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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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roots and the survival in forest substrates, no information is available on the presence and persistence of *P. ramorum* in root balls of symptomless commercial *Rhododendron* plants. Also, an easy, non-destructive assay to test a large number of such plants is essential, but has not been developed. Furthermore, the persistence of *P. ramorum* in inoculated leaves under European weather conditions when buried in potting medium or forest substrates has not yet been assessed. Therefore, the specific objectives of this study were to: (i) determine the pathogenicity and survival capacity of *P. ramorum* in the root ball of containerized *Rhododendron* plants using a novel non-destructive baiting technique; (ii) apply the traditional as well as the novel baiting techniques to determine the presence of *P. ramorum* in a commercial shipment of containerized *Rhododendron* plants over time; and (iii) determine the survival capacity of *P. ramorum* in different rootless substrates, including commercial potting medium and forest substrates.

Materials and methods

Survival of *P. ramorum* in the root ball of containerized *Rhododendron* plants

The objectives of the first experiment were to determine whether artificial *Rhododendron* root ball inoculation with *P. ramorum* can cause visual symptoms above ground and to determine the persistence of *P. ramorum* in the potting medium using a novel baiting technique. Experiments relating to these objectives were conducted at the Institute for Agricultural and Fisheries Research (ILVO).

Root balls were inoculated with *P. ramorum* isolate PR/D/02/880, originally isolated from an infected *Rhododendron* plant from a Flemish nursery in 2002. It is of A1 mating type and belongs to the main EU1MG1 microsatellite genotype group (Vercauteren *et al.*, 2010). Three types of inoculum were used in the assays: zoospores, sporangia or infected leaves. Sporangia suspensions were produced by flooding 2-week-old cultures grown on diluted V8 (80 mL L^{-1} clarified V8 and 15 g L^{-1} agar) with 20 mL distilled water as described in De Dobbelaere *et al.* (2010). Zoospores were generated by controlled chilling of the sporangia suspensions (De Dobbelaere *et al.*, 2010). Zoospore and sporangia concentrations were determined using a haemocytometer and diluted with sterile distilled water to the desired concentrations. Thirty millilitres of the zoospore or sporangium inoculum were applied onto the surface of the root ball, at approximately 5 cm from the stem base. Infected leaves of *Rhododendron* cv. Cunningham's White were generated by dipping them in a 10^4 zoospores mL^{-1} suspension for 1 min and incubating them for 10 days at 17°C.

Infected leaves or leaf pieces were buried in the potting medium, approximately 1 cm below the surface. Each type of inoculum was applied at two concentrations (low and high), as described in Table 1. Containerized *Rhododendron* plants cv. Madame Masson were obtained from a commercial nursery and maintained at 20°C and a 12 h photoperiod in a biosafety growth chamber. No fungicides were applied during the course of the experiments. The potted plants were individually placed in plastic saucers and manually watered every 2–3 days via the saucer to prevent water splashing onto the stems or foliage. There were six replicate plants per treatment, placed in a completely randomized design. The resultant treatment combinations are shown in Table 2. The occurrence of disease symptoms on the upper parts of the plants was assessed visually on a weekly basis.

Table 1 Description of the three types of *Phytophthora ramorum* inoculum, at two concentrations each, that were used for inoculating containerized *Rhododendron* plants

	High inoculum concentration	Low inoculum concentration
Infected leaves	5 whole leaves	5 × 1 cm^2 leaf pieces
Sporangia	30 mL of 10^4 sporangia mL^{-1}	30 mL of 10^2 sporangia mL^{-1}
Zoospores	30 mL of 10^5 zoospores mL^{-1}	30 mL of 10^3 zoospores mL^{-1}
Control	30 mL sterile deionized water (for both inoculum levels)	

A novel non-destructive leaf bait test was developed to detect *P. ramorum* in root balls of *Rhododendron* container plants, either at the end of the experiment (experiment 1) or at monthly intervals (experiment 2). Each root ball was carefully lifted from its pot and four wounded bait leaves of *Rhododendron* cv. Cunningham's White were placed on the bottom of the pot. The plants were placed back in their pots and the water level (rain water) in each saucer was raised to approximately 2 cm during 3–5 days, after which the water was removed from the saucer and the bait leaves were recovered from each pot. Bait leaves were carefully washed under tap water and if lesions were observed, whether at the wounded site or not, pieces of lesioned leaf material were excised, surface-sterilized (30 s in 1% NaOCl followed by 2 × 30 s in sterile deionized water), and plated onto semiselective PARP medium (Jeffers & Martin, 1986). If no lesions were observed then the bait leaves were incubated at room temperature for a further 5–7 days between moist paper towels, after which they were re-evaluated and processed as described above. Plated pieces of bait leaves were incubated at 20°C and the presence of *P. ramorum* among the outgrowing cultures was evaluated microscopically.

The experiment was conducted twice. In the first experiment (inoculation in July 2007), the pot size was 1 L and the experiment lasted 4 months. In the second experiment (inoculation in September 2007), larger (4 L) containerized plants were used and the experiment was maintained for 8 months. Additionally, 7 months post-inoculation, the hypothesis that wounding could result in visible symptoms on the upper part of the plant was tested. Roots of three out of six *Rhododendron* plants of

Table 2 Recovery of *Phytophthora ramorum* from root balls and roots of inoculated *Rhododendron* cv. Madame Masson potted plants via baiting and direct microscopy (recovery experiment 2). There was no recovery of *P. ramorum* from any of the control plants (data not shown)

		Monthly *in situ* baiting of root ball (mpi)							Final baiting of roots		
Inoculum (type and level)	Replicate plant	1	2	3	4	5	6	7	Water-rinsed	Surface-sterilized	Chlamydospores in roots
Sporangia											
Low	1										
	2	+	+	+	+	+				+	
	3	+	+	+					+	+	+
	4										
	5	+	+	+	+	+	+	+	+	+	+
	6		+	+		+	+				+
	Freq.	0·50	0·67	0·67	0·33	0·50	0·33	0·17	0·33	0·50	0·50
High	1		+	+	+		+		+	+	+
	2										
	3	+	+	+		+	+	+	+		+
	4		+	+	+	+	+	+	+	+	+
	5	+	+	+	+			+	+	+	+
	6	+	+	+	+		+				+
	Freq.	0·50	0·83	0·83	0·66	0·33	0·66	0·50	0·66	0·50	0·83
Infected leaves											
Low	1						+				+
	2										
	3		+	+	+	+	+	+	+	+	
	4						+		+		+
	5	+	+	+	+	+		+	+	+	+
	6										
	Freq.	0·17	0·33	0·33	0·33	0·33	0·50	0·33	0·50	0·33	0·50
High	1				+	+		+	+		+
	2		+	+	+	+		+		+	+
	3		+	+	+		+	+	+	+	+
	4		+				+		+		+
	5	+	+	+	+	+			+	+	+
	6	+	+	+		+	+		+		+
	Freq.	0·33	0·83	0·67	0·67	0·67	0·50	0·50	0·83	0·50	1·00
Zoospores											
Low	1										
	2	+		+	+	+	+	+	+	+	+
	3				+			+	+		
	4										
	5	+		+	+			+	+		+
	6										
	Freq.	0·33	0·00	0·33	0·50	0·17	0·17	0·50	0·50	0·17	0·33
High	1		+	+							+
	2	+	+		+			+	+	+	+
	3		+	+	+					+	+
	4	+	+	+	+		+		+		
	5		+	+	+	+		+	+	+	+
	6		+	+					+		
	Freq.	0·33	1·00	0·83	0·67	0·17	0·17	0·33	0·67	0·50	0·67
Overall freq.		0·36	0·61	0·61	0·53	0·36	0·39	0·39	0·58	0·42	0·64

+: recovery; mpi: months post-inoculation; freq.: frequency.

each treatment were wounded by making two vertical incisions (approximately 5 cm from the stem base, 15 cm wide, and 20 cm deep), one on each side of the root ball. One month later (8 months post-inoculation), a destructive baiting technique was used to detect *P. ramorum* in a subsample of removed and rinsed-only roots (5 min continuous tap water rinse) and in rinsed and surface-sterilized roots (rinsed in tap water as described above, followed by 30 s in 1% NaOCl and 2 × 30 s in sterile deionized water). Each rinsed or rinsed and surface-sterilized root sample was placed in an ethanol-sterilized plastic container (20 × 20 × 6 cm), together with 600 mL sterile water and five *Rhododendron* cv. Cunningham's White bait leaves, similar to the methods described by

Themann *et al.* (2002a,b) and Themann & Werres (1998). After 5–7 days, bait leaves with lesions were processed as described above.

To confirm the latent infection of the roots, root tissue was cleared and stained using a modification of the method described by Phillips & Hayman (1970), which allowed subsequent microscopic assessment of the presence of mycelium and chlamydospores. Approximately 20 g rinsed roots were incubated in 10% KOH at 60°C for 7 days. Cleared roots were rinsed twice with tap water and placed in diluted HCl (5·6% v/v) for 20 min. After draining, roots were stained in 0·05% (w/v) trypan blue in lactoglycerin at 60°C for 30 min. The staining solution was removed and roots were destained and stored in lactoglycerin until microscopic evaluation for the presence or absence of chlamydospores at the ×115 magnification setting of an Olympus SZX10 stereomicroscope. The effects of inoculum dose, inoculum type, and time after inoculation and their interactions were determined using a logistic regression model ($n = 6$) at $P = 0{\cdot}05$ in GenStat v. 13.0 (VSN Int.).

Detection of *P. ramorum* in root balls of *Rhododendron* from commercial nurseries

At the Julius Kühn-Institute (JKI), a total of 205 symptomless *Rhododendron* plants (in 1·5 L pots) of five cultivars (Catawbiense Boursault (five plants), Catawbiense Grandiflorum (48), Cunningham's White (51), Brigitte (51) and Sneezy (50)) were obtained from the German nursery trade in October 2008. The plants originated from plots that were found free of *P. ramorum* during the last two official inspections, following EU emergency phytosanitary measures 2007/201/EC. Presence of *P. ramorum* in the root balls was evaluated monthly over a period of 2 years, using different sampling techniques at different time points. In method 1, small volumes of substrate were taken manually at four different parts of the root ball (two at the bottom and two at the upper level of each plant) from each plant and pooled for 5–27 plants (Table 3). In method 2, samples consisting of root pieces and substrate were taken with a cork borer (1·5 cm diameter) at two opposite sides of the pot (sampling depth in the root ball approximately 4 cm). For this method, samples from 5 to 26 plants were pooled (Table 3). Method 3 consisted of the novel root ball baiting technique described above and was performed on individual plants. The root ball samples from methods 1 and 2 were baited with *Rhododendron* leaves and incubated at 20°C and 16 h light according to Themann *et al.* (2002b). After 10 days, the number of leaves with characteristic symptoms was calculated and three tissue pieces per leaf were cut out for isolation. The outgrowing colonies were tested using PCR with the *Phytophthora* genus-specific primers FMPh-8b/FMPh-10b (Martin *et al.*, 2004) and the *P. ramorum*-specific primers Phyto 1/4 and Phyto 2/3 (Davidson *et al.*, 2003) using the PCR conditions described by the respective authors.

Survival in rootless potting medium and forest substrates

The survival capacity of *P. ramorum* in rootless potting medium and forest substrates was assessed at an outdoor quarantine facility. Forest substrates were sampled at 0–50 cm depth in sand to loamy sand soils at the Grotenhout forest near Turnhout, Belgium (N 51°17′, E 4°52′). Four types of forest substrate were sampled near *Rhododendron ponticum* plants under either *Quercus robur*, *Q. rubra*, *Fagus sylvatica* or *Pinus sylvestris* trees. For each substrate, six columns were extracted in April 2008 as follows. A 60 cm long polyvinyl chloride (PVC) tube with an internal diameter of 10 cm was hammered into the soil. The columns were then removed by digging out the PVC pipe and placing a plastic cover over the bottom of each column base to retain the soil. The potting medium consisted of 85% white peat and 15% coco fibre mixture, intended for commercial production of Ericaceous plants (pH 4–4·5). A set of six replicate PVC pipes was filled with this substrate. Survival of *P. ramorum* in the commercial and forest substrates was assessed via the recovery rate from infected leaf pieces that were buried in the substrate columns. Leaves of *Rhododendron* cv. Cunningham's White were infected as described above. Leaf disks (5 mm diameter) from *P. ramorum*-infected leaves were placed in small (20 × 40 mm) nylon mesh bags, 15 per bag. Eight bags were buried at 4 cm depth in each substrate column in April 2008. Contents of six control bags were analysed immediately for *P. ramorum* as described further (time point 0). Fifty control bags were stored without substrate in a box at 4°C, together with a moist paper towel to avoid desiccation. The substrate columns were randomly placed in a 150 × 150 × 80 cm (height) container constructed from waterproof multiplex wood. The vertical walls of the container were insulated with 5 cm polystyrene foam board. The plastic covers at the bottom of each substrate column were perforated (approximately 30 holes of 2 mm diameter) to allow water drainage. Before placement of the substrate columns in the container, the bottom of the container was filled with a 7 cm layer of gravel for optimal water drainage. The container was further filled with sandy soil surrounding the soil columns, so that the level of soil outside the columns corresponded to the level of substrate inside the columns. The wooden container contained a drain hole and was placed outdoors in a quarantine nursery plot at an ornamental research station (Proefcentrum voor Sierteelt – PCS, Destelbergen, Belgium). In this quarantine plot, drain water was captured, disinfected, and checked for absence of live *P. ramorum* propagules before disposal. A single mesh bag was removed from each column at 2, 4, 6, 8, 10, 14, 21 and 33 months after burial. Until 10 months after burial, all leaf disks were plated individually on PARP medium at each sampling point. The percentage of leaf disks from which *P. ramorum* could be recovered was counted (evaluation method 1). Starting 14 months after burial, when leaf disks had started to disintegrate,

Table 3 Detection of *Phytophthora* spp. in the root balls of commercially produced *Rhododendron* plants (five cultivars) over a 2 year period. Three sampling methods (1, 2 and 3) were used over time (see Materials and Methods). For methods 1 and 2 the plants were tested in pools (labelled with Roman numerals within each cultivar) and the results refer to the bulked material of that pool. For method 3, individual plants were sampled and a positive result is preceded by the number of positive plants in that pool. Due to security reasons the plants had to be moved to a different glasshouse at the end of February 2009. The plant pools for CW had to be redefined in March 2009 due to the new distribution pattern of the plants

	Rhododendron cultivars												
	CB (*n* = 5)	CG (*n* = 48)		CW (*n* = 53)					B (*n* = 51)		S (*n* = 50)		Sampling
Sampling date	I (5)[a]	I (24)	II (24)	I (6)	II (8)	III (12)	IV (27)		I (26)	II (25)	I (25)	II (25)	method
10 November 2008	n	n	n	n[b]			n		n	n	n	n	1
1 December 2008	n	n	n	n[c]		n	n		n	n	n	n	1
5 January 2009	n	n[d]	n	Pr[c]		n	n		n	n	n	n	1
2 February 2009	n	n	n	Pr	n	Pr	n		n	n	n	n	1
	I (5)	I (23)	II (24)	V (5)	VI (6)	VII (6)	VIII (12)	IX (24)	I (26)	II (25)	I (26)	II (24)	
2 March 2009	n	n	n	n	n	n	n	n	n	n	n	n	1
30 March 2009	n	n	n	n	n	n	n	n	n	n	n	n	1
27 April 2009	n	n	n	n	n	n	n	n	n	n	n	n	1
25 May 2009	n	n	n	n	n	n	n	n	n	n	n[e]	n	1
22 June 2009	n	n	n	n	n	n	n	n	n	n	n	n	1
27 July 2009	n	n	P	n	n	n	n	n	n	Pr	n	n	2
24 August 2009	n	n	n	n	n	n	n	n	n	n	n	n	2
21 September 2009	n	n	n	n	n	n	n	n	n	n	n	n	2
19 October 2009	n	n	n	n	n	Pr	n	n	n	n	n	n	2
16 November 2009	n	n	n	n	n	n	n	n	n	n	n	n	2
11 January 2010	n	n	n	n	n	n	n	n	n	n	n	n	2
8 February 2010	n	n	n	n	n	Pr	n	n	n	n	n	n	2
9 March 2010	n	n	n	n	n	Pr	n	n	n	n	n	n	2
6 April 2010	n	n	Pr	n	n	n	n	Pr	n	n	n	n	2
3 May 2010	n	n	n	n	n	n	n	n	n	n	n	n	2
1 June 2010	n	n	n	n	n	n	n	n	n	n	n	n	2
28 June 2010	n	n	n	n	n	n	n	n	n	n	n	n	2
26 July 2010	n	n	n	n	n	n	n	n	n	n	n	n	2
8 + 21 October 2010	–	–	–	n[c]	n	n	1 Pr	1 P	–	–	–	–	3
8 + 15 November 2010	–	3 P	n	–	–	–	–	–	–	–	–	–	3
22 + 29 November 2010	–	–	–	–	–	–	–	–	–	–	n	n	3
6 + 13 December 2010	–	–	–	–	–	–	–	–	n	n	–	–	3
20 December 2010	n	–	–	–	–	–	–	–	–	–	–	–	3

Pr: *P. ramorum*; P: *Phytophthora* sp. other than *P. ramorum*; n: no *Phytophthora* sp. detected; –: test not conducted.
Rhododendron cultivars – CB: Catawbiense Boursault; CG: Catawbiense Grandiflorum; CW: Cunningham's White; B: Brigitte; S: Sneezy.
[a]Number of plants pooled.
[b]Pools I, II and III were sampled together for this sampling point.
[c]Pools I and II were sampled together for this sampling point.
[d]One plant in this pool showed disease symptoms on the upper plant parts and was separated from the other plants on 28 January 2009.
[e]Number of plants in pool is 24 instead of 26 for this sampling point.

the total content of each mesh bag was plated on PARP medium and the number of colonies per mesh bag was counted (evaluation method 2). Finally, the percentage of mesh bags from which *P. ramorum* was recovered was determined for each time point and soil type (evaluation method 3). The data ($n = 90$ for evaluation method 1; $n = 6$ for evaluation methods 2 and 3) were analysed using a logistic regression model including time after burial, substrate type and their interaction term in GenStat v. 13.0 (VSN Int.).

During the experiment, hourly temperature data and daily precipitation data were logged at the PCS weather station and used to calculate weekly minimum, maximum and average temperature data and weekly total precipitation data.

Results

Survival of *P. ramorum* in the root ball of containerized *Rhododendron* plants

In the first inoculation experiment, no disease symptoms were observed on the roots nor on the above-ground plant parts. However, baiting of the root balls confirmed the presence of *P. ramorum* in 11% of the plants, 4 months after inoculation. *Phytophthora ramorum* was detected in the root balls when the pots were inoculated with infected leaves (one plant out of six for the low as well as the high inoculum level) or with the sporangia suspension (two plants out of six for the high inoculum level only), but not when inoculated with zoospores.

Even higher recovery rates were obtained in the second experiment. No disease symptoms were observed during the entire experiment, while baiting of the root balls confirmed the presence of *P. ramorum* in 77·7% of the inoculated potted plants (Table 2). There was a decrease in the recovery rate over time, but it was not statistically significant ($P = 0{\cdot}30$). Inoculum type had no influence on the recovery rate of *P. ramorum* in the root balls ($P = 0{\cdot}26$). In contrast, the high inoculum dose resulted in a significantly higher recovery rate than the lower one ($P = 0{\cdot}01$). There were no statistically significant interactions between inoculum dose, inoculum type and time after inoculation. Root balls that baited positive, on average did so 4·2 times out of seven, with a standard deviation of 1·7. Wounding at 7 months post-inoculation did not induce above-ground symptom development. Baiting of water-rinsed roots and rinsed and surface-sterilized roots at the end of the experiment confirmed the presence of *P. ramorum* in 58·3% and 41·6% of the root samples, respectively (Table 2). Microscopical analysis of cleared and stained roots revealed the presence of chlamydospores in 63·9% of the root samples (Table 2). All positive root samples were from plants that had a positive root ball bait test.

Detection of *P. ramorum* in root balls of *Rhododendron* from commercial nurseries

Although none of the plants showed disease symptoms when they arrived at JKI in October 2008, at the beginning of January 2009 one *Rhododendron* cv. Catawbiense Grandiflorum plant showed disease symptoms on the upper plant parts (Table 3). The plant was separated from the remaining plants immediately. PCR-based testing of the plant parts showing symptoms was positive for *P. ramorum*. Up to the end of the experiment no other plants developed any kind of disease symptoms, neither on the twigs and leaves nor on the roots.

Phytophthora ramorum could be detected in at least one pooled sample from the root balls of each cultivar, except *Rhododendron* cv. Catawbiense Boursault and *Rhododendron* cv. Sneezy (Table 3). However, except for *Rhododendron* cv. Cunningham's White, this was only at a single sampling date and in a single pool of plants. Baiting from individual plants (method 3) was only performed once for each pool but resulted in *P. ramorum* detection for one of the cultivars (cv. Cunningham's White). There was no correspondence between the findings with the different methods. Other *Phytophthora* species were baited as well, even in the same pool in which *P. ramorum* was detected (pools CG II and CW IX).

Survival in rootless potting medium and forest substrates

A seasonal effect was observed in the recovery of *P. ramorum* from the buried leaf disks. Using the number of *P. ramorum* positive mesh bags (evaluation method 3) as input data in the logistic regression model, 'time after burial' had a significant ($P < 0{\cdot}001$) effect, while 'substrate type' and the interaction term did not ($P > 0{\cdot}05$). Recovery of *P. ramorum* from infested leaf disks in the different substrates dropped significantly 6 months after burial ($P = 0{\cdot}005$; Fig. 1). However, a large increase in recovery was detected in February 2009 (after 10 months) ($P = 0{\cdot}002$), following a period of cold temperatures (down to −11°C). During the summer, recovery levels of *P. ramorum* dropped again significantly ($P < 0{\cdot}001$) and increased again in January 2010 (after 21 months; $P = 0{\cdot}01$). Although at decreasing incidence for all forest substrates, the pathogen could easily be recovered from all substrates after each cold period, at least until 33 months after burial (Table 4). Significant differences between the different substrates ($P < 0{\cdot}001$) and interaction between the time after burial and the substrates ($P < 0{\cdot}001$) were detected using the survival data of *P. ramorum* in individual leaf disks (evaluation methods 1 and 2; Table 4). Recovery of *P. ramorum* was significantly higher ($P < 0{\cdot}05$) from disks in potting medium than from disks in forest substrate from *F. sylvatica* and *Q. robur* for evaluation method 1 (up to 10 months after burial). For evaluation method 2 (from 14 to 33 months after burial), recovery from disks in potting medium was significantly higher ($P < 0{\cdot}05$) and recovery from disks in *Q. robur* forest substrate was significantly lower than recovery from disks in all other substrates.

Discussion

The first series of experiments demonstrated the ability of *P. ramorum* to survive latently for at least 8 months in the root ball of inoculated *Rhododendron* plants. Inoculum of *P. ramorum* could be baited from the root balls and *Rhododendron* roots were infected, while no plant mortality or any above-ground symptoms were observed, even after several months. The baiting experiment of plants from commercial nurseries also demonstrated latent survival, as *P. ramorum* was detected in root balls of symptomless and non-inoculated *Rhododendron* plants. *Phytophthora ramorum* was baited from these root balls up to 2 years (October 2008–October 2010) after the plants were acquired. This is in line with other studies, which found that *P. ramorum* has the potential to survive latently in root balls for significant periods of time without causing any visual symptoms. Linderman & Davis (2006) could recover *P. ramorum* from the root balls of *Rhododendron* cv. Nova Zembla potted plants up to 12 months after inoculation but did not observe any symptoms. In irrigation experiments with *P. ramorum*-contaminated water, the pathogen could be detected in the root balls of symptomless *Rhododendron* and *Viburnum* plants at the beginning of the following year (Werres *et al.*, 2007). Symptomless root infections have also been observed previously in containerized plants (Riedel *et al.*, 2008; Tjosvold *et al.*, 2009) as well as in unmanaged and cleared woodlands (Fichtner *et al.*, 2011). Shishkoff (2007) demonstrated that *P. ramorum* could be recovered from inoculated excised roots of

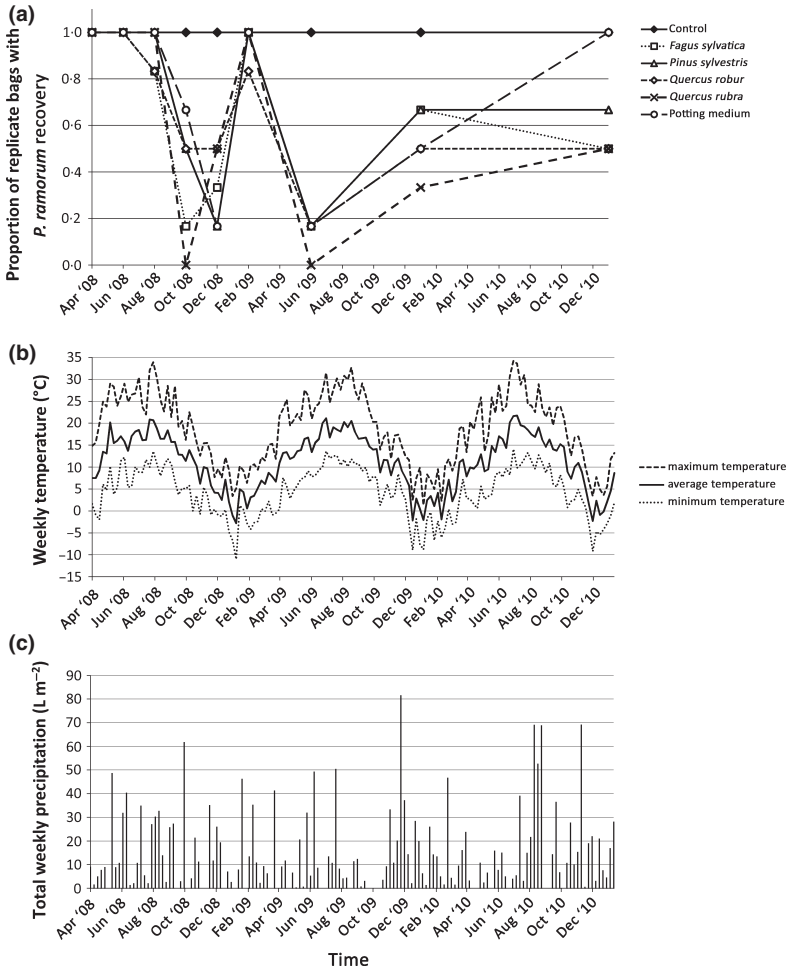

Figure 1 (a) Recovery of *Phytophthora ramorum* over time from infected *Rhododendron* leaf disks buried 4 cm below the surface in four different forest substrates (main tree species listed) or potting medium and stored at an outdoor quarantine facility. Data are the proportions of nylon mesh bags (each containing 15 infected leaf disks) from which the pathogen could be recovered (six replicate bags). The control treatment consisted of leaf disks stored in a moist chamber at 4°C. (b) Minimum, maximum and average weekly temperature (in °C) during the experiment. (c) Total weekly precipitation (L m^{-2}) during the experiment. The time axis is aligned for the three parts and labelled with the month and year.

different host plants at least 8–11 months after burial and infected roots were able to produce sporangia. These symptomless infections are in contrast with the studies from Parke (2007) and Roubtsova & Bostock (2008), in which *Rhododendron* plants died following artificial infestation of the potting medium only, especially in the case of additional salt stress. Although these last two studies show that above-ground symptoms can developed after infection of the roots, especially in the case of stress, latent survival of *P. ramorum* in root balls seems to occur more often. Even in the case of symptomless presence in the root balls, this can probably lead to delayed above-ground infections under commercial conditions, via the production of zoospores during intense rain events, which can drain from the pot and get splashed onto above-ground plant parts. Presence of plants with symptomless root infections can explain the continued or intermittent findings of unique multilocus genotypes of *P. ramorum* in specific nurseries (Vercauteren *et al.*, 2010). It can also explain the dispersal of specific multilocus genotypes within and between continents, presumably via commercial shipments of latently infected plants (Goss *et al.*, 2009, 2011). This latent survival and spread hampers the symptom-based eradication effort and helps explain why it is difficult to further reduce the number of infected nursery sites in several European countries.

Several techniques have been used to detect *Phytophthora* propagules in root balls of containerized plants. Direct plating of roots or dilution plating of soil or potting medium onto semiselective media is possible but these techniques are destructive, labour-intensive, not very sensitive and cannot be used for routine screening of hundreds of plants (Mitchell & Kannwisher-Mitchell, 1992; Themann *et al.*, 2002a). Baiting of roots or potting

Table 4 Recovery of *Phytophthora ramorum* over time from infected *Rhododendron* leaf disks buried 4 cm under the surface of four different forest substrates (main tree species listed) or commercial potting medium and stored outdoors

	Recovery over time[a]						
	Number of *P. ramorum* positive leaf disks per bag (out of 15)					Number of *P. ramorum* colonies per bag	
Substrate (forest)	2 (June 2008)	4 (August 2008)	6 (October 2008)	8 (December 2008)	10 (February 2009)	14 (June 2009)	21 (January 2010)	33 (January 2011)
Fagus sylvatica	12·33 ± 1·26a[b]	7·50 ± 2·51a	1·17 ± 1·17a	1·00 ± 0·68a	8·17 ± 2·01ab	0·17 ± 0·17a	4·83 ± 2·37a	1·33 ± 0·67a
Pinus sylvestris	11·50 ± 0·76a	12·00 ± 1·65b	1·33 ± 0·80a	0·33 ± 0·33a	12·00 ± 1·37b	0·33 ± 0·33a	5·67 ± 2·60a	2·67 ± 1·73a
Quercus robur	12·00 ± 1·24a	8·17 ± 2·15ab	1·00 ± 0·52a	1·83 ± 1·14a	4·83 ± 1·58a	0·17 ± 0·17a	1·83 ± 1·11a	1·17 ± 0·54a
Quercus rubra	10·83 ± 1·96a	10·17 ± 0·70ab	0·00 ± 0·00a	1·00 ± 0·52a	9·00 ± 1·73ab	0·00 ± 0·00a	3·17 ± 2·46a	1·83 ± 0·87a
Potting medium	13·67 ± 0·88a	10·17 ± 1·56ab	3·00 ± 1·55a	1·33 ± 1·33a	11·83 ± 1·22b	0·17 ± 0·17a	3·50 ± 1·88a	7·33 ± 2·38b

Data are the average number of leaf disks from which the pathogen could be recovered ± standard error for months 2–10 (evaluation method 1) and the average number of colonies per 15 leaf disks ± standard error for months 14–33 (evaluation method 2). There were six replicates with 15 leaf disks per replicate.

[a]Time as number of months after burial, with actual month and year in brackets.

[b]Within each time point, recoveries marked with the same letter are not significantly different based on the least significant difference (LSD; $P > 0{\cdot}05$) using a logistic regression model including time, substrate and their interaction term.

medium is a sensitive alternative, but it is also destructive, quite labour intensive, and not applicable to a large number of plants. In irrigated systems, run-off drain water can be captured and baited for *Phytophthora* species, allowing the monitoring of a large number of pots. However, such detection is only possible when sufficient drain water is present and cannot be applied to an *ex situ* shipment of plants under commercial transport conditions. The new leaf bait test described here is based on a combination of partial flooding and baiting, and plating onto semiselective medium. Wetting the soil induces germination of chlamydospores and results in an increase of the inoculum concentration (Lutz & Menge, 1986). Baiting exploits the selective pathogenicity of the pathogen to living host tissue such as apples, pears and *Rhododendron* leaves. For *P. ramorum* in particular, *Rhododendron* leaf baits were most sensitive and are therefore used in the bait test described in this study (Fichtner *et al.*, 2007a). From the infected bait tissue, *Phytophthora* species can then be successfully isolated by plating disinfected bait material taken from the leading edge of the lesion on media semiselective for *Phytophthora*, such as PARP. After incubating the plates for 2–5 days (Davidson *et al.*, 2002), the presence of *P. ramorum* can be confirmed by microscopic examination for its unique morphological characteristics. Alternatively, the presence of a specific *Phytophthora* species could be detected directly in the bait leaf via molecular techniques.

The baiting technique used here has the main advantages of being non-destructive, sensitive, and applicable to tens or hundreds of (*ex situ*) samples. Its applicability in research on latent survival of *P. ramorum* was demonstrated as it outperformed baiting of roots (rinsed and surface-sterilized) and direct microscopic detection of cleared roots. The application of the baiting technique is not restricted to the detection of *P. ramorum*; for example, it can also be applied in plant disease clinics for the detection of a variety of *Phytophthora* species from the root balls of different host plants. Using this technique has, for example, baited *P. cactorum, P. cinnamomi*, *P. plurivora*, *P. cryptogea*, *P. multivora* and *P. syringae* from *Fagus*, *Taxus*, *Azalea*, *Buxus*, *Syringa* and *Rhododendron*, respectively (data not shown). If leaf pieces instead of whole leaves are used as baits, semiselective media are necessary. For *Phytophthora* species susceptible to the supplements in the semiselective media it is often possible to isolate them from entire *Rhododendron* leaf baits using non-selective media. *Pythium* species will occasionally also be baited using this technique. However, due to the differences in culture characteristics, distinction from *Phytophthora* species is usually not difficult. Also, by only leaving the baits under the root balls for 3 days and further incubating them outside the pots, the selectivity of this protocol for *Phytophthora* species was found to be at least as good as the traditional methods discussed earlier (data not shown).

Survival of *P. ramorum* in rootless substrates was found to be quite extensive: the pathogen could be recovered from infected *Rhododendron* leaf pieces buried in

different soil substrates for at least 33 months. The soil phase of *P. ramorum* has previously been demonstrated in natural ecosystems. Fichtner *et al.* (2009) suggest that *P. ramorum* can survive up to 1 year in leaf debris in US forests, even during the summer. In the UK, *P. ramorum* survived in soil of naturally infested sites for at least 28 months (Turner *et al.*, 2008). Although the survival is extensive, a striking seasonality was observed in the recovery rate, with lower recovery during the summer and autumn and higher recovery during and after the winter. The lower recovery during the warmer periods could be the result of the lower soil humidity during these periods. Seasonal differences were also found in *P. ramorum* recovery from soil under infected oaks in a Californian mixed evergreen forest (Fichtner *et al.*, 2009). Recovery rate was highest during the winter rainy season. After the rains ceased, recovery was low or not possible, suggesting that the pathogen is either not viable, or viable but unrecoverable due to its low population density or the presence of dormant propagules (Fichtner *et al.*, 2009). Moisture content also plays a role in the differential survival within the soil profile. Fichtner *et al.* (2007a, 2009) found enhanced survival in leaf disks incubated in soil compared to litter. They report that soil with enhanced soil moisture retention as a result of a higher organic matter content, like redwood-associated soils, may promote survival of *P. ramorum*. Natural perturbation processes resulting in inoculum burial into the soil may promote long-term pathogen survival. Better survival in the soil layer was also reported for other *Phytophthora* species (Nesbitt *et al.*, 1979; Konam & Guest, 2002), most probably due to the higher moisture content compared to the soil surface. Higher moisture content could also explain the higher recovery rate of *P. ramorum* in potting medium compared to natural soils in this study.

This study has shown that *P. ramorum* can easily overwinter outdoors in Belgium, in forest soils as well as in commercial potting medium. This is in line with Werres *et al.* (2007) and Browning *et al.* (2008), who showed that *P. ramorum* is very robust in its ability to survive cold temperatures, especially when compared to several other *Phytophthora* species (Sneh & McIntosh, 1974; Benson, 1982; Kuske & Benson, 1983). Actually, not only soil humidity but especially cold temperatures may play a crucial role in the survival and particularly in the recoverability of *P. ramorum* from soil. Cold storage of soils at 4°C for 1 month prior to baiting has recently been shown to increase the recovery of *P. ramorum* via the enhancement of chlamydospore germination and the subsequent production of sporangia (Tooley & Carras, 2011). The cold temperatures probably also broke the dormancy of the chlamydospores in the winter samples in the present study, and together with the higher moisture content can explain the higher recovery rate in these samples. This is in agreement with the higher recovery of *P. ramorum* from naturally infested UK soil samples collected during the winter by Turner *et al.* (2008). It is also in line with Lutz & Menge (1986) who found a higher recovery rate in the winter months compared to the summer months for *P. parasitica*.

Whether the majority of the survival of *P. ramorum* inside the root balls of plants from commercial nurseries is due to chlamydospores in latently infected roots or in decomposing infected leaf debris cannot be ascertained from the current study. Nevertheless, the presence and survival of *P. ramorum* in root balls has been demonstrated and may play a much larger role in the latent dissemination of *P. ramorum* in the commercial nursery system than previously thought. The advantages of the novel baiting method make it a practical tool for nurserymen and inspection services to monitor the presence of *P. ramorum* in the root balls of commercial plants and may substantially help the *P. ramorum* eradication effort.

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References

Benson DM, 1982. Cold inactivation of *Phytophthora cinnamomi*. *Phytopathology* **72**, 560–3.

Bienapfl J, Zanzot J, Murphy S, Garbelotto M, Rizzo D, 2005. Isolation of a new lineage of *Phytophthora ramorum* from asymptomatic stems and roots of a commercial lot of rhododendron in California. *Phytopathology* **95**, S9–10.

Brasier CM, Webber J, 2010. Plant pathology: sudden larch death. *Nature* **466**, 824–5.

Browning M, Englander L, Tooley PW, Berner D, 2008. Survival of *Phytophthora ramorum* hyphae after exposure to temperature extremes and various humidities. *Mycologia* **100**, 236–45.

Colburn GC, Shishkoff N, 2006a. Density of *Phytophthora ramorum* chlamydospores in soil necessary to cause infection. *Phytopathology* **96**, S25.

Colburn GC, Shishkoff N, 2006b. Detection of *Phytophthora ramorum* chlamydospores in soil. *Phytopathology* **96**, S190.

Colburn GC, Sechler K, Shishkoff N, 2005. Survivability and pathogenicity of *Phytophthora ramorum* chlamydospores in soil. *Phytopathology* **95**, S20.

Davidson JM, Rizzo D, Garbelotto M, Tjosvold SA, Slaughter GW, 2002. *Phytophthora ramorum* and Sudden Oak Death in California: II. Transmission of *Phytophthora ramorum* via *Umbellularia californica* (California bay) leaves in California oak

woodlands. In: Standiford RB, McCreary D, Purcell KL, eds. *Proceedings of the Fifth Symposium on Oak Woodlands: Oaks in California's Changing Landscape*. San Diego, CA, USA: USDA Forest Service: Gen. Tech. Rep. PSW-GTR-184, 741–9.

Davidson JM, Werres S, Garbelotto M, Hansen E, Rizzo DM, 2003. Sudden Oak Death and associated diseases caused by *Phytophthora ramorum*. *Plant Health Progress*, doi: 10.1094/PHP-2003-0703-01-DG.

Davidson JM, Wickland AC, Patterson HA, Falk KR, Rizzo DM, 2005. Transmission of *Phytophthora ramorum* in mixed-evergreen forest in California. *Phytopathology* **95**, 587–96.

De Dobbelaere I, Vercauteren A, Speybroeck N *et al.*, 2010. Effect of host factors on the susceptibility of *Rhododendron* to *Phytophthora ramorum*. *Plant Pathology* **59**, 301–12.

Fichtner EJ, Lynch SC, Rizzo DM, 2007a. Detection, distribution, sporulation, and survival of *Phytophthora ramorum* in a California redwood-tanoak forest soil. *Phytopathology* **97**, 1366–75.

Fichtner EJ, Rizzo DM, Lynch SC, Davidson JM, Buckles G, Parke JL, 2007b. Summer survival of *Phytophthora ramorum* in California forests. *Phytopathology* **97**, S36.

Fichtner EJ, Lynch SC, Rizzo DM, 2009. Survival, dispersal, and potential soil-mediated suppression of *Phytophthora ramorum* in a California redwood-tanoak forest. *Phytopathology* **99**, 608–19.

Fichtner EJ, Rizzo DM, Kirk SA, Webber JF, 2011. Root infections may challenge management of invasive *Phytophthora* spp. in UK woodlands. *Plant Disease* **95**, 13–8.

Goss EM, Larsen M, Chastagner GA, Givens DR, Grunwald NJ, 2009. Population genetic analysis infers migration pathways of *Phytophthora ramorum* in US nurseries. *PLoS Pathogens* **5**, e1000583.

Goss EM, Larsen M, Vercauteren A, Werres S, Heungens K, Grunwald NJ, 2011. *Phytophthora ramorum* in Canada: evidence for migration within North America and from Europe. *Phytopathology* **101**, 166–71.

Jeffers SN, Martin SB, 1986. Comparison of two media selective for *Phytophthora* and *Pythium* species. *Plant Disease* **70**, 1038–43.

Konam JK, Guest DI, 2002. Leaf litter mulch reduces the survival of *Phytophthora palmivora* under cocoa trees in Papua New Guinea. *Australasian Plant Pathology* **31**, 381–3.

Kuske CR, Benson DM, 1983. Overwintering and survival of *Phytophthora parasitica*, causing dieback of *Rhododendron*. *Phytopathology* **73**, 1192–6.

Linderman RG, Davis E, 2005. Survival of *Phytophthora ramorum* in potting mix components or soil and eradication with aerated steam treatment. *Phytopathology* **95**, S61.

Linderman RG, Davis EA, 2006. Survival of *Phytophthora ramorum* compared to other species of *Phytophthora* in potting media components, compost, and soil. *HortTechnology* **16**, 502–7.

Lutz A, Menge J, 1986. Breaking winter dormancy of *Phytophthora parasitica* propagules using heat-shock. *Mycologia* **78**, 148–50.

Martin FN, Tooley PW, Blomquist C, 2004. Molecular detection of *Phytophthora ramorum*, the causal agent of sudden oak death in California, and two additional species commonly recovered from diseased plant material. *Phytopathology* **94**, 621–31.

Mitchell DJ, Kannwisher-Mitchell ME, 1992. Phytophthora. In: Singleton LL, Mihail JD, Rush CM, eds. *Methods for Research on Soilborne Phytopathogenic Fungi*. St Paul, MN, USA: APS Press, 31–8.

Nesbitt HJ, Malajczuk N, Glenn AR, 1979. Effect of organic matter on the survival of *Phytophthora cinnamomi* Rands in soil. *Soil Biology and Biochemistry* **11**, 133–6.

Parke JL, 2007. Root and stem infection of rhododendron from potting medium infested with *Phytophthora ramorum*. *Plant Disease* **91**, 1265–70.

Parke JL, Bienapfl J, Oh E *et al.*, 2006. Natural infection of tanoak seedling roots by *Phytophthora ramorum*. *Phytopathology* **96**, S90.

Phillips JM, Hayman DS, 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society* **55**, 158–60.

Riedel M, Wagner S, Gotz M, Belbahri L, Lefort F, Werres S, 2008. Studies of tissue colonization in *Rhododendron* by *Phytophthora ramorum*. In: Frankel SJ, Kliejunas JT, Palmieri KM, eds. *Proceedings of the Sudden Oak Death Third Science Symposium*. Albany, CA, USA: USDA Forest Service: Gen. Tech. Rep. PSW-GTR-214, 485–7.

Rizzo DM, Garbelotto M, Davidson JM, Slaughter GW, Koike ST, 2002. *Phytophthora ramorum* as the cause of extensive mortality of *Quercus* spp. and *Lithocarpus densiflorus* in California. *Plant Disease* **86**, 205–14.

Roubtsova TV, Bostock RM, 2008. Impact of episodic root stress on the susceptibility of *Rhododendron* sp. and *Viburnum tinus* to *Phytophthora ramorum*. *Phytopathology* **98**, S136.

Shishkoff N, 2007. Persistence of *Phytophthora ramorum* in soil mix and roots of nursery ornamentals. *Plant Disease* **91**, 1245–9.

Shishkoff N, 2008. Sporulation on plant roots by *Phytophthora ramorum*. *Phytopathology* **98**, S145.

Shishkoff N, Senesac A, 2005. Susceptibility to *Phytophthora ramorum* of roots and shoots of common container weeds. *Phytopathology* **95**, S96.

Sneh B, McIntosh DL, 1974. Studies on behavior and survival of *Phytophthora cactorum* in soil. *Canadian Journal of Botany* **52**, 795–802.

Themann K, Werres S, 1998. Verwendung von Rhododendronblättern zum Nachweis von *Phytophthora*-Arten in Wurzel- und Bodenproben. *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes* **50**, 3745.

Themann K, Werres S, Diener HA, Luttmann R, 2002a. Comparison of different methods to detect *Phytophthora* spp. in recycling water from nurseries. *Journal of Plant Pathology* **84**, 41–51.

Themann K, Werres S, Luttmann R, Diener HA, 2002b. Observations of *Phytophthora* spp. in water recirculation systems in commercial hardy ornamental nursery stock. *European Journal of Plant Pathology* **108**, 337–43.

Tjosvold SA, Chambers DL, Fichtner EJ, Koike ST, Mori SR, 2009. Disease risk of potting media infested with *Phytophthora ramorum* under nursery conditions. *Plant Disease* **93**, 371–6.

Tooley PW, Carras MM, 2011. Enhanced recovery of *Phytophthora ramorum* from soil following 30 days of storage at 4°C. *Journal of Phytopathology* **159**, 641–3.

Turner J, Jennings P, Humphries G *et al.*, 2008. Natural outbreaks of *Phytophthora ramorum* in the U.K.: current status and monitoring update. In: Frankel SJ, Kliejunas JT, Palmieri KM,

eds. *Proceedings of the Sudden Oak Death Third Science Symposium*. Albany, CA, USA: USDA Forest Service: Gen. Tech. Rep. PSW-GTR-214, 43–8.

Vercauteren A, De Dobbelaere I, Grünwald NJ *et al.*, 2010. Clonal expansion of the Belgian *Phytophthora ramorum* populations based on new microsatellite markers. *Molecular Ecology* **19**, 92–107.

Werres S, Marwitz R, Veld WAMI *et al.*, 2001. *Phytophthora ramorum* sp. nov., a new pathogen on *Rhododendron* and *Viburnum*. *Mycological Research* **105**, 1155–65.

Werres S, Wagner S, Brand T, Kaminski K, Seipp D, 2007. Survival of *Phytophthora ramorum* in recirculating irrigation water and subsequent infection of *Rhododendron* and *Viburnum*. *Plant Disease* **91**, 1034–44.