characterization of potato fungal pathogens using FTIR-ATR spectroscopy
Commercial Potato crops are susceptible to a large variety of fungal pathogens.

Each year fungal pathogens cause great crop damages worldwide, resulting in losses of billions of dollars and euro.
Research approach

- Spectral analysis may help evaluate the association of different fungus isolates to various properties.
- We aim to determine different characteristics of fungal pathogens using their absorption spectrum.
Research goals

- Classification of fungi isolates according to their characteristics will allow better treatment.
- Developing the Fourier Transform Infra-Red spectroscopy (FTIR) as a quicker and cheaper method (comparing to the biochemical and molecular methods used today).
Each matter absorbs part of the electromagnetic waves in a specific way. The absorption depends on the materials functional groups and its structure and can be considered as a “fingerprint” of the matter.

Biologic materials absorb infra-red (IR) radiation through changes in the vibrational and rotational levels of the molecules in the matter.
The FTIR spectrometer transmits a broad ray of IR light through a specimen and measures the amount of light absorbed in each frequency. Applying Fourier transform on the measured data provides an absorption spectrum typical to the specimen.
Absorption spectrum
Absorption spectrum

Absorption [A.U.]

Wavenumber [cm$^{-1}$]

Lipids
Amides
Nucleic acids
Carbohydrates
Principal component analysis

- Original spectrum is composed of about 500 points (dimensions).
- To decrease the magnitude of the problem we define a small number of Principal Components (PC’s).
In order to restore the original spectrum each PC must be multiplied with a certain weight (coefficient) and be added to the rest of the PC’s. 

PC1 has the major characteristics of the original spectrum, and so its weight is the highest.
These weights or coefficients, are now the describers of the spectrum.

Instead of the 500 dimension spectrum we started with, we now have a 10-50 dimension spectrum.
Spectrum PCA restoration

- Absorption [A.U]
- Wavenumber [cm\(^{-1}\)]
- Restored
- Measured

Graph showing absorption against wavenumber with restored and measured data indicated.
Results
First characteristic we examined is classification of isolates into Vegetative Compatibility Groups or VCGs.

VCGs are isolate groups which have a common genetic similarity. These groups may make sub-population of common physiologic and pathogenic characteristics.

This information is helpful in assessing the pathogenicity level of a certain isolate found in the field.
Colletotrichum coccodes has been reported worldwide on many different hosts, primarily Solanaceae and Cucurbitaceae. It is the causal agent of black dot disease on potatoes.

Verticillium dahliae which is the causal agent of Verticillium wilt in over 400 plant species including herbaceous, perennials, and woody species.
VCG classification – *C. coccodes*
VCG classification - *C. coccodes*
VCG classification – V. dahliae

![Graph showing VCG classification for V. dahliae]
Phytophthora infestans, is the causal agent of late blight in potato. This pathogen has devastated the potato crops in Ireland 150 years ago and is still causing great losses worldwide.

Although fungicides controlling *P. infestans* are used successfully for almost 100 years, some strains have developed resistance to most common fungicides.

Identification and characterization of these resistant strains, is required for better control of the disease.
Fungicide resistance - Bands

Absorption [A.U.]

- Blue: Resistant
- Red: Non-resistant
- Pink: Overlap

Wavenumber [cm$^{-1}$]

- Lipids
- Amide I
- Phosphate I
- Carbohydrates
Fungicide resistance - Average

(a) Non-resistant vs. Resistant
(b) Lipids vs. Carbohydrates
(c) Lipids vs. Amide I
(d) Lipids vs. Phosphate I
Fungicide resistance - Actual

(a) Non-resistant vs. Resistant carbohydrates
(b) Non-resistant vs. Resistant lipids
(c) Non-resistant vs. Resistant lipids
(d) Non-resistant vs. Resistant phosphate I
# Fungicide resistance

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Applying Linear Discriminant Analysis (LDA) on the PCA manipulated data allows better distinction between the two groups of isolates.

Using LDA has resulted in increasing the sensitivity to 81% and the specificity to 83%.
It is possible to determine characteristics of various fungi isolates, using FTIR spectroscopy.

This can be done almost “at a push of a button”.

Today we continue the research and aim to look into other fungi characteristics.

We aim to diagnose fungi in situ in the field directly on the plant, using a portable FTIR probe.

We believe that FTIR technology could be used as a diagnostic tool for recognition and classification of phytopathogenic fungi.
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Questions