New Disease Reports

A new report of *Phaeoacremonium viticola* and *P. hispanicum* causing grapevine trunk disease in Castilla y León, Spain

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Until recently, *Phaeoacremonium aleophilum* (*Pal*) was the only species of its genus associated with grapevine decline in Castilla y León (Spain). In September 2009, isolates of *Phaeoacremonium*, morphologically different from *Pal*, were isolated from the roots and rootstock of an adult grapevine (*Vitis vinifera*) and from the rootstock of a young plant. The scions of both plants belonged to cv. Tempranillo; the rootstocks were both of type 110R. Only the adult plants collected in the Ribera de Duero area, showed external 'esca disease' symptoms (interveinal chlorotic leaves and internal black wood streaking). Single conidial isolates were obtained and grown on potato dextrose agar (PDA) at 25°C in darkness. Isolate Y271-03-1d reached a radius of 2.7-3.2 mm after eight days. The colony, which was at first yellowish-white, showed an entire margin. After three weeks, the aerial mycelium was reddish violet and the reverse reddish brown. The conidiophores were short, 12-35 µm long (mean 24 µm), erect and unbranched. The conidia were hyaline, aseptate and reniform, 3-5.2 x 1.25-2 µm (mean 4.0 x 1.5 µm), and the L/W ratio was 3.03. Based on these descriptions *Phaeoacremonium viticola* was identified (Dupont *et al*., 2000). This identification was confirmed by partial sequencing of the β-tubulin (primers T1 and Bt2b) and actin (primers ACT-512F and ACT-783R) genes. Sequences for each fragment were deposited in the GenBank database under Accession Nos. HQ700718 and HQ700719; they showed 100% similarity to corresponding sequences from *P. viticola* deposited under DQ173105 and DQ173128 respectively. Part of the calmodulin gene was sequenced for the first time using primers CAL-228F and CAL-737R (Accession No. HQ700720). Pathogenicity tests were conducted on seven detached canes of current season growth and on seven one-year-old plants, both belonging to cv. Tempranillo. All were inoculated with a PDA plug containing*P. viticola;* controls were treated with agar only. After two and four months, *P. viticola* was re-isolated from internal brown lesions in 72% and 43% of the inoculated canes and plants respectively. Control plants showed no symptoms and *P. viticola* was not recovered.

Using the same methodology, isolate Y549-09-3b reached a radius of 4.5-5.3 mm after eight days at 25°C on PDA. It initially showed a whitish brown entire margin. The aerial mycelium turned from a honey to a darker buff colour after three weeks, while the reverse appeared browner. The conidiophores were short, 18-40 µm long (mean 29.5 µm), erect and unbranched with inflated bases. The conidia were hyaline, oblong and ellipsoidal, 4-5.7 x 1.5-2.5 µm (mean 4.5 x 2 µm), and the L/W ratio was 2.33. Based on these descriptions *P. hispanicum* was identified (Gramaje *et al*., 2009). Partial sequencing of the β-tubulin and actin genes confirmed this identification (HQ700715 and HQ700716); these showed 100% and 97% similarity to the corresponding sequences with *P. hispanicum* deposited in GenBank under FJ517164 and FJ517156 respectively. As above, part of the calmodulin gene was sequenced and the result deposited in GenBank (HQ700717). Koch's postulates were satisfied as described above for *P. viticola.* After two and four months, *P. hispanicum* was re-isolated from internal brown lesions in 86% and 27% of inoculated canes and plants respectively. Control plants remained healthy and *P. hispanicum* was not recovered. To our knowledge this is the first report of *Phaeoacremonium viticola* and *P. hispanicum* in Castilla y León (Spain).

References

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