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Survival of *Phytophthora ramorum* in *Rhododendron* root balls and in rootless substrates

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This study assesses the survival of *Phytophthora ramorum* in the root ball of *Rhododendron* container plants as well as in different rootless forest substrates and a horticultural potting medium. Following inoculation of the root balls, the above-ground plant parts stayed symptomless, whilst the pathogen could be recovered with a novel non-destructive baiting assay from the root balls until at least 8 months post-inoculation. Plating of surface-sterilized roots and direct microscopic analysis confirmed the presence of *P. ramorum* in the roots. *Phytophthora ramorum* could also be baited from the root balls of symptomless *Rhododendron* plants from commercial nurseries, even 2 years after acquisition. Survival of *P. ramorum* in rootless media was assessed after burying disks of infected leaf material below the soil surface in columns filled with four different undisturbed forest substrates or a potting medium, and incubated at an outdoor quarantine facility. *Phytophthora ramorum* could be recovered at least 33 months after burial from all substrates, with a significant increase in recovery after the winter period. These data suggest the possibility for long-term symptomless presence of *P. ramorum* in root balls of commercial *Rhododendron* plants as well as survival in potting medium and different forest substrates under western European climate conditions. Symptomless presence in root balls can contribute to latent spread of this pathogen between nurseries. The novel baiting test, being non-destructive, simple and applicable to a relatively large number of plants, can offer a valuable tool to test plants for the presence of *Phytophthora* species in root balls.

Keywords: baiting, detection, latent survival, sudden oak death

Introduction

Phytophthora ramorum, a heterothallic oomycete plant pathogen, has caused extensive tree mortality on several oak species on the west coast of the USA (where it is known as sudden oak death; Werres *et al.*, 2001; Rizzo *et al.*, 2002). In Europe, *P. ramorum* is mostly associated with *Rhododendron* and *Viburnum* nursery plants, on which it causes leaf necrosis and twig dieback (Werres *et al.*, 2001). More recently, it has also been associated with extensive dieback and mortality of plantations of Japanese larch (*Larix kaempferi*) in the UK and Ireland (Brasier & Webber, 2010). Since 2002, the risk of an epidemic disease in the European natural environment has led to the establishment of EU emergency phytosanitary measures. These involve surveys of commercial host

plants, which are based on visual symptoms, as *P. ramorum* is mostly considered a pathogen of aerial plant parts (Rizzo *et al.*, 2002). However, multiple lines of evidence suggest that *P. ramorum* may also have a soil phase, questioning the efficiency of the current phytosanitary measures in limiting the spread of the pathogen. *Phytophthora ramorum* has been detected in container media and field soil of contaminated nurseries (Colburn *et al.*, 2005; Davidson *et al.*, 2005; Linderman & Davis, 2005, 2006; Colburn & Shishkoff, 2006a,b; Fichtner *et al.*, 2007a,b, 2009; Shishkoff, 2007; Tjosvold *et al.*, 2009). Furthermore, it has been recovered from symptomless roots under laboratory or greenhouse conditions (Bienapfl *et al.*, 2005; Colburn *et al.*, 2005; Shishkoff & Senesac, 2005; Colburn & Shishkoff, 2006b; Parke *et al.*, 2006; Parke, 2007; Shishkoff, 2007, 2008) as well as from outdoor plants irrigated with water artificially contaminated with *P. ramorum* (Werres *et al.*, 2007). Given the lack of root symptoms, the below-ground phase of *P. ramorum* is likely to be connected to its survival. However, due to the transport of containerized plants, it may also play a significant role in its long-distance dispersal.

Although several of the above-mentioned studies have established the presence of *P. ramorum* in symptomless

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