THE MICROSCOPE Vol 56:4 p.157-162 (2008)

Palynological Investigation of Post-Flight Solid Rocket Booster Foreign Material

David M. Jarzen* and Linda A. Nelson**
Florida Museum of Natural History and United Space Alliance, LLC

KEYWORDS

Space Shuttle, pollen, *Schinus terebinthifolius*, Brazilian pepper tree, Asteraceae, pollen collection, bees, JEOL 6480LV Scanning Electron Microscope, Fourier Transform Infrared (FTIR)

ABSTRACT

Investigations of foreign material in a drain tube from the Solid Rocket Booster (SRB) of a recent Space Shuttle mission was identified as pollen. The source of the pollen is from deposits made by bees, collecting pollen from plants found at the Kennedy Space Center, Cape Canaveral, Florida. The pollen is determined to have been present in the frustum drain tubes before the shuttle flight. During the flight, the pollen did not undergo thermal maturation.

INTRODUCTION

After every NASA Space Shuttle mission, the Solid Rocket Boosters (SRBs) are recovered and observed for missing and abnormal material. After a recent shuttle mission, STS-120, an unknown orange-yellow material was observed in a small cavity and a corresponding Teflon™ drain tube (Figure 1). This cavity allows seawater to drain from the interior of the structure after recovery from the Atlantic Ocean. The material was collected for identification and to determine the time frame of introduction. The material was comprised of sea water and pollen grains of primarily two taxa, with minor amounts of other taxa. This report describes both the initial investigation and the subsequent analysis of the pollen recovered and the parent plant habit, habitat and pollination syndromes.

MATERIALS AND METHODS

Five samples were collected from the SRB frustum: (1) liquid from the inner cavity; (2) solid clumped material from the inner tube vent; (3) solid gelatinous plant tissue material from the lower right outer vent drain tube; (4) liquid from the lower right drain tube; and (5) solid gelatinous material from the lower left outer vent drain tube.

Initial Investigation (United Space Alliance, LLC)

The residue and liquid samples were examined using an Olympus BX51 light microscope equipped with dark field, polarized light, and UV-excitation fluorescence illumination (Figure 2). The residue and dried liquid samples were also examined using a JEOL 6480LV Scanning Electron Microscope (SEM) in low vacuum mode. Images and EDS spectra were acquired using an accelerating voltage of 20 KeV with a chamber pressure of 30 Pa and 9mm working distance.

Fourier Transform Infrared (FTIR) analysis was performed on liquid from the drain tube using a Thermo-Electron 4700 FT-IR Spectrometer equipped with a DTGS detector and a Pike MIRacle™ Diamond reflectance accessory. Interferograms were collected as double-sided and bi-directional. The spectra were obtained by signal averaging 32 scans collected at a reso-

* University of Florida, Gainesville, FL 32611
** 8550 Astronaut Boulevard, Cape Canaveral, FL 32920

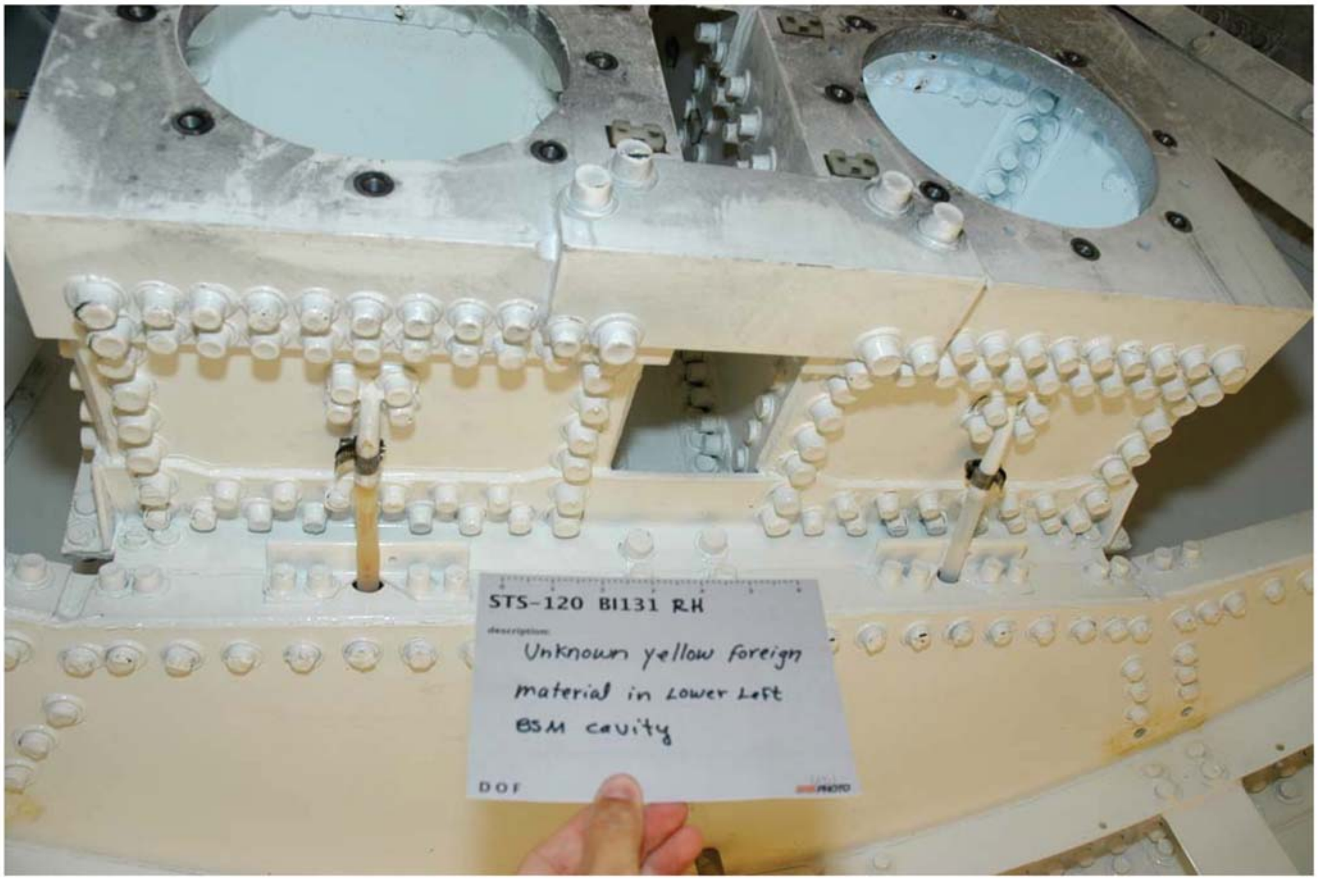

Figure 1. Yellow unknown material in frustum drain tube

lution of 4 cm^{-1}. A medium apodization function was applied to the interferogram data.

FT-IR analysis of the liquid samples (drain tube and BSM cavity) confirmed that liquid was primarily water along with some inorganic salts. Both liquid samples contained a yellow-orange material that was not dissolved in the water. Aliquots of the liquid samples were placed on a microscope slide and heated gently on a hot plate to dissolve the water component. The slides were first observed under the light microscope under various illumination conditions. The material consisted of translucent crystals and yellow particles of two different morphological types: one was round with triangular spines radiating outward measuring about 20 microns in diameter; the other was oblong with a smooth surface measuring about 20 microns by 15 microns. The material resembled pollen grains (Figure 2). The material was consistent regardless of whether the source was the cavity or the drain tube. The samples were then prepared for further observation using the SEM.

The same pollen grains were observed in the SEM specimens as well as several visually different crystalline materials (Figure 3). Each crystalline type was analyzed using Energy Dispersive Spectroscopy (EDS). The EDS analysis found that the crystalline materials present in both the BSM cavity and drain tube samples were consistent with those expected in seawater; i.e., primarily sodium chloride along with chlorides, chlorates and sulfates of magnesium, potassium, and calcium.

Pollen Analysis (University of Florida)

All five samples were processed using standard palynological techniques, following Traverse (1) with modifications outlined in Jarzen (2). Slides were prepared using glycerin jelly as the mounting medium. Storage of slides, residues and unused portions of the samples are stored with the Paleobotany and Palynology Collection, Florida Museum of Natural History, Gainesville, Florida.

Photography was done using a Nikon Coolpix 4500™ digital camera, mounted on a Leitz Dialux 20™

Figure 2. UV fluorescence (excitation) microscopy image at 500x magnification, showing the two common pollen types.

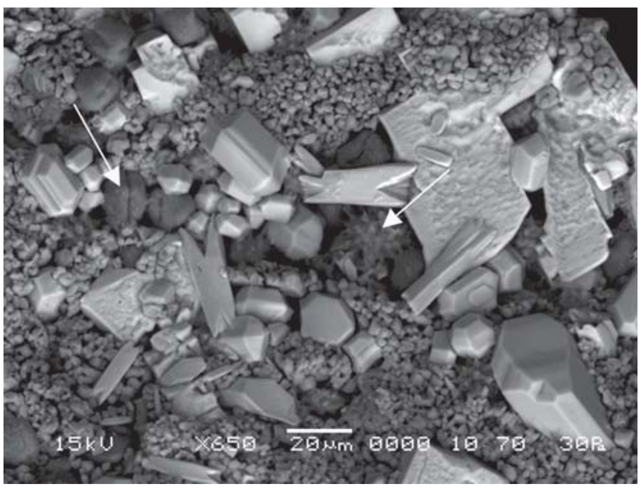

Figure 3. SEM Image showing salts and pollen grains at 650x magnification. Arrows indicate pollen grains.

research microscope. The location of illustrated specimens is given by reference to the sample name and number (1 through 5) followed by a slide number (A-1, A-2…etc.) and England Finder Slide (EFS) location. Identification of the recovered pollen was made using the Modern Pollen and Spore Reference Collection of 7100 taxa at the Paleobotany and Palynology Laboratory, Florida Museum of Natural History, Gainesville, Florida, U.S.A., and through literature sources (3,4,5).

POLLEN DESCRIPTIONS

Family Anacardiaceae Lindley 1830

Genus *Schinus* Linnaeus 1753
***Schinus terebinthifolius* Raddi 1820**
Figure 4, A-C

Pollen occurring in monads, prolate to subprolate, amb in polar view nearly circular, tricolporate; colpi meridionally arranged, extending nearly to the poles, narrow, with smooth margins; pores elongated equatorially (lalongate), with rounded to jagged margins; surface very finely foveolate, fovea aligned in striate pattern; exine two-layered, sexine of equal thickness to nexine, exine about 2.5 µm thick; dimensions polar axis 24.0 (28.3) 31.2 µm, equatorial axis 21.5 (24.5) 26.4 µm, p/e ratio 1.155 (10 grains measured).

Family Asteraceae Martinov 1820
Figure 4, D,E

Pollen of the Asteraceae (Compositae) is generally tricolporate, with an echinate sculpture. The spines are either short and nearly wart-like to elongated and sharply pointed. The pollen recovered occurs in monads, is spherical in shape, tricolporate with short colpi and indistinct pores; the surface is echinate with spines measuring up to 5.0 µm long and 2.5 µm wide at the base; exine 2.5 to 3.0 µm thick. Diameter of grain including the spines measures 36.0 (38.4) 40.8 µm (10 grains measured).

Family Fabaceae Lindley 1836
Genus *Vigna* Savi 1824
***Vigna luteola* (Jacq.) Bentham 1859**
Figure 4, F-H

Pollen occurring in monads, triporate, amb rounded triangular with nearly straight to convex sides; pores situated on the equator, circular, up to 10 µm in diameter; surface reticulate, with large lumina, 6.5 to 12 µm in largest dimension, muri 1.5 µm wide, sinuous; exine two-layered, sexine thicker than nexine, exine thickness 2.5 – 3.0 µm; equatorial diameter 48 (54.7) 60 µm (10 grains measured).

Pollen grains of Poaceae (Figure 4, J; two grains observed) and *Pinus* sp. (Figure 4, I; three grains observed), were also recovered from the SRB samples. These grains are not described here because they are anemophilous (wind pollinated) and could have entered the SRBs at any time before or after the flight.

RESULTS AND DISCUSSION

All samples contain salt crystals consistent with seawater and well-preserved pollen of primarily two taxa. The pollen is fresh in appearance, with cellular contents (observed before the acetolysis treatment),

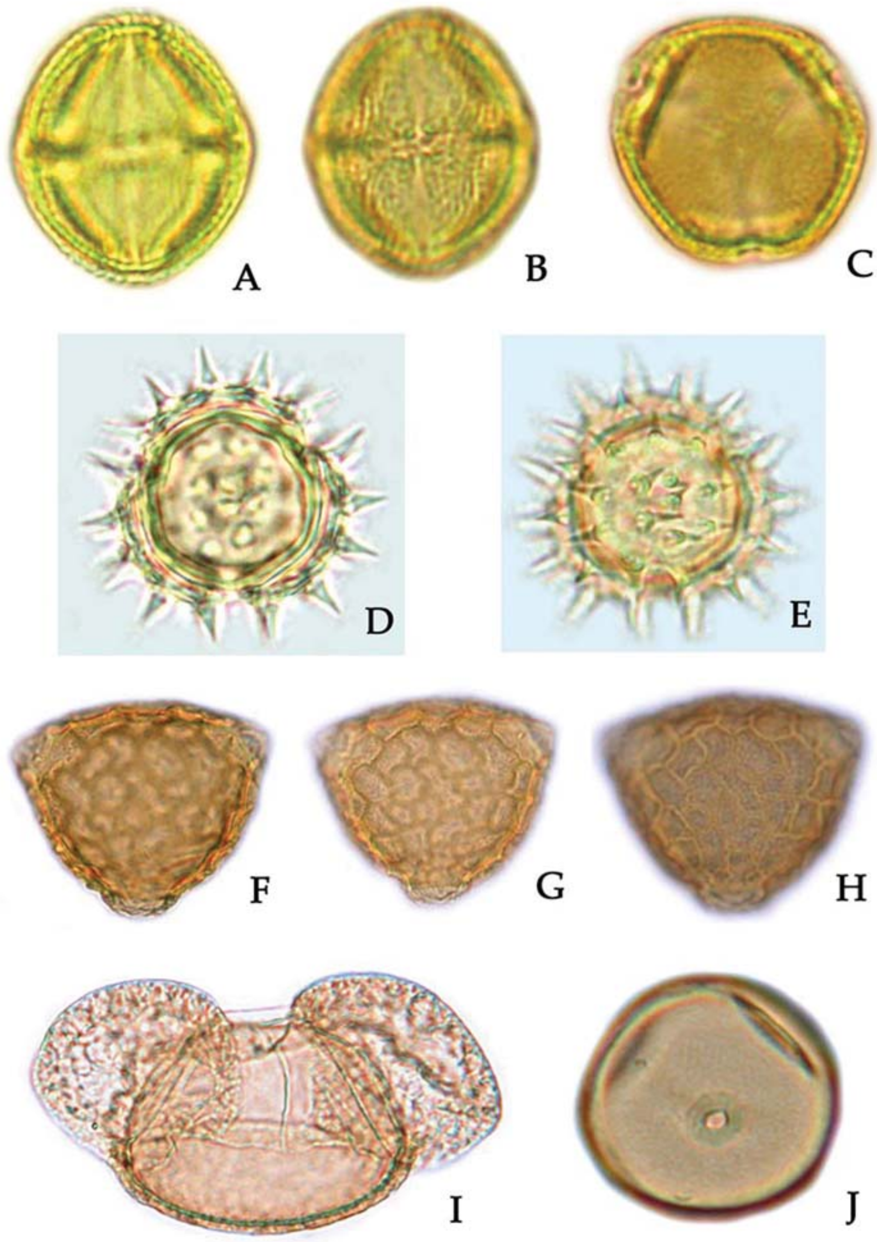

Figure 4, A-J. Selected palynomorphs recovered from the SRB samples. Following the taxon name, the information provided includes slide number, "England Finder" coordinate (prefixed EFS), and size information.

A. Schinus terebinthifolius, equatorial view optical section. Inner tube vent, Slide A-1, EFS L28/2, long axis = 26 µm.
B. Schinus terebinthifolius, equatorial view surface showing striate pattern. Inner tube vent, Slide A-1,EFS L28/2, long axis = 26 µm.
C. Schinus terebinthifolius, polar view, showing two wall layers. Inner tube vent, Slide A-1, EFS R27/4, diameter = 25 µm.
D. Asteraceae/Compositae polar view, optical section, showing long spines. Inner tube vent, Slide A-1, EFS R32/4, diameter = 37 µm.
E. Asteraceae/Compositae polar view, surface focus. Inner tube vent, Slide A-1, EFS R32/4, diameter = 37 µm.
F. Vigna luteola polar view optical section. Lower right drain tube water. Slide A-1,EFS M40, diameter = 50 µm.
G. Vigna luteola polar view surface showing reticulate pattern. Lower right drain tube water. Slide A-1, EFS M40, diameter = 50 µm.
H. Vigna luteola polar view showing lumina granules. Lower right drain tube water. Slide A-1, EFS M40, diameter = 50 µm.
I. Pinus sp. Pine pollen with two reticulate air bladders. Lower right outer vent drain tube. Slide A-1, EFS J21/1, longest dimension = 75 µm.
J. Poaceae. Grass pollen showing a single pore. Lower right outer vent drain tube. Slide A-1, EFS E23, diameter = 40 µm.

and light-to-medium yellow in color. The taxa recovered are *Schinus terebinthifolius* Raddi and a member of the Asteraceae family (Aster family). Rare occurrences of pollen of *Vigna luteola* (Jacq.) Bentham, *Pinus* sp. (pine) and Poaceae (grass family) were also encountered. Relative percentages of the taxa identified from the five samples are recorded in Table I.

Schinus terebinthifolius, the Brazilian pepper tree is an exotic, invasive plant occurring from Volusia and Levy counties along the coast of Florida, southward. It is present at the Kennedy Space Center, Cape Canaveral (6). In southern Florida, it grows on a broad range of moist to mesic sites, sometimes forming nearly monotypic stands, including tropical hardwood hammocks, bay heads, pine rocklands, sawgrass marshes, *Muhlenbergia* prairies, and the salt marsh-mangrove transition zone (7).

Schinus terebinthifolius thrives on disturbed soils created by natural disruptions, e.g., hurricanes, and is especially invasive in areas affected by human activities. Brazilian pepper does not become established in deeper wetland communities and rarely grows on sites inundated longer than three to six months. In Everglades National Park, it is absent from marshes and prairies with hydroperiods exceeding six months as well as from tree islands with closed canopies (8). The Brazilian pepper plant flowers from September through October, with a second flowering period of less than 10% of the population from March through May (7). Pollination is by diurnal insects including honey bees, syrphid flies, and Lepidoptera. Cassani and coauthors discuss the insect pollinators of the Brazilian pepper tree in south Florida (9).

The Asteraceae is a large family, with pollen of the many species being very similar. It is quite difficult to separate the 23,000 species based on pollen morphology alone. Pollen is both insect and wind dispersed. At the Kennedy Space Center 108 species of Asteraceae have been identified (6). The pollen recovered from the SRB is most comparable to the long-spined pollen of the sunflower group of plants (Tribe Heliantheae), which are entomophillous, dispersed by a variety of insects, especially bees. The pollen recovered from the SRBs are identical to each other, suggesting they are all from the same parent plant species.

Vigna luteola (hairy cowpea), a member of the legume family, is widely distributed in the southeastern United States from Texas south to the Florida Keys, the Caribbean, Central America and parts of South America. It primarily inhabits pinelands, coastal areas and disturbed sites. The pollen of *V. luteola* is insect pollinated. Very few grains of *Vigna luteola* were recov-

Sample No./Name	*Schinus terebinthifolius*	Asteraceae	*Vigna luteola*	TOTAL COUNT
1. Inner Cavity water	179 (60%)	121 (40%)	--	300
2. Inner Tube vent	246 (82%)	54 (18%)	P	300
3. Lower Right outer vent drain tube	134 (45%)	166 (55%)	P	300
4. Lower Right drain tube water	137 (46%)	163 (54%)	P	300
5. Lower Left outer vent drain tube	143 (48%)	157 (52%)	--	300

Table I. Counts and relative percentages of identified pollen from five samples collected from STS-120 solid rocket boosters. "P" indicates pollen present, but not encountered in counts.

ered from the SRB samples and do not figure into the percentages of pollen counts shown in Table I.

The five samples collected from the SRBs and processed for their pollen content are comprised of the pollen of primarily two insect-pollinated plants known to occur commonly at the Kennedy Space Center. The fact that the samples are comprised of pollen in great numbers and from only two species suggests that the pollen was deposited in the drain tube vents by insects. Wind dispersed pollen is more random in nature, and would likely contain pollen of many species. Likewise, the absence of fungal spores (mold) suggests that wind-dispersed pollen and spores are not a factor in the pollen assemblage recovered from the SRB samples.

Graham (10) illustrated that pollen which has undergone temperature changes as low as 100° C will show a darkening, or thermal maturation, of the pollen wall (exine). Thermal maturation of the pollen was not observed. Rather the pollen recovered from the SRBs is light yellow in color suggesting that the cavity area and Teflon tubing were protected from high temperature changes. Elucidation of the type of insect(s) which made the deposits is unknown at this time. Observations made at the launch pad site have revealed the presence of many bees in and around the booster rocket assembly.

The timing of the placement of the pollen material in the tubing is probably prior to launch, as the booster rockets are brought to the launch pad several weeks before the actual launch, certainly time enough for bees or other insects to locate vent tube openings and to make deposits of pollen. Additionally, the flowering of the Brazilian pepper tree coincides with the time period during which this particular Shuttle Transportation System was exposed at the launch pad. Once the SRBs are returned to Earth following a launch, the time between recovery and inspection may be too brief to allow insects to deposit pollen-bearing materials. The splashdown of the SRBs into the Atlantic Ocean most likely dislodged the pollen from the interior drain tube and subsequently blocked the outer drain hole opening, thereby retaining the material and seawater in the cavity.

ACKNOWLEDGEMENTS

Appreciation is expressed to Vaughn Bryant Jr., David Dilcher and Terry Lott for helpful discussions regarding the processing and interpretation of the results. Susan Jarzen assisted with sample preparation and manuscript details.

REFERENCES

1. Traverse, A. *Paleopalynology*. Springer, The Netherlands, 2007.

2. Jarzen, D.M. "Guide to the Operations and Procedures Adopted in the Palynology Laboratory," Florida Museum of Natural History, University of Florida, Gainesville, Florida, 2006.

3. Jones, G.D., Bryant, V.M., Lieux, M.H., Jones, S.D., and Lingren, P.D. "Pollen of the Southeastern United States: With Emphasis on Melissopalynology and Entomopalynology." *American Association of Stratigraphic Palynologists Contribution Series*, **30**, p 76, 1995.

4. Kapp, R.O., Davis, O.K., and King, J.E. *Ronald O. Kapp's Pollen and Spores* (2nd ed.). American Association of Stratigraphic Palynologists Foundation Publication, Dallas, Texas, 2000.

5. Willard, D.A., Cooper, S.R., Gamez, D., and Jensen, J. "Atlas of Pollen and Spores of the Florida Everglades." *Palynology*, **28**, pp 175-227, 2004.

6. Schmalzer, P.A., Foster, T.E., and Duncan, B.W. "Revised Flora and List of Threatened and Endangered Plants for the John F. Kennedy Space Center Area, Florida." National Aeronautics and Space Administration Document NASA/TM-2002-211175, 2002.

7. Ewel, J.J., Ojima, D.S., Karl, D.A., and De Busk, W.F. "*Schinus* in Successional Ecosystems of Everglades National Park." South Florida Research Center Report T-676, Everglades National Park, 1982.

8. La Rosa, A.M., Doren, R.F., and Gunderson, L. "Alien Plant Management in Everglades National Park: An Historical Perspective." pp 47-63 in Stone, C.P., Smith C.W. and Tunison, J.T. (eds.). "Alien Plant Invasions in Native Ecosystems of Hawaii: Management and Research." University of Hawaii Cooperative National Park Resources Studies Unit, Honolulu, 1992.

9. Cassani, J.R., Maloney, D.R., Habeck, D.H., and Bennett, F.D. "New Insect Records on Brazilian Pepper Tree, *Schinus terebinthifolius* in South Florida." *Florida Entomologist*, **72**, pp 714-716, 1989.

10. Graham, A. "Forensic Palynology and the Ruidoso, New Mexico Plane Crash — the Pollen Evidence II." *Journal of Forensic Science*, **42**, pp 391-393, 1997.