



Confirmation of *Oculimacula yalundae* causing eyespot of cereals in Tunisia

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In 2010, reports were received of severe lodging of wheat in many fields located in the northeastern cereal growing area in Tunisia, leading some farmers to harvest early for silage. This area is characterised by a sub-tropical Mediterranean climate, with more than 500 mm of annual rainfall, primarily during winter. A survey conducted in 2011 found eyespot lesions in 17 of 19 durum and bread wheat fields, with incidence up to 94%. Lesions, mostly seen near the ground, were straw yellow in colour, often with black pupil-like dots in the centre bordered by greenish-brown to dark-brown rings (Fig. 1). The stems often broke at the lesion point. In the fields, heads on affected tillers were small, poorly filled and matured early, producing white heads.

Attempts to isolate the pathogen from adult plants collected in May 2011 failed. However in 2012, recovery of the fungus was successful when the isolation was made from young plants collected in early March, even from very discrete lesions. Typical colonies of *Oculimacula* spp. (syn. *Pseudocercospora herpotrichoides* and *Tapesia* spp.; Crous *et al.*, 2003), characterised by an olive grey colour with a dome in the centre were obtained on potato dextrose agar (PDA). To further identify at the species level, sequence analyses of the ribosomal DNA of two isolates were carried out. DNA extraction was conducted on mycelium following the protocol of Möller *et al.* (1992). PCR amplification was performed using primers ITS1 and ITS4 (White *et al.*, 1990). The BLAST search revealed that the sequence obtained from these isolates (GenBank Accession Nos. KF961611 and KF977547) had 99% and 98%, respectively, sequence identity with an *O. yalundae* isolate from Germany (JF412009.1).

A pathogenicity test was conducted in pots on wheat plants at the two-leaf stage. Sterilised, 2 cm wheat stem fragments were colonised by the pathogen by placing them on individual PDA plates bearing the two isolates. The colonised fragments were then placed close to the stems and maintained in position with a wooden toothpick (Fig. 2). Pots were placed

in a greenhouse with a temperature approximately 16°C during the day and 8°C at night and were irrigated daily. After eight weeks, typical symptoms of eyespot were observed in almost all of the treated plants (Fig. 3). The fungus was re-isolated from the lesions. The control plants inoculated with fungus-free stem fragments remained healthy.

Eyespot disease of cereals is widespread throughout the temperate regions of the world. The disease has been reported to occur in Tunisia, but little information is given (CABI, 1981). In addition, it has been reported previously in northern Mediterranean regions, including Italy and France (Crous *et al.*, 2003). Another species, *O. acufomis* is also associated with eyespot disease. It differs from *O. yalundae* in its pathogenicity and distribution (Crous *et al.*, 2003). The occurrence of this species in Tunisia and its relative importance to *O. yalundae* will be investigated in a more extensive survey.

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Figure 1



Figure 2

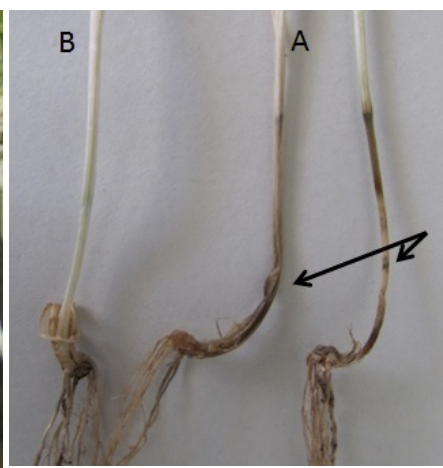


Figure 3

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