New Disease Reports

First report of *Neofabraea kienholzii* causing bull's eye rot of apple in the UK

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Apple rot surveys have been conducted since the 1930's in the UK. Together they help build a picture of how the rot profile has changed over time. In surveys conducted in the 1930's and the 1960's (Wilkinson, 1954; Preece, 1967), *Neofabraea* was the dominant causative agent of rot, but losses due to this pathogen became negligible in the 1980's (Berrie, 1989). More recent surveys found a resurgence of this disease in susceptible varieties (including cv. Cox's Orange Pippin) prompting identification of the species of *Neofabraea* responsible. Total actual losses due to rots have reduced significantly over the surveying period (for example total losses in cv. Cox's Orange Pippin have reduced 10 fold from 26.3% in the 1930's to 2.6% in the 2010's) thanks to advances in storage technologies. Rots attributed to *Neofabraea* spp. have reduced from nearly 50% to 15% in the same period but this still constitutes an economic loss to growers and packers (Saville *et al*., 2015).

Apple samples that exhibited discreet, circular "cheek" lesions which were light to dark brown, flat or slightly sunken, and firm, were collected from the packhouse during 2013 and 2014 surveys. Samples were returned to the lab and rinsed with sterile water and surface disinfected with 70% ethanol. Tissue from the leading edge beneath the skin was isolated and placed on potato dextrose agar. Plates were incubated at 20°C with a 16 hr photoperiod. Isolates were slow growing producing colonies of varying colour but with colony morphology characteristics typical of *Neofabraea* species (Fig. 1). DNA was extracted from axenic, single spore derived cultures, β-tubulin and ITS regions were amplified and sequenced. BLAST analysis revealed that in addition to *N. perrenans* and *N. vegabunda* isolates, which have been previously reported in the UK (Edney, 1983), a single isolate (G48) out of 49 tested matched *Neofabraea kienholzii* with 100% identity. Repeating the above with a collection curated during the 2015 rot survey (growing season 2014), using the more phylogenetically informative β-tubulin region only, a single isolate (R4/15_2) out of 20 isolates tested had 100% identity to *Neofabraea kienholzii*. The sequences have been deposited in GenBank: Accession Nos. MF977989 (G48, ITS), MF983810 (G48, β-tubulin) and MF983809 (R4/15_2, β-tubulin).

To prove pathogenicity, Koch's postulates were conducted with isolate G48 (Fig. 2). Apples cv. Cox's Orange Pippin were surface sterilised, wounded with a sterile hypodermic needle and inoculated with 20 µl of conidial suspension (3×10^4 conidia ml^{-1}). Inoculation points were coated with petroleum jelly to maintain humidity. After 60 days incubation at 2°C, simulating commercial storage conditions for cv. Cox's Orange Pippin, lesions had developed on all inoculated apples characteristic of those caused by *Neofabraea* (Fig. 3). Non-inoculated control fruit showed no symptoms. Isolations from the leading edge of the lesion, as described above, confirmed the presence of *Neofabraea*.

This is the first report of *Neofabraea kienholzii* in the UK, where before only *N. perrenans* and *N. vegabunda* isolates were believed to be present (Edney, 1983). Previously named '*Neofabraea* sp. nov.', *Neofabraea kienholzii* was first described by Spotts *et al.* (2009) and has since been reported in many apple (and pear) growing territories around the world. This data is important to contribute to a global picture of the distribution of this genus and so that control strategies can be tailored for the UK industry to mitigate losses due to apple rot diseases.

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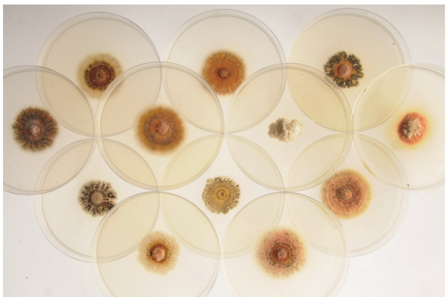

Figure 1

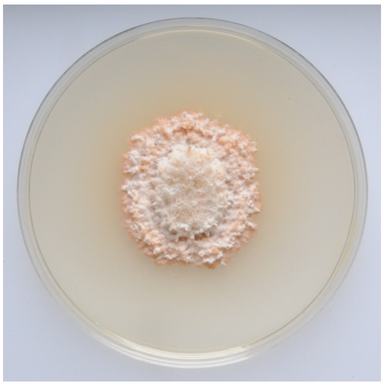

Figure 2

Figure 3

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