First report of *Diaporthe gulyae* systemic seed infection* of sunflower – implications for DSC management

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*paper submitted for review

The Australian *D. gulyae* outbreak triggered a seed infection investigation……

- **2009/10 mid-stem lodging and yield losses**
- **Symptoms on leaves and stems almost identical to those of *D. helianthi (exotic)***
- **Seed infection without visual symptoms on the capitulum**
- **Pycnidia emerged from the seed after incubation**

Lodging and lesions typical of Phomopsis Stem Canker, seed infection in heavily infected plants

Diaporthe gulyae……most virulent new species

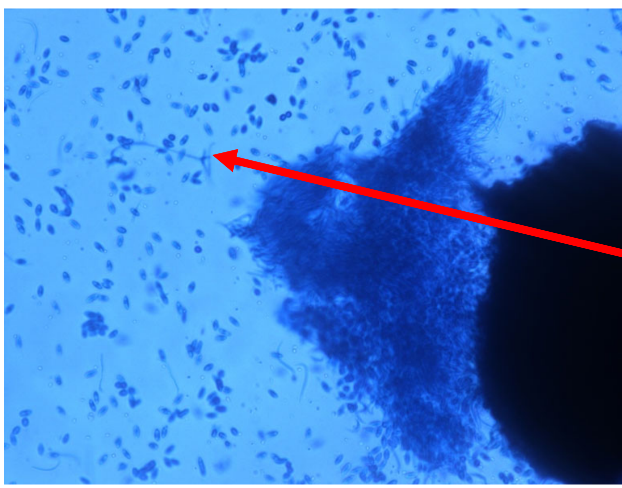
Etymology: In recognition of Dr. Tom Gulya (USDA-ARS) for his outstanding contributions to sunflower pathology research and enduring mentoring roles in the USA, Europe and Australia.

First identified as a new species in Australia after a damaging stem canker outbreak on sunflower in 2009/10.

***Described in 2011 (Thompson et al. 2011) as:**
Diaporthe gulyae **RG Shivas, SM Thompson & AJ Young**

***Thompson SM, Tan YP, Young AJ, Neate SM, Aitken EAB, Shivas RG (2011a). Stem cankers on sunflower (*Helianthus annuus*) in Australia reveal a complex of pathogenic *Diaporthe (Phomopsis)* species. Persoonia 27: 80–89.**

With the exception of conidia, *D. gulyae (alpha conidia)* symptoms on sunflower very similar to those of *D. helianthi* (beta conidia)....



Pycnidia in leaf, alpha conidia, *D. gulyae* type generated from leaf infection

Stem lesion colour gradient

Seed infection – oozing pycnidia

Stem lesions – the most commonly seen symptoms.

D. gulyae **stem symptoms similar to those of *D. helianthi.***
Accurate differentiation of the species in the field is not recommended; Use molecular technologies to ID.

D. gulyae virulence*……

*<u>Associated </u>with 14 hosts in Australia – 5 crops and 9 weeds**

***Pathogenic on multiple crops: eg. safflower, sunflower, soybean, mungbean, lupin and **peanut (papers in prep).**

***Pathogenic on multiple weeds: eg. *Xanthium* sp., *Physalis* sp., *H. annuus* (wild-type), *Sonchus oleraceus* (papers in prep).**

Highly virulent on sunflower and other crops and weeds

Virulence rating 4 or 5 on a 1-5 rating scale

D. gulyae isolates collected from leaves, stems, stem base/roots, capitula, seeds

***PhD thesis: Brumpton Thompson S. 2020.**
Diaporthe species association with sunflower and other crops and weeds in eastern Australia. PhD Thesis, School of Agriculture and Food Services, The University of Queensland, Australia. https://doi.org/10.14264/uql.2020.779

**** Thompson et al. 2018**

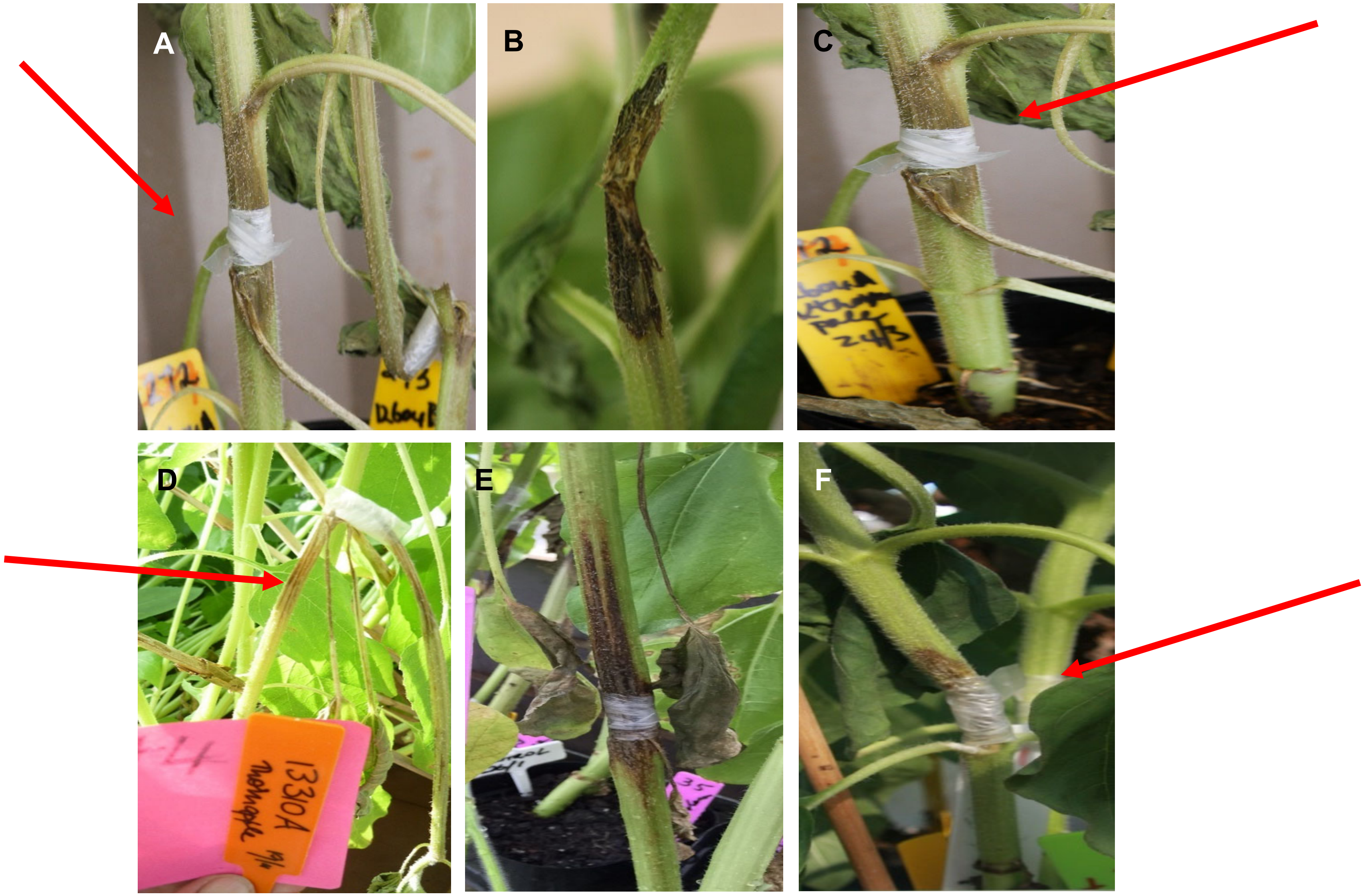
Stem slit inoculations – a harsh test, so consider the results with the biology of the *Diaporthe* genus in mind …………… opportunistic colonisation

Virulence ratings = degree of severity of infection

Rating			Pathogenicity, virulence
1			Not pathogenic
2			Not pathogenic, or pathogenic with low virulence, or latent
3			Pathogenic, moderate virulence
4			Pathogenic, high virulence
5			Pathogenic, very high virulence

^pathogenic VR2-5, keep in mind plant age, time after inoc

Diaporthe gulyae isolates from a range of hosts – pathogenicity testing, lesion colour variation…



Diaporthe spp (7) identified from sunflower seed in Australia…….virulence on sunflower (1-5 rating^)

- ***D. ambigua*……………………………………………… *VR1/2***
- ***D. goulteri*……………………………………………… *VR2***
- ***D. gulyae*……………………………………………… *VR4/5***
- ***D. masirevicii*……………………………………… *VR3***
- ***D. ueckeri* syn. *miriciae*……………… *VR3***
- ***D. serafiniae*……………………………………… *VR2/3***
- ***Diaporthe* sp. nov. 14 (not tested)**

D. gulyae

^pathogenic VR2-5, keep in mind plant age, time after inoc

Seed infection experiments……

- **1. Laboratory experiments – visual inspection of germination a range of D. gulyae infected seeds**

- **2. Glasshouse experiments – no other infected plants in G/H or within 20km**

  Match-stick inoculation of *D. gulyae* isolate at the 4th node

  Stem lesions developed within 2 -5 dai

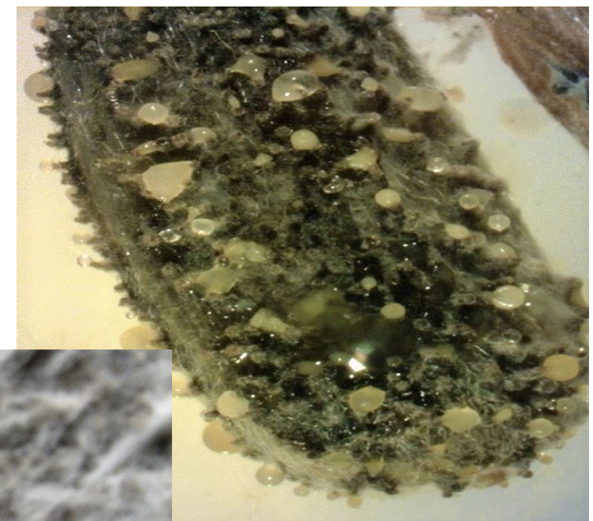
  Heads were bagged at budding

  Plants harvested 21 days after physiological maturity and any head infection evaluated

- **3. Glasshouse pot trials - evaluating emergence of infected seeds**

1. Lab assessment of germination of infected seeds…..

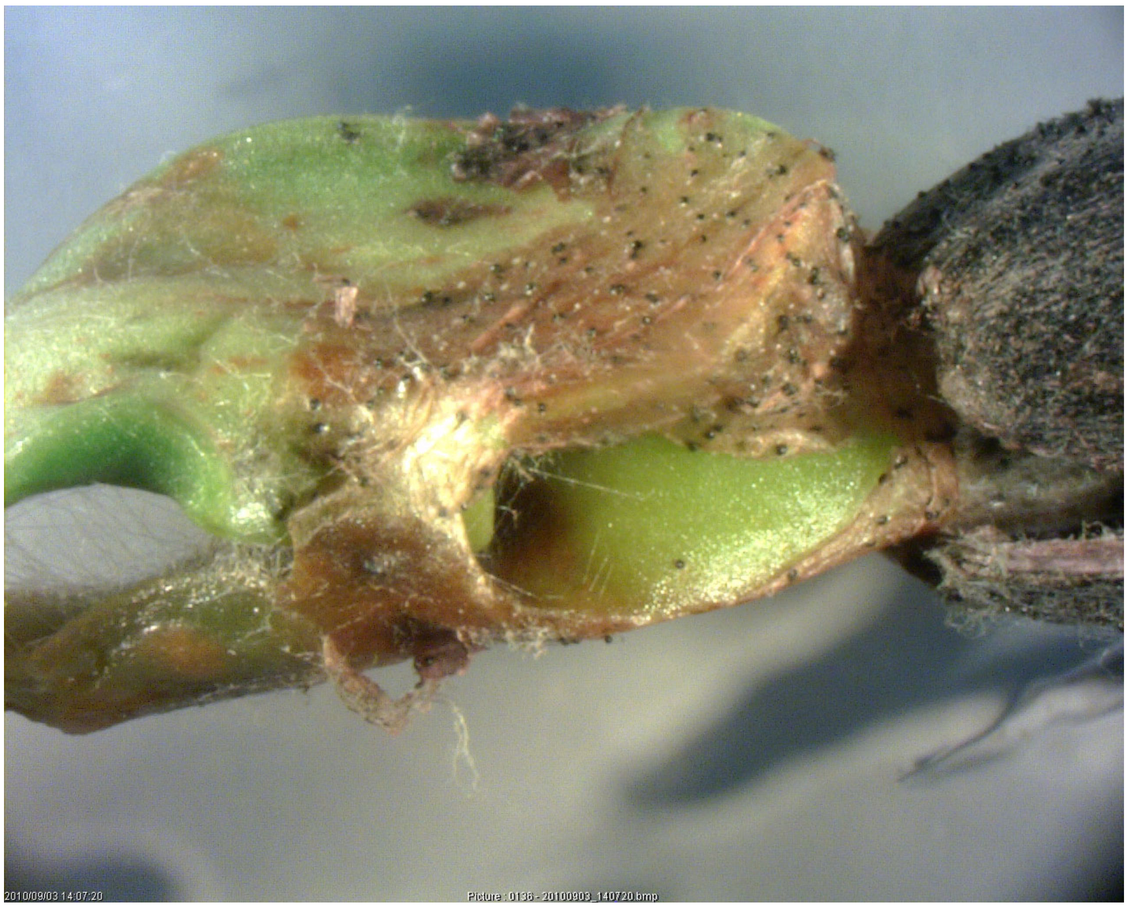
- Highly infected seeds do not germinate on WAS

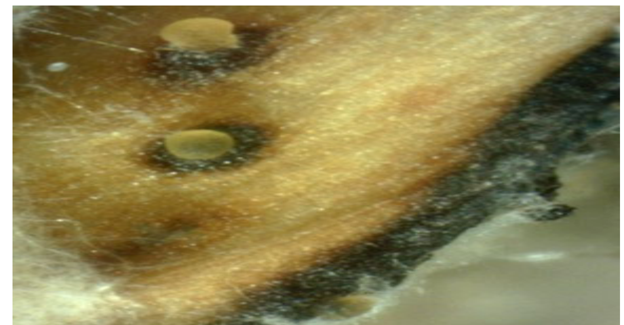


1. Infected seed germination (lab)……

Some seeds (with fewer pycnidia observed) germinated on WAS then developed necrosis of cotyledons

Necrosis spread from the cotyledons to the emerging radicle





2. Investigating systemic infection……

- **Sunflower plants grown in pots**
- **Inoculated at V10-R1 growth stage, match stick**
- ***D. gulyae* isolate of the type (BRIP 54025, MB561569)**
- **Progression of hyphal growth upwards in the stem was monitored by destructive sampling of a selection of inoculated plants**
- **Heads bagged during budding**
- **Surviving plants were harvested 21 days after physiological maturity**
- **Capitula pieces, seed and husks incubated**

Proof of systemic infection via the stem……

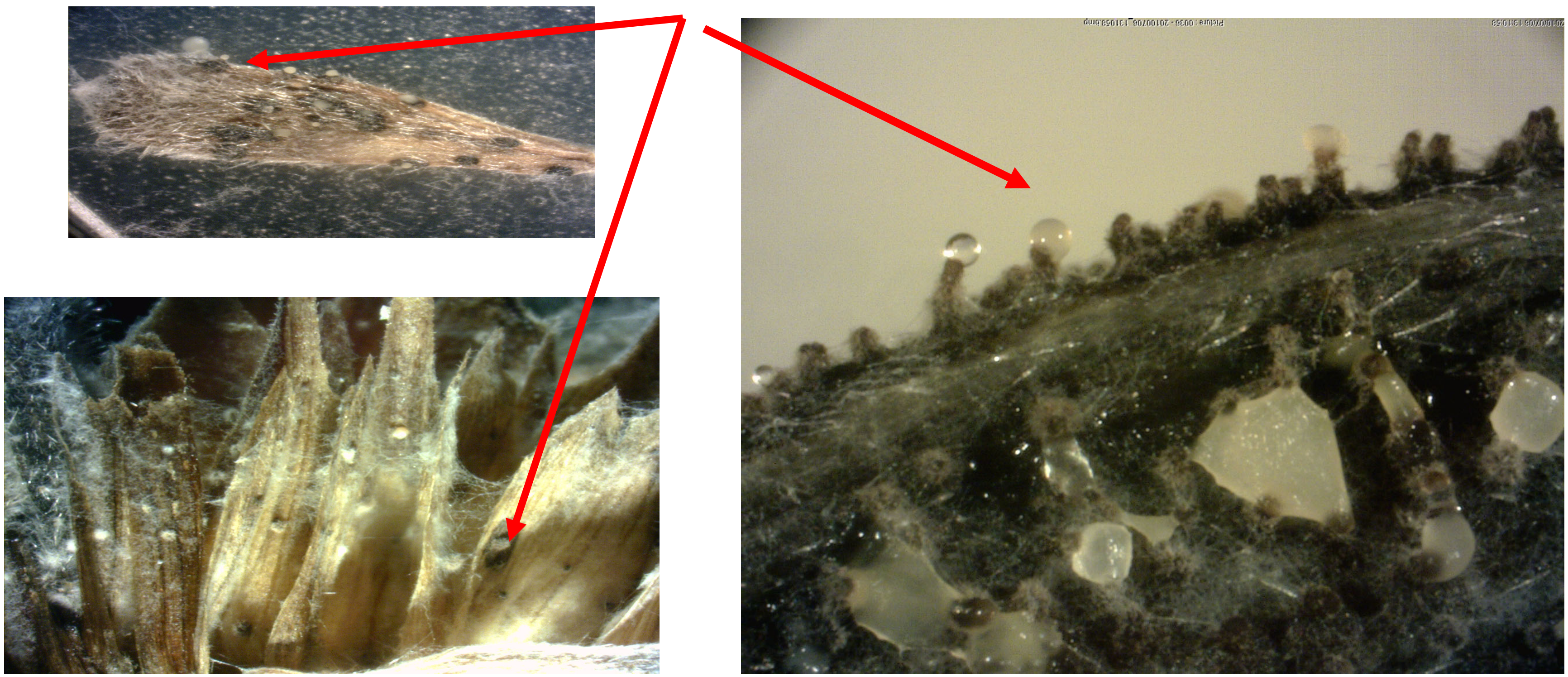
- A lesion developed at the site of inoculation
- Lesions elongated over time
- Destructive sampling of the stems revealed hyphal colonisation of the stem tissues above the lesion
- Sampling occurred at +35, +49 and +56dai
- No signs or symptoms had developed on the capitula by the time of harvest at +21 days after phys maturity
- Harvested seeds were asymptomatic prior to incubation

Results*……..Capitula, husk and seed infection following stem inoculation (*Paper submitted for review)

- ***D. gulyae*** **hyphae and pycnidia emerged from incubated capitula pieces (approx. 71%), husks and seed (approx. 64%).**

- **Infected seeds were found in all surviving inoculated plants**

  No infection was seen in mock inoculated controls



3. Infected seed germination and emergence in pot trials.....

- **Most seeds with <u>few pycnidia</u> germinated and appeared disease free until V2-V3 in pot trials**
- **Some planted seeds emerged with infected <u>pericarp intact and pycnidia viable</u>**
- **Patches of <u>brown necrotic tissue</u> could be seen on the cotyledons of some emerged seedings – *D. gulyae* isolated**
- **Such infected seeds are a <u>possible inoculum reservoir</u> and source of new outbreaks**
- **Further investigation of <u>*D. gulyae* colonisation</u> of emerged seedlings and older plants suggested**

Sunflower seedling emergence habit may assist *D. gulyae* survival on emerging seedlings…

- **<u>Epigeal</u> emergence - cotyledons are pulled rather than pushed (hypogeal) from the soil**

- **Then cotyledons are extended above the soil surface by elongation of the hypocotyl**

- **Pericarp halves (whether infected or not) can be retained on emerged cotyledons or dropped onto the soil surface after emergence.**

Seed infection of *D. gulyae* vs *D. helianthi*

- **Both species are known to infect sunflower seeds**
- **Unclear whether *D. helianthi* infects capitulum directly (ascospores) - some reports of this occurring.**
- **Also reports suggesting systemic seed infection of *D. helianthi* via the stem**
- **Opinions vary as to the significance of *D helianthi* seed infection in broadening distribution**
- **Our results are clear – *D. gulyae* can infect seeds systemically following stem infection**
- **Some infected seeds can germinate and produce a viable seedling**
- **Infection on emerged cotyledons can occur**
- **Viable *D . gulyae* pycnidia were found on attached pericarp pieces after seedling emergence**
- ***D. gulyae* seed infection has the potential to broaden distribution**

Implications for management……

Growers and advisors, seed companies – know the biology of pathogens, symptoms and timing of infection; understand the survival strategies of each pathogen; be vigilant, know your crops and paddocks, be aware of the disease levels and biology of your pathogens

Have a Whole of Farming System Overview

Seed companies – vigilance, inspect nurseries, bury or process infected stubble, be aware of any disease outbreaks; utilize seed testing

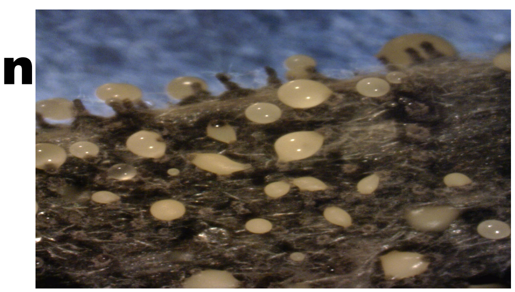
<u>**Harvest sunflower as soon as possible after physiological maturity**</u>

Seed infection has the potential to broaden distribution of *D. gulyae* to new fields, regions, countries

Importance of biosecurity protocols

Suggested further studies.....

- *D. gulyae* ascospore production and infection of the capitulum needs further investigation
- Investigate possible *D. gulyae* endophytic colonisation of seedlings emerged from infected seeds
- Evaluate temperature, humidity and timing of infection parameters vs incidence and/or severity of seed infection (similar to those identified for soybean?)
- Investigate *D. gulyae* hyphal colonisation of host tissues – same as for *D. helianthi*?
- Investigate *D. gulyae* hyphal and pycnidial development within the seed.
- Numbers of pycnidia vs seed germination
- Fungicides/ seed treatments



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Publications........

Thompson SM, Tan YP, Young AJ, Neate SM, Aitken EAB, Shivas RG (2011a). Stem cankers on sunflower (*Helianthus annuus*) in Australia reveal a complex of pathogenic *Diaporthe (Phomopsis)* species. Persoonia 27: 80–89.

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Thompson S, Grams R, Neate S, Shivas R, Ryley M, Tan YP, et al. (2018a). First reports of *Diaporthe kongii*, *D. masirevicii* and *D. ueckerae* associated with stem and peg dieback on peanut in Australia. *Plant Disease*: 102: 1459.

Thompson SM, Tan YP, Neate SM, Shivas RG, Lindbeck K, Aitken EAB (2018b). *Diaporthe novem* isolated from sunflower (*Helianthus annuus*) and other crop and weed hosts in Australia. European Journal of Plant Pathology 152: 823-831.

Brumpton Thompson S. 2020. *Diaporthe* species association with sunflower and other crops and weeds in eastern Australia. PhD Thesis, School of Agriculture and Food Services, The University of Queensland, Australia. https://doi.org/10.14264/uql.2020.779