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

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Key words

Diaporthe gulyae
Diaporthe kochmanii
Diaporthe kongii
ITS phylogeny
sunflower taxonomy
TEF-1α

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 eustromatic or pycnidial conidiomata containing elongated phialides with cylindrical, well-developed collarates 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 filliform 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 *Diaporthe* 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 Nickerk 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 Nickerk 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 (Muntañola-Cvetkovic et al. 1981). Yield reductions of up to 40 % have been recorded in Europe (Masi- revic & Gulya 1992) including the former Yugoslavia as well as France where it was considered a major pathogen of sunflower (Battilani et al. 2003, Debakee 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. Montañola-Cvetkovic 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.
Diaporthe gulyae
53158  Goran Lake, NSW  stem  Wild H. annuus  4  JF431284  JN645799
53166  Premer, NSW  seed  Ausagold 62  4  JF431289  JN645801
53172  Premer, NSW  seed  Hylœcle 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  JF431297  JN645805
54026  Ryeford, Qld  leaf  Sunbird 7  5  JF431298  JN645804
54025  Ryeford, Qld  leaf  Sunbird 7  4  JF431299  JN645803

Diaporthe kochmanii
54033  Gatton, Qld  stem  Experimental  2  JF431295  JN645809
54034  Gatton, Qld  stem  Experimental  3  JF431296  JN645810

Diaporthe kongii
54032  Childrens, Qld  stem  Female  3  JF431300  JN645798
54031  Childrens 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.

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 cultures. 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 23 °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 character states 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 (OA) (Oxoid) 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 (Finzymes). The PCR products were purified with the QiAquick 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 Hylœcle 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
### Table 2  Reference isolates used in the phylogenetic analyses.

<table>
<thead>
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<th>Species</th>
<th>Isolate no.</th>
<th>Host</th>
<th>GenBank accession numbers</th>
<th>Reference</th>
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<td>DQ286249</td>
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<td>Vitis vinifera</td>
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<td>Crotalaria spectabilis</td>
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<td>Helianthus annuus</td>
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<td>F. vulgare</td>
<td>AY485746</td>
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<td>Cosmos bipinnatus</td>
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<td>Glycine max</td>
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<td>HQ335305</td>
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<td>Plantago lanceolata</td>
<td>GQ292519</td>
<td></td>
</tr>
<tr>
<td>Phomopsis viticola</td>
<td>CBS 114016</td>
<td>V. vinifera</td>
<td>FJ889454</td>
<td>GQ250351</td>
</tr>
</tbody>
</table>

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-1alpha.
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 sequences. 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 consistent with the ITS phylogramme, but with a stronger bootstrap value (65 %) (Fig. 2).

The phylogenetic analysis of the ITS dataset included 31 isolates of D. helianthi sourced from five publications (Says-Lesage 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 appeared 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 appearance 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 incubated 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 analysis, 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, sp. nov. — MycoBank MB561569; Fig. 3

Conidiomata pycnidialia, sparsa in PDA, subglobosa, usque ad 3 mm diametro, 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 cylindraceae, 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.

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![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.](image-url)
Conidiomata pycnidial, 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 septate, subhyaline, up to 6 µm diam. Conidiogenous cells cylindrical, hyaline, 7–18 × 1.5–2.5 µm. Alpha conidia globose, subglobose, ellipsoid, 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 the centre, scant aerial mycelium, reverse buff with a slightly darker centre; on OA covering the entire plate after 10 d, adpressed with scattered tufts of greyish mycelium, greyish sepia, with a fuscous black central zone 1 cm diam, reverse greyish sepia with a fuscous black central zone.

Specimens examined. AUSTRALIA, Queensland, Ryeford near Clifton, on Helianthus annuus hybrid Sunbird 7, 29 Nov. 2010, S.M. Thompson (holotype BRIP 54025, includes ex-type culture); Ryeford near Clifton, on Helianthus annuus hybrid Sunbird 7, 29 Nov. 2010, S.M. Thompson, paratypes BRIP 54026, 54027.

Notes — Based on molecular phylogenetic inference, D. gulyae was placed near to the ex-type specimens of D. angelicae, D. stewartii and P. dauci, as well as a strain of P. subordinaria (Fig. 1, 2). Morphologically there is little difference between these species but unique fixed nucleotides accurately differentiate D. gulyae. Diaporthe gulyae differs from D. stewartii in two loci: ITS position 24 (T) and 98 (A); TEF-1α position 19 (A), 324 (T), 30 (T), 46 (T), 47 (A) and 315 (T). Diaporthe gulyae differs from D. angelicae and P. dauci in two loci: ITS position 59 (C), 90 (T), 136 (A) and 457 (A); TEF-1α position 30 (T) and 47 (A).

Diaporthe gulyae causes a severe stem canker on sunflower and saffron thistle. On the basis of pathology and substrate preference D. gulyae differs from D. angelicae, which is found on the decaying stems of hosts in the Apiaceae (Castlebury et al. 2003); D. stewartii, which causes stem blight of Cosmos bipinnatus (Asteraceae) (Harrison 1935); P. dauci, which causes infection blight of Daucus carota (carrot, Umbelliferae) (von Arx 1951); and D. adunca (P. subordinaria), which attacks the scapes of Plantago lanceolata (Plantaginae) (Meijer et al. 1994).

Diaporthe konigii R.G. Shivas, S.M. Thompson & A.J. Young, sp. nov. — MycoBank MB561570; Fig. 4a, c, e

Conidiomata pycnidial, sparsa in PDA, subglobose, usque ad 2 mm diametro, rostris ostiolatis levibus ad apicem et saepe tectis hyphis brevis inramosis usque ad 200 µm, a few minute black stroma, the central zone and patches have 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 PDA, 1 Dec. 2010, S.M. Thompson (holotype BRIP 54031, includes ex-type culture); Childers, on Helianthus annuus hybrid PDA, 1 Dec. 2010, S.M. Thompson, paratype BRIP 54032.

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

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

Perithecia formata in PDA et in caulis esterilis apricifloris post octo hedemodas, subglobose, usque ad 350 µm diametro, plerumque solitaria in agaro vel aggregata in fasciculis in caulis, cincta ectostroma nigro, uno vel pluribus collis cymatibus nigris ostiolatis usque ad 2 mm hau ductis ab eis in pycnidia. Asci unilocuantes, globosae, 33–41 × 5–7 µm, hyalini, octosphori, biseriati, annulo cincto refracto apiacali. Ascospores hyalinae, mediane septicatae, ovales ad cymatibus, hau ductis ad septum, guttula in quacue cellulae, 9–10 × 2.5–3.5 µm, leves. Conidiomata pycnidialia, sparsa in PDA, nigra, subglobose, usque ad 2 mm diametro, uno vel pluribus collis cymatibus nigris ostiolatis usque ad 2 mm. Conidiophora facta e strato interiore pariets locularis, polyanugiliarum, interdum ramosa et sep tata, subhyalina ad brunnea oleicrana, usque ad 6 µm diametro. Cellulae conidioengenes cylindraceae ad obclavatas, hyalinae, 5–10 × 1.5–3 µm. Alpha conidia ovata ad cylindracea, (5–)5.5–(7–)5.5 × 2–3 µm. Beta conidia flexuosa ad lunata, plerumque curvata per 45–90°, hyalina, 11–17 × 1.5–1.5 µm.

Etymology. In recognition of Dr Gary Kong who pioneered the investigation of rust races on sunflower in Australia and his widely recognised contributions to sunflower pathology.

Perithecia formed on PDA and on sterilised stems of sunflower after 8 wk, subglobose, up to 350 µm diam, usually solitary in the agaro or aggregated in clusters on the stems, surrounded by a black ectostroma, with 1 or more cylindrical, black, ostiolate necks up to 2 mm, indistinguishable from those on pycnidia. Asci unilocuantes, cylindraceae, 33–41 × 5–7 (av. = 37 × 6 µm), hyalina, 8-sporae, biseriatae, with conspicuous refractive apiacai. Ascospores hyalinae, mediane septicatae, ovalis ad cylindraceae, hau ductis ad septum, not constricted at the septum, with a guttula in each cell, 9–10 × 2.5–3.5 µm (av. = 9.5 ± 3 µm), smooth. Conidiomata pycnidial, scattered on PDA, black, subglobose, up to 2 mm diam, with 1 or more cylindrical black ostiolate necks up to 2 mm long. Conidiophores formed from the inner layer of the locular wall, polyanugiliarum, sometimes branched and septate, subhyalinae ad palae olivaceae brown, up to 6 µm diam. Conidiogenous cells cylindrical to obclavates, hyalinae, 6–12 × 1.5–4 µm. Alpha conidia ovale ad cylindraceae, biguttulata, hyalina, 5.5–7(–7.5) × 2–2.5(–3) µm. Beta conidia sigmoide ad lunata, mostly curved through 90–180°, hyalina, 13–23 × 1.5–1.5 µm.

Etymology. In recognition of Dr Gary Kong for his innovative contributions to sunflower pathology in Australia, specifically his investigation of the genetics of resistance to Puccinia helianthi and Alternaria helianthi.

Conidiomata pycnidial, scattered on PDA, subglobose, up to 2 mm diam, with short (less than 0.5 mm) ostiolate beaks smooth towards apex and often covered with short unbranched hyphae up to 200 µm, surrounded by a black ectostroma. Conidiophores formed from the inner layer of the locular wall, polyanugiliarum, sometimes branched and septate, subhyalinae ad palae olivaceae brown, up to 6 µm diam. Conidiogenous cells cylindrical

Etymology. In recognition of Dr Tom Gulya for his outstanding contributions to sunflower pathology and enduring mentoring roles in the USA, Europe and Australia.

Conidiomata pycnidial, 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 septate, subhyaline, up to 6 µm diam. Conidiogenous cells cylindrical
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.
Notes — Based on phylogenetic inference of the TEF-1α sequence data D. kochmanii is closest to D. kongii. Morphologically these two species cannot be reliably separated. Diaporthe kochmanii differs from D. kongii in the TEF-1α locus position 60 (A), 82 (C), 184 (C), 215 (C), 240 (T), 260 (G), 266 (C), 268 (T), 280 (G), 284 (T), and 288 (T).

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