Green and Brown Bridges of crop and weed residues aid survival of new pathogenic *Phomopsis/Diaporthe* spp. from sunflower, soybean and other crops and weeds in Australia.

Dr Sue Thompson[1,3], Prof Stephen Neate[1,4], Prof Roger Shivas[1,2], Dr Yu Pei Tan[2], Prof Liz Aitken[3]

[1]University of Southern Queensland, Toowoomba, Australia (Ret).
[2]DAF, Biosecurity Qld, Ecosciences Precinct, Brisbane, Australia.
[3]University of Queensland, St Lucia, Brisbane, Australia.
[4]University of Adelaide, South Australia

GRDC Grains Research & Development Corporation

The University Of Queensland

AOF

University of Southern Queensland

Australian Sunflower Association

NSA Reseach Forum .

Jan 14, 2021

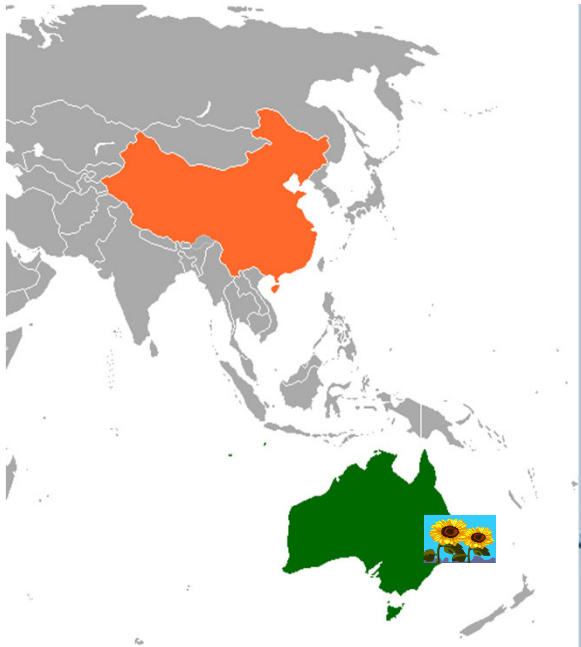
- **GRDC funded *Project 000186:* Diseases of broadacre summer crops of the GRDC Northern Region**
- **USQ Toowoomba, Australia.**
- ***R&D, Extension, Diagnostics***
- **Sunflower, soybeans, sorghum, mungbeans, chickpeas, maize.**

PhD component - Phomopsis/Diaporthe spp

- ***ID of causal spp., Survival on stubble, Identification of alternative hosts.; Management strategies***

- **Sue Thompson – Aus Sunflower Assoc Comm; US Sunflower Pathology Group**
- **Roger Shivas - Mycology, Taxonomy, PhD supervisor DAFQ**
- **Yu Pei Tan - Molecular Analyses**
- **Stephen Neate - PhD supervisor USQ**
- **Liz Aitken - PhD supervisor UQ**

Down under.....



Population

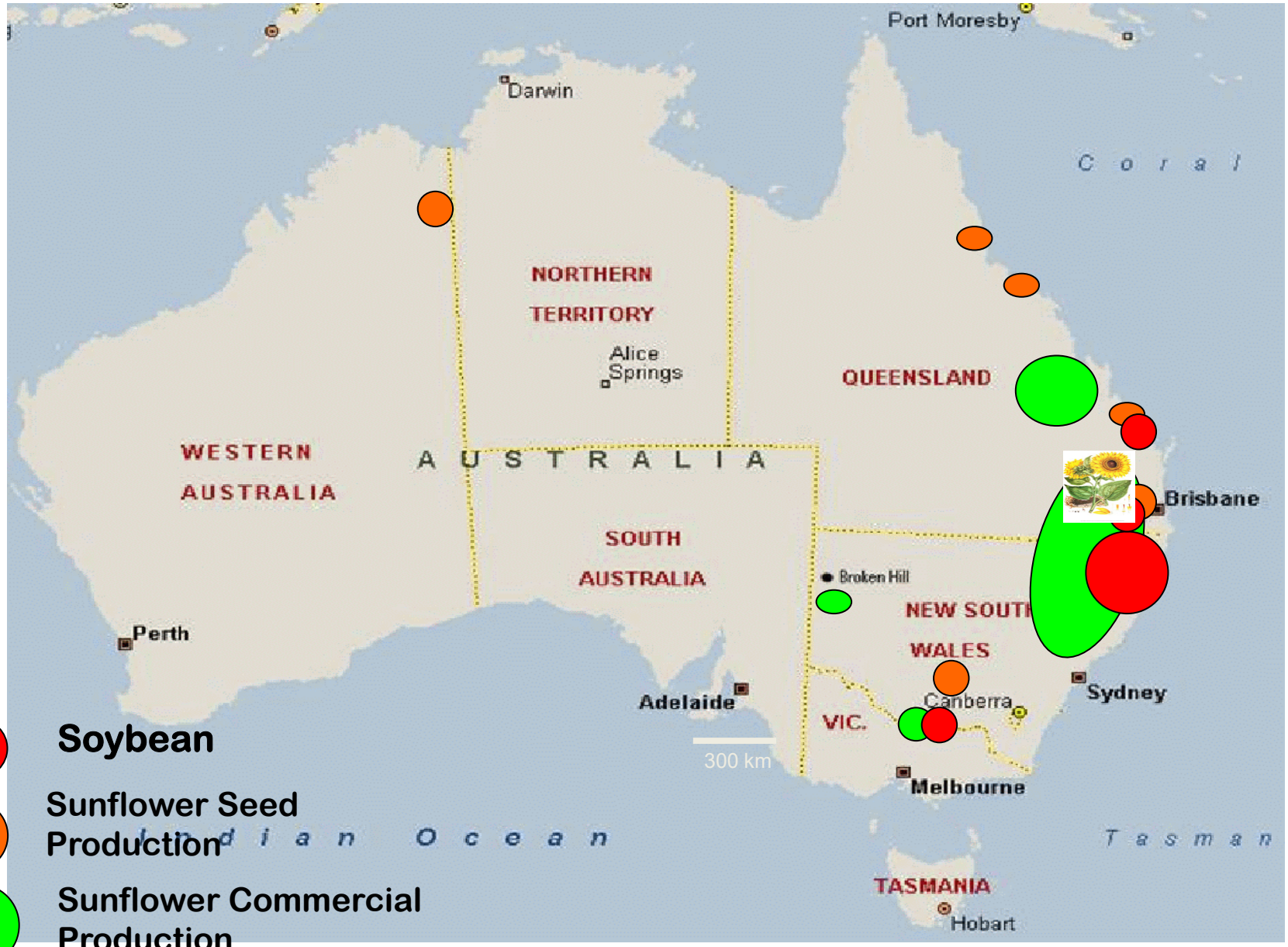
Australia: 25 mill

California: 39 mill

Texas: 27 mill

USA: 320 mill

Sunflower and soybean production areas



30+ yrs of Zero and Min Till – the upside……

- Home for beneficial insects ✓✓
- Moisture savings ✓✓
- Energy savings ✓✓
- Compaction reduction ✓✓
- Possible nematode suppression ✓
- Possible disease suppression ✓
- Weed control (herbicides) – stubble can inhibit emergence ✓

Moree, NSW

Zero and Min Till – the downside….

- **Herbicide - resistance x**
  Environmental concerns x
- **Protection for insect pests eg. Lucerne Crown Borer x**
- **Protection and increased survival for multiple pathogens x**

- ***Pathogens aided by stubble:***
- **Phomopsis stem and pod cankers (*Diaporthe* spp.) - sunflower, soybean, lupin, grapevine, citrus**

- ***Sclerotinia minor, S. sclerotiorum, Athelia rolfsii – 200+ hosts***

- ***Fusarium* spp. – sorghum stalk rot, corn cob rot, wheat crown rot, cotton root rot**

- ***Macrophomina phaseolina* – charcoal rot of soybeans, sorghum, sunflower**

Introducing Sam Markell to *A rolfsii* in Aus

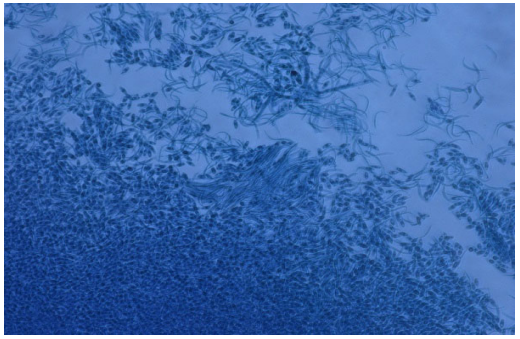
Genus Diaporthe , a case study of survival on green and brown bridges…..

- Wide host range: saprobes, endophytes, pathogens
- Woody shrubs, woody weeds, broadacre crops - soybean, sunflower, grapes; tree crops - junipers, peach, plum, rooibos; weeds- saffron thistle, burrs
- *Sunflower: Phomopsis Stem Canker PSC*
- *Soybean: Phomopsis Pod and Stem Blight, Stem Canker*
- *Phomopsis* sp. - name given to the anamorph, asexual state
- *Diaporthe* sp. - name given to the teleomorph, sexual state

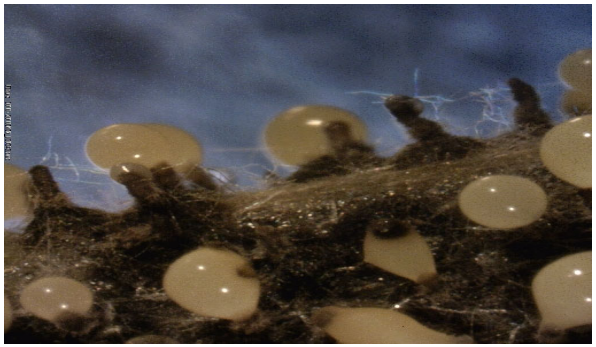
•One Fungus – One Name

International Code for Botanical Nomenclature

Perithecia of a *Diaporthe* sp.



Alpha and/or beta conidia



Pycnidia of *D gulyae* on seed

*This Australian green and brown bridge story – started with the first PSC outbreak on sunflower in 2009/10**

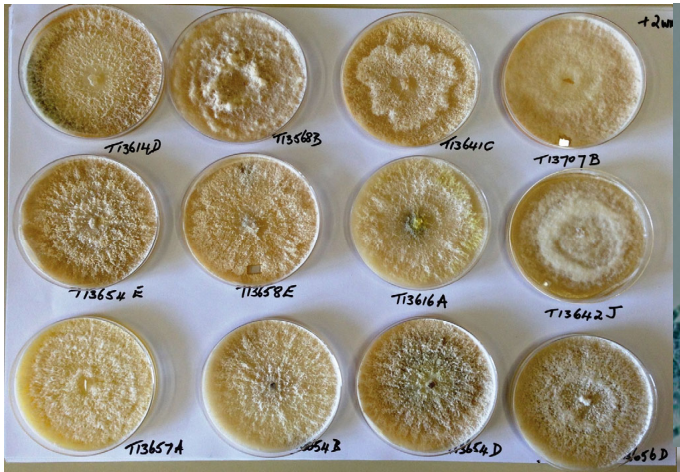
- **Symptoms and host based ID** → ***D. helianthi? Exotic to Aus.***

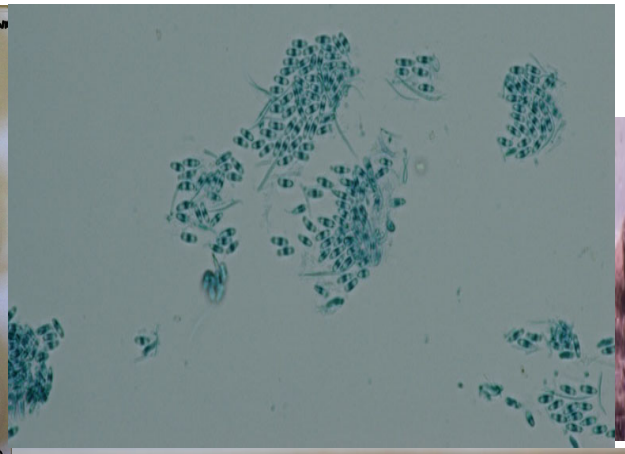
- ***Causal pathogen (s) of the Australian outbreak?***
- ***Where did it come from?***

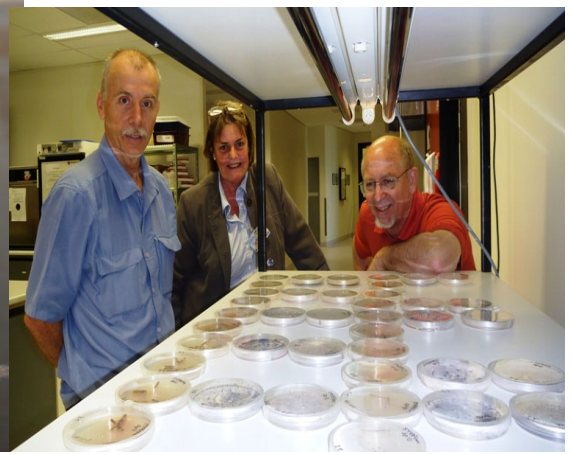
Lodging and lesions typical of Phomopsis Stem Canker

Step 1. ID the pathogen(s) from sunflower and other isolates

- Three-pronged diagnostic approach
- Morphological characterisation
- Molecular phylogeny *eg* ITS, Tef-1$^{\alpha}$, BT, CAL, others
- Followed by pathogenicity testing on selected crops and weeds







Pathogenicity testing of isolates associated with sunflower.....

Start with sunflower and other crops in the rotation....
Green bridges or potential hosts?
Also, test isolates on weeds.

Sunflower

Mungbean

Chickpea

Soybean

Early results – three new Diaporthe species on sunflower:*

- ***D. gulyae*** *(RG Shivas, SM Thompson & AJ Young)*

  Most damaging species….. *Virulence Rating 5

D. kongii *(RG Shivas, SM Thompson & AJ Young)*

D. kochmanii *(RG Shivas, SM Thompson & AJ Young),*

(syn. D. sojae)

- **ID based on host no longer valid for *Diaporthe* spp. on sunflower *virulent species on sunflower not D helianthi .***

- **New molecular technologies are revealing previous errors in identification of species, a multitude of undescribed species**

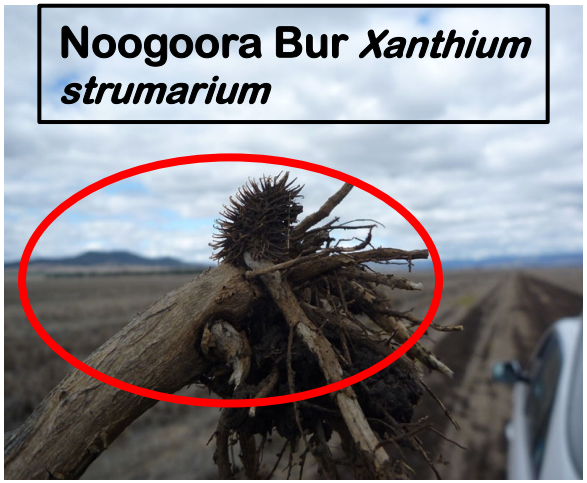
- ***Finding a new species and host associations is just the beginning*……. ‘associated with’ does not mean ‘pathogen of’.**

*Thompson et al. 2011. Persoonia 27:80-89

Step 2. Where did it come from?

Brown bridges?

Stubble washed across paddock



Viable pycnidia on crop and weed stubble

Step 2. Live weeds as alternative hosts/ green bridges?

Carthamus lanatus (Saffron Thistle), Ash et. al. 2009)

Xanthium strumarium (noogoora bur): molecular, path testing -

First record for Australia

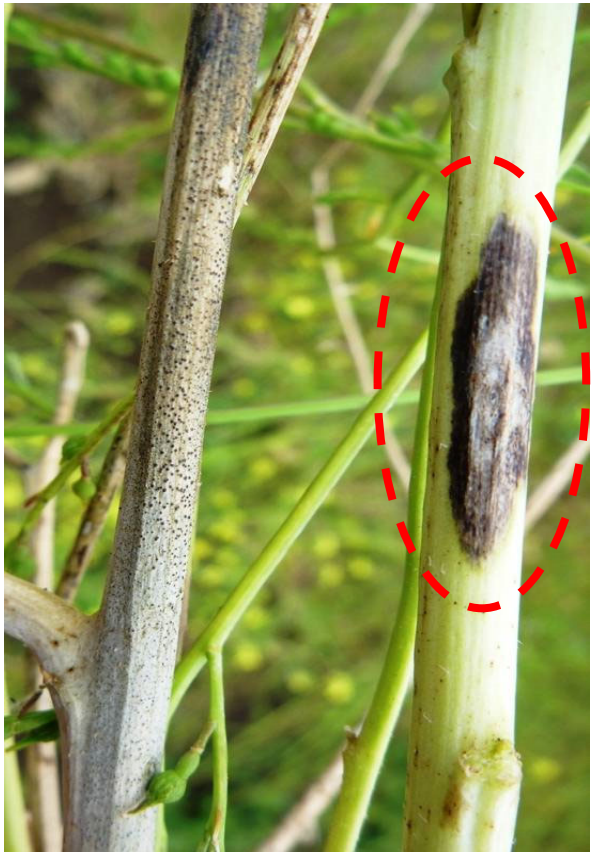
D. gulyae

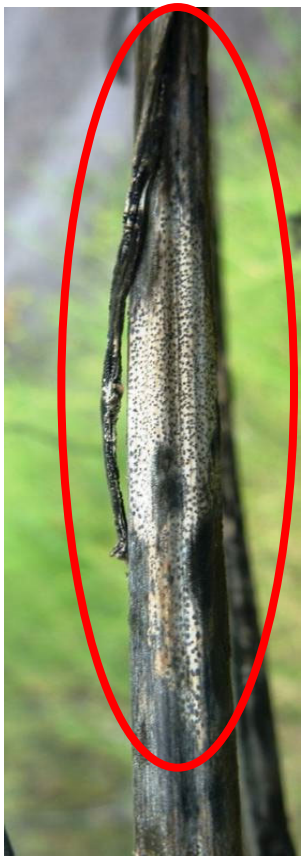
Noogoora Bur *Xanthium strumarium*
Lesions and developing fruiting bodies

Diaporthe spp on other weeds …..live (green) and dead (brown) bridges?

Turnip Weed *Rapistrum rugostrum alongside a sunflower nursery.*

Live plant, black lesion - D. kongii;
Dead plants – D. kongii plus multiple Diaporthe spp.





Where did it come from? .....

Lodging site 1, Qld

- 20+ years no sunflowers grown
- Lesions at flowering
- Lodging in patches as heads filled

Which species?

<u>Sunflower IDs</u> – ***D. gulyae, D. novem , D. ambigua, D. masirevicii***

***Thompson et al. 2018**

Investigation of the live weeds at the site…

Sunflower : ***D. gulyae, D. novem , D. ambigua, D. masirevicii***

Weeds: ***Malva parviflora*** **(small flowered mallow) –** ***D. novem*** **(tip dieback),** ***Sochus oleraceus*** **(sow thistle, stem), noogoora bur (stem),** ***Bidens pilosa*** **(cobblers peg, stem)……*D. gulyae***

D. gulyae on Malva sp

Green and *brown* bridges.....site 2, *Ryeford. Qld*

- **Sunflower crop – *D. gulyae***
- **Sunflower stubble – *D. gulyae, D. masirevicii***

- **Sesbania, live, tip dieback – *D. gulyae*, *D. sojae* *(syn D kochmanii)***
- ***Sesbania stubble* - *D. masirevicii***

- **Noogoora bur live, stem lesions – *D. gulyae***
- **Datura sp (thornapple) stubble – *D. gulyae***
- **Misc stubble – *D. masirevicii, other spp.***

Sesbania tip dieback

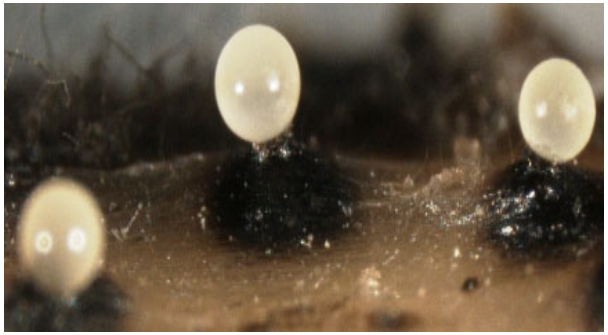
Site 3. Brown bridges…Caroona NSW

Live sunflower, previous crop: *D. gulyae*

Sunflower stubble: *D. gulyae*

Noogoora Bur stubble: *D. gulyae*

Wild sunflower stubble: *D. gulyae*



Site 4 - Moree, Northern NSW

Live: *V sativa* (vetch) – *D novem, D leucospermi*
Stubble: *Vicia faba*, *X strumarium*, *H annuus* (wild) – *D gulyae*

Isolates from this site were pathogenic on chickpea mungbean, soybean, sunflower, lupin …

To support pathogenicity test results by inducing natural infection, a green bridge - alternative crop host trial 1, 2014

Thanks Pacific Seeds!
(Advanta)

Chickpea stem infection

Soybean petiole infection

Mungbean infection at node

Natural infection, green bridge trial 2, Qld 2015

An additional outcome – a further eight new species.....*

Names chosen to honour significant contributers to sunflower in Australia and internationally

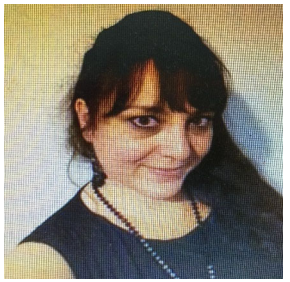
D. charlesworthii.....

D. goulteri.....

D. macintoshii.....

D. masirevicii.....

D. middletonii.....



D. miriciae.....

D. sackstonii.....

D. serafiniae.....

*Thompson et al. 2015

Summary: 14 *Diaporthe* spp. associated with sunflower in Australia....

Associated with: the species is isolated from plant parts of dead and/or live sunflower plants.

Saprobe, endophyte, pathogen

'associated with' does NOT mean 'pathogenic on'

Pathogenic: the species can infect/colonise plant tissues, cause necrosis, eg. a lesion

Virulence: the severity of infection.... eg. lesion length.
*severity rating 1-5

(*Thompson et al. 2011, Brumpton Thompson 2020)

Examples of Diaporthe sp x crop assoc....

Diaporthe gulyae* – identified as a highly virulent pathogen of multiple crops and weeds

- ****Associated with 6 crops – sunflower, soybean, sorghum, mungbean, maize and fababean**
- ****Associated with 15 weeds**
- ****Pathogenic (potential pathogen): sunflower, soybean, mungbean, chickpea, peanut, lupin, safflower, canola, noogoora bur, gooseberry, sow thistle, wild sunflower**

The very novel Dr Tom Gulya

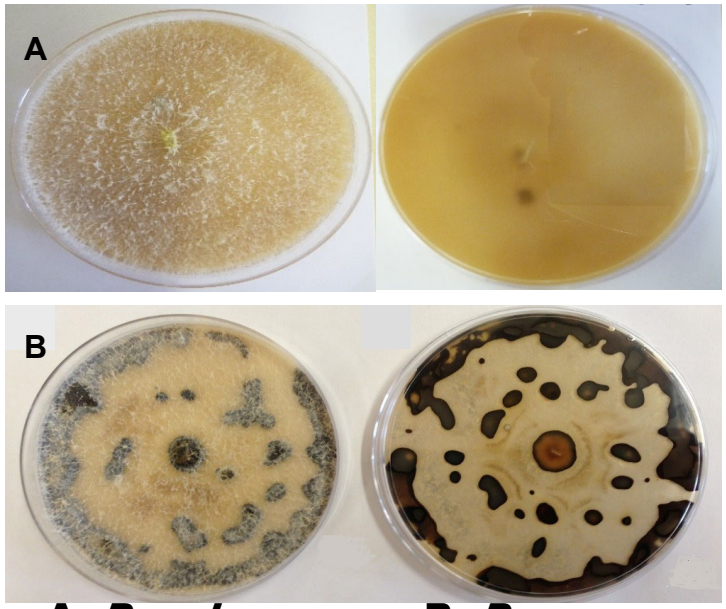
D. gulyae on sorghum seed, sunflower stem and mungbean stem

** Thompson et al. 2011. **Papers published and/or in prep; Brumpton Thompson 2020*

*Diaporthe novem**.....

- **Recorded in Europe on sunflower and soybean**
- **One of 14 species identified on sunflower in Australia**

- **16 host associations including:**
- **Sunflower – stem, live**
- **Soybean – stem live**
- **Lupin – stem, mature**
- **Sesbania cannabina – tip dieback**
- **Sorghum bicolor – seed**



A. *D. gulyae* **B. *D. novem***

- ****Pathogenic on sunflower, soybean, mungbean, chickpea, lupin, the weed *Physalis* sp. - a broad ranging potential crop pathogen.**
- **Majority are first records** *Thompson et al. 2018; ** *papers in prep*

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

***Diaporthe* species virulence on selected crops**

	Crops							
	Peanut *Arachis hypogaea*	Chickpea *Cicer arietinum*	Fababean *Vicia faba*	Maize *Zea mays*	Mungbean *Vicia radiata*	Sorghum *Sorghum bicolor*	Soybean *Glycine max*	Sunflower *Helianthus annuus*
***Diaporthe* spp.**								
ambigua								x
aspalathi	x				x		x	
charlesworthii	x							
goulteri								x
gulyae			stubble	leaf	stem, petiole	seed	stem, seed	base, stem, leaf, capitulum, seed
infecunda			stubble		x	seed		
kongii	x	x		leaf	x		x	x
leucospermi		x			x			
longicolla							x	
masirevicii	x			leaf	x		x	x
micheliae							x	
middletonii		x			x		x	
novem		x	stubble		x	seed	x	stem
sackstonii		x			x		x	x
serafiniae		x						x
sojae syn. *kochmanii*							x	x
ueckeri syn. *miriciae*	x	x			x	seed	x	x
Diaporthe spp. nov. 13-31	x	x			x		x	
Total species	5	8	3	3	14	4	14	14

Some *Diaporthe spp associated with a selection of weeds in Australia*

	Abutilon sp	*Bidens pilosa*	*Circium vulgare*	*Datura stramonium*	*Helianthus annuus*	*Hibiscus trionum*	*Macroptylium lathyroides*	*Malva parviflora*	*Physalis sp*	*Rapistrum rugosum*	*Sonchus oleraceus*	*Xanthium strumarium*	
	Abutilon	Cobblers Peg	Spear Thistle	Common Thornapple	Wild Sunflower	Bladder Ketmia	Phasey Bean	Small Fl Mallow	Gooseberry	Turnip Weed	Sow Thistle	Noogoora Bur	
	Malvacea	Asteraceae	Asteraceae	Solonaceae	Asteraceae	Malvacea	Fabaceae	Malvacea	Solonaceae	Brassicaceae	Asteraceae	Asteraceae	Total
ambigua				stem dead	stem live						x	stem live	6
azadirachtae						x							1
charlesworthi													1
fraxini-augustifoliae		x											2
goulteri					x								3
gulyae		x	x	x	stem					x	x	x	15
kongii		x					x			x			4
leucospermi	x			x						x			6
macintoshii										x			1
masirevicii	x	x					x	x		x		x	8
middletonii										x			2
novem				x	x			x		x	x	x	10
sackstonii	x												2
serafiniae		x		x			x	x		x			6
ueckeri syn. *miriciae*							x			x	x		5
Total	**3**	**9**	**1**	**6**	**5**	**1**	**9**	**4**	**0**	**11**	**7**	**5**	

Live weed alternative hosts - *Diaporthe gulyae (pathogenic)**

Total 15 D. gulyae x weed host associations

First records

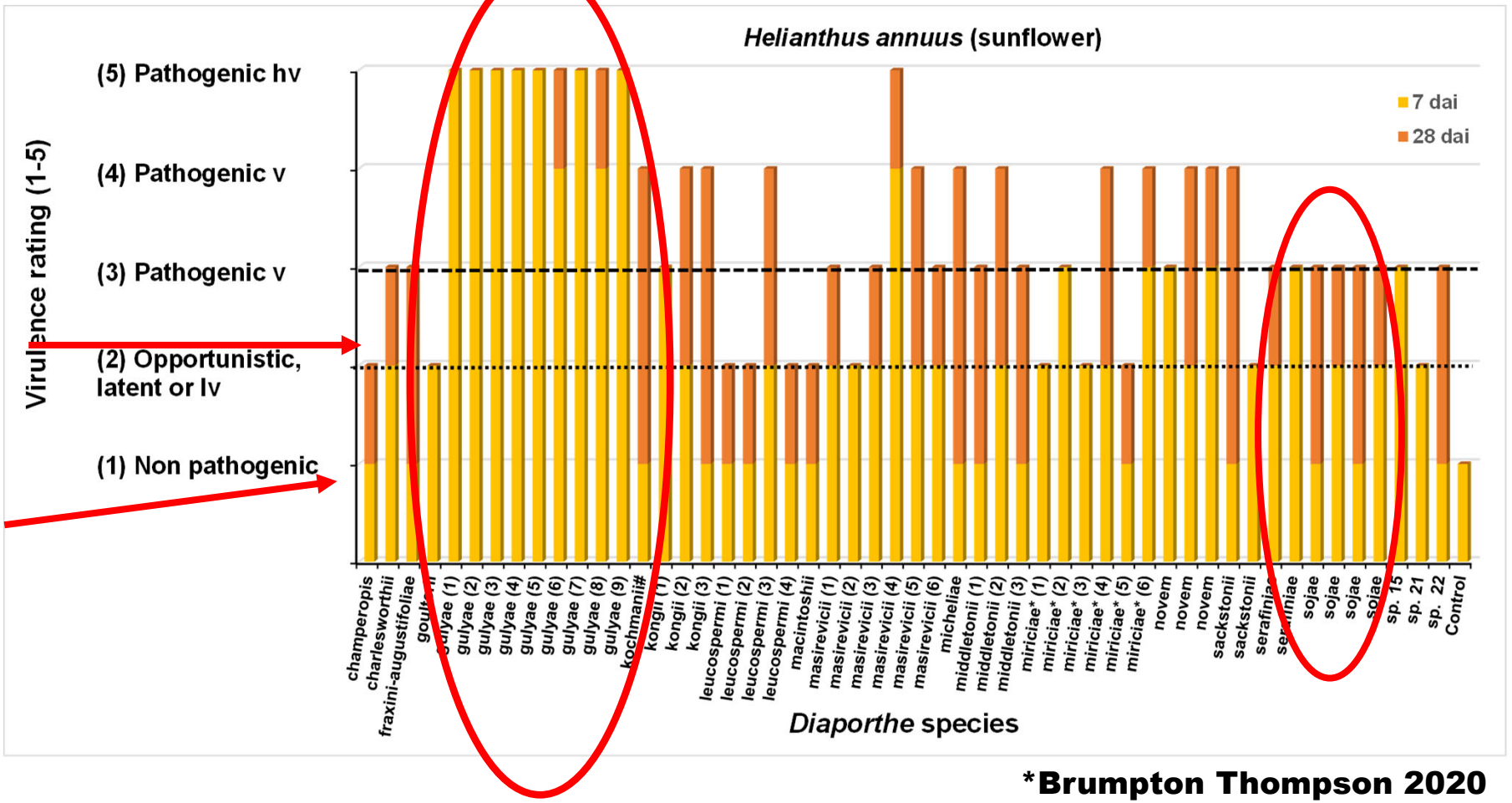
- **Noogoora Bur** ***Xanthium strumarium***
- **Saffron Thistle** ***Carthamus lanatus .... Ash et al. 2009***
- **Wild Sunflower** ***Helianthus annuus***
- **Sow Thistle** ***Sonchus oleraceus***
- **Gooseberry** ***Physlis sp (no associations to date)***

Noogoora Burr *Xanthium strumarium*

Wild *Helianthus annuus*

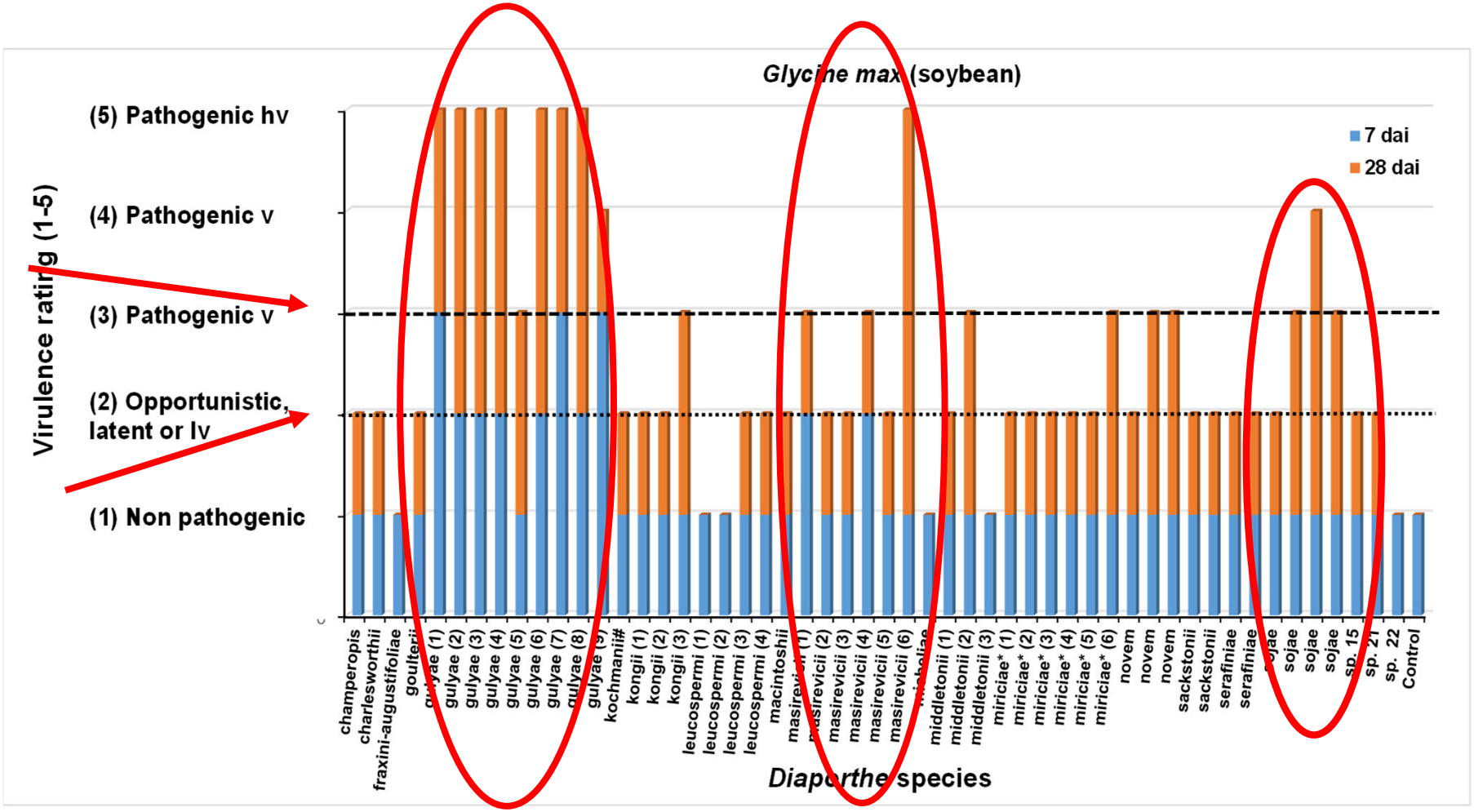
Pathogenicity and virulence – not always straight forward……influence of plant age, time of rating after inoc, collection host…..

Disease Progression Ladders – show virulence of *Diaporthe* spp on <u>Sunflower</u> (B89)* at 7 and 28dai after stem slit inoculations



*Brumpton Thompson 2020

Disease Progression Ladders - Virulence of *Diaporthe* spp on Soybean cv Bunya* at 7 and 28dai after stem slit inoculations



*Brumpton Thompson 2020

Novel species and previously unrecognised associations in green and brown bridges.....*

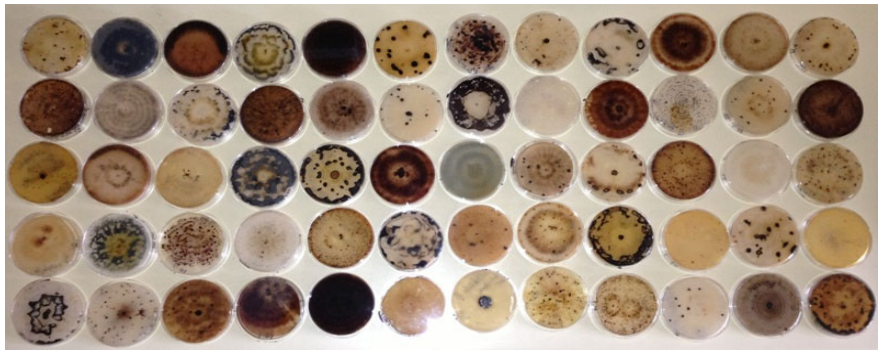
- *Total 49 Diaporthe spp identified*
- ***32 novel Diaporthe species (11 now described)***
- ***76 new crop x Diaporthe spp associations***
- ***89 new weed x Diaporthe spp associations***

Fungal biodiversity largely unexplored

- ***Diaporthe ambigua*** **- potential pathogen of stonefruit, multiple tree hosts (sunflower stubble, noogoora burr)**

- ***D. aspalathi*** **– potential pathogen of lupin, isolated from peanut, soybean, mungbean**

- ***D. leucospermi*** **– potential pathogen of protea, isolated from mungbean, vetch**
- ***D. novem*** **– hosts include sunflower, soybean; isolated from sunflower, lupin seed, sorghum seed, vetch, turnip weed, Datura spp.**



Diaporthe **spp. cultural differences**

***Brumpton Thompson 2020**

More alternative 'hosts' of other species and genera ……

Pathogen	*Common Name*	*Live Hosts*	*Symptoms*	*Other 'Hosts'/Stubble/Residues*
Diaporthe gulyae	Stem Canker	Sunflower, soybean, mungbean chickpea	Lesions and lodging, early senescence	Live and dead noogoora bur, bathurst burr, thistles, live and dead cobblers peg, sorghum seed, dead thornapple, live and dead wild sunflower, sunflower seed, maize leaf
Diaporthe kongii	Stem Canker	Sunflower, soybean, chickpea, mungbean	Lesions and lodging, early senescence	Lantana, cotton, dead and live turnip weed, maize leaf
Fusarium graminearum	Gibberella Cob Rot, Stalk Rot	Maize	Cob Rot, Stalk Rot	Maize stubble, maize leaf, soybean roots, lupin seed, soybean seed
	Head Blight, Crown Rot	Wheat	Head death, stalk rot	
Fusarium andiyazi	Fus Stalk Rot	Sorghum	Stalk Rot	Vetch, live - leaf spot, Moree

Other genera.....

- *Alternaria spp*
- *Alternaria brassicae*
- *Alternaria tenuissima*
- *Aspergillus tubinensis*
- *Bionectria sp*
- *Bionectria ochroleuca*
- *Botryotinia fuckeliana*
- *Cochliobolus sativus*
- *Colletotrichum boninense s.l.*
- *Colletotricum gloeosporioides*
- *Colletotricum truncatum*
- *Colletotricum trifolii/destructivum*
- *Fusarium incarnatum*
- *Fusarium oxysporum*
- *Fusarium proliferatum*
- *Fusarium sp*
- *Haematonectria haematoccocca*
- *Lasiodiplodia theobromae*
- *Leptosphaeria biglobosa 'candanen..*
- *Macrophomina phaseolina*
- *Monographella cucumerina*
- *Monographella sp*
- *Myrothecium sp*
- *Nectria sp*
- *Neofusicoccum luteum*
- *Neofusicoccum parvum*
- *Paraphaeosphaeria sp*
- *Pestalotiopsis sp*
- *Phlebiopsis sp*
- *Phoma spp*
- *Pithomyces chartarum*
- *Plectosporium sp*
- *Stagonospera sp*
- *Stemphylium sp*
- *Stenocarpella maydis*

- Isolated from Turnip Weed
- Damaging pathogen of brassicas

- Isolated from sunflower stubble
- Pathogen of wheat – root rot

- Isolated from soybean stubble, unidentified weed residues
- Causes fruit rots on avocados and mangos, stem and pod blight soybeans

- Isolated from multiple crop and weed residues
- Significant pathogen of summer field crops – sorghum, soybean, maize, sunflower.

- Isolated from maize residues
- Pathogen of maize

To minimise build-up of a pathogen reservoir:

- **Growers and advisors - familiarise yourself with the pathogens of all crops in the rotation**
- **Understand pathogen biology and survival mechanisms**
- **Take a whole of farming system approach**
- **Know your Green Bridges – around silos, fencelines, pumpsheds, roadsides, waterways**
- **Beware the Brown Bridges– dead weed 'hosts', crop and weed residues/stubble left on the surface and along paddock edges, volunteers, may be aiding survival of multiple pathogens in the Green and Brown Bridges**
- **Rotation, rotation, rotation.....consider strategic tillage**

What does it all mean…?

- Green and brown crop and weed bridges are contributing to inoculum reservoirs of multiple *Diaporthe* species in Australia, regardless of presence of crop hosts

- Some of those species will be pathogenic on at least one of the crops in the farming system. Or other cropping systems…..

- Some live weeds in non cropping ecosystems also harbour significant crop pathogens in a green bridge eg. garden plants, trees

- Dead 'non-host' weeds as well as crop stubble can be a inoculum reservoir for future disease in a brown bridge

- Zero and min tillage systems are contributing to survival of some pathogens and insects.

Acknowledgements

- Australian Sunflower Assoc (ASA), Aust. Oilseeds Federation (AOF)
- Grains Research and Development Corporation (GRDC)
- Department of Agriculture and Fisheries, Queensland (DAFQ)
- Growers and advisors in all regions
- Loretta Serafin: NSW DPI, Tamworth, NSW
- Mal Ryley: USQ Emeritus, Toowoomba, Qld
- Sam Markell and Bryan Hensen: NDSU, Fargo, ND
- Seed Companies: Avosun, KB Ornamentals, Nuseed, Pacific Seeds (Advanta)
- Tom Gulya: USDA (Ret. Fargo ND), CA.
- University of Southern Queensland (USQ)
- University of Queensland (UQ)

Contact details:

Sue Thompson
botanica1104@gmail.com

Nuseed Ausigold 62. Norwin, Qld. Australia.

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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