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Pollen Composition in a Crashed Plane's Engine

REFERENCE: Lewis, WH. Pollen composition in a crashed plane's engine. J Forensic Sci 1997;42(3):387–390.

ABSTRACT: Pollen removed from engine tubing of a crashed plane near Ruidoso, New Mexico, which had been stored outdoors in partially broken boxes from May to October following a December crash, consisted almost entirely of insect-pollinated types at the near exclusion of wind-pollinated pollen. Plants producing both groups of pollen were found immediately adjacent to the stored engine parts, with many wind-pollinated species shedding abundant atmospheric pollen during the flowering season. There is no known mechanism whereby insect-pollinated types could selectively have been filtered from the air at the exclusion of the predominant wind-pollinated ones. Thus, the pollen part of the mass found in the tubing could not have accumulated either suddenly or over time while the plane was in operation, and therefore the mass must have been a post-crash accumulation. Furthermore, pollen taken from the same mass initiated pollen tube expansion in a sucrose solution and both pollen cytoplasm and walls appeared normal in TEM sections in contrast to pollen charred for only 5 min at 250°C (the crashed engine burned for many hours reaching at least 1000°C). Pollen examined from the engine tubing could only have been deposited after the crash occurred.

KEYWORDS: forensic science, December, 1989 Ruidoso, NM plane crash, forensic botany, pollen composition, insect-pollinated, wind-pollinated

A mass of biological material was removed from engine tubing known as the B2 elbow of a plane which had crashed and burned for many hours near Ruidoso, New Mexico. Following the December crash, parts of the engines were stored outdoors in open and broken boxes at the Ruidoso airport storage site from May to October. A suit was filed claiming that a design flaw in the engine allowed particulate matter to build up in the B2 elbow, and thus a resolution of the case involved determining if the matter were a pre- or post-crash accumulation. Given that part of this matter consisted of pollen, could a resolution of the pollen content assist in determining when pollen accumulation took place? Were there other pollen data which could assist in determining the critical time of pollen deposition?

Materials and Methods

Pollen obtained from a small part of the mass in the engine tubing, from various plants growing in the vicinity of the plane's outdoor storage area, and from herbarium specimens at the Missouri Botanical Garden, was acetolyzed (1,2) to provide permanent slides for analysis. In brief, anthers or small flowers were placed in a centrifuge tube containing about 2.5 mL mixture of eight parts acetic anhydride and one part concentrated sulfuric acid. The mixture was brought to nearly 100°C in a water bath, stirred gently for about 2 min, cooled, centrifuged, and decanted. The sediment was rinsed in 95% ethanol, filtered, and rinsed further in tap water. The pollen remaining in the tube was mounted in glycerin jelly and made permanent using melted paraffin. Other material for whole pollen comparisons was stained in dilute basic fuchsin, centrifuged, decanted, left overnight in 50% glycerin, and mounted in glycerin jelly. A Zeiss compound microscope with Plano 40× and 100× objectives was used for all observations.

Additional collections were further examined using scanning (SEM) and transmission (TEM) electron microscopy. Again in brief, pollen for SEM was air dried, and placed on a double-stick Scotch tape which had been stuck on aluminum stubs. The material was then sputter-coated with a thin layer of gold and examined using a Hitachi S-450 operated at 20 Kv. To prepare for TEM, charred and uncharred pollen was fixed for 15 min in 4% or 2.5%, respectively, glutaraldehyde in 0.1M PO_4 buffer, centrifuged, washed in 0.1M PO_4 for 30 min, and dehydrated in 50% through 100% (twice) at 10 min intervals. The pollen was embedded in three changes of Spurr's low-viscosity resin, 30 min each, and kept overnight in a 60°C oven. Sectioning by diamond knife was completed using a Hitachi-600 operated at 75 Kv. Photomicrographs were taken on either Polaroid 55P/N or Kodak Ektapan film.

Germination experiments were conducted by placing pollen from the B2 elbow or a central heater stored at the outdoor storage site in a 10% sucrose solution overnight at which time light photomicrographs were taken.

Charring experiments subjected pollen of *Melilotus officinalis* (L.) Pall. taken from plants in the immediate vicinity of the storage site to 250°C for about 5 min. TEMs of these charred pollen were then compared with those taken directly from the B2 tubing of the crashed plane's engine and with other pollen examined by light microscopy and SEM.

Results and Discussion

Pollen Composition of B2 Elbow Mass

As provided in Table 1, five pollen types were recovered from the biological mass found in the plane's engine tubing. Only two types dominated, *Grindelia squarrosa* (Pursh) Dun. (62.24%) and *Melilotus officinalis* (36.56%), and together they represented 98.8% of the sample examined. Pollen from the other three plants were comparatively very rare.

Both kinds of pollen making up the great majority of those found in the elbow tubing are insect-pollinated. Two of the remainder, *Verbesina encelioides* (Cav.) Bentham & Hooker and *Sphaeralcea coccinea* (Pursh) Rydb., are also insect-pollinated, although their very low frequency suggests a serindipedous accumulation. All

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Received 29 May 1996; accepted 10 Sept. 1996.

TABLE 1—*Composition of pollen mass in engine elbow tubing (B2).*

Plant Source	Pollinating	Percent Pollen*
Asteraceae		
Grindelia squarrosa (curlycup gumweed)	July–Oct.	62.24
Verbesina encelioides [probably] (crownbeard)	May–Oct.	0.27
Fabaceae		
Melilotus officinalis (yellow sweetclover)	May–Oct.	36.56
Malvaceae		
Sphaeralcea coccinea (scarlet globemallow)	May–Oct.	0.82
Pinaceae		
Pinus edulis (pinyon pine)	April–May	0.10

*Based on a sample of 2,913 pollen.

four species grow commonly in the immediate vicinity of the outdoor storage site where parts of the crashed plane were stored, including the B2 elbow. Pollen of these insect-pollinated types totaled 99.9% of the pollen mass sampled; only 0.1% was representative of wind-pollination (*Pinus edulis* Englm.). This imbalance could not be more marked, particularly when many airborne pollen types are found around the storage site and pollinate from May through October, such as numerous trees, grasses, and weeds (Table 2). Moreover, an airborne pollen sampling reported southeast of Ruidoso at Hobbs, New Mexico (*3*), summarized in Table 3 and Fig. 1, confirmed the common atmospheric occurrences of tree pollen like *Juniperus* and of grasses and weeds during the months of exposure of the engine parts at Ruidoso, yet none of these pollen types were represented in the pollen mass from the tubing.

To my knowledge, there is no known mechanism whereby the two major insect-pollinated pollen types could selectively have been filtered from the air to the exclusion of pollen from the diverse wind-pollinated plants which abound in the immediate vicinity of the outdoor storage area and whose wind-borne pollen types are characteristically common in the ambient air. These observations reinforce the conclusion that the pollen part of the mass could not have accumulated either suddenly or over time while the plane was in operation, and therefore that the mass must have been a post-crash accumulation.

TABLE 2—*Genera of common plants in the vicinity of the Ruidoso airport outdoor storage facility (wind-pollinated in bold, others insect-pollinated).*

Trees
Juniperus, Pinus, Quercus
Grasses
Bothriochloa, Bouteloua, Panicum, Poa, etc.
Herbaceous Dicotyledons
Amaranthaceae: ***Amaranthus***
Asteraceae: ***Ambrosia, Artemisia,*** *Cirsium, Conyza, Erigeron, Grindelia, Lactuca, Senecio, Verbesina*
Brassicaceae: *Lepidium*
Chenopodiaceae: ***Kochia, Salsola***
Fabaceae: *Lathyrus, Melilotus*
Geraniaceae: *Erodium*
Malvaceae: *Sphaeralcea*
Solanaceae: *Solanum*
Verbenaceae: *Verbena*

TABLE 3—*Aeropollen sampled 1989–90 for one year at Hobbs, New Mexico.*

Plant Type	Percent Pollen*	Major Pollen Source, Percent, Major Months Shed
Trees/ Shrubs	70.2	*Morus,* 41.2 (April)
		Cupressaceae, mostly *Juniperus,* 13.7 (Feb., Oct.)
		Pinaceae, mostly *Pinus,* 7.1 (May)
		Quercus, 3.2 (April)
		Broussonetia, 2.6 (April)
Grasses	11.1	Poaceae (Apr., Sept.–Oct.)
Weeds	18.3	*Amaranthus*/Chenopodiaceae, 14.7 (Oct.)
		Ambrosia, 3.0 (Sept.–Oct.)

*Percent of 13,104 pollen sampled.

Gemination of Melilotus Officinalis *Pollen*

Representing the two major pollen types obtained from the B2 tubing mass, Fig. 2 shows germinating tubes emerging from apertures of *Grindelia squarrosa* (A) and *Melilotus officinalis* (B); similarly, pollen from plants of *M. officinalis* collected at St. Louis and in the heater at the storage site showed limited pollen tube expansion at apertures. Given that the heat from the crashed plane generated temperatures in excess of 1000°C for many hours, under no circumstances could the pollen from the B2 tubing subjected to this extreme condition be showing cytoplasm extending beyond the sexine. These pollen grains of both species must have been deposited in the B2 tubing of the engine after the crash occurred. The presence of flowering plants adjacent to the engine for months during the summer provides a readily available source for the pollen forming part of the biological mass.

Charred Pollen of Melilotus Officinalis

Pollen of *M. officinalis* removed from the B2 elbow tubing was sectioned using TEM, and a polar view, equatorially cut, shows the three apertures with one in particular having a slightly protruded early tube with the cytoplasm breaking and extending through the sexine with little covering (Fig. 3). Note numerous organelles, organized columellae of the sexine wall, and the well-represented intine below the sexine, all of which are typical of untreated but preserved pollen. Contrast this micromorphology with that of pollen removed from plants adjacent to the storage site subjected to charring at only 250°C for about 5 min (Fig. 4). A similar view and cut clearly illustrates the destruction of cytoplasm masses at each aperture, leaving only cavities and remnants of the sexine wall. The intine is gone, organelles are restricted and reduced, and the columellae are barely visible. Moderate heat induces changes in protein-based cytoplasm much as observed when the white (protein) part of an egg is boiled. No such pollen or cytoplasm-state was observed from the pollen mass of the B2 elbow tubing either in pollen section (Fig. 3) or culture (Fig. 2). Therefore, pollen found in the B2 elbow could not have been subjected to burning.

Based on evidence from pollen content in which selected concentrations of two major entomophilous pollen types have been found, at the exclusion of virtually all wind-dispersed pollen which is found in abundance in the atmosphere, I know of no mechanism whereby these two insect-pollinated types could selectively have been filtered from the air to the exclusion of the numerous anemophilous pollen types found in the nearby atmosphere. Further, the initiation of pollen tube development with intact cytoplasm

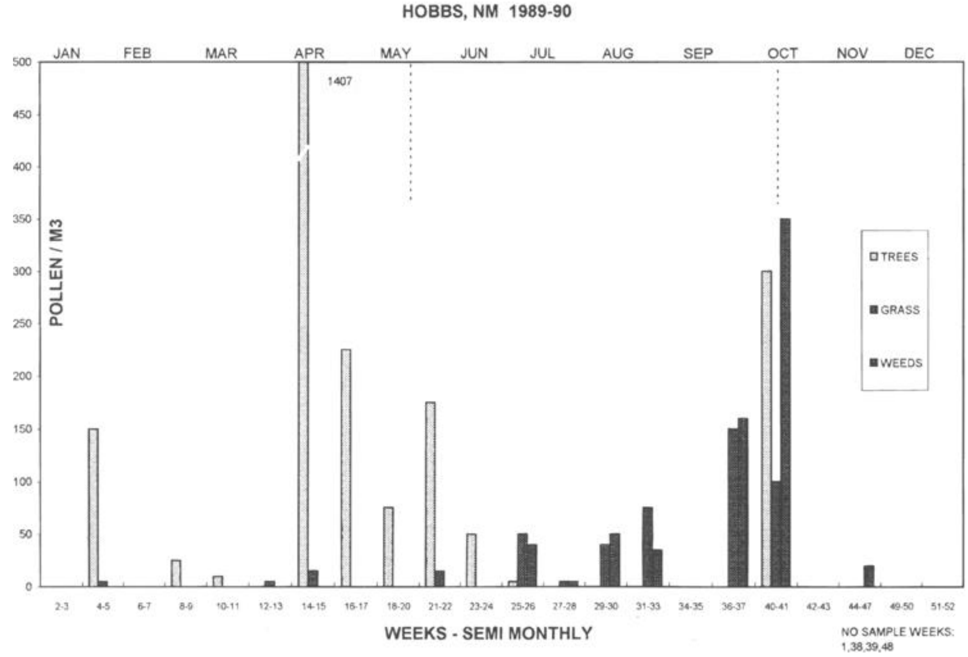

FIG. 1—*Graph of major aeropollen types sampled for one year at Hobbs, New Mexico (modified from three).*

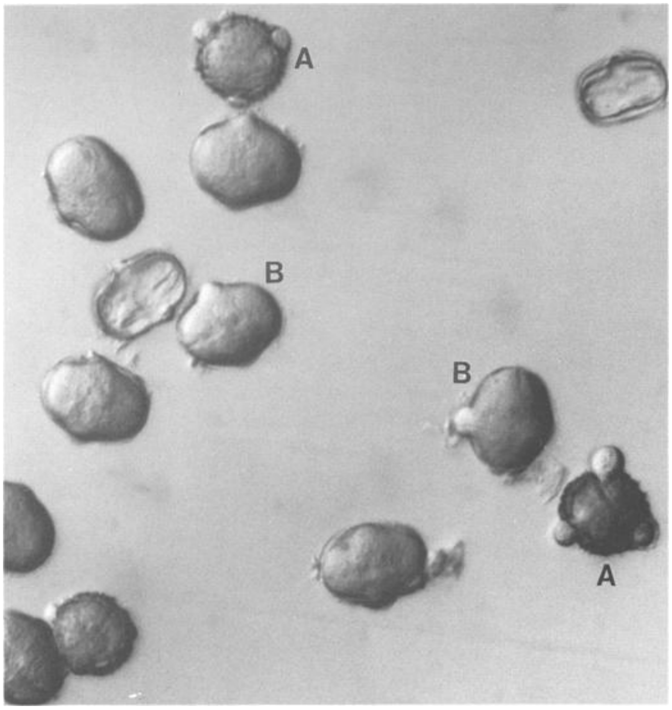

FIG. 2—*Germinating pollen of* Grindelia squarrosa *(A) and* Melilotus officinalis *(B) in 10% sucrose solution obtained from the B2 tubing of the crashed plane's engine, light photomicrograph ×830.*

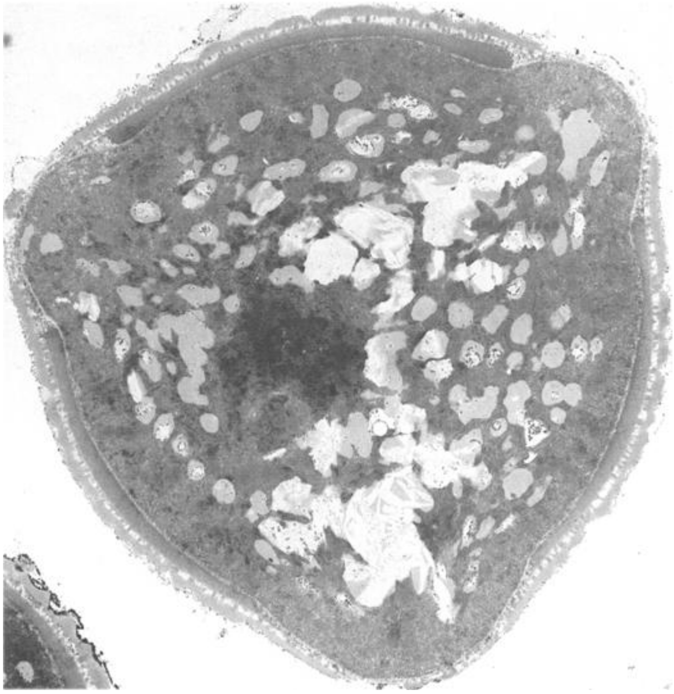

FIG. 3—*TEM section of* Melilotus officinalis *pollen obtained from the B2 tubing of the crashed plane's engine, ×7500.*

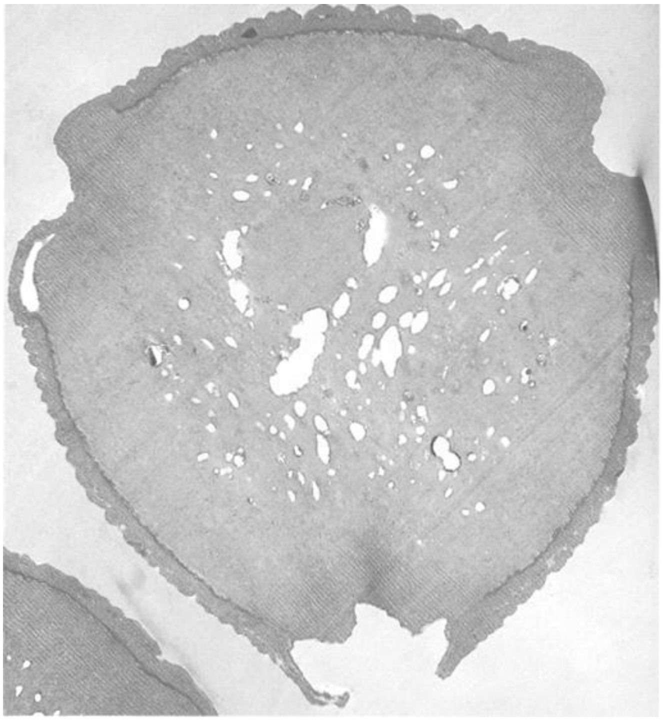

FIG. 4—*TEM section of* Melilotus officinalis *pollen obtained from plants at the storage site subjected to charring at 250° C for about 5 min, ×7500.*

obtained from the B2 elbow tubing pollen mass, and the presence of normal appearing cytoplasm and walls of sectioned pollen from the same mass compared to a highly altered pollen morphology following burning, reinforce the conclusion that the pollen part of the mass in the engine's tubing must have accumulated after the plane had crashed.

Acknowledgment

Light, scanning, and transmission photomicrographs were taken by G. Michael Veith, Senior Microscopist, Department of Biology, Washington University, whose significant contribution to this research is gratefully acknowledged.

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