A Study of the Factors that Influence the Infrared Spectra of Pollens

Jacqui Cloud and George Parodi
Saint Anselm College

RESULTS

For heat treatments or exposure to electrical discharge the pollen changed color, from yellow to black. The photoacoustic infrared spectra of these samples demonstrated changes, particularly in the amide I peak. The bottom spectrum in each panel is the difference spectrum: treated ragweed minus untreated ragweed. When the pollen was treated with heat or a voltage, the amide I peak increased in relative intensity.

Since the exine is made up of carotenoid and carotenoid esters, there are no nitrogen atoms present, meaning the amide I peak is from the intine. Treating the ragweed with enough heat or power results in a larger amide I peak. This effect is reproducible both for different types of pollen and for ragweed on different occasions.

No differences were observed when spectra of solvent extracted pollen samples were compared to untreated sample spectra. Results of the experiment designed to isolate the sporopollenin exine are still being analyzed. Initial indications are that the amide I peak is smaller in the isolated material, but not entirely absent as shown in the solvent workup panel below.

DISCUSSION

These treatments have successfully altered the spectra of ragweed. New peaks are being exposed when the pollen is treated with heat or a high voltage. Either the sporopollenin is being broken down, the intine is changing in some way, or new nitrogenous material is being produced. Our belief is that the sporopollenin is being broken down to some extent but not enough to be able to identify the different types of pollen.

The efforts in dissolving the intine to leave behind sporopollenin are not completely successful, because there are still some nitrogen peaks in the sporopollenin spectrum. Different methods of dissolving the intine might need to be explored.

For future research it would be useful to use Raman spectroscopy, considering the amount of literature using Raman spectroscopy to identify pollen. Using Raman with the pretreatments described above would be the next logical step in trying to differentiate between different types of pollen.

REFERENCES

1. Dickerson, P. Application of Chromatography to the Identification of Pollen Samples by FTIR-PAS. St. Anselm College, Manchester, 2005.

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INTRODUCTION

Classifying pollens using Fourier Transform Infrared photoacoustic spectroscopy (FTIR-PAS) with minimal preparation would be a valuable tool because of the ease and the efficiency that would result. Previous research has demonstrated the feasibility of classifying pollens by FTIR transmission (KBr pellet) or by Raman spectroscopy. In the Raman studies, however, a high power laser pretreatment was employed to reduce interfering fluorescence and allow observation of Raman peaks.

Proposed Research:

Difficulties in classifying pollens using FTIR-PAS led us to investigate the factors that influence pollen spectra. In particular we wanted to understand what changes were effected by high power laser pretreatment.

Hypothesis:

Treating pollen with heat, solvents, or electrical discharge should alter the IR spectrum in a manner that might mimic the effects of laser pretreatment.

Past Results:

Past FTIR-PAS experiments demonstrated little ability to differentiate different types of pollen.

MATERIALS AND METHODS

Photoacoustic Spectroscopy:

Discovered by Alexander Graham Bell in 1880. The sample in a closed cell is irradiated with a chopped beam of light. If light is absorbed, the sample becomes a heat source and produces an acoustic or sound wave. The microphone then converts the acoustic wave to an electrical signal, which is read by the detector.

In FTIR-PAS the light source and chopper are replaced by the beam from a Fourier-transform infrared spectrometer.

Data Collection:

The sample compartment was filled with a continuous stream of helium, to enhance the PAS signal and to avoid influence from carbon dioxide or water vapor. Using a plastic bag around the sample compartment traps more helium and the spectra have better S/N and reproducibility. Spectra were recorded at 0.1 cm/s OPD velocity and a resolution of 8 cm⁻¹.

Pollen:

Pollen has two layers, intine and exine. The intine is made up of cellulose and genetic material and the exine is made up of sporopollenin which is composed of carotenoids and carotenoid esters.

Treatments:

Ragweed pollen (Greer) was treated by heating at 160°C, heating at 200°C under oxygen, sparking with a Tesla coil, or extracting with a variety of solvents. In a separate experiment an attempt was made to dissolve the intine and isolate the exine.