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Isolation and identification of *Pseudomonas syringae* pv. *aesculi* causing bleeding canker of horse chestnut in the UK

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Since 2003 the incidence of stem bleeding on horse chestnut (*Aesculus hippocastanum*) has risen markedly in the UK. Symptoms include rusty-brown, or black, gummy liquid seeping from bark (Fig. 1), and necrotic phloem with a mottled orange-brown colour (Fig. 2). Some bleeding cankers have been so extensive that infected tissue encircled branches or trunks, causing dieback or tree mortality in 3-4 years. Previous episodes of horse chestnut bleeding canker were caused by *Phytophthora*, but considered uncommon and only recorded in southern England (Strouts & Winter, 2000). However, reports made to Forest Research Disease Diagnostic Advisory Service indicate the disorder is now present in England, Scotland and Wales, with a similar upsurge in bleeding canker also recorded in the Netherlands, Belgium and France.

Culturing from the margins of the necrotic phloem on to various selective media rarely yielded *Phytophthora*. Instead a Gram-negative bacterium was isolated consistently. Ten isolates were characterised and were fluorescent on King's medium B, produced levan on sucrose nutrient agar, were negative for oxidase and arginine dihydrolase, non-pectolytic and induced a hypersensitive response in tobacco (LOPAT+---+). Fatty acid analysis and sequencing of the gyrase B (*gyrB*) gene (Sarkar & Guttman, 2004) indicated a single strain of *P. syringae*. The *gyrB* sequence was identical to that of *P. syringae* pv *aesculi* (NCPPB3681) isolated from foliage of *A. indica* in India (Durgapal & Singh, 1980) and to a *P. syringae* strain isolated from leaf spot symptoms on *A. indica* in Surrey in 2005. REP PCR analysis (Schaad et al., 2001) showed all UK isolates shared identical profiles. Of the 25 amplicons identified using BOX-, ERIC- and REP PCR, 20 were common to NCPPB3681, indicating that all the strains were closely related.

Suspensions of four UK isolates (10^7 CFU/ml) were inoculated through stem cuts into 5-yr old *A. hippocastanum* saplings. After four months, necrotic bark lesions (3-7 cm^2) formed around the inoculation points with some bleeding; no necrosis occurred with the water controls. Re-isolation and identification confirmed Koch's postulates. This is the first report of *P. syringae* pv. *aesculi* outside India, and the impact of the bacterium on *A. hippocastanum* in the UK appears to be widespread and damaging.

Figure 1: Bark cracking and bleeding canker on horse chestnut caused by *Pseudomonas syringae* pv. *aesculi*

Figure 2: Necrotic phloem and mottled discoloration underneath an area of bleeding bark on horse chestnut caused by *Pseudomonas syringae* pv. *aesculi*

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