Seed health testing in Spinach seed by multispectral imaging.

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Seed health testing in Spinach

- No accredited method

- Microscopy
  - need trained people
  - time consuming

- Ring test
  - lack of repeatability
Purpose

Is multispectral imaging a potential tool for seed health testing of Spinach samples?

- Separation of uninfected and infected seeds
- Distinguish between five common pathogenic fungi
Seed borne fungi

Common pathogenic fungi on spinach

- *Verticillium dahliae*
- *Fusarium oxysporum*
- *Stemphylium botryosum*
- *Cladosporium variable*
- *Colletotrichum dematium*

Fungi that can be confused with the above

- *Alternaria spp.*
- And 5-6 others genus
Experimental set up

- Sterilization of sample from one seedlot
  Obtain clean isolate of the five fungi

- Artificial inoculation of sterile spinach seeds

- Evaluate seed by freeze-blotter test and microscopy
  Compare fungi growth with natural infected seeds

- Imaging – 10 pictures with 6 x 3 seeds (artificial)
  4 pictures with 25 seeds (natural)
Development stages of the fungi

After one week we obtain the best picture representing the pathogenic fungi at following development stages:

- Conidia
- Conidiophores
- Mycelium
- Resting stage
  - Pseudeothecia
  - Microsclerotia
Multispectral imaging

VideometerLab

- 19 light emitting diodes with specific wavelength ranging from 395-970 nm
- Image size: 1280 x 960 pixels
- Measurement of non uniform color, texture and chemical composition

An image is captured from each wavelength in less than 30 sec.
Raw images

Fusarium is different from the others in visual light (VIS)

Fusarium is similar to uninfected seeds, but different from the others in near infrared light (NIR)

VIS
450nm

NIR
870nm
Features extraction

- A spectral signature
  Histogram showing pixel distribution

- Classification - Pair wise comparison
  Jefferies-Matusita distance (JM-distance)

- Calibration of model - CDA segmentation
  Transformation and threshold
  -> binary image (1 band)
  -> use morphological tools to improve segmentation
Histogram of pixel value

In the low wavelength, no clear separation

In the high wavelength, separation in three groups

450nm

870nm

Pixel intensity

Number of pixels

Alternaria spp.  Fusarium spp.
Cladosporium spp.  Stemphylium spp.
Verticillium spp.  Uninfected
Classification by JM-distance (converted to %)

<table>
<thead>
<tr>
<th></th>
<th>Uninfected</th>
<th>Alternaria</th>
<th>Cladosporium</th>
<th>Stemphylium</th>
<th>Verticillium</th>
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<tbody>
<tr>
<td>Alternaria</td>
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<tr>
<td>Cladosporium</td>
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<td></td>
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<td>100</td>
<td>96.8</td>
<td>56.3</td>
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<td>100</td>
<td>90.6</td>
<td>56.3</td>
<td>93.1</td>
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<tr>
<td>Fusarium</td>
<td>99.6</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>93.1</td>
</tr>
</tbody>
</table>

Histrograms after CDA transformation

JM = 90.6

JM = 100
CDA segmentation

Detection of uninfected seeds

Raw picture | Segmentation after transformation | Binary morphology

- Erosion
- Opening
Count number of objects (seeds) and measurement of area (square pixels)

The particle analyzer finds 5 objects below 100 pixels and 2+1 objects bigger than 700 pixels in area (uninfected seeds)
Verification of model

Test of 30 uninfected and 5 x30 artificially inoculated seeds
- 20% uninfected by VideometerLab
- 17% uninfected by microscope

Test of 100 naturally infected seeds
- 84% uninfected by VideometerLab
- 71% by microscope
Conclusion

Multispectral imaging is a potential tool in seed health testing

- Infected and uninfecte4d seeds can be separated
  - Both VIS and NIR wavelength are needed

- Separation within infected seeds need further work
Thanks

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- Supervisors and colleagues