Review of Palaeobotany and Palynology 259 (2018) 142–158

Contents lists available at ScienceDirect

Review of Palaeobotany and Palynology

journal homepage: www.elsevier.com/locate/revpalbo

Effects of stepped-combustion on fresh pollen grains: Morphoscopic, thermogravimetric, and chemical proxies for the interpretation of archeological charred assemblages

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ARTICLE INFO

Article history:
Received 2 June 2018
Received in revised form 5 October 2018
Accepted 6 October 2018
Available online 11 October 2018

Keywords:
Pollen
Bee-breads
Polar – equatorial diameters
Brightness
Tectum
GC–MS
IR analysis

ABSTRACT

Macroscopic charred remains have long since been studied in archeology, archaeobotany, and palaeoenvironmental research. Despite this long tradition, very little attention has so far been given to microscopic charred botanical remains, apart from microcharcoal. The discovery of extraordinarily well-preserved fossil bee-breads and fragments of charred honeycombs in an Etruscan settlement in northern Italy prompted us to perform a combustion experiment on fresh bee-breads, aimed to study the main morphological, structural and chemical modifications induced by heat on pollen grains and whole bee-breads. The experiment enabled the observation and quantification of changes in overall shape and size, brightness, *tectum* structure and sculptures of pollen grains, as well as changes in the chemical composition of whole bee-breads. Data obtained on fresh material were then applied to archeological samples from the Etruscan Forcello settlement for inferences on the combustion temperatures reached during the fire that destroyed the site.

1. Introduction

1.1. The potential of pollen carbonization in charred assemblages

Carbonization is one of the most common processes behind the preservation of macroscopic organic remains in the palaeobotanical and archeological records (Spicer 1991; Jacomet 2007). The study of macroscopic charred remains has become a routine activity in most archeological projects, providing insights on past subsistence, economy, cultural processes, and natural environments (Dincauze 2000; Jacomet 2007). Several decades of research provide a solid methodological and theoretical framework for the study of charred macroremains, their role in human activities, and syn- and post-depositional processes (e.g. Hally 1981; Asouti and Austin 2005).

Despite the attention long since paid to charred macroremains, less interest is given to microscopic ones. Most researchers are skeptical about even the possibility of finding charred pollen and spores, abandoning the potential for palynological information, especially in archeological contexts. Even if present, charred palynomorphs may not be recognized as such during routine counting; palynologists usually work on acetolyzed samples, a procedure that stains pollen grains to facilitate their identification (Erdtman 1960) but uniforms their brightness, canceling or reducing differences that might have been present when they were first deposited.

A spectacular example of preservation of charred palynomorphs comes from an Etruscan site in northern Italy – Forcello near Bagnolo san Vito (Mantua province). At this site, in ca. 510–495 BCE, a violent fire destroyed a wooden-constructed building complex (phase F houses), including an artisan workshop housed therein. The destruction level (su 476) was soon sealed after the fire by a thick layer of pure clay, thus preserving the fire debris and the house's furnishings in situ and in an undisputable stratigraphic position (Castellano et al. 2017). The temperatures of the fire were clearly very high, as indicated by the color alteration (green to black) of a Greek Cypriot jasper scarab, indicating an exposure to temperatures above 400/500 °C (Devoto 2007). Given these premises, no one expected to find pollen in such a context. The extremely high temperatures to which the level was exposed rendered the context futile for palynological investigations until fragments of charred honeycombs and bee products (melted honey-wax, bee-breads, and honeybees) were discovered scattered on the floor of the aforementioned artisan workshop. Analysis of non-acetolyzed charred

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https://doi.org/10.1016/j.revpalbo.2018.10.005
0034-6667/

bee-breads (a fermented mixture of pollen, honey, and bee saliva used by worker bees as food for larvae and for young bees to produce royal jelly) recorded pollen grains at different charring degrees – from slightly singed exine to completely charred, black and opaque grains. Castellano et al. (2017) related such differences to varying combustion temperatures reached by bee-breads during the fire that destroyed the building where they were originally stored and later found. These findings encouraged us to set up a combustion experiment on modern fresh bee-breads, mostly made by *Hedera helix* pollen, to document changes in the morphology of pollen grains and in the chemical composition of whole bee-breads. The main goal of this paper is to provide an experimental framework for the study of charred palynomorphs from archeological samples through a comparison with experimental data obtained from fresh material.

1.2. Color of organic matter as an index of its thermal history

To the best of our knowledge, this contribution represents the first experimental-based study of charred palynomorphs from archeological contexts. Research in the field of petroleum geology traditionally deals with properties of organic matter (color, reflectance and fluorescence), depending on maturation induced by carbonification during burial (Gutjahr 1966). Palynomorphs are considered to be organic geothermometers where variations in color can be related to their thermal history (Ujiié, 2001). Gutjahr (1966) and Staplin (1969) focused on objective ways to define palynomorph color, a topic that was continuously studied up to the application of Color Image Analysis by Yule et al. (1998), who set up the procedure, at first, on artificially heated fresh *Lycopodium* spores and then on fossil material for inferences on their thermal maturation. This research has been further developed by Ujiié (2001), with experiments on fresh and fossil material and the definition of a stTAI (statistical Thermal Alteration Index), i.e., the mean value of mode brightness measured in 100 grains per sample.

Although carbonification (conversion of organic matter into coal over time) and carbonization (conversion of organic matter into carbon through pyrolysis or distillation) are different processes, operating on different time scales and rates, we borrow from petroleum geology the idea of a thermal alteration index and test its applicability both on artificially combusted palynomorphs and on archeological charred remains. Interestingly, the fact that the archeological samples considered in this paper contain other species than the artificially combusted ones turned out to be not relevant as far as the brightness of pollen is concerned, as general criteria for inter-specific comparisons could still be defined (Section 5.1).

2. Materials

2.1. Fresh pollen

Pollen grains of *Hedera helix* were chosen as the object of the heating experiment due to their medium-thickness exine (more details in Section 2.1.1), comparable to that of a large number of pollen types. Pollen of *Hedera helix* accounts for 99% of the grains contained in fresh bee-breads provided by a beekeeper raising honeybees in the Italian Alps.

2.1.1. Pollen morphology of *Hedera helix* L.

A thorough description of *Hedera helix* pollen is given by van Helvoort and Punt (1984), who examined five ivy populations from Belgium, the Netherlands, England, Italy, and Malta. This plant produces tri-zonocolporate pollen grains, with circular to slightly elliptical shape in equatorial view and triangular outline in polar view (see, later on, Plate I). Ornamentation is reticulate, with simpli- to multicolumellate *muri* and *lumina* angular, and it is irregular in size and outline. *Lumina* are smaller near the colpi, which are bordered by a distinct and broad *margo*. In glycerine jelly, polar diameter ranges between 34 – (36.5) – 41 μm and equatorial diameter between 28 – (32.5) – 37 μm. P/E ratio is 1.02 – (1.12) – 1.28, and exine thickness is between 2.5 and 3.5 μm. Slightly different values are reported by Beug (2004) for polar diameter (31.9 – (36.7) – 42.1 μm) and exine thickness (1.9–2.2 μm), which were collected from the measurements of a total number of 50 pollen grains from four localities.

2.2. Archeological samples

We used 10 archeological samples (Table 1) among the material considered by Castellano et al. (2017).

Table 1
The archeological samples considered in this paper.

Full sample names	Sample name used in text and figures	Sample type	Interpretation of the pollen assemblage	Pollen content
FORus476R18b18_b1 FORus476R18b17_b2 FORus476R18b17_b FORus476R18e16_b	b18_b1 b17_b2 b17_b e16_b	Bee breads from a charred honeycomb	Pollen contained in bee breads is the result of intentional foraging by honey bees and do not represent atmospheric pollen deposition (Castellano et al. 2017)	Only pollen of *Nymphoides peltata* type
FORus476R18b16_bb2	b16_bb2			*Nymphoides peltata* type, *Centaurea jacea* group, *Mentha* type, *Torilis nodosa* type. Rare grains of *Tussilago farfara* type, *Verbena*, *Cirsium* and *Nymphaea*.
FORus476R18c16_1	c16_1			*Centaurea jacea* group, *Mentha* type, *Tussilago farfara* type
FORus476R18b17_b1	b17_b1			mostly *Nymphoides peltata* type with rare *Mentha* type, *Torilis nodosa* type, *Tussilago farfara* type, *Verbena* and *Cirsium*.
FORc389, sample #1	charred clot #1	Clots of a vitrified porous mixture of fossil honey and wax	Samples formed as a result of fire-induced honeycomb melting. Fluid honey, wax and other material (bee breads, fragments of *Apis mellifera* bodies, etc.) dispersed on the floor of house F2, mixed with mineral particles from the pavement and clotted (Castellano et al. 2017).	*Centaurea jacea* group, *Mentha* type, *Tussilago farfara* type, *Aster* type, *Vitis*
FORc389, sample #2	charred clot #2			*Centaurea jacea* group, *Mentha* type, *Tussilago farfara* type, *Aster* type, *Vitis, Malva silvestris* type, *Cirsium*
FORc511	FORc511	Clay infill from a channel bordering house F2	Charred material and ashes covered the channel bordering house F2 and, after the fire, those levels were sealed with clay. Sample FORc511 might be an admixture of those different materials, therefore containing pollen from pure atmospheric deposition and pollen focussed in the channel after the fire.	Gramineae, Cyperaceae, Cichorioideae, Caryophyllaceae, *Centaurea jacea* group, *Malva silvestris* type, *Corylus*, *Tilia*, *Alnus*