Green and brown bridges between weeds and crops reveal novel *Diaporthe* species in Australia

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**Abstract** *Diaporthe* (syn. *Phomopsis*) species are well-known saprobes, endophytes or pathogens on a range of plants. Several species have wide host ranges and multiple species may sometimes colonise the same host species. This study describes eight novel *Diaporthe* species isolated from live and/or dead tissue from the broad acre crops lupin, maize, mungbean, soybean and sunflower, and associated weed species in Queensland and New South Wales, as well as the environmental weed bitou bush (*Chrysanthemoides monilifera* subsp. *rotundata*) in eastern Australia. The new taxa are differentiated on the basis of morphology and DNA sequence analyses based on the nuclear ribosomal internal transcribed spacer region, and part of the translation elongation factor-1α and β-tubulin genes. The possible agricultural significance of live weeds and crop residues (‘green bridges’) as well as dead weeds and crop residues (‘brown bridges’) in aiding survival of the newly described *Diaporthe* species is discussed.

**INTRODUCTION**

*Diaporthe* (syn. *Phomopsis*) species are well-known saprobes, endophytes or pathogens on a wide range of hosts. Species in this genus are well-known in the plant pathology literature as the cause of many significant plant diseases worldwide, including stem cankers, leaf and pod blights, and seed decay (Rehner & Uecker 1994, Santos et al. 2011, Udayanga et al. 2011). Further, *Diaporthe* species have been recorded as opportunistic saprobes on decaying leaves, twigs and stem residues, as well as endophytes on healthy leaves, stems, seeds and roots (Muralli et al. 2006, Gomes et al. 2013).

The recent use of DNA-sequencing-based methods and the application of the Genealogical Concordance Phylogenetic Species Recognition (GCPsr) criteria have resulted in a rapid increase in the discovery of cryptic species in several large genera of plant pathogenic fungi, such as *Colletotrichum* (Damm et al. 2012a, b, Weir et al. 2012), *Diaporthe* (Shivas & Cai 2012, Udayanga et al. 2014) and *Fusarium* (O’Donnell et al. 2009, 2012). This approach also provides a more stable taxonomy for *Diaporthe*, from which a clearer understanding about the host range of particular species is emerging. It is known that many species of *Diaporthe* have wide host ranges (Mengistu et al. 2007, Santos et al. 2011, Udayanga et al. 2011, Gomes et al. 2013) and multiple species can colonise the same host (Farr et al. 2002, Crous & Groenewald 2005, van Niekerk et al. 2005, Thompson et al. 2011).

It is well documented in plant pathology literature that live weeds and volunteer crop plants serve as alternative hosts for a range of pathogens, including *Diaporthe* species, by providing a ‘green bridge’ that facilitates pathogen survival between crop phases. Following the first outbreaks of *Diaporthe helianthi* (syn. *Phomopsis helianthi*) on sunflower in the former Yugoslavia (now Serbia), Mihaljevic & Muntafola-Cvetkovic (1985) recovered *Diaporthe* species from 15 plant species, including the weeds *Xanthium italicum* and *X. strumarium*. Subsequent studies by Vranečić et al. (2010) confirmed *Arctium lappa*, *X. italicum* and *X. strumarium* as weed hosts for *D. helianthi*.

Alternative weed hosts have been suspected to play a role in the epidemiology of three species, *D. gulyae*, *D. kochmannii* and *D. kongii*, recently found associated with sunflower canker in eastern Australia, eight novel species were identified based on GCPsr criteria, from both live crop and weed hosts as well as crop stubble and weed residues in Queensland (Qld) and New South Wales (NSW). Dead standing weeds and residues are common amongst crop stubble in Australian broad acre and low tillage cropping systems, where herbicides are often used for weed control. Additionally, one of the new *Diaporthe* species was also identified from a study into the cause of dieback of the coastal environmental weed *Chrysanthemoides monilifera* subsp. *rotundata* (bitou bush) in northern NSW. All eight species of *Diaporthe* are described and illustrated here.

**MATERIALS AND METHODS**

**Isolates**

Isolates from broad acre cropping regions

Plant material was collected from a range of summer crops including lupin, maize, mungbean, soybean and sunflower, as well as major weed species and plant residues on the soil surface...
across the broad acre cropping regions of Qld and NSW (Table 1). The material included necrotic lesions or visible pycnidia on stems, leaves, petioles, heads and seeds from live plants and/or dead plants. Specimens from plant residues were only selected from material for which the inflorescence was present so that the plant species could be identified.

Small pieces (10–30 mm) of tissue or entire seeds were surface sterilised in 1% sodium hypochlorite solution for 3 min, then rinsed with sterile distilled water. The surface sterilised tissue was placed onto 9 cm diam Petri plates containing water agar and surface sterilised either by: i) immersing in 2% NaOCl for 2–4 min. Tissue pieces were rinsed three times in sterile distilled water, blotted dry with paper towel, then cut longitudinally with a sterile scalpel and placed on the surface of fresh plates of PDAS and incubated as above.

Isolates from bitou bush

Stems of live bitou bush plants affected by dieback were collected from Bongil Bongil National Park and Bellingen Head State Park in northern NSW (Table 1). Pieces of stem tips at the margin between healthy and dead tissue, immersed in 70% ethanol for 30 s followed by 2% sodium hypochlorite for 2–4 min. Tissue pieces were rinsed three times in sterile distilled water, blotted dry with paper towel, then cut longitudinally with a sterile scalpel and placed on the surface of fresh plates of PDAS and incubated as above.

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1/2 PDAS under the same conditions as above. All isolates recovered were deposited in the Plant Pathology Herbarium (BRIP), Brisbane, Australia.

**Morphology**

To determine morphological characteristics, isolates were grown on water agar with pieces of sterilised wheat stems placed on the surface (WSA) and incubated under a 12 h photoperiod with near ultraviolet light (NUV) (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. At least 20 measurements of selected structures were made and, means and standard deviations (SD) calculated. 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 were grown for a further 7 d under 12 h of PDA and oatmeal agar (OMA) (Oxoid) that had been grown at 23 °C. Colony colours (surface and reverse) were described according to the colour charts of Rayner (1970). Nomenclatural novel- ties were deposited in MycoBank (Crous et al. 2004) (www. mycobank.org).

**DNA isolation, amplification and analyses**

For isolates from broad acre cropping regions, 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. For isolates from bitou bush, genomic DNA was extracted from mycelia scraped off 1/2 PDAS cultures using Mo-Bio Ultraclean Microbial DNA Isolation Kit.

The internal transcribed spacer (ITS) region of the nuclear ribosomal genes was amplified with the primers ITS4 (White et al. 1990), and V9G (de Hoog & Gerrits van den Ende 1998) or ITS1F (Gardes & Brun 1995) for the isolates from broad acre cropping regions and bitou bush, respectively. For all isolates, the primers EF1-728 F (Carbone & Kohn 1999) and EF2 (O’Donnell et al. 1998) were used to amplify part of the translation elongation factor 1-α (TEF) gene, and the primers T1 (O’Donnell & Gignelik 1997) and Bt2b (Glass & Donaldson 1995) were used to amplify part of the β-tubulin (BT) gene.

The ITS region of the bitou bush isolates was amplified with Platinum Taq (Invitrogen) according to manufacturer’s instructions and the PCR conditions were 95 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min × 25 cycles. PCR products were purified with the Agencourt AMPure XP system (Beckman Coulter).

The ITS region of the broad acre cropping regions and the BT and TEF loci of all isolates in this study were amplified with the Phusion High-Fidelity PCR Master Mix (Finnzymes) and the PCR conditions were 98 °C for 30 s, followed by 30 cycles of 98 °C for 10 s, 55 °C (ITS and TEF) or 60 °C (BT) for 30 s, 72 °C for 30 s, and a final extension at 72 °C for 5 min. The PCR products were purified with the QIAquick PCR Purification Kit (QIAGEN), and sequenced by Macrogen Incorporated (Seoul, Korea) using the amplification primers.

All unique sequences from different host-isolate combinations generated in this study were assembled using Vector NTI Advance 11.0 (Invitrogen). The ITS sequences were initially aligned with representative Diaportha species from recent studies (Thompson et al. 2011, Udanyaga et al. 2012, Gomes et al. 2013) using MAFFT alignment algorithm (Katoh et al. 2009) in the software Geneious (Biomatters Ltd). Diaporthella corylina (CBS 121124) was selected as outgroup taxon in the phylogenetic analyses based on its position as sister genus in Diaporthales (Vasilyeva et al. 2007).

A Neighbour-Joining (NJ) analysis using the Kimura-2 parameter with Gamma distribution was applied (data not shown), and the closest phylogenetic neighbours were selected for a combined analyses using BT, TEF and TEF genes. The sequences of each gene were aligned separately and manually adjusted where needed. Alignment gaps were treated as missing character states, and all characters were unordered and of equal weight. Bayesian analysis was performed with MrBayes v. 3.2.1 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003) in Geneious. The Markov Chain Monte Carlo (MCMC) analysis used four chains and started from a random tree topology. The sample frequency was set at 200 and the temperature of the heated chain was 0.3. Burn-in was set at 25 % after which the likelihood values were stationary. Maximum Likelihood (ML) analysis, including 1 000 bootstrap replicates, were run using RAXML v. 7.2.8 (Stamatakis & Alchiotis 2010) in Geneious. The nucleotide substitution model chosen was General Time Reversible (GTR) with a gamma-distributed rate of variation.

The concatenated alignment and resulting tree were deposited in TreeBASE (study S15707). Unique fixed nucleotides positions are used to characterise and differentiate two species from closely related phylogenetic species. For each species that was described, the closest phylogenetic neighbour was selected and this focused dataset was subjected to single nucleotide polymorphisms (SNPs) analyses. These SNPs were determined for each aligned data partition using DnaSP v. v.5.10.01 (Librado & Rozas 2009).

**RESULTS**

**Isolates**

More than 500 Diaportha isolates were recovered from live or dead plant tissues or seeds, from the crops sunflower, soybean, mungbean, lupin, maize, as well as from a range of weed species in the broad acre cropping regions of Qld and NSW (Table 2). Of these isolates, 147 could not be assigned to known taxa based on ITS sequence BLASTn search results against the GenBank database. Many of the remaining isolates recovered from a number of crop and weed hosts were identified as one of three recently described species from sunflower, namely: D. gulyae, D. koehmani and D. kongii (Thompson et al. 2011) (data not shown). Fifteen Diaportha isolates were recovered from the bitou bush material, including eight isolates of D. kongii (data not shown).

**Phylogenetic analyses**

Approximately 600 bases of the ITS region were sequenced from the isolates investigated in this study and initially aligned against 116 sequences from 106 Diaportha species, most of which were from ex-type cultures. The evolutionary relationships of these sequences were analysed using the NJ method (data not shown; TreeBASE study S15707). From this NJ phylogenetic tree, 19 Diaportha taxa closest to the isolates in this study were selected for a combined analyses using the ITS, TEF and BT sequences. The combined sequence (ITS, TEF and BT) alignment for the Bayesian and ML analyses contained 1 642 characters from 35 isolates (including the outgroup taxon) (Table 1). The Bayesian analysis lasted 1 100 000 generations, and the consensus tree with posterior probability was calculated from 4 951 trees left after 110 000 trees were discarded at the burn-in phase. The tree topology and bootstrap values of the ML analysis supported the trees obtained from the Bayesian analysis. The multilocus phylogenetic tree (Fig. 1), along with mor-
Table 2  Crops and weeds from which the novel *Diaporthe* spp. species described in this paper were isolated.

| Plant host | Host family  | *Diaporthe* spp. |
|------------|--------------|------------------|
| Glycine max | Fabaceae      | *Diaporthe charlesworthii* |
| Helianthus annuus | Asteraceae | *Diaporthe goulteri* |
| Lupinus alba | Fabaceae | *Diaporthe macintoshii* |
| Vigna radiata | Fabaceae | *Diaporthe middletonii* |
| Zea mays | Poaceae | *Diaporthe masirevicii* |
| *Bidens pilosa* | Asteraceae | *Diaporthe miriciae* |
| *Chrysanthemoides monilifera* subsp. rotundata | Asteraceae | *Diaporthe sackstonii* |
| *Datura ferox* | Solanaceae | *Diaporthe serafiniae* |
| *Gaura parviflora* | Onagraceae | *Diaporthe stictica* |
| *Malva parafaflora* | Malvaceae | *Diaporthe stictica* |
| *Rapistrum rugosum* | Brassicaceae | *Diaporthe stictica* |
| *Sesbania cannabina* | Fabaceae | *Diaporthe stictica* |
| *Solanum nigrum* | Solanaceae | *Diaporthe stictica* |

1 Material from which the fungi were isolated is indicated in table: L = live stem (including leaf or petiole) tissue; D = dead stem (including petiole) tissue; S = seeds.

Fig. 1  Phylogenetic tree based on the combined multilocus (ITS, TEF and BT) alignment. The tree with the highest log likelihood (-8570) is shown. Bayesian posterior probabilities (pp) and RAxML bootstrap values (bs) are given at the nodes (pp/bs). Only those with bs percentage of greater than 60 are shown. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.4745)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Ex-type cultures are indicated by an asterisk (*).
phological examinations (see below), support the establishment of eight novel Diaporthe species, which are described below.

**Taxonomy**

*Diaporthe charlesworthii* R.G. Shivas, S.M. Thomps. & Y.P. Tan, *sp. nov.* — MycoBank MB808668; Fig. 2a–f

**Etymology.** In recognition of Australian sunflower grower Kevin Charlesworth (Ryeford Qld), for his contributions to the sunflower industry and passionate advocate of research.

*Conidiomata* pycnidial and multilocular, scattered, abundant on PDA, OMA and WSA after 4 wk, subglobose, up to 1 mm diam, ostiolate, necks absent or up to 1 mm. *Conidiophores* formed from the inner layer of the locular wall, 0–2-septate, branched at septa, hyaline to subhyaline, cylindrical, 15–35 × 1.5–3 μm. *Conidiogenous cells* cylindrical to flexuous, tapered towards the apex, hyaline, 10–25 × 1.5–3.0 μm. *Alpha conidia* abundant, fusiform to cylindrical, rounded towards the apex, hyaline, (6–)7–9.5(–11) × 2–2.5 μm. *Beta conidia* abundant amongst the alpha conidia, flexuous to J-shaped, hyaline, 25–35 × 1.0–1.5 μm. *Perithecia* not seen.

*Cultural characteristics* — Colonies on PDA after 10 d reaching the edge of the plate, margin coralloid with feathery branches, adpressed, without aerial mycelium, with numerous irregularly zonate dark stromata up to 2 mm diam, isabelline becoming lighter towards the margin; reverse similar to the surface with zonations more apparent. On OMA covering entire plate after 10 d, with little aerial mycelium and numerous scattered pale mouse grey irregular stromata up to 1.5 cm diam, pale isabelline between the stromata; reverse irregularly mottled, cinnamon to isabelline.

**Specimen examined.** Australia, Queensland, Gatton, from stem of Rapsitrum rugosum, 24 Nov. 2011, S.M. Thompson (T12757Z), holotype BRIP 54884m (includes ex-type culture).

**Notes** — The multigene analysis of isolate BRIP 54884m was not significantly homologous to any sequences in GenBank. No morphologically similar isolates are known from Rapsitrum rugosum. Therefore, this isolate is designated as representative of a new taxon. *Diaporthe charlesworthii* is one of three novel species isolated in this study from dead stems of *R. rugosum* (*Brassicaceae*), a widely distributed weed in eastern Australia.

*Diaporthe goulteri* R.G. Shivas, S.M. Thomps. & Y.P. Tan, *sp. nov.* — MycoBank MB808669; Fig. 2g–j

**Etymology.** In recognition of Australian scientist Ken Goulter, for his significant contributions to Australian sunflower pathology including the differentiation of sunflower rust races and early studies on the diversity of *Diaporthe* species.

*Conidiomata* multilocular, rare on PDA after 4 wk, abundant on OMA and WSA after 4 wk and often on a thin layer of dark *textura angularis* 50–100 μm thick with sharp margins on irregularly patches up to 1 cm diam, ostiolate, necks absent or less than 250 μm on PDA and OMA after 4 wk, necks up to 1.5 mm on wheat straw pieces on WA after 4 wk, abundant pale yellow conidial droplets exude from ostioles, sienna coloured droplets form on thin dark patches of *textura angularis*. *Conidiophores* formed from the inner layer of the locular wall, reduced to conidiogenous cells or 1-septate, hyaline to pale yellowish brown, filiform, 10–30 × 1.5–3 μm. *Conidiogenous cells* cylindrical to flexuous, tapered towards the apex, hyaline, 5–15 × 1.5–2.5 μm. *Alpha conidia* abundant, fusiform to cylindrical,

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**Fig. 2** Diaporthe spp. — a–f: *Diaporthe charlesworthii* (ex-type BRIP 54884m) after 4 wk. a. Culture on PDA (top) and OMA (bottom); b. conidiomata on OMA; c. conidiomata on PDA; d. conidiophores; e. alpha conidia and beta conidia; f. beta conidia. — g–j: *Diaporthe goulteri* (ex-type BRIP 55657a) after 4 wk. g. Culture on PDA (top) and OMA (bottom); h. conidiomata on sterilised wheat straw; i. conidiomata on OMA; j. alpha conidia. — Scale bars: a, g = 1 cm; b, c, h, i = 1 mm; d–f, j = 10 μm.
Fig. 3 Diaporthe spp. — a–e: Diaporthe macintoshii (ex-type BRIP 55064a) after 4 wk. a. Culture on PDA; b. pycnidia on sterilised wheat straw; c. pycnidia on OMA; d. conidiophores; e. alpha conidia and beta conidia. — f–j: Diaporthe masirevicii (ex-type BRIP 57892a) after 4 wk. f. Culture on PDA; g. conidiomatum on OMA; h. alpha conidia; i. conidiophores; j. alpha conidia and beta conidia. — k–p: Diaporthe middletonii (ex-type BRIP 54884e) after 4 wk. k. Culture on PDA (top) and OMA (bottom); l. pycnidia on sterilised wheat straw; m. conidiophores; n. alpha conidia; o. pycnidia on OMA; p. beta conidia. — q–u: Diaporthe miniciae (ex-type BRIP 54736j) after 4 wk. q. Culture on PDA; r. conidiomata on sterilised wheat straw; s. conidiophores; t. section across conidiomatum; u. alpha and beta conidia. — Scale bars: a, f, k, q = 1 cm; b, c, g, l, o, r = 1 mm; d, e, h–j, m, n, p, s, u = 10 μm; t = 100 μm.
rounded at the apex, slightly narrowed towards the base, hyaline, (6–)6.5–8(–9) × 2–2.5(–3) μm. Beta conidia not seen.

Diaporthe macintoshii R.G. Shivas, S.M. Thomps. & Y.P. Tan, sp. nov. — MycoBank MB808670; Fig. 3a–e

Etymology. In recognition of Australian agronomist Paul McIntosh, for his indefatigable and gregarious service to the Australian sunflower industry over 30 years.

Conidiomata pycnidial, solitary or aggregated in small groups, scattered, abundant on PDA, OMA and WSA after 4 wk, subglobose, up to 0.5 mm diam, ostiolate, necks absent, cream conidial droplets exuded from some ostioles. Conidiophores formed from the inner layer of the locular wall, 0–2-septate, hyaline to subhyaline, cylindrical, 10–20 × 1.5–3.5 μm. Conidiogenous cells cylindrical to flexuous, tapered towards the apex, hyaline, 10–15 × 1.5–2.5 μm. Alpha conidia abundant, fusiform to oval, narrowed towards apex and base, hyaline, (6.5–)8–11(–15) × 2–3(–3.5) μm. Beta conidia abundant amongst the alpha conidia, flexuous to hamate, hyaline, 15–30 × 1.0–1.5 μm. Perithecia not seen.

Cultural characteristics — Colonies on PDA covering entire plate after 10 d, adpressed, white to buff; reverse buff. On OMA covering entire plate after 10 d, white tinged with pale vinaceous, with several scattered circular mouse grey patches up to 1 cm diam, these patches are sometimes confluent and at the centres have oliveaceous mycelium with droplets of cinnamon coloured exudate and one or a few funicular columns of white mycelium up to 3 mm high; reverse uniformly buff.

Specimen examined. AUSTRALIA, Queensland, Ryeford, from a seed of Helianthus annuus, 15 Feb. 2011, S.M. Thompson (T12996A); holotype BRIP 55657a (includes ex-type culture).

Notes — Cultures of D. goulteri produced a cinnamon coloured exudate under the conditions described here. It is not known if this phenotypic characteristic is taxonomically useful. A BLASTn search with the ITS sequence showed the closest match was to HQ44993 from Solidago canadensis in China, with 99% identity (2 bp difference).

Diaporthe masirevicii R.G. Shivas, J. McIntosh, S.M. Thomps. & Y.P. Tan, sp. nov. — MycoBank MB808672; Fig. 3k–p

Etymology. In recognition of Australian plant pathologist Keith Middleton, for his innovative contributions to plant pathology of summer crops, especially his early studies of sunflower rust (Puccinia helianthi) and Rhizopus sp. infection in sunflower.

Conidiomata pycnidial, up to 300 μm diam on PDA and WSA after 4 wk, aggregated in scattered groups or multicellular on a 50–100 μm thick layer of dark textura angularis with sharp margins that irregularly covers most of the agar surface on OMA after 4 wk, subglobose, ostiolate, necks absent or about 200 μm, cream conidial droplets exuded from a few ostioles. Conidiophores formed from the inner layer of the locular wall, reduced to conidiogenous cells or 1-septate, hyaline to pale yellowish brown, cylindrical, 10–25 × 1.5–3.5 μm. Conidiogenous cells cylindrical, hyaline, 5–20 × 1.5–2.5 μm. Alpha conidia abundant, fusiform to cylindrical, rounded at the apex, obconically truncate at base, mostly biguttulate, hyaline, (5–)6.0–7.5(–8) × 2–2.5(–3) μm. Beta conidia scarce abundant, flexuous, mostly J-shaped, hyaline, 20–35 × 1.0–1.5 μm. Perithecia not seen.

Cultural characteristics — Colonies on PDA covering entire plate after 10 d, with scant aerial mycelium and numerous scattered dark stromata visible as black dots, buff; reverse similar to the surface. On OMA covering entire plate after 10 d, with scattered funiculosum mycelium to 1 cm high, surface mostly leaden black with irregular faintly pale vinaceous patches
towards the edge of the plate; reverse buff. Rosy vinaceous pigment produced in WA around colonised wheat straw pieces after 4 wk.

*Specimens examined.* **AUSTRALIA,** Queensland, Gatton, from stem of *Raphanus**; reverse uniformly rosy buff. Mouse grey patches up to several cm diam associated with entire plate after 10 d, ropey with a few scattered funiculose up to 2 mm diam, buff; reverse rosy buff. On OMA covering entire plate after 10 d, adpressed, white numerous scattered dark stromata up to 2 mm diam; reverse uniformly mottled white to uniformly isabelline.

**Diaporthe myricae** R.G. Shivas, S.M. Thomps. & Y.P. Tan, *sp. nov.* — MycoBank MB808673; Fig. 3q–u

**Etymology.** Named after Australian scientist Elizabeth Miric, who first recognised diversity in the Australian isolates of *Diaporthe* (*Phomopsis*) on sunflower in her PhD thesis entitled: 'Pathological, morphological and molecular studies of a worldwide collection of the sunflower pathogens *Phomopsis helianthi* and Phoma macdonalda' (University of Queensland, 2002).

**Conidiomata** pycnidial or multilocular, scattered or aggregated on PDA, OMA and WSA after 4 wk, solitary, ostiolate with necks up to 1 mm, pale yellow conidial droplets exuded from some ostioles. **Conidiophores** formed from the inner layer of the locular wall, reduced to conidiogenous cells or 1–2-septate, hyaline to subhyaline, cylindrical to obclavate, 10–20 × 1.5–3 μm. **Conidiogenous cells** cylindrical to obclavate, tapered towards the apex, hyaline, 5–12 × 1.5–3 μm. **Alpha conidia** abundant, fusiform to oval, rounded at the apex, narrowed at the base, hyaline, 6–7.5–(9) × 2.5–(3) μm. **Beta conidia** scattered or in groups amongst the alpha conidia, flexuous to hamate, hyaline, 20–35 × 1.0–1.5 μm. **Perithecia** not seen.

**Cultural characteristics —** Colonies on PDA covering entire plate after 10 d, adpressed, with a few scattered dark stromata up to 1 mm diam surrounded by patches of white sparse mycelium, buff; reverse isabelline with a few dark scattered stromata up to 3 mm diam. On OMA covering entire plate after 10 d, white tinged with pale vinaceous with pale mouse grey patches, with many scattered dark stromata mostly up to 4 mm diam; reverse uniformly cinnamon.

**Notes.** — **A BLASTn** search with the ITS sequence of the type isolate, BRIP 54884e, showed 100 % match to EF88935 from *Coffea arabica* in Hawaii, USA; 99 % identity (3–5 bp difference) to EU878434 from *Luehea divaricata* in Brazil; 99 % identity to JQ936257 from *Glycine max* cv. *Conquesta*; and 99 % identity to KP467129 from *Centrolobium ochrophyllum* in Ecuador.

**Diaporthe sackstonii** R.G. Shivas, S.M. Thomps. & Y.P. Tan, *sp. nov.* — MycoBank MB808674; Fig. 4a–e

**Etymology.** Named after the eminent Canadian plant pathologist Walde-mar E. Sackston, for his pioneering contribution to sunflower disease research on an international scale from the 1950s to the 1990s.

**Conidiomata** pycnidial or multilocular, solitary, scattered, scarce on PDA after 4 wk, abundant on OMA after 4 wk on a thin 50–100 μm thick layer of dark *textura angularis* with sharp margins that irregularly covers much of the agar surface, abundant on WSA after 4 wk, up to 1 mm diam, ostiolate, necks up to 0.5 mm, cream conidial droplets exuded from some ostioles. **Conidiophores** formed from the inner layer of the locular wall, reduced to conidiogenous cells or septate, filiform, 15–40 × 1.5–3 μm, hyaline to pale yellowish brown. **Conidiogenous cells** cylindrical to lageniform, tapered towards the apex, hyaline, 10–15 × 1.5–3.0 μm. **Alpha conidia** abundant, fusiform, rounded at the apex, obconically truncate at base, hyaline, 6–7(–8) × 2–2.5 μm. **Beta conidia** not seen. **Perithecia** not seen.

**Cultural characteristics —** Colonies on PDA covering entire plate after 10 d, adpressed, with a few scattered dark stromata up to 1 mm diam surrounded by patches of white sparse mycelium, buff; reverse isabelline with a few dark scattered stromata up to 3 mm diam. On OMA covering entire plate after 10 d, white tinged with pale vinaceous with pale mouse grey patches, with many scattered dark stromata mostly up to 4 mm diam; reverse uniformly cinnamon.

**Notes.** — The phylogenetic inference from the combined sequence data showed *D. sackstonii* clustered next to *D. infe-cunda* (Gomes et al. 2013), as well as the newly described *D. serafinae*. In culture, *D. sackstonii* produced abundant pyc-nidia on PDA and OMA, compared to *D. infe-cunda*, which was sterile. *Diaporthe* *sackstonii* differs from *D. serafinae* in three loci: ITS positions 40 (C), 78 (C) and 85 (G); TEF 91 % match (Identities 263/290, Gaps 8/290); BT 98 % match (Identities 635/649, Gaps 3/649).

**Diaporthe serafinae** R.G. Shivas, S.M. Thomps. & Y.P. Tan, *sp. nov.* — MycoBank MB808675; Fig. 4f–j

**Etymology.** Named after the dedicated Australian agronomist Loretta Serafin, for her research on sunflower crop production and who provided the samples from which this species was isolated.

**Conidiomata** multilocular, scattered, abundant on PDA, OMA and WSA after 4 wk, up to 2 mm diam, ostiolate, with necks up to 1.5 mm, cream conidial droplets exuded from most ostioles. **Conidiophores** formed from the inner layer of the locular wall, 1-septate, hyaline to pale yellowish brown, fusiform, 15–25 × 1.5–3.5 μm. **Conidiogenous cells** cylindrical to flexuous, tapered towards the apex, hyaline, 5–20 × 1.5–2.5 μm. **Alpha conidia** abundant, fusiform, rounded at the apex, narrowed towards the base, biguttulate, hyaline, 5.5–7(–8) × 1.5–2.5–(3) μm. **Beta conidia** not seen. **Perithecia** not seen.

**Cultural characteristics —** Colonies on PDA covering entire plate after 10 d, adpressed, white numerous scattered dark stromata up to 2 mm diam; reverse uniformly mottled white to buff. On OMA covering entire plate after 10 d, adpressed, with numerous scattered dark stromata up to 4 mm diam; reverse uniformly isabelline.
Fig. 4 Diaporthe spp. — a–e: Diaporthe sackstonii (ex-type BRIP 54669b) after 4 wk. a. Culture on OMA; b. conidiomata on sterilised wheat straw; c. conidiomata on OMA; d. conidiophores; e. alpha conidia. — f–j: Diaporthe serafiniae (ex-type BRIP 55665b) after 4 wk. f. Culture on PDA; g. conidiomata on sterilised wheat straw; h. conidiomata on OMA; i. conidiophores; j. alpha conidia. — Scale bars: a, f = 1 cm; b, g, h = 1 mm; c = 100 µm; d, e, i, j = 10 µm.

Specimens examined. Australia, Queensland, Glenore Grove, from seed of an ornamental variety of Helianthus annuus, 1 Apr. 2012, S.M. Thompson (T13010A), holotype BRIP 55665b (includes ex-type culture); New South Wales, from stem of Lupinus albus ‘Rosetta’, L. Serafin (T12568A), BRIP 54136.

Notes — The phylogenetic inference from the combined sequence data showed D. serafiniae clustered close to D. infecunda (Gomes et al. 2013) (Fig. 1). In culture, D. serafiniae produced abundant pycnidia on PDA and OMA, compared to D. infecunda, which was sterile.

DISCUSSION

The application of principles of genealogical concordance species concepts based on multigene phylogenetic analysis has led, in recent years, to the discovery of many new cryptic species in some important genera of plant pathogenic fungi, e.g. Colletotrichum (Damm et al. 2012a, b, Weir et al. 2012), Phyllosticta (Wikee et al. 2013) and Diaporthe (Gomes et al. 2013, Tan et al. 2013). There are about 2 000 names for Diaporthe (including Phomopsis) species in the literature (Gomes et al. 2013). Many epitypes have been recently designated for species of Diaporthe (Udayanga et al. 2012, Gomes et al. 2013), which has helped to stabilise the taxonomy of this genus. However, many Diaporthe species still lack ex-type (including epitype and neotype) cultures from which DNA is easily extracted for molecular phylogenetic analysis. Gomes et al. (2013) proposed two approaches to resolve the taxonomy of Diaporthe species – either recollect and redescribe all the existing species (which is impractical) or start again. A new start is not as daunting as it seems when the nomenclatural code that governs the naming of fungi has a tool that facilitates this approach in provision for lists of rejected as well as protected names (McNeill et al. 2012). In reality, plant pathologists and mycologists seem to have embraced a new start, as since 2010 there have been approximately 40 new species of Diaporthe described (see MycoBank, www.mycobank.org), including 12 from Australia (Thompson et al. 2011, Crous et al. 2011, 2012, Tan et al. 2013).

Colonisation of the same host plant by multiple Diaporthe species has been reported before (Farr et al. 2002, Crous & Groenewald 2005, van Niekerk et al. 2005, Thompson et al. 2011) and appears to be quite common in nature. Five of our new species were isolated from live sunflower stems. Of these five species, D. masirevicii and D. miriciae were also associated with cankers on live soybean and mungbean plants. Some new species appeared to be endophytic such as the species found on asymptomatic live maize plants and some may play a role in the dieback disease of bitou bush and tip dieback symptoms on hosts such as Sesbania cannabina and Bidens pilosa. Another group, which includes D. charlesworthi and D. macintoshii, may be primarily saprophytic, having only been isolated from decaying plant material. Detailed investigations of the pathogenicity and host range of all species are required to shed light on their ecology.

The presence of D. goulteri, D. masirevicii, D. miriciae and D. serafiniae in live crops as well as crop stubble and weed residues, highlights the potential of decaying plant material on the soil surface to act as a reservoir of inoculum for subsequent crops. It is well recognised that crop stubble aids the survival of Diaporthe species, such as D. toxica on lupins (Cowling et al. 1987), D. phaseolorum var. caulivora on soybeans (Kmetz et al. 1979), and D. helianthi on sunflower (Maširević & Gulya 1992). The role of broadleaf weed residues as an aid to survival
is not well documented for many pathogenic fungal species. Our results indicate that dead weeds at the edges of cultivated fields and waterways as well as unburned weed residues, on the soil surface and amongst crop plants in low tillage systems, create a ‘brown bridge’ of dead plant material that may harbour multiple pathogenic fungal species. More sampling of maize is required to confirm its possible role in the epidemiology of Diaporthe species that are pathogens of broadleaf rotational crop species. These findings support the observation by Delaye et al. (2013) and Malcolm et al. (2013) that the complex infection and survival associations between fungi and plants, including endophytic associations are poorly known.

Two species of Diaporthe isolated from sunflower, D. kongii (Thompson et al. 2011) and D. masirevicii, were also recovered from bitou bush, which is invasive in coastal dune vegetation (Vranjic et al. 2012) away from the inland broad acre cropping regions in Qld and NSW. This provides evidence that the distribution, life style and host range of many Diaporthe species may be broader than expected and more complex than currently known. Both sunflower and bitou bush belong to the Asteraceae, and whether this is significant with respect to the possible hosts and distribution of these fungi is not known.

There have been 20 species, including those from this study, of Diaporthe described from Australia since 2010 (Thompson et al. 2011, Crous et al. 2011, 2012, Tan et al. 2013). Some have been identified as significant plant pathogens although the ecological significance of most is not known. This study starts to address the case that Hyde et al. (2010) made to reassess and revise plant-associated pathogens, especially Diaporthe, in order to preserve the effective role that biosecurity agencies play in keeping unwanted plant pathogens out of Australia. Although the host range and pathogenicity of these eight newly described Diaporthe species is largely unknown, our study highlights the importance of both ‘green bridges’ and ‘brown bridges’ in the epidemiology of Diaporthe species.

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