Palynology: instrument of research for the relics of the Shroud of Turin and the Sudarium of Oviedo

Palynology is the science that studies pollen, the element that contains the male gamete and which is generated exclusively in the flowers of angiosperm and gymnosperm plants, while spores are produced by ferns and mushrooms for the same end of reproduction. During the flowering, the period of pollination begins; by means of this process the pollen reaches the egg, which contains the female gamete, stored in the ovary; once this is fertilized the fertile seed is produced. The process goes unnoticed and unseen by us; even so, the plants carry it out to perfection. The pollen grain is made up of a germ cell, which remains alive only during the time of pollination. During the dispersion, so as not to succumb to natural phenomena such as solar radiation, humidity, or the attack of fungi, the grain is provided with a coating that is resistant, meaning that it is not destroyed by the passage of time. This strong coating, called the exine, is formed of sporopolenine, a substance of oxidative polymers of carotenoids. This too, in its external part, is provided with ornamental elements and sculptural features; these make pollen dispersion easier, according to the particular type of pollination. The properties of the exine allow pollen to be highly resistant, although bacteria and fungi are able to attack and can degrade it, as can strong oxidizing processes or fire. The different structures, forms and ornamentations of the exine have particular importance in Palynology, because they represent the common distinctions within the same botanical species; the observation of the specific type is crucial in the task of recognizing the taxonomic unit that produced it.

Pollen grains are produced in abundance, depending both on the botanical species and on the type of pollination. Plants with wind pollination produce large amounts of pollen to meet the inevitable waste due to the randomness of transport; entomophilous plants, which employ insects, produce smaller amounts, and the transport process is more accurate.

The dispersion, transport and deposition of pollen are dependent on variables of biological species (intrinsic) and environmental variables (extrinsic) related, for example to the presence of suitable substrates that trap pollen. The mass of pollen floating in the air is

distributed more or less homogeneously over an area (greater or smaller in extension) around the source, and it is transported and deposited as a result of currents, in the form of pollen rain. Pollen grains are found in all environments, transported by animals or by air currents; these enable them to move away from the place of origin.

Pollen is ubiquitous, and in general does not deteriorate; it is preserved in varying amounts and over a very long time (in soils, archaeological layers, honey, hair, clothes, etc). Thanks to these characteristics, the search for pollen is of great help in scientific investigation, especially in forensic science and archaeology. Pollen identified on an object or a place is valid evidence in real cases of research; it helps to understand whether the samples are caused by human or natural phenomenon, as long as there is correct and clear interpretation. The identification of pollen allows a link to be established with a geographic environment where the plant species has developed; it could also define a possible specific area or uses made of plants of anthropic interest.

Palynology is an exact science that works in conjunction with other branches of botany or scientific collections, following a common clue; this makes it possible to validate the general results of the overall investigation.

Interest in palynological studies in forensics and archaeology is important for the following reasons:

a. Pollen has a perfect morphological structure, which is fixed in each species; when analyzing a large magnification, it is possible to distinguish the botany hierarchy, the family level, gender, and finally the specific plant species.

b. Pollen is the most common element of the plants, which is found on a surface only if there is a cause, such as direct contact with flowers, on the part of insects, animals or humans, in the case of entomophilous pollen; pollen which is transported by the wind is moved by air currents.

c. The exine of pollen is not destroyed in a terrestrial environment; it remains unchanged for thousands of years in an archaeological or fossil context.

d. The pollen found also allows links to be made with other scientific evidence from the scene. The discovery of entomophilous pollen, especially in fossil records or in association

with particular archaeological and botanical species, enables detection of possible 'natural contamination' derived from contact with plant parts, or from other anthropic uses with plants.

Considerations of the palynological studies of the Shroud

If we transfer these principles to previous studies of the relics of the Shroud of Turin and the Sudarium of Oviedo, it is likely that these have palynomorphs held within them from the very first moments of their history. Pollen gives indirect traces that may indicate that is has been there for many centuries. This is the primary presence of pollen, which can be distinguished from pollen presence resulting from natural contamination and a secondary adhesion. A very interesting thing could be found, as indicated by Scannerini and Caramiello (1989), the oldest pollen that may be immersed or embedded in grease, so it could be difficult to identify specifically in the case of the Shroud. Several studies, including some from the areas of Forensics or Archaeology, have successfully registered pollen. These findings, in different pieces of research, have helped to clarify the meaning of these pollens. Examples include studies of mummies of the second century AD (Ciuffarella, 1998), Roman incineration vases of I - III century AD (Buosi et al., 2013), or mummies of the Renaissance in Italy (Giuffra et al., 2011), which discovered entomophilous pollen that indicated possible use of plants in the processes associated with the historical items.

The conclusions of palynological studies of the Shroud carried out by the criminologist Frei are wildly inaccurate, as any scientist would testify (Frei 1976, 1979a, 1979b, 1982, 1983, 1985; Ghio 1986). The approximately 50 species of pollen which were identified have appeared in a list, divided into sectors that show the long journey of the relic from the East to the West of Europe, including the stay in different countries, according to historical knowledge. Pollen was definitely identified, but was forcibly made to match a probable geographic origin in order to demonstrate the long journey made by the relic. In this work, the relic was considered as a kind of flag that would have collected all the pollen in its path; no one remembers, however, that this was a valuable object which was protected and hidden while being transported. The conclusions raise doubts about the interpretation made of pollen found, because it is very difficult and almost impossible for there to have been so much variability in pollen, which strangely somehow also remained attached to the fibres over the centuries. I also doubt that Frei, who was not a specialist in

Palynology, was able to study the morphology of pollen from all over Europe and Asia as he attempted to advance in the right direction of his research. He would have needed several years to study the morphological characteristics of pollen with an electron microscope; that would have meant studying thousands of species from Israel to France and Italy. It would have required a collection of control samples from all species in bloom, or waiting for the flowering time of each to compare them with samples from the Shroud, given that in the 70's Palynology was in its infancy.

The easiest thing for Frei to do was to publish the least possible number of images of original Shroud pollen, then make them match, and link with, plants growing in places along the route the Shroud was known to have passed; this was the only way his research would make an impact. I believe it was research that was full of inaccurate ideas and preconceptions which, instead of checking and confirming if the relic was original, has even led to speculation today on whether there was actually any presence at all of these pollens types in the Shroud. But these issues have not been exposed by researchers, including botanists and palynologists, who analyzed some original samples; these include Danin and Baruch, Litt or Maloney. Despite the fact that the research of Frei may seem disappointing, pollen must of course exist, but the issue is that it has to be interpreted properly. It is surprising that the criticism of pollen from the Shroud has been written by historians and other lay-critics; for this reason, I feel their assessment lacks value. It may seem unlikely that the microscopic pollen can adhere to the linen fibres and stay there for a long time, but if you use oily substances, as they did in the customs of the early centuries AD, it is very likely that the ancient pollen could still remain stuck to the cloth.

Samples of powder from the Shroud were collected in the seventies, with a simple method of contact using an adhesive tape on the canvas; if entomophilous pollen were the most common, it must have reached the relic in a different way and not by the wind. It may represent evidence of natural contaminants, due to the use of specific plants, or proximity to them. Some specific cause, a contact or botanical use, must be responsible, a source which still remains to be ascertained. The remaining pollen could have arrived by wind, indicating secondary contamination, in a micro-trace that could have detached itself from the fabric at any time. One particular anemophilous type of pollen, which would have been an

interesting trace, but that could not be found, is olive pollen; this is a very common species in Israel and it blooms from March onwards (Waisel et al., 1997). Evidence of olive pollen may have been lost, or have become detached, or it may not have been perfectly identified. The critical studies of Frei were based on many aspects, such as on the methodology, on the adhesive tape, the abnormal pollen concentration in the initial segment, or the falsity of the photos, etc.

In the 90's the Frei original samples were analyzed by Danin and Baruch (Danin et al., 1999), who confirmed the same species discovered by Frei. Finally, in the year 2000, some authors such as McCrone (1990), Litt (Danin & Guerra, 2008), Bryant (2000), and Boi (2012), cast doubt upon these identifications of pollen; the findings are accepted also by Danin himself (Danin & Guerra, 2008), who prefers to continue his investigation into imaginary images of plants on the Shroud. My deduction is that the pollen examination and interpretation were analyzed in an inconsistent way, and were wrongly interpreted. This part of the research was later abandoned, with Frei turning his attention to the negative photo of the Shroud, looking for images of the plants associated with the same pollen he had identified. These results without scientific validity are associated with the phenomenon of pareidolia (Di Lazzaro et al., 2013).

All these results, interpreted without palynological knowledge, have led to unscientific conclusions which are difficult to support, both from the point of view of scientific discussion and from the perspective of the historical context.

Until a few years ago, no researcher had checked the identification of pollen found, to see if it was correct. Litt, after observing some samples (Danin & Guerra, 2008), and Bryant (2000), who looked at the images published, have found that the most abundant type of pollen does not belong to the species identified by Frei or Danin & Baruch as *Gundelia tournefortii*, but to a kind from the Asteraceae family. They are the first clues that eventually make it clear that initial identification of this pollen is not recognized by scientists.

I can also affirm that several photos published by Frei, taken with electronic microscopy, do not correspond to the species mentioned; neither do they not correspond to original pollen, but rather to pollen used for experimental control; only a few original images of the Shroud pollen have been published. The optical microscope analysis does not allow the vast majority of specific pollen levels to be recognized; for this reason Frei carried out his research with electron microscopy too. In the case of the large family of Asteraceae, to which *Gundelia tournefortii*, also belongs, an analysis with high magnification is needed in order to determine the differences from other similar taxa of this extended family. This taxon was photographed by Frei with scanning electron microscopy; it is very likely that the pollen samples do belong to the Shroud, as they were in poor condition and found in isolation (not joined to others). The taxon has been wrongly identified as *Ridolfia segetum*, from the Apiaceae family. Another mistake is the pollen of *Pistacia* spp., identified as *Anemone coronaria*. Considering the importance of the relic, these mistakes are inexcusable and offer a sad clue to support the idea that Frei had not sufficient preparation in the field of palynology.

To confirm the identification of the pollen species of the Shroud, knowledge of the characteristics of the botanical families of pollen is required. This needs to be compared to the flora of Europe, Asia Minor and North Africa; only in this way can the pollen present be established. Currently there is a pollen atlas that makes this type of research easier; in the 70's, however, Palynology was only just beginning; and the material of comparison was not available. It would have been necessary to collect flowering plants of each geographical area, and then identify them; preparation of samples with known pollen (slide collection) would have been required, because of the lack of a photographic atlas and identification keys. The research would have taken decades, if we also add the need for analysis with light and electron microscopy.

The list of Shroud pollens drafted by Frei has been illustrated, in the main, with observations in optical microscopy, which reach 1000 magnification. Though the Shroud powders were removed by and attached to an adhesive tape, this does not offer the best view of the details and morphological characteristics required for the palynological study. The collection method used by Frei should be enough in itself to understand that a new pollen collection is necessary, with an in-depth analysis with electron microscopy to confirm the true plant species.

After 40 years of research by Frei, the existence of pollen on the Shroud remains a mystery,

due to inaccuracies in the research, which does not follow the principles and dynamics of the science of Palynology which the pollen present in the relic deserves. The original samples of the tapes glued to the slides, including original manuscripts of Frei, have been lost to us, so we find ourselves once more at the starting point of the Shroud studies.

The sample material collected by Frei fact was sold illegally to the ASSIST in the USA, making it practically inaccessible. To continue the studies we need to have original material, if we are to be able to support any conclusions and carry on researching into the types of pollen present. A couple of years ago I scrutinised some electron microscope images of pollen made by Morano, Frei's technician; these photos are preserved in Italy. The collection of original photos is composed mostly of photos of pollen control and testing; images of pollen of the relic are very few in number. The test used to identify the pollen, with regard to the old photos, would be unlikely to be matched in the group if the pollen were to have been damaged or covered in dust or grease. In some photos taken with a scanning electron microscope and published by Frei, there are the original types, but he preferred to insert contemporary and nearly-perfect pollen, instead of showing the original pollen; that is what leads us to believe that these latter types of particles are not in fact present on the linen. From the scientific point of view the pollen is definitely present, but the results published come from mere speculation, as well as incompetence, both as regards botany and in palynological terms.

There is much misinformation, and there are many inaccuracies; there is no excuse for failing to interpret the evidence present. Thus the veracity of the experiment has collapsed. (This is an affirmation that is also supported by palynological reports discussed by other Shroud researchers).

Pollens exist, but researchers have previously failed to discover what may have been left adhered to the linen, apart from why and how it got there.

Much of the research on the pollen has generated criticism about its serious shortcomings, of which the most relevant are:

- The method of sample collection with an adhesive tape is aggressive to the fibres of the linen, which at the same time makes it difficult to identify the specific pollen.

- The search method and the results are not set out and reasoned through in a scientific way; the impression is that the results were reached far too quickly, and that they leaned heavily on other previous bibliographic studies.

- The palynological research is not scientifically clear, explicit and well-defined; I cannot but doubt whether the species have been identified correctly.

- The lack of a valid scientific method along with the corresponding lack of reference material. The researchers' enthusiasm, though laudable, was not enough to compensate for these failings and correctly identify the pollen.

- In most cases, the analysis carried out with light microscopy, while being able to determine the level of genus and botanical family does not make it possible to establish the exact plant species. For this reason, it is logical that errors will come about.

- The results mention the discovery of pollen of extinct plants; two millennia in the evolution of the plant is not a long time; that period of time may have altered only the distribution area of certain plants.

- The studies published show what are essentially images of modern pollen, possibly of fresh, control group pollen, which makes us doubt whether these types were ever really present on the cloth.

- There are serious mistakes in the identification of pollen, confusion at the level of the family and even confusion of fungal spores with pollen; this evidence leads us to confirm that there was not sufficient expertise to carry out this delicate investigation.

- There is no clarification of why the entomophilous pollen type is the most abundant, and how it could have reached the relic.

- The research to be undertaken prior to the study of the pollen itself would involve collecting samples of plants, identifying these and waiting for flowers to bloom so that pollen could be gathered. These processes are of course simplified nowadays, thanks to the presence of the reference material.

- The conclusions that affirm that the presence of pollen gives evidence of the journey

taken by the Shroud are too precise to be confirmed with the use of light microscopy alone; most of the palynomorphs which remained stuck to the adhesive can hardly provide results of great significance.

- Conclusions have been drawn from with samples that were later sold to the ASSIST; it was wrong to sell them like this; the original samples are evidence that belongs to the Shroud and to the scientific community. They are not the property of the researchers.

- The conclusions of the previous research are based on assumptions that led to errors of judgment being committed, so I believe that further research should be undertaken.

Considerations as regards the palynological studies of the Shroud of Oviedo

In 1978 Frei took microscopic particles from the surface of the Shroud of Oviedo with the simple method of using segments of adhesive tape; he thereby reaches partial and ambiguous conclusions (Ricci, 1985).

In the 90's, the Spanish Centre of Sindonology (CES) carried out fresh collections with non-invasive methods, so as not to damage the fabric; these are currently being studied. Initial research of the analysis with light microscopy was presented by the palynologist Gómez Ferreras (1994), in which 141 pollen types belonging to Mediterranean taxa were identified. Studies are presently being undertaken, analyzing the vacuumed powder with electron microscopy. Pollen is not abundant, but essentially the presence of entomophilous types is interesting; these appear embedded in dissolved resins and incense, and that fact has sometimes not made specific recognition possible. On the other hand, other pollen appears which does not have coatings. These tests lead us to reflect on the fact that even the Sudarium of Oviedo possibly underwent treatment with oils and essences for its cleansing and so that it might contain the body fluids of the deceased. Surprisingly, in the analysis of blood stains, Doctor Sánchez Hermosilla discovered an entomophilous pollen type of Asteraceae, possibly *Helichrysum*, attached and embedded in blood. This discovery, a few months ago, determined that pollen became adhered when the fluid was still fresh and not yet solidified, demonstrating an original presence from the very first moment that the blood came in contact with the fibres of the flax. This pollen would have been present in oils and ointments, because only from the pressing of fresh flowers is the oil of Helichrysum produced. The discovery explains and justifies there being particular pollens found on the Shroud, confirming the presence of botanical products used in ancient funeral rituals, along with Ferula and Pistacia. For historical reasons, cultural rituals with botanical products were used only a few centuries BC and until the third century A.D.

It seems impossible that pollen could offer so much information, but it is the only element that with certainty can reach the present day without being completely destroyed, and it is the only element that can be identified exactly.

Current research that is performed with the powder of the Shroud can be applied to the

Shroud of Turin. This unification of the methods of study could discover possible relationships, counter other evidence, and find relevant links to historical facts, as well as to ancient customs.

Possible studies to be carried out on the Shroud

a) Establish a committee of palynologists to prepare a protocol for sample collection and other micro-traces that are present.

b) Carry out a microscopic control to determine the state of conservation and deterioration of the fibres of the linen.

c) Due to previous sample-taking and to the change of position of the relic, it is likely to have lost much of the oldest micro-traces; it is still advisable and desirable to conduct a careful examination to find all the particles which are still stuck to the fabric. Since the relic came into contact with the air, and it was examined by scientists without using measures that avoided contamination, it can be supposed that recent pollen and spores will also be found.

d) Proceed to the collection of new samples, by means of non-evasive methods that do not compromise the state of the fibre, such as controlled aspiration.

e) The appearance of a single pollen-encrusted stain of biological fluid could prove to be the oldest pollen presence on the Shroud, along with the micro-traces that appear to be the most damaged and covered with hardened substances.

f) Consider the risk of the presence of spores, which if they develop fungal hyphae can threaten the current state of the linen fibres, as well as of other micro-traces.

g) Carry out a sampling of sewn mending carried out in the XVIth century on the burnt parts of the original cloth; identification of pollen could help to date the most recent historical events. Compare the types of pollen, both entomophilous and anemophilous, with those in the original cloth.

h) The powders can be analyzed by electron microscopy without further chemical preparatory treatments.

i) Create a database and images of large magnification of all the particles tracked, identifying the area where each was collected.

I) The previous sampling did not allow the complete pollen spectrum to be prepared; all the palynomorphs found must be compared with present plant species.

m) The result will make it possible to associate the pollen with cultural uses or with a specific geographical area, allowing an association to be made between the Shroud of Turin and the Sudarium of Oviedo.

n) In Palynology it is essential to have patience and perseverance; in general it has been confirmed to be a valid science, due to the extraordinary accuracy it reaches in its findings, as well as because it does not use invasive methods.

o) Microscopy with large magnification will help to discover the micro-traces present. The investigation of pollen does not distance the Shroud from its history, but rather contributes to demonstrating, through scientifically exact studies, the history of the relic.

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