Development of molecular diagnostics for sugarcane downy mildew

Nicole Thompson\textsuperscript{1}, Rob Magarey\textsuperscript{1}, Lastus Kuniata\textsuperscript{2} and Barry Croft\textsuperscript{1}

\textsuperscript{1} BSES Limited, Australia \hspace{0.5cm} \textsuperscript{2} Ramu Agri-Industries Limited, Papua New Guinea

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Outline of presentation

- Introduction
  - Downy mildew
  - History of DM in Australia
- Molecular diagnostic design
- Results and conclusions
- Future work
Introduction

- Found in many sugarcane growing regions of the world (SE Asia)

- Downy mildew
  - Caused by Oomycete pathogen in genus *Peronosclerospora*
    - *P. sacchari*, *P. philippinensis*, (others?)
    - Classical taxonomy difficult
Downy mildew symptoms

- Classic leaf stripe
  - White or red in colour
  - Down is visible on underside of leaf in morning

- Leaf shredding
  - May/may not be accompanied with classic symptoms
History lesson

- Downy mildew in Australia in 1910’s
- Eradicated from commercial cane in late 1950’s by intervention and use of resistant varieties
- BSES ceased resistance screening in 1980’s
- Since that time ...
  - No selection for DM resistant varieties = reduction of number of DM resistant canes
  - Estimated 60% - 80% of the crop now has susceptibility
Biosecurity project

- SRDC funded project in collaboration with Ramu Agri-Industries Limited, PNG
- Primary aim: screen Australian varieties for resistance to downy mildew, Ramu stunt and *Sesamia* borer.
- Project also allows for some basic research into sugarcane downy mildew
  - Surveys
  - Molecular diagnostics
Geography lesson
Disease surveys
Molecular diagnostic test

- 2009 visited USA labs to discuss and work on downy mildew (USDA and Texas A&M)
- Gained valuable information including unpublished DNA sequence
- Designed several sets of primers to show differences between *Peronosclerospora* species
  - 4 genome regions targeted
Molecular diagnostic test

- Designed several sets of primers that could show differences between *Peronosclerospora* species

<table>
<thead>
<tr>
<th>Species</th>
<th>Cox-1</th>
<th>EF-1a</th>
<th>B-tub</th>
<th>ITS-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>P sacchari*</td>
<td>185/295</td>
<td>470?</td>
<td>?</td>
<td>100</td>
</tr>
<tr>
<td>P philippinensis*</td>
<td>?</td>
<td>420?</td>
<td>?</td>
<td>100</td>
</tr>
<tr>
<td>P maydis</td>
<td>?</td>
<td>410?</td>
<td>400?</td>
<td>100</td>
</tr>
<tr>
<td>P sorghii</td>
<td>317</td>
<td>?</td>
<td>400?</td>
<td>100</td>
</tr>
</tbody>
</table>
Primer test/optimisation

Cox-1

EF-1A

B-tub

ITS-1
Primers tested on known *Peronosclerospora*

- P. species of same type grouped together
- Proof of concept for Cox-1 primer pair

Neighbour joining, 1000 bootstraps (Mega 5)
# Cox-1 PCR: first PNG samples

<table>
<thead>
<tr>
<th>Marker</th>
<th>Sample Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>S. edulis</td>
</tr>
<tr>
<td>2.</td>
<td>S. edulis</td>
</tr>
<tr>
<td>3.</td>
<td>S. edulis</td>
</tr>
<tr>
<td>4.</td>
<td>S. edulis</td>
</tr>
<tr>
<td>5.</td>
<td>S. robustum</td>
</tr>
<tr>
<td>6.</td>
<td>S. spontaneum</td>
</tr>
<tr>
<td>7.</td>
<td>B72-177</td>
</tr>
<tr>
<td>8.</td>
<td>Commercial</td>
</tr>
<tr>
<td>9.</td>
<td>Commercial</td>
</tr>
<tr>
<td>10.</td>
<td>R570</td>
</tr>
<tr>
<td>11.</td>
<td>Q127</td>
</tr>
<tr>
<td>12.</td>
<td>Q136 (ls)</td>
</tr>
<tr>
<td>13.</td>
<td>Q136 (c)</td>
</tr>
<tr>
<td>14.</td>
<td>Commercial</td>
</tr>
<tr>
<td>15.</td>
<td>Negative</td>
</tr>
</tbody>
</table>
## Cox-1 analysis of survey samples

<table>
<thead>
<tr>
<th>50 bp marker</th>
<th>S. edule</th>
<th>S. robustum</th>
<th>S. spontaneum</th>
<th>R570</th>
<th>Maize</th>
<th>S. officinarum</th>
<th>Miscanthus</th>
<th>S. robustum</th>
<th>Maize</th>
<th>S. robustum</th>
<th>Miscanthus</th>
<th>S. robustum</th>
<th>Maize</th>
<th>Miscanthus</th>
<th>S. robustum</th>
<th>S. edule</th>
<th>S. robustum</th>
<th>Commercial cane</th>
<th>Healthy</th>
<th>Water</th>
<th>50bp marker</th>
</tr>
</thead>
</table>

![Droplet gel image with samples and markers]
Sequencing of Cox-1 amplicons

3 variants of Cox-1 that are different from previously characterised sequences

S. edule and commercial

Miscanthus and S. officinarum

S. rob. and S. spont
Most Ramu Valley samples
All Sugarcane samples

Most highlands S robustum samples

Most Ramu Valley S robustum and S spontaneum samples

Australian historic samples and Madang S edule

Most highlands samples from Miscanthus, S officinarum.
One Australian accession.
Ramu Agri-Industries plantation results

- Possibly 2 variants of Peronosclerospora present in the Ramu Valley
  - Predominantly one Cox-1 variant in the commercial plantation
  - Predominantly one symptom in the commercial plantation (classic symptoms)
    - One variety (Q136) had both symptoms, but only one Cox-1 variant.
  - Cox-1 variant does not appear to be correlated with symptoms
Results from survey

- Miscanthus and *S. robustum* leaf shredding is amplified by Cox-1 primers
- Not a lot of classical symptoms observed outside of Ramu Valley
  - Only on *S. officinarum, S. edule* and maize
  - Reported on *S. robustum*, but not found unless associated with leaf shredding
DM variation in PNG

- Sequence of Cox-1 revealed loose groupings:
  - *S. robustum* from highlands (highest point)
  - *S. officinarum* and *Miscanthus* from highlands
  - *P. philippinensis* clustered amongst commercial varieties on RAIL estate
  - *P. sacchari* fell outside the clusters
  - DM sample on *S. edule* near Madang is most similar to the herbarium specimen from Qld in 1980’s.
Conclusions

- Cox-1 primer pair good for determining differences in DM
  - Species? Need more type specimens
- Variation due to location
  - Secondary due to host species
Future work

- Survey sample analysis and sample collection
- Analysis of key samples from other countries
- Microscopic analysis of *Peronosclerospora* species in PNG
Acknowledgments

- Ramu Agri-Industries Limited
  - Dr. Lastus Kuniata, Kaile Korowi
  - Field staff and technicians

- BSES
  - Dr. Rob Magarey, Dr. Kathy Braithwaite, Dr. Peter Samson

- Biosecurity Queensland (Qld DEEDI)
  - Dr. Roger Shivas (Herbarium)

- Funding from SRDC
Call for specimens!

- If you have access to sugarcane downy mildew specimens and are able to send them
  - Especially if you have some known/type specimens
- All costs of shipping covered
- nthompson@bses.com.au