

## CREATION RESEARCH SOCIETY STUDIES ON PRECAMBRIAN POLLEN, PART III: A POLLEN ANALYSIS OF HAKATAI SHALE AND OTHER GRAND CANYON ROCKS

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### Abstract

*Samples of Grand Canyon formations of Hakatai Shale, Hermit Shale and Supai Formation were collected using special care to avoid field contamination by atmospheric pollen grains. These samples were subjected to pollen extraction both with and without the use of hydrofluoric acid. The pollen extracts were examined and objects therein photographed under light and scanning electron microscopes. Pollen grains of Pinus, other gymnosperms, and angiosperms were recovered from certain Hakatai Shale samples, together with what appear to be fungal and algal spores.*

*Slides covered with open drops of water or oil were exposed to the atmosphere at various laboratories for a total of 400 days. Objects present on those slides were photographed. Not one of the objects found on these exposed slides was positively identified as a pollen grain. It is concluded that pollen-bearing plants existed while the lithification of Precambrian Hakatai strata occurred—a conclusion which fits with the young earth catastrophist view of origins but conflicts with uniformitarian macroevolutionism.*

### Introduction

C. L. Burdick (1966, 1972) reported finding pollen grains of seed plants in Precambrian Hakatai shale of the Grand Canyon. A. V. Chadwick *et al.* (1973) also admitted finding pollen grains in Hakatai shale while using Burdick's extraction techniques. In a later paper, however, Chadwick (1981) reported that he was unable to recover any pollen grains from other Hakatai shale samples processed by a different procedure involving hydrofluoric acid (HF). He implied that the pollen grains previously recovered by Burdick (and presumably by himself?) were of atmospheric origin and had probably contaminated the rock samples during collection or transportation and were not ". . . authentic examples of Precambrian pollen . . ." (1981, p. 11).

The Research Committee of the Creation Research Society (CRS) authorized E. L. Williams, G. F. Howe, G. T. Matzko, and W. Lammerts to repeat Burdick's research. In February, 1984, rock samples were collected in Grand Canyon. Later pollen extractions were made and analyzed microscopically.

In Howe *et al.* (1986) a preliminary account of these findings was presented. Papers on the history of CRS pollen research and the problem of atmospheric contamination (Parts I and II of this three-part series) have been published—Howe (1987) and Lammerts and Howe (1987).

### Methods and Materials:

#### Collection of Samples

A permit to collect samples from rock exposures of Hermit Shale, Supai Formation and Hakatai Shale

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along the South Kaibab Trail was secured from the National Park Service. The samples were collected on February 10, 1984, a winter day when relatively few species of plants were pollinating in the Grand Canyon. The Hakatai Shale layer was chosen because it was from such material that Burdick (1966 and 1972) and Chadwick *et al.* (1973) had previously extracted pollen grains and that Chadwick (1981) had later reported failure to find pollen. Hermit Shale was also selected to see if similar grains could be recovered from other shales and the Supai Formation was included as a control.

Rock samples were taken after first chipping back three to four inches under the rock surface with a hammer and chisel. A new plastic bag was removed from its carton and opened for only a few seconds during rock sample collection. As inner fragments were chipped loose, they fell directly into the open plastic bag which was held directly beneath the chiseled surface. These precautions were taken to preclude field contamination with atmospheric pollen during sample collection.

After a sample was bagged, the plastic sack was immediately closed, labeled, sealed and placed inside its own plastic canister on which a duplicate label and seal were applied. Thus there was no chance of confusing the samples once they had been taken. A list of the samples and specific details of their collection is given in Table I. Samples were shipped to G. T. Matzko who processed them and performed pollen extractions in the chemistry laboratory at Bob Jones University, Greenville, South Carolina after E. L. Williams had performed some preliminary separations to observe the action of HCl and HF on the mineral samples.

#### An Experiment on Airborn Contaminants in the Microscopy Laboratory

Since some of the microscope work was done by G. F. Howe in two different laboratories at Reese Academic Center (RAC), of The Master's College, we wanted to estimate how likely it was that samples might have become contaminated with atmospheric pollen during slide preparation and microscopic observation. We wished to know what types of objects

**Table I**

Information pertaining to rock samples collected, South Kaibab Trail, Grand Canyon, 2/10/84. Altitude readings were taken directly from NPS altitude markers or with a pocket altimeter calibrated to each altitude marker appearing along the trail. Slight distances in altitude were estimated.

Layer	Sample No.	Approximate Altitude (ft.)	Collection Data
Hermit Shale	H 1	6300	50 feet vertically below Hermit-Coconino contact line.
Hermit Shale	H 2	6200	80 feet vertically below Hermit-Coconino contact line.
Hermit Shale	H 3	6200	At a lookout area under a rock ledge.
Supai	S 1	6000	150 feet vertically below Supai-Hermit contact.
Supai	S 2	5900	About midway into the Supai region. Mixed grey and red material.
Supai	S 3	5200	At lower Supai region. Whitish sandstone.
Hakatai	HK 1	3500	At the upper section of Hakatai rock exposure. Flakey, red, shaley material.
Hakatai	HK 2	3300	About half-way down the Hakatai trail area. Hard, granular quartzitic material with whitish and pink grains.
Hakatai	HK 3	3200	Considerable distance below HK 2 collection site. Brownish shaley material.

would fall on glass slides exposed to the air of these particular laboratory rooms for periods of time longer than those previously reported by Lammerts and Howe (1987).

Six slides were placed on tables in three different laboratory rooms—the two rooms in which slide preparation and light microscope observation were accomplished (RAC 11 and RAC 19, Table III) and one extra room in which none of the analysis was performed (RAC 17). Three of the slides in each room had a drop of microscope oil added to them before the exposure and the other three in each room had drops of tap water added at intervals throughout the exposure period. At the end of 7-9 days, 14 days, and to 38-57 days one oil and one water slide from each room were examined for the presence of contaminant objects and a photographic record was made—see Figures 3-7. Although these slides were untouched during their exposure, the rooms where they were exposed were being used daily for classes and laboratory sessions, presenting ample opportunity for contamination by pollen grains if such is likely.

#### Sample Processing and Pollen Extraction

The methods we used were modifications of the method briefly described by C. L. Burdick (1966). Certain subsamples or separations were first ground in a micromill for five minutes after cleaning under running water with a brush and abrasive cleaner—see Table II. Other samples were of loose material which was neither washed nor ground in the mill. Scrupulous care was taken throughout the processing to avoid contamination of the sample by atmospheric pollen in the laboratory.

One gram of each sample was placed in a 50 ml centrifuge tube where it was covered with Varsol (being thoroughly wetted). Five drops of non-ionic detergent were added and the mixture was stirred.

The sample was then covered with distilled water in the centrifuge tube and vibrated in an ultrasonic bath as follows: 10 minutes for samples a-f and 20 minutes for samples g-p, (Table II). The tubes were then each centrifuged for three minutes at 1500 rpm. The solution was decanted and the samples were washed by varying combinations of 10-minute HCl washes and/or 10-minute water washes, as noted in Table II.

Samples o and p, Table II, had one 10 minute wash (10% HCl) followed by a 90 minute digestion in HF and then two water washes. The HF digestion of extracts o and p was carried out by adding HF drop by drop to the sample and stirring at five minute intervals with a polyethylene stirring rod in a 250 ml polyethylene beaker. A vigorous reaction was evident when HF was first added and CaF<sub>2</sub> formation was evident in the sample.

After centrifuging and decanting, a zinc bromide solution of specific gravity 2.2 was added and the mixture stirred. Each sample was then placed into the centrifuge and rotated at 1500 rpm for three minutes. Since pollen of angiosperms and gymnosperms and spores of algae, fungi and other cryptogams (non-seed producing plants) have a specific gravity of about 1.5, they float in the zinc bromide solution. The technique of Chadwick (1980) was used for removing the float from the tubes. The float was then transferred to 15 ml centrifuge tubes where distilled water was added so that pollen grains and spores present would sink.

The precipitate was next washed twice in water and centrifuged for five minutes. In most samples 5 ml of glycerin was added, centrifuged and decanted. This glycerin separation was then shipped in labeled vials for analysis under light microscopy. In samples o and p, however, a pH 7.00 buffer [0.05 molar potassium phosphate (monobasic)-sodium hydroxide buffer] was added to the float material for shipment. In a few samples the pollen grain extract was left in water for analysis with scanning electron microscope (SEM) (completely eliminating the glycerin step) because we found that glycerin inhibited SEM analysis. In all instances a fresh disposable dropper was used to make the final transfer to new glass shipping vials with plastic screw caps.

#### Making Light Microscope Slides and Photographs

The formica or painted wood tops of laboratory tables used to prepare slides were washed with water and wiped dry before each work period. In some cases a dissecting needle was used to transfer small drops of glycerin or water from a given sample vial (Table II) to a freshly cleaned glass slide. The dissecting needle was thoroughly cleaned with soap and a scrubbing pad, flamed red over a Bunsen burner, and allowed to cool before using. On other occasions the transfer was made with a fresh disposable glass dropper, the tip of which had been heated and drawn to a narrow point to fit into the sample vial.

Each new slide was cleaned before use, rinsed in water, rinsed in 95% alcohol, flamed dry, and then allowed to cool. A fresh glass cover slide was used and

**Table II**

Information pertaining to pollen extracts made from the various rock samples collected, South Kaibab Trail, Grand Canyon. Each rock sample carries the same notation established in Table I. These extracts were made in the Chemistry Department at Bob Jones University. The light microscope slides were made and examined at The Master's College.

Pollen extract	Rock Sample as per Table I	Sample Texture	No. 10-min. acid given to sample followed by No. of water washes	No. slides first, No. of slides with pollen second
a. <sup>o</sup>	HK 1	solid chunks	2 : 1	3 : 0
b.	H 1	loose material	2 : 1	1 : 0
c.	H 2	loose material	2 : 1	1 : 0
d.	H 3	loose material	2 : 1	2 : 0
e.	S 1	loose material	2 : 1	2 : 0
f.	S 2	loose material	2 : 1	1 : 0
g.	S 3	loose material	2 : 1	1 : 0
h.	HK 1	loose material	3 : 1	1 : 0
i.	HK 2	loose material	3 : 1	1 : 0
j. <sup>o</sup>	HK 1	solid chunk	3 : 1	1 : 0
k. <sup>o</sup>	HK 2	solid chunk	1 : 2	1 : 0
l. <sup>o</sup>	HK 3	solid chunk	0 : 3	5 : 0
m.	HK 1	loose material	0 : 2	11 : 8
n.	HK 1	loose material	0 : 2	2 : 0
o. <sup>oo</sup>	HK 1	loose material	1:90 min HF:2	1 : 0
p. <sup>oo</sup>	IHK 1 and III	loose material	1:90 min HF:2	9 : 8

<sup>o</sup>Had five minutes micromill treatment.

<sup>oo</sup>Had HF treatment as noted between the acid and final water washes.

the mount was examined at 100 magnifications. Any object resembling a pollen grain or spore of a non-seed plant in size, shape, or overall appearance was analyzed carefully at higher magnification (400X). A time period of 15 minutes or longer was spent examining each slide with the microscope. Sometimes a slide was re-examined after a period of time ranging from only a few hours in most cases to four days in one instance.

Forty-three light microscope slides were prepared from the 16 samples as shown in Table II. Eleven out of the 43 slides were made from the 5-22-85 Hakatai I extraction (sample m). Eight of these 11 slides showed pollen of seed plants and/or cells of cryptogams. The size of these objects was measured by using an optical micrometer that had been calibrated against a stage micrometer.

Nine additional slides were made from sample p which had been digested in HF. The closed, screw-capped vial containing an extract from HK-3 had lost its water by evaporation in shipment and storage but the dried sediment remained in the tightly capped vial. Liquid from sample o was removed by clean dropper and put into the vial of dry HK-3 extract just mentioned so that sample p actually consisted of the buffer fluid from Hakatai I (sample o) mixed with dry sediment inside a vial of HK-3 extract. Thus pollen grains on such slides were from the HK-3 sediment or from the liquid on the HK-1 sample.

Photographs of biological grains were taken by dark field microscopy, see Figure 1 and following.

**Scanning Electron Microscope (SEM) Techniques**

SEM analysis and photography of various samples was performed by E. L. Williams. Each preparation for SEM study was taken from the top of the glycerin extract and washed in acetone to dissolve the glycerin. It was then filtered on filter paper with methyl alcohol to dissolve or wash away any residual glycerin and to retrieve the sample on the paper.

The filter paper with the sample was allowed to dry following which the sample was scraped from the filter

paper onto double-coated tape where it was gold-palladium coated and photographed using SEM.

SEM photographs were also taken of some fresh pollen grains of pine and oak (Figures 43 and 45 respectively), of fresh pollen that was subjected to HF treatment (Figures 44 and 46), and of pollen extracted from the HK-1 rock sample (loose material which had not been subjected to micromill treatment—Figures 13-17). This sample was given no acid washes but two water washes—a treatment identical to extract m, Table II. One pollen grain, one possible pollen grain, and some cellular objects were found in these SEM analyses—see Figures 13-16. Also, some objects were found by SEM analysis of another HK-1 sample that was subjected to HF extraction—see Figures 38-42.

**Results**

Our results are in the form of photographs of numerous objects found in rock samples, (Figures 1-32 and 38-42). In each figure caption the dimensions are given in micrometers (µm) together with the color of the object and other descriptive remarks.

Certain of our pollen grains and/or spores as the case may be were examined by an accomplished palynologist. While he worked on them he was not aware that the pictures were taken of objects present in extracts of Precambrian Hakatai shale. Following the words "palynologist's discussion," his remarks are printed under each picture that he analyzed. Qualified workers who wish to continue this research can request color enlargements of all or various figures in this paper from Howe.

**Discussion**

**Original "Contamination"**

Because of their small size, pollen grains of seed plants and spores or cells of cryptogams are readily carried by currents of air or moisture. When the various rock layers of the Grand Canyon were undergoing lithification, pollen grains present as original contami-

**Table III**

A survey of contaminant materials present on slides exposed to laboratory air in Reese Academic Center, The Master's College for varying periods of time. The symbol "nr" stands for "not recorded." Slides either had one drop of microscope immersion oil (o) or several drops of water (w) during the exposure. The number of water drops is indicated after the symbol "w," in column two. Although tracheids, trichomes, fungal spores, epidermal cells, and even cells resembling red blood cells or fungi were found several times, only three possible pollen grains (see slides with asterisks) appeared on the slides representing a total of 400 days slide exposure to air in laboratories that were experiencing daily class usage.

Room Number	Material on slide	Minutes spent analyzing slide	Days exposed to atmosphere	Objects present at time of study
*19	o	45	9	1 fungal spore or red blood cell(?) 1 possible disaccate pollen grain
19	w - 7	30	7	4 fungus spores
19	o	nr	14	1 fungus spore
19	w - 8	nr	14	4 fungal spores
*19	o	30	39	2 little spores 1 very dark opaque object pollen? 1 fungus spore cluster
19	w - 13	30	39	1 plant trichome 1 fungus spore
11	o	nr	7	1 plant trichome 2 fungus spores
11	w - 13	nr	7	3 fungus spores 1 epithelial cell
11	o	15	14	1 fungus object
11	w - 16	15	14	no cellular objects
11	o	30	38	2 red blood cells(?) or fungal cells(?) 3 tracheids or vessel fragments 2 fungal spores 1 epithelial cell?
11	w	30	38	5 fungus spore clusters
17	o	30	9	3 red blood cells(?) or fungal spores(?)
17	w - 8	40	9	2 fungus spores 1 plant trichome
17	o	nr	14	2 small sausage-shaped cells 1 fungus spore
17	w - 8	15	14	3 fungus spores
*17	o	45	57	4 epithelial cells 5 sausage-shaped spore 1 possible pollen grain 1 fungus spore cluster
17	w - 13	30	57	1 narrow strand of cells—algae? 10 double spores (15 x 9 m) 1 small cell (12 x 19) 1 small cell—algal spore? 2 fungal spore clusters

nants became part of the rock matrix. Without this original "contamination" of the rock with pollen, palynology would be impossible. Thus a palynologist tries to bend every effort to be certain that objects recovered from rock extracts are part of this original process.

#### Contamination after Lithification?

Even in the catastrophists' scale of time, however, Precambrian rocks such as the Hakatai shale are believed to have existed for thousands of years since their solidification and have thereby been exposed to the atmosphere for long time periods after lithification. During these periods of time after the rock layers were formed, they were exposed to atmospheric pollen. How can we be certain that pollen present in these rocks did not enter the rock long after lithification?

The structure of Hakatai shale rock is fine enough that it would certainly not allow objects the size of pollen grains or even considerably smaller to infiltrate the strata once the rock matrix had formed. Seams in the superficial layers, however, might allow water-borne pollen to penetrate certain areas of Hakatai shale at the surface of rock exposures. Pollen may have contaminated the rock along these seams after the time when the stratum formed and before the sample was collected. By chipping back three or four inches in solid areas which appeared to have no obvious seams or cracks, we have avoided using contaminated rocks and we believe that we thereby circumvented what could be called "post-lithification contamination." Two other workers—Burdick (1966) and Chadwick (1973)—both likewise encountered pollen grains in Hakatai shale samples which were also collected by using techniques aimed at minimizing the chance of collecting rock that had become contaminated with recent pollen. Thus in three instances where workers have attempted to get unweathered samples of rock, pollen was present in Hakatai Shale.

#### Contamination during Sample Collection in the Field?

There is the further possibility that rock samples from the Grand Canyon routinely get contaminated during collection. As noted earlier, we opened collecting bags only very briefly and allowed freshly chipped pieces of rock to fall directly into the bags which were then twice labeled and sealed. Based on previous studies by Lammerts and Howe (1987) we suggest that the chance of field contamination occurring during this brief collection time is very low. The chance of contaminating samples during collection was further lowered by our sampling date, February 10, 1984, as there was snow at the top of the canyon and the entire length of the south Kaibab trail was so cold that we were forced to wear gloves and heavy clothing. It was so cold that all the shrubs and trees were dormant. Thus fresh pollen would have been absent from the air and pollen from the previous year would have settled.

#### Contamination in The Laboratory?

The next step at which a sample might become admixed with recent air-borne pollen would be during the time it is opened in the chemistry laboratory for pollen extractions or in the microscope lab for final analysis. To examine this possibility we exposed slides

for a total of 400 laboratory slide-days in busy teaching laboratory rooms.

Figures 33-37 show representative objects encountered on these exposed slides. Other objects not shown include plant epidermal hairs, tracheids, xylem vessels, possible soot particles, charred carbon particles and fly ash (see Table III). These experiments simply show that when exposed to the air of laboratories where classes were being conducted, no pollen grains could be found on the exposed slides even though many other types of objects did fall on them. Accordingly it is extremely doubtful if any of the pollen grains or spores of Figures 1-32 and 38-42 could be the result of contamination from the atmosphere of the room where slides were examined.

On these exposed slides some tracheids, xylem vessel elements, and plant trichomes (epidermal hairs) appeared. The room in which much of the microscopic analysis was done had been used on occasion for botany classes and this might explain such contaminant items on slides from that lab.

The tracheid found in one of our Hakatai rock samples (Figure 30) most likely came from that rock sample. Another tracheid on top of the cover slide (Figure 31) may have gotten there by an accidental placement of pollen extract on top of the cover slide or it may have been a laboratory contaminant.

It is impossible to absolutely disprove pollen contamination in the laboratory but based on this study and previous data by Lammerts and Howe (1987), we conclude that the likelihood of even one or more pollen grains entering our sample vials or slide preparations is extremely small. It was actually difficult for exposed slides to become contaminated after many days of exposure in busy laboratories.

There is reasonable certainty that pollen grains discovered in this study entered the Hakatai Shale rock during its original period of lithification, having been produced by plants that were living at the time the rock was formed. The palynologist studied the pictures of our laboratory contaminants (Figures 33-37 and others) and concluded as follows:

I don't think you have a contamination problem in the lab. It may be a bit dusty, but the dust does not appear to be pollen.

#### Why Were Two Extraction Methods Used?

Chadwick (1981) has asserted that Burdick's method was inadequate. Chadwick recommended a different method of pollen extraction, involving HF (Doher, 1980). We found that pollen grains were present in samples produced either by the Burdick method (extract m, Table II), or by the Chadwick method (extract p, Table II).

One wonders why Chadwick was unable to extract pollen using the HF method which would presumably remove silicates from the samples thereby making fossil pollen more visible. On p. 4 of her own methods section, L. I. Doher had this word of caution in such use of HF:

Continue agitation until the sample is disaggregated. The length of time that sample is left in HF will depend upon the volume of the sample and the lithology. Most samples will be disaggregated within 1-2 hours. Since HF seems to have a

corrosive action on pollen and spores it is best to stir constantly until the sample is disaggregated and wash immediately. (Some samples may have to remain in HF overnight or sometimes for days to disaggregate. These samples, however, do not usually contain very good pollen or spores.) Doher (1980, p. 4)

Neither Chadwick (1973) nor Solomon and Morgan elaborated on the length of time that their samples remained in hydrofluoric acid. The possibility exists that their negative results were caused by pollen destruction in an elongated HF phase of treating the samples. Chadwick admitted having found pollen grains earlier (1973) in Hakatai shale when using the Burdick (1966) method which involved only HCl with no HF treatment at all. We found that it was not necessary to use hydrochloric acid in the procedure we developed as with our most productive extractions. (See Table II, sample m where no acid wash was employed.) But we also noted that there were numerous pollen grains present in one of the samples we treated with HF—p, Table II.

In their own study of pollen in Twiggs Clay of the Georgia Coastal Plain, Schmidt and Wise (1979) (working with silicate-containing materials) employed no HF in extracting pollen. In private correspondence to E. L. Williams, Wise (1985) stated that HF has been used with mediocre results to extract coccoliths. Evidently it is not necessary to use HF even if silicates are present. Furthermore, the use of HF brings with it the potential problem of destroying fossil pollen grains even though the cell wall of such grains is somewhat resistant to HF. Again it should be remembered that pollen grains were present even after HF treatment in the present study so that we recovered pollen grains both with the Burdick (no HF) and the Chadwick (HF) techniques. Thus our positive results differed with those of Solomon and Morgan (1973, p. 10) who used the HF extraction method on Hakatai Shale and discovered that their results were "... total palynological sterility: i.e. no pollen grains or land-plant spores of any kind were seen."

#### Summary

We have recovered the following objects from samples of Hakatai Shale: pine pollen, Ephedra-like pollen, angiosperm-type pollen, fungal spores and possible algal cells. Our findings support the belief that pollen-bearing plants, algae and fungi existed at the time when Hakatai shale lithification occurred. Such a finding supports Burdick's claims (1966) and casts considerable doubt on the uniformitarian evolutionists' age estimate of billions or even hundreds-of-millions of years for the dating of Hakatai Shale.

One of our Hakatai Shale pollen grains even shows evidence of preserved nuclei (Figure 23). Some of the objects recovered have the bright green color of chlorophyll—Figures 17 and 18. On this basis we also suggest that Hakatai Shale strata were formed relatively recently. Such a conclusion fits squarely with the assumptions of scientific creationism.

Further research will involve analysis of additional extracts from our rock samples with a more extensive examination of the Supai and Hermit material. Qualified workers may receive subsamples of our material

for their own use and/or we invite critical experimenters to collect their own Hakatai Shale samples to repeat this study.

#### Acknowledgments:

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Figure 1. Hakatai shale sample m, Table II. [32x 58  $\mu$ m] Clear, light brown color. This item is very much like Burdick's (1972) Figure 4, p. 29. Palynologist's discussion: "This is most certainly *Pinus*. One of the bladders is damaged but the other is more or less intact."

Figure 2. Hakatai shale sample m, Table II. [32 x 61  $\mu$ m] Clear, light reddish-brown coloration. Palynologist's discussion: "This is probably the same type as Figure 1: it looks somewhat different because of the different alignment of the grain."

Figure 3. Hakatai shale, sample m., Table II. [approximately 25 x 60  $\mu$ m] Palynologist discussion: "This is the saccate type again, probably *Pinus*. The grain has dried out causing the 2 bladders to fold in onto the body of the grain."

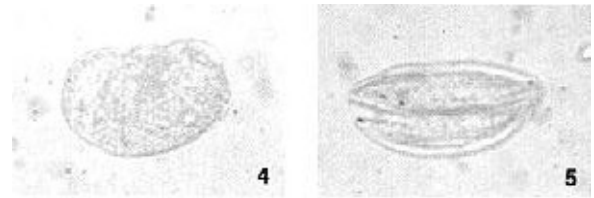


Figure 4. Hakatai shale, sample m, Table II. [25 x 56  $\mu$ m] Clear. Palynologist's discussion: "Probably the same as Figure 3. As you suspect one of the bladders has been lost."

Figure 5. Hakatai shale, sample m, Table II. [25 x 56  $\mu$ m] Clear. Closely resembles Burdick's (1972) Figure 5, *Ephedra* pollen and his (1966) Plate I, Figure 3 designated "*Ephedra* species." Palynologist's discussion: "Unfortunately this is not a very good image but I would hazard a guess that the grain is *Ephedra* (mormon tea). This pollen type, may have 5 to 15 parallel ridges running 'north-south' along the surface. In this case the ridge number is low. *Ephedra* pollen is capable of long distance transport and therefore means little in a paleoecological sense unless it is found in quantity."

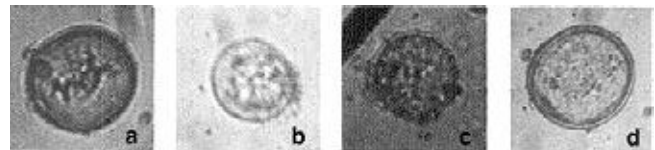


Figure 6. Hakatai shale, sample m, Table II. Four objects with diameters as follows: a-31  $\mu$ m, b-25  $\mu$ m, c-26  $\mu$ m, d-32  $\mu$ m. Each is clear with a slight yellowish color. Palynologist's discussion of a and b: "These appear to be similar although you record different diameters. Accurate identification on the basis of the photographs alone is impossible. I would say though that they are probably not pollen grains and are more likely to be spores of some kind (fungal or algal?)."

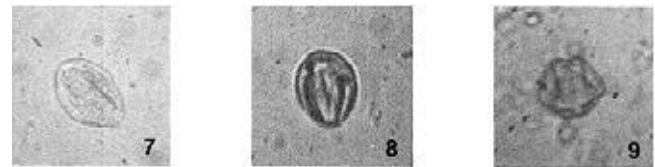


Figure 7. Hakatai shale sample m, Table II. [18 x 25  $\mu$ m] Clear. Palynologist's discussion: "This almost certainly is a pollen grain—I would guess it is a tricolpate or tricolporate type. Unfortunately, this group is a large and difficult one and without a closer look I couldn't be certain what family is involved."

Figure 8. Hakatai shale sample m, Table II. Clear. [18 x 22  $\mu$ m] Palynologist's discussion: "This is similar to Figure 7 and again shows the tricolpate aperture arrangement (3 furrows). *Acer* (maple) or *Quercus* (oak) might be represented here but I would have to look at the slide to be sure."

Figure 9. Hakatai shale sample m, Table II. [21  $\mu$ m] Clear. This photograph was not examined by the palynologist but resembles the grains of Figures 7 and 8.

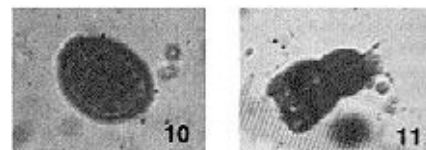


Figure 10: Hakatai shale sample m, Table II. [22 x 29  $\mu$ m] Dark orange coloration, clear. Palynologist's discussion: "This could be a pollen grain but again identification is impossible because of poor resolution of the photograph."

Figure 11. Hakatai shale sample m, Table II. [18 x 29  $\mu$ m] Dark brownish-orange color. Clear. Ocular micrometer is also seen. Palynologist's discussion: "This is a fungal spore possible *Alternaria*. The surface is rough and minutely verrucate (warty) and this is typical of *Alternaria*."



Figure 12. Hakatai shale sample m, Table II. [21 x 38  $\mu\text{m}$ ] Blackish brown color. Opaque. Palynologist's discussion: "This is a fungal spore (2 celled). The taxonomy of fungal spores is notoriously difficult. A similar 2 celled type that is occasionally encountered in Quaternary sediments is *Pullularia* (black yeast)."

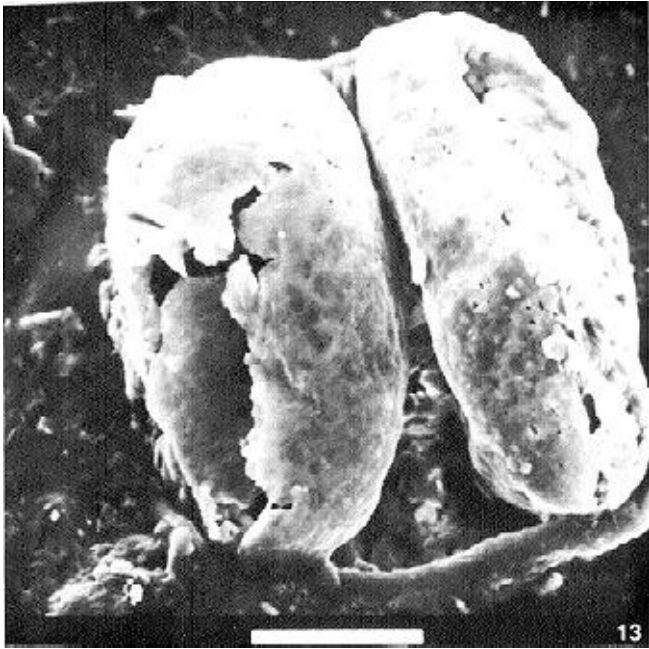


Figure 13. Scanning electron photomicrograph of an object from Hakatai shale. [30 x 35  $\mu\text{m}$ ] Note white bar on this figure is 10  $\mu\text{m}$ . Palynologist's comments: "This is a badly corroded saccate pollen grain, probably referable to the genus *Pinus* (pine). I base this on the relatively small size of the specimen, the body of the grain being only about 30  $\mu\text{m}$ . Unfortunately, identification to species or even subgenus is not possible here."

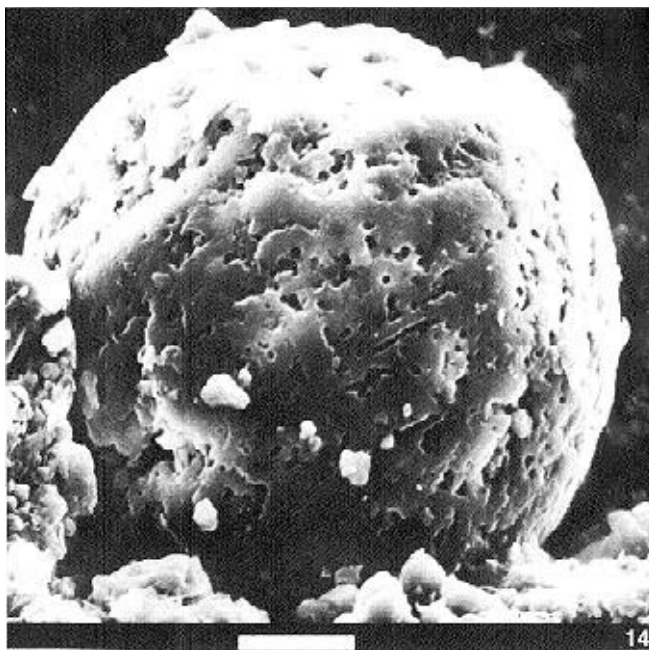


Figure 14. Scanning electron photomicrograph of an object from Hakatai [53  $\mu\text{m}$ ] White bar is 10  $\mu\text{m}$ . Palynologist's discussion: "This is not a pollen grain but most likely a microscopic article of wood charcoal. Another possibility would be a small piece of volcanic material (Pumice?) or perhaps fly ash (industrial produced smoke particles)."

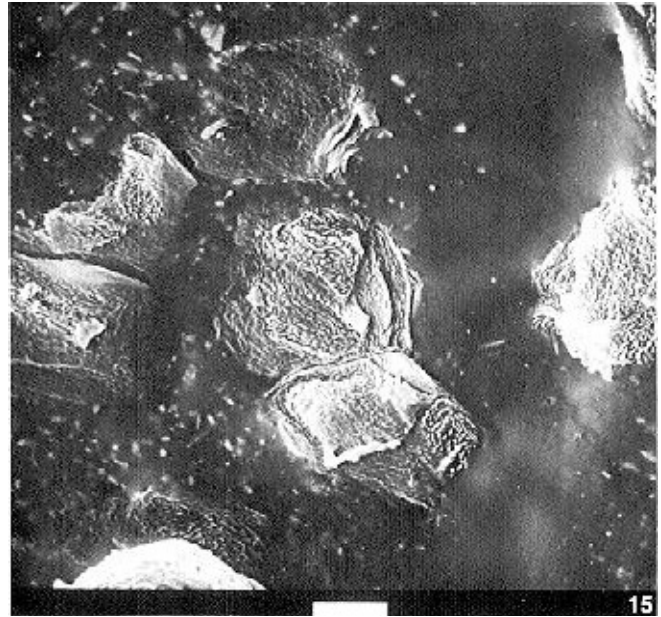


Figure 15. Scanning electron photomicrograph of objects from Hakatai shale. Object in center diameter about 26  $\mu\text{m}$ . White bar is 10  $\mu\text{m}$ . Palynologist's discussion for this figure and the one following: "These enigmatic 'things' are somewhat similar to *Tsuga* (hemlock) pollen. The fine detail of the surface on Figure 16 looks very much like the rugulate surface of *Tsuga* pollen. However, *Tsuga* pollen is usually spherical in shape and at least 60 micrometers in diameter, so I am not sure about the identification here."



Figure 16. Scanning electron photomicrograph of objects from Hakatai shale. Object at lower center is about 20  $\mu\text{m}$  diameter. Magnification is 1400x. For discussion see figure caption for Figure 15.

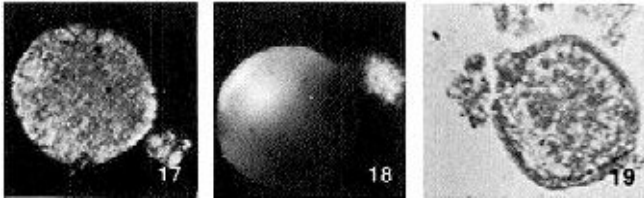


Figure 17. Hakatai shale sample p, Table II. Sidelight (darkfield) picture. Bright green color, speckled with yellow. Opaque. Granular surface. [40  $\mu\text{m}$  diameter]. Palynologist's discussion: "This object is not a pollen grain. Fresh pollen is usually yellow in color and although the cytoplasm may be visible in untreated material, it does not look like the object on the photograph. My guess is that this is an algal cell."

Figure 18. Hakatai shale sample p, Table II. Sidelight (darkfield) Grass-green color. Opaque. Granular surface. [44  $\mu\text{m}$ ] diameter. Rock crystals visible at left and top. Palynologist's discussion: "I am afraid you have got me with this one. Possibly freshwater algae? Definitely not a pollen grain."

Figure 19. Hakatai shale sample p, Table II. Grey-orange color, clear. Estimated diameter, 40  $\mu\text{m}$ . Palynologist's discussion: "This could be a pollen grain—the exine (cell wall) is evident. However, I would rather not speculate as to what species is represented."

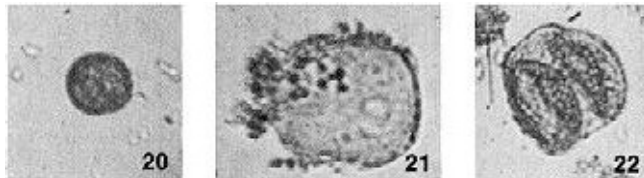


Figure 20. Hakatai shale sample p, Table II. Grey color, opaque. [19  $\mu\text{m}$  diameter]. Palynologist's discussion: "Impossible to tell without more resolution."

Figure 21. Hakatai shale sample p, Table II. Brownish-orange color, clear. [43 x 35  $\mu\text{m}$ ]. Palynologist's discussion: "This appears to be a periporate pollen type . . . looks very much like *Plantago lanceolata*. What you are calling the 'nucleus' is actually a pore. Another possibility here might be *Ulmus*, although elm pollen only has pores around the equator. *Plantago lanceolata* is, of course, a European introduction to North America."

Figure 22. Hakatai shale sample p, Table II. Brownish tint. Clear. [35  $\mu\text{m}$ ]. Saccate type, bladders folded. Palynologist's discussion: "This is almost certainly *Pinus*."

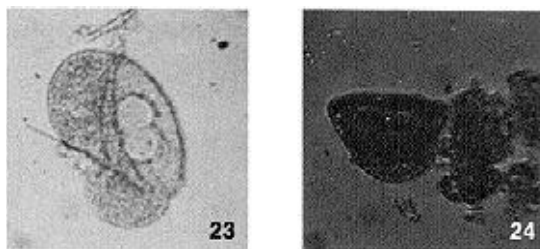


Figure 23. Hakatai shale sample p, Table II. Brownish-almost colorless. Clear. [34 x 53  $\mu\text{m}$ ]. Saccate type. Note cell at top showing cell contents (nuclei?). Palynologist's discussion: "This is also a *Pinus*. You could be right about the nuclei. However, this would indicate that the pollen grain was very 'young.' Cytoplasm is never preserved in fossil material of the kind I work with."

Figure 24. Hakatai shale sample p, Table II. Brownish color. Clear. [26 x 37  $\mu\text{m}$ ]. Saccate type. Side view, bladders folded. Concerning Figures 25-29 the palynologist had these words to say. "In my opinion these are all *Pinus*. They are all saccate, but in some cases the bladders have folded back on the body of the grain. The differences in size suggest that more than one species is represented here; however, pine pollen taxonomy is notoriously difficult and even with the best equipment it is rarely possible to separate different species."

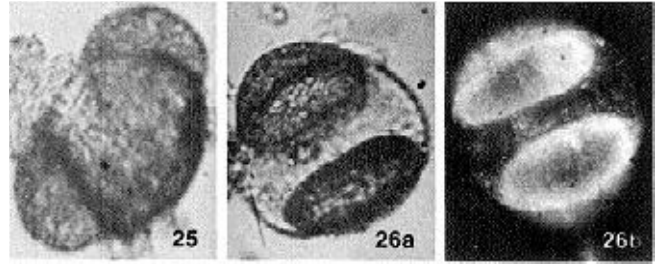


Figure 25. Hakatai shale sample p, Table II. Yellowish-brown color. Cloudy. [50 x 76  $\mu\text{m}$ ]. Saccate type pollen. See caption Figure 24 for discussion.

Figure 26a. Hakatai Shale sample p, Table II. Saccate type pollen. [37 x 56  $\mu\text{m}$ ]. See caption Figure 24 for discussion.

Figure 26b. Same pollen grain as in Figure 26a with darkfield photography. See caption Figure 24 for discussion.

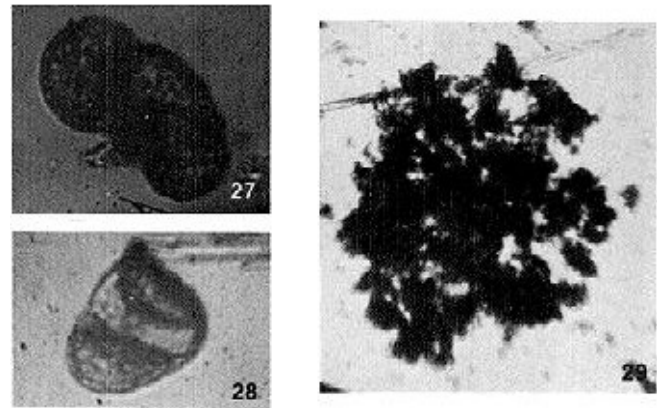


Figure 27. Hakatai Shale sample p, Table II. Saccate type pollen. See caption Figure 24 for discussion.

Figure 28. Hakatai Shale sample p, Table II. Saccate type pollen. See caption Figure 24 for discussion.

Figure 29. Hakatai Shale sample p, Table II. A view of the rock material visible after use of the HF procedure.

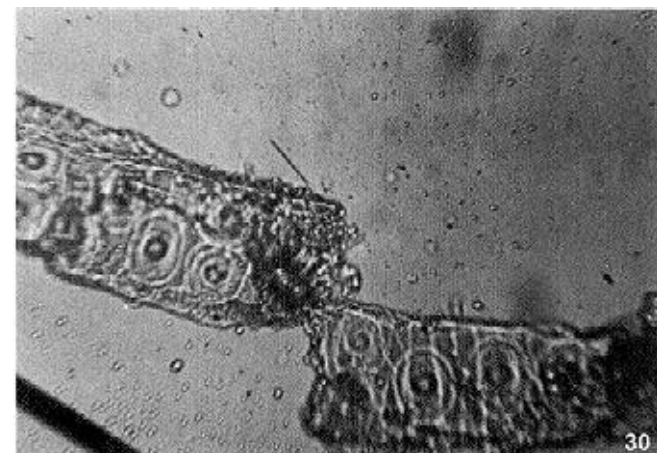


Figure 30. Hakatai Shale sample p, Table II. A tracheid with bordered pits. Tracheid width 51  $\mu\text{m}$ . Width of bordered pit 26  $\mu\text{m}$ . Width of pore in center of pit 6  $\mu\text{m}$ . Length of tracheid 294  $\mu\text{m}$ . Palynologist's discussion: "You are correct, these are tracheids. The bordered pits suggest that gymnosperms are involved."





Figure 31. Found on top of the cover slip on a slide made from Hakatai Shale sample p, Table II. Tracheid with bordered pits. Pit 20  $\mu\text{m}$  diameter, pore 6  $\mu\text{m}$  diameter. Palynologist's discussion: "... it appears some spiral thickening is present in the cell wall—perhaps *Pseudotsuga* (Douglas fir) or *Larix* (larch)?" Note, since this one was found on top of the coverslide it may have got there by accidental placement of pollen extract on top of the cover slide or it may have been a laboratory contaminant.

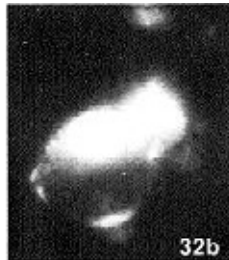


Figure 32a. Hakatai Shale sample p, Table II. Golden-brown color, opaque. Palynologist's discussion: "This is a mystery to me! It is definitely not a pollen grain, however, pollen grains are rarely smooth-walled and spherical." Note bud-like projection on cell.

Figure 32b. Same object as in figure 32a with darkfield photography.

Figure 33. Object present on slide with seven water drops exposed to atmosphere for seven days in RAC Room 19—see Table III. [58 x 15  $\mu\text{m}$ ]. There were many fungus spores like this one found on the various slides exposed to the laboratory air. Palynologist's discussion: "This is definitely a fungal spore. What species is involved I can't say."

Figure 34. Object present on slide with eight water drops exposed to atmosphere in RAC Room 17 for nine days—see Table III. The large object is 15 x 25  $\mu\text{m}$ . Palynologist's discussion of this and Figures 35-37: "These are also fungal structures of various kinds."



Figure 35. Object present on slide with 13 water drops exposed to atmosphere in RAC Room 11 for seven days—see Table III. [78x 18  $\mu\text{m}$ ]. See caption Figure 34 for discussion.

Figure 36. Object present on slide with 13 water drops exposed to atmosphere in RAC Room 17 for 57 days—see Table III. [24 x 13  $\mu\text{m}$ ]. See caption Figure 34 for discussion.

Figure 37. Object present on slide with eight water drops exposed to atmosphere in RAC Room 17 for 14 days—see Table III. [7x 19  $\mu\text{m}$ ]. See Figure 34 caption for discussion.

Figure 40. Object present (SEM) from Hakatai Shale treated with HF. [70 x 60  $\mu\text{m}$ ]. White bar—10  $\mu\text{m}$ . Palynologist said this looked like a charred carbon particle. →

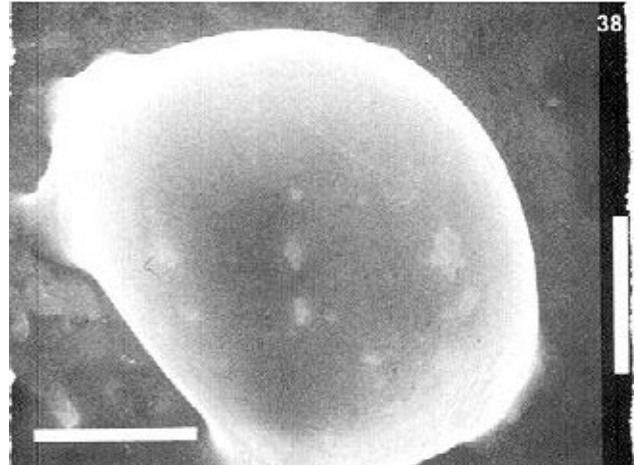


Figure 38. Object resert (SEM) from Hakatai Shale treated with HF. 26 x 35  $\mu\text{m}$ . White bar—10  $\mu\text{m}$ . Palynologist's discussion of this one: "38 appears to be covered with coating medium. I can't help very much with your friend's SEM images."

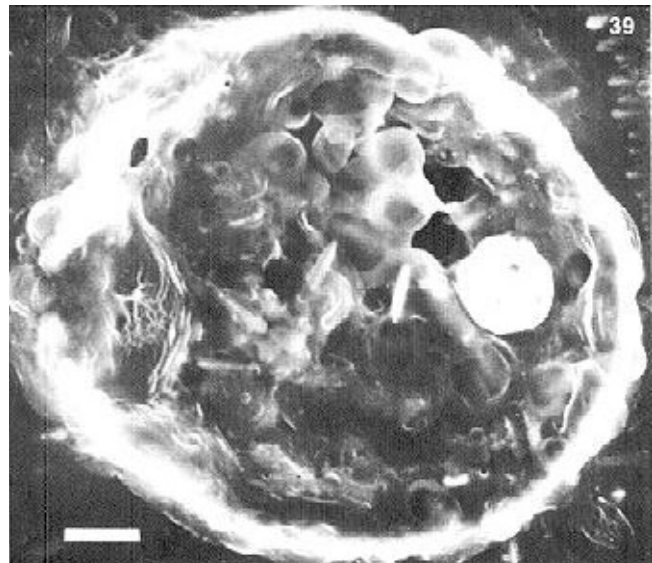
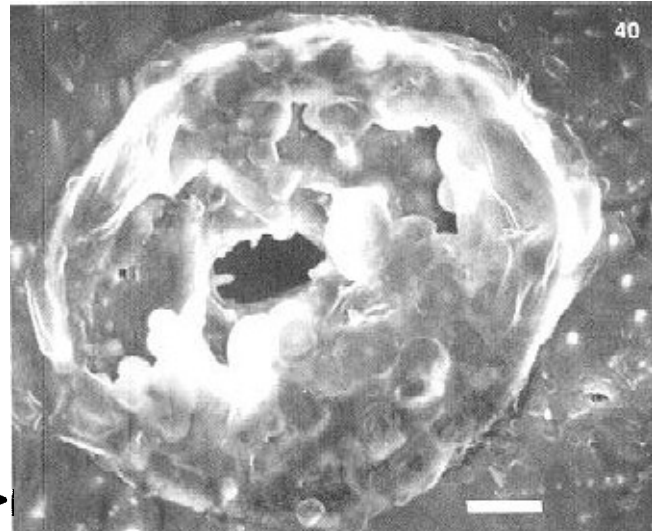


Figure 39. Object present (SEM) from Hakatai Shale treated with HF. [80 x 70  $\mu\text{m}$ ]. White bar—10  $\mu\text{m}$ . Palynologist said this looked somewhat like a charred carbon particle.



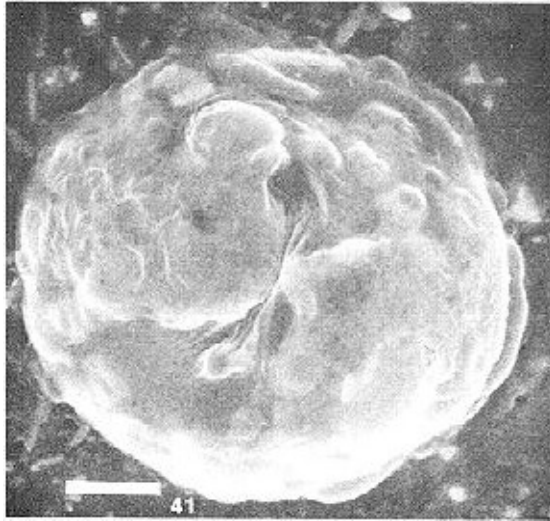


Figure 41. Object present (SEM) from Hakatai Shale treated with HF. [Approximately 50  $\mu\text{m}$ ] diameter. White bar—10  $\mu\text{m}$ . Palynologist said this was a mystery to him.

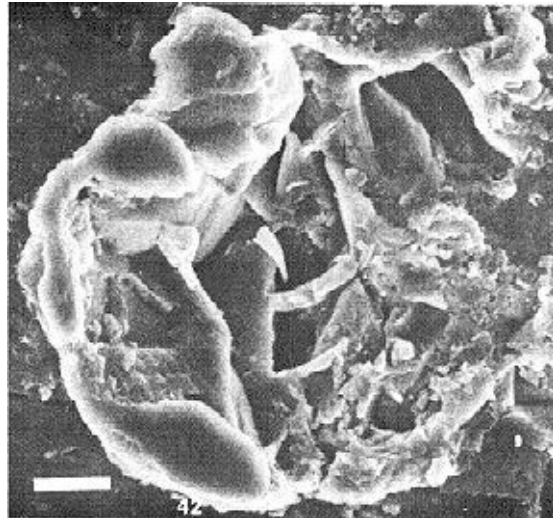


Figure 42. Object present (SEM) from Hakatai Shale treated with HF. [Approximately 70  $\mu\text{m}$ ] diameter. White bar—10  $\mu\text{m}$ .

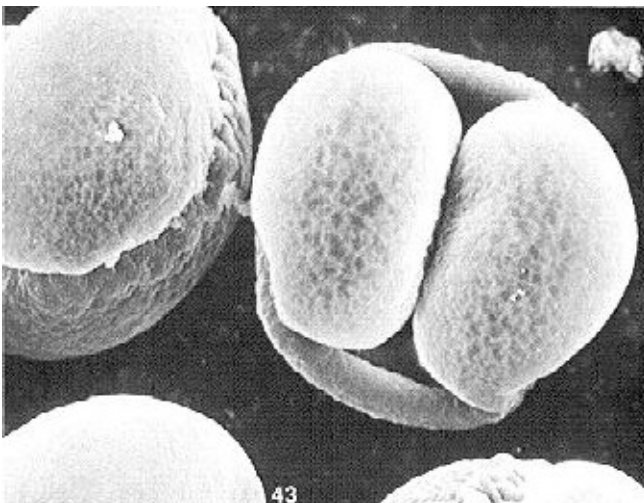


Figure 43. Fresh *Pinus* pollen gold coated, photographed before HF exposure.

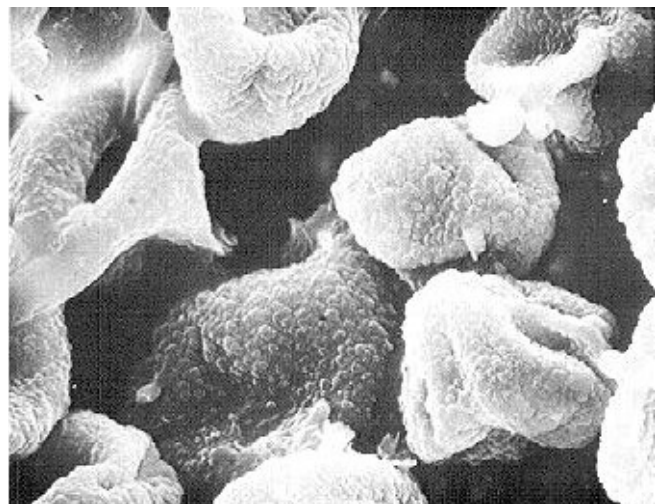
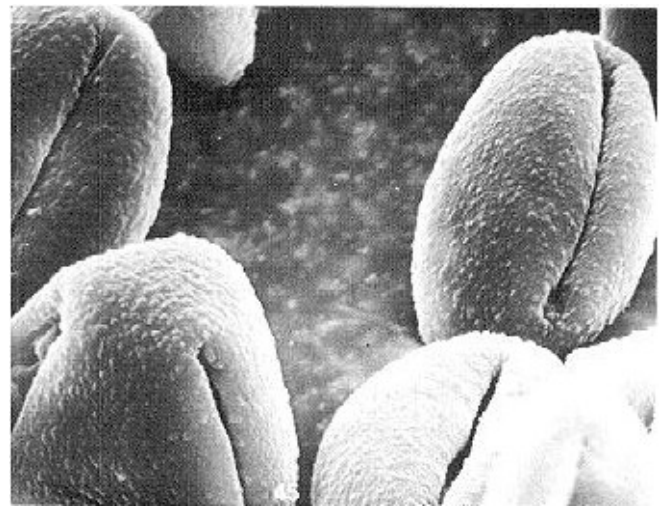
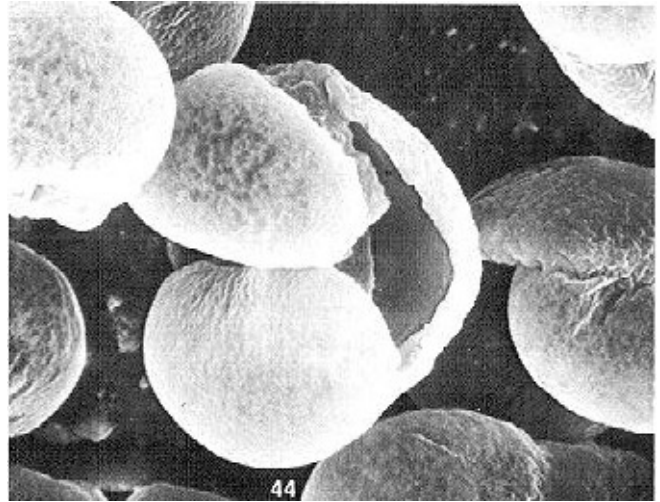


Figure 44. *Pinus* pollen gold coated, photographed after HF exposure.

Figure 45. *Quercus agrifolia* (coast live oak) pollen, fresh, gold coated, photographed before HF exposure.

Figure 46. *Quercus agrifolia* (coast live oak) pollen after exposure to HF, gold coated.