Neoscochyta species cause leaf scorch on wheat in Australia

H. Golzar¹ · G. Thomas¹ · K. W. Jayasena² · D. Wright¹ · C. Wang¹ · M. Kehoe¹

Received: 5 July 2018 / Accepted: 2 December 2018 / Published online: 2 January 2019
© Australasian Plant Pathology Society Inc. 2019

Abstract

A conspicuous leaf scorch of wheat associated with Didymellaceae family was observed on commercial wheat varieties in Western Australia during recent years. Two fungal species isolated from leaf lesions were identified based on morphological characteristics and DNA sequencing. *Neoscochyta europaea* was isolated from symptomatic leaf lesions on the wheat varieties Trojan and Wyalkatchem also *Neoscochyta graminicola* was detected on Calingiri variety. Pathogenicity tests were conducted and Koch's postulates were fulfilled by reisolating of these fungal species. This is a first report of *Neoscochyta europaea* and *Neoscochyta graminicola* causing leaf scorch on wheat in Australia.

Keywords Didymellaceae · Didymella · Neoscochyta · Ascochyta · Leaf scorch · Wheat

The Didymellaceae family consist of a wide range of fungal species, including *Ascochyta* spp. and *Neoscochyta* spp., causing serious economic losses on various crop species. Plant species from the family Poaceae are known to be infected by Didymellaceae species (Punithalingam 1979; Mel'nik 2000). The economic importance of these fungal pathogens is not clear however damaging disease symptoms on wheat and barley have been reported (Cormey et al. 1994; Perelló and Moreno 2003; Bockus et al. 2010; Kosiada 2012).

Didymella exitialis is a common pathogen on wheat and barley in Europe (Punithalingam 1979) and has been reported on wheat causing various leaf scorch severities on different wheat varieties in New Zealand (Cromey et al. 1994; Braithwaite et al. 1998).

In addition the occurrence of *A. tritici* on wheat, *A. sorghi* on brome grass and wheat, *A. hordei* on barley and *A. avenae* on oat has been reported in New Zealand (Braithwaite et al. 1998; Kosiada 2012). *A. tritici*, *A. graminicola* and *A. sorghi* are considered the cause of ascochyta leaf spot in wheat (Bockus et al. 2010).

The occurrence of *A. hordei* var. *europaea* on wheat has been reported to be significant in Argentina and under artificial inoculation up to 45% of leaf area became necrotic (Perelló and Moreno 2003).

The taxonomy of Didymellaceae family was revised based on its phylogenetic analysis because of lacking teleomorphic stage and morphological differentiation. Therefore *Neoscochyta* was identified to be the correct genus for some of the *Ascochyta* species that have previously been reported (Chen et al. 2017).

Leaf necrosis was observed on the commercial wheat varieties confused with the septoria leaf blotch symptom in recent years (Fig. 1a). Leaf samples from wheat varieties Wyalkatchem and Calingiri grown in wheat belt regions in 2014 and Trojan in Albany 2017 were tested to clarify this confusion. Isolation was made using sections of leaf tissue surrounding the lesions and surface-sterilised by immersion in a 1.25% aqueous solution of sodium hypochlorite for 2 min, rinsed in sterile water and dried in a laminar flow cabinet. The sterilised leaf pieces were then either placed on potato dextrose agar (PDA) and incubated at 22 ± 3 °C, then fungal colonies sub-cultured onto oat meal agar (OMA) and then single-spored to obtain pure cultures; or placed on moist filter paper and incubated at 25 °C with a 12 h dark and light for inducing growth and sporulation.

Morphological characteristics of the isolated fungi were determined and *N. europaea* and *N. graminicola* were identified from symptomatic leaf lesions (Figs. 1, 2, 3 and 4).

Colonies of *N. europaea* on OMA were olivaceous grey, 30 to 40 mm in diameter after 7 d. Pycnidia ostiolate, semi-

✉ H. Golzar
hgolzar@agric.wa.gov.au

¹ Department of Primary Industries and Regional Development, 3 Baron-Hay Court, South Perth 6151, Western Australia

² Albany, Western Australia

Fig. 1 *Neosascochyta europaea*. Leaf scorch bearing pycnidia and pseudothecia structures embedded in the naturally infected leaf tissues of Trojan variety (a), asci and ascospores (b) and pycniospores (c). Bars = 5 mm (a) and 10 μ m (b and c)

Fig. 2 *Neosascochyta europaea*. Responses to artificial inoculation on Trojan variety, 48 h postinoculation (a), and 3 weeks after inoculation in comparison to control pink sign (b). Bars = 5 mm

Fig. 3 *Neoscochyta graminicola*. Pycnidial structures on the naturally infected of Calingiri variety leaf tissues (**a**) and pycniospores (**b**). Bars = 50 μ m and 10 μ m respectively

immersed in the media, globose, 85–215 μ m, conidia hyaline fusoid to cylindrical, guttulets and one septate 12.5–19.5 $\times$ 3–5 μ m. *Pseudothecia* dark brown, round, ostiolate, solitary or in-group immersed in the surface of infected leaf tissues 120–250 μ m in diameter. Asci bitunicate, cylindrical to clavate, ascospores fusoid, hyaline and guttulets, one septate, constricted at the septum 16.5–22.5 $\times$ 3.5–5 μ m in diameter.

Colonies of *N. graminicola* on OMA were olivaceous grey 18–24 mm in diameter after 7 d. Pycnidia dark brown 135–240 μ m, conidia ellipsoidal to cylindrical, hyaline to light brown and one septate 14–20 $\times$ 3–5.5 μ m in diameter. Teleomorph of *N. graminicola* was not detected.

The morphological characteristics of these species are identical to similar species described in previous references (Punithalingam 1969, 1979).

The representative isolates of morphologically identified fungi were grown on PDA for 10 days at 22 $\pm$ 3 $^{\circ}$ C. Genomic DNA was extracted from fungal mycelium with a DNeasy Plant Mini Kit (Qiagen) according to the manufacturer's instructions. The ITS region was amplified with V9G (De Hoog and Gerrits van den Ende 1998) and ITS4 (White et al. 1990), the LSU region with LR0R (Rehner

and Samuels 1994) and LR7 (Vilgalys and Hester 1990), the *tub2* region with Bt2a and Bt2b (Glass and Donaldson 1995) and the *rpb2* region with fRPB2-5F and fRPB2-7cR (Liu et al. 1999). PCR products were purified using the QIAquick PCR purification kit (Qiagen) and sent to the Australian Genome Research Facility for direct sequencing. Sequences were analysed and subjected to analysis by BLAST against the available sequences in Genbank using Geneious 9.1.7 (Biomatters). WAC13728 and WAC14075 were confirmed to be a 100% match for *N. europaea* across the ITS, LSU, *rpb2* and *tub2* regions. WAC13710 was unable to be determined by the ITS and LSU regions, however gave a 100% match to *N. graminicola* for the *rpb2* and *tub2* regions.

A replicated trial was conducted for individual fungal isolates using Wyalkatchem, Calingiri and Trojan varieties. These pathogenicity tests were performed at three leaf stage of seedlings under glasshouse condition using representative isolates of *N. europaea* and *N. graminicola*. Conidial suspension (10^6 spores/mL) was prepared in sterile water using a 21-day-old culture of each of the isolates grown on OMA. The conidial suspension was sprayed on the leaf surface to

Fig. 4 *Neosascochyta graminicola*. Responses to artificial inoculation on Calingiri variety, 2 weeks post-inoculation (**a**), in comparison to control (**b**). Bars = 5 mm

run-off. Controls were sprayed using sterilised water. The plants were misted for 48 h and then moved to a glasshouse at 22 ± 3 °C. The expression of disease symptoms were evaluated two and 3 weeks post-inoculation. Control plants remained asymptomatic (Figs. 2 and 4). The preliminary infection was evident 48 h post inoculation (Fig. 2a) however there was slow development of disease expression and infection did not develop on the upper leaves in the glasshouse condition (Fig. 2b). The wheat samples received for disease diagnostic and in a survey conducted in the southern regions of WA, the leaf scorching of mature plants was evident (Fig. 1). It seems that environmental factors and monoculture of susceptible wheat varieties are important in disease development. This hypothesis has been supported by the previous research (Perelló and Moreno 2003; Kosiada 2012).

Koch's Postulates were fulfilled by re-isolation and confirmation by morphological characteristics and molecular identification of *N. europaea* and *N. graminicola*. Cultures of *N. europaea* (WAC14075 and WAC13728) that were isolated on the wheat varieties Trojan and Wyalkatchem and *N. graminicola* (WAC13710) from Calingiri variety deposited in the Western Australia Plant Pathogen Collection. To our

knowledge, there are no previous reports of *N. europaea* and *N. graminicola* causing leaf scorch on wheat in Australia.

Acknowledgements The authors would like to thank Ms. Yu Pei Tan and Dr. Roger Shivas for their comments on molecular identification of the isolated fungi.

We also would like to acknowledge the support and funding by the Grains Research Development Corporation through the project DAW00229.

References

- Bockus WW, Bowden RL, Hunger RM, Morrill WL, Murray TD, Smiley RW (2010) Compendium of wheat diseases and pests, 3rd edn. American Phytopathological Society, St. Paul
- Braithwaite M, Alexander BJR, Adams RLM (1998) Nationwide survey of pests and disease of cereal and grass seed crops in New Zealand. 2. In: Fungi and bacteria Proceed. 51st N.Z. Plant Protection Conf, pp 51–59
- Chen Q, Hou LW, Duan WJ, Crous PW, Cai L (2017) Didymellaceae revisited. Stud Mycol 87:105–159 Available online at www.studiesinmycology.org
- Cromey MG, Ganey S, Braithwaite M, Boddington HJ (1994) *Didymella exitialis* on wheat in New Zealand. N Z J Crop Hortic Sci 22:139–144

- De Hoog GS, Gerrits van den Ende AHG (1998) Molecular diagnostics of clinical strains of filamentous Basidiomycetes. *Mycoses* 41:183–189
- Glass NL, Donaldson G (1995) Development of primer sets designed for use with PCR to amplify conserved genes from filamentous ascomycetes. *Applied and Environmental Microbiology* 61:1323–1330
- Kosiada T (2012) In vitro growth of some species of *Ascochyta* Lib. *Cent Eur J Biol* 7:1076–1083
- Liu YJ, Whelen S, Hall BD (1999) Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. *Mol Biol Evol* 16:1799–1808
- Mel'nik VA (2000) Key to the fungi of the genus *Ascochyta* Lib. (Coelomycetes). In: Mel'nik VA, Braun U, Hagedorn G (eds) *Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft. Heft 379*. Perley Buchverlag, Berlin
- Perelló AE, Moreno MV (2003) Occurrence of *Ascochyta hordei* var. *europaea* on wheat (*Triticum aestivum*) leaves in Argentina. *Australas Plant Pathol* 32:565–566
- Punithalingam E (1969) Studies on *Sphaeropsidales* in culture. *Mycol Pap* 119:1–24
- Punithalingam E (1979) Graminicolous *Ascochyta* species. *Mycol Pap* 142:1–214
- Rehner SA, Samuels GJ (1994) Taxonomy and phylogeny of *Gliocladium* analysed from nuclear large subunit ribosomal DNA sequences. *Mycol Res* 98:625–634
- Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *J Bacteriol* 172:4238–4246
- White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) *PCR protocols: a guide to methods and applications*. Academic Press, San Diego, pp 315–322