First record of verticillium wilt (*Verticillium longisporum*) in winter oilseed rape in the UK

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Additional key words: soil-borne pathogen, fungal plant disease

Verticillium longisporum is an important pathogen of oilseed rape (OSR) and vegetable brassicas in several European countries, but has not been reported previously in the UK (Karapapa *et al*., 1997; Steventon *et al.*, 2002). In 2007, Verticillium wilt was suspected in UK crops of winter OSR (W-OSR) on cv. Castille in Romney Marsh, Kent and on cv. Barrel near Hereford. At these two locations, 32 and 10% of the plants, respectively, appeared to be affected, but the presence of stem canker may have masked some infections. Symptoms were first seen as the crops began to ripen (seeds green-brown to brown, Growth Stage: 6,4-6,5) and included brown and dark grey vertical bands on the stems from soil level into the branches, and premature ripening of some branches (Fig. 1).

Microsclerotia were observed on stem samples collected in the field (Fig. 2), suggesting *V. longisporum* as the causal agent. Cultures were prepared from field samples by immersing stem pieces in 5% sodium hypochlorite solution for one minute, washing twice in sterile distilled water and plating onto potato dextrose agar containing 25 mg/l streptomycin sulphate. Isolates from three plants per outbreak were identified morphologically as *V. longisporum*. Mean conidial dimensions (25 spores per isolate) were 8.80-9.65 µm (length) and 2.50-2.85 µm (width) and all isolates produced elongated microsclerotia, characters typical of *V. longisporum* (Karapapa *et al.,* 1997). The identity was confirmed by PCR using species-specific primers (Steventon *et al*., 2002) and, as a member of the α sub-group, by direct sequencing of the amplicons from primer pairs ITS4-ITS5 and DB19-DB22 (Collins *et al.*, 2003; 2005). Sequences for isolate 003 from Kent were deposited in GenBank (Accession Nos. HQ702376 and HQ702377). All isolates tested from 2008 and 2009 were identical with previously deposited sequences for European OSR isolates (*e.g.* AF363992 and AF363246 respectively). Pathogenicity was confirmed by inoculating three OSR cv. Castille seedlings per isolate using the root dip technique with 1 x 10^6 spores/ml (Karapapa *et al.,* 1997) under heated glasshouse conditions at 19°C. Leaf yellowing and blackening of the leaf veins were found 26 days after inoculation (Fig. 3). Yellowing affecting the three oldest leaves increased for seven to nine days. After five weeks the final mean leaf area affected was 63-78% with no differences between isolates. No leaf yellowing occurred in the controls. After five weeks, *V. longisporum* was re-isolated from all the inoculated seedlings, but not from the non-inoculated controls.

In June 2008, infection of W-OSR crops in different fields on the same farms was found on cv. Es Astrid in Kent (56% incidence) and on cv. Lioness in Hereford (15% incidence). The Kent farm had been growing W-OSR alternating with winter wheat for at least 10 years whilst the Hereford farm had grown W-OSR one year in four. These short rotations of OSR may be contributing to the appearance of this disease. This study confirms the identification of *V. longisporum* on any host in the UK, through molecular studies and detailed spore measurements that were not reported in an earlier review (Gladders, 2009). This pathogen occurs in several European countries and, since OSR may be traded freely, following a Defra consultation, no statutory plant health action is to be taken.

Acknowledgements
The authors gratefully acknowledge the support of BBSRC, Defra, HGCA and EC for work on *V. longisporum*

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Figure 1: Verticillium wilt causing brown stripe symptoms in a field crop pre-harvest.

Figure 2: Grey microsclerotia on mature oilseed rape stems affected by Verticillium wilt.

Figure 3: Leaf yellow symptoms of Verticillium wilt in the pathogenicity test.