

# Detection and Diversity of *Phytophthora* species in UK gardens



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## Introduction

The RHS tests around 300 plants every year with root and stem rot, branch die-back and stem canker symptoms for possible *Phytophthora* infection. During 2006-2009 two *Phytophthora* detection techniques were compared to determine a robust method to detect *Phytophthora* species from varied hosts and sample type.

Sixty percent of invasive plants in the UK are garden escapes. With over 15 million UK gardens, equating to 270,000 hectares of land, it seems gardens may represent an important pathway from cultivated land into natural ecosystems.

Quarantine *Phytophthora* spp. found in UK nurseries and woodlands, such as *P. ramorum* and *P. kernoviae*, highlight the need for fast and sensitive detection methods from soil and *in planta* samples and also demonstrate the invasive potential of this pathogen.

## Methods

The RHS advisory service deals with a wide range of hosts and potentially any *Phytophthora* spp., this study compares two detection methods for their reliability within a diagnostic clinic.

Symptomatic plants and associated rhizosphere soils were tested for *Phytophthora* by baiting the pathogen into apples (Mitchell and Kannwischer-Mitchell, 1992). Sporangia production in pond water provided confirmation of the pathogen. By way of comparison, total genomic DNA was extracted from samples alongside baiting.

An ITS nested PCR protocol (Bonants *et al.*, 1997; Cooke and Duncan, 1997) was used for amplification producing a confirmatory 900bp band. Species information was obtained by sequencing the 900bp amplicons for both bait derived cultures and directly extracted DNA samples (Figure 1)

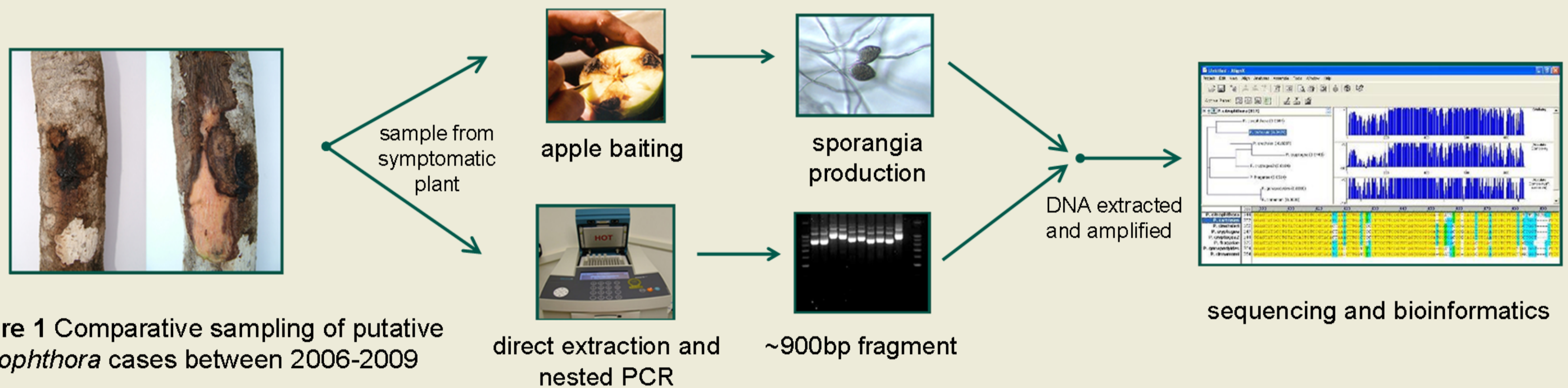


Figure 1 Comparative sampling of putative *Phytophthora* cases between 2006-2009

STEM n=291	bait positive	bait negative
direct PCR positive	25	191
direct PCR negative	4	71

ROOT n=998	bait positive	bait negative
direct PCR positive	70	694
direct PCR negative	9	225

SOIL n=841	bait positive	bait negative
direct PCR positive	55	461
direct PCR negative	32	293

Table 1 Combined data from 2006-2009 (broken down in to sample types stem, root and soil) illustrating 70% positive results through direct PCR compared to only 9% using baiting.

## Results and Discussion

Comparison of the results are seen in Table 1. The nested PCR protocol was developed to detect *Phytophthora* spp. but also detects species within the orders Peronosporales and Pythiales. Sequencing results for directly extracted samples identified over 570 samples with *Phytophthora* (70% of stem sequences, 50% of root sequences and 45% of soil sequences) and over 650 samples identified with *Pythium*. Whereas, through apple baiting, only 200 samples were confirmed as *Phytophthora* following sequencing.

Over 25 *Phytophthora* spp. (or species complexes) were identified during 2006-2009 associated with dead or dying plants. All the top identified species, *P. cryptogea*, *P. plurivora* and *P. cinnamomi*, have wide host ranges. Among the new species recorded in the U.K. were *Phytophthora* taxon 'niederhauserii' on *Grevillea*, *P. tropicalis* on *Viburnum* and *Telopea* and *P. austrocedrae* on *Juniperus*. More than 80 host plants were recorded, representing approximately 50 host families. *Taxus*, followed by *Rhododendron* and *Viburnum* were the most frequently recorded hosts, all of which are common garden plants.

The direct extraction and nested PCR method tested proved more sensitive and faster than baiting. It also resulted in increased detection of rare or new *Phytophthora* species records for the RHS. *Phytophthora* spp. detected in this study were widely distributed throughout UK gardens (Figure 2).

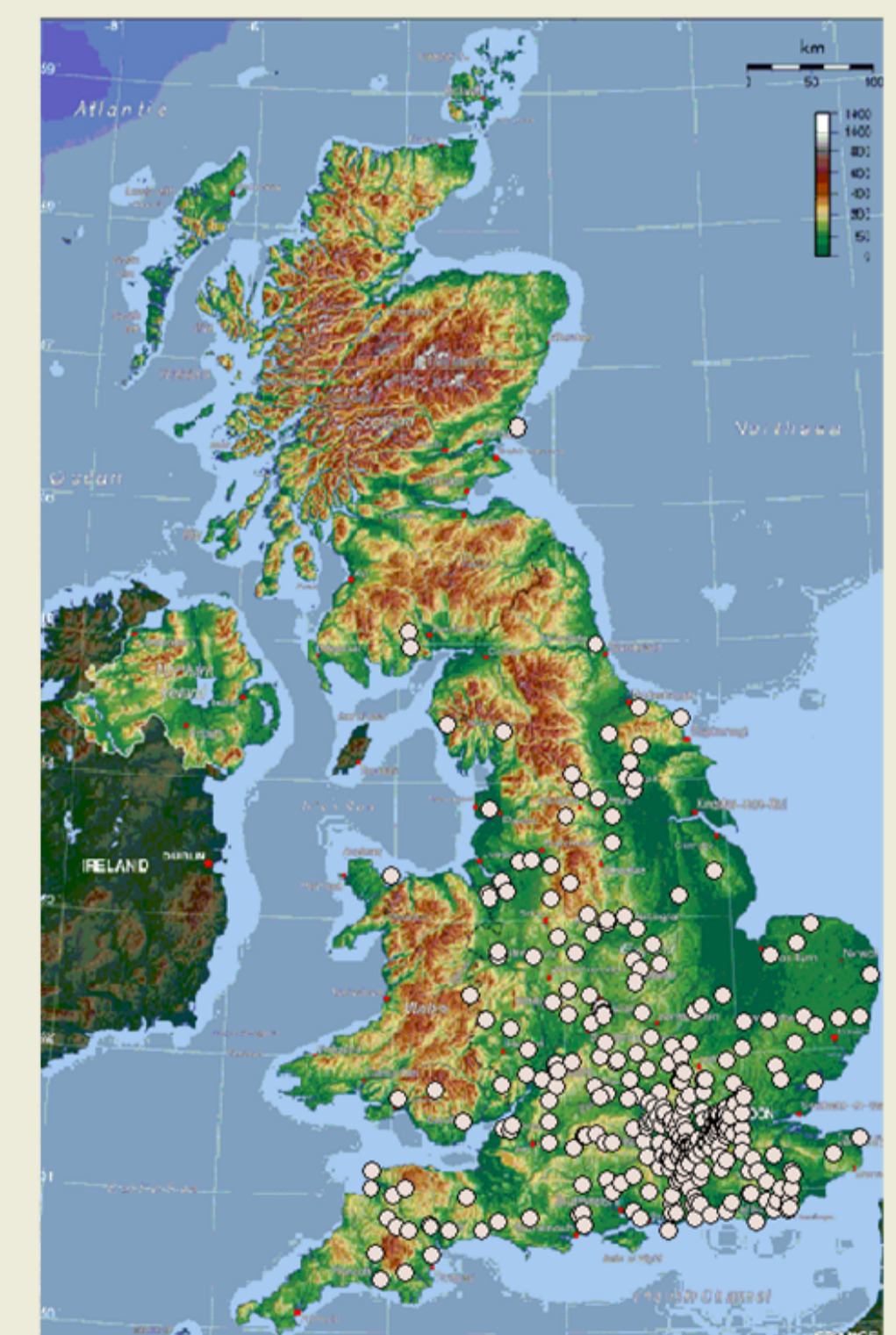


Figure 2 A distribution map of *Phytophthora* across the UK, collated from positive tests (2006-2009), highlights their wide dispersal in gardens.

## References

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