowledge of this order among amateur and professional specialists, as well as numerous publications and reference articles for their identification, with some knowledge of their distribution and diet.

Two groups (each having an associated inventory method) are in particularly wide use in tropical environments:

- Nymphalidae, and especially the subfamily Charaxinae, which are attracted by traps containing fermenting bait (cf. the *Charaxes* trap method discussed below). The number of species suitable for sampling with this method is relatively realistic: between 40 and 100 species depending on the region. These butterflies are territorial, and are often associated with the canopy and woody host plants (Henning, 1988). Certain species are encountered in the savanna and even in manmade environments. This group is sensitive to environmental fragmentation (Daily and Ehrich, 1995) and given a degree of knowledge of the local fauna, the heritage value of forest remnants can be evaluated (Joly, 2003). This technique is effective in all continental tropical forest environments.
- Heterocera (moths), captured by light trapping. The families and the method used are comparable to those discussed for temperate environments. Given the extreme diversity of Heterocera in the tropical environment and the effectiveness of light traps, which will attract over 2,000 moths per collection night, it is necessary to target a small number of easily identifiable groups. In French Guiana, Sphingidae were used in a Petit-Saut dam impact study (Cerdan *et al.*, 1993). This group contains around 120 species in Guiana and a comparable number in other tropical regions (D'Abreu, 1986). Sphingidae have a great dispersal capability and are very sensitive to being attracted by light sources, which can attract them far away from their preferred habitat. However, various studies have shown that they have fairly strong affinities with environment type, on condition that a qualitative analysis is done of the composition of the samples rather than adopting a simple presence vs. absence approach



(Chey *et al.*, 1997; Touroult and Le Gall, 2001b). Depending on the available capabilities for species identification and interpretation, many other groups of Heterocera can successfully be used: e.g. Geometridae in the mountainous areas of the Andes (Hilt *et al.*, 2006).

**Comment:** The transect-based method of monitoring daylight Lepidoptera (cf. Part II.1.1 of Chapter 2 and Part III of Chapter 4) can also be employed in tropical environments. A high degree of skill is required for species recognition in the field. Wood and Gillman (1998) have shown in Trinidad that the results of an evaluation of species richness will differ sharply between counts based on transects and on the use of ‘*Charaxes* traps’. This latter type of trap provides a more selective view, capturing species requiring a closed canopy, whereas the transect method samples forest edge and undergrowth species. The *Conservation international* organisation recommends sampling of the ‘*Charaxes* trap’ type for the ecological monitoring of Rhopalocera in tropical environments (Batra, 2006).

## ***I.2 - Coleoptera Scarabaeidae***

This large family of Coleoptera is a useful group for inventories. It includes a large number of species that are relatively well known and easily sampled. The species are not directly linked with host plants but have a varied range of lifestyles according to their subfamilies (sometimes considered to constitute fully fledged families) and are sensitive to habitat type. They therefore provide more detailed ecological data than a simple link with richness of vegetation.

### **Coprophagous Coleoptera Scarabaeidae**

In temperate zones, coprophagous Scarabaeidae are relatively uncommon in forests and tend to be associated with open pastures containing large domesticated herbivores (Lumaret, 1990). In tropical zones, numerous species are forest-dwelling, lend themselves easily to inventories and can be used in ecological studies (Hanski and Cambefort, 1992). In Africa, areas of savanna are significantly richer than forest areas, reflecting a clear link with the large mammal fauna; in America and Asia, the forests are host to richer communities than open environments.

The forest species are good indicators because they are associated with the presence of a fauna providing a food resource (vertebrate dung, dead vertebrates and invertebrates), are sensitive to microclimate (forest undergrowth conditions) and in most cases cannot move around in open environments (Scheffler, 2005). The ecological requirements and preferences of these species are well known in a number of contexts: West Africa (Cambefort, 1984; Davis and Philips, 2005), Central Africa (Walter, 1978), Central America (e.g. Estrada *et al.*, 1993), Amazonia (Scheffler, 2005) and Southeast Asia (e.g. Sabu *et al.*, 2006). One of the problems in using this group is the determination of the large numbers of small species (3-6mm) given the lack of any comprehensive reference work (the documentation is fragmented, and takes the form of taxonomic articles).

Two main methods can be used for these inventories:

- Bait trapping. According to the bait used, this method will permit targeted, repeatable sampling of coprophagous beetles.
- Interception trapping. This method is very effective for the capture of forest-dwelling coprophagous beetles, which usually fly around close to ground level. It can provide a fairly reliable picture of the community of species present and their abundance, independently of any effect linked to food resources.

### **Coleoptera Cetoniinae (or Cetoniidae depending on authors)**

This taxonomic group, which includes species indicative of forest ecosystem quality in France (Brustel, 2004), can be a good indicator group in Africa (Touroult and Le Gall, 2001a) and probably in Asia. This is because the number of species lies within a range that can realistically be ‘handled’ (between 80 and 300 species in African forest countries), they are fairly easy to identify and can be captured using standard methods. For determination of African species, reference can be made to the publications devoted to them in the collection *Coléoptères du Monde* (e.g. Rigout and Allard, 1992), the iconography of Sakai and Nagai (1998) and more recent revisions of certain genera in journals such as *Coleoptera* or *Les Cahiers Magellanes*. In America, the number of species is smaller (thirty or so species per region) and they are less easy to sample, which makes them a less practical but nevertheless useable group for ecological studies (Solis, 2004).



Cetoniinae are not associated with specific host plants but are frequently restricted to particular habitats (some can be found only in the forest, others in the savanna, etc.) and micro-habitat (larval development in tree cavities, epiphyte humus, rotten wood, litter, and so on). Although the precise ecology of each species in tropical environments is not known, the communities vary significantly between environments and can be fairly easily interpreted. In Benin, it was possible to identify species that move around in the dark undergrowth, avoiding clearings and forest edges (cf. Table 12).

Sampling involves the use of aerial traps with fermented bait, these being comparable with the traps used in Europe.

**Table 12: Trapping results in southern Benin, in a dense semi-deciduous forest zone, illustrating the diversity in terms of Cetoniinae and species characteristic of different environments (according to Touroult and Le Gall, 2001a).**

Results for each environment	Forest (traps at height)	Forest (traps in under-growth)	Palm stands	Farmland with trees	Fields	All
Number of traps	6	2	10	2	4	24
Average number of Cetoniinae captured per trap in 10 days' trapping	29	13	4	42	6	15
Species richness	22	9	12	13	12	26
Diversity (Fisher's Alpha index)	4	2,2	1,6	2,3	2,9	4,23
Percentage of most common species (Berger-Parker dominance index)	41%	53%	26%	76%	38%	44%
<b>Some characteristic species (numbers of individuals per environment)</b>						
<i>Paralleucosma glycyphanooides</i> (Moser, 1908)	52	1	0	0	0	53
<i>Caelorrhina thoreyi</i> (Schaum, 1841)	35	4	0	0	0	39
<i>Stethodesma strachani</i> Bainbridge, 1840	14	0	0	0	0	14
<i>Myodermum alutaceum</i> Afzelius, 1817	231	75	5	5	2	318
<i>Tmesorrhina iris</i> (Fabricius, 1781)	71	46	0	3	0	120
<i>Chordodera quinquelineata</i> (Fabricius, 1781)	0	6	0	0	0	6
<i>Pachnoda tridentata</i> (Olivier, 1789)	2	0	23	17	18	60
<i>Diplognatha gagates</i> (Forster, 1771)	1	0	41	7	17	66
<i>Chlorocala africana</i> (Drury, 1773)	138	3	40	58	27	266
<i>Plaesiorrhinella cinctuta</i> (Voet, 1779)	410	1	31	372	56	870
<i>Marmylida marginella</i> (Fabricius, 1775)	1	0	2	5	9	17
<i>Gametis sanguinolenta</i> (Olivier, 1789)	6	0	3	8	7	24
Totals for 14 other uncommon species	38	9	15	14	12	88
Total for each environment	999	145	160	489	148	1941

### ***1.3 Coleoptera Cicindelidae***

This family of Coleoptera is well represented in tropical zones, with a reasonable number of species. They are usually easy to identify and can be used for studies of all environments: open, coastal, with-outtrees or forested (Rivers-Moore and Samways, 1996; Clark and Samways, 1997). In particular, there is a very good monograph on African Cicindelidae (Werner, 2000). Cicindelidae are predatory Coleoptera, with most species living on the ground. They are sensitive to soil disturbance and availab-

ility of prey and are often micro-habitat specific, preferring for example river banks, forest clearings and undergrowth (Pearson and Vogler, 2001). There is no effective trapping method for them, and they must be captured directly with a net, in daylight, while moving along a transect.

#### ***1.4 Saproxyllic Coleoptera***

These various families can be sampled using the same methods as those used in temperate environments: interception traps and emergence enclosures are the two most effective techniques, to which light traps can be added. Nevertheless, members of many small families are not identifiable and knowledge of their life-history is virtually non-existent. These techniques and groups can be used for inventories but they are difficult to interpret. The use of Coleoptera Cerambycidae (longhorn beetles), which are fairly easy to inventory and determine, can be envisaged for assessments of the conservation status of saproxyllic insects in degraded forests and in plantations. This is so because they are sensitive to the availability of food resources (numbers of tree species, quantity, diameters) and to their continuity. Knowledge of their specific ecological requirements can enable more detailed analysis of the results (e.g. certain Prioninae are associated with large quantities of dead wood).

Various groups of scarabs, the majority saproxyllic, are fairly well known in certain tropical zones. This is true for Dynastidae in Central America for example (Ratcliffe, 2003) and for Rutelidae in the whole of the neotropical zone (cf. Soula, 2005 for example). They can be good markers for biodiversity linked to wood in advanced stages of decomposition. They can be sampled using light traps, fermenting bait traps and interception traps, as well as by examining rotting wood for larvae and adults.

## **II – SOME METHODS SUITABLE FOR USE IN TROPICAL ENVIRONMENTS**

Generally speaking, the capture methods described for temperate environments also function in tropical environments (coloured trays, Malaise traps, and so on). It is worth noting that Barber (pitfall) traps are not very effective in catching tropical Carabidae, which are in any case less numerous in tropical forests than in temperate environments at ground level.

Further methods and methods suitable for tropical environments are discussed below.

### ***II.1 Interception traps***

#### **The window flight trap and its variants**

(cf. also Part III.2 of Chapter 2)

As is also the case in Europe, this relatively recent technique is very effective in catching Coleoptera in tropical forests, based on our experience in French Guiana, Martinique and Brazil (Dégallier, *personal communication*). In Guiana, the type of trap described below will catch between 200 and 500 coprophagous scarabids every week.

In tropical forests, compared with temperate environments, problems are caused by leaves regularly falling into the traps and obstructing the collection receptacles, in addition to intense precipitation that may dilute the preserving fluid during periods conducive to insect activity.

The most widely used model is the single-vane type: a sheet of transparent perspex 1.2m or more in length by 80cm in height, positioned across an insect travel 'corridor'. The sheet is fixed between two trees at a height suited to the target species (Photo 100).



**Photo 10: Single-vane window flight trap (French Guiana).**

A trough is fixed either to the perspex sheet or hung separately below the sheet. The trough has a small number of holes for overflow drainage during very rainy weather. The mix in the trough is a saturated salt and water solution, which prevents the decomposition of the sample and invasion by water beetles and other aquatic insects. During the rainy season, it is useful to position a canvas cover over the trap to limit dilution of the preserving fluid and obstruction by falling leaves.

The trap can be emptied every 8-10 days and samples packed in plastic envelopes with some 70° alcohol.

Size is one of the limitations of these traps when they need to be set up at a distant location, in addition to their cost (around €80 a trap), and the resulting large quantity of captured material to be sorted, with numerous families whose species cannot be reliably determined. This type of trap is also ill-suited to installation in sunny, open environments due to the fast evaporation of the preserving fluid.

#### **Variant**

For the capture of low-flying species, notably coprophagous scarabs and Histeridae (cf. Solis, undated; Dégallier, 2004) a more 'flexible' method exists. This involves stretching a sheet of mesh fabric (similar to a robust mosquito net) tightly above ground level and placing small rectangular trays in position as collection receptacles (sorting tray type) or digging the soil to a shallow depth and laying down a hermetic tarpaulin.

This method has an advantage in that it provides a trap that is easy to carry and relatively tightly targeted on 'heavy' flying Coleoptera such as scarabs.

#### **A special interception method: the 'mist' net**

This is based on the interception of travelling insects by means of an acrylic fibre fabric similar to 'Cryldé®' as used in agriculture (to protect orchards). It is employed particularly in the neotropical zone and especially by entomologists in French Guiana, but it will work anywhere, even in temperate environments.

The method is effective for Coleoptera and Hemiptera more than 6mm in size. It can catch very discreet species and especially those rarely caught in window flight traps (Buprestidae most notably) and insects flying in open, sunny areas.

Among the limitations of this method are its cost (purchase of the synthetic fabric), the difficulty of setting it up and the necessity for checking it frequently (ideally every other day). It is not really open to standardisation and it should therefore be used mainly for biodiversity inventories.



**Photo 11: Interception ‘mist’ net or synthetic ‘spider’s web’ (Panama).**

Various types of capture fabrics can be used. One of the simplest is the artificial spider’s web material sold in Halloween joke shops! In the field it should be stretched vertically across a route assumed to be used by insects, in most cases in areas of windfall, or on vertical poles dug into the soil (Photo 11).

### ***II.2 - Fogging***

(cf. also Part II.1.1 of Chapter 2)

This is a method used mainly for fundamental research into tropical environments and involves spraying low-remanence insecticide over a tree and collecting all the species in collection receptacles (a tarpaulin or funnel) placed on the ground in the treated area (Chey *et al.*, 1998). This technique can provide a snapshot of the fauna present but should be ruled out for repetitive studies and inventories because it is both non-selective and complicated to use.

### ***II.3 - Emergence enclosures***

One particularly effective method for catching saproxylic Coleoptera involves taking a sample of wood and branches that have been invaded by larvae and leaving them to ‘incubate’ in a closed enclosure until the adult insect emerges. This technique will collect numerous species that are highly discreet, small in size or whose period of flight is brief. It is also very practical in certain tropical regions with limited seasonality, allowing regular monitoring with limited time spent in the field. This straightforward, inexpensive method provides knowledge of the environment in which species develop, including their host plants if care is taken to make a note of plant species when collecting the material.

A few figures will illustrate the effectiveness of this method for neotropical longhorn beetles:

- In Guadeloupe, it enabled us to capture 80% of the island’s Cerambycids in 16 months (compared with less than 40 % using conventional direct collection methods)!
- In French Guiana, based on three years’ experience, 15 cubic metres of varied types of wood carefully selected and renewed every year enabled some 10,000 longhorn beetles to be collected each year, representing around 500 different species (or approximately one quarter of the estimated total fauna).

The drawbacks of this method include the cumbersome nature of the enclosures, the time needed to obtain specimens (over a year in some cases), the need for virtually daily monitoring and the difficulty of obtaining comparable data from sample to sample (the person collecting the substrate has a major influence).

There are two methods for collecting wood for this kind of insect ‘farming’:

- Production of dead wood by cutting it oneself and leaving it for two months in the forest to ensure that xylophiles lay their eggs in it. This method offers certainty as to the host plant



(Tavakilian *et al.*, 1997). It is for example possible to suspend bundles of freshly cut lianas or branches (Photo 12).

- Branches, small tree trunks and lianas already dead for some weeks or months can be gathered in the field. In this case, it is often more problematic to determine the plant species collected. All sorts of wood may be host to the larvae of saproxylic Coleoptera but we have already observed that the fauna is richer on dead branches that have remained suspended in the tree and exposed to sunlight. The presence of larvae in the substrate can be confirmed by observing a cross-section of the branch (cut with a saw or secateurs), revealing larvae by partially breaking up the wood or observing deposits of ‘frass’ (larval waste) under the branches.



**Photo 12: Suspended wood bundle awaiting oviposition by saproxylic Coleoptera (French Guiana).**

The pieces of wood are then left to dry for a few days to ensure that they have not absorbed too much water, before being placed in a cardboard or plastic box, taking care to remove the spiders, ants and cockroaches of various kinds that might eat the adults on emergence. This box should then be covered with a bin bag in thick plastic. The bottom of a mineral water bottle can be used with absorbent paper to collect the insects, which will be attracted by the light (Photo 13 and Photo 14). A tight seal between bag and bottle can be provided by means of elastic bands (Chalumeau and Touroult, 2005).



**Photo 13: Dead wood before being placed in the emergence enclosure (French Antilles).**



**Photo 14: Emergence enclosure with collection receptacle (a plastic half-bottle).**

Other systems are possible to avoid frequent build-up of insects at the joint between bag to bottle: screw-fitting collection bottles whose pierced lids can be stuck to the plastic boxes (method of Giugliaris and Dalens, *personal communication*). The collection bottles must have an open portion covered in wire mesh to avoid a situation in which the enclosures are completely filled by gases from fermentation and excessive fungal growth.

The boxes should be kept in a room protected from excessive heat with the transparent collection receptacle oriented towards the brightest lit area. Ideally, it is desirable to inspect the receptacle towards midday and at nightfall and to check the interior of the box on occasion because certain species will stay at a respectable distance from the light.

To conclude, there are also the 'traditional' emergence cages with wire mesh on all sides. The main advantage of this is that it prevents excess humidity and fermentation but the insects that emerge are less visible and the substrate tends to dry out.

#### **II.4 - The light trap**

(cf. also Parts II.1.2 and III.4 of Chapter 2)

This technique is based, as has already been described for temperate environments, on the fact that many nocturnal insects are attracted by light at night.

In tropical environments, automated traps are little used, the main reason being the sheer quantity of insects attracted. The method in most widespread use is the following, with virtually as many variants as there are entomologists (see Hequet, 1996, for example).

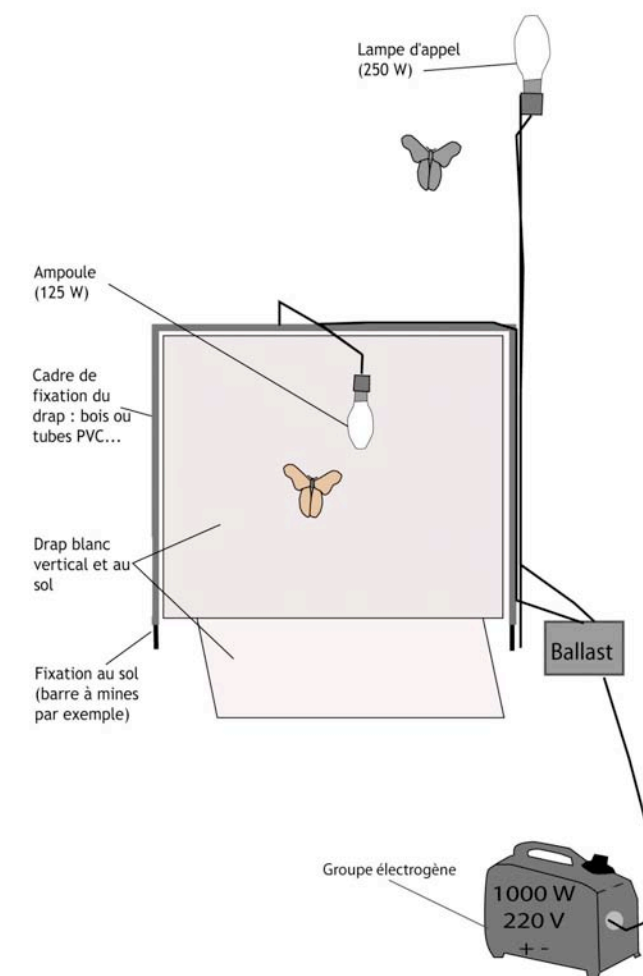
It involves placing a light system in an unobstructed location. The device, which is fairly complex, is usually composed of three bulbs producing a spectrum rich in ultraviolet. The most powerful, the 'beacon', is positioned at a height of three to six metres on a mast; the two others are positioned 1.6 metres from the ground and these serve to guide the insects to a white sheet measuring 1.8 metres by 2 metres stretched vertically (Figure 5, Photo 15, Photo 16 and Photo 17). Another white sheet is laid out on the ground to make it easier to see the insects landing on the ground. The bulbs are powered by a generator. The insects are collected on both sides of the sheet, on the ground and in the immediate surroundings of the trap, since some insects will land only at a certain distance from the light.



**Photo 15: A light trap in French Guiana.**



**Photo 16: A light trap combining a mercury vapour bulb and a blacklight tube (Zambia).**



**Figure 5: Diagram of a light sheet trap.**

The type of bulb and the power used vary widely between entomologists and protocols:

- They range from mercury vapour lamp assemblies rated at around 1,000 watts (two 400W bulbs as a beacon light and two 80W bulbs near the sheet) to simpler devices with a single 125W lamp.
- When sampling the fauna in a given environment, we would tend to recommend a compromise: a 250W beacon light and two 125W bulbs low down. This makes it possible to attract insects from a fair distance without having a repellent effect on certain species that tend to avoid excessively powerful light sources. This system also reduces fuel consumption to less than six litres a night.
- Ballasts should be used to avoid bulbs overheating and exploding during rain.
- Low-power fluorescent tubes of 'blacklight' type are effective and attract rather different fauna; they are widely used by North American entomologists and especially in Central America. Some entomologists combine both types of light source near the light sheet (Photo 16).
- Ancillary traps using low-power lamps emitting different light spectra are routinely used as back-ups to the main trap in order to widen the variety of captures and attract species that the main trap may have repelled.

#### Advantages and drawbacks

This collection technique will easily attract large quantities of Heterocera, Coleoptera, Neuroptera, Hymenoptera, Hemiptera, etc. It is widely used in ecology but it is not perfect. One of the main criticisms is that two consecutive collection nights at the same location can yield very different results in



terms of abundance and composition. The variation in attractive power for different species and for the same species at different sites is a serious drawback (Southwood, 1978). Yéla and Holyoak (1997) have studied the effects of weather conditions on light trap captures. The higher the temperature and the denser the cloud cover, the more insects are caught, with hot, stormy nights being particularly productive. Conversely, the nearer the moon is to full, the smaller the number of insects. It is worth noting that depending on the hour at which the moon rises, it is possible to collect insects at the beginning of the night, including lunar phases very close to full moon.



**Photo 17: A light trap in the very early morning (Guiana).**

Given all these limitations, comparison of the results from night to night has little meaning. It is better to compare two sites on the basis of a cumulative figure for several collection nights (e.g. at least four nights split between two favourable lunar phases).

Another problem with the light trap is that it will sometimes attract insects from great distances. When it is impossible to locate the trap in an area that is homogeneous, insects always come in from adjacent environments. For this reason, caution is needed in analysing the results for species that are good fliers (moths in particular).

Once attracted to the collection sheet, the insects targeted in the inventory are picked up for later determination. One inventory and evaluation method could be tested: photographing the sheet at regular times and identifying the Lepidoptera from the photographic image, calibrating the method by comparing the image with the complete sample of specimens.

## ***II.5 - Traps with scent lures***

### **The 'Charaxes' aerial trap**

A 'Charaxes trap' is a trap that attracts butterflies; it was developed most notably by J. Plantrou for studies of African Charaxes (Plantrou, 1983).

This type of selective trap is used to catch Nymphalidae, and especially Charaxinae, which are very sensitive to baits, along with other groups such as Euphaedra and certain Satyridae.

The trap is composed of a tulle fabric cylinder 25cm in diameter and 65cm in height with a suspended tray underneath containing bait (Figure 6 and Photo 18). The trap itself is hung by cord at a certain height (more than 5m) in a tree. The captured fauna will differ according to the height of the trap and its exposure to light (undergrowth species or canopy species). The butterfly enters by slipping through the gap between the tray and the tulle cylinder to consume the bait. When it leaves, the butterfly's reflex is to fly upwards and it is then unable to find its way out.

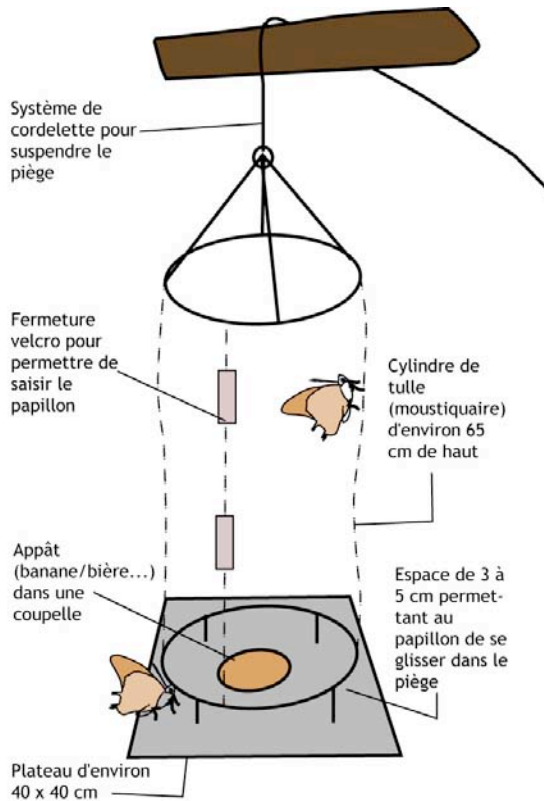
Where the bait is concerned, banana fermented with alcohol (beer, rum or palm wine) can be used, or in Africa, fresh dung from a carnivore such as a lion.

Charaxes traps need to be checked every day (or even several times a day) to prevent the butterflies causing damage to themselves. Moreover, there is always a risk that they may escape if the gap between the tulle cylinder and the bait tray is too large.

When collecting the capture, the trap must be brought down gently, and the butterflies should then be stunned by applying pressure to the thorax before being extracted via the Velcro-closed opening. The bait should be renewed regularly.

This type of trap must be set up in brightly-lit locations. Hot, dry weather will enhance their attractiveness (Henning, 1988). In Africa, Nymphalidae attracted by such traps are more frequently captured at the end of the rainy season and during the first half of the dry season.

The drawbacks of this method are the cost of making the trap and the need to check it daily.



**Figure 6: Diagram of a Charaxes trap.**

**Photo 18: A Charaxes trap suspended at a height of 8 metres (Benin).**

### The 'Cetoniinae' aerial trap

There are many types of trap for Cetoniinae but all are based on the same principle. They will also catch other families of insect, especially Cerambycidae and Rutelidae in tropical America. They consist of fermented bait placed in a container and suspended in a tree.

A model that is very effective in Africa is composed of a 3-litre bucket containing a mix of one half crushed banana to one half palm wine (Touroult and Le Gall, 2001a). The trap is then suspended at a certain height using a cord (Photo 19) or by bending down a young tree. The Cetoniinae attracted drown in the liquid and the trap needs emptying only once a week.



**Photo 19: A Cetoniinae trap: a suspended bucket containing a mix of banana and palm wine (Benin).**

Various plastic bottles can be reused by cutting a side opening (approximately 6cm by 6cm). The baits in frequent use include the following as used in Europe: red wine, possibly with added sugar (this does not yield good results in Africa), a mix of ripe banana, beer and sugar, fruit juice (e.g. banana, peach) (Photo 20). If salt is added to the mix this will limit the fermentation of insects in the trap and allow visits to it to be made at longer intervals (up to a fortnight).



**Photo 20: An aerial trap baited with banana juice + rum + sugar + salt (Guiana).**

This is a very effective trap, but that effectiveness is compromised by rain, which dilutes the bait mix. The most attractive traps are those set up in the sun, at a certain height and in a place relatively free of obstructions, but sites should be varied to obtain undergrowth species also.

Based on our experience, this trap is more selective for saproxylic Coleoptera in tropical environments than in temperate zones. In Africa, it will catch Cetoniinae in the main, with the exception of a few butterflies; in the neotropical zone it mainly captures longhorn beetles, cantharids, Cetoniinae and Rutelidae.

This is among the most effective traps in terms of yield: it is inexpensive, straightforward, easy to check and relatively selective.



### The coprophage trap

This trap attracts species into a buried container (Barber or pitfall type) flush with the soil surface and containing a lure. Coprophage traps will exert strong attraction at 20 metres and possibly even further up to 50 metres (Cambefort, 1984). Most coprophagous beetles wait on low plants or fly at a height of 20cm to a metre above ground level in search of food. There are two daily peaks in activity depending on the species: at dawn and at nightfall; other less numerous species are active in daylight or at night.



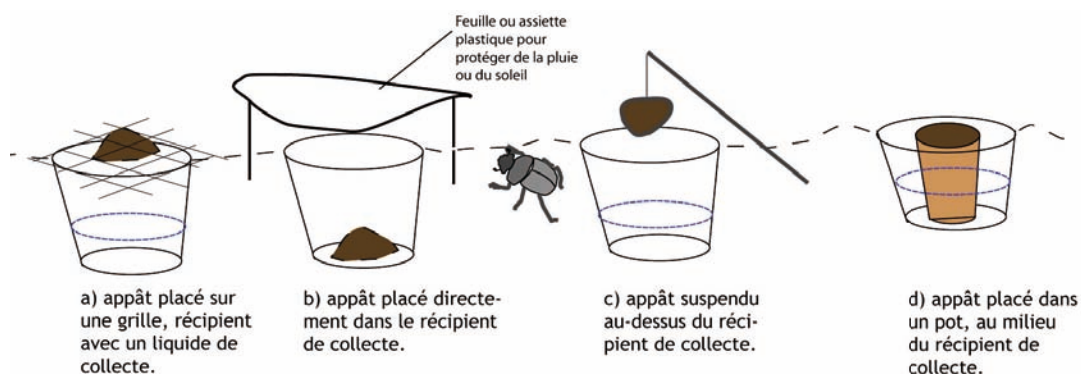
**Photo 21: A coprophage trap – the bait is in the suspended gauze bag.**

Various versions of this type of trap have been developed (Streit, 2004) (Figure 7):

- With the bait placed directly in the collection receptacle. Simple but makes for problematic cleaning and sample sorting.
- With the bait placed in a bag suspended over the collection receptacle containing soapy water; the bag is intended to ensure good diffusion of the odour (Photo 21).
- With the bait placed in a second container positioned at the centre of the collection receptacle containing soapy water.

The last two versions are more complicated to set up but provide samples that are clean and easy to sort.

In any event, although Cambefort (1984) recommends that the bait should be exposed directly within sight of the scarabids, it appears to be preferable to protect the trap from rain and sun with a 'roof' consisting of a large leaf held over the trap by two twigs, or alternatively a plastic cover.



**Figure 7: Examples of different versions of the coprophage beetle trap.**

This type of trap should be emptied every other day at most given the speed with which the bait degrades and the fragility of the Coleoptera, which die rapidly and whose pronotum is easily detached.

Various baits can be used: various types of dung, crushed diplopods, the bodies of small vertebrates, including reptiles and amphibians, seafood, etc. The communities will vary according to the type of bait. Broadly speaking, a distinction can be made between herbivore dung species (low nitrogen content), including those associated with herbivores that browse woody materials (elephants), species that prefer omnivore and carnivore dung, and species that tend more towards necrophagy (Hanski and Cambefort, 1992). Human excrement is among the most effective lures, with a relatively wide attraction spectrum.

The dead animals and pieces of meat will attract scavengers that may destroy the trap. In this case, a grill should be positioned over the bait container (Streit, 2006).

This method of trapping is fairly straightforward, highly selective and will capture large quantities of material. It does however have burdensome constraints in terms of renewing bait supplies for the trap and daily checks. For these reasons we recommend the use of single-vane interception traps placed near the ground for the sampling of coprophage scarabs and forest necrophages.

### III – TAKING SEASONALITY INTO ACCOUNT

Unlike the situation in Europe, where the periods conducive to insect inventories are well known, largely restricted to the spring and summer, things are less clear in the tropics. This is an essential factor for targeted and effective inventories and studies. Where a more exhaustive inventory of biodiversity is the aim, or a search for new species, collections can be carried out in less favourable periods.

Generally speaking, in tropical regions with sharply differing seasons (e.g. Sudanese Africa), insects are largely present during the rainy season, with an emergence peak in the first month. The greater the contrast with the dry season, the more insect emergence is concentrated in the rainy season.

Conversely, in an equatorial or tropical climate with no distinctive dry season (with at least 50mm precipitation in its driest month), insects are present all year round with variations that may be more or less easy to interpret.

For example, in French Guiana, a light trap study (Dégallier *et al.*, 2004) has confirmed the year-round presence of a varied entomofauna, with differences of taxonomic nature and between canopy and undergrowth.

In general terms, the study shows a peak in insect density in October and November and a trough in April and May (1/5<sup>th</sup> in terms of biomass). These results and those summarised in Table 13 should be treated with caution since rain has an influence on insect movement towards the trap or even on the latter's attractiveness, and these results are expressed in biomass and not in terms of diversity or abundance. The study was conducted on a single secondary forest site over a year. As is the case in temperate environments, annual fluctuations can be large, and there can be wide-amplitude fluctuations over several decades according to the experience of entomologists regularly collecting insects. The results obtained by Dégallier *et al.* (2004) do not match 'expert' knowledge of Coleoptera since these insects seem generally to be more abundant in light traps at the beginning of the dry season (August and September).

**Table 13: Abundance peaks for different insect orders in French Guiana, evaluated in terms of their light trap biomass from September 1978 to October 1979 (according to Dégallier et al., 2004).**

	Undergrowth abundance peak	Canopy abundance peak
Coleoptera	November-December (end of the dry season)	June-July (end of the rainy season)
Nocturnal Lepidoptera	October-November (dry season)	September-October (dry season)
Orthoptera	September and January	July to October
Homoptera	November-December	July, August and December
Auchenorrhyncha		
Heteroptera	November-December	September-October
Hymenoptera	September to December	November, January and March
'Small orders'	November (end of the dry season)	November (end of the dry season)
'Micro-arthropods'	November to February	October to December

In the Lesser Antilles, Coleoptera are more abundant in the dry season from March to June, (according to expert observation and based on data from emergence enclosures and interception traps) and nocturnal Lepidoptera more abundant during the rainy season (F. Deknuydt, *personal communication*).

#### IV – LIMITATIONS AND FUTURE PROSPECTS

The description of the taxonomic groups and methods in this section on tropical forest environments is far from exhaustive. Many other groups may turn out to be suitable for inventory purposes (e.g. ants, Odonata or Orthoptera).

One method that has not been developed in this chapter involves the harvesting of certain groups of insects by the inhabitants of villages in the study area in return for monetary reward and a minimum of training on the target groups. This method is employed especially in Africa. Although it has limitations in terms of comparability between samples, it can prove very effective in establishing inventories rapidly for individual localities and it allows direct collection effort to be approximated (number of harvesters, for example).

Among the methodological difficulties there is the fact that small island environments such as the Lesser Antilles or Reunion raise problems for inventories: their fauna has a reduced species richness which does not allow for effective sampling of certain taxonomic groups. Techniques such as light trapping for nocturnal Lepidoptera or emergence enclosures for saproxylic Coleoptera do nevertheless yield results that lend themselves to interpretation.

Given the problems of identification the researcher faces in tropical environments, some occasionally make use of the notion of the 'morphospecies' when assessing species richness in comparative studies of environments (Clark and Samways, 1997). This involves the visual determination of the species of collected insects but without a precise identification of genus or species. However, going beyond inventories aimed at obtaining straightforward lists of species and assessments of species richness (Magurran, 1988), it is especially important to develop tools for a more qualitative evaluation of the heritage value of geographical areas. This evaluation may be sought at the biogeographical level for large naturally formed areas (affinity and biogeographical originality of the species present) or at the smaller level of the site (whole forests, plantations or secondary environments) in order to better understand the role of such spaces in maintaining local biodiversity.

With this in mind, it appears preferable to use groups of species that are habitat and micro-habitat specific and which are not directly linked to richness of plant species alone, since information on such species is usually correlated with the flora.

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## ***CHAPTER 4***

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### ***TARGET INSECT GROUPS IN TEMPERATE FORESTS***



It should be remembered that the immense diversity of insects makes an exhaustive inventory impossible. It is for that reason that the Inv.Ent.for. working group proposes to focus mainly on five insect groups with varying levels of diversity: Coleoptera Carabidae, saproxylic Coleoptera, syrphid Diptera, Lepidoptera and red wood ants (Hymenoptera). The choice of these groups is based on criteria of diversity, ecological role and representativeness in the forest ecosystem and associated ecosystems (clearings, pre-forest heath, etc.) according to knowledge of their biology and taxonomy, and lastly the existence of a reliable and practical sampling method. This chapter describes each group and suggests the most useful methods for sampling them.

## I - COLEOPTERA CARABIDAE

(Christophe Bouget)

### *I.1 - Presentation of the group*

#### Taxonomic diversity

To date, over 40,000 species of Carabidae have been described (Lövei and Sunderland, 1996). Nearly 2,700 are present in Europe, making them the largest family of Coleoptera Adephaga and the third largest family of Coleoptera, after the Staphylinidae and Curculionidae. The carabid family has a little over 1,000 member species in France; for comparison, Italy has 1,292 (Minelli *et al.*, 1995). The following table illustrates this diversity at different geographical scales. As an example, at more local levels, we have trapped a hundred or so species in neutrocline to acidiline oak-hornbeam forest in Brie, thirty or so species by stand type on control sites and around fifteen in each study plot in Montargis forest (Bouget, 2001).

**Table 14: Some examples of the diversity of Coleoptera Carabidae at different spatial levels.**

Spatial level	Example	Species richness	References
Country	France	# 1000	
Region	Rhône-Alpes	549	Coulon <i>et al.</i> (2000)
	Alsace	352	Callot and Schott (1993)
Département	Indre-et-Loire	306	Cocquempot <i>et al.</i> (1997)
Forest	Fontainebleau State-owned forest	312	Cantonnet <i>et al.</i> (1997)
	Grésigne State-owned forest	158	Casset and Toda (2001) Rabil (1992)

The Carabidae thus offer fairly high levels of local taxonomic diversity and abundance.

#### Ecological diversity

The Carabidae occupy the majority of forest habitats: some are associated with wet forests, others with forest banks and paths. Others are linked to specific micro-habitats: tree stumps, mossy areas, the bases of large trees, etc.

Most are terricolous, but a number of groups are at least partly tree-dwelling (most Lebiinae, certain Platynini, Calosoma, *Carabus intricatus*, etc.). Depending on their relationship to temperature, humidity levels and light, plus their annual phenology (summer diapause, reproduction in spring or autumn), their specific habitat preferences vary. They may be nocturnal, diurnal, winged, brachypterous or apterous, and have a wide variety of lifestyles.

Most of these species are predators (with varying degrees of prey specialisation: springtails, annelids, gastropods, etc.), and certain genera (e.g. *Amara*, *Harpalus*) are at least partially seed and plant-eating.

### *I.2 - Interest*

The Carabidae (or carabids) are Coleoptera that vary in size, ranging from *Carabus* at 4cm to small Tachyiini a few millimetres long, but nevertheless with a fairly homogeneous and easily recognisable habitus. Their long legs allow them to run energetically along the ground and some species (Lebiinae most notably) will climb up plants; others can fly.

Coleoptera Carabidae are widely used as response indicators in numerous ecosystems in both hemispheres (New, 1998; Rainio and Niemelä, 2003). Their sensitivity to forest management, and especially tree felling, has been frequently studied (Niemelä, 1999).

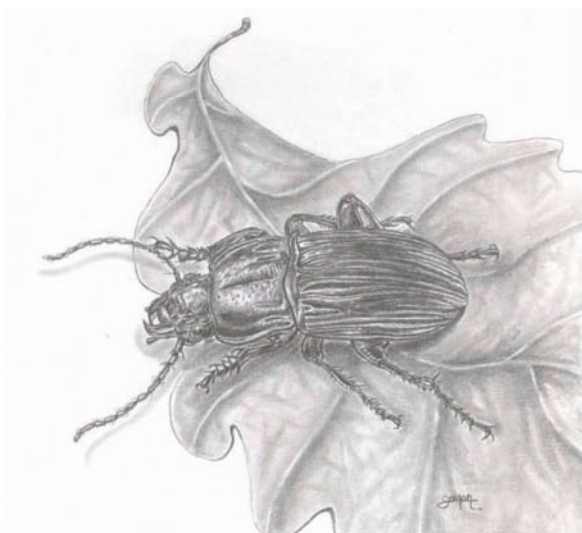
Among the epigeic arthropods (e.g. Formicidae, Araneids, Myriapoda), study of the carabids is justified by the following:

- Their taxonomy and ecology are relatively well known.
- They have a wide geographical distribution.
- They have a variable degree of habitat specialisation, ranging from eurytopic generalists to stenotopic specialists.
- Their responses reflect those of other groups, especially spiders and the other epigeic Coleoptera (Rushton *et al.* 1989; Niemelä *et al.*, 1996) as well as other Coleoptera (Scarabaeidae and Pselaphidae) (Oliver and Beattie, 1996). However, this assumption is in fact unreliable: in the Swiss agricultural environment, carabid diversity does not seem to be correlated with that of other groups (Duelli and Obrist, 1998).
- Their functional role in the forest ecosystem (see below).
- Sampling of carabids with pitfall traps is a tried and tested method and yields large captures (see section on 'pitfall traps').

### **Functional role in the forest ecosystem**

The Carabidae are numerically abundant in forest litter. When studying annual ground populations in a chestnut coppice, Yon (1983) counted 91 individual Carabidae per square metre, or 1.3% of the total population of epigeic arthropods, whereas the Araneids represented 3.5% and Chilopoda 8.6% of that same total. The three groups are equivalent in terms of biomass.

As predators in the main, the carabids are seen as auxiliary species in the forest environment, actively regulating phytophages and xylophages. They represent an intermediate trophic group predated by tertiary consumers (insectivore vertebrates) and secondary consumers of small invertebrates, and are in this respect representative of the network's upstream and downstream links.



**Figure 8: *Abax* sp. (drawing by G. Goujon).**

### **Available systematic resources**

The literature on identification of carabid species is fairly copious. Works on fauna are well supplemented by recent monograph articles on individual genera or species groups and enable french fauna to be identified. Identification is based in most cases on external morphology. However, for certain groups, extraction of the male (aedeagus) and female (spermatheca) genitalia is crucial.

A supplement updating Jeannel's work on French fauna (1941-1942) is currently being written by J. Coulon (*personal communication*). In many regions of France, amateur entomologists have made contributions on the Carabidae. In the majority of regional entomological societies there are entomo-

logists in possession of reference collections for this group. There are a small number of national experts and they can be contacted as a last resort.

### **Ease of trapping**

The carabids, whether they crawl or fly, can be caught using interception and attraction traps at ground level (pitfall traps) or in the air (window flight and light traps). Capture on sight in targeted micro-habitats is also effective but difficult to standardise.

The phenology of these species is spread over the whole year.

### ***I.3 - Sampling***

The pitfall trap is relatively effective for epigeic carabids that travel along the ground, but such traps will capture few species that are active fliers or move little on the ground. Window and pitfall traps provide extra information on assemblages of Carabidae and pitfall traps provide a very patchy representation of the carabid fauna circulating locally (cf. see insert below).

#### **Insert 11: Carabid sampling methods and assemblages**

As an example, the sampling of Carabidae in three hardwood forests in the Seine-et-Marne with 130 pitfall traps and 62 window flight traps (2 to 4 pitfall traps and 1 or 2 window traps per study plot), emptied monthly from April to September, yielded the following cumulative figures: a total of 93 species, 35 (37.5%) in both traps, 35 (37.5%) in window traps only and 23 (25%) in pitfall traps only. Light traps capture effectively species less commonly found in the Barber traps (notably *Ophonus*, *Bradycellus*, etc.).

#### **Choice of pitfall trap and preservative liquid** (cf. Table 15)

In our experience, it is best to use the following (cf. Chapter 2, Part III.1):

- Cylindrical receptacles of standard size in polythene (Unipak type) (diameter 85mm, height 110mm);
- Liquid: monopropylene glycol (MPG) 50% + 10% salt (rather than monoethylene glycol (MEG), which is more toxic, or MEG antifreeze sold in retail outlets, but which is too dilute (25%).

**Table 15: Preserving/attraction liquids for pitfall traps and their constraints in terms of sampling objectives.**

Objective	Liquid	Trap emptying frequency	Constraints asuable products
Quantitative (passive trapping: random interception)	Brine + detergent Water + detergent	7-15 days < 7 days	No
	MPG (or MEG) 50% + detergent + salt MPG (or MEG) 50% + detergent Vehicle antifreeze (MEG 25%) + detergent + salt	30 days 15 days 15 days	Yes
Quantitative to Qualitative?	Vinagar Beer/Vin + detergent Beer/wine + detergent + salt Formol 5 à 8 %	7-15 days < 7 days 7-15 days 15 days	No Yes

#### **Number of traps per study plot**

The minimum number of traps will depend on the objective and on the degree of heterogeneity in the environment. Insert 12 sets out some examples taken from the literature.

We recommend six traps per site (1 site = 1 study plot).

This figure, which can be divided by two or three, makes it possible to provide a balanced number of replications for each expression of a variable sub-factor on a given site, as 3 + 3 (e.g. 6 traps in a forest block, of which three will be near a tree stump and three distant from any tree stump) or 2 + 2 + 2 (unlike 5, which can be divided only by 5).



### **Insert 12: Number of traps and biodiversity estimation.**

In a forest block covering five hectares, Obrtel (1971) demonstrated the following:

- Five traps (at least 15m apart) suffice to capture half of all species trapped with the 25 pitfall traps initially placed, including the dominant species.
- Twenty traps are required to capture 90% of all species trapped with the aforementioned 25 traps.
- Between 10 and 12 traps suffice for an estimation of species abundance.

In the boreal forest environment, Niemelä *et al.* (1986) have shown that sampling limited to 15 traps 2-3m apart (i.e. not independent) yield a rarefied species richness very similar to trapping based on double the number (30) or three times the number of traps (45).

In a study of carabid species richness in ten wetland areas, Brose (2002) calculated non-parametric richness estimators and showed that the number of samples could be reduced to five pitfall traps per site for a minimum sampling programme without significant variation in the richness estimation.

Dauffy-Richard (2007) has shown that four pitfall traps per study plot constitute a highly inadequate sampling effort in young and open forest stands. These results are consistent with a study conducted earlier in an open environment that recommended a strict minimum of six traps for measurement of the species richness of pastures (Desender and Pollet, 1998).

### **Spatial arrangement of replications**

If the cumulative sample figures are to be informative, it is necessary to ensure that the replications/traps are mutually independent. This is so because where traps are too close together it is often the case that one of them will attract a large part of the local fauna, depleting the catch in the other traps.

### **Insert 13: How far apart should traps be?**

Above a certain trap density threshold, interactions between traps are observed, along with a loss of effectiveness for individual traps (Drach *et al.*, 1981). Comparative research has shown that it is possible to assume that traps are independent from 10 metres (Scheller, 1984, Niemelä *et al.*, 1986), or 15 metres (Obrtel, 1971) or even 50m (Digweed *et al.*, 1995). The capture variograms of Moore *et al.* (2002) suggest that traps placed at intervals of 15m are not independent. We have compared trap intervals of 14m and 50m (Bouget, 2001) and observed no difference in trap interaction.

An inter-trap distance greater than 20m appears reasonable.

The spatial arrangement of the traps will vary along with the objective:

- Regular array:
  - grids with square or hexagonal cells (1 trap at each apex),
  - linear transects (traps laid at a constant interval along a line),
  - groups: a triangle, a square or a circle of traps in each forest block.
- Irregular array: the traps are placed in proximity to landmarks in the environment that are considered to be preferred micro-habitats for the insects being studied (a tree stump, windfall, the base of large trees, patches of bryophytes, etc.).

### **Repetition in time**

#### **A year-related effect?**

Due to climatic accidents and species abundance cycles, years are rarely similar. As is true for any other faunistic method or assemblage, repetition will be necessary over several years in order to average out such variations.

#### Insert 14: Annual variations in carabid populations.

Many carabid studies are based on a single annual campaign. If the survey is repeated in a second year, the results are often highly correlated between micro-habitats (Antvogel and Bonn, 2001), or between types of forest (Niemelä *et al.*, 1992).

Judas *et al.* (2002) have shown that the spatial distribution of forest species did not change significantly over four years during the period studied. However, variations between years due to climatic or biotic causes (cyclical population dynamics) have been illustrated by several pieces of research:

- In Canadian forests certain forest carabids varied in abundance by a factor of 2 to 8 in the space of two years (Niemelä *et al.*, 1992);
- A population of *Carabus auronitens* in a German oak forest varied by a factor of 2 to 19 during a six-year study (Klenner, 1989).

#### Seasonal programming and simplification

It should be remembered that many carabids follow a cycle based around a summer diapause and a winter diapause and that there are insects that reproduce in spring and others in the autumn.

According to the inventory objective and available physical, human and financial resources, it is possible to opt for one of the two types of trapping described below:

- Continuous trapping ('year-catch')

To ensure that an inventory is exhaustive, this type of programming is to be preferred: insofar as it is feasible, the campaign should cover the period from April to October (with variations according to region) in order to include the period of activity of the majority of species.

- Discontinuous and targeted trapping

In order to economise on sampling, sampling effort can be focused on a small number of periods during the year. Trapping can thus be done in the spring and autumn. If just one season is chosen, spring (April-June) is to be preferred.

This approach permits insect immigration to regenerate local communities between trapping campaigns. However, there is a risk that the trapping will fall at unfavourable times for weather-related reasons and thus miss insect activity peaks, which are difficult to predict.

Decisions need to be taken on the duration of trapping and intervals between trap checks to reflect difficulties encountered in the field, the preserving capacity of the liquid and the probability of trap disturbance due to human activity or wild boars (cf. example below).

When studying changes in abundance levels over time, it must be borne in mind that observed variations may be due to elimination of fauna by the preceding campaign.

#### Insert 15: Repeated occasional trapping: some references.

Despite the studies of Niemelä *et al.* (1990), Rümer and Mühlenberg (1988) for example, continuous trapping ('year-catch'; Niemelä, 2000) was preferred to repeated occasional trapping, this method being very sensitive to insect phenology and changes in its timing due to weather conditions in any one year. The effectiveness of occasional trapping campaigns has been tested in the context of trials of minimum sampling programmes aimed at reducing the overall trapping campaign duration. With three traps per habitat (meadowland and forest) in May and June, Rümer and Mühlenberg (1988) captured 60% of the expected species. Niemelä *et al.* (1990) compared continuous trapping with trapping at the beginning and end of the season of activity for 5 + 5, 10 + 10 and 14 + 14 days. They concluded that the dominant species were sampled with sufficient accuracy with 10 + 10 days. Most of the species unrepresented in this discontinuous programme were rare in the continuous programme (< 10 individuals).

#### *I.4 - In the field*

##### Trap installation precautions

Choice of site is important: traps should be set up close to important micro-habitats, slopes and hollows should be avoided (and thereby flooding of the collection jar by water run-off).

Installation: dig a cylindrical hole using a pedological auger or a planting trowel, insert the jar and make sure it is perfectly flush with the ground surface by tamping soil into the gap between the surrounding soil and the edge of the jar.

Where the soil is hard, the hole can be kept in good working order by leaving a double receptacle open at both ends permanently in the ground and inserting the trapping receptacle.

Trap installation will lead to an initial disturbance effect (Digweed *et al.*, 1995): see Chap. 2, Part III-1 for practical recommendations and precautions.

### **Collection protocol**

(cf. also Chapter 4)

We propose the following protocol for emptying the trap and bagging the samples:

- Filter the trap through a fine-mesh strainer in the field (and recover the used liquid if it is toxic).
- Store the content dry in a ziplock bag (e.g. Mini-grip® freezer bags) labelled and placed in a freezer. The label must specify the date and stand and trap numbers in an accurate and durable manner (take care to ensure that the ink is long-lasting).
- The preserving fluid should be renewed each time the trap is emptied.

It is not advisable to do any preliminary sorting of the trap contents at the site given that small species may be hidden in the waste or caught up in slug mucus for example.

<b>PROPOSALS BY INV.ENT.FOR.</b>		
	<b>Objective 1: Qualitative (Inventory of fauna)</b>	<b>Objective 2: Comparative</b>
NUMBER OF SITES	N/A	5 to 10 per context
NUMBER OF TRAPS	6 per site	2 to 5 depending on number of sites
DISTANCE BETWEEN TRAPS	20m	20m
PERIOD	March to October	Intensive: March to October Extensive: April-May and September
FREQUENCY	15 to 30 days*	15 to 30 days*
PRESERVING FLUID	*	*

\* If it is possible to recover the liquid after use, use a mix of MPG 50% + water 50% + 10% salt by weight; frequency of trap emptying: up to 30 days.  
If reuse is not possible, use saturated brine or a 4% solution of copper sulphate: water + 10% salt; frequency of trap emptying: 7-15 days.

### Insert 16: Handbooks for the identification of Coleoptera Carabidae.

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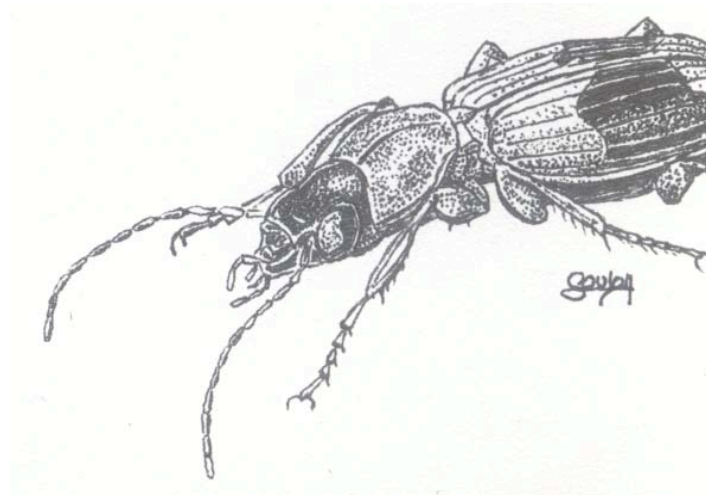
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**To find out more:**

Some websites devoted to Coleoptera Carabidae:

- *Carabus* photo gallery
  - <http://volny.cz/midge/carabus/carabus.htm>
- German society for applied carabidology (*Gesellschaft für angewandte Carabidologie*)
  - <http://www.carabidae.de/> ou <http://www.laufkaefer.de/>
- Alsatian Coleoptera Carabidae
  - <http://claude.schott.free.fr/Carabidae/Carabus/Carabidae-liste-planches.html>
- Ground beetles of Ireland
  - <http://www.habitas.org.uk/groundbeetles/>
- Illustrated key to Carabidae in Germany, designed by Arved Lompe (text in German)
  - <http://coleo-net.de/coleo/texte/carabidae.htm>



**Figure 9: *Badister* sp. (drawing by G. Goujon).**



## II - SAPROXYLIC COLEOPTERA

(Christophe Bouget and Hervé Brustel)

### II.1 - Presentation of the group

Dead wood is a key component of the forest habitat in providing a trophic or spatial resource for saproxylic organisms, which “depend during part of their life cycle on dead or dying wood from dying or dead trees, still standing or fallen, or wood fungi, or the presence of other saproxylic organisms” (Speight, 1989).

Saproxylic organisms thus depend on a broad gradient of micro-habitats and trophic resources provided by dead wood and old trees: windfall, snags and standing dead trees, windthrow and raw wood fragments on the ground, dead branches in living crowns (primary branches), tree stumps, micro-habitats associated with old trees: areas of necrosis, decay, cavities, bark fungus carpophores, dripping sap, etc.



Figure 10: Cerambycidae, tree snag and bracket fungus (drawing by G. Goujon).

### Importance and taxonomic diversity

The saproxylic cortège represents between 20% and 25% of all forest species (taking flora and fauna together) and is dominated by fungi (30%) and Coleoptera (20% of species) (Stokland *et al.*, 2004). In France, it appears that 20% of all Coleoptera species belong to the saproxylic cortège (over 2,300 species, or over half of all forest Coleoptera (Bouget *et al.*, 2008).

Although several families are emblematic of this group (Lucanidae, Cerambycidae, Buprestidae, Curculionidae Scolytinae), in fact nearly 71 families of Coleoptera include at least one saproxylic species (Table 5).

However, a large number of those families are difficult to access for the non-specialist entomologist.

### Ecological diversity

Saproxylic Coleoptera show great ecological diversity at the larval and adult stages. At the trophic level their diet may be of the following types: (i) primary xylophage, in the case of species developing on healthy living wood, (ii) secondary xylophage: species developing on living wood that is dying or freshly dead wood, (iii) xylomycetophage: the taxa of epicortical carpophores (e.g. bracket fungus), (iv) xylomycophage: living off subcortical myceliums, (v) zoophage: predators active in xylophage tunnels or under bark, (vi) saprophage: detritiphages and microphages inhabiting tunnels and consum-



ing exuviae and sundry organic residue, (vii) opophage: small groups associated with sap runs from damaged trees (Bouget *et al.*, 2005).

At the imago stage numerous species live outside the dead wood, feeding little if at all and living off the reserves of fat stored by the larvae, or seeking out sources of carbohydrates to meet their energy needs (sap, flower nectar) and pollen for egg maturation.

In addition to their great taxonomic and ecological diversity, Coleoptera predominate in the saproxylic invertebrate biomass (up to 95%; Dajoz, 1966).

**Table 16: Coleoptera families comprising at least one saproxylic species (order classification according to Lawrence and Newton, 1995).**

<b>ADEPHAGA Schellenberg, 1806</b>	CUCUJIFORMIA Lameere, 1938
CARABOIDEA Latreille, 1802	LYMEXYLOIDEA Fleming, 1821
RHYSODIDAE Laporte, 1840	LYMEXYLIDAE Fleming, 1821
CARABIDAE Latreille, 1802	CLEROIDEA Latreille, 1802
<b>POLYPHAGA Emery, 1886</b>	PHLOIOPHILIDAE Kiesenwetter, 1863
STAPHYLINIFORMIA Lameere, 1900	TROGOSITIDAE F., 1801
HYDROPHILOIDEA Latreille, 1802	CLERIDAE Latreille, 1802
SPHAERITIDAE Shuckard, 1839	ACANTHOCNEMIDAE Crowson, 1964
HISTERIDAE Gyllenhal, 1808	MELYRIDAE Leach, 1815
STAPHYLINOIDEA Latreille, 1802	CUCUJOIDEA Latreille, 1802
PTILIIDAE Erichson, 1845/Motschulsky, 1845	SPHINDIDAE Jacquelin du Val, 1860
LEIODIDAE Fleming, 1821	NITIDULIDAE Latreille, 1802
SCYDMAENIDAE Leach, 1815	MONOTOMIDAE Laporte, 1840
STAPHYLINIDAE Latreille, 1802	PHLOEOSTICHIDAE Reitter, 1911
SCARABAEIFORMIA Crowson, 1960	SILVANIDAE Kirby, 1837
SCARABEOIDEA Latreille, 1802	CUCUJIDAE Latreille, 1802
LUCANIDAE Latreille, 1806	LAEMOPHLOEIDAE Ganglbauer, 1899
TROGIDAE MacLeay, 1819	CRYPTOPHAGIDAE Kirby, 1837
SCARABAEIDAE Latreille, 1802	LANGURIIDAE Crotch, 1873
ELATERIFORMIA Crowson, 1960	EROTYLIDAE Latreille, 1802
SCIRTOIDEA Fleming, 1821	BIPHYLIDAE LeConte, 1861
EUCINETIDAE Lacordaire, 1857	BOTHRIDERIDAE Erichson, 1845
CLAMBIDAE Fischer, 1821	CERYLONIDAE Billberg, 1820
SCIRTIDAE Fleming, 1821	ALEXIIDAE Imhoff, 1856
DASCILLOIDEA Guérin-Méneville, 1843 (1834)	ENDOMYCHIDAE Leach, 1815
BUPRESTOIDEA Leach, 1815	CORYLOPHIDAE LeConte, 1852
BUPRESTIDAE Leach, 1815	LATRIDIIDAE Erichson, 1842
BYRRHOIDEA Latreille, 1806	TENEBRIONOIDEA Latreille, 1802
ELMIDAE Curtis, 1830	MYCETOPHAGIDAE Leach, 1815
DRYOPIDAE Billberg, 1820 (1817)	CIIDAE Leach in Samouelle, 1819
ELATEROIDEA Leach, 1815	TETRATOMIDAE Billberg, 1820
CEROPHYTIDAE Latreille, 1834	MELANDRYIDAE Leach, 1815
EUCNEMIDAE Eschscholtz, 1829	MORDELLIDAE Latreille, 1802
THROSCIDAE Laporte, 1840	ZOPHERIDAE Solier, 1834
ELATERIDAE Leach, 1815	TENEBRIONIDAE Latreille, 1802
LYCIDAE Laporte, 1836	PROSTOMIDAE C.G. Thomson, 1859
CANTHARIDAE Imhoff, 1856 (1815)	OEDEMERIDAE Latreille, 1810
BOSTRICHIFORMIA Forbes, 1926	STENOTRACHELIDAE C.G. Thomson, 1859
DERODONTOIDEA LeConte, 1861	PYTHIDAE Solier, 1834
DERODONTIDAE LeConte, 1861	PYROCHROIDAE Latreille, 1807
BOSTRICOIDEA Latreille, 1802	SALPINGIDAE Leach, 1815
NOSODENDRIDAE Erichson, 1846	ADERIDAE Winkler, 1927
DERMESTIDAE Latreille, 1804	SCRAPTIIDAE Mulsant, 1856/Gistel, 1856
ENDECATOMIDAE LeConte, 1861	CHRYSOMELOIDEA Latreille, 1802
BOSTRICHIDAE Latreille, 1802	CERAMBYCIDAE Latreille, 1802
ANOBIIDAE Fleming, 1821	CURCULIONOIDEA Latreille, 1802
	ANTHRIBIDAE Billberg, 1820
	BRENTIDAE Billberg, 1820
	CURCULIONIDAE Latreille, 1802

NB: The reorganisation by subfamily in this classification masks former families such as the Scolytidae (Curculionidae), Ptinidae (Anabiidae) and Lyctidae (Bostrichidae).

## II.2 - Interest

### The functional role of saproxylic organisms

Saproxylic organisms recycle nutrients and play a direct part in maintaining the fertility of forest soil. It is estimated that in a natural forest one third of the minerals released in the upper levels of the soil come from the activity of saproxylic species (Swift, 1977). Certain cavicolous saproxylophages are also able, by means of endosymbiotes that fix atmospheric nitrogen, to enrich the surrounding substrate (Jönsson *et al.*, 2004). All the by-products of consumption by saproxylic species are reused in the next tree growth cycle (e.g. decomposing wood providing a basis for the regeneration of mountain forests) (Vallauri, 2005).

Within a diversified saproxylic cortege, predators and parasitoids regulate pest populations.

### A group under threat

As early as 1988, the Council of Europe recommended that national governments should “consider the desirability of making a survey of saproxylic organisms when assessing the quality of forests for nature conservation purposes” (Recommendations R (88) 10 and 11, Committee of Ministers).

Insect species dependent on dead wood seem to have suffered significant losses over the last few millennia. Five thousand years of human activity and several centuries of forest management have had the following main effects: fragmentation of previously continuous forests, reductions in ancient forest areas, diversity of tree species and volumes of dead wood, increases in the area occupied by even-aged stands, and changes in the dynamics of natural disturbance (Esseen *et al.*, 1997).

A large number of species are on lists of insects under threat of extinction in various European countries. Twenty per cent of species of saproxylic Coleoptera are endangered in Finland (Berg *et al.*, 1994), 35% in Germany (Köhler, 2000); 17 species of saproxylic Coleoptera are said to have become extinct between 4900 BP and the present day in the United Kingdom (Buckland and Dinnin, 1993) due to anthropogenic degradation of forest habitats. And seventeen species of saproxylic Coleoptera have disappeared from Finland’s forests since 1800 (Martikainen, 2003).

### **Insert 17: Saproxylic Coleoptera species with conservation status.**

Seven species of saproxylic Coleoptera in France are listed in Annex II or IV of the European Habitat Directive: *Rosalia alpina*, *Osmoderma eremita*, *Limoniscus violaceus*, *Lucanus cervus*, *Cerambyx cerdo*, *Stephanopachys linearis*, *Stephanopachys substriatus*, *Phryganophilus ruficollis* and *Rhysodes sulcatus*.

A large number of species are on regional lists of species of determining importance for ZNIEFF status.

Brustel (2004) has set in train work to characterise a heritage index for each species in order to assist assessment of the conservation status of French forests.

## II.3 - Sampling

### Choice of method

Depending on the skills available, an approach to saproxylic Coleoptera can be defined at three levels:

- An approach limited to a diversified, accessible family: Cerambycids.  
Methods: Window flight traps and beer traps supplemented by beating, capture on sight and emergence enclosures for colonised wood (qualitative inventory).
- An extended approach including a small number of dominant families for which identification handbooks are available: Cerambycidae, Scolytidae, Buprestidae, Lucanidae, Scarabaeidae, Elateridae, Cleridae.  
Methods open to standardisation: window flight and beer traps.
- An exhaustive approach covering all families of saproxylic Coleoptera.  
Method open to standardisation: window flight trap supplemented by emergence traps.

Studies of saproxylic fauna are easier to standardise where the mobile component is concerned if window flight traps are used. In order to be able to associate saproxylic species with their micro-habitats, it is necessary to sample the emerging fauna in micro-habitats using emergence traps.

On a number of practical grounds (smaller volume of preserving fluid, greater robustness, reduced dimensions, quick set-up and easier transportation when broken down into separate parts, cross-vane window flight traps, and especially the standard Polytrap™ (Brustel, 2004), are to be preferred to single-vane window flight traps.

These traps should be suspended from a natural support (e.g. a branch in the tree crown) at head height, a position that can be adjusted to suit the density of the sampled stratum and the risks of disturbance by game animals.

### **Trapping duration and period**

Sampling campaigns from April to September will cover the major part of the period of activity of saproxylic Coleoptera (Brustel, 2004; Wermelinger *et al.*, 2002). As an example, in the case of one of the dominant groups in the saproxylic cortège, the Rhizophaginae, Thieren *et al.* (2003) have demonstrated that 93% of the 1,098 individual specimens captured from May to October had been caught before the beginning of August. For the group as a whole, seasonal capture profiles show species richness and abundance declining rapidly after extreme values in early June to mid-July, according to site and year, in both lowlands and uplands (Insert 18). However, a few species, particularly the mycetophiles, are associated with the end of the biological season (Brustel, 2004).

### **Spatial disposition**

- If the objective is to make comparisons between sites:

We recommend setting up two traps per study plot separated by a distance sufficient to ensure independent replication (20m, in the absence of experimental results on this). In addition we also advise that:

- traps should be placed on the different sites in similar conditions (height, exposure, stand density),
- the use of chemical lures should be avoided for comparisons of open environments and closed environments,
- similar devices should be used on the various sites (colour, shape, area),
- a pair of traps will cover heterogeneity in the study plot more effectively, as well as reducing the risk of a total failure to gather data due to trap malfunction.

For information, in an ecological study in Rambouillet forest (Yvelines, France) with 60 study plots each with two traps, we showed that:

- on average, a trap will cover **69.8% (± 2.9%)** of the species richness contributed by both traps together;
- **63.6 (± 6.1%)** of the species are present in just one of the two traps on the study plot.

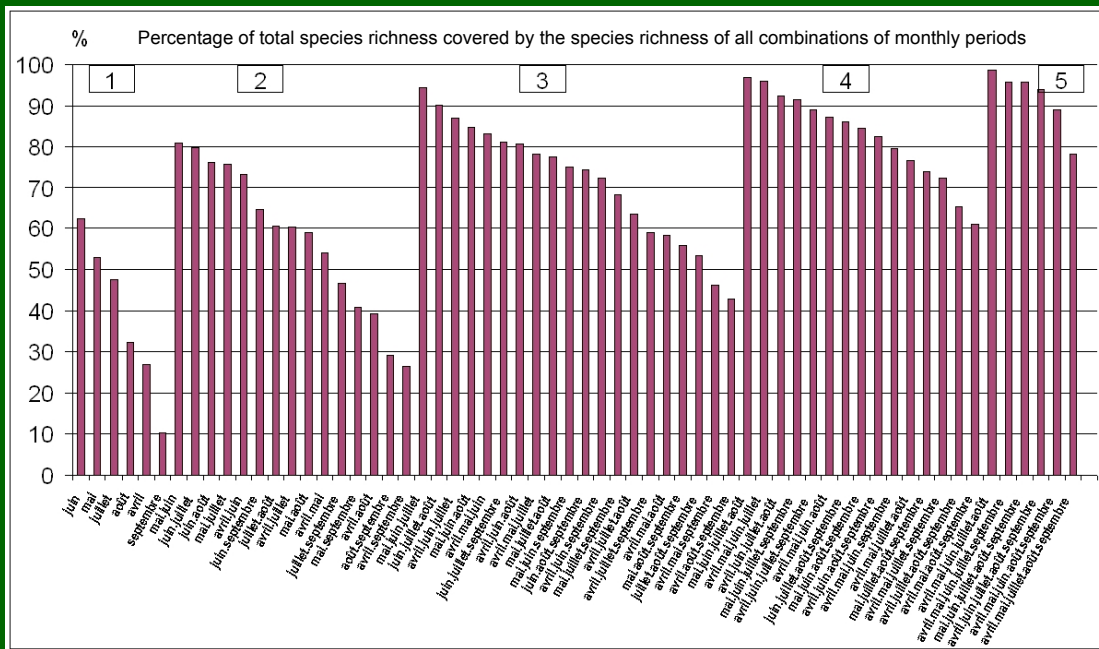
- Where the aim is to build an inventory of a site (forest block, reserve):

In order to cover the environmental gradient across the whole of the site and given that it is costly to use continuous trapping on all *N* micro-sites representative of the heterogeneity of the site, a compromise is possible whereby a rotation of *n* traps ( $n < N$ ) is established between periods and between different situations representative of the heterogeneity of the site. Where comparison is not the aim, it is recommended to go further and apply traps only to the extreme situations, installing traps where there is certainty of capture (e.g. local concentrations of dead wood, micro-clearings in closed stands, on snags, under windthrow), the whole being accompanied by multiyear repetitions.

## Insert 18: Capture seasonality and optimisation of trap inspection schedules

In the context of a report linked to technical support provided by Cemagref to ONF (Bouget, 2008), we studied the basic contribution to species richness of each trapping campaign and combination of campaigns in relation to the totality of the sampling season. For this analysis, thirteen data sets relating to monthly sampling from lowland hardwood forests (e.g. Rambouillet, Brie, Tronçais) or highland forests (Pyrenees, etc.) were compiled.

As the graph below shows, the best scores come from continuous trapping (rather than trapping by discontinuous periods), with a central focus on the period of maximum activity (June). Four or five monthly campaigns do not make a significantly greater contribution than three. Of the combinations of three monthly campaigns, May-June-July is on average the best combination, ahead of June-July-August, April-June-July and May-June-August.



The following table contains a summary of the results:

Number of monthly campaigns	Best score	% of total species richness obtained	Number of data sets available for analysis
1 campaign	June	62%	9
2 campaigns	May-June	81%	6
3 campaigns	May-June-July	94%	6
4 campaigns	April-May-June-July = May-June-July-August	96%	3 4
5 campaigns	April-May-June-July- August	98%	2

The advantages of multiyear replication of a programme can be discussed in this context.

Martikainen and Kaila (2004) have shown that over 75% of common species captured over a whole period of ten years of trapping were already detected after three years. On the other hand, the detection of 'rare' species is much slower. For rare species, the similarity of the faunistic composition in two consecutive years of trapping is less than 40%, whereas the figure is near 70% for common species.

Using a very limited French data set (three years' monitoring using study plots in the Eastern Pyrenees), our assessment was that a second and third year of sampling contributed gains in species richness of 50% and 100% respectively compared with the first year. In other words, only 50% of the number of species captured after three years were detected by the end of the first year.

### II.4 - In the field

The traps should be suspended from a natural support (e.g. branch in the crown) at head height, a position that should be adjusted in accordance with obstructions or their absence in the stratum to be sampled and the risks of disturbance by game animals (if this is the case, hang the trap higher).

The collection fluid (e.g. brine) should be prepared the day before each time the trap is visited in order to ensure that the salt has dissolved before the trap is set again. The contents of the bottle can be recovered using a household strainer with a fine plastic mesh. The insects can be stored in a bag (e.g.

Mini-grip® freezer bags) that should be numbered (according to the location) and dated using a paper label; one bag should be used for each ‘trapping method’.

Any lures used should be recharged and the traps set once again. The samples should be kept in cool conditions while awaiting dispatch (several days) or deep-frozen while awaiting processing. Freeze-thaw-refreeze cycles cause fewer problems than physical shocks to deep-frozen material.

Captures must be processed in the laboratory.

### II.5 - Laboratory work: sorting and identification

(cf. Chapter 4)

Coleoptera should be identified by family and then by species for a proportion of families that will depend on the skill level of the team and its network of specialists.

PROPOSALS BY INV.ENT.FOR			
Level of approach:	Approach 1 (Minimalist)	Approach 2 (Extended)	Approach 3 (Exhaustive)
Target group	Cerambycidae	Cerambycidae, Scolytidae, Buprestidae, Lucanidae, Scarabaeidae, Cleridae, Elateridae	All families
Methods	Beating, capture on sight, window flight, beer, emergence traps	1 Window flight trap per site 2 beer traps per site	2 window traps per site, emergence traps

#### Insert 19: Some key references for identification of the main families of saproxylic Coleoptera.

We recommend that works of vulgarisation not be used for identification (use as simple aide-mémoires or as rough guides to narrow the choice down for more suitable publications).

Coleoptera families can be identified using:

- Delvare, G., Aberlenc, H.P., 1989. Les Insectes d’Afrique et d’Amérique Tropicale. - Clés pour la reconnaissance des Familles. C.I.R.A.D., Prifas, Acridologie opérationnelle - Ecoforce internationale, 298 p.
- Unwin, D.M., 1984. A Key to the Families of British Beetles. Publisher Field Studies Council (FSC), Volume 66, 197 p.
- Hürka K., 2005. Brouci České a Slovenské republiky. Beetles of the Czech and Slovak Republics. Editions Kabourek, 70 photographic plates, 390 p.

Although out-dated in terms of the number of taxa, the four Portevin volumes still constitute a basic reference for the study of French Coleoptera. Although the paper edition is now virtually impossible to find, scanned versions (pdf files) are circulating in the entomological microcosm, as is true of many other ‘impossible to find’ reference works.

- PORTEVIN, G., 1929. Histoire Naturelle des Coléoptères de France. Volume I - Adephaga, Polyphaga: Staphylinoidea. *Lechevalier, P., Paris*, 649 p.
- PORTEVIN, G., 1931. Histoire Naturelle des Coléoptères de France. Volume II - Polyphaga: Lamellicornia, Palpicornia, Diversicornia. *Lechevalier, P., Paris*, 542 p.
- PORTEVIN, G., 1934. Histoire Naturelle des Coléoptères de France. Volume III - Polyphaga: Heteromera, Phytophaga. *Lechevalier, P., Paris*, 374 p.
- PORTEVIN, G., 1935. Histoire Naturelle des Coléoptères de France. Volume IV - Polyphaga: Rhyncho-phora. *Lechevalier, P., Paris*, 500 p.

Another series of key publications is the *Die Käfer Mitteleuropa* (or ‘DKM’) collection, now updated but with some gaps, particularly where Mediterranean taxa are concerned. The *Handbooks for the Identification of British insects* are easy to use but notoriously incomplete when it comes to many families of French fauna.

- FREUDE, F., HARDE, K.W., LOHSE, G.A., 1979. Die Käfer Mitteleuropa - Volume 6 - Diversicornia. *Goecke and Evers, Krefeld*. 366 p.
- FREUDE, F., HARDE, K.W., LOHSE, G.A., 1967. Die Käfer Mitteleuropa - Volume 7 - Clavicornia. *Goecke and Evers, Krefeld*. 310 p.
- FREUDE, F., HARDE, K.W., LOHSE, G.A., 1969. Die Käfer Mitteleuropa - Volume 8 - Terebrantia, Heteromera, Lamellicornia. *Goecke and Evers, Krefeld*. 388 p.
- LOHSE, G.A., LUCHT, W.H., 1992. Die Käfer Mitteleuropa - Band 13 - 2. Supplement with catalogue section. *Goecke and Evers, Krefeld*. 375 p.

**Table 17: The principal families of saproxylic Coleoptera, some of their characteristics and their references for identification.**

Family	No. saproxylic species			Total no. species	Issues		Difficulty	Major reference work
	No	Yes	?		Heritage	Frequency		
ACANTHOCNEMIDAE		1	1	1	+	-	?	Alonso-Zarazaga <i>et al.</i> , 2003
ADERIDAE		13	13	13	+/-	+/-	+/-♂+♀	Gompel and Barrau, 2002
ALEXIIDAE		4	4	4	?	-	+/-	Portevin, 1931; DKM
ANOBIIDAE		121	2	123	+/-	+	+/-♂+♀	Laclos and Buche (2008-2009)
ANOBIIDAE PTININAE		14	42	56	+/-	+	+	Belles, 1990, 1996, 2002
ANTHRIBIDAE	1	28		29	+	+/-	+/-	Hoffman, 1945
BIPHYLIDAE		3		3	+	+/-	-	Portevin, 1931
BOSTRICHIDAE		18		18	+	+/-	+/-	Lesne, 1901 to 1906
ENDECATOMIDAE		1		1	+	-	+/-	Portevin, 1931
BOSTRICHIDAE LYCTINAE		8		8	-	-	+	Portevin, 1931
BOTHRIDERIDAE		17		17	+	-	+/-	Dajoz, 1977
BRENTIDAE		1		1	+	-	-	Portevin, 1935
BUPRESTIDAE	1	89	7	97	+	+	+/-	Schaefer, 1949, 1955, 1983; Verdugo, 2005
BUPRESTIDAE Agrilinae	27	44	1	72	+/-	+	+	Farrugia, 2007
CANTHARIDAE Malthininae		55	1	56	?	+	+	Portevin, 1931; DKM
CARABIDAE Trechinae		1		1	-	-	+/-	Cf. chapter on Carabidae
CERAMBYCIDAE	33	206	3	242	+	+	-	Villiers, 1978; Bense, 1995
CEROPHYTIDAE		1		1	+	-	-	Portevin, 1931
CERYLONIDAE		8	3	11	+/-	+	+	Dajoz, 1976
CIIDAE		44		44	?	+	+	Portevin, 1931
CLAMBIDAE		2		2	?	-	+	Portevin, 1929
CLERIDAE	13	20		33	+	+	+/-	Gerstmeier, 1998
CORYLOPHIDAE		14	1	15	?	+/-	+	Bowstead, 1999
CRYPTOPHAGIDAE	3	78	42	123	?	+	+	Falcoz, 1929; DKM
CUCUJIDAE		3		3	+	-	+/-	Portevin, 1931
CURCULIONIDAE	41	117	4	162	+	+	+	Hoffmann, 1950, 1954, 1958; Tempere <i>et al.</i> , 1989
CURC. Platypodinae		2		2	+	+	-	Portevin, 1935
CURC. Scolytinae	15	136	3	154	+/-	+	+	Balachowsky, 1949
DERMESTIDAE	0	25	37	62	+/-	+	+	Portevin, 1931; DKM
DERODONTIDAE		2		2	+	-	-	Portevin, 1931; DKM
DRYOPIDAE		17		17	?	-	?	Portevin, 1931; DKM
ELMIDAE		1		1	?	-	?	Portevin, 1929; DKM
ELATERIDAE	148	69	10	227	+	+	+	Leseigneur, 1998
ENDOMYCHIDAE		11	10	21	+	+/-	+	Portevin, 1931
EROTYLIDAE		15		15	+	+/-	+/-	Portevin, 1931; Dajoz, 1985
EUCINETIDAE		2		2	+/-	-	+/-	Portevin, 1931
EUCNEMIDAE		24		24	+	+	+/-	Leseigneur, 1978; Barthe, 1928.
HISTERIDAE	94	41	12	147	+	+	+	Vienna, 1980; Yelamos, 2002
LAEMOPHLOEIDAE		23		23	+	+	+	DKM; Lechanteur, 1994
LANGURIIDAE		1	4	5	?	-	+/-	DKM
LATRIDIIDAE			95	95	?	+	+	Bouget and Vincent, 2008; Rucker sous presse
LEIODIDAE Cholevinae	182	4		186	?	+	+	Portevin, 1929; DKM
LEIODIDAE Leiodinae	60	20		80	+	+	+	Portevin, 1929; DKM
LUCANIDAE		11		11	+	+	-	Paulian and Baraud, 1982
LYCIDAE		8		8	+	-	+/-	Allemand and Brustel, 2005
LYMEXYLIDAE		2		2	-	+	-	Portevin, 1931
MELANDRYIDAE		38		38	+	+/-	+/-	Houlbert and Barthe, 1935
MELYRIDAE Dasytinae		66		66	?	+	+	Constantin, 2007, 2008;

								Liberti, 2004
MELYRIDAE Malachiinae		7	73	80	?	+/-	+	Portevin, 1931; Plata and Santiago, 1990; DKM
MONOTOMIDAE Monotominae	9	4		13	+	-	+	Peacock, 1977; DKM
MONOT. Rhizophaginae		15		15	+	+	+	Bouget and Moncoutier, 2003
MORDELLIDAE	4	8	71	83	+	+	+	DKM
MYCETOPHAGIDAE		22		22	+	+	+/-	Portevin, 1934; Rogé, 1992; Bouyon and Vincent, 2003
NITIDULIDAE	10	69	2	83	+	+	+	Audisio, 1993
NOSODENDRIDAE		1		1	+	-	-	Portevin, 1931
OEDEMERIDAE		39		39	+	+	+/-	Vazquez, 2002
PHLOEOSTICHIDAE		1		1	+	-	-	Portevin, 1931
PHLOIOPHILIDAE		1		1	+	-	-	Portevin, 1931
PROSTOMIDAE		1		1	+	-	-	Portevin, 1931
PTILIIDAE		78		78	?	-	+	Portevin, 1929; DKM
PYROCHROIDAE Agnathinae		1		1	+	-	-	Portevin, 1934
PYRO. Pyrochroinae		3		3	+	+/-	-	Portevin, 1934
PYTHIDAE		1		1	+	+/-	-	Portevin, 1934
RHYSODIDAE		2		2	+	-	-	Dajoz, 1975
SALPINGIDAE Agleninae			1	1	-	-	-	Dajoz, 1977
SALPINGIDAE Salpinginae		17		17	+	+	+	Iablokoff, 1985; DKM
SCARABAEIDAE Cetoniinae	2	16	4	22	+	+	-	Paulian and Baraud, 1982
SCARAB. Dynastinae		7		7	+	-	-	Paulian and Baraud, 1982
SCIRTIDAE	26	1		27	?	+/-	+	Portevin, 1931; DKM
SCRAPTIIDAE		34		34	?	+	+	Portevin, 1934; DKM
SCYDMAENIDAE	113	13		126	?	+/-	+	Portevin, 1929; DKM
SILVANIDAE	11	5	1	17	+	+	+/-	Portevin, 1931
SPHAERITIDAE		1		1	+	-	-	Portevin, 1929
SPHINDIDAE		4		4	+	+/-	+/-	Freeman <i>et al.</i> , 2003
STAPHYLINIDAE	581	248	716	1545	?	+	+	Coiffait, 1972 to 1984; DKM
STAPH. Pselaphinae	263	50		313	?	+	+	Jeannel, 1950; DKM
STENOTRACHELIDAE		2		2	?	-	?	DKM
TENEBRIONIDAE	55	63	4	122	+	+	+	Portevin, 1934
TENEBR. ALLECULINAE		27		27	+	+	+	Portevin, 1934
TETRATOMIDAE		4		4	+	+/-	-	Portevin, 1934
THROSCIDAE	13	2		15	+	+	+	Leseigneur, 1996, 1997, 2005; VanMeer, 1998
TROGIDAE	8	2		10	+	-	+/-	Paulian and Baraud, 1982
TROGOSSITIDAE	1	11		12	+	+/-	+/-	Portevin, 1931
ZOPHERIDAE (COLYDIDAE)		22	7	29	+	+	+	Dajoz, 1977
Total	1732	2193	1174	5083				

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**Vallauri, D., 2005.** Le bois dit mort, une lacune des forêts en France et en Europe. In *Bois mort et à cavités. Une clé pour des forêts vivantes* (ed. D. Vallauri, J. André *et al.* ), pp. 9-17. Chambéry, France: Lavoisier, Tec et Doc.

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### **To find out more:**

- Databases on the ecology of saproxylic Coleoptera:
  - <http://frisbee.nogent.cemagref.fr/fr/frisbee/accueilFr>
  - <http://www.saproxylic.org/>
- Collections of photographs:
  - Nitidulidae: <http://www.koehleroptera.de/gallery2/nitidulidae/nitidulidae.html>
  - Buprestidae: <http://www.volny.cz/midge/buprang/jewelbeetles.htm>
  - Buprestidae: <http://utenti.romascuola.net/bups/jewel.htm>
  - Elateridae: <http://www.elateridae.com/>
  - Cerambycidae: <http://www.uochb.cas.cz/~natur/cerambyx/cerambyx.htm>
  - Laemophloeidae: <http://fsca.entomology.museum/Coleoptera/Mike/LaemophloeidaeLink.html>
  - Miscellaneous families: <http://www.koleopterologie.de/gallery/index.html>
  - Miscellaneous families: <http://www.hlasek.com/ccbrouci1an.html>
  - Miscellaneous families: [http://www.aegaweb.com/fot\\_map/](http://www.aegaweb.com/fot_map/)
  - Miscellaneous families: <http://culex.biol.uni.wroc.pl/cassidae/Colpolon/lista%20rodzin.htm>



**Photo 22: Spotted longhorn beetle (*Leptura maculata*, Cerambycidae).**



### III – LEPIDOPTERA

(Philippe Bonneil)

#### III.1 - Presentation of the group

Lepidoptera are the second largest insect order after Coleoptera in terms of taxonomic diversity (between 150,000 and 500,000 species, approximately, in the world, of which more than 5,000 can be found in France and Europe; Chinery and Cuisin, 1994; Heppner, 1998; Solis and Pogue, 1999). These insects, described etymologically as having ‘scaly’ wings, are probably the most popular and familiar for the non-specialist public due to their beauty and elegance. They are conventionally divided into two groups, Rhopalocera being the best known. A distinction is made between the latter and the Heterocera on the basis of the club-like termination of their antennae (the latter being the origin of the names given to the groups) and their daytime lifestyle, whereas the second group has antennae in a variety of shapes and are usually active at night (although several species are active in the day). The taxonomic diversity of the Heterocera is however very much greater than that of the Rhopalocera (over 95% of the total number of species) and the diet of many Heterocera associates them with forest ligneous or herbaceous species.



Photo 23: A butterfly: the Black-Veined White (*Aporia crataegi*, Pieridae).



Photo 24: A moth: the Nun Moth (*Lymantria monacha*, Lymantriidae).

Although the Rhopalocera also include a very large number of species that live in a forest habitat, relatively few are associated with trees and bushes. The Heterocera, less well known, are also an extremely interesting group to study in the forest context.

The Lepidoptera are also generally classified in two groups (on arbitrary, non-phylogenetic grounds):

- The micro-Lepidoptera, which include only families of Heterocera of which the majority are small in size (e.g. Tortricidae, Crambidae, Micropterigidae) or, alternatively, large (Cossidae, Hepialidae, Limacodidae);
- And the macro-Lepidoptera, with families of larger species (Heterocera Geometridae, Noctuidae, Notodontidae, etc. and the Rhopalocera).

#### III.2 - Interest

##### Diversity

Very great diversity is encountered in forests: for example, 1,638 species have been counted in Fontainebleau forest, more than 1,400 of which are Heterocera (Gibeaux, 1999).

##### A major role in the forest ecosystem

The abundance of Lepidoptera in forests is colossal: one hectare of Polish oak forest will contain between two and eight million caterpillars according to Witkowski *et al.* (1992). These phytophagous insects play a major role in the plant population dynamic, the organisation of plant communities, biogeochemical cycles and canopy-atmosphere-soil interactions (Schowalter *et al.*, 1986; Schowalter and

Lowman, 1999). The adults contribute to pollination. These insects are also an important resource for a number of predators (insects, birds, chiroptera, and other small mammals) and parasitoid insects.

### **Sensitivity to changes in their environment**

Generally speaking, Rhopalocera and Heterocera are considered to be good indicators of environmental change (Erhardt, 1985; Erhardt and Thomas, 1991; Luff and Woiwod, 1995). Because of their high sensitivity to environmental conditions, Heterocera are deemed to be good indicators of the degree of forest disturbance and degradation. Specifically, recent research has shown that several families of macro-Heterocera (Geometridae, Noctuidae, Notodontidae, Lymantriidae, Arctiidae, etc. whose size is medium to large, with some exceptions however) are good indicators of the influence of human activity on the forest habitat (e.g. felling, fragmentation) (Summerville *et al.*, 2004). Taken as a whole, the macro-Heterocera are sensitive to fairly intensive tree felling and to the forestry management cycle that follows (Bonneil, 2005).

### **Taxonomic stability and relatively easy identification**

The taxonomy of the Lepidoptera is well established and a list of species exists at national level to which reference is possible as a taxonomic database (Leraut, 1997).

The identification of the Rhopalocera, which is based on morphology, is fairly easy and made easier still by publications in French that are both numerous and highly accessible (Insert 20).

The identification of Heterocera is also relatively practical, especially for a large number of macro-Heterocera species and genera (e.g. Geometridae, Noctuidae, Notodontidae, Lymantriidae, Arctiidae). There are a small number of guides to macro-Heterocera, these also being based on morphology and wing patterns (Skinner, 1998; Waring *et al.*, 2003; Robineau, 2007) (cf. Insert 20). As a last resort and for validation purposes, certain experts can be contacted in entomological associations and societies.

Lastly, identification of many micro-Heterocera (e.g. Tortricidae, Crambidae, Pyralidae, Incurvariidae) is more problematic, especially for certain families (the Tortricidae or Tortrix Moths for example). Furthermore, there is no guide to the whole of this large group; handbooks are specific to a family or subfamily (Insert 20). The use of specialist skills is recommended.

## ***III.3 - Sampling***

### **Sampling Rhopalocera**

- **Inventories**

In the case of Rhopalocera, movement along a pre-determined route in a given habitat or environment, identifying species on sight or capturing them with a net if necessary is a reliable and well-established method. This technique can be supplemented by trapping using a range of lures (honeydew or other bait recipes).

- **Comparative studies and monitoring**

In this case, the transect method developed by Pollard and Yates (1993) and applied in the “Butterfly Monitoring Scheme” in the United Kingdom, is a tried and tested method for comparing fauna in Rhopalocera populations, involving several visits during the active season (every two or three weeks from April to September). This technique is well suited to monitoring changes in open environments using butterflies as bio-indicators (Demergès, 2002), but it is more problematic in closed forest environments. Nevertheless, it can prove useful for monitoring ‘open’ forest environments such as fire-breaks, clearings, peat bogs, etc. The reader is referred to public reports published by *Réserves Naturelles de France* for more information on this transect-based method (Demergès, 2002, Langlois and Gilg, 2007) with the proviso that if they are to be comparable, the resulting data sets must be expressed in terms of the same units (e.g. numbers of individuals or species observed every 100m along the transect).

## **Sampling Heterocera**

- **Inventories**

In the case of Heterocera, inventories also require more than one technique, including attractive trapping (light traps especially, and bait traps). Light attraction is widely used to attract nocturnal insects, Lepidoptera Heterocera in particular. This type of trapping is the most frequently used and will capture the largest number of species from all families. Traps with lures (nectar, mixes of fermented fruit, sugar and alcohol) can obtain further species and are particularly effective in catching species of Noctuidae (Süssenbach and Fiedler, 1999).

The mutual complementarity of hunting and trapping techniques is of crucial importance in any inventory but is limited by physical and human resources, as well as the time that can be devoted to it. In any event, the time required to build as exhaustive an inventory as possible is a whole year in order to obtain the whole range of lepidopterological fauna and a number of consecutive years (a minimum of two) in order to correct for inter-year variations.

- **Comparative studies and monitoring**

Comparison of Heterocera populations is commonly done using automatic light traps equipped with an interception and collection system (cf. Chapter 2, Part III.4). This type of trapping, which is widely used by scientists, is considered a standardised method for sampling populations of Lepidoptera Heterocera (Bonneil, 2005). In addition to a large number of species of Heterocera, this type of trap will also attract other insect orders, including numerous Diptera, Hymenoptera, Coleoptera, etc. Bait traps can also be used, especially for sampling Noctuidae (Süssenbach and Fiedler, 1999).

### **Advice on setting up a trapping scheme for comparative studies and Heterocera monitoring**

One of the problems of entomological studies is the quantity of equipment, manpower and time available to set up traps, sort the results and identify the species. One solution is to optimise trapping campaigns in order:

- To provide a maximum of repetitions per aspect to be sampled (e.g. an environment, a forest management method, a forest management stage);
- To collect the maximum number of species;
- To use the minimum amount of equipment for the minimum amount of time;
- To collect sufficient data without spending unreasonable amounts of time sorting and identifying the individuals captured.

For studies of Lepidoptera Heterocera, we recommend that trapping campaigns should be concentrated in only part of the year during the period when a maximum of species are present as adults, i.e. in spring or summer, from early June to the end of August for example. Trapping campaigns should be spread over periods when the moon is new in order to ensure optimum effectiveness (which gives three or four trapping dates).

Our experience (Bonneil, 2005) is that there is a high level of variation in captures between different nights, even if they are consecutive (because of the high level of mobility of individuals or changing weather conditions). As a consequence, it is highly desirable to do all the trapping on the same night or nights (assuming that enough traps are available to sample all planned sites at the same time).

#### ***III.4 - In the field***

Where comparative sampling of Heterocera is concerned, automatic light traps are suspended by a cord over a tree branch at a certain distance from the trunk (be careful of the shadow that will be cast) at the same height (maximum three metres from the ground, with one metre being preferable). The batteries will need to be protected from rain by a plastic bag.

The collecting jars should be filled with pieces of egg box and accompanied by a small suspended bottle containing ethyl acetate, the odour of this being diffused via a cotton wick.

The greatest care must be taken when collecting the jars the next day (in the morning, as early as possible): certain individuals will have landed on the trap (window, roof, cord) or on adjacent branches or tree trunks. An attempt should be made to capture them in the jar. Care should also be taken not to allow particularly active individuals to escape.



We recommend that the ethyl acetate bottle should be removed before the jar is closed to ensure that its movement or spillage of liquid during transportation does not damage the captured individuals.

### III.5 – In the laboratory

(cf. also Chapter 5)

Store the jars in a refrigerator or freezer if possible until sorting can be carried out.

After sorting, store the individuals in a freezer on carded cotton between layers of cardboard (bristol board type). Do not forget to note the collection date and site (or study plot) on the cardboard layers.

As far as possible, all identified individuals should be laid out and kept in reference boxes.

PROPOSAL BY INV.ENT.FOR.		
	Inventory	Comparative Studies / Monitoring
Daylight Lepidoptera (Rhopalocera)	Capture on sight with net + baited traps (nectar and other lures)	Predetermined transects (cf. Demergès, 2002)
Nocturnal Lepidoptera (Heterocera)	Light traps (sheet and standardised automatic types) + bait traps (nectar, pheromones and other lures)	Standardised automatic light traps (periods when moon new, three traps minimum per environment on the same night)

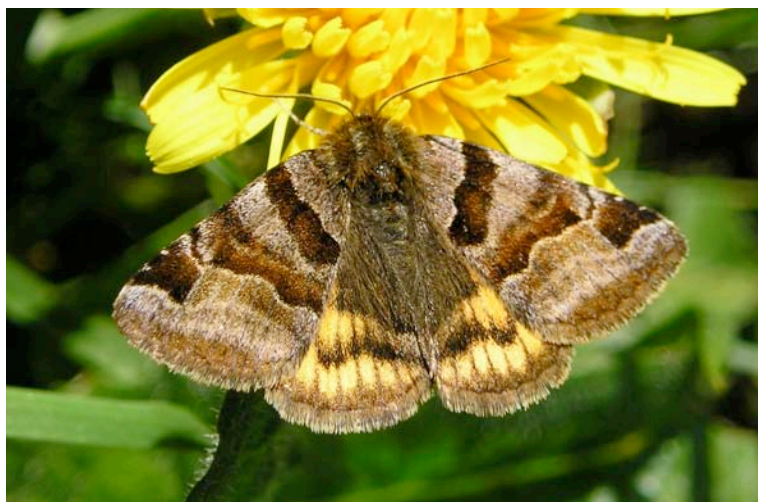


Photo 25: The Burnet Companion (*Euclidia glyphica*, Noctuidae).

## Insert 20: Handbooks for the identification of Lepidoptera

### ▪ Lists of species:

Leraut P., 1997. Liste systématique et synonymique des Lépidoptères de France, Belgique et Corse. Supplement for Alexanor, 2<sup>nd</sup> ed., Paris, 526 p.

LHOMME L., 1923-1949. *Catalogue des Lépidoptères de France et de Belgique* I. MacroLépidoptères, 800 p ; II. MicroLépidoptères, 1253 p (L. Lhomme publ. Le Carriol par Douelle).

### ▪ Rhopalocera:

Chinery M., Cuisin, M., 1994. Les Papillons d'Europe (Rhopalocères et Hétérocères diurnes). Delachaux and Niestlé publishers, 323 p.

Higgins, L.; Hargreaves, B.; Lhonoré, J., 1991. *Guide complet des papillons d'Europe et d'Afrique du Nord*; Delachaux and Niestlé, p. 270.

Lafranchis T., 2000. Les papillons de jour de France, Belgique et Luxembourg et leurs chenilles. Coll. Parthénope, Biotope publishers, Mèze (France), 448 p.

P. Whalley and R. Lewington, 2003. Tous les papillons de France et d'Europe, Octopus, 168 p

Ligue Suisse pour la Protection de la Nature (LSPN), 1987a. Les papillons de jour et leurs biotopes, espèces : dangers qui les menacent. Protection. Vol. Fototar publishers, Bâle, 512 p.

Ligue Suisse pour la Protection de la Nature (LSPN), 1987b. Les papillons de jour et leurs biotopes, espèces : dangers qui les menacent. Protection. Vol. 2, Bâle, 667 p.

Tolman T., Lewington R., 1999. Guide des Papillons d'Europe et d'Afrique du nord. Delachaux and Niestlé publishers, Neuchâtel-Paris, 320 p.

### ▪ Heterocera:

- For all macro-Heterocera:

Leraut P., 1997. *Les papillons dans leur milieu*, Bordas, 256 p.

Leraut P., 2006. *Papillons de nuit d'Europe. Bombyx, sphinx, écailles... Volume 1*, NAP Editions, 400 p.

Skinner B., 1998. *The Colour Identification Guide to Moths of the British Isles*, London, Penguins books Ltd., 276 p.

Robineau R. (Eds), 2007. *Guide des papillons nocturnes de France*, Delachaux and Niestlé, 287 p.

Waring P., Townsend M. and Lewington R., 2003. *Field guide to the Moths of Great Britain and Ireland*. British Wildlife Publishing.

- For further information, publications for more precise determination of Heterocera families (according to David Demergès):

#### ➤ Geometridae:

Leraut P., 1997. *Les papillons dans leur milieu*, Bordas, 256 p. (except for genera *Idea* and *Eupithecia*), Collection: "The Geometrid Moths of Europe" publishers Apollo Books. (several volumes relating to one or more families)

#### ➤ Sphingidae, Lasiocampidae, Lymantridae, Notodontidae, Axiidae, Drepanidae:

Leraut P., 1997. *Les papillons dans leur milieu*, Bordas, 256 p.

#### ➤ Arctiidae:

Leraut P., 1997. *Les papillons dans leur milieu*, Bordas, 256 p.

de Toulgoët H., 1952. Contribution à l'étude des Eilema français (Arctiidae Lithosiinae), *Revue Française de Lépidoptérologie*, 13, 11-12, .

#### ➤ Noctuidae:

Collection "Noctuidae Europeae" (directed by M. Fibiger) published by Apollo Books.

Nowacki J., 1998. *The Noctuids (Lepidoptera, Noctuidae) of Central Europe*, Franisek Slamka, 144 p.

Books on the best known families of micro-Lepidoptera (identifications to be checked by specialists in all cases):

#### ➤ Pyralidae:

Leraut P., 2003. *Le guide entomologique*, Paris, Delachaux and Niestlé, 527 p.

#### ➤ Sesiidae (difficult to identify in the absence of attraction using special synthetic pheromones):

Bertaccini E. and Fiumi G., 2002. Bombici e Sfini d'Italia. Volume 4: Lepidoptera Sesiioidea, Giuliano Russo, 181 p.

#### ➤ Zygaenidae:

Drouet E. and Faillie L., 1997. *Atlas des espèces françaises du genre Zygeana*, J.M. Desse, 74 p.

Faillie L., 1994. Guide pour l'identification des espèces françaises du genre Zygeana, J.M. Desse, 52 p.

#### ➤ Tortricidae:

Razowski J., 2002. Tortricidae of Europe. Volume 1: Tortricinae and Chlidanotinae, Franisek Slamka, 247 p.

Razowski J., 2003. Tortricidae of Europe. Volume 2: Olethreutinae, Franisek Slamka, 301 p.

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- Summerville K.S. and Crist T.O.**, 2002. Effects of timber harvest on forest Lepidoptera: Community, guild, and species responses. Ecological Applications, 12, 3, p. 820-835.
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## **To find out more:**

Some websites of interest for Lepidoptera:

- <http://www.lepinet.fr/>
- <http://ukmoths.org.uk/> (in English)
- <http://www.leps.it/> (in English)
- <http://www.ukleps.org/index.html> (in English)

## IV - SYRPHIDS

(Anne Vallet and Jean-Pierre Sarthou)

### *IV.1 – Presentation of the group*

The syrphids are a family in the Diptera order. They have a particular feature in that many mimic wasps and bees. Their size varies between a few millimetres and approximately 20mm. This family is characterised by the presence of a ‘spurious vein’ on the wing. The flight of all males and the females of some species (Iliff, 2005) includes phases in which they hover (hence ‘hoverflies’, their common name in English), which differentiates them from the majority of other flying insects. They usually have no common name in French, with the exception of two genera:

- genus *Eristalis* or ‘Éristales’ in French;
- genus *Volucella* or ‘Volucelles’ in French.

The adults feed on nectar, pollen and aphid honeydew. The larvae are zoophagous (especially aphids) (30%), phytophagous (20%) or saprophagous (30%), the diet of the remainder being mixed (Castella, 2008).

This family of Diptera has been relatively closely studied for the following reasons:

- Approximately 80 species have saproxylic larvae dependent on dead wood in various forms. Certain larvae live in wet cavities in old trees. This makes it possible to use this group as a bio-indicator for ancient forests of great natural heritage value (Speight, 1989, Good and Speight, 1996). In the case of France, no less than 150 are found in forests (Castella, 2008).
- In a large number of species the larvae predate on aphids. This family is therefore a subject of research in the area of biological pest control.
- They play a not insignificant role in pollination: the adults feed on pollen and nectar, visiting large numbers of flowers.

None of these species is currently on any French or European protection list.

### *IV.2 - Interest*

#### **Taxonomic diversity and ecological diversity:**

Syrphid Diptera include very many species (over 510 in France and approximately 850 in Europe).

The diversity of their diets enables them to occupy all terrestrial habitats other than in caves and under water.

This larval specialisation goes hand in hand for each food type with high-amplitude ecological valence, ranging from stenoecious (species highly specialised in terms of their larval and adult habitats) to euryoecious (generalist, ubiquitous species).

#### **A familiar group:**

These important intrinsic characteristics are accompanied with other major extrinsic assets:

- Less than 5% of these species pose problems of identification to specialists; the criteria for identification are currently relatively stable and reliable.
- Their ecology is well understood: larval micro-habitats, adult macro-habitats, duration of larval development, number of generations per year, migration, and so on.
- Their European and national distribution is understood despite gaps in a few regions (work in progress).
- All of these characteristics confer upon the Diptera Syrphidae the advantage of being a good bio-indicative taxon for the quality of natural environments (Speight, 1986).

#### **A well-established study protocol coupled with a database:**

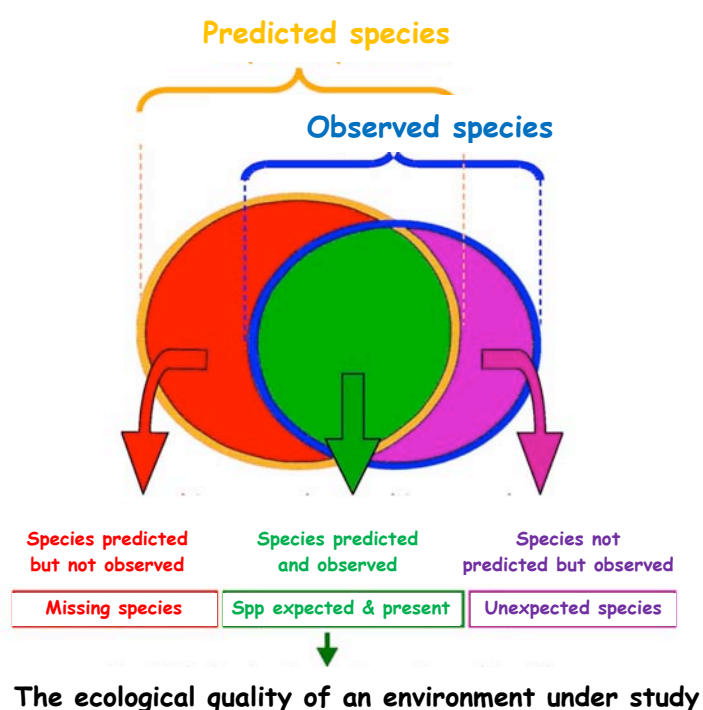
All the elements described above have been gathered together in a large database (“Syrph-The-Net”). This database has required nearly fifteen years of information-gathering, diagnostic work, systematics, ecology and biogeography for the hundreds of European species. Coupled with a standardised trapping protocol, it will permit precise bio-evaluation and ecological diagnostic analysis (Speight *et al.*, 2000).

### IV.3 – Studying syrphids with Syrph-The-Net

The main features of this method are summarised here, but for more details, the reader is referred to the original text (Speight *et al.*, 2000).

#### **Basic principle**

The method is based on the absence or presence of species in a given environment. The list of syrphids caught on the study site is compared, by means of Syrph-The-Net, with the list of species potentially present in an identical environment for a given region and period (Figure 11). The percentage of species effectively present (*i.e.* predicted and observed) is thus considered to indicate the ecological integrity of the relevant environment. This model also makes it possible to interpret in functional terms the list of species that are missing (*i.e.* predicted but not observed) in order to link the lifestyle characteristics of those species with the corresponding ecological characteristics assumed also to be missing from the habitat. And lastly, the unexpected species (*i.e.* not predicted but observed) provide information on undetected characteristics of the site (an additional habitat, trends from another habitat) and/or the presence of neighbouring habitats.



**Figure 11: The use of SyrphTheNet to assess the ecological quality of an environment (according to Sarthou, Third International Symposium on the Syrphidae - Leiden, Netherlands - September 2-5, 2005).**

#### **A range of possible study objectives**

##### ➤ Objective 1: Faunistic studies

#### **Faunistic research:**

The aim here is to arrive at a representation as complete as possible of the species present on a site. The number of traps to be set up will depend on the complexity and the extensiveness of the area to be inventoried. At least one trap must be installed for each habitat represented on the site. The trapping season must cover the whole plant growth season. Such trapping can be continued over several years and coupled with other capture methods (e.g. net, yellow trays, emergence traps).

### **Heritage research:**

The aim in this case is to try to seek out species of possible heritage interest. A species of especial interest is so only for a given region. In the case of the syrphids, there is a website: 'Syrfid' (<http://syrfid.ensat.fr>) which provides a list of species present in France by territorial *département* and the numbers of times each species is mentioned in the literature. This makes it easy to gain an idea of how rare a species is in a region. Some regions are in the process of creating or updating their own lists of heritage species.

Syrfid also indicates the degree of vulnerability in France and across Europe of each species present in France.

- Level 1: species justifying special surveillance
- Level 2: species in sharp decline
- Level 3: species threatened with extinction

### ➤ Objective 2: Ecological studies

#### **The species richness of functional groups**

Syrph-The-Net describes the biology of all European syrphids. It is therefore easy to classify captured syrphids in accordance with their role in the environment being studied. In a forest, various types of stand and/or forestry management protocols applied in the past must be sampled to gain an idea of their species richness. For example, a small number of species associated with dead wood might indicate that management is too intensive.

#### **The ecological integrity of habitats**

The integrity of a habitat can be measured by comparing the species actually found there with the species that could potentially be present. In this way, if less than 50% of syrphids predicted by Syrph-The-Net are identified during a specifically targeted sampling programme (Speight *et al.*, 2000), the habitat can be considered to be degraded.

#### **Prediction of the future population development due to habitat changes**

The data entered in Syrph-The-Net can also predict changes in syrphid populations due to planned modifications of the environment (Speight *et al.*, 2002). However, Syrph-The-Net data cannot be used to develop models of the type with which we are familiar. The predictions provided by this database are dependent on the initial observation of the populations and the variables entered in the database. The habitat categories included in Syrph-The-Net have also been chosen because syrphids respond to them. Prediction entails the association of each of the species present initially with the planned habitat modifications.

#### **Analysis of the results**

Syrph-The-Net is used to produce a list of all the species potentially present on the site in accordance with the habitats observed. This basic list is drawn up from the complete list of all European syrphids. It will then need further refinement to reflect:

- The syrphids known to be present in the region;
- The trapping date, which must correspond to the species' flight period.

Next, this list of predicted species is compared and contrasted with the list of species found in the traps. According to the habitats observed, a list of species that are absent (predicted but not observed), present as expected (predicted and observed) or unexpected (not predicted but observed).

Following this, the same can be done for each functional group or each of the habitats (macro- or micro) observed in order to fine-tune the diagnostic analysis.

The following are some examples of analysis of the results of application of Syrph-The-Net available for consultation:

- An analysis of subalpine meadowland using Syrph-The-Net (Castella and Speight, 2005).



- An assessment, using Diptera Syrphidae, of the impact of traditional forest management on upland beech-pine woods (Sarhou *et al.*, 2005; Larrieu, 2005);
- The relationship between a community of syrphid species and wooded areas in the forests of south-west France (Ouin *et al.*, 2006).

#### ***IV.4 - Sampling***

##### **The Malaise trap**

Syrphids can be conveniently sampled at the adult stage using a standardised interception trap: the Malaise trap. This lightweight device can operate unattended for two or three weeks and will ensure continuous sampling over the whole period of flight. It is particularly effective and will capture all sorts of insects (and not only syrphids) during their travels. A trap will cost somewhere between €150 and €200. They can be used for three to five years before exposure to ultraviolet light makes the fabric brittle. The insects are collected in a jar filled with 70° alcohol which also serves as a preserving fluid.

Malaise traps are particularly well suited to catching syrphid fauna. They have the advantage of operating unattended and will capture species flying early in the day. These traps can be set up and the insects collected by inexperienced staff, which is not the case for capture on sight. Unlike methods requiring human intervention such as net capture, trap-based sampling can provide quantitative data on species abundance.

##### **Other possible sampling methods**

Each insect capture technique has its own sources of bias and will not reflect totally faithfully the local insect population. According to the desires of the researcher, it is possible to combine the use of Malaise traps with other methods for catching syrphids: nets, coloured traps, emergence traps, and so on.

##### **Sampling plan**

The first task is to list all the macro-habitats present on the site to be studied. Those habitats must match the list suggested by Syrph-The-Net. The second task is to set up at least two Malaise traps per type of habitat to be sampled.

In order to arrive at a better representation of the syrphid community on the site, it will be necessary to undertake trapping over at least two periods. The first should be timed for the spring (at the peak of the flowering period). The second should be in the summer. Trapping dates and durations can be adjusted to suit the weather conditions in the survey area. It is advisable to leave the traps in place for at least fifteen consecutive days.

The duration of trapping can be adjusted to suit the chosen objectives. For example, for an inventory of heritage species, the traps must be in place throughout the entire plant growth season in order to capture both early and late syrphids. Syrphids can also be sought out for several consecutive years in order to gain a more precise idea of the community and its populations. If the most exhaustive possible survey possible is desired, at least three years of study will be required.

#### ***IV.5 - In the field***

The syrphid flight period is dependent on a large number of factors, among them weather, time of day and topography. The location of a Malaise trap and its orientation will have an effect on its effectiveness. Insect flight corridors are highly dependent on the site's micro-topography and dominant winds. Malaise traps can be set up on travel corridors (forest edges or borders). Such locations will maximise syrphid captures as they fly between different habitats, in addition to migrations (some syrphid species are migratory). The reverse is also true – if traps are set up outside such travel corridors, this will be conducive to the capture of local syrphids. For the purposes of comparison between sites, it is preferable to orient all the traps identically, using a compass. Trapping over long periods can highlight capture peaks that will differ significantly depending on the flowering of nearby plant species.

It is recommended that the collecting jars in place on the traps should be checked at least once a fortnight and once a week in periods of strong wind or high temperatures. Damage due to animals is an exceptional occurrence. Collection requires only that the collecting jar be changed on the trap.



#### IV.6 – In the laboratory

The collecting jar taken from a Malaise trap can be used to store the insects for at least a year. Sorting should be done with a binocular microscope, using a Petri dish to extract the specimens. This work will be more or less time-consuming according to the objective: perhaps an hour per sample to isolate the syrphids from the rest, two hours to take out the syrphids, Apoidea, Coleoptera, Lepidoptera and Hemiptera, and anything up to a dozen hours to separate all the families.

Then the syrphids need to be identified. In the current state of our knowledge, it will not be possible to identify some genera down to species level, and others will be identifiable only on the basis of the male genitalia.

Syrphids are conserved in the same way as other insects: the enemies of any dry collection are damp (mould) and detritivore insects (*Dermestes* in particular). The drawbacks of a collection kept in alcohol (which is necessary with regular use of Malaise tents) are the slow evaporation of the alcohol in any container that is other than totally hermetically sealed (the small 'Eppendorf' plastic tubes are convenient for storing one to three individuals) and the fairly rapid discolouration of alcohol-soaked teguments when exposed to light.

#### Insert 21: Handbooks for the identification of syrphids.

Stubbs, A. E. and Falk, S. J., 1983. British hoverflies: an illustrated identification guide. Br. Ent. Nat. Hist. Soc., London, 253 p. (in English).

Bradescu, V., 1991. Les Syrphidés de Roumanie (Diptera, Syrphidae), Clés de détermination et répartition. Trav. Mus. Hist. nat. Grigore Antipa, 31, 7-83.

Verlinden, L., 1994. Faune de Belgique : Syrphidés (Syrphidae). 1-298. Inst. Roy. Sci. Nat. Belg., Brussels.

Van Veen M., 2004, *Hoverflies of Northwest Europe, identification keys to the Syrphidae*, Utrecht, Netherlands, KNNV Publishing, 254 p.

The website of Cyrille Dussaix, which has numerous photos, will enable fine-grained species determination <http://perso.wanadoo.fr/cyrille.dussaix>

Speight, M.C.D., Castella, E., Obrdlik, P. and Ball, S. (eds.) *Syrph the Net, the database of European Syrphidae*, Syrph the Net publications, Dublin. <http://www.iol.ie/~millweb/syrph/syrphid.htm>

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**Good J.A. and Speight M.C.D.**, 1996. Saproxylic invertebrates and their conservation throughout Europe. *Convention on the Conservation of European Wildlife and their Natural Habitats*. 52 p. Council of Europe, Strasbourg.

**Iiff, D.**, 2005. Hovering activity of female Syrphinae (Diptera, Syrphidae). In: Menno Reemer M. and John T. Smit J.T. 3rd International Symposium on Syrphidae, 2 – 5 September 2005 Leiden, Netherlands. p. 21.

**Larrieu L.**, 2005. Étude Biodiversité Hèches – Syrphidés. CRPF Midi-Pyrénées. <http://www.crfp-midi-pyrenees.com/vousinformer/publication1-1.htm>.

**Ouin A., Sarthou J.P., Bouyjou B., Deconchat M., Lacombe J.P. and Monteil C.**, 2006. The species-area relationship in the hoverfly (Diptera, Syrphidae) communities of forest fragments in southern France. *Ecography* 29, 183-190.

**Sarthou J.P., Laurent Larrieu L. and Delarue A.**, 2005. Ecological assessment with Syrph the Net: the case of four stands in a Fagus-Abies forest in Hautes-Pyrénées (South-Western France). In: Menno Reemer M. and John T. Smit J.T. 3rd International Symposium on Syrphidae, 2 – 5 September 2005 Leiden, Netherlands. p. 31.

**Speight M.C.D.**, 1986. Criteria for the selection of insects to be used as bioindicators in nature conservation research, in anonymous (Eds), *3<sup>rd</sup> Eur. Cong. Ent.*, p. 485-488.

**Speight M.C.D.**, 1989. Les invertébrés saproxyliques et leur protection. *Conseil de l'Europe, coll. Sauvegarde de la nature*, 42, 1-78,

**Speight M.C.D., Castella E., Obrdlik P. and Ball**, 2000. Syrph-The-Net: the database of the European syrphidae (Diptera). In: Speight, M.C.D., Castella, E., Sarthou, J.-P. and Monteil, C. (eds.) Syrph the Net, the database of European Syrphidae, Vol. 25, 77 p., Syrph the Net publications, Dublin.

**Speight, M.C.D.; Good, J.A. and Castella, E.**, 2002. Predicting the changes in farm syrphid faunas that could be caused by changes in farm management regimes (Diptera, Syrphidae). – *Volucella* 6, 125-137. Stuttgart.

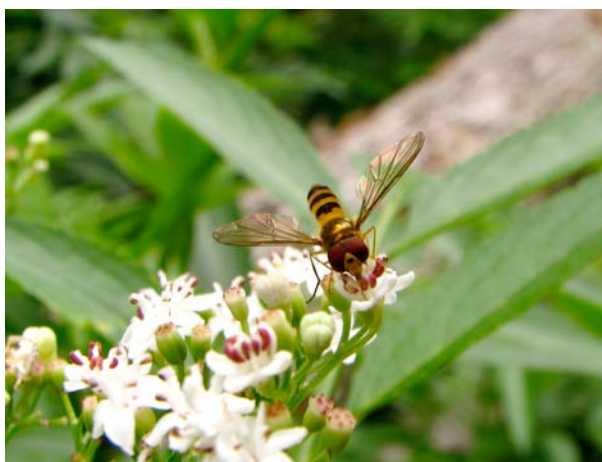
### To find out more:

#### Websites (in French):

- <http://perso.wanadoo.fr/cyrille.dussaix>: has numerous photos of syrphids to assist identification.
- <http://syrfid.ensat.fr/>: list of species by French territorial *département*.

#### Foreign websites (in English in most cases):

- <http://www.iol.ie/~millweb/syrph/syrphid.htm> (Syrph The Net): website demonstrating the use of the Syrph-The-Net database, run by Martin C.D. Speight.
- <http://www.naturkundemuseum-bw.de/stuttgart/volucella/> (Volucella): presentation of an essential journal for all syphidologists, created in 1995, and appearing annually or biannually.
- <http://www.syrphidae.com> (The world of Syrphidae...): a generalist site on Syrphidae (bibliography, hyperlinks, national lists, etc.).
- [http://home.hccnet.nl/mp.van.veen/hf\\_index.html](http://home.hccnet.nl/mp.van.veen/hf_index.html): website with keys for identifying the species in 25 genera in north-western Europe; an extension of the book by M. van Veen, which is regularly updated and useful for certain species and genera.
- <http://www.faunaeur.org> (Fauna Europea): the building (work in progress) of a database containing complete scientific names and distribution details for all multicellular animals living in terrestrial and aquatic environments in Europe.
- <http://www.nottingham.ac.uk/~plzfg/>: personal website of Francis Gilbert containing numerous documents that can be downloaded as PDF files.
- <http://www.ufz.de/index.php?de=1901>: personal website of Frank Dziock containing numerous links to other websites.
- <http://www.geller-grimm.de/address/europe.htm>: a site created and maintained by Fritz Geller-Grimm containing a database list of individuals working on Syrphidae, with details of their specialisations and contact details (postal address, telephone, email address).



**Photo 26: A syrphid.**

## V – RED WOOD ANTS

(Louis-Michel Nageleisen)

### V.1 - Presentation of the group

Ants (Hymenoptera, Formicidae) form a particularly interesting family, with approximately 180 species in France. They have colonised every potential terrestrial biotope and are omnipresent throughout the natural world. Moreover, they are at the top of the trophic chain (as opportunistic predators), which means that they are highly sensitive to any deterioration in the environment.

An evaluation of the number of species on a site would be an indicator of great interest for assessments of the quality of the environment. However, ants are a group that has been studied relatively little by entomologists, either amateur or professional, and those specialising in this family in France are very few in number.

On the other hand, the family includes a group of species under the general heading of ‘wood ants’ comprising individuals of fairly large size and whose species are relatively easy to identify. They live essentially in the forest environment. They build mounds of twigs that are easily spotted in the forest, making it very straightforward to characterise populations without the need for specialist entomological skills.

Red wood ants (*Formica rufa sensu lato*) constitute a complex of species of which five (or perhaps seven) are present in French forests:

- *Formica rufa* Linné 1758
- *Formica polyctena* Foerster 1850
- *Formica lugubris* Zetterstedt 1840, and perhaps *Formica paralugubris* Seifert 1996
- *Formica aquilonia* Yarrow 1955
- *Formica pratensis* Retz, and possibly *Formica nigricans* Emery 1909.

While *F. rufa* is well represented throughout France, *F. polyctena* is a species inhabiting lowlands and uplands. *F. lugubris* is frequently encountered in all mountainous areas up to the tree line. *F. paralugubris*, very similar to *F. lugubris* and recently described in the Western Alps, may be more widespread than is presently known. As for *F. aquilonia*, a very typical Northern Alpine species, it is more common in Fennoscandia; in Germany, it is present only in the eastern part of the Bavarian Alps. In France, it seems to exist only at high altitude (the subalpine zone and above the tree line in the Alps). Lastly, *F. pratensis* is a species inhabiting open meadowland and is therefore less frequently found in forests than the species previously mentioned. Whereas the other species may build large mounds more than a metre high, *F. pratensis* builds only small mounds a dozen or so centimetres in height. *F. nigricans* is considered by various authors to be a form of *F. pratensis* with longer, denser pilosity; it is however definitely deemed to be a species by Collingwood (1979).

### V.2 - Interest

In the forest ecosystem, the wood ant group stands nearly at the top of the trophic chain. The abundant literature describes the group’s fundamental role in the functioning of the ecosystem and its sensitivity to environmental disturbance (Adlung, 1966; Frouz *et al.*, 1997; Kahru, 1998; Masson, 1975; etc.). Authors (Gosswald, 1984; Nageleisen, 1999; Pavan, 1961; Torossian, 1977; etc.) agree to state that in a relatively undisturbed pine or mixed forest (a mix of hardwood and softwood species) a population of wood ants will consist of large epigeic nests (mounds) with a minimum density of four per hectare.

In order to evaluate the status of wood ant populations, and indirectly the degree to which a forest is degraded, a method has been developed on the basis of a nest (mound) census.

### V.3 – Sampling

Sampling is not based on trapping as in the case of other insect groups. It involves counting the mounds of twigs and describing their distribution, volume, interrelations, etc.

Such a census can be undertaken in several phases:

- An initial survey of users of the geographical area to be studied (land managers, owners, etc.) will usually allow zones of especial interest to be located.

- Next, a survey along all the traffic routes in the forest (roads, paths, logging tracks, boundaries between blocks) will provide a linear index of abundance (number of nests per kilometre).
- Finally, in order to characterise wood ant populations more precisely, it is possible, using previous surveys as a basis, to evaluate the density of mounds in areas where their presence is confirmed, using either the quadrat method or the transect method.

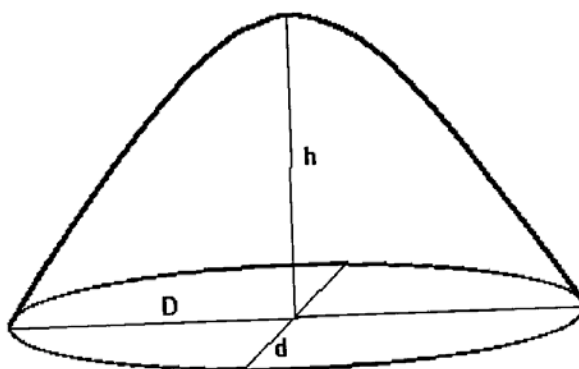
#### ***V.4 - In the field***

Once the mounds are located, a number of parameters should be recorded:

- GPS coordinates for precise mapping.
- Mound dimensions for determination of volume.
- Determination whether neighbouring mounds belong to the same colony (polycalic colony) or not.
- Mound environment (slope, exposure, stand cover, tree species present within a radius of 10m, and so on).

Each mound should be described by means of three numerical characteristics: its height plus two diameters along perpendicular axes at the base, allowing the epigeal volume to be estimated.

When calculating mound volume, the mound is treated as a paraboloid with an elliptical footprint, which means that a cross-section of the nest parallel with the ground would yield an ellipse and a perpendicular vertical cross-section would give a parabola (figure 12).



**Figure 12: Diagram of a red wood ant nest and the dimensions to be measured.**

The formula is as follows:  $V = \frac{2}{3} \times \pi \times \frac{D}{2} \times \frac{d}{2} \times H$

where:  $D$  is the large diameter  
 $d$  is the small diameter  
 $H$  is the height of the mound.

The volume figure produced by application of this formula is of course no more than an estimate. Various factors can distort volume evaluations. The mound may for example be built on a tree stump, on rocks or on a bank, etc. Nevertheless, the precision obtained for the population as a whole is quite sufficient.

Where there is a slope, when assessing mound height the level passing through the summit of the mound should be used in order to minimise calculation errors.

For each mound, four or five workers should be sampled and placed in a jar filled with alcohol for species determination (cf. Insert 22).

Where several mounds are close to one another ( $d < 20m$ ), it is advantageous to check whether the mounds belong to a single (polycalic) colony or to more than one colony. This is easy to verify by placing a few workers from each mound in a container or transferring the contents of one jar filled with workers from a mound into a jar containing workers from another mound. Lack of aggression between workers from two mounds will confirm that both mounds belong to a single polycalic colony.

### V.5 – Characterisation of wood ant populations.

For a given site, the wood ant population can be characterised by:

- Nest density (per hectare or per kilometre);
- Average nest volume (Table 19);
- Distribution of the small, medium-sized, large and very large nests (cf. Table 18);
- Total pseudo-biomass per hectare (sum of the epigeal volumes of all mounds expressed per hectare);
- The existence of polycalic colonies and their size (number of related nests, surface area covered by the colony, etc.).

Since the mounds are relatively durable structures (despite the sometimes major variations in volume during any given year) it is also possible to describe mounds that are no long active in order to evaluate the population dynamic.

As an indication, Torossian (1984), working in the Alps and Pyrenees, describes four population types based on these parameters:

Forest type:	A	B	C	D
Pseudo-biomass	15 and over	7	< 0.5	1 to 10
Large nests	>90%	< 50%	0%	Variable
Medium-sized nests		> 50%		
Density	11	45	10 to 15	Variable
Mean volume	>1	0.2	0.04	0.2

A – Forests with very dense populations

B – Forests with dense populations

C – Forests with deficient populations

D – Forests with unstable mean populations

Caution is required when interpreting the absence of wood ant mounds. This is because wood ants are particularly closely associated with softwood species such as firs and spruce which provide them with an abundance of the aphids with which the ants have specific symbiotic relationships. For this reason, in stands made up exclusively of hardwood species wood ant populations are naturally very limited, but not non-existent. Wetlands and slopes with little exposure to the sun provide microclimatic conditions of little interest to wood ants. They will often be absent from such contexts.

It is therefore the evolution of populations in a given area or synchronic comparison of areas favourable in principle to wood ant populations that can be interpreted as an indication of the quality of the forest environment. For this reason, it is important to establish a zero baseline for such populations.

The existence of polycalic colonies (several nests forming a single colony) that are large in terms of the number of related nests constitutes an additional parameter revelatory of the quality of the environment and low levels of anthropogenic disturbance.

**Table 18: Nest classes (according to Torossian, 1984).**

Class	Volume (dm³)	Nest size
1	< 1	Small
2	1 to 2	Small
3	2 to 4	Small
4	4 to 8	Small
5	8 to 16	Small
6	16 to 32	Small
7	32 to 64	Medium
8	64 to 128	Medium
9	128 to 256	Medium
10	256 to 500	Medium
11	500 to 1,000	Large
12	1,000 to 2,000	Large
13	2,000 to 4,000	Very large
14	Over 4,000	Very large

## Insert 22: Handbooks for the identification of wood ants:

BERNARD F., 1968. Les Fourmis d'Europe occidentale et septentrionale. Masson et C<sup>ie</sup> publishers, 73p.  
COLLINGWOOD C.A., 1979. The Formicidae ( Hymenoptera) of Fennoscandia and Denmark. Fauna Entomologica Scandinavia, Vol. 8.  
SEIFERT B., 1996. Ameisen beobachten, bestimmen. Naturbuch publishers, Augsburg.  
To find out more: <http://antarea.fr/projet/index.html>

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### To find out more:

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**Table 19: Wood ant mound volume calculation chart.**

**Lines: mound height in decimetres. Columns: the average in decimetres of the two base diameters. Volume expressed in cubic decimetres.**

	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7</b>	<b>8</b>	<b>9</b>	<b>10</b>	<b>11</b>	<b>12</b>	<b>13</b>	<b>14</b>	<b>15</b>	<b>16</b>	<b>17</b>	<b>18</b>	<b>19</b>	<b>20</b>	
<b>1</b>	1	1	2	2	3	3	4	4	5	5	6	6	7	7	8	8	9	9	10	10	<b>1</b>
<b>2</b>	2	4	6	8	10	13	15	17	19	21	23	25	27	29	31	33	36	38	40	42	<b>2</b>
<b>3</b>	5	9	14	19	24	28	33	38	42	47	52	57	61	66	71	75	80	85	89	94	<b>3</b>
<b>4</b>	8	17	25	33	42	50	59	67	75	84	92	100	109	117	126	134	142	151	159	167	<b>4</b>
<b>5</b>	13	26	39	52	65	79	92	105	118	131	144	157	170	183	196	209	222	236	249	262	<b>5</b>
<b>6</b>	19	38	57	75	94	113	132	151	170	188	207	226	245	264	283	301	320	339	358	377	<b>6</b>
<b>7</b>	26	51	77	103	128	154	180	205	231	256	282	308	333	359	385	410	436	462	487	513	<b>7</b>
<b>8</b>	33	67	100	134	167	201	234	268	301	335	368	402	435	469	502	536	569	603	636	670	<b>8</b>
<b>9</b>	42	85	127	170	212	254	297	339	382	424	466	509	551	593	636	678	721	763	805	848	<b>9</b>
<b>10</b>	52	105	157	209	262	314	366	419	471	523	576	628	680	733	785	837	890	942	994	1047	<b>10</b>
<b>11</b>	63	127	190	253	317	380	443	507	570	633	697	760	823	887	950	1013	1076	1140	1203	1266	<b>11</b>
<b>12</b>	75	151	226	301	377	452	528	603	678	754	829	904	980	1055	1130	1206	1281	1356	1432	1507	<b>12</b>
<b>13</b>	88	177	265	354	442	531	619	708	796	884	973	1061	1150	1238	1327	1415	1504	1592	1680	1769	<b>13</b>
<b>14</b>	103	205	308	410	513	615	718	821	923	1026	1128	1231	1333	1436	1539	1641	1744	1846	1949	2051	<b>14</b>
<b>15</b>	118	236	353	471	589	707	824	942	1060	1178	1295	1413	1531	1649	1766	1884	2002	2120	2237	2355	<b>15</b>
<b>16</b>	134	268	402	536	670	804	938	1072	1206	1340	1474	1608	1742	1876	2010	2144	2278	2412	2545	2679	<b>16</b>
<b>17</b>	151	302	454	605	756	907	1059	1210	1361	1512	1664	1815	1966	2117	2269	2420	2571	2722	2874	3025	<b>17</b>
<b>18</b>	170	339	509	678	848	1017	1187	1356	1526	1696	1865	2035	2204	2374	2543	2713	2883	3052	3222	3391	<b>18</b>
<b>19</b>	189	378	567	756	945	1134	1322	1511	1700	1889	2078	2267	2456	2645	2834	3023	3212	3401	3590	3778	<b>19</b>
<b>20</b>	209	419	628	837	1047	1256	1465	1675	1884	2093	2303	2512	2721	2931	3140	3349	3559	3768	3977	4187	<b>20</b>
<b>21</b>	231	462	692	923	1154	1385	1616	1846	2077	2308	2539	2769	3000	3231	3462	3693	3923	4154	4385	4616	<b>21</b>
<b>22</b>	253	507	760	1013	1266	1520	1773	2026	2280	2533	2786	3040	3293	3546	3799	4053	4306	4559	4813	5066	<b>22</b>
<b>23</b>	277	554	831	1107	1384	1661	1938	2215	2492	2768	3045	3322	3599	3876	4153	4429	4706	4983	5260	5537	<b>23</b>
<b>24</b>	301	603	904	1206	1507	1809	2110	2412	2713	3014	3316	3617	3919	4220	4522	4823	5124	5426	5727	6029	<b>24</b>
<b>25</b>	327	654	981	1308	1635	1963	2290	2617	2944	3271	3598	3925	4252	4579	4906	5233	5560	5888	6215	6542	<b>25</b>
<b>26</b>	354	708	1061	1415	1769	2123	2476	2830	3184	3538	3892	4245	4599	4953	5307	5660	6014	6368	6722	7075	<b>26</b>
<b>27</b>	382	763	1145	1526	1908	2289	2671	3052	3434	3815	4197	4578	4960	5341	5723	6104	6486	6867	7249	7630	<b>27</b>
<b>28</b>	410	821	1231	1641	2051	2462	2872	3282	3693	4103	4513	4924	5334	5744	6154	6565	6975	7385	7796	8206	<b>28</b>
<b>29</b>	440	880	1320	1760	2201	2641	3081	3521	3961	4401	4841	5281	5722	6162	6602	7042	7482	7922	8362	8802	<b>29</b>
<b>30</b>	471	942	1413	1884	2355	2826	3297	3768	4239	4710	5181	5652	6123	6594	7065	7536	8007	8478	8949	9420	<b>30</b>
<b>31</b>	503	1006	1509	2012	2515	3018	3520	4023	4526	5029	5532	6035	6538	7041	7544	8047	8550	9053	9556	10058	<b>31</b>
<b>32</b>	536	1072	1608	2144	2679	3215	3751	4287	4823	5359	5895	6431	6967	7503	8038	8574	9110	9646	10182	10718	<b>32</b>
<b>33</b>	570	1140	1710	2280	2850	3419	3989	4559	5129	5699	6269	6839	7409	7979	8549	9119	9688	10258	10828	11398	<b>33</b>
<b>34</b>	605	1210	1815	2420	3025	3630	4235	4840	5445	6050	6655	7260	7865	8470	9075	9680	10285	10890	11494	12099	<b>34</b>
<b>35</b>	641	1282	1923	2564	3205	3847	4488	5129	5770	6411	7052	7693	8334	8975	9616	10257	10898	11540	12181	12822	<b>35</b>
<b>36</b>	678	1356	2035	2713	3391	4069	4748	5426	6104	6782	7461	8139	8817	9495	10174	10852	11530	12208	12887	13565	<b>36</b>
<b>37</b>	716	1433	2149	2866	3582	4299	5015	5732	6448	7164	7881	8597	9314	10030	10747	11463	12180	12896	13612	14329	<b>37</b>
<b>38</b>	756	1511	2267	3023	3778	4534	5290	6046	6801	7557	8313	9068	9824	10580	11335	12091	12847	13602	14358	15114	<b>38</b>
	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7</b>	<b>8</b>	<b>9</b>	<b>10</b>	<b>11</b>	<b>12</b>	<b>13</b>	<b>14</b>	<b>15</b>	<b>16</b>	<b>17</b>	<b>18</b>	<b>19</b>	<b>20</b>	

## VI - OVERVIEW OF SAMPLING METHODS ACCORDING TO THE GROUPS TO BE STUDIED IN THE FOREST CONTEXT

The present Chapter 4 provides a non-exhaustive list (the only limitation on exhaustiveness being the entomologist's imagination) of the methods for sampling insects. Specifically, it contains a more comprehensive description of a few methods and a small number of groups of insects that it is preferable to use and to survey (and as a strict minimum) when conducting entomological studies of forest areas. For an easier overview of the whole, they are summarised in the following table.

**Table 20: Insect groups and sampling methods recommended by the Inv.Ent.For. working group**

		Lepidoptera	Coleoptera		Diptera	Hymenoptera
			Carabids	Saproxylic	Syrphids	Wood ants
Sight surveys without insect collection	Line transect	Daylight				
	Mapping					
Active collection	Sight survey	Daylight				
	Beating					
	Debarking					
Emergence traps	On site					
	Off site					
Interception trap	Pitfall trap					
	Malaise trap					
	Window flight trap					
Attractive traps	Light traps	Nocturnal				
	Coloured traps					
	Chemical lure traps	Some nocturnal species (pheromones)		'Beer' traps		

Green: method recommended by the Inv.Ent.For. working group  
 Yellow: other methods possible

***CHAPTER 5***

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***MANAGING TRAP CATCHES***

**(Thierry Noblecourt)**



## I – STORAGE OF SAMPLES AFTER COLLECTION

The present recommendations are valid for all trapping techniques and the groups sampled (with the exception of fragile insects such as Lepidoptera).

No sorting should be done in the field except to remove large pieces of debris (plant or other), which should be rinsed off with water above a sieve. The material recovered in the sieve should be gathered together with the initial sample.

Pass the material collected through a sieve with very fine mesh and then transfer the contents thus retained to a Ziploc® or Minigrip® type freezer bag with a hermetic closure, which should then be correctly labelled with details of location and date. The bag can then be placed directly in the freezer. This is light in weight that takes up little space. If the bags need to be sent by postal mail, put a little 95° ethanol in the bag before closing it.

When collecting very small insects (e.g. micro-Hymenoptera in a Malaise trap) that might pass through the mesh of the sieve, pour the content of the collection jar into a filter (a coffee filter for example), place the filter directly in the freezer bag after draining off the liquid, and then store it in the freezer.

Each bag should correspond to the material collected in a given trap on a given date. It is imperative to slip a label (see below) into the bag; this should state the place, type and number of the trap, the date, and so on (take care to use high-quality ink that will last for as long as necessary).

## II – PREPARATION FOR SAMPLE SORTING

Before beginning any processing of the samples, two documents need to be prepared:

- The sorting form.
- Sheets of labels to match the trapping site.

**The sorting form** (cf. Figure 13)

This must be suitable for the targeted species but a number of data fields need to be filled in (territorial *département*, municipality, stand, GPS coordinates, trap set-up and collection dates, trap type or capture method, etc.).

The form should be numbered in a continuous series by place and year (e.g. ‘Tronçais 2007/01’, ‘Tronçais 2007/02’, ..., ‘Tronçais 2008/01’, ...).

The entire monitoring process for sampling is based on these forms, which should be completed as the identifications proceed. Certain identifications may be made and then not recur until several years later, which means that it is necessary to number the form and to put this number on the labels accompanying the species to be identified. Form-based monitoring makes data management easier when the programme is spread over more than one year.



<b>FORET : Tronçais (F-03-St Bonet)</b>		<b>DATE DE POSE 31.V. 2005</b>		<b>N° de Fiche : Tronçais2005/08</b>	
<b>STATION : Futaie Colbert</b>		<b>DATE DE RELEVÉ 14.VI.2005</b>		<b>N° de Fiche DFF :</b>	
<b>Coord GPS : N --° --' --" E --° --' --" alt : ---- m</b>		Type coordonnées : WGS84		<b>Type de piège : Inter transparent</b>	
<b>BUPRESTIDAE</b>		<b>LUCANIDAE</b>		<b>Nombre :</b> 1	
				<b>Position :</b> bas	
				<b>Manipulateur :</b> TN	
				<b>Date de dépouillement :</b> 05/08	
				<b>Durée :</b> 15'	
		<b>OEDEMERIDAE</b>		<b>HYMENOPTERA</b> 5	
				<b>HYMENOPTERA SYMPHYTA</b> 1	
<b>CARABIDAE</b>		<b>HISTERIDAE</b>		<b>DERMAPTERA</b> 1	
				<b>ODONATA</b>	
				<b>EPHEMEROPTERA</b>	
				<b>PLECOPTERA</b>	
				<b>DIPTERA</b> 5	
		<b>SILPHIDAE</b> 1		<b>LEPIDOPTERA</b>	
		Xylodrepa 4punctata 1		<b>BLATTOPTERA</b>	
<b>CERAMBYCIDAE</b> 3				<b>MECOPTERA</b>	
Gramoptera ruficornis 3		<b>STAPHYLINIDAE</b>		<b>NEUROPTERA</b>	
				<b>ORTHOPTERA</b>	
				<b>HEMIPTEROIDEA</b>	
				<b>AUTRES INSECTES</b> 1	
				<b>ATHROPODES</b> 2	
				<b>MORDELIDAE-ANASPIDAE</b>	
<b>CETONIDAE</b> 1				<b>MONOTOMIDAE</b>	
Gnorimus nobilis 1					
		<b>SCARABAEIDAE</b>		<b>SALPINGIDAE</b> 5	
				Rhinosimus ruficollis 3	
				Rhinosimus planirostris 2	
<b>CLERIDAE</b> 4				<b>COLYDIIDAE</b>	
Thanasimus formicarius 3		<b>ANOBIIDAE</b>			
Tillus elonfatus 1					
<b>ELATERIDAE</b> 4		<b>TENEBRIONIDAE</b>		<b>RHIZOPHAGIDAE</b> 2	
Dalopius marginatus 3					
Nothodes parvulus 1					
<b>EUCNEMIDAE</b> 1		<b>ALLECULIDAE - LAGRIIDAE</b>		<b>NITIDULIDAE</b>	
<b>SCOLYTIDAE-PLATYPODIDAE</b>		<b>ENDOMYCHIDAE</b>		<b>CUCUJIDAE-SYLVANIDAE</b> 1	
				Uleiota planata 1	
		<b>LYCIDAE</b>			
<b>HELODIDAE</b>		<b>LYMEXYLIDAE</b>		<b>CRYPTOPHAGIDAE</b>	
				<b>CERYLONIDAE</b>	
				<b>MALACHIIDAE-CANTHARIDAE</b>	
				<b>LATHRIDIIDAE</b> 2	
		<b>EROTYLIDAE</b>		<b>COCCINELLIDAE</b>	
				<b>THROSCIDAE</b>	
<b>CURCULIONIDAE</b>				<b>CIIDAE</b> 1	
				<b>PTINIDAE</b> 1	
				<b>CHRYSOMELIDAE</b>	
		<b>MYCETOPHAGIDAE</b> 2		<b>MELYRIDAE</b>	
		Litargus connexus 2		<b>MELOIDAE</b>	
<b>ANTHRIBIDAE</b>		<b>MELANDRYIDAE</b>		<b>AUTRES COLEOPTERES</b>	
<b>BOSTRYCHIDAE</b>					

Figure 13: A typical sorting form.

## Labels

These are conventional location labels also containing the number of the record form (Figure 14).

Form TRONCAIS: 2005/08 F-03-Tronçais Strict Biological Reserve / Managed Biological Reserve /Managed Transparent/ <del>black</del> trap Date: 31.V. to 14.VI.2005 ONF Noblecourt leg	Form TRONCAIS: 2005/08 F-03-Tronçais Strict Biological Reserve / Managed Biological Reserve /Managed Transparent/ <del>black</del> trap Date: 31.V. to 14.VI.2005 ONF Noblecourt leg
Form TRONCAIS: 2005/ F-03- Tronçais Strict Biological Reserve / Managed Biological Reserve /Managed Transparent/black trap Date: ONF Noblecourt leg	Form TRONCAIS: 2005/ F-03- Tronçais Strict Biological Reserve / Managed Biological Reserve /Managed Transparent/black trap Date: ONF Noblecourt leg

Figure 14: Typical insect collection labels.

The most convenient and logical approach is to prepare the designations of the various trapping techniques and the various localities in advance on the same label. Then that all that needs to be done is to cross out the entries that do not apply and add the form number and date in indelible ink. Labels should be prepared on 160g/m<sup>2</sup> bristol board using a laser printer (if an inkjet printer is used the print will disappear when it comes into contact with alcohol).

## III – SORTING SAMPLES

Allow the sample (the content of a bag) to thaw out slowly because some insects will break up if they warm up too rapidly.

The content should be diluted in a thin layer of water in a shallow tray.

After having cleaned the sample by removing leaves and twigs, it should be **sorted by family (according to group)**. This sorting procedure must necessarily be carried out using a binocular magnifier. The various families can be separated out into different dishes (Photo 27) and then, according to the skills available, either identified or put back into storage for later identification or despatch to a specialist. This storage should be in bottles containing weak alcohol solution (45°) for re-examination in the short term or 70° for long-term storage, or on layers of filter paper. Particular attention needs to be paid to labelling all new batches deriving from sorting in order to maintain sample traceability.

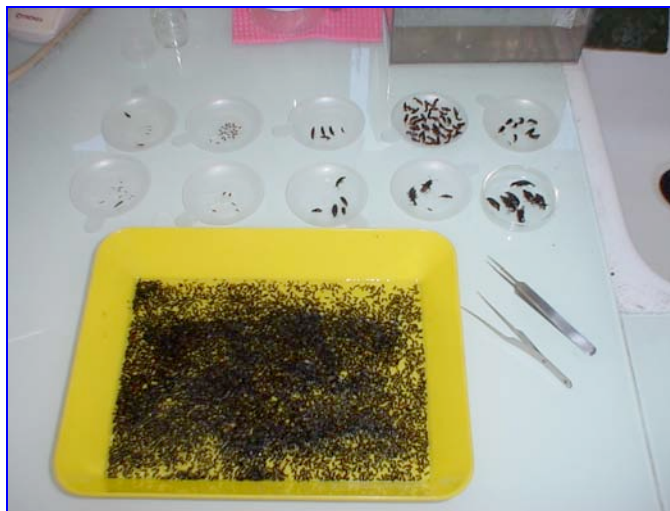


Photo 27: Sorting by family (picture: Arnaboldi/ONF).

## IV – IDENTIFICATION

Species identification can be carried out using identification keys available in the specialist publications usually available for given families (e.g. the *Faunes de France* collection<sup>5</sup>) (cf. identification handbooks by target group in Chapter 4). A reference collection is particularly useful in assisting this identification process. It can be built up gradually over the years and needs to be checked (i.e. validated) by an entomologist with thorough knowledge of the family concerned.

An example of each identified species must be retained in a collection. This applies especially to difficult-to-identify and uncommon species. It is a quality assurance approach enabling identifications to be checked after the fact. All the species found in samples and placed in the collection must be associated with the number of a sample record form noted on the location label: it must be possible to trace back to the form from the species (if there is a change in identification) and to find the species using the form.

### **Submissions to specialists**

In the event that identification proves impossible or is uncertain, it will be necessary to call on outside expertise, initially at regional level. Preliminary contact should be made before sending a sample in order to ensure that entomologists already staggering under a heavy workload with numerous insects to identify are not submerged further in samples. It is standard practice to sacrifice a few individuals when seeking the advice of a specialist.

The species should be mounted either according to the indications of the specialist or stored in a weak solution of alcohol (45°). In any event, the location labels should be enclosed with the insects.

### **Repackaging**

Hermetic tubes should be preferred (e.g. an Eppendorf type 1.5ml micro-tube for small insects, cf. photo below). Place the label inside the tube, checking that it is easily read from outside (take care to use high-quality ink that will last for as long as necessary).



**Photo 28: Typical tubes for storing samples (left: an Eppendorf tube, ref. Bioblock B51633; right: a Micrew screw tube with seal, refs Bioblock B14437 and B14457).**

NB: In the case of butterflies, they should not be kept in alcohol; it is preferable to place them in paper screws or on layers of cardboard (bristol card type) with carded cotton, after first drying them out.

### **Insects not in groups targeted by the inventory**

When carrying out an inventory, it is usual to collect in traps numerous species that are in groups that do not correspond with the initially defined objectives. All captured insects should be retained as a

<sup>5</sup> Cf. [www.faunedefrance.org](http://www.faunedefrance.org)

matter of principle. Following sorting by order or family, non-target insects should be stored in a bottle filled with 45° alcohol with a detailed label setting out complete details on the capture method (see above). These samples can then be studied at a later time.

## V - CONSERVATION

Samples should be kept in a dry place away from direct sunlight (discolouration risk). When they are stored in bottles or tubes filled with alcohol, the level of the liquid should be checked regularly (topping up if evaporation has occurred in order to ensure that the insects remain immersed).

Dry storage is not advisable other than for insects mounted in collection cases (mounted on pins or stuck to light cardboard bases). This is because insects, once dried out, can become highly brittle and appendages (legs, antennae, etc.) essential to identification often break off from the body during prolonged storage unless the conditions are extremely favourable, on carded cotton in a closed case (cf. photo below). If there is insufficient space available, it is a good idea to contact a regional museum of natural history in order to store certain reference collections in their reserves.



**Photo 29:** An example of storage in a rectangular case on a carded cotton base (Caubère case, ref. 546, measuring 60mm x 45mm x 8mm, or ref. 756 measuring 80mm x 65mm x 8mm).

## VI – MAKING USE OF NEW DATA

When submitting material for identification to specialist entomologists, they must be able to make use of data they judge to be of interest. Thus, in the event of a major scientific discovery (a species new to France or a species new to science) the person identifying that information must be able to publish it in the scientific journal of their choosing, including mention of the person or entity commissioning the study and the nature of the latter. Where the species is new to science, the person who first describes it is obliged to deposit at least one type (a holotype, and if possible an allotype) at the National Museum of Natural History in Paris.

## VII – MANAGING INVENTORY DATA

It is necessary to make provision for data computerisation from the outset. This is a key phase that will add value to the inventory and allow the sharing and pooling of the raw results at various levels (regional, national). Each inventory in a given forest is a new brick in the building of knowledge of the species (status, distribution, biology, phenology, etc.).

Various software programs are available for the management of data gathered by naturalists and scientists. In some cases organisations and private individuals have developed their own databases for personal use, while others have developed very comprehensive databases that they make available to

other users. Examples of this are the 'Data Fauna Flora' program of the University of Mons-Hainaut (Belgium), the *Réserves Naturelles de France* 'Serena' database and the ONF Naturalist Database.

In addition to storing the data, these databases allow them to be centralised and exchanged between users on the same network (*Réserves Naturelles*, ONF local offices, etc.), as well as the transfer of data to the regional environmental directorates (DIREN) to which they are attached.

## VIII – PROFESSIONAL ETHICS

In most cases, studies involve capturing insects in nature. Entomologists have a duty to focus studies on improving our knowledge of the insect world while at the same time contributing to the preservation of the environments in which those insects live.

Given the deterioration in the natural environment, while it is clear that the destruction of biotopes is a much more serious threat than the collection of insects, such capture as a goal in itself has ceased to be acceptable. The collection of material must always be justified by the pursuit of scientific (research, inventories, monitoring of entomological fauna, etc.) or pedagogical objectives.

For these reasons, it is appropriate:

- to limit the collection of specimens to a strict minimum on the basis of a sampling plan aligned with the study objectives;
- to use non-selective automated traps for long durations in the same sector only as an exceptional measure and to limit the number of such traps to the strict minimum necessary to meet the requirements of the relevant study;
- to optimise trap selectivity to target the group being studied in order to avoid wastage of biological material and elimination of fauna;
- to refrain from the deliberate capture of protected insects;
- to preserve the integrity of the biotopes being surveyed;
- to protect large fauna by using substances whose toxicity is the minimum necessary, and to reduce as far as possible the capture of micromammals and amphibians (protective grids);
- to conserve the captured material for later studies and to entrust material that is not studied to other specialists.

### **Obedience to regulations**

Entomologists must adhere to national regulations regarding the protection of nature on the national territory of the French Republic and European regulations where they are directly applicable to the Member States. No practice that runs counter to existing regulations can be envisaged without prior authorisation from the competent official departments. In particular, all inventories of protected areas (nature reserves, core areas of national parks, and the like) are subject to prior authorisation from their scientific services. Applications for permission to capture protected species are examined by the regional environmental directorates (DIREN). Some considerable time may be required to obtain such permission and account should be taken of this in drawing up the timetable for the study concerned.

### **Ownership of data, publication**

Raw data belong to the surveyor, or 'finder', who must retain the scientific benefit thereof (De Beaufort and Maurin, 1988) and the ability to use such data for later scientific work. Inventory data should be compiled in a final report submitted to the person or entity commissioning the study. They may then be published with inclusion of a reference to the sources of the scientific data (finder, identifier, scientific confirmation) as well as to the persons or entities commissioning the work and funding it, where applicable.

Ownership of the specimens collected must be clearly defined in an agreement signed prior to conducting the inventory. The building of a reference collection for a given site to be retained by the manager for teaching purposes for example can be beneficial, on condition that proper storage is provided, along with satisfactory upkeep. Otherwise, it is preferable for the specimens to be kept by the entomologists.

**To find out more:**

- Websites of suppliers of naturalist data management software:
  - ‘Serena’ software, *Réserves Naturelles de France*: <http://www.sciena.org/serena/>
  - ‘Data Flora Fauna’ software: <http://zoologie.umh.ac.be/dff/>
  
- Websites of equipment suppliers:
  - Eppendorf and Microw tubes from Bioblock: [www.bioblock.com](http://www.bioblock.com)
  - Caubère boxes and cases: [www.caubere.fr](http://www.caubere.fr).

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