# FIELD AND LABORATORY TECHNIQUES IN PLANT COMPRESSIONS: AN INTEGRATED APPROACH

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**Abstract.** This paper presents and discusses field and laboratory techniques applied to fossil plant compressions, with examples and details related to the the current practice at the Laboratory of Paleobotany within the Research Centre of Coal Geology and Environmental Sciences, University of Bucharest. Field techniques include plant collecting, while laboratory techniques include mechanical techniques (splitting, breaking, and detaching), storage techniques (cabinets and drawers), chemical techniques (cuticle analysis), recording techniques (cards and databases for hand specimens and taxa), investigation and illustration techniques (microscopy, photography and drawing), and library techniques (books, reprints organization, reference management and online publication). Both field and laboratory sets of techniques are approached in order to emphasize the need of preserving preliminary data and results in an open, standardized, logic and integrate manner, so that access to any data sets, to preliminary results and to published results should be granted anytime, in effortless and ethical conditions. Integrating these techniques implies also careful labelling and storing paleobotanical material, cards, databases, and granting open access to the whole system for the scientific community.

**Keywords**: Field techniques, laboratory techniques, compressions, Paleobotany, integrated approach.

## INTRODUCTION

Field and laboratory techniques applied in Paleobotany were (and still are) discussed and published consistently. Jones and Rowe (1999) edited a synthesis of paleobotanical and palynological methods, with historical insights and updates. Fossil plant collections (hand specimens, biological slides, thin sections, etc.), field documents, cards, photographs,or databases represent the repository of preliminary data sets and of preliminary results, granting a coherent base for valuable, published scientific results. Preserving collections, data sets and preliminary results is essential to demonstrate the accuracy of published results, such as new species, new phytostratigraphy, new paleoecological results etc. Therefore, preserving them is a necessary ethical attitude and a necessary measure against any involuntary academic misconduct. In this paper, a series of examples are given related to the current practice at the Laboratory of Paleobotany within the Research Centre of Coal Geology and Environmental Sciences, University of Bucharest.

## FIELD TECHNIQUES

Collecting fossil plants in the field requires techniques allowing maximizing the number of samples and recording, in the highest detail, the information yielded by the sedimentary and structural context of outcrops. Ideally, when time resources, collecting (weather, quality of outcrops, accessibility, rock types, and conservation status) and transport conditions are optimal, the highest possible number of samples gathered is reached only when the outcrop has no more fossils to offer. In situ collecting in underground mining works, open cast mines or natural outcrops should always be preferred to sterile dump collecting, as the stratigraphic and paleoecological context can thus be recorded in detail. Nevertheless, waste dumps may offer wide opportunities for collecting, especially when they yield a rich or a well preserved flora. An introduction to collecting methods in the field was given by Rowe and Jones (1999).

Precise *in situ* collecting can only be undertaken by detailed counting of sedimentary layers, followed by detailed photography and drawing, in order to record the sedimentological and paleoecological information. Sample counting and labelling can be done using pre-labeled stickers with printed inventory numbers (McElwain et al., 2007), or it can be done by using the site number. The first method is indicated for time controlled collecting, while the second is preferred for collecting without time constraints.

Splitting hand specimens in outcrops should always follow the best preservation technique for the fossil fragments; this operation should be postponed when more delicate tools, such as needles or fine chisels are needed. The use of geological hammers for sedimentary rocks (such as those produced by Estwing) and chisels of various sizes increase the quality of the collected material and decrease the fatigue related to vibrations during splitting specimens.

Wrapping hand specimens with newspapers proved to be the best solution, especially when it was done using duct tape. Fine samples, such as very thin sandstone samples, should be carefully wrapped and kept for transportation in rigid boxes filled with supplementary paper or plastic flakes. Labelling the wrapped specimens is important, for recording the site and notebook number; it should be done only with a permanent, waterproof marker. It is also recommended to write these numbers in several places on the newspaper covering the specimens.

## LABORATORY TECHNIQUES

Laboratory techniques in fossil plant compressions include the following:

1. mechanical techniques (splitting, breaking, and detachment);
2. storage techniques (cabinets and drawers);
3. chemical techniques (cuticle analysis);
4. recording techniques (cards and databases for hand specimens and for taxa);
5. investigation and illustration techniques

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(microscopy, photography and drawing);

6. library techniques (books, reprints organization, reference management and online publication).

**1. Mechanical techniques**

Splitting hand specimens and detaching the plant compressions is essential for obtaining the best information, including anatomical and morphological characters, from plant fragments. Fairon-Demaret et al. (1999) detailed the mechanical methods used for detachment, including those using chisel and needle. A pocket knife is very useful for fine splitting of sandstone samples, while needles are essential for fine and very fine detachment, as well as for cuticle and sporangia sampling. Needles can be used for extracting leaf material in compressions, for cuticle analysis, in order to macerate the leaf fragments. Fine, sharp pointed tweezers are very useful for handling compression fragments. A pointed chisel with a pyramidal, three-surface tip is extremely useful for separating sandstone microlayers when hitting perpendicularly to the surface. This is an ideal tool when opening new surfaces of plant fragments, as the microlayers can be finely removed even from large areas of hand specimens. White glue for wood works quite well for assembling together sandstone fragments containing plant rests. Collecting cuticle fragments can be improved by using a small plastic sheet loaded with static electricity, so that the cuticles will stick to its surface.

**2. Storage**

Storage of hand specimens can be organized in drawers or trays, arranged in cabinets of various sizes and shapes. Compactors are the most efficient storage solutions, but they are also the most expensive. Hand specimen boxes are very practical, although they are not essential. Putting together hand specimens from the same occurrence in groups of designated drawers is a pre-requisite for easier handling and searching. For easy curatorship, each drawer should receive a unique number, visibly written and irrespective to the cabinet number, so that finding a selected hand specimen should be a quick operation. Plastic covers for hand specimens inside the drawers are helpful for anti-dust protection. Plastic trays with various depths are very useful as temporary storage solutions in the lab or while transporting material, after collecting it. Keeping electronic record of drawers and of their contents (using Excel software, for example) is simple; this stage speeds a lot the search for labelled and recorded hand specimens.

**3. Chemical techniques**

Cuticle analysis includes maceration of plant compressions and mounting of cuticles in biological slides, or SEM stubs (Kerp, 1990, Kerp and Krings, 1999). After isolating the compression fragment from its sedimentary matrix, a mild attack with hydrofluoric acid (HF) and hydrochloric acid (HCl), separated by successive water treatments can ease the main attack with reagents such as the Schulze Reagent or with nitric acid ($HNO_3$), used in order to oxidize and dissolve the coal matter and to separate the cuticles. The main attack with Schulze Reagent implies the treatment of the leaf fragments with a mixture of nitric acid ($HNO_3$, between 20-70 % concentration) and potassium chlorate ($KClO_3$) in a glass tube, under constant survey in order to avoid dissolving the cuticles. The mixture can be used either in the form of an aqueous solution (wet Schulze Reagent) or it can be obtained on spot by pouring nitric acid and a few potassium chlorate crystals (dry Schulze Reagent) over the leaf fragments. After the oxidizing attack the cuticles are washed, neutralized with potassium hydroxide (KOH, 5 % concentration), washed again and mounted on biological microscope slides or on SEM stubs. Plant fragments react very differently to Schulze Reagent, therefore it is necessary to continuously check the oxidation process in order to avoid complete dissolution. Sometimes, depending on the maturation of the organic matter, staining cuticles with safranin is necessary. Kaiser Jelly is a recommended solution for mounting cuticles even though it is a toxic substance, because the classic gelatine jelly is less resistant in time. Sealing the biological slides can be done with wax or with nail polish for avoiding fungal attacks. A good fume hood with HF standards is necessary for the chemical treatment of fossils, with an efficient ventilating system active inclusively in ventilated cabinets for acid storage.

**4. Recording techniques of hand specimens, slides and taxa**

Labelling hand specimens in the lab can be done using the notebook data in the following order: site number, field notebook number, layer number and sample number (Popa, 1998), such as P40/C2/S5b/23A meaning Site (“point” or outcrop) no. 40, Popa field notebook no. 2, base of Layer no. 5, hand specimen no. 23, counterpart A (if the specimen was split and both part and counterpart were kept). This technique has two advantages: 1. it records the precise occurrence of the fossils within the inventory itself, and 2. it always provides a unique inventory number for any hand specimen collected, therefore for each leaf fragment. To this code a curator can also add an inventory number for the scientific collection, if needed. Labelling hand specimens using white Tipex paint is ideal, as such paint can support all types of markers, including pencils. Long lasting writing markers should be preferred, that can also smooth surfaces when they are too rough for writing.

Recording the hand specimens on cards is indispensable for correct, clean records of scientific collections. Cards represent a physical support for the collection’s inventory and they can always be digitalized either by scanning or by copying their contents in a database. Basically, a card should include: 1. the record number or inventory code; 2. a simple sketch of the hand specimen, with the numbers of identification of each fossil fragment on it (in this way, each plant fragment receives a unique number, such as P40/C2/S5b/23A/F3); 3. a list of taxa assigned to each fossil fragment on the hand specimen; 4. occurrence information; 5. publication information (if the hand specimen belongs to a type collection); 6. preservation degree; 7. sampled fragments for subsidiary analysis. Such information can be easily stored on cards half the size of an A4 paper or a third of a Letter sized paper (http://mepopa.com/forms), organized as a single table, with the sketch containing the fragment numbers on its back.

Recording biological slides containing macerated cuticles, *in situ* spores and pollen, disperse cuticles or palynomorphs require generally simple tables printed on A4 paper, bound together. Such records should always contain: 1. taxon, 2. slide number; 3. hand specimen and plant fragment number; 4. observations. Boxes with biological slides can be kept on bookshelves, in designated areas, preferably next to the microscopes.

Various database software solutions can be used for hand specimens records (*e.g.* cards), such as Microsoft Access, Excel or Filemaker.

Recording taxa is essential for correct taxonomic and systematic results and for indentifying the nomenclatural correct, valid names. Previously published taxa can be recorded on individual, pre-formatted cards for both genera and species names (http://mepopa.com/forms). These cards should include: 1. the names according to the Botanical Code of Nomenclature; 2. systematic affinities, 3. occurrence; 4. synonymy lists; 5. lists of junior synonyms; 6. reference lists; 7. personal observations and notes; 8. drawings; 9. lists of hand specimens containing the specific taxon recorded. These forms can be easily printed on A4 paper. Another useful type of form is represented by pre-formatted, blank differential tables for each genus, which can be produced by listing characters versus species; it can be printed on A3 paper for easily connecting tables for genera with numerous papers. Differential tables are particularly useful for identifying the valid species when listed all together with their characters. Tables can be designed for specific macroscopic and microscopic characters of every genus, choosing to list all or the most significant characters for identification of the species.

Oriented, specific information such as taxa lists, plant occurrences, collection information and illustration information should be kept in separate files and carefully recorded.

## 5. Investigation and illustration techniques (microscopy, photography and drawing)

Microscopy in the study of compressive fossil plants includes optical, SEM and TEM techniques. Optical microscopy is essential in cuticle analysis and in spore and pollen investigation (Kerp and Krings, 1999), accomplished with several methods: Bright field (BF), phase contrast (PC) and epifluorescence (EF), while dark field (DF) is rarely used. Optical microscopy using a transmitted light microscope is usually carried out with objectives with magnifications such as 5X, 10X, 20X, 40X, 63X and 100X (for the last one, immersion oil is used). In the Laboratory of Paleobotany, we use a Carl Zeiss Axioscope A1 microscope with these 6 objectives, and a Carl Zeiss Stemi 2000-C dissecting microscope. Both tools include dedicated Canon cameras, attached with tubular mounts to the microscopes and connected at their turn to a graphic workstation. Bright field (BF) investigation works perfectly with cuticles and palynomorphs, as well as with any thin section for calcareous algae or permineralized tissues. Phase contrast can be sometimes useful for particular cuticles or palynomorphs (PC), while, for some palynomorphs, dark field investigation (DF) is used occasionally. Epifluorescence (EF) is suitable for compressions, if their fluorescence reaction is high. When this is possible, epifluorescence microscopy can be spectacular, with results similar even to SEM. In the Paleobotany Lab, the Axioscope A1 microscope is equipped with an epifluorescence source delivering enough ultraviolet light for very good results in surveying Triassic, Jurassic, Cretaceous, Miocene and Pliocene cuticles. Even low ranked coals, such as lignites from Oltenia show an excellent epifluorescence reaction, while higher ranked coals such as bituminous coals or anthracites from the South Carpathians show a weaker epifluorescence reaction. Objectives with a lower magnification for the dissecting microscope are better for surveying pinnules and venation. SEM techniques in Paleobotany require mounting the macerated cuticles of *in situ* spores and pollen on stubs, in order to be coated with gold (Collinson, 1999; Popa and McElwain, 2009). Mounting the fragments on stuns require also getting the samples dry before introducing them in a gold sputter. TEM methods applied for cuticle ultrastructures (Taylor, 1999) are more complex, as detailed by Guignard et al. (2004).

Microscopy software is particularly useful for image processing and for archiving data acquired with both types of microscopes. Carl Zeiss produces Axiovision software, designed for image analysis, enhancement and storage, with several modules of which two are of particular importance for fossil plants: Extended Focus and Asset Archive. Extended Focus permits to acquire photographs taken at several focus levels of the same microscopic field, each of them having clear areas depending to the focus used to capture the photographed microscopic subject. These photographs are then automatically compiled through several mathematical algorithms for obtaining a clear field all over the final micrograph, thus extending the depth of focus for the whole image. Asset Archive stores linked images and text within the same files with the ".zvi" extension in a database which can be searched through keywords, species names, occurrence, age, inventory numbers or biological slide numbers. Moreover, it is a flexible tool for extended use. Such a database for microscopy and macroscopic information is helpful for rapid queries and selection of the best shots of fossil plants, in order to rapidly prepare plates and text-figures. Axiovision also permits direct annotations and graphs on photographs, for temporarily emphasizing various anatomical characters. Axiovision, with its modules, is only a solution for data processing and archiving in Paleobotany and Palynology, among many others produced by Leica, Nikon, Olympus or any other major microscope manufacturer. The graphic station in the Paleobotany Lab is equipped with two LCD monitors, for an enhanced and fast use of all commands in Axiovision.

Macrophotography is a widely used technique in Paleobotany (Rowe, 1999), and it is essential for illustrating fossil plants, although compressions are notoriously difficult to photograph due to lack of contrast between fossil fragments and their sedimentary matrix. Contrast, brightness and white balance can be finely tuned through various methods, ranging from illumination, lenses, and cameras to software enhancement. Using a sturdy copy stand eases the photographic work, especially when the camera is attached to a strong, mobile holder. Kaiser produces several types of copy stands, to which lateral lights can be attached. Illumination is essential for good quality photographs, and at least two lateral lights should be used. Lateral illumination increases image quality as it can increase the contrast for venation, leaf margin or reproductive structures, and induces shades for any micro-relief representing morphological characters. Personally, I use two lateral, fluorescent lights bought from Ikea, mounted as close as possible to the hand specimen, so that all morphological characters will be visible through micro shading. Light intensity is not important as long as there is no indirect light coming from windows or from artificial light sources. Photographing in a dark room with lateral lights is the best method, as it avoids any parasitic light. Sometimes, the best illumination is provided by direct sun light. Distilled water is useful for low contrast specimens; in this case submerging them in distilled water can be very efficient. The lowest aperture is ideal to use, as it maximizes the depth of focus, even when photographing dark specimens, which require long exposure times. This is why a good quality copy stand is necessary too, as well as a wired trigger for the camera, for avoiding any shakes while taking a long exposure photograph. High exposure times permit also to select a lower film sensibility in order to avoid digital noise and ghosts. A DSLR professional camera is preferred, rather than compact cameras, although the second type recently comes with excellent lenses which allow taking so-called super macro photography. This is also the case of Canon Powershot S3IS, which I used extensively during the last years, both in the field and in the lab, with good results. A Panasonic Lumix DMC-L10 DSLR camera which we acquired for the lab is also a good choice for macrophotography, with a proprietary Four Thirds mount for macro lenses such as the Olympus Zuiko Macro 35mm, a clear and simple dedicated, macro lens which I also successfully used. A camera LCD monitor which can be tilted and swivelled is very useful for cameras mounted on a copy stand, as it permits a quick and easy view over the photographed object. Cameras with LCD monitor that cannot tilt and swivel are difficult to use when mounted on a copy stand. Using filters, such as the circular polarizing filters, also increases contrast (Pott and McLoughlin, 2009), especially in the field, for obtaining better pictures of outcrops, in both shade and sun. I have rarely used tripods in the field, while in the Paleobotany Lab I found them useless when compared to a copy stand.

Drawing using vector methods over fossil plant photographs usually provides high accuracy reconstructions and anatomical drawings. The ordinary softwares for vector drawing such as CorelDraw! and Adobe Illustrator imply importing a bitmap image (usually a jpg file) of a fossil in a vector file, defining several layers of objects, drawing over photographs and then deleting the bitmap image. The result is always a detailed, fine vector drawing emphasizing the type characters in systematics and taxonomy. The same technique is used for drawing occurrence maps and stratigraphic logs in paleobotanical or geological manuscripts.

### 6. Library techniques

A professional Paleobotany library is basically a research tool as essential as any other tool used in Paleobotany such as hammers, chisels, microscopes, lenses or cameras. Keeping a well organized library equals to keeping microscopes sharp and well maintained and tweezers clean, and should be treated with maximum care. Books and reprints management includes the proper storage of printed or digital paleobotanical information such as books, reprints, periodicals, reports, maps, manuscripts, etc., so that search and retrieval for this kind of information should be fast and efficient, yet friendly to the paper material.

Large, dry bookshelves are essential for keeping a good, functional library. Reprints, and sometimes books, can be kept in cardboard boxes, labelled with the author's name and initials, for better organization, for easier queries and for keeping them out of dust reach. Separators should be used between sections containing different author initials within each cardboard box. A library should be split in various subdivisions, for easy reach to the printed material, especially when it is used for systematic identification of plant material. Examples of sections can be Palaeophytic Paleobotany, Mesophytic Paleobotany, Neophytic Paleobotany, Palynology, Paleobotanical and Palynological Methods, Biographies, Abstract volumes, Paleobotanical illustrations, and Paleobotany newsletters.

A reference management software is essential for a lucrative, well kept Paleobotany library, as well as for any other type of scientific, professional library. Several options such as EndNote, Mendeley, and Biblioscape as retail solutions or Zotero and Refbase as open source solutions are essential not only for the management of the printed and digital information of the library, but also for easier manuscript preparation and for online, global search for scientific information. Personally, I have been using Endnote since 1995, when the second version was released, feeding it constantly with references and systematic information (lists of genera and species included in the recorded references). A software of this type permits to: 1. record any type of printed or digital source of information, manually or automatically; 2. copy and link in its own database any type of file related to the work recorded, which comes extremely convenient when dealing with PDF files of image files; 3. search online for original references in all major scientific databases and libraries, 4. link to Researcher ID, Web of Knowledge, Web of Science and to a personal, online storage account (on Endnoteweb.com); 5. prepare and automatically format reference lists in manuscripts of research papers or in any other types of papers, depending on the chosen publisher. Moreover, configuring properly and feeding Endnote with keywords and taxa published in each recorded work, allows searching and finding, in the shortest possible time, the right papers needed for precise taxonomical studies. For example, when searching for papers recorded in the library dealing with species of the genus *Weltrichia*, Endnote would retrieve an alphabetically ordered list of printed or digital works dealing with all species of *Weltrichia* in its database, indicating the cardboard box in which the reprints or books are kept, or showing the pdf files of various articles dealing with any species of *Weltrichia*. Such a search can be afterwards extended online, for finding even more information (pdf files included) than that recorded from the own, internal library.

Posting online research papers and books in pdf format increases the number of citations and it improves the way the published works are used by the scientific community, therefore increasing the scientific impact of publications. Web design software is very diverse, such as Adobe InDesign, Macromedia Dreamweaver MX or Microsoft Frontpage, and it allows easy editing for any kind of scientific websites. An example related to online paleobotanical expression is *Mihai's Paleobotany Chronicles*, at http://mepopa.com , where research articles, books, systematic information, occurrence of fossil plants, taxonomy and educational materials are uploaded and maintained periodically. An online solution maintained by an author eases the dissemination of scientific information, and increases the educational success. Special attention should be given to copyright, as the major science publishers usually oppose to uploading copyrighted articles.

## CONCLUSIONS

Preserving original data sets, fossil collections and preliminary paleobotanical results in an organized, permanent manner is essential for rigorous scientific research and advancement in the field. Fossil plant compressions imply a series of specific techniques in the field, such as typical collecting, and in the lab, such as mechanical ones, storage, chemical ones, recording, investigation and illustration, as well as library techniques; all these should be approached in an integrative manner in order to preserve data sets and results for an open, public, and ethical access. Scientific collections, documents, records, databases and libraries should be kept organized, mapped, standardized, online and open for the scientific community.

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