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INTRODUCTION

The following protocol describes a method for acquiring spectra from herbarium samples using near-infrared spectrometry (NIRS). This analytical technique measures the absorption of infrared radiation by organic matter, providing information about the chemical composition of samples. Measurements can be made in transmission (light passing through the sample) or reflection (light reflected), with spectra obtained for different wavelengths (800 to 2500nm for the near infrared). This method is particularly suitable for herbarium specimens, which are fragile and must be handled with care. Analysis is performed on the whole tissue, without destruction. The analysis is performed on the whole tissue, without destruction. The portability and speed of the instrument allows a wide range of analyses to be carried out directly on different herbarium specimens

MATERIALS

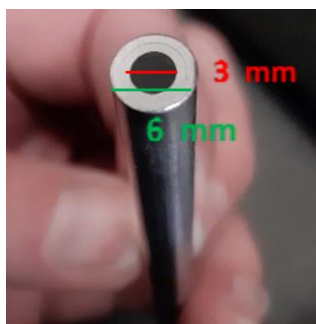
Instrument: Spectrophotometer ASD LabSpec Pro (350-2500nm)

Probe: Pen-shaped probe 3mm diameter

Background: Black paper 100% absorbant - [Metal Spectral](#)™

Spectralon® for white reference

Software for data acquisition: Indico® pro



METHODS

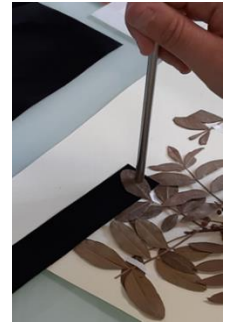
References

The white reference is measured every 15 minutes using Spectralon® (99% reflectance).

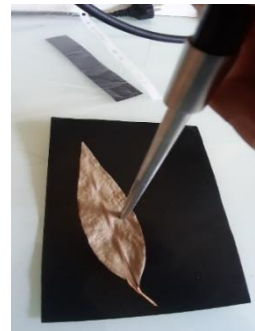
The temperature of the room in which measurements are taken can affect the results obtained. It is therefore necessary to note the temperature before starting the measurements, in order to be able to monitor it in the event of different or aberrant results from one measurement session to the next.

Specimen preparation

- If the specimen is fixed with gummed strips, use a strip of black paper which is slipped between the leaf and the paper at the point where the probe takes the measurement. Clean the paper strip before with precision wiper.



- If detached leaves or fragments are available in a folder, use a larger sheet of black paper and place the leaves directly on top of it.



Spectrum encoding

Particular attention must be paid to the way in which spectral data are encoded with a unique ID.

For example, we use a combination of a 5-letter code for the species (inspired by the [EPPO code](#)) and the unique barcode of the specimen (PIAATMPU673541: *Pistacia atlantica* Desf. from the herbaria of the University of Montpellier with a specimen barcode MPU673541).

The use of the herbarium barcode allows to keep some information in the name of the spectrum. It also facilitates the link with the collection data.

Measurement

Among the parameters of the spectrum acquisition software, the sample count is set to 30. It means that each recorded spectrum was the average of 30 individual scans.

Measurements are made on the adaxial side of the leaf, outside the main vein whenever possible. The probe should be held vertically and in contact with the sample. However, do not apply too much pressure as this may damage the leaf.

Spectra are collected on 3 to 5 leaves per specimen, with 10 points per leaf. It leads to a total of 30 to 50 points per specimen.

Note on the advantages of spectrometer portability

An important advantage is that the instrument is easy to transport, and the measurements are fast. So, we have been able to carry out measurements directly in various herbaria.

Some specimens can't be loaned and sent, such as types and some of the oldest specimens. This method provides an opportunity to access a large number of specimens in a short time without having to ask for the specimens to be sent.



At Paris Museum herbarium (P)



At the Montpellier university herbarium (MPU)