Stem cankers on sunflower (Helianthus annuus) in Australia reveal a complex of pathogenic Diaporthe (Phomopsis) species

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Diaporthe kongii
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Abstract The identification of Diaporthe (anamorph Phomopsis) species associated with stem canker of sunflower (Helianthus annuus) in Australia was studied using morphology, DNA sequence analysis and pathology. Phylogenetic analysis revealed three clades that did not correspond with known taxa, and these are believed to represent novel species. Diaporthe gulyae sp. nov. is described for isolates that caused a severe stem canker, specifically pale brown to dark brown, irregularly shaped lesions centred at the stem nodes with pith deterioration and mid-stem lodging. This pathogenicity of D. gulyae was confirmed by satisfying Koch’s Postulates. These symptoms are almost identical to those of sunflower stem canker caused by D. helianthi that can cause yield reductions of up to 40 % in Europe and the USA, although it has not been found in Australia. We show that there has been broad misapplication of the name D. helianthi to many isolates of Diaporthe (Phomopsis) found causing, or associated with, stem cankers on sunflower. In GenBank, a number of isolates had been identified as D. helianthi, which were accommodated in several clades by molecular phylogenetic analysis. Two less damaging species, D. kochmanii sp. nov. and D. kongii sp. nov., are also described from cankers on sunflower in Australia.

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INTRODUCTION

Phomopsis species are widespread and occur on a diverse range of host plants as pathogens, endophytes or saprobes (Uecker 1988). The morphological characters that define Phomopsis are dark eutromatic or pycnidial conidiomata containing elongated phialides with cylindrical, well-developed collarettes that form two types of hyaline conidia: 1-celled α-conidia that are biguttulate, fusiform and easily germinate on artificial media, and β-conidia that are filiform and rarely germinate (Wehmeyer 1933, Sutton 1980). Species of Phomopsis represent anamorphs of Diaporthe (Ascomycota, Diaporthales, Valsaceae) with at least 180 connections given by Uecker (1988), which represents about 80 % of named Phomopsis species. The name Diaportha Nitschke (1870) precedes Phomopsis Sacc. & Roum. in Saccardo (1894).

Host association has often been the basis for species identification in Diaporthe and Phomopsis, as morphological and culture characteristics are inadequate or unreliable for species differentiation (van Rensburg et al. 2006). Recent studies have demonstrated that a number of Phomopsis species have wide host ranges (van Niekerk et al. 2005, Santos & Phillips 2009, Ash et al. 2010), and more than one species can occur on a single host (Mostert et al. 2001, Santos & Phillips 2009).

Molecular phylogenies, especially those derived from DNA sequence analyses of the ribosomal internal transcribed spacer (ITS) regions of the nuclear ribosomal RNA genes and translation elongation factor-1α (TEF-1α) have been used to identify species (Mostert et al. 2001, van Niekerk et al. 2005, van Rensburg et al. 2006, Santos & Phillips 2009, Ash et al. 2010). The polyphyletic status of D. helianthi has been recognised by Rekab et al. (2004). Hyde et al. (2010) suggested that discarding the host-based species concept was the first step in the development of a useful and reliable classification for Phomopsis and highlighted that there had been much confusion around the application of species names, drawing particular attention to the name D. helianthi.

Stem canker attributed to D. helianthi (anamorph P. helianthi) has become one of the most important diseases of sunflower (Helianthus annuus) worldwide since first described from the former Yugoslavia (Munțănoiu-Cvetković et al. 1981). Yield reductions of up to 40 % have been recorded in Europe (Masićrević & Gulya 1992) including the former Yugoslavia as well as France where it was considered a major pathogen of sunflower (Battilani et al. 2003, Debaeke et al. 2003). Diaporthe helianthi is also widespread in the sunflower growing regions of the USA (Gulya et al. 1997) but has not been reported from Australia. Munțănoiu-Cvetković et al. (1985) found that multiple Phomopsis species were associated with cankers on sunflower in the former Yugoslavia, although only P. helianthi was responsible for the serious disease outbreaks. Gulya et al. (1997) suggested that pathogenic Phomopsis species on sunflower might consist of more than one species or biotype with apparent biological differences between the isolates from Europe and the USA. Miric et al. (2001) raised the possibility that several pathogenic Phomopsis species occurred on sunflower in Australia.

In 2009, lodging and premature senescence caused significant damage to sunflower crops in New South Wales (NSW), and to a lesser extent in Queensland (Qld), Australia, after extended periods of wet weather. The symptoms included pith damage behind elongated, brown to brown-black lesions, which weakened stems and led to mid-stem lodging as the heads filled. The aim of this study was to use morphological, molecular and pathogenicity studies to clarify the identity of the Diaporthe (Phomopsis) species occurring on sunflower in Australia.
**MATERIAL AND METHODS**

**Isolates**

Over 300 isolates of *Diaporthe* (*Phomopsis*) were obtained from stems, leaves and seed of both cultivated and wild sunflower plants exhibiting symptoms of stem canker across NSW and Qld. Small excised stem and leaf pieces with brown or brownish black lesions were surface-sterilised by dipping into 90% ethanol and flaming briefly prior to placement on 1.5% water agar amended with 100 µg/mL streptomycin sulphate (WAS) in 9 cm diam Petri dishes. Cultures that grew from this tissue were incubated for up to 3 wk to induce pycnidial formation. For seed isolations, seeds harvested from infected crops and individual plants were incubated without surface sterilisation on WAS in Petri dishes for up to 14 d to allow pycnidia to develop. For all isolations, conidia oozing from pycnidia were streaked onto potato-dextrose agar (Oxoid) (PDA) amended with 100 µg/mL streptomycin sulphate (PDAS). Hyphal tips were then taken from all isolates and grown on PDAS to establish pure isolates. Cultures were incubated for 7 d under ambient light at 23–25 °C. For pathogenicity experiments, 7 d old cultures were used to provide inocula. Fourteen selected isolates representing a range of virulence symptoms and morphological characteristics were deposited in the Plant Pathology Herbarium (BRIP), Brisbane, Australia as both living and dried cultures (Table 1).

**Morphology**

For fungal morphology, isolates were grown on PDA with pieces of sterilised wheat stems placed on the surface and incubated under 12 h near-ultraviolet light / 12 h dark (Smith 2002) at 25 °C. Fungal structures were mounted on glass slides in lactic acid (100 % v/v) for microscopic examination after 28 d of incubation. Means and standard deviations (SD) of selected structures were made from at least 20 measurements. Ranges were expressed as (min.–) mean–SD – mean+SD (–max.) with values rounded to 0.5 µm. Images were captured with a Leica DFC 500 camera attached to a Leica DM5500B compound microscope with Nomarski differential interference contrast.

For colony morphology, 3 d old cultures on 9 cm diam plates of PDA and oatmeal agar (Oxoid) (Oatmeal) that had been grown in the dark at 23 °C were grown for a further 7 d under 12 h near-ultraviolet light / 12 h dark. Colony colours (surface and reverse) were rated according to the colour charts of Rayner (1970).

**DNA isolation, amplification and analyses**

Mycelia were scraped off PDA cultures and macerated with 0.5 mm glass beads (Daintree Scientific) in a Tissue Lyser (QIAGEN). Genomic DNA was then extracted with the Gentra Puregene DNA Extraction kit (QIAGEN) according to the manufacturer’s instructions. The primers ITS1 and ITS4 (White et al. 1990) were used to amplify the ITS region of the ribosome genes. To further differentiate *D. angelicae*, *D. stewartii*, *D. gulyae* and *P. dauci*, the primers EF1-728F (Carbone & Kohn 1999) and EF2 (O’Donnell et al. 1998) were used to amplify part of the translation elongation factor-1alpha (TEF-1α) gene. Both the ITS and TEF loci were amplified with the Phusion High-Fidelity PCR Master Mix (Finnzymes). The PCR products were purified with the QiAgic PCR Purification Kit (QIAGEN) and sequenced on the 3730xl DNA Analyzer (Applied Biosystems) using the amplifying primers. The sequences generated in this study were assembled using Vector NTI Advance v. 11.0 (Invitrogen) and deposited in GenBank (Table 2). These sequences were aligned with sequences from representative *Diaporthe*/Phomopsis species from GenBank (Table 2) in MEGA v. 5.05 (Tamura et al. 2011).

The sequences of *Leucostoma persoonii* and *Valsa cerato­sparma* were used as outgroups in the ITS dataset, whilst sequences of *Leucostoma niveum* and *Valsa ambiens* were used as outgroups in the TEF-1α dataset. Alignment gaps were treated as missing character states and all characters were unordered and of equal weight.

The ITS and TEF-1α phylogenetic trees were inferred in MEGA v. 5.05 by Maximum Likelihood (ML). Modeltest in MEGA v. 5.05 determined that the K2+G and HKY+G models were the most suitable nucleotide substitution models for ITS and TEF-1α, respectively. Bootstrap support values with 1 000 replications were calculated for tree branches. The sequences obtained from GenBank are listed by their taxon names followed by strain numbers in the trees (Fig. 1, 2). Nomenclatural novelties were deposited in MycoBank (www. MycoBank.org) (Crous et al. 2004).

**Pathogenicity**

Pathogenicity was determined by inoculating plants of the sunflower hybrid Hyoleic 41 at the V6–V8 (Schneiter & Miller 1981) growth stage and grown in a cabinet under a 25 °C 12 h light / 20 °C 12 h dark cycle using two methods, wound inoculation and mycelium contact. The wound inoculation method (adapted

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**Table 1** Diaporthe cultures isolated from sunflower investigated in this study.

| Species | Isolate number (BRIP) | Locality | Source | Sunflower Hybrid/Wild | Virulence Rating¹ | GenBank Accession numbers |
|---------|-----------------------|----------|--------|-----------------------|-------------------|--------------------------|
| *Diaporthe gulyae* | 53158 | Goran Lake, NSW | stem | Wild H. annuus | 4 | JF431284 JN645799 |
| | 53166 | Premer, NSW | seed | Ausigold | 4 | JF431289 JN645801 |
| | 53172 | Premer, NSW | seed | Hyoleic 41 | 5 | JF431290 JN645802 |
| | 53159 | Premer, NSW | seed | Advantage | 5 | JF431291 JN645800 |
| | 54030 | Nobby, Qld | stem | Sunbird 7 | 5 | JF431292 JN645808 |
| | 54029 | Hermitage, Qld | stem | Sunbird 7 | 5 | JF431293 JN645807 |
| | 54028 | Hermitage, Qld | stem | Sunbird 7 | 5 | JF431294 JN645806 |
| | 54027 | Ryeford, Qld | leaf | Sunbird 7 | 5 | JF431295 JN645805 |
| | 54026 | Ryeford, Qld | leaf | Sunbird 7 | 5 | JF431296 JN645804 |
| | 54025 | Ryeford, Qld | leaf | Sunbird 7 | 4 | JF431297 JN645803 |
| *Diaporthe kochmanii* | 54033 | Gatton, Qld | stem | Experimental | 2 | JF431295 JN645809 |
| | 54034 | Gatton, Qld | stem | Experimental | 3 | JF431296 JN645810 |
| *Diaporthe kongii* | 54032 | Childers, Qld | stem | Female | 3 | JF431300 JN645798 |
| | 54031 | Childers, Qld | stem | Female | 3 | JF431301 JN645797 |

¹ Ex-type cultures are in **bold**.

² At 14 d after inoculation where 0 = no discolouration or very slight discolouration or scarring at site of inoculation; 1 = low level discolouration at site of inoculation; 2 = very small lesion or slight discolouration 1–2 mm diam; 3 = necrotic lesions 2–5 mm, some light stem streaking, leaf wilting and twisting; 4 = lesions 5–10 mm diam, significant necrosis and dark stem streaking, leaf and plant wilting, stunting, and some lodging; 5 = very severe necrosis and lesions, dark streaking, leaf necrosis, twisting and wilting, stunting, lodging or plant death.
Table 2  Reference isolates used in the phylogenetic analyses.

| Species                        | Isolate no. | Host                | GenBank accession numbers | Reference                      |
|-------------------------------|-------------|---------------------|---------------------------|--------------------------------|
| Diaporthe alleghaniensis      | CBS 495.72  | Betula alleghaniensis | FJ889444                  | GQ250298 Santos et al. 2010    |
| Diaporthe ambiguа             | CBS 114015  | Pyrus communis      | AF230767                  | GQ250299 Mostert et al. 2001   |
| Diaporthe angelicaе            | CBS 111592  | Heracleum spondylium | AY196779                  | GQ250302 Santos et al. 2010    |
| Diaporthe aspalathi           | CBS 117169  | Aspalathus linearis | DG286275                  | GQ286249 van Rensburg et al. 2006 |
| Diaporthe australafricana     | STE-U 2655  | Vitis vinifera      | AF230744                  | Mostert et al. 2001            |
| Diaporthe crotalariaе         | CBS 162.33  | Crotalaria spectabilis | FJ889445                  | GQ250307 Santos et al. 2010    |
| Diaporthe helianthi           | Ar            | Arctium lappa       | FJ841859                  | GQ250308 Santos et al. 2010    |
| Diaporthe hickoriaе            | CBS 592.81  | Helianthus annuus   | AY705842                  | GQ250308 Santos et al. 2010    |
| Diaporthe lusitanicaе         | CBS 123212  | V. vinifera         | AY485745                  |                                |
| Diaporthe melonis              | CBS 507.78  | Foeniculum vulgare  | EU878427                  |                                |
| Diaporthe neotheicolaе         | CBS 123208  | F. vulgarе           | EU814477                  |                                |
| Diaporthe perjunctа            | CBS 109745  | Ulmus glabra        | AY485785                  |                                |
| Diaporthe stewartii           | CBS 193.36  | Cosmos bipinnatus   | FJ889448                  |                                |
| Diaporthe strumella var. longispora | CBS 194.36  | Ribes sp.            | FJ889449                  |                                |
| Diaporthe vaccinii            | CBS 160.32  | Oxyccocus macrocarpus | AY952141                  |                                |
| Diaporthe viticolaе          | CBS 113201  | V. vinifera         | AY485750                  |                                |
| Diaporthe sp.                 | DAR 7381    | Carthamus lanatus   | EU311607                  |                                |
| Phomopsis amygdali            | CBS 126679  | Prunus dulcis       | GQ281791                  |                                |
| Phomopsis cotoneastri         | CBS 439.82  | Cotoneaster sp.     | FJ889450                  |                                |
| Phomopsis cuppateа             | CBS 117499  | Aspalathus linearis | AY339322                  |                                |
| Phomopsis dauci                | CBS 315.49  | Daucus carota       | AY339354                  |                                |
| Phomopsis longicolla          | SSLP-1       | Glycine max         | HQ333500                  |                                |
| Phomopsis phoenicicola        | CBS 161.64  | Areca catechu       | FJ889452                  |                                |
| Phomopsis sclerotiordei       | CBS 296.67  | Cucumis sativus     | AF439626                  |                                |
| Phomopsis subordinaria        | CBS 104.84  | Plantago lanceolata | GQ222519                  |                                |
| Phomopsis viticolaе            | CBS 114016  | V. vinifera         | FJ889445                  |                                |

1 CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; Ph- & Di-: culture collection housed at Centro de Recursos Microbiológicos, Caparica, Portugal.
2 ITS: internal transcribed spacer.
3 TEF-1α: translation elongation factor-1 alpha.
4 Di-C004/5 is also recorded as CBS 123208.
5 Ex-type cultures are in **bold**.
Fig. 1. Phylogenetic tree resulting from the alignment of 540 characters of the ITS region. The phylogenetic tree was inferred using the Maximum Likelihood method based on the Kimura 2-parameter model. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1 000 replicates) are shown next to the branches. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.3209)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Species described in this work are highlighted. Ex-type cultures are in bold.
from Herr et al. 1983 and van Rensburg et al. 2006) required the placement of a 5 mm cube of colonised WAS into a 5–10 mm long slit made in the stem at a node. This wound was then sprayed with distilled water and wrapped with permeable film (Parafilm™). Control plants were wounded with a 5–10 mm long slit at the nodes as for the treated plants, then wrapped with permeable film without placing an agar cube in the wound. Both inoculated and control plants were sprayed with distilled water, placed in a dew chamber and incubated at 25 °C 12 h light / 20 °C 12 h dark for 48 h then returned to a growth cabinet under the light and temperature regime described above. This test was replicated five times for each isolate.

The less invasive mycelium contact method (Miric 2002) was used as a secondary test for pathogenicity of selected isolates. A 5 mm cube of inoculated agar was placed in contact with the stem at a node, sprayed with distilled water, wrapped with permeable film and incubated as described above. Plants were assessed for lesion development at 14 d after inoculation on a scale of 1 to 5 (Table 1).

**RESULTS**

**Phylogenetic analysis**

For the ITS region, approximately 540 bases were sequenced for the isolates in this study and added to the alignment. The alignment included sequences from 58 Diaporthe/Phomopsis species (including two outgroups), of which 23 were from ex-type cultures.

For the TEF-1α region, approximately 580 bases were sequenced for the isolates in this study. However, only 350 bases
could be used to compare with the GenBank-retrieved sequenc-
es. The alignment included sequences from 24 Diaporthe/
Phomopsis species (including two outgroups), of which 20
were from ex-type cultures. Evolutionary relationships of these
sequences were analysed using the ML method based on a
K2+G model for ITS, and a HKY+G model for TEF-1α, as
determined by Modeltest in MEGA v. 5.05.

The phylogramme of the ITS region showed that the Australian
isolates of Diaporthe from stem cankers on sunflower formed
three well-supported clades, which indicate novel species (Fig.
1). One of these clades was close to ex-type strains of three
species, namely D. angelicae, D. stewartii and P. dauci, as
well as an isolate of P. subordinaria. Furthermore, this clade
included an isolate (DAR 73811) identified by Ash et al. (2010)
as Phomopsis sp. that was pathogenic on Carthamus lanatus
(saffron thistle, Asteraceae). To improve the resolution between
this clade and D. angelicae, D. stewartii and P. dauci, an ML
analysis was conducted on the TEF-1α dataset, which is con-
sistent with the ITS phylogramme, but with a stronger bootstrap
value (65 %) (Fig. 2).

The phylogenetic analysis of the ITS dataset included 31 iso-
lates of D. helianthi sourced from five publications (Says-Les-
age et al. 2002, van Niekerk et al. 2005, Bernardi-Wenzel et
al. 2010, Santos et al. 2010, Vrandecic et al. 2010) and formed
three distinct clades (Fig. 1). One clade included the ex-type
culture of D. helianthi (CBS 592.81), while two other clades ap-
ppeared to represent novel Diaporthe species (Fig. 1, Diaporthe
sp. 1 and 2).

Pathogenicity

The 14 selected isolates inoculated onto sunflower caused a
range of symptoms (Table 1), which divided them into two main
groups. Ten isolates causing the most severe symptoms, rated
4 or 5 for virulence, originated from stems, seeds and leaves of
infected sunflower plants from both NSW and Qld. Four isolates,
causing less severe symptoms and rated 2 or 3 were collected
from stems of infected plants in Queensland.

Using the wound inoculation method, tan to brown elongated
lesions were evident above and below the point of inoculation
after 3–7 d for the most virulent isolates, (those rated 4 or 5)
with lesions expanding rapidly upwards causing plant death
after 7–14 d. Earliest symptoms at 1–3 d after inoculation
for the most virulent isolates (rated 4 or 5) included brownish
streaks moving upwards from the inoculation site, wilting of
leaves at the node closest to the site of inoculation as well as
leaves directly above the site. At times, wilting of leaves above
the site of inoculation occurred without obvious stem streaking.
Generally, affected leaves developed a water-soaked appear-
ance sometimes associated with twisting.

Two to four weeks after inoculation, stem pieces above and
below the site of the wound were excised from all plants with
lesions, surface sterilized as previously described, and incu-
bated on WAS at 23–25 °C for up to 3 wk. Pycnidia developed
between 7–21 d. Conidia oozing from pycnidia were streaked
onto PDAS and the cultures compared with those of the original
isolates. Isolates were re-inoculated onto sunflower plants to
confirm their pathogenicity and to complete Koch’s Postulates.

A comparison of wound and mycelium contact inoculation
methods showed similar results for pathogenicity for individual
isolates after 14 d, although wound inoculated plants displayed
symptoms 1–7 d earlier than those inoculated by the mycelium
contact method.

Taxonomy

Based on morphology, pathogenicity and DNA sequence analy-
sis, three undescribed species of Diaporthe were recognised.
Although two of the new fungi only produced an anamorphic
stage, all have been described in Diaporthe (1870), which has
priority over Phomopsis (1884).

Diaporthe gulyae R.G. Shivas, S.M. Thompson & A.J. Young,
s. nov. — MycoBank MB561569; Fig. 3

Conidiomata pycnidialia, sparsa in PDA, subglobosa, usque ad 3 mm dia-
metro, interdum rostris ostiolatis usque ad 1 mm longis, cinctis ectostromate
nigro. Conidiophora facta e strato interiore parietis locularis, interdum ramosa
et septata, subhyalina, usque ad 6 µm diametro. Cellulae conidiogenae cylin-
draceae, hyalinae, 7–18 × 1.5–2.5 µm. Alpha conidia globosa, subglobosa,
ellipsoidea, ovalia vel obovoidea, hyalina, (6–)6.5–9.0(–10) × 2.5–3.5 µm.
Beta conidia haud conspecta.

Fig. 3 Diaporthe gulyae (ex-type BRIP 54025). a. Cultures on PDA (left), OA (right) after 7 d (top) and 28 d (bottom); b. pycnidial beaks on sterilised wheat
straw; c. alpha conidia; d. conidia and conidiophores. — Scale bars: b = 100 µm; c, d = 10 µm.
**Etymology.** In recognition of Dr Gary Kong for his innovative contributions to sunflower pathology research and enduring mentoring roles in the USA, Europe and Australia.

**Conidiomata** pycnidal, scattered on PDA, subglobose, up to 3 mm diam, occasionally with ostiolate beaks up to 1 mm long, surrounded by a black ectostroma. *Conidiophores* formed from the inner layer of the locular wall, sometimes branched and septe, subhyaline, up to 6 µm diam. *Conidiogenous cells* cylindrical, hyaline, 7–18 × 1.5–2.5 µm. *Alpha conidia* globose, subglobose, ellipsoidal, oval or obvoid, hyaline, (6–)6.5–9.0 (–10) × 2.5–3.5 µm. *Beta conidia* not seen.

**Culture characteristics —** Colonies on PDA covering entire plate after 10 d, buff, ropey near the margin and adpressed in towards apex and often covered with short unbranched hyphae up to 200 µm, surrounded by a black ectostroma. *Conidiomata* formed from the inner layer of the locular wall, polyangular, sometimes branched and septe, subhyaline to pale olivaceous brown, up to 6 µm diam. *Conidiogenous cells* cylindrical to obclavate, hyaline, 6–12 × 1.5–4 µm. *Alpha conidia* oval to cylindrical, biguttulate, hyaline, 5.5–7 (–7.5) × 2–2.5 (–3) µm. *Beta conidia* sigmoid to lunate, mostly curved through 90–180°, hyaline, 13–23 × 1–1.5 µm.

**Culture characterises —** Colonies on PDA covering entire plate after 10 d, ropey with a conspicuous ring 2.5 cm in diam of tufted aerial mycelium and abundant tufts towards the margin, white to greyish white with scattered amber patches, with several scattered minute black stroma, reverse with an isabelline ring, paler towards the margin; on OA covering the entire plate after 10 d, adpressed, rosy-buff, with an irregular grey olivaceous central zone about 4.5 cm diam and smaller irregular grey olivaceous patches towards the margin containing a few minute black stroma, the central zone and patches have yellowish margins, reverse rosy buff with irregular isabelline patches.

**Specimens examined.** Australia, Queensland, Childers, on *Helianthus annuus* hybrid PDAS, 1 Dec. 2010, S.M. Thompson (holotype BRIP 54031, includes ex-type culture); Childers, on *Helianthus annuus* hybrid PDAS, 1 Dec. 2010, S.M. Thompson, paratype BRIP 54032.

**Notes** Based on phylogenetic inference from the ITS sequence data (Fig. 1), *D. kongii* is closely related to *P. cuppata*, which was isolated from plants of *Asclepias lineata* (Fabaceae) with die-back (van Rensburg et al. 2006). Morphologically *D. kongii* has smaller conidia than those of *P. cuppata*, which measure (10–)12–13 (–14) µm.

**Diaporthe kochmannii** R.G. Shivas, S.M. Thompson & A.J. Young, sp. nov. — MycoBank MB561571; Fig. 4b, d–h

Perithecia formata in PDA et in caulibus sterilis apicifloris post octo hebdomades, subglobose, usque ad 350 µm diametro, plerumque solitaria in agaro vel aggregata in fasciculis in caulibus, cineta ectostromate nigro, uno vel pluribus collis cylindraceis nigris ostiolatis usque ad 2 mm haud distinctis ab eis in pycnidis. Asci uncinati, cylindracei, 33–41 × 5–7 µm, hyalini, octospori, biseriati, annulo conspicuo refractivo apicali. Ascospores hyalini, medieae septe, ovasales ad cylindraceas, haud constrictae ad septum, guttula in quaque cellula, 9–10 × 2.5–3.5 µm, leves. Diaporthe kochmannia, sparsa in PDA, nigra, subglobose, usque ad 2 mm diametro, uno vel pluribus collis cylindraceis nigris ostiolatis usque ad 2 mm. Diaporthe facta e strato interiore pariets locularis, polyangularia, interdum ramosa et septata, subhyalina ad brunnea olivacea, usque ad 6 µm diametro. Celulae conidioigenae cylindraceae ad obclavatas, hyalinae, 5–10 × 1.5–3 µm. Alpha conidia ovalia ad cylindracea, (5–)5.5–7 (–7.5) × 2–3.5 µm. Beta conidia flexuosa ad lunata, plerumque curvata per 45–90°, hyalina, 11–17 × 1–1.5 µm.

**Etymology.** In recognition of Dr Joe Kochman who pioneered the investigation of rust races on sunflower in Australia and his widely recognised contributions to sunflower pathology.
Culture characteristics — Colonies on PDA covering entire plate after 10 d, ropey with abundant tufts of mycelium, pale mouse grey, lighter towards the margin, with abundant scattered minute black stroma, reverse smoke grey with a darker central zone 5 cm diam; on OA covering the entire plate after 10 d, adpressed with scant tufted aerial mycelium, pale rosy vinaceous, with irregular pale olivaceous grey patches up to 1 cm wide containing minute black stroma, reverse pale rosy vinaceous with pale greyish areas where stroma form.

Specimens examined, Australia, Queensland, Lawes, on *Helianthus annuus* Experimental Line, 25 Nov. 2010, S.M. Thompson (holotype BRIP 54033, includes ex-type culture); Lawes, on *Helianthus annuus* hybrid PDAS, 25 Nov. 2010, S.M. Thompson, paratype BRIP 54034.

Fig. 4  *Diaporthe kongii* (ex-type BRIP 54031) and *D. kochmanii* (ex-type BRIP 54033). a. *Diaporthe kongii* cultures on PDA (left), OA (right) after 7 d (top) and 28 d (bottom); b. *Diaporthe kochmanii* cultures on PDA (left), OA (right) after 7 d (top) and 28 d (bottom); c. pycnidial beaks of *D. kongii* on sterilised wheat straw; d. perithecial necks of *D. kochmanii* on sterilised wheat straw; e. alpha and beta conidia of *D. kongii*; f. alpha and beta conidia of *D. kochmanii*; g. beta conidia of *D. kochmanii*; h. asci and ascospores of *D. kochmanii*. — Scale bars: c, d = 1 mm; e–h = 10 µm.
Diaporthe helianthi derived from an ex-type isolate, clustered in a clade with isolates from the former Yugoslavia and France (Fig. 1). Diaporthe helianthi has also been recorded from hosts other than sunflower in Croatia (Vrandecic et al., 2010), which was part of the former Yugoslavia. All records of D. helianthi from hosts other than sunflower, without comparison to sequence data from ex-type cultures, should be treated with caution (e.g. van Niekerk et al., 2005, Bernardi-Wenzel et al., 2010). Unintentional misapplications of the name D. helianthi have resulted from the absence and inaccessibility of cultures derived from type material, which are needed for molecular comparison.

Based on the localities of previous Diaporthe collections in Australia from sunflower, soybean (Glycine max), Noogoora burr (Xanthium pungens) (Miric 2002), saffron thistle (Ash et al., 2010) plus herbarium records, we expect that future surveys will broaden the host and distribution ranges of these newly described species. We also anticipate that more species associated with stem cankers on sunflower in Australia will be identified.

The results of our study highlight the need for the re-evaluation of the identification and classification of Diaporthe (Phomopsis) species (Farr et al., 2002, Hyde et al., 2010, Santos et al., 2010, Udayanga et al., 2011). Accurate and reliable methods of identification for Diaporthe species is a major concern for biosecurity agencies in many countries, including Australia. In this regard, D. helianthi has not been identified from sunflower in Australia and remains a biosecurity threat.

Advances in molecular identification techniques are helping to further define species boundaries by providing more specific genetic evidence in support of taxonomic differences (Udayanga et al., 2011). The combination of pathology (host range and pathogenicity), taxonomic descriptions and molecular analyses will certainly result in the identification and description of more Diaporthe species from a range of host plants worldwide.

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