Development of a detached bioassay to determine cross-pathogenicity and pathogenic variation of *Botryosphaeriales* isolates on macadamia

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Introduction to Macadamias

- Macadamia spp. native to subtropical eastern Australia
- Australia home to all 4 species
- M. integrifolia & M. tetraphylla grown for edible kernel
- Australia currently largest producer worldwide

In Australia 2016:

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Production area (Ha)</td>
<td>19 000</td>
</tr>
<tr>
<td>Production (t)</td>
<td>15 558</td>
</tr>
<tr>
<td>Production ($m)</td>
<td>$247.3</td>
</tr>
<tr>
<td>Export value ($m)</td>
<td>$253.2</td>
</tr>
</tbody>
</table>

Production Regions Worldwide
Production Regions Australia

- Current growing region from Atherton Tablelands to mid-north coast of NSW
- Also areas in WA, Emerald & Mackay
Background to *Botryosphaeriales*-induced dieback

- Branch dieback
- Discolouration of internal tissue of stem
- Point of gummosis
- Leaves of affected branches become brown remaining attached
- Infection of main trunk results in tree decline & death
Purpose of Study

- Increase in incidence and severity of branch dieback in last 10 years
- Unknown how many of the *Botryosphaeriales* species affect macadamia
- Also unknown what cultivars are susceptible
- Wide host range – Implications on neighbouring crops?
- Unable to carry out field trials – Bioassay
Research Aims

a) Determine if *Botryosphaeriales* isolates are cross-pathogenic on macadamia.

b) Determine pathogenic variation and aggressiveness of *Botryosphaeriales* isolates on macadamia.

c) Develop a rapid, detached bioassay for macadamia cultivars.
<table>
<thead>
<tr>
<th>Accession no.</th>
<th>Identity</th>
<th>Source of isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAC1705*</td>
<td>Botryosphaeriaceae sp.</td>
<td>Macadamia integrifolia</td>
</tr>
<tr>
<td>MAC1706*</td>
<td>Botryosphaeriaceae sp.</td>
<td>Macadamia integrifolia</td>
</tr>
<tr>
<td>BRIP53573*</td>
<td>Lasiodiplodia pseudotheobromae</td>
<td>Macadamia integrifolia</td>
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<tr>
<td>BRIP53574*</td>
<td>Neofusicoccum parvum</td>
<td>Macadamia integrifolia</td>
</tr>
<tr>
<td>BRIP55401*</td>
<td>Neofusicoccum cryptoaustrale</td>
<td>Persea americana</td>
</tr>
<tr>
<td>BRIP59728*</td>
<td>Neofusicoccum australie</td>
<td>Persea americana</td>
</tr>
<tr>
<td>BRIP64718*</td>
<td>Lasiodiplodia theobromae</td>
<td>Passiflora edulis</td>
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<tr>
<td>BRIP64091</td>
<td>Neofusicoccum parvum</td>
<td>Vaccinium sp.</td>
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<td>DAR83073</td>
<td>Neofusicoccum parvum</td>
<td>Vaccinium sp.</td>
</tr>
<tr>
<td>DAR83074</td>
<td>Neofusicoccum parvum</td>
<td>Vaccinium sp.</td>
</tr>
<tr>
<td>DAR83076*</td>
<td>Neofusicoccum kwambonambiense</td>
<td>Vaccinium sp.</td>
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<td>DAR83079</td>
<td>Neofusicoccum kwambonambiense</td>
<td>Vaccinium sp.</td>
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<tr>
<td>DAR83082</td>
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<td>Neofusicoccum parvum</td>
<td>Musa</td>
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<td>BRIP63679</td>
<td>Neofusicoccum parvum</td>
<td>Mangifera indica</td>
</tr>
<tr>
<td>BRIP55370</td>
<td>Lasiodiplodia pseudotheobromae</td>
<td>Citrus limon</td>
</tr>
</tbody>
</table>

* Isolates used in pathogenicity study on macadamia.
Methods

*Macadamia Leaf Bioassay – Cultivars ‘HAES 246’, ‘HAES 344’, ‘HAES 741’, A16*

- Mid rib wounded and wound placed on inoculum plug in Petri dish with moist cotton wool mount
- Stored at 25°C, 100% humidity, 24 h photoperiod
Methods

Macadamia Seedling Assay – Cultivars HAES 246, HAES 344, A16, HAES 741

- Wound made using a round file to remove bark and expose internal tissue, followed by placing an inoculum plug on wound and sealing it
Methods

- Blueberry Leaf Bioassay – OPI
  - Petiole of leaves inserted into inoculum plug, moist vermiculite added creating microcosm
  - Stored at 25°C, 100% humidity, 24 hr photoperiod
Results – Blueberry Leaves

- Susceptible to all isolates regardless of the host
- Minimal pathogenic variation within species e.g. *N. parvum*
- Most aggressive isolates from *Macadamia integrifolia*

Disease progression curve of selected isolates on leaves over 9 days
Results – Macadamia Leaves

- Isolate from *Macadamia integrifolia* most aggressive
- Some susceptibility to species from other hosts
- Slow disease progression

246 inoculated with *Botryosphaeriaceae* sp.

Average diseased leaf surface area (%) 28 days post-inoculation
Results – Macadamia Seedlings

Inoculated with *Botryosphaeriaceae* sp.; top 2 at 2 weeks, Right at 3 weeks. Right images Cv. 246, top image Cv. 741.
Seedling lesion length – 4 weeks

- **Botryosphaeriaceae sp.**
- **N. cryptoaustrale**
- **N. parvum**
- **Botryosphaeriaceae sp.**
- **Control**
Findings & Future Research

- *Botryosphaeriales* isolates pathogenic on both hosts, with isolate 1705 (*Botryosphaeriaceae* sp.) causing rapid dieback of macadamia seedlings.
- Same species isolated from different hosts showed pathogenic variation.
- Symptoms observed on blueberry leaves were similar to those observed on macadamia leaves.
- Seedling assays required to verify the results obtained by leaf assays in future studies.
- Further research may aim to determine the host-pathogen interaction of this fungi, particularly in regards to disease progression.
- Current gap in knowledge about infection progression of woody pathogens in different crops.
Acknowledgements

Supervisors:
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- Associate Professor Vic Galea

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