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-sequence-based methods and the application of the Genealogical Concordance Phylogenetic Recognition System (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. 2013).

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 & Muntañola-Cvetković (1985) recovered Diaporthe species from 15 plant species, including the weeds Xanthium italicum and X. strumarium. Subsequent studies by Vrandečić 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. kochmani and D. kongii, recently found associated with sunflower stem canker in eastern Australia (Thompson et al. 2011). During recent investigations to identify alternative hosts of the Diaporthe species that cause 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.
Table 1 Diaporthe spp., and the outgroup taxon Diaportheorya corylina, included in the phylogenetic analysis of this study. Newly described taxa and deposited sequences are in **bold**.

<table>
<thead>
<tr>
<th>Species</th>
<th>Isolate no.</th>
<th>Host</th>
<th>Locality</th>
<th>GenBank accession no.</th>
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<td>Ficus carica</td>
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<td>Diaporthe corylina</td>
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<td>Corylus sp.</td>
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</table>

- **BRIP**: Plant Pathology Herbarium, Dutton Park, Queensland, Australia; CBS: Centraalbureau voor Schimmelmclturen, Utrecht, The Netherlands.
- **NSW**: New South Wales; Qld: Queensland; USA: United States of America.
- **Other than those in bold**: all sequences were downloaded from GenBank and published in van Rensburg et al. (2006), Santos et al. (2010), Udayanga et al. (2011, 2012), Gomes et al. (2013) and Tan et al. (2013).
- **Ex-type or ex-epitype culture.**

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 amended with 200 µg/mL streptomycin sulphate (1/2 PDAS) or on acidified PDA (one drop of 25 % lactic acid added per plate when pouring) in 9 cm diam Petri dishes. Plates were incubated at room temperature under 24 h fluorescent lights.

The bark of stem pieces (c. 1–3 cm diam and 12–15 cm long), cut near the base of wilting bitou bush plants was removed, using a sharp, surface-sterilised knife, over more than half of the circumference of the pieces and for c. 7–8 cm long in the middle of the pieces. A surface-sterilised wood chisel was then used to remove thin slices (up to c. 30, each c. 1–3 cm long) from the wood (xylem) in 9 cm diam Petri dishes. Plates were incubated at room temperature under 24 h fluorescent lights.

Pieces of hyphae at the margin of colonies that grew from the middle of the pieces and for c. 7–8 cm long in the middle of the pieces. A surface-sterilised wood chisel was then used to remove thin slices (up to c. 30, each c. 1–3 cm long) from the wood (xylem) in 9 cm diam Petri dishes. Plates were incubated at room temperature under 24 h fluorescent lights.

**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 with necrotic symptoms were cut in 1–2 cm long sections, including 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 1/2 strength PDA amended with 200 µg/mL streptomycin sulphate (1/2 PDAS) or on acidified PDA (one drop of 25 % lactic acid added per plate when pouring) in 9 cm diam Petri dishes. Plates were incubated at room temperature under 24 h fluorescent lights.
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 determined for colony morphology, 3-d-old cultures on 9 cm diam plates with Nomarski differential interference contrast.

For colony morphology, 3-d-old cultures on 9 cm diam plates with Nomarski differential interference contrast. For each isolate the number and size of colonies were recorded. For further growth characteristics, isolates were grown at 16 °C and 23 °C on PDA for 7 d under 16 h photoperiod with NUV light at 23 °C (Thompson et al. 2011). 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 1993) 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 & Cigelnik 1997) and Bt2b (Glass & Donaldson 1990), and V9G (de Hoog & Gerrits van den Ende 1998) were used to amplify part 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 1993) 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 & Cigelnik 1997) and Bt2b (Glass & Donaldson 1990) 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 isolates 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 Diaporthe species from recent studies (Thompson et al. 2011, Udayanga et al. 2012, Gomes et al. 2013) using MAFFT alignment algorithm (Katoh et al. 2009) in the software Geneious (Biomatters Ltd). Diaporthe 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, ITS 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 were 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. 5.10.01 (Librado & Rozas 2009).

### RESULTS

**Isolates**

More than 500 Diaporthe 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. kochmanii* and *D. kongii* (Thompson et al. 2011) (data not shown). Fifteen *Diaporthe* 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 *Diaporthe* 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 *Diaporthe* 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.

<table>
<thead>
<tr>
<th>Plant host¹</th>
<th>Host family</th>
<th>Diaporthe spp.</th>
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<tr>
<td>Helianthus annuus</td>
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<tr>
<td>Lupinus alba</td>
<td>Fabaceae</td>
<td></td>
</tr>
<tr>
<td>Vigna radiata</td>
<td>Fabaceae</td>
<td></td>
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<td>Zea mays</td>
<td>Poaceae</td>
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</table>

<table>
<thead>
<tr>
<th>Crop</th>
<th>Host family</th>
<th>Diaporthe spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bidens pilosa</td>
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<td>Chrysanthemoides monilifera subsp. rotundata</td>
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<td>Rapistrum rugosum</td>
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<td>Solanum nigrum</td>
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</table>

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.

Diaporthe masirevicii

BRIP 57330 Chrysanthemoides monilifera subsp. rotundata
BRIP 57892a Helianthus annuus
BRIP 54296 Glycine max
BRIP 54120c Zea mays

Diaporthe kongii BRIP 54031*

Diaporthe endophytica CBS 133811*

Diaporthe phaseolorum CBS 116019

BRIP 56918a Vigna radiata
BRIP 54736* Helianthus annuus
BRIP 55662c Glycine max

Diaporthe sojae CBS 180.55*

Diaporthe melonis CBS 507.78

Diaporthe batalas CBS 122.21
Diaporthe helianthi CBS 592.81*

Diaporthe hordei CBS 481.92

BRIP 54136 Lupinus albus 'Rosetta'
BRIP 55665a Helianthus annuus

Diaporthe infecunda CBS 133812*

BRIP 57329 Chrysanthemoides monilifera subsp. rotundata
BRIP 54884* Rapistrum rugosum
BRIP 54669b* Helianthus annuus

Diaporthe saccstonii

Diaporthe helianthiaceae BRIP 54792*

Diaporthe gulyae BRIP 54025*
Diaporthe neoarctii CBS 109490*

Diaporthe cuppatae CBS 117499*

Diaporthe stictica CBS 370.54
Diaporthe elseaeaei CBS 504.72

BRIP 55064a* Rapistrum rugosum
Diaporthe anakardi CBS 720.97
Diaporthe feonculacea CBS 123208
Diaporthe cinerascens CBS 719.96

BRIP 54884m* Rapistrum rugosum

Diaporthe ronimayaporum CBS 133182

BRIP 55657a* Helianthus annuus

Diaporthe macintoshii

Diaporthe charlesworthii

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 at the apex, narrowed towards the base, (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.

**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, HLBABD.

**Specimen examined.** **AUSTRALIA.** Queensland, Gatton, from stem of *Rapistrum 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 *Rapistrum 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** is one of three novel species isolated in this study from dead stems of *R. rugosum* (*Brassicaceae*), a widely distributed weed in eastern Australia.

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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 miniciei* (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, (6.5–8–9) × 2–2.5(–3) μm. Beta conidia not seen. 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 olivaceous mycelium with droplets of cinna- mon coloured exudate and one or a few funiculose 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 col-oured 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 macintoshii R.G. Shivas, S.M. Thoms. & 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 after 10 d reaching the edge of the plate, margin coralloid, adpressed, with scattered dark stromata up to 1 mm diam, isabelline with low tufts of off white mycelium; reverse mottled buff to isabelline with darker patches corresponding to stromata. On OMA covering entire plate after 10 d, adpressed with some funiculose mycelium towards the margin, ropey, dark mouse grey, with numerous scattered dark stromata up to 2 mm diam; reverse mottled buff with irregular dark patches.

Specimen examined. AUSTRALIA, Queensland, Toowoomba, from stem of Rapistratum rotundata, 6 Dec. 2011, S.M. Thompson (T12768A); holotype BRIP 55664a (includes ex-type culture).

Notes — Diaporthe macintoshii is one of three novel species isolated in this study from dead stems of R. rugosum. This species was also found on Chrysanthemoides monilifera subsp. rotundata, which is an important weed of coastal dune vegetation in eastern Australia.

Diaporthe middletonii R.G. Shivas, L. Morin, S.M. Thoms. & 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 Rhihospur sp. infection in sunflower.

Conidiomata pycnidial, up to 300 μm diam on PDA and WSA after 4 wk, aggregated in scattered groups or multilocular 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 funiculose mycelium up 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 Raphiolepis indica, 24 Nov. 2011, S.M. Thompson (T12757H), holotype BRIP 54884a (includes ex-type culture); New South Wales, Bongil Bongil National Park, from stem of Chrysanthemoides monilifera subsp. rotundata, 1 June 2011, L. Morin (056), BRIP 57329.

Notes — Diaportha middletonii is one of three novel species found on R. rugosum, as well as one of three novel species found on Chrysanthemoides monilifera subsp. rotundata, which is an important weed of coastal dune vegetation in eastern Australia. A BLASTn search with the ITS sequence of the type isolate, BRIP 54884a, 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. Conquistor; and 99 % identity to KF467129 from Centrolobium ochroleucon in Ecuador.

Diaportha miriciae 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 Diaportha (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 macdonaldii’ (University of Queensland, 2002).

Conidionomata pycnial or multicellular, 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 pale yellowish brown, fusiform, 15–25 × 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) × 2–2.5(–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, apressed, with a few scattered dark stromata up to 1 mm diam covered 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.

Specimens examined. Australia, Queensland, Clermont, from a petiole of Helianthus annuus, 10 June 2011, S.M. Thompson (T12667B); holotype BRIP 54669b (includes ex-type culture).

Notes — The phylogenetic inference from the combined sequence data showed D. sackstonii clustered next to D. infecunda (Gomes et al. 2013), as well as the newly described D. serafiniae. In culture, D. sackstonii produced abundant pycnidia on PDA and OMA, compared to D. infecunda, which was sterile. Diaportha sackstonii differs from D. serafiniae 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).

Diaportha serafiniae 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.

Conidionomata multicellular, 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, bipartulate, 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, apressed, white numerous 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.
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 as 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 unburied 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, saprobic or endophytic species of *Diaporthe*. We suggest that the ‘brown bridge’ of weed residues plays a significant role in aiding the survival of *Diaporthe* species. This is comparable to the ‘green bridge’ of alternative live weed hosts, such as those that facilitate survival of pathogenic *Diaporthe* species between cropping phases (Mihaljevic & Muntaňola-Cvetković 1985, Roy et al. 1997, Li et al. 2001, 2010, Vrandečić et al. 2010).

Of added significance for disease management is the isolation from maize of *D. gulyae*, a highly virulent pathogen on sunflower (Thompson et al. 2011). *Diaporthe gulyae* was isolated from asymptomatic maize plants, indicating endophytic colonisation. Maize is often recommended as a rotation crop to follow broadleaf crops such as sunflower, soybean and mungbean, which are susceptible to a number of damaging stem and pod cankers caused by *Diaporthe* 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 broadacre 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, Croux 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.

Acknowledgements Broad acre component: The authors would like to acknowledge the Queensland Department of Agriculture, Fisheries and Forestry (DAFFQ), the Grains Research and Development Corporation (GRDC), the University of Queensland (UQ), as well as the generous assistance of growers and advisors. Additionally, we acknowledge Drs Tom Gulya (USDA-ARS), Alistair McTaggart (UQ), Vu Tuan Nguyen (DAFFQ), Malcolm Ryley (DAFFQ), and Ms Ella Trembiczki (DAFFQ) for their technical and philanthropic support of this study. Bitou bush component: This study was supported by CSIRO, NSW National Parks and Wildlife Service, and the Australian Government National Weeds and Productivity Research Program administered by the Rural Industries Research and Development Corporation. We thank Mr Shamsul Hoque (CSIRO Plant Industry) and Ms Ruth Andrew (CSIRO Earth Science and Resources) for technical assistance, and also acknowledge the range of stakeholders for their support and/or permission to collect samples on their land.

REFERENCES


