Chapter 8

Sampling arthropods from the canopy by insecticidal knockdown

by

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Abstract

Insecticidal knockdown or canopy fogging is an easy-to-apply method to explore the canopy habitat, which harbours an abundant and diverse fauna of arthropods but which is still largely neglected in research. The method is sufficiently developed that a large proportion of the canopy fauna can be collected semi-quantitatively without causing much spatiotemporal disturbance. This requires the use of natural pyrethrum diluted in a paraffin-like carrier substance. Natural pyrethrum is highly specific to arthropods and quickly destroyed in sunlight without leaving persistent toxic substances in the ecosystem. The large dependence of the fogging method on the weather conditions are more than just compensated by the faunistic data allowing a tree specific analysis of the diversity, structure and dynamics of arboreal communities. Today, fogging produces more than descriptive data but is used in experimental research, like the biodiversity exploratories established in Germany, which aim at investigating the relation between biodiversity and ecosystem functioning.

Key words: Fogging, community, natural pyrethrum, pyrethroids

1. Introduction

I remember how astonished I was when I heard in a lecture about forest ecology at the beginning of my study how little we still know about the functioning of forest ecosystems and how far we still have to go before we might be able to use such complex ecosystems in a sustainable way. Evidence for this comes from the regularly occurring gradations of phytophagous or saproxylic insects, which cause enormous economic damage every year. How is this possible in a country with such a long history in forest research (Küster, 1998) I thought? Today, after 18 years of forest research I think part of the answer can be found in the canopy, a habitat that has simply been forgotten in the past.

One can say that the basics of modern canopy research lies in tropical rain forests where species diversity shows a maximum (Stork *et al.*, 1997; Linsenmair *et al.,* 2001; Basset *et al.,* 2003a). This was demonstrated by Erwin's work on canopy arthropods and his estimation of global species richness (Erwin, 1982). He applied the canopy fogging method, which was until then largely unfamiliar (Southwood, 1961; Southwood *et al.,* 1982), to individual trees of a lowland rain forest focusing on beetles in his analysis. From his data he concluded that global species richness must be much higher than the assumed two million species of plants and animals. His two-page paper caused a reorientation of biodiversity research, which focused on tropical forests for the next two decades.

Approximately since 10 years it is known that also trees in the temperate zone harbour a diverse and abundant fauna of arthropods. For example, in 705 fogging samples from individual tree crowns in Europe the number of free living arthropods varied between some hundred and 40.000 specimens (Floren, own data). Extrapolating these numbers to a single hectare of mixed deciduous European forest resulted in a conservative estimation of at least 1 million arthropods living in the canopy (Floren, 2008). These numbers alone suggest that the canopy fauna is of large importance for ecosystem processes and can not be neglected when analysing biotic interactions, energy fluxes etc., although this is still often praxis (Ellenberg *et al.,* 1986; Floren & Schmidl, 2008).

New and adopted methods were required and developed during the last years (Basset *et al.,* 2003b). Probably most often used are eclectors, flight interception traps and canopy fogging. The advantage of the fogging lies first in a semi-quantitative collection of arthropods that move on leaf and branches and second in a much better chance to assign the collected species to a particular tree allowing to picture the tree specific fauna with hitherto impossible accurateness (Sprick & Floren, 2007; 2008). The results of the different methodical approaches are difficult to compare because eclectors and flight interception traps collect only few specimens per day and need to be installed several months in order to get a representative faunistic sample (*e.g.* Floren & Schmidl, 2003; Müller *et al.,* 2008).

2. The operating mode of the fogging machine

As its very name indicates, insecticidal knockdown uses an insecticide as a killing agent, which is applied in the tree crown by a special fogging machine.

The machine itself is rather simple and easy to handle: a fuel-air mixture is ignited in a combustion chamber generating a gas column in the resonator pipe, which oscillates ca. 90 times per second. The insecticide is injected at the end of the pipe and dispersed into fine droplets of less than 10 micrometers (Fig. 1). Because the fog is warm and expels with high velocity it raises high enough to penetrate the canopy of temperate European trees.

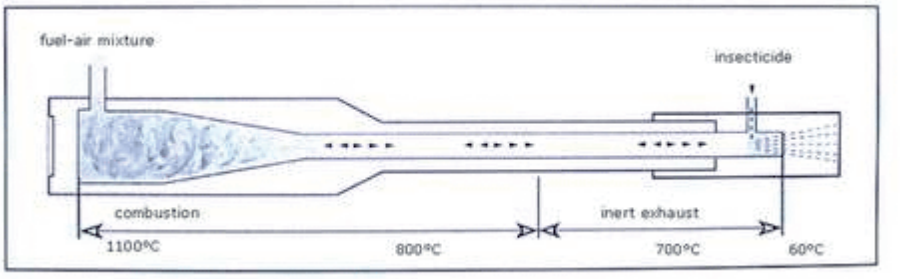

Fig. 1. The fogging machine (here an SN-50, operating manual SN-50, Swingtec GmbH, Germany) and its mode of operation.

The fogging machine is very loud (even when a noise protection device is used) and ear muffs are a must for anyone. Moreover, the machine is getting hot during fogging and often the burned patterns on the forearms of student helpers document their participation at a fogging project.

A one millimeter diameter of the nozzle through which the insecticide is injected produces a thick fog (Fig. 2) without depleting the fuel tank too quickly, allowing fogging several trees. During fogging the machine is usually held upwards. This, however, prevents a continuous fuel supply finally resulting in a disquietingly operating machine until it extinguishes. If this occurs, the expelled fuel-oil mixture inflames at the hot exhaust.

2.1. Insecticide and carrier substance

The insecticides used in fogging studies are mostly synthetical pyrethroids (*e.g.* Permethrin), which derive structurally from pyrethrins the main components of natural pyrethrum (NP). The first pyrethroids synthesized were photosensitive and broken apart by sunlight quickly. Therefore, pyrethroids became economically successful only after the development of photostability. Pyrethroids are contact poisons and characterised by a high knockdown – but a low knockout capacity. They were designed to replace organochlorine pesticides, organophosphates and carbamates, which are toxicologically and ecologically much more hazardous (Fromme, 2005). They persist in the ecosystem for several weeks but do not accumulate in the food web (Forth *et al.,* 2005).

Chemically, pyrethroids are esters derived from chrysanthemic and pyrethroic acids and an alcohol (Schulz *et al.,* 1993, Forth *et al.,* 2005). Due to the quick metabolisation of pyrethroids synergists are added as stabilizers and effect

enhancers, like Piperonylbutoxid (PBO), which inhibits the enzymatic metabolism in the arthropod. PBO itself is not toxic for insects and toxicity for mammals is low (Perkow & Ploss, 2007). The insecticide is usually diluted in diesel oil, which also contains synthetic additives serving as a carrier substance. Highly raffinated white oil can be used instead (for example Essobayol 82). The oil also causes the good visibility of the fog allowing to visually controlling the effectiveness of the fogging (Fig. 2).

From an ecological point of view, the negative implications of synthetic pyrethroides (next paragraph) can be avoided by using NP as an insecticide. NP is an old insecticide known for several thousand of years. In the medieval times it was known as Persian- and later as Dalmatian insect powder. Its characteristic properties: it is highly specific to arthropods, liphophilic, has a low vapor, and is quickly destroyed in sunlight due to its photosensitivity. It is only little poisonous to endothermic organisms and does not affect groundwater but it is toxic to fish. Main components are Pyrethrines (ca. 40%), Cinerin (ca. 10%) and Jasmolin (ca. 5%) (Schulz *et al.,* 1993). NP is extracted from various species of dried chrysanthemums (*Tanacetum cineraifolium* (Trevir.) Sch.-Bip. and *T. coccineum* (Willd.) Grierson. (Asteraceae)). They are cultivated and harvested on a grand scale in East-Africa. The price depends on the world market but is significantly higher than the price for synthetic pyrethroids (a 16 kg drum around 1500 €).

3. Effectiveness of natural pyrethrum on arthropods and endotherms

Pyrethroids are highly effective neurotoxins. The mechanism of action requires direct contact with the arthropod and is based on the blockage of sodium movement into nerve cells via inhibition of the enzymes adenosine triphosphate and acetylcholinesterase and the gamma-aminobutyric acidA receptor (Katz *et al.,* 2008). NP is highly specific to arthropods and possesses a high knockdown capacity while simultaneously having only a low knockout capacity. Furthermore, NP is highly repellent and used to antagonize hidden living arthropods. Pyrethroids do not or little affect plant pathogenic mites and well protected scale insects (Herve, 1985). Resistance to pyrethroids was observed after widespread application in the field and is based on an overproduction of esterases and an increase of mixed function oxidase activity (Khambay, 2002; Schröder *et al.,* 2009). In humans dermal absorption over the integument is poor. Pyrethroids are most effective when inhaled but quickly destroyed by hydrolases, enzymes that are lacking in arthropods. Pyrethroids are not stored in body tissue. Acute exposure causes reddening and irritations of skin, mucosa and the respiratory passages (Forth *et al.,* 2005). Pyrethroids may cause contact allergies (Fromme, 2005). The central nervous system might be affected from chronic exposure (Erikson & Frederiksson, 1993). Using synthetic pyrethroids requires therefore the abidance of safety measures, like wearing a respirator.

Fig. 2. Applying the fogging in the field (Photos by A. Floren).

4. Dosage of the insecticide and duration of the fogging

The great dependence of the fogging method on the weather conditions is problematic. Fogging cannot be carried out during rain, when there is too much dew or strong air currency. Generally, the best time is shortly after sunrise or before sun set when there is no thermal up wind. Due to this, fogging is rarely possible during the day. Attention should be paid that some groups of arthropods are more active in late warm afternoon, what may affect the results. Depending on the local conditions fogging is applied between three to ten minutes. In order to guarantee full impact of the insecticide, the exposure time of the fog in a tree crown should be at least three minutes.

Most trees in Europe reach heights of 30 to 40 meters, which could be reached by the fog under favourable conditions (Fig. 2A, B). However, such heights are not reached when air currencies prevent the fog from rising vertically. Given such conditions, fogging should be performed in the tree crown (Fig. 2D), from large ladders (Fig. 2C), or if necessary from a larger distance so that the fog can slowly travel to the tree tops. Pointing the fogging machine along another tree trunk may serve the expelling fog to ascent a few meters higher.

A 1% concentration of the actual insecticide is sufficient to guarantee a high knockdown effect (Adis *et al.,* 1998). Very quickly, small soft skinned arthropods come down, like wasps (Hymenoptera), various groups of Diptera and Psocoptera. Spiders try to escape at their silky thread, only to end up in the collecting sheets. Larger beetles and grasshoppers can be heard dropping down still after one and a half hour following fogging. Therefore, an insect dropping time of two hours should be allowed before all specimens are collected with a fine brush and a kitchen shovel and stored in 70% ethanol. A concentration less than 1% will only numb robust arthropods temporally and they recover quickly, indicating that fogging can also be used to collect living arthropods (see Paarmann & Kerck, 1997). As some arthropods run hectically around after dropping down, the collecting sheets should be suspended so that specimens skid to the centre (Fig. 2).

Regularly it is criticized that the arthropods, obtained by fogging, are not preserved in an adequate way, because all are stored in ethanol. However, the necessity to process all samples immediately (usually several samples a day) make it impossible to treat different groups of arthropods in different ways.

Furthermore, some of the arthropods are too small to be visible and can only be sorted in the lab using a stereomicroscope. This is the only way to guarantee that all specimens will finally reach the specialist. The storage in ethanol is therefore the only feasible way when specialists are not on site to pick their favourite groups personally.

Fig. 3. Due to the high knockdown capacity of natural pyrethrum the 'insect rain' starts immediately after fogging. The fogging of this oak tree resulted in more than 40 000 arthropods (Photo by A. Floren).

5. The study area, installation of collecting sheets and tree selectivity

The ground vegetation beneath the study trees must be cleared from high vegetation. Ideally, the collecting sheets should be suspended preventing soil arthropods from entering the sheets. Thus one can avoid later discussions whether ecologically interesting species were in fact sampled from the canopy. For the same reason the collecting sheets should be cleaned after usage.

A word about the collecting sheets: while collecting funnels were round or rectangular and suspended on ropes installed above the ground at the beginning (Erwin, 1983; Stork, 1987; Floren & Linsenmair, 1997), I am using only stable plastic sheets (mainly pieces of 4 x 5 meter), which are easy to transport and quickly mounted (Floren & Schmidl, 2003). Their plain surface prevents arthropods from getting caught with their tarsal claws. The use of large plastic sheets is not only quicker but makes it also easier to cover most of the crown projection (80-90% can be achieved mostly). This is desirable in order to get a reliable subsample of the arboreal fauna. However, many studies still use only few square meters, loosing most of the dropping specimens and therefore a lot of valuable information. At the sides of hills and mountains or in savannahs where wind is coming up quickly after having performed the fogging, the suspended collecting sheets must be fixed on the ground in order to prevent them from being turned upwards thereby loosing all the arthropods. This method is preferred to

weighting the collecting sheets with stones or pieces of wood because in this case contamination with soil arthropods can occur.

In temperate forests tree selectivity is achieved by simply sparing out branches from neighbouring trees and exact positioning of the collecting sheets beneath the study tree. This is quite important because species abundances often allow inferring on tree specific association (Floren & Gogala, 2002; Sprick & Floren, 2007, 2008). Guaranteeing tree specificity can be a larger problem in tropical forests, however, where several species of trees can grow within a few meters. In order to exclude collecting arthropods from different neighbouring trees or trees of the higher canopy that may partly cover the crown of the study tree, I stretched out a large cotton cloth above the study tree the day before fogging in previous studies (Floren & Linsenmair, 1997). This approach has proven very efficient but the amount of work is large. Alternatively, the search time for suitable tree species is much higher and can usually been carried out only with the help of a botanist.

Fig. 4. Fogging a young tree by using a large cotton sac. (Photo by A. Floren).

The fogging can also be used to collect the arthropods from young trees or bushes. This requires to carefully installing the collecting funnels beneath the tree without causing disturbance. A cotton sac is then quickly put on the plant and the fog blown inside for a few seconds from below (Fig. 4). After shaking the tree the arthropods can be sampled a few minutes later.

6. Disturbance generated by fogging

Fogging was considered to be a mass destructive method for a long time. This negative label can be adjusted when the fogging experiment is applied professional, including the usage of natural pyrethrum. After being applied to the tree, the fog mixes with the higher air and is quickly blown away by the wind so that already a few minutes after the application, nothing indicates that a fogging experiment was performed. During inversions, which are sometimes observed, the fog can be pushed downwards again. Such situations can look ghastly and although no harm emanates from the NP, people might feel threatened. The disturbance caused by the fogging is spatiotemporally limited and the effect of the insecticide decreases quickly with increasing distance from the study tree; already in hundred meters distance from the place of fogging specimens recover quickly (Floren & Schmidl, 2003).

The question how quickly tree specific communities recover after fogging has not gained much attraction. The few results available show a large variability; for example Stork (1991) collected only 20% of the original number of specimen after re-fogging a tree in Borneo, an effect that might be caused by the persistent insecticide, however. On the other hand approximately the same number of specimens was collected in a re-fog 10 days after the initial fog in a forest in Peru (cited after Stork & Hammond, 1997). In a more comprehensive study Horstmann *et al.* (1999) found that the re-colonization of fogged trees by small Hymenoptera in a lowland rain forest of Borneo was still incomplete after periods of 7-19 month. Generally, re-fogging data vary largely indicating that the rearrangement of communities is largely unpredictable (Floren, 2003, 2008). In contrast, communities of arboreal arthropods in temperate regions with their pronounced seasonality seem not to be distinguishable from those collected in the following year.

Due to high visibility of the fog one should bear in mind to inform the local fire brigade in order to prevent a needless move out as I had experienced a few times. As there is no fire brigade in tropical regions it is all the more important to inform the people living in the surrounding area about the project and the harmless of the fog.

7. Comparability of fogging investigations

Adis *et al.* (1998) published recommendations for the standardisation of fogging experiments arguing for better comparability of data. One can assume, however, that fogging, if applied properly, produce comparable data independent whether it was carried out in the tree or from the ground or what type of collecting sheets were used. More important is the underlying question of the study. For example, are data on seasonal effects comparable with those on stratification? How did the local weather conditions affect the quality of the data etc.? Comparison of absolute numbers of arthropods (like specimens per square meter) are more difficult to interpret because species abundances depend on small scale factors, which are difficult to measure, like microclimatic conditions, differences in habitat structure etc. Furthermore, such comparisons require the consideration of tree

specific parameters like crown size, crown volume, percent leaf cover, diameter of trunk in breast height etc. Leaf cover, is of particular importance. It can be measured as the relative proportion of leaf area against the sky (Floren & Linsenmair, 1998). The standardised number of arthropods (SA) is then:

SA = (arthropods/sqm) * 100/rel. proportion of leaf cover.

A canopy community is not sampled completely by insecticidal knockdown. Indispensable failures derive from arthropods drifting away during their way down or which miss the sheets, specimens that skitter away or those that remain on the leaf or in bark crevices. One can assume to collect another 5 to 20% of arthropods when the collecting sheets remain in place until the other day (Floren & Schmidl, 2003). It must also be mentioned that fogging does certainly not sample arthropods living in epiphytes or in suspended soils, like detritus accumulations, ferns etc. (Yanoviak *et al.,* 2004).

8. Which groups of arthropods are sampled reliably?

It is not surprising that fogging collects mainly arthropods that live free in the tree, while endophytic species are undersampled (mainly species of the voluminous wooden body – stem, branches and bark – and small species that stay in bark crevices or in flowers etc.). Mites, Collembola and Thysanoptera vary greatly between fogged trees and they are certainly much more numerous than reflected in the fogging samples (references in Floren & Schmidl, 2008). As fogging is not the most appropriate method of trapping these groups, I do not consider them in community level analysis.

Time and again the question comes up whether fogging samples also large animals, like stag beetles or fast flying insects. The answer is yes, there are good-flying insects in the samples, like horseflies (Diptera), but it is not known whether they are collected quantitatively. Fogging does not sample large butterflies, simply because they are rarely found in the crowns, while small moth can be quite numerous. In this context one should consider that a fogging experiment is a brief operation and that the sampled part of the canopy is rather small reducing the chance to collect less frequent specimens.

9. Concluding remarks

Insecticidal knockdown makes it possible to collect arboreal free living arthropods in a semi-quantitative way, allowing characterising tree specific communities in their diversity, structure and dynamics. In this respect fogging is unique. Although arthropod abundance in the trees is high one can just ask as well why species do not reach even higher numbers. For example, common species, like phytophagous *Rhynchaenus fagi* (Curculionidae, Coleoptera), can be collected with more than 3000 individuals per tree, but in relation to all leaf of an individual tree this number is comparatively low, too.

While fogging was used to collect and to characterise the arboreal fauna of different trees during the last years (references in Floren & Schmidl 2008) it is applied today also in experimental research, for example to analyse

recolonisation dynamics, in predator exclusion experiments or to analyse changes in canopy communities after manipulation of resources (http://www.biodiversity-exploratories.de).

Following the recommendations given in this paper we can assume that canopy fogging produces a representative picture of the canopy assemblage. In Europe this requires to fog between 5 and 10 trees per tree species and arthropod groups in most cases. Tables summarising the advantages and disadvantages of insecticidal knockdown have been already published (Adis *et al.;* 1998 Stork & Hammond 1997; Basset *et al.*,2003b). Therefore, I do not want to add another list but make the following general remarks:

- Fogging is a highly effective method of collecting canopy arthropods but one can make the best of the data only when the whole community of canopy arthropods is sampled. By doing so a surprisingly high efficiency is achieved as demonstrated by a study of canopy spiders in a SE-Asian lowland rainforest, where different forest types could be distinguished by singletons alone by using advanced statistical methods (Floren & Müller, submitted).
- One should avoid false expectations. Insecticidal knockdown is not universally applicable but has, as any other method, its pros and cons. It allows a quick characterisation of the canopy community but can not replace other approaches like selective searching for *e.g.* cryptic or endophytic species.
- Finally, it should be noted that due to the large dependence on the weather conditions a fogging experiment can not be forced and field work is more unpredictable than applying different methods.

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