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Field Sampling and Chemical Analysis

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ABSTRACT: The mass of material plugging the B2 elbow was 4.1 mm in diameter and 0.6 mm thick. It consisted of both light and dark colored organic material mixed with stellate trichomes and the pollen of several plants found near where the aircraft wreckage was stored. The mass was colonized by fungi such as *Alternaria* and *Cladosporium*. The organic matrix consisted of two dissimilar materials: A dark "gum-like" material and a lighter, golden-colored "nectar" and was subjected to FT-infrared (FTIR) spectroscopic analyses. Both reflectance and transmission FTIR techniques were used in the analyses, but the best results were obtained from transmission FTIR. This required that opaque substances such as the organic matrix be prepared as thin films. Several materials were collected from near the storage site as reference materials and these were also analyzed by FTIR spectroscopy. The reference materials included leaf and sap from *Sphaeralcea coccinea* and *Grindelia squarrosa* growing around the storage site. Commercial honey, cleaning solvents, and other organic fluids used in the aviation industry were also analyzed as well as gummy deposits found in equipment unrelated to the aircraft wreckage but stored near the aircraft parts. Mixed FTIR spectra were obtained from the organic matrix found in the B2 elbow, but showed a significant carbohydrate component. Comparison of these spectra to the reference materials clearly showed that the organic matrix was composed of macerated *Sphaeralcea* leaves mixed with a honey-like nectar.

KEYWORDS: forensic science, December, 1989 Ruidoso, NM plane crash, fourier transform infrared spectrometry, non-destructive analysis, bee nest

The mass found in the B2 elbow consisted of a heterogeneous liquid/resinous material mixed with stellate trichomes and pollen, as well as several insect parts, and was colonized by fungi such as *Alternaria* and *Cladosporium* species. The mass was circular, 4.1 mm in diameter and only 0.63 mm thick. The primary objective of field sampling and chemical analyses of the B2 mass was to obtain samples from various sources that could be chemically shown, within reasonable doubt, to have the same composition as the B2 mass. The hope, therefore, was to provide further supporting evidence to the existing physical evidence. Due to the nature of the mass and the fact that insect parts were found in the mass, as well as whole insects in other complete nests similar to the B2 mass, our hypothesis was that the mass constituted one end of a solitary bee nest and was cemented together with masticated leaves, stellate trichomes, nectar, and pollen. We therefore sampled the area around the yard, where the wreckage had been stored for over a year, for materials that may have been used in the construction of the nest. We also tested several other materials, such as lubricants and cleaning fluids, as well as a commercial bottle of honey, in order to test this hypothesis. Our objective was to describe the mass in some detail both microscopically and chemically in a non-destructive fashion, and to provide evidence for the identity of the mass forming part of a solitary bee nest.

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Materials and Methods

Sampling

Samples were collected with the primary aim to test our hypothesis that the mass was part of a bees nest by collecting samples that may provide evidence either supporting or challenging the hypothesis. Our first goal was to examine the storage yard, where the wreckage had been stored, to find other pieces of equipment, unrelated to the aircraft wreckage that had been stored in the same area at the same time as the wreckage. We hoped these may also contain masses similar to the mass found in the B2 elbow. It didn't take long to locate an old aircraft engine heater that was bristling with open ended tubes of 3–5 mm internal diameter. These tubes were filled with many nests and masses similar to the B2 mass. We took samples of these materials as well as samples of plant material and sap from plants surrounding the storage yard. We collected leaf and stem samples from *Sphaeralcea coccinea* (Pursh) Dunal. and *Grindelia squarrosa* ((Pursh) Rydb. which were very common in and around the storage yard. We also collected sap samples from the closest trees to the storage yard. These included two Pinyon pines (*Pinus edulis* Engelm.), 82 and 107 m from the storage yard, and three Junipers (*Juniperus* L. sp.), 43, 44, and 46 m from the storage yard (Fig. 1).

Further samples of engine cleaning fluids and solvents were obtained from various manufacturers to determine if the mass could potentially be an accumulation of residual cleaning fluid that was improperly used. The cleaners and solvents were: Turco®, Burlin-512M®, and Turbo Clean®. Finally, we obtained a small jar of commercial honey from a local supermarket to use as a standard for bee nectar.

FTIR and Microscopic Analysis

Analysis of the samples was confined to non-destructive tests because the B2 mass was court evidence and could not be legally destroyed. We therefore used binocular and compound light microscopes as well as scanning electron microscopes (SEM) and fourier transform infra-red spectroscopy (FTIR) (1,2). These devices allowed the collection of high quality information with little or no disturbance to the material being examined. We were permitted to remove tiny pieces of the B2 mass for transmitted light microscopy and FTIR. This approach provided the very best information on the nature of the mass; however, much of the characterization

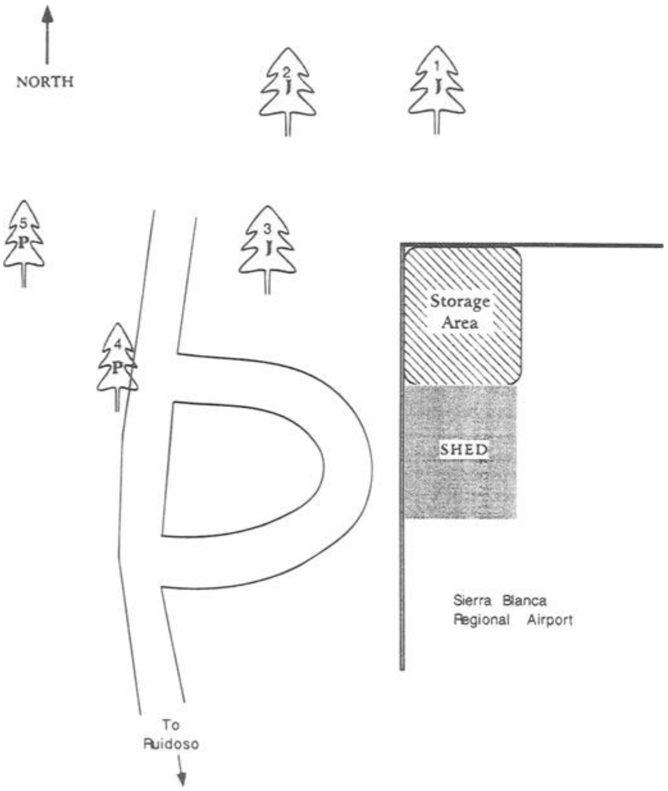

FIG. 1—*Map of the storage area at Sierra Blanca Regional airport, Ruidoso, New Mexico where the aircraft wreckage was stored and the proximity of Pinyon pine and Juniper trees that were sampled in this study.*

FIG. 2—*FTIR spectrogram of* Sphaeralcea coccinea *leaves and the dark "gum-like" material from the B2 elbow.*

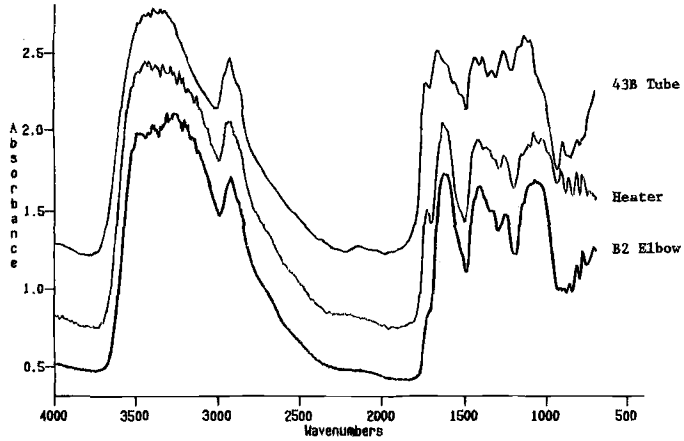

FIG. 3—*FTIR spectrogram of dark "gum-like" material from the B2 elbow, a bee's nest in the heater tubes, and a hydraulic tube from the aircraft (43B).*

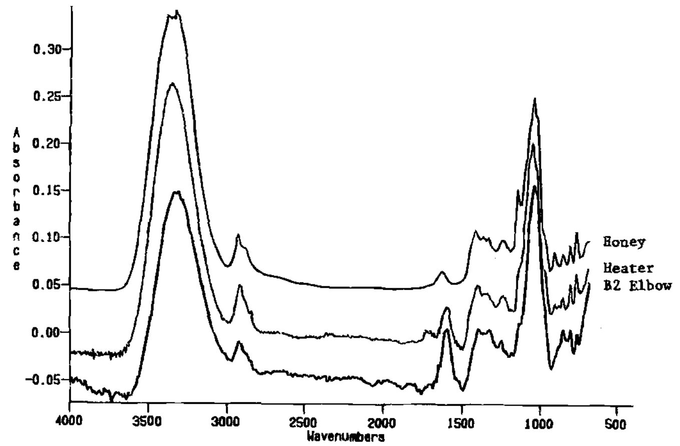

FIG. 4—*FTIR spectrogram of the golden-colored liquid from the B2 elbow, a bee's nest in the heater tubes, and commercial honey.*

was performed using incident light microscopy, SEM, and reflectance FTIR.

All incident light microscopy, SEM, and FTIR was performed on completely undisturbed samples. Transmitted light microscopy and FTIR was performed on thin smears of material placed either on a glass slide for microscopy or a sodium chloride block for FTIR. There was no staining of material.

Results

The B2 mass consisted of a mixture of stellate trichomes, pollen, insect parts, and a dark "gum" interspersed with a hyaline golden-colored fluid. The identity of the trichomes, pollen, and insect parts form the subject of other contributions in this series; however, the FTIR identification of the dark material and the golden-colored fluid proved pivotal to the final verdict. FTIR transmission spectra of the "gum" showed that it consisted primarily of *Sphaeralcea* leaves, which was consistent with the trichome evidence because the trichomes were also from *Sphaeralcea coccinea* (Fig. 2). The samples from the heater tubes also proved to be composed of *Sphaeralcea* leaves (Fig. 3). The golden colored fluid proved to be nectar based on transmission FTIR spectra identical to commercial honey (Fig. 4). There was no evidence based on the FTIR analysis that the solvents and cleaning fluids were found in the B2 mass at all (Fig. 5). The analysis was a simple comparison of spectra because this has significant visual impact, but also because the complex mixture being analyzed could not be readily decomposed into base constituents. In addition, the abundant biological evidence ultimately made more detailed chemical analysis unnecessary. The key to the analysis was three sets of peaks that unequivocally identified the gum and the nectar. The number of peaks between wave number 2500 and 3500 corresponding to hydroxyl groups in the B2 "gum" material are broader than found in the cleaners and solvents (Fig. 5), but it is identical to the breadth of the peaks found in the *Sphaeralcea* samples. The peak at wave number 2900 is the C-H stretch and found in all samples although this peak is much larger in the mostly hydrocarbon cleaners and solvents. The

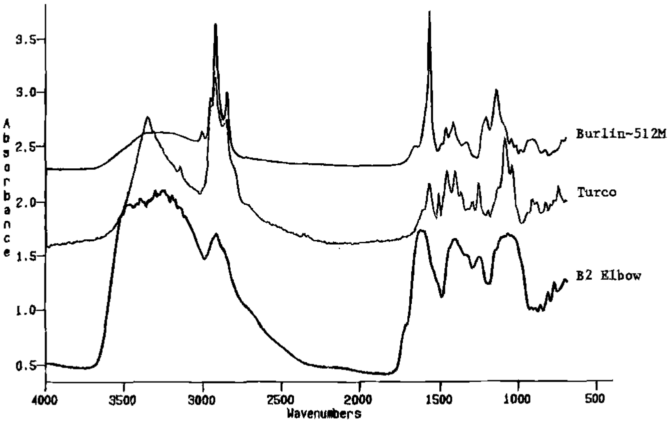

FIG. 5—*FTIR spectrogram of the "gum-like" mass in the B2 elbow and two aircraft engine cleaners, Turco and Burlin-512M.*

breadth of the peaks between wave number 1000 and 1700 covering the C-O stretch also matches the *Sphaeralcea* sample better than any other (Fig(s). 2 and 5). Finally, the most convincing evidence comes from a set of four small peaks found between wave number 700 and 900. These peaks were found in all samples suspected of being bee nests and also in the commercial honey samples (Fig(s). 2, 3, and 4). Indeed, when the golden-colored liquid from the B2 mass was carefully analyzed by a narrow FTIR beam, it was found to have an identical spectrum to the commercial honey (Fig. 4).

Discussion

The chemical analyses joined with the trichome, pollen, and entomological evidence to show that the B2 Mass was indeed the bottom wall of a solitary bee's nest. The mass consisted of masticated *Sphaeralcea* leaves, mixed with honey-like nectar, trichomes of *Sphaeralcea,* and pollen of several species. The highly variable nature of these mixed biological materials and the lack of sample material due to legal issues makes comparison of the FTIR spectra to published libraries impossible although it is clear that despite the limitation of the procedure that clear patterns emerged allowing identification of the materials making up the B2 mass beyond any reasonable doubt.

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