Plant Pathology (2010) **59**, 301–312 Doi: 10.1111/j.1365-3059.2009.02212.x

Effect of host factors on the susceptibility of *Rhododendron* to *Phytophthora ramorum*

I. De Dobbelaere[ab], A. Vercauteren[ab], N. Speybroeck[c], D. Berkvens[c], E. Van Bockstaele[ab], M. Maes[a] and K. Heungens[a]*

[a]*Institute for Agricultural and Fisheries Research (ILVO), Plant Sciences Unit - Crop Protection, Merelbeke;* [b]*Ghent University, Faculty of Bioscience Engineering, Department of Plant Production, Ghent; and* [c]*Institute of Tropical Medicine Prince Leopold, Antwerp, Belgium*

Phytophthora ramorum causes sudden oak death (SOD) in western coastal forests of the USA. In Europe, the pathogen is mainly present in the nursery industry, particularly on *Rhododendron*. Because of the primary role of *Rhododendron* as a host and potentially as a vector, the effect of *Rhododendron* host factors on *P. ramorum* susceptibility and sporulation was investigated. Inoculation methods using either wounded or non-wounded detached leaves were applied to 59 *Rhododendron* cultivars and 22 botanical species, replicated in three separate years. All *Rhododendron* species and cultivars were susceptible when using wounded leaves, but not when using non-wounded leaves, suggesting a resistance mechanism operating at the level of leaf penetration. Using a regression tree analysis, the cultivars and species were split into four susceptibility classes. Young leaves were more susceptible than mature leaves when wounded, but less susceptible when non-wounded. This effect was not correlated with leaf hydrophobicity or the number of leaf hairs. The presence or the type of rootstock did not affect the cultivar susceptibility level. Sporangia and chlamydospore production in the leaf lesions varied widely among *Rhododendron* cultivars and was not correlated with the susceptibility level. The susceptibility to *P. ramorum* correlated well with the susceptibility to *P. citricola* and *P. hedraiandra* × *cactorum*, suggesting that the resistance mechanisms against these species are non-specific. Susceptibility to *P. kernoviae* was low for most cultivars. These findings have implications for detection, spread and disease control, and are therefore important in pest risk assessment.

Keywords: host resistance, leaf age, leaf hairs, *Phytophthora kernoviae,* rootstock, sporulation

Introduction

In 1993, a new leaf and twig blight of *Rhododendron* was observed in ornamental nurseries in Germany and the Netherlands (Werres & Marwitz, 1997; Werres *et al.*, 2001). Since 1995, a canker disease, commonly referred to as sudden oak death (SOD), has lead to extensive mortality of oak trees (mainly *Lithocarpus densiflorus* and *Quercus agrifolia*) on the west coast of the USA (Rizzo *et al.*, 2002). SOD infection killed an estimated 235 000 trees in the Big Sur region of California alone (Meentemeyer *et al.*, 2008). Both diseases are caused by a new *Phytophthora* species, *P. ramorum* (Werres *et al.*, 2001). *Phytophthora ramorum* causes a number of distinct disease symptoms on a wide range of hosts in 45 plant families (APHIS, 2008). Shoot tip dieback and leaf blight are the most common symptoms in Europe, mostly occurring on shrubs and ornamental species. *Rhododendron* is the main host, but other genera including *Viburnum*, *Camelia*, *Syringa*, *Kalmia*, *Pieris* and *Taxus* are also affected (Davidson *et al.*, 2003; Beales *et al.*, 2004;Lane *et al.*, 2004). In Europe, bleeding cankers on trees have been identified in the United Kingdom and in the Netherlands, but mortality has been limited to less than 50 trees (Brasier *et al.*, 2004; Denman *et al.*, 2005a; De Gruyter & Steegs, 2008; Tracy, 2009). The concern for the spread of *P. ramorum* within Europe has resulted in EU emergency phytosanitary measures since 2002 (2002/757/EC and amended by decisions 2004/426/EC and 2007/201/EC) and EU pest risk assessment efforts (http://rapra.csl.gov.uk/pra/index.cfm). In the framework of US and EU pest risk assessments, various research groups have conducted susceptibility assays on plant species from different plant genera. For *Rhododendron*, a limited number of species and/or cultivars have been tested (Tooley *et al.*, 2004; McDonald *et al.*, 2006; Kaminski & Wagner, 2008). This is in part because *Rhododendron* is considered a known susceptible host and the focus has been on identifying other

*E-mail: kurt.heungens@ilvo.vlaanderen.be

Published online 8 December 2009

Plant Pathology

genera that are at risk. *Rhododendron* is part of the Ericaceae family and contains more than 1000 species, divided into eight subgenera: *Hymenanthes* (elepidote rhododendrons), *Rhododendron* (lepidote rhododendrons), *Tsutsusi* (evergreen azaleas) and *Pentanthera* (deciduous azaleas) are the four major subgenera and *Azaleastrum*, *Candidastrum*, *Mumeazalea* and *Therorhodion* are the four minor subgenera (Chamberlain *et al.*, 1996). *Rhododendron* breeding has led to approximately 28 000 cultivars, usually hybrids (Leslie, 2004). It is a popular ornamental plant, with an annual production in Europe estimated at seven million plants (Bruggeman, 2004). *Rhododendron* has been the most frequently encountered host of *P. ramorum* during the surveys mandated as part of the EU phytosanitary measures. Due to its susceptibility to *P. ramorum* and its international trade, *Rhododendron* is a potential vector for spread of the pathogen throughout the nursery trade and into public green areas and home gardens, from which it might spread to the environment. Because of its primary role as a host and potentially as a vector in Europe, more information on the effect of *Rhododendron* host factors on *P. ramorum* susceptibility and sporulation was needed. This information has implications for detection, spread and disease control, and is therefore important in pest risk assessment. A similar analysis has been conducted with a number of *Rhododendron* species and cultivars that are relevant to the USA (Tooley *et al.*, 2004), but information about species and cultivars relevant to the EU, as well as the effect of other host factors on susceptibility, was not yet available. Thus, the specific objectives of this research were: (i) to determine the susceptibility of a series of *Rhododendron* species and cultivars, selected for botanical classification as well as European commercial importance; (ii) to determine the sporulation characteristics in a range of cultivars with differential susceptibility; (iii) to study the effect of other host factors such as leaf age and the presence of rootstock on host susceptibility; and (iv) to determine whether the susceptibility to *P. ramorum* is correlated with the susceptibility to other *Phytophthora* species causing leaf and stem blight.

Materials and methods

Host plants

A total of 22 botanical *Rhododendron* species and 59 *Rhododendron* cultivars (hybrids) were selected (Table 1). The species were representatives of the four largest subgeneric groups within the genus *Rhododendron*, including lepidote and elepidote *Rhododendron* species, as well as deciduous and evergreen azaleas. Economic importance in Europe was the main criterion for cultivar selection. At least two plants of each *Rhododendron* cultivar tested were purchased at commercial nurseries in Flanders, Belgium in the spring of 2004 or 2005, and planted together in a shaded area at the ILVO research site. Cultivar Vireya and the three evergreen azaleas, which are sensitive to frost damage, were maintained in the greenhouse. Leaf material from *Rhododendron* species was collected from 10 + -year-old plants at a private arboretum in Lochristi, Belgium. *Rhododendron* plants were not treated with fungicides. Plants at the ILVO site received 10 g of Compo Basacote® Plus 6M fertilizer per plant in the spring of each year. Due to quarantine restrictions, inoculation tests were performed on detached leaf material in an L2-Q research facility. The leaves were fully developed (> 3 months old), but from the most recent year's growth.

Pathogen isolates

Phytophthora ramorum isolate PR/D/02/880 was the main isolate used in all experiments. It was isolated from a *Rhododendron* cv. John Walter at a commercial nursery during the 2002 EU-mandated survey. It is of A1 mating type and belongs to the main microsatellite genotype group of the EU1 lineage (Ivors *et al.*, 2006; Grünwald *et al.*, 2009; Vercauteren *et al.*, 2010). Preliminary pathogenicity tests using four different EU1 *P. ramorum* isolates (two from *Rhododendron* and two from *Viburnum*) did not show pathological variation on several cultivars (data not shown), supporting the use of a single isolate in the tests.

For the other *Phytophthora* species, the following isolates were used: *P. citricola* PC/D/05/89, isolated from *Rhododendron* cv. Nova Zembla in July 2005, Belgium; *P. hedraiandra* × *cactorum* PH/M/05/32, isolated from *R. catawbiense* in July 2005, Belgium; and *P. kernoviae* CC2300 and CC2246, isolated from *Rhododendron* in the UK, and obtained from Fera (formerly CSL), UK.

Inoculum production and inoculation methods

Two inoculation methods were used, with either mycelial disks or zoospores as inoculum. Mycelial disks (6 mm) were removed from the margin of growing cultures on clarified V8 agar (Campbell Foods). For zoospore production, sporangia of *P. ramorum* were washed from two-week-old cultures on diluted clarified V8 agar (80 mL L^{-1} clarified V8 and 15 g L^{-1} agar). The sporangial suspension was placed at 11°C for 1 to 2 h to induce zoospore release. Zoospores were filtered through a 10 μm nylon mesh and their concentration determined with a haemacytometer. The concentration was adjusted to 1×10^4 zoospores mL^{-1} and zoospores were kept at 1 to 4°C during the inoculation assay.

For *P. citricola*, *P. hedraiandra* × *cactorum*, and *P. kernoviae* zoospore production, mycelial mats were produced in clarified V8 broth in Roux flasks and incubated at 20°C for 4 to 6 days. Mycelial mats were washed with sterile deionized H_2O and incubated for 4 to 6 days in soil extract at 17°C and 16 h per day fluorescent light to induce sporangial formation. They were rinsed with sterile deionized H_2O and incubated at 12°C for 2 h to induce zoospore release, after which the zoospore

Table 1 Parentage and susceptibility of 59 *Rhododendron* cultivars to *Phytophthora ramorum* based on detached leaf inoculation assay using non-wounded and wounded leaves

			Non-wounded leaves				Wounded leaves			
Cultivar	Parentage[a]	Sub-genus[b]	No.[c]	RLLA[d] (%)	SEM	Class[e]	No.[c]	Lesion diam. (mm)	SEM	Class[e]
Marianne	(*wardii* x Alice Street) X Mareike	E	60	89·8	1·5	4	30	13·3	0·9	2
John Walter	*catawbiense* X *arboreum*	E	60	88·4	1·2	4	28	17·6	0·9	3
Harvest Moon	Mrs. L. Smith X *campylocarpum* hybrid	E	60	84·7	1·3	4	30	17·5	1·4	3
ponticum Variegatum	hybrid of *ponticum*	E	60	84·1	2·4	4	30	19·1	0·9	3
Grace Seabrook	the hon. J. M. de Montague X *strigillosum*	E	60	82·9	2·1	4	28	11·4	0·7	1
Cheer	C. White X red *catawbiense* seedling	E	60	79·2	2·2	4	30	14·9	0·4	2
Mme Masson	*catawbiense* X *ponticum*	E	280	75·0	1·5	3	140	18·8	1·5	3
Scarlet Wonder	Essex Scarlet X *forrestii*	E	60	74·8	2·7	3	30	10·7	0·8	1
Belkanto	Mrs. Millais X (*wardii* x A. Street) x (Omega x *wardii*)	E	60	72·4	3·3	3	30	12·9	0·9	2
Polaris	Y. ''Koichiro Wada'' X Omega	E	40	71·5	3·8	3	20	17·4	1·1	3
Marcel Ménard	parentage unknown	E	60	69·7	3·1	3	30	11·5	1·3	1
Etoile de Sleidinge	probably *ponticum* hybrid	E	60	69·4	2·9	3	30	15·8	1·0	2
Germania	(A. van Welie x *williamsianum*) X C. van Tol	E	61	68·9	3·1	3	30	16·4	0·9	2
Blue Tit	*impeditum* X *augustinii*	L	70	68·7	3·4	3	30	15·0	0·9	2
Boursault	hybrid of *catawbiense*	E	60	66·4	3·7	3	29	17·2	0·9	2
Blue Peter	possibly hybrid of *ponticum*	E	60	63·0	3·1	3	30	17·2	0·7	2
Bengal	E. Scarlet X for. subsp. *forrestii* Repens	E	40	61·4	2·9	3	20	10·5	1·2	1
Goldkrone	(*wardii* x Alice Street) X (Omega x *wardii*)	E	60	61·0	3·0	3	30	11·9	0·9	2
Chevalier Félix de Sauvage	*caucasicum* X un-named Hardy Hybrid	E	60	60·5	4·0	3	30	15·1	0·7	2
President Roosevelt	hybrid of *limbatum*	E	60	59·4	4·0	3	30	17·4	1·0	3
Vulcan	Mars X *griersonianum*	E	60	58·3	4·1	3	30	13·5	1·4	2
Roseum Elegans	hybrid of *catawbiense*	E	59	57·7	4·8	3	29	15·6	1·3	2
Britannia	Queen Wilhelmina X Stanley Davies	E	60	57·4	3·0	3	29	16·1	0·9	2
Albert Schweitzer	parentage unknown	E	60	57·3	2·9	3	28	17·8	0·6	3
catawbiense Grandiflorum	hybrid of *catawbiense*	E	60	55·5	3·6	3	30	14·5	0·9	2
Delta	parentage unknown	E	40	54·8	3·3	3	20	13·0	0·8	2
Lord Roberts	probably hybrid of *catawbiense*	E	60	53·3	4·7	3	30	15·3	0·7	2
Praecox	*ciliatum* X *dauricum*	L	58	53·1	4·3	3	30	19·0	0·9	3
Gomer Waterer	Madame Carvalho X Pink Pearl	E	60	52·5	3·7	3	30	17·3	1·0	3
Rocket	*meddianum* X *strigillosum*	E	60	50·0	3·9	3	29	16·3	1·1	2
Pink Pearl	George Hardy X Broughtonii	E	62	48·6	4·4	3	30	14·4	0·4	2
Wilgen's Ruby	Britannia X John Walter	E	60	46·3	4·0	3	30	13·5	0·2	2
Cosmopolitan	Cunningham's White X Vesuvius	E	60	43·5	4·2	2	30	14·6	0·6	2
Bad Eilsen	Essex Scarlet X *forrestii* Repens group	E	60	43·0	2·9	2	30	13·8	0·6	2
Baden-Baden	Essex Scarlet X *forrestii* Repens group	E	65	42·2	3·1	2	29	13·6	1·0	2
Nova Zembla	Parsons Grandiflorum X unknown. hardy. red H.	E	60	39·5	4·4	2	30	12·6	0·6	2
Dora Amateis	minus *carolinianum* group X *ciliatum*	L	60	34·7	3·8	2	39	13·0	1·0	2
Cunningham's White	*caucasicum* X white-flowered *ponticum*	E	480	31·9	1·2	2	256	14·2	1·0	2
Kalinka	Morgenrot X (Mars x Y. 'Koichiro Wada'')	E	65	29·1	2·8	2	30	15·6	0·3	2
Anna Rose Whitney	*griersonianum* X Countess of Derby	E	60	19·0	2·7	2	30	13·5	0·6	2
Double Date	parentage unknown	E	60	16·8	2·1	1	29	11·2	0·5	1
Saxon Glow	Hot Topic X *saxifragoides* (Vireya hybrid)	L	65	14·7	2·8	1	30	30·9	0·8	4
Anna Baldsiefen	Gable's Pioneer	L	37	14·4	2·8	1	20	15·0	0·8	2
Virginia Richards	(*wardii* x F.C. Puddle Group) X Mrs. Robertson	E	60	13·5	2·3	1	29	9·0	0·7	1
Tortoiseshell Orange	Goldsworth Orange X *griersonianum*	E	40	9·8	2·0	1	20	7·7	1·1	1
Halfdan Lem	the hon. Jean Marie de Montague X Red Loderi	E	40	9·2	2·7	1	20	10·0	0·9	1
Percy Wiseman	*Yakushimanum* X Fabia Tangerine	E	40	9·2	1·5	1	30	12·7	0·3	2
Lem's Monarch	Anna X Marinus Koster	E	58	4·1	0·6	1	29	10·2	0·5	1
Morgenrot	*Yakushimanum* ''Koichiro Wada'' X Spitfire	E	60	3·9	0·9	1	30	13·5	0·6	2
Golden Torch	bambi X (Grosclaude group x *griersonianum*)	E	40	3·8	0·8	1	20	10·4	0·6	1

Table 1 (Continued)

Cultivar	Parentage[a]	Sub-genus[b]	Non-wounded leaves				Wounded leaves			
			No.[c]	RLLA[d] (%)	SEM	Class[e]	No.[c]	Lesion diam. (mm)	SEM	Class[e]
Red Jack	(Wilgen's Ruby x May Day Group) X *forrestii* H	E	60	3·7	0·8	1	29	14·3	0·4	2
Fantastica	Mars X *yakushimanum* 'Koichiro Wada''	E	60	3·4	0·7	1	29	14·3	0·3	2
Morning Cloud	*yakushimanum* X Springbok	E	60	2·1	0·6	1	30	11·5	0·6	1
Shamrock	*keiskei* (dwarf form) X hanceanum Nanum G.	L	60	2·0	0·7	1	30	19·0	0·4	3
Gartendirektor Rieger	Adriaan Koster X *williamsianum*	E	240	1·7	0·3	1	128	7·7	0·6	1
Helmut Vogel	hybrid of simsii	DA	60	1·3	0·6	1	30	13·6	0·5	2
Otto	hybrid of simsii	DA	65	0·7	0·4	1	30	16·7	0·7	2
Mrs.Kint	hybrid of simsii	DA	65	0·7	0·3	1	30	20·3	0·4	3
Albatross Townhill White	Loderi Group X *fortunei subsp. discolor*	E	60	0·3	0·1	1	30	10·6	0·2	1

[a]Based on Leslie (2004).
[b]The four major subgenera of the *Rhododendron* genus: *Hymenanthes* (elepidote rhododendrons) (E), *Rhododendron* (lepidote rhododendrons) (L), *Tsutsusi* (evergreen azaleas) (EA), and *Penthanthera* (deciduous azaleas) (DA).
[c]Total number of leaves used in the three replicate years (for the eight cultivars with a reduced number of replicates: total number of leaves used in two replicate years)
[d]RLLA = relative lesioned leaf area (see Material and methods).
[e]Susceptibility class, based on an RT analysis. Class 1 contains the most resistant cultivars, Class 4 contains the most susceptible cultivars.

suspension was filtered through a 10 μm nylon mesh. Zoospore concentration was determined with a haemacytometer and the following concentrations were established via dilution with sterile deionized H_2O: 2×10^4, 5×10^4 and 5×10^3 zoospores mL^{-1} for *P. citricola*, *P. hedraiandra* × *cactorum* and *P. kernoviae*, respectively. These concentrations resulted in a desired number of leaf lesions during preliminary tests, except for *P. kernoviae*, for which zoospore concentration was determined by the maximum concentration produced.

Inoculation methods were modified from Tooley *et al.* (2004). For the inoculation method using wounded leaves, 10 detached leaves of each *Rhododendron* species or cultivar were individually puncture-wounded with a needle, after which a mycelium plug (6 mm) was placed on top of each wound and covered with a droplet of 0·2% water agar. Inoculated leaves were placed in between moist paper towels and laid inside plastic boxes. The boxes were wrapped in large plastic bags and placed at 17°C for 6 days, at which point the lesion diameter (in mm) was measured in the longitudinal direction of each leaf with a ruler.

For inoculation of non-wounded tissue, 20 replicate leaves of each *Rhododendron* cultivar or species were dipped in the zoospore suspension for either 1 min (*P. ramorum*) or 2 min (other *Phytophthora* species). Care was taken not to immerse the petiole, in order to avoid pathogen penetration via the cut wound. After inoculation, the leaves were incubated as described for the method using wounded leaves. Digital pictures of the leaves were taken and analysed with Assess 1·0 (APS) to determine the relative lesioned leaf area (RLLA), being the area of necrotic tissue on each leaf as a percentage of the total leaf area.

Susceptibility of species and cultivars to *P. ramorum*

Screening assays for susceptibility were performed from July to mid-September of each year from 2004 to 2007. Most *Rhododendron* species or cultivars were tested three times, in three separate years. Batches containing 10 to 15 species and cultivars were tested at roughly weekly intervals. A reference cultivar (*Rhododendron* Cunningham's White) was included in each batch. The leaves of the *Rhododendron* cultivars were detached on the morning of the inoculation day, gently rinsed with tap water, and kept under moist paper towels until inoculation. Leaves of the *Rhododendron* species were detached the day before inoculation and kept in a plastic bag with a moist paper towel at 4°C until they were processed in the same way as the leaves from the cultivars.

Sporulation on cultivars with different levels of susceptibility

Twelve cultivars were selected that represent a range of susceptibility. Listed from more resistant to more susceptible, chosen cultivars were *R.* G. Rieger, *R.* Red Jack, *R.* Fantastica, *R.* T. Orange, *R.* Kalinka, *R.* Cunningham's White, *R.* Britannia, *R. c.* Grandiflorum, *R.* Mme Masson, *R.* Germania, *R. p.* Variegatum and *R.* M. Menard. Leaves (24) from each cultivar were inoculated with the method using wounded leaves. Production and quantification of sporangia was performed as follows: the upper paper towels and the mycelium disks (inoculum) were removed after 6 days of incubation. The leaves were then returned to the plastic boxes with their abaxial surface facing

upwards, the plastic bags were replaced, and the leaves were exposed to a 16 h per day light regime at 17°C in a growth chamber. The leaves were misted carefully with sterile distilled water daily to ensure that the leaf surface remained moist at all times. After 4 days of additional incubation, each leaf surface was gently scraped with 1 mL sterile distilled water and a glass rod to dislodge the sporangia. The leaves were rinsed twice with 0·5 mL sterile distilled water. The scrapings and rinse water were captured and transferred into a 2 mL microtube with a Pasteur pipette, and a drop of Trypan blue stain (in lactoglycerin) was added. The microtubes were kept at 4°C until further processing. Depending on the number of sporangia in the scrapings, sporangia were either counted directly or after 3 min centrifugation at 2655 **g**, removal of all but approximately 100 μL of the supernatant, and resuspension of the spores. The number of sporangia in three drops of 20 μL were counted microscopically at 100× magnification and the number of sporangia per leaf was calculated according to the (remaining) total volume of the leaf scrapings, which was determined with an automatic pipette. The area of the leaf lesion was quantified using digital photography, a reference measure, and Assess 1·0 (APS), allowing calculation of the number of sporangia per unit of lesion area.

Chlamydospores were counted microscopically in cleared leaf lesions. The protocol for clearing and staining plant parts for visualization of the fungal structures was modified from Philips & Hayman (1970). Per leaf, one disk (6 mm diameter) was taken from the centre of the necrotic tissue of the 10-day-old lesions with a cork borer and cleared in 5 mL 10% KOH during 7 days at 60°C. The KOH was replaced daily. Chlamydospores were counted in cleared leaf tissues at 115× magnification using a stereo microscope. The number of chlamydospores was also expressed per lesion area, using the area of the leaf disks or the actual area of the lesion if it was smaller than the leaf disk.

Effect of leaf age on susceptibility to *P. ramorum*

Two sets of tests were conducted to determine the effect of leaf age on the susceptibility to *P. ramorum*. The first set involved a single cultivar (*R.* Cunningham's White), for which the susceptibility of one-year-old leaves versus new (current year) leaves was determined every two weeks, until the new leaves had fully developed and no longer showed obvious visual differences with the leaves from the previous year (usually after four months). This type of test was performed in 2006 and 2007, using the two inoculation methods mentioned above.

To determine if the effects observed in the first set of tests were cultivar-dependent, and to test whether leaf hydrophobicity (water drops on hydrophobic leaves have a contact angle >90°) or the number and type of leaf hairs at different leaf ages were correlated with susceptibility, a second set of susceptibility tests involving one-year-old versus new leaves of 15 different *Rhododendron* cultivars was conducted. Each cultivar was tested twice, in May and June of 2007. The number of lesions (and not the lesion area) on each of 10 replicate non-wounded leaves was determined, 5 to 7 days after inoculation. The presence of hairs on the abaxial surface of the leaves was evaluated microscopically for each cultivar. Hydrophobicity of the abaxial surface was determined with a leaf droplet test (Brewer *et al.*, 1991).

Effect of rootstock on susceptibility to *P. ramorum*

Inoculation tests were performed on four *Rhododendron* cultivars from the two most susceptible classes (*R.* Mme Masson, *R.* John Walter, *R. catawbiense* Grandiflorum and *R.* Lord Roberts) that were either produced directly from rooted cuttings, or that were grafted onto *R.* Cunningham's White or *R. ponticum* rootstock. A total of 20 leaves per cultivar (from five replicate plants) were tested using the method with non-wounded leaves. Plants were produced by a commercial *Rhododendron* grower starting in November 2006 and were tested in June 2008.

Susceptibility to different *Phytophthora* species

To test whether the susceptibility level to *P. ramorum* was correlated with the susceptibility level to other *Phytophthora* species, the standard assays with wounded and non-wounded leaves were performed twice on 12 cultivars with variable resistance to *P. ramorum*. The same cultivars were used as for the determination of the sporulation characteristics. Tests were performed in the summer of 2008.

Data analyses

The data on the susceptibility of *Rhododendron* leaf material to *P. ramorum* were analysed separately for species and cultivars, given the difference in origin and age of the plants used in these two groups, and the slightly different incubation conditions before inoculation.

Cultivars and species were split into four susceptibility classes based on a regression tree (RT) analysis using the standard settings of the CART© software. RT analysis creates homogeneous groups from an initial population of values, providing cut-off values. Details on the methodology can be found in Speybroeck *et al.* (2004), Saegerman *et al.* (2004), Thang *et al.* (2008) and Yewhalaw *et al.* (2009). Linear relationships (e.g. susceptibility to *P. ramorum* versus susceptibility to *P. citricola*) were calculated using the linear regression model in STATISTICA 8·0 (Statsoft). Comparisons between groups (e.g. different types of rootstock) were conducted using the general linear model in STATISTICA 8·0, using appropriate categorical factors. Susceptibility data from individual leaves (instead of averages) were used in the statistical analysis where possible.

Some responses are expressed as proportions, e.g. the RRLA data. These non-normally distributed outcomes were transformed using the arcsine square root transformation.

Averages are reported together with the corresponding standard error (avg ± sterr).

Results

Susceptibility of different species and cultivars to *P. ramorum*

The inoculation of non-wounded detached leaves of different *Rhododendron* cultivars resulted in a wide range of susceptibility to *P. ramorum*. Using an RT analysis, the cultivars were split into four susceptibility classes (Table 1). The susceptibility of *Rhododendron* species also varied widely when non-wounded leaves were tested, although the overall level of susceptibility was smaller (Table 2). The *Rhododendron* species were also split into four classes based on an RT analysis. In contrast, pathogen growth was observed in all cultivars and all species when using wounded leaves. The difference in susceptibility was also smaller between most cultivars and between most species when using wounded leaves (Tables 1, 2). There was a significant ($P < 0{\cdot}0001$) linear relationship between the susceptibility observed using the two inoculation methods, but the coefficient of determination was low ($R^2 = 0{\cdot}121$). In practice, inoculation of non-wounded leaves was most informative and most relevant for determination of the susceptibility level. Therefore, further reference to host susceptibility relates to the method using non-wounded leaves, unless specifically mentioned otherwise.

Representatives of the elepidote and lepidote *Rhododendron* subgenera were found in all susceptibility classes, but the majority of the lepidote *Rhododendron* cultivars and species (79%) were grouped in the more resistant classes 1 and 2. The three evergreen azalea cultivars (*R. simsii* hybrids) were resistant (class 1) based on the non-wounded method (RLLA < 1·5%). However, when wounded, they developed average-size lesions (13·6–20·3 mm). The representatives of the deciduous azaleas showed intermediate resistance (class 2). In 2005 and 2006, *R.* Mme Masson and *R.* G. Rieger were included as extra controls during each test batch, serving as a susceptible and a resistant reference cultivar, respectively. The differences in susceptibility between the three control cultivars remained stable during the different assays (data not shown).

Table 2 Susceptibility of 22 *Rhododendron* species to *Phytophthora ramorum* based on detached leaf inoculation assays using non-wounded and wounded leaves

		Non-wounded leaves				Wounded leaves			
Rhododendron species	Sub-genus[a]	No.[b]	RLLA[c] (%)	SEM	Class[d]	No.[b]	Lesion diam. (mm)	SEM	Class[d]
russatin	L	65	85·3	1·64	4	30	18·7	0·05	4
dichroantum	E	40	69·9	4·08	3	20	8·5	0·11	2
ponticum	E	60	68·0	3·63	3	30	13·4	0·04	3
wardii	E	60	63·0	2·84	3	30	13·4	0·05	3
campylocarpum	E	40	55·1	4·46	3	20	9·2	0·05	2
catawbiense	E	51	53·7	5·06	3	31	17·7	0·08	4
dichroantum subsp. *scyphocalix*	E	40	53·3	4·13	3	20	10·4	0·02	2
fortunei	E	60	46·9	3·45	3	30	6·4	0·04	1
caucasicum	E	60	22·0	3·12	2	29	11·6	0·10	2
occidentale	DA	63	21·6	2·30	2	30	8·5	0·07	2
molle ssp. *japonicum*	DA	59	20·7	2·70	2	29	7·4	0·04	1
carolinianum	L	68	13·8	2·46	2	20	7·8	0·04	2
campylogynum var. *myrtilloides*	L	68	7·4	1·98	1	34	12·6	0·03	3
racemosum	L	70	4·6	0·89	1	30	14·7	0·11	3
arboreum	E	40	1·7	0·59	1	20	3·9	0·04	1
ambiguum	L	65	1·2	0·30	1	30	9·6	0·04	2
keiskei	L	65	1·1	0·21	1	30	13·1	0·09	3
yakushimanum	E	60	0·7	0·20	1	30	10·5	0·05	2
williamsianum	E	60	0·7	0·09	1	30	7·2	0·04	1
cinnabarinum	L	63	0·4	0·24	1	30	11·8	0·17	2
impeditum	L	65	0·3	0·13	1	30	7·0	0·04	1
insigne	E	60	0·1	0·04	1	29	4·0	0·03	1

[a]The four major subgenera of the *Rhododendron* genus: *Hymenanthes* (elepidote rhododendrons) (E), *Rhododendron* (lepidote rhododendrons) (L), *Tsutsusi* (evergreen azaleas) (EA), and *Penthanthera* (deciduous azaleas) (DA).

[b]Total number of leaves used in the three replicate years. For four species (with n = 40 in the assay with non-wounded leaves), data represents total number of leaves in two replicate years.

[c]RLLA = relative lesioned leaf area (see Material and methods).

[d]Susceptibility class, based on an RT analysis (see Table 1 and Materials and methods).

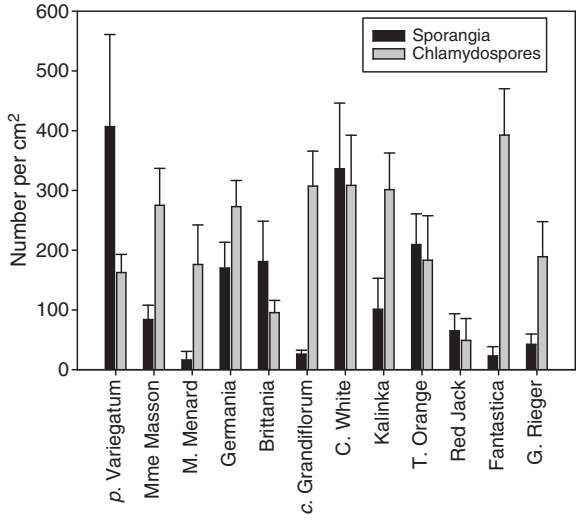

Figure 1 Average number of sporangia and chlamydospores of *Phytophthora ramorum* per cm^2 in 12 *Rhododendron* cultivars. Cultivars were chosen and are sorted based on their different levels of symptom expression in the assay with non-wounded leaves (Table 1). Error bars represent standard errors of the mean.

Sporulation of *P. ramorum* on cultivars with different levels of susceptibility

Production of sporangia and chlamydospores on and in the leaf lesions of the cultivars from the different susceptibility classes is presented in Fig. 1. Sporangia production ranged from 0 to 3384 sporangia cm^{-2}, with an average of 138·6 ± 19·7 and a median of 25·1 sporangia cm^{-2}. Chlamydospore production ranged from 0 to 1484 chlamydospores cm^{-2}, with an average of 231·1 ± 17·1 and a median of 113·1 chlamydospores cm^{-2}. There was no significant linear relationship between the sporangial density and the lesion area (P = 0·88). The linear relationship between chlamydospore density and lesion area was significant (P = 0·032), but the coefficient of determination was low (R^2 = 0·017). There was no significant (P = 0·40) linear relationship between the sporangial density and the chlamydospore density.

Effect of leaf age on susceptibility to *P. ramorum*

Young leaves of *R.* 'Cunningham's White' were consistently, and in most cases, significantly (P < 0·05 for 11 out of 13 time points) less susceptible to *P. ramorum* than mature leaves when inoculated using non-wounded leaves (Fig. 2a, b). Averaged over the two years, the RLLA was 27·0 ± 1·8 and 49·9 ± 1·9% for young and mature leaves, respectively. A different effect was observed when wounded leaves were inoculated (Fig. 2c, d). Until the middle (2007) or end (2006) of June, the diameter of the lesions in young leaves was up to 333% the size of those in mature leaves. These differences were significant at all time points (P < 0·001). In the second part of the season, the young leaves showed lesions with a diameter only as large as or slightly larger than (up to 143%) mature leaves. At four out of six time points, these differences were no longer significant. General linear models that contained the factors leaf age, part of the season (as described above), and the interaction term were significant (P < 0·001) for all factors in both years, which confirmed these observations.

Even more pronounced differences were observed between young and mature leaves when only scoring the number of lesions, and not the lesioned area, in the non-wounded-leaf assay on 15 *Rhododendron* cultivars (including *R.* Cunningham's White) (Table 3). Averaged over the 15 cultivars and representing all susceptibility classes, 93·8% of young leaves had less than 10 lesions,

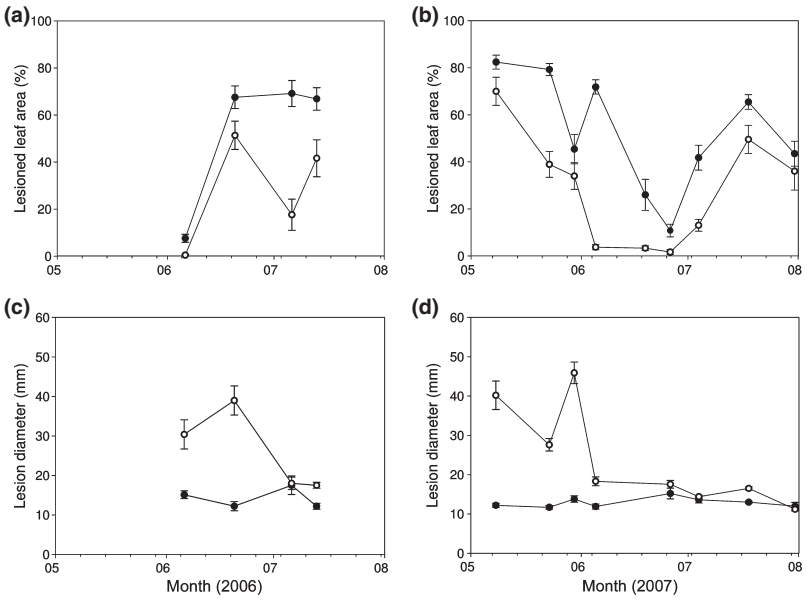

Figure 2 Susceptibility (percentage leaf area with lesions) of young (current year, empty symbols) and mature (previous year, filled symbols) *Rhododendron* leaves cv. Cunningham's White to *Phytophthora ramorum* in 2006 (a, c) and in 2007 (b, d), by inoculating non-wounded leaves (a, b) or wounded leaves (c, d). Error bars represent standard errors of the mean.

Table 3 Infection characteristics and leaf properties of young versus mature leaves of various *Rhododendron* cultivars using a non-wounded inoculation method

			Young leaves			Mature leaves		
Cultivar	RLLA[a]	Class[b]	Drop test[c]	No. leaf hairs	% leaves with <10 lesions per leaf	Drop test[c]	No. leaf hairs	% leaves with <10 lesions per leaf
John Walter	88·4	4	>90	many	95	60–90	many	8
Mme Masson	75·0	3	<30	average	82	<30	average	6
Germania	68·9	3	60–90	average	95	60–90	average	50
Blue Peter	63·0	3	60–90	average	95	60–90	average	35
Goldkrone	61·0	3	>90	average	80	>90	average	5
President Roosevelt	59·4	3	60–90	average	65	60–90	average	15
Roseum Elegans	57·7	3	60–90	average	100	60–90	average	5
c. Grandiflorum	55·5	3	30–60	few	100	60–90	average	40
Rocket	50·0	3	60–90	average	100	60–90	average	65
Cunningham's White	31·9	2	60–90	average	95	60–90	average	25
Kalinka	29·1	2	>90	indumentum	100	>90	indumentum	95
Virginia Richards	13·5	1	>90	average	100	60–90	average	100
Percy Wiseman	9·2	1	>90	many	100	60–90	many	60
Morgenrot	3·9	1	>90	indumentum	100	>90	indumentum	95
Fantastica	3·4	1	>90	indumentum	100	>90	indumentum	100
Average					93·8			46·9

[a]RLLA = Relative Lesioned Leaf Area. Average data per cultivar (see Table 1).

[b]Susceptibility class, based on an RT analysis (see Table 1 and Materials and methods).

[c]Contact angle of water droplets on the leaves (in degrees). Hydrophobic leaf surfaces result in larger contact angles.

while this was only 46·9% for mature leaves. The difference between young and mature leaves was most obvious in the cultivars of the two most susceptible classes 3 and 4 (90·2 versus 25·4% of leaves with less than 10 lesions, respectively), as the proportion of leaves with less than 10 lesions is intrinsically high in the more resistant classes. Although the most susceptible classes showed a more pronounced difference in the number of leaf lesions between young and mature leaves, none of the 15 *Rhododendron* cultivars showed more lesions in the young leaves than in the mature leaves. The results of the general linear model that included the factor susceptibility (RLLA), the categorical factor leaf age, and the interaction factor was significant for the factor susceptibility ($P < 0{\cdot}000001$) and the interaction factor ($P < 0{\cdot}001$). This indicates a significant negative relationship between RLLA and the number of old leaves with few lesions, while this effect was not observed for young leaves.

There was no clear difference in the number of leaf hairs on young versus mature leaves, although for some cultivars, young leaves were slightly more hydrophobic than mature leaves. The number of leaf lesions was not correlated with the number of leaf hairs or the hydrophobicity of the leaf surface, although it was clear that cultivars that possess indumentum (a dense leaf hair covering of the abaxial leaf surface), and are therefore very hydrophobic, are less susceptible to zoospore-mediated infection (Table 3).

Effect of rootstock

Averaged over the four cultivars, the RLLA was very similar when plants were grown directly from cuttings (92·2 ± 0·9%) versus grafted on *R. ponticum* (92·6 ± 1·0%) or grafted on *R.* Cunningham's White (92·4 ± 0·7%) rootstock. As expected, in the general linear model with the categorical variables rootstock and cultivar (and the interaction term), the factor rootstock was not significant ($P = 0{\cdot}83$). However, the interaction term was significant ($P < 0{\cdot}001$) because on Cunningham's White rootstock, cvs Lord Roberts and Grandiflorum had a lower susceptibility than when grown on ponticum rootstock or when grown straight from cuttings, while the opposite was true for cvs John Walter and Mme Masson. The biological relevance of these differences is considered minor.

Susceptibility to different *Phytophthora* species

There were large differences in lesion area among the twelve *Rhododendron* cultivars when using non-wounded leaves of *P. hedraiandra* × *cactorum* or *P. citricola* (Fig. 3). A significant linear relationship was observed between the susceptibility to these *Phytophthora* species and the susceptibility to *P. ramorum* ($R^2 = 0{\cdot}59$, $P = 0{\cdot}004$ and $R^2 = 0{\cdot}81$, $P = 0{\cdot}00006$, respectively). Similarly, there was a significant linear relationship between the susceptibility to *P. hedraiandra* × *cactorum* and *P. citricola* ($R^2 = 0{\cdot}77$, $P = 0{\cdot}0002$). There was no significant ($P = 0{\cdot}10$ to $0{\cdot}13$) linear relationship between the susceptibility to *P. kernoviae* and any of the other *Phytophthora* species tested. However, for most *Rhododendron* cultivars, the necrotic area caused by *P. kernoviae* was small, limiting the ability to determine such a relationship.

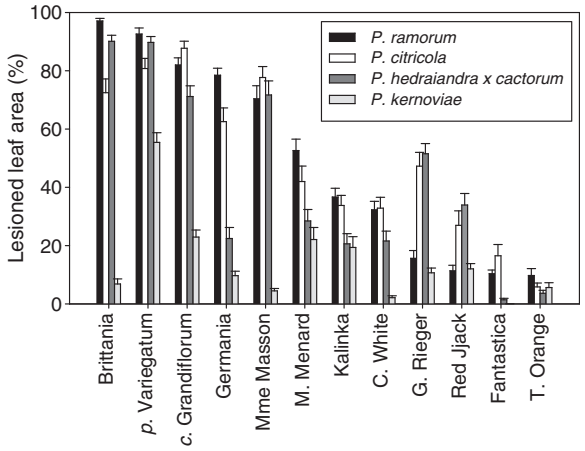

Figure 3 Percentage leaf area with lesions of 12 *Rhododendron* cultivars after separate inoculation with four different *Phytophthora* species. Cultivars were chosen based on their different levels of symptom expression in the main assay with inoculation of non-wounded leaves (see Table 1). They are sorted based on the percentage lesioned leaf area for *P. ramorum* in this assay. Error bars represent standard errors of the mean.

Infection of wounded leaves caused only small differences in lesion diameter between the different *Rhododendron* cultivars, independent of the *Phytophthora* species used (data not shown). Because of this, no biologically relevant correlation coefficients could be calculated. Averaged over the 12 cultivars, *P. citricola* caused the largest lesions (19·0 ± 0·5 mm). Smaller lesions were observed with *P. ramorum* (16·1 ± 0·3 mm) and *P. hedraiandra* × *cactorum* (13·3 ± 0·3 mm), while *P. kernoviae* caused the smallest lesions (8·9 ± 0·2 mm). The lesion diameter was significantly different between each of the four *Phytophthora* species ($P < 0{\cdot}05$), except between *P. ramorum* and *P. hedraiandra* × *cactorum* ($P = 0{\cdot}056$).

Discussion

The effect of the host factors genotype, leaf age and rootstock were tested on *P. ramorum* susceptibility and sporulation in botanical and commercial representatives of the genus *Rhododendron*. A correlation between the host susceptibility to *P. ramorum* and other *Phytophthora* species was also tested for. Two types of detached leaf assays were used. The test using wounded leaves is indicative of the ability of the pathogen to grow inside the host tissue, while the test using non-wounded leaves is mainly indicative of the pathogen's ability to penetrate and infect the host tissue starting from epiphytic zoospores. A preliminary study (De Dobbelaere *et al.*, 2006) also involved inoculation of wounded and non-wounded stems, but these tests provided little additional information. The use of detached plant material instead of whole plants was mostly prompted by biosecurity regulations, but other studies have shown a good correlation between assays on detached versus non-detached leaves (Parke *et al.*, 2002, 2005; Hansen *et al.*, 2005). Considering that *P. ramorum* sporangia (and zoospore) release is water- dependent (Moralejo *et al.*, 2006) and *Rhododendron* plants are frequently irrigated under commercial practice, sporangia and especially zoospore-mediated infection (Widmer, 2009) is probably of primary importance for *Rhododendron*. Although pruning is a common practice in *Rhododendron* culture, and therefore wounded tissue is occasionally created, this is limited in time and usually does not coincide with wet conditions, which are needed for successful infection. Therefore, the inoculation method involving non-wounded leaves is probably the most relevant method for estimating the field susceptibility level. Although the relative susceptibility levels between different *Rhododendron* species was maintained throughout the testing periods, the absolute susceptibility levels can vary from week to week for the method involving non-wounded leaves (data not shown). To compensate for this effect, the assays were repeated in three different years.

The comparison of cultivar and species susceptibility was performed on leaves that were at least 3 months old. Differences in susceptibility between young leaves were much less pronounced than differences between mature leaves, as most young leaves have reduced susceptibility when not wounded.

A single isolate of *P. ramorum* was used throughout this study. Preliminary tests had demonstrated no differences when using two isolates from *Rhododendron* and two isolates from *Viburnum* (data not shown). Tooley & Kyde (2004) also observed similar results with the two isolates they used, even when they belonged to the EU1 versus NA1 lineage of *P. ramorum*. Recent AFLP and SSLP data have confirmed that the isolate used in this study belongs to the main microsatellite genotype in Europe, and that the European population of *P. ramorum* is near-clonal (Vercauteren *et al.*, 2010). Therefore, no isolate-dependent variation is expected.

The inoculation of non-wounded leaves showed considerable variation in susceptibility between the different species and cultivars, as compared to the method with wounded leaves. This would suggest that when resistance is present, it is most likely expressed at the level of tissue penetration. Most of the lepidote rhododendrons, which tend to have scales on their leaves, occur in the more resistant classes 1 and 2. These scales may be direct physical barriers. They also make the leaves very hydrophobic, which probably prevents the zoospores from reaching the leaf surface. Most of the elepidote species were more susceptible to *P. ramorum*. Elepidote species that have an indumentum, such as those from the *Yakushimanum* subgroup (including *Rhododendron* cvs Kalinka, Fantastica and Morgenrot) and the species *R. insigne*, *R. arboreum* and *R. caucasicum* were more resistant. *Rhododendron* species with scales or indumentum also tend to suffer less insect damage than species with glabrous leaf types (Valla, 1980), implying that these leaf structures may have a general protective effect. Not only morphological structures but also physiological products can play a role

in plant defence: glandular scales of lepidote species play a role in plant defence against insects by containing or secreting volatile materials (Clarke & Bell, 1978). The elepidote species *R. smirnovii* and *R. williamsianum* also produce a large amount of the same secretions, whose production can be associated with the leaf hairs (Clarke & Bell, 1978; Doss, 1980, 1984). As some of these compounds may also affect zoospores, the role of glandular secretions on the resistance to *Phytophthora* species deserves more attention.

In the group of the evergreen azaleas (*R. simsii*), very few lesions were observed using the method with non-wounded leaves. Similar results were observed in other studies (Tjösvold *et al.*, 2002; Tooley *et al.*, 2004; Kaminski & Wagner, 2008). The deciduous azaleas were more susceptible than the evergreen azaleas, as was also reported by Tjösvold *et al.* (2002), but the susceptibility level of the deciduous azaleas was still relatively small (RLLA < 22%) in this study.

The considerable level of difference in susceptibility between different cultivars creates opportunities for pathogen management. Grünwald *et al.* (2008) observed cultivar differences within a host species when testing the susceptibility of *Viburnum* species. Cross-checking cultivar susceptibility data with the parentage of the cultivars indicated that inheritance of resistance may be possible. For example, *R.* Mme Masson is a hybrid of two susceptible species and is also highly susceptible, whereas *R.* Fantastica is resistant, and is a hybrid of two resistant species. However, controlled crossing experiments and evaluation of the resistance level in the progeny are needed to determine the inheritance mechanisms. Even if a limited number of genes are responsible for resistance, assessment of the susceptibility level of individual cultivars remains necessary if the resistance genes in the resistant parent(s) are not homozygous. This could for example explain why *R.* Polaris is susceptible, even though one of its parents is resistant.

Although the cultivars and species were grouped into four susceptibility classes, based on a relevant statistical method, this grouping is still somewhat artificial when comparing cultivars or species that are situated at opposite ends of neighbouring susceptibility classes. Most other susceptibility assessment studies provide a similar artificial grouping (Denman *et al.*, 2005c; Kaminski & Wagner, 2008), which does allow straightforward presentation of the data and is useful in pest risk assessments. However, inclusion of reference cultivars allows cross-experimental comparisons.

Using similar methods, Tooley *et al.* (2004) evaluated the susceptibility to *P. ramorum* of 51 ericaceous ornamental hosts, including 31 *Rhododendron* species and cultivars. Four *Rhododendron* species, *R. catawbiense*, and cultivars *R.* Cunningham's White, *R.* Nova Zembla and *R.* Roseum Elegans were also tested in the present study: *R. catawbiense* and *R.* Roseum Elegans were ranked as being relatively more susceptible in this study, while they ranked as relatively less susceptible in the study by Tooley *et al.* (2004). This might indicate that multiyear data may be needed to obtain a reliable ranking of the average susceptibility. If so, it would also indicate that within a given year, the susceptibility rank may be variable for cultivars in classes 2 and 3. In general, Tooley *et al.* (2004) observed a smaller lesion area in the *Rhododendron* species and cultivars they used, which may be due to small differences in inoculation and incubation methods.

Young leaves were less susceptible to *P. ramorum* infection as long as they are not wounded. This confirms the presence of resistance mechanisms at the level of leaf penetration. The hypothesis that young leaves have more leaf hairs and a more hydrophobic leaf surface, which restricts access of the zoospores to the leaf surface, was not supported by the data. A second hypothesis, in which young leaves are associated with the presence of a zoospore-encysting product, possibly in their leaf hairs, deserves further testing. When *P. ramorum* accesses young leaves via wounds, pathogen growth was substantially larger in young leaves than in older leaves possibly because of the succulent nature of young tissue or by a difference in expression of defence mechanisms or nutrient concentrations between growing and older tissue. Meristems are primary sinks of translocated nutrients obtained from storage structures and older leaves (Fife & Nambiar, 1984; Kaitaniemi & Honkanen, 1996). The higher susceptibility of growing tissues is reported for several pests and diseases. Similar effects of leaf age on *P. ramorum* susceptibility of wounded tissue has also been reported with other hosts (Denman *et al.*, 2005b,c; Hansen *et al.*, 2005).

The presence or the nature of the rootstock had no biologically relevant effect. The resistance mechanisms that result in reduction of tissue penetration apparently are not affected by the rootstock.

As in the study of McDonald *et al.* (2006), significant differences were observed in sporangia and chlamydospore production in the necrotic areas of the different cultivars, over a range of approximately 10^1 to 10^3 units cm^{-2}. Denman *et al.* (2006b) also observed sporangia formation on *Rhododendron* in the range of 1×10^1 to 4×10^2 sporangia per leaf lesion, depending on the time of year the leaves were collected. The correlation between the sporulation density and lesion area (or cultivar susceptibility) was low. Therefore, as susceptible cultivars may have a low sporulation density and vice versa, not only the size of the lesion but also the sporulation capacity per surface area should be considered when evaluating the capacity of a cultivar to generate inoculum.

Susceptibility of *Rhododendron* cultivars to three different *Phytophthora* species that are commonly isolated from leaf and twig blight was well-correlated suggesting that resistance mechanisms at the level of pathogen penetration are non-specific. Use of less susceptible cultivars is therefore not only recommended in the framework of *P. ramorum* quarantine measures, but also for the control of other *Phytophthora* species.

Phytophthora kernoviae was included in this study, as it has been listed as a potentially invasive exotic

Phytophthora species (Brasier *et al.*, 2005; Denman *et al.*, 2006a; Tracy, 2009), and in that respect bears resemblance to *P. ramorum*. Only small lesions were produced by the two isolates of *P. kernoviae* on the cultivars tested. The limited lesion area may in part be due to the inability to generate large numbers of zoospores with this species, but lesions were limited even when wounded leaves were inoculated via a mycelium plug. *Phytophthora kernoviae* lesion size on *Rhododendron* cultivars may therefore not be correlated with its pathogenicity on *Rhododendron ponticum* in woodland settings, which is reported to be at least equivalent to that of *P. ramorum* (Denman *et al.*, 2006a). Interestingly, the RLLA of *P. kernoviae* on *R. ponticum* cv. Variegatum was significantly larger than on the other cultivars in the test. Further tests should be conducted to confirm that *P. kernoviae* is more aggressive on *R. ponticum* and its cultivars, as this may affect pest risk assessment of this pathogen.

Based on the assay using wounded leaves, *P. citricola* was the most aggressive of the *Phytophthora* species tested. This species is among the most aggressive *Phytophthora* species worldwide and has been reported in association with hundreds of different plant species (Erwin & Ribeiro, 1996; Balci *et al.*, 2008). It is also the most frequently isolated *Phytophthora* species during Belgian surveys in the framework of the EU mandated emergency measures (data not shown). *Phytophthora hedraiandra* × *cactorum* is a recently described hybrid species (De Cock & Lévesque, 2004). It also causes leaf blight and twig dieback on *Rhododendron*, and is currently isolated more frequently than *P. cactorum* during the EU mandated surveys (data not shown).

The data presented in this paper not only provide information on the nature and extent of the resistance of *Rhododendron* to *P. ramorum* and other *Phytophthora* species but may permit more targeted detection of *P. ramorum* by plant health inspectors, allow nursery owners to better manage *Phytophthora* species and identify resistant parents for future breeding programmes.

Acknowledgements

We are grateful to H. Goossens for providing *Rhododendron* species plant material. We thank C. Matthijs for producing the plants for the rootstock experiment. We are very grateful to T. Van Vooren for her excellent technical assistance, and to B. Gehesquière for his help with the sporulation assays. This research was partly supported by the 'ramorum' grant and the NRL agreement10-ILVOC-RA-PLANTEN from the Belgian Federal Agency for the Safety of the Food Chain (FAVV) and grant LO-040692 from the Institute for the Promotion of Innovation by Science and Technology in Flanders (IWT).

References

APHIS, 2008. *APHIS List of Regulated Hosts and Plants Associated with* Phytophthora ramorum. United States Department of Agriculture Animal and Plant Health Inspection Service. [http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/downloads/pdf_files/usdaprlist.pdf].

Balci Y, Balci S, McDonald WL, Gottschalk KW, 2008. Foliar susceptibility of eastern oak species to *Phytophthora* infection. *Forest Pathology* **38**, 320–31.

Beales PA, Schlenzig A, Inman AJ, 2004. First report of ramorum bud and leaf blight (*Phytophthora ramorum*) on *Syringa vulgaris* in the UK. *Plant Pathology* **53**, 525.

Brasier CM, Denman S, Rose J *et al.*, 2004. First report of ramorum bleeding canker on *Quercus falcata*, caused by *Phytophthora ramorum*. *Plant Pathology* **53**, 804.

Brasier CM, Beales PA, Kirk SA, Denman S, Rose J, 2005. *Phytophthora kernoviae* sp nov., an invasive pathogen causing bleeding stem lesions on forest trees and foliar necrosis of ornamentals in the UK. *Mycological Research* **109**, 853–9.

Brewer CA, Smith WK, Vogelmann TC, 1991. Functional interaction between leaf trichomes, leaf wettability and the optical properties of water droplets. *Plant Cell and Environment* **14**, 955–62.

Bruggeman A, 2004. Rhododendron market in Europe. *International Azalea and Rhododendron Conference*. Kruishoutem, Belgium.

Chamberlain D, Hyam R, Argent G, Fairweather G, Walter KS, 1996. *The Genus* Rhododendron *: It's Classification & Synonymy*. Edinburgh, UK. Royal Botanic Garden, Edinburgh.

Clarke RG, Bell HT, 1978. Resistance among Rhododendron species to obscure root weevil feeding. *Journal of Economic Entomology* **71**, 869–70.

Davidson JM, Werres S, Garbelotto M, Hansen EM, Rizzo DM, 2003. Sudden oak death and associated diseases caused by *Phytophthora ramorum*. *Plant Health Progress* Online [http://www.plantmanagementnetwork.org/pub/php/diagnosticguide/2003/sod/.

De Cock AWAM, Lévesque CA, 2004. New species of *Pythium* and *Phytophthora*. *Studies in Mycology* **50**, 481–7.

De Dobbelaere I, Heungens K, Maes M, 2006. Susceptibility levels of *Rhododendron* species and hybrids to *Phytophthora ramorum*. In: Frankel SJ, Shea PJ, Haverty MI, eds. *The Sudden Oak Death Second Science Symposium: The State of Our Knowledge, 2005*. Albany, NY, USA: USDA PSW-GTR-196, 79–81.

De Gruyter J, Steegs MHCG, 2008. Managing *Phytophthora ramorum* in the Netherlands. *EPPO Bulletin* **36**, 399–401.

Denman S, Kirk SA, Brasier CM, Barton VC, Hughes KJD, Webber JF, 2005a. *Phytophthora ramorum* on *Quercus ilex* in the United Kingdom. *Plant Disease* **89**, 1241.

Denman S, Kirk SA, Brasier CM *et al.*, 2005b. Foliar infection of sweet chestnut (*Castanea sativa*) by *Phytophthora ramorum* in the UK. *Plant Pathology* **54**, 581.

Denman S, Kirk SA, Brasier CM, Webber JF, 2005c. *In vitro* leaf inoculation studies as an indication of tree foliage susceptibility to *Phytophthora ramorum* in the UK. *Plant Pathology* **54**, 512–21.

Denman S, Kirk S, Whybrow A, Orton E, Webber JF, 2006a. *Phytophthora kernoviae* and *P. ramorum*: host susceptibility and sporulation potential on foliage of susceptible trees. *EPPO Bulletin* **36**, 373–6.

Denman S, Orton E, Kirk S, Brasier C, 2006b. Sporulation potential of *Phytophthora ramorum* on detached leaves of some susceptible UK trees *in vitro*. In: Brasier C, Jung T, Oßwald W,

eds. *Progress in Research on Phytophthora Diseases of Forest Trees. Proceedings of the Third International IUFRO Working Party S07.02.09*. Farnham: Forest Research, 75–8.

Doss RP, 1980. Investigation of the bases of resistance of selected *Rhododendron* species to foliar feeding by the obscure root weevil (*Sciopithes obscurus*). *Environmental Entomology* **9**, 549–52.

Doss RP, 1984. Role of glandular scales of lepidote rhododendrons in insect resistance. *Journal of Chemical Ecology* **10**, 1787–97.

Erwin DC, Ribeiro OK, 1996. *Phytophthora Diseases Worldwide*. St. Paul, MN, USA: The American Phytopathological Society.

Fife DN, Nambiar EKS, 1984. Movement of nutrients in radiata pine needles in relation to the growth of shoots. *Annals of Botany* **54**, 303–14.

Grünwald NJ, Gos EM, Ivors K *et al.*, 2009. Standardizing the nomenclature for clonal lineages of the sudden oak death pathogen, *Phytophthora ramorum*. *Phytopathology* **99**, 792–5.

Grünwald NJ, Kitner M, McDonald V, Goss EM, 2008. Susceptibility in *Viburnum* to *Phytophthora ramorum*. *Plant Disease* **92**, 210–4.

Hansen EM, Parke JL, Sutton W, 2005. Susceptibility of Oregon forest trees and shrubs to *Phytophthora ramorum*: a comparison of artificial inoculation and natural infection. *Plant Disease* **89**, 63–70.

Ivors K, Garbelotto M, Vries IDE *et al.*, 2006. Microsatellite markers identify three lineages of *Phytophthora ramorum* in US nurseries, yet single lineages in US forest and European nursery populations. *Molecular Ecology* **15**, 1493–505.

Kaitaniemi P, Honkanen T, 1996. Simulating source-sink control of carbon and nutrient translocation in a modular plant. *Ecological Modelling* **88**, 227–40.

Kaminski K, Wagner S, 2008. *In vitro* inoculation studies for estimating the susceptibility of ornamental plants to *Phytophthora ramorum*. *Journal of Phytopathology* **156**, 480–6.

Lane CR, Beales PA, Hughes KJD, Tomlinson JA, Inman AJ, Warwick K, 2004. First report of ramorum dieback (*Phytophthora ramorum*) on container-grown English yew (*Taxus baccata*) in England. *Plant Pathology* **53**, 522.

Leslie AC, 2004. *The International Rhododendron Register and Checklist*. 2nd edn. London, UK: The Royal Horticultural Society.

McDonald V, Grünwald NJ, Linderman R, 2006. Evaluation of infection potential and sporulation of *Phytophthora ramorum* on five *Rhododendron* cultivars. *Phytopathology* **96**, S75.

Meentemeyer RK, Rank NE, Shoemaker DA *et al.*, 2008. Impact of sudden oak death on tree mortality in the Big Sur ecoregion of California. *Biological Invasions* **10**, 1243–55.

Moralejo E, Garcia Munoz JA, Descals E, 2006. *Phytophthora ramorum* sporulation: epidemiological and evolutionary implications. *EPPO Bulletin* **36**, 383–8.

Parke JL, Linderman R, Hansen EM, 2002. Assessing the susceptibility of Pacific Northwest nursery plants to *Phytophthora ramorum* using a detached leaf assay. *Sudden Oak Death First Science Symposium: The State of Our Knowledge, Monterey, CA, USA, 15 December, 2002*.

Parke JL, Roth ML, Choquette DJ, 2005. Detached leaf assays with *Phytophthora ramorum*: are they valid? *Sudden Oak Death Second Science Symposium: The State of Our Knowledge, Monterey, CA, USA, 18 January, 2005*.

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.

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.

Saegerman C, Speybroeck N, Roels S, Vanopdenbosch E, Thiry E, Berkvens D, 2004. Decision support tools for clinical diagnosis of disease in cows with suspected bovine spongiform encephalopathy. *Journal of Clinical Microbiology* **42**, 172–8.

Speybroeck N, Berkvens D, Mfoukou-Ntsakala A *et al.*, 2004. Classification trees versus multinomial models in the analysis of urban farming systems in Central Africa. *Agricultural Systems* **80**, 133–49.

Thang ND, Erhart A, Speybroeck N *et al.*, 2008. Malaria in central Vietnam: analysis of risk factors by multivariate analysis and classification tree models. *Malaria Journal* **7**, 28.

Tjösvold SA, Koike ST, Davidson JM, Rizzo DM, 2002. Susceptibility of Azalea (Rhododendron) to *Phytophthora ramorum*. *Sudden Oak Death First Science Symposium: The State of Our Knowledge, Monterey, CA, USA, 15 December, 2002*.

Tooley PW, Kyde KL, Englander L, 2004. Susceptibility of selected ericaceous ornamental host species to *Phytophthora ramorum*. *Plant Disease* **88**, 993–9.

Tracy DR, 2009. *Phytophthora ramorum* and *Phytophthora kernoviae*: the woodland perspective. *EPPO Bulletin* **39**, 161–7.

Valla DM, 1980. *An Evaluation of Resistance to the Black Vine Weevil* Otiorhynchus sulcatus *(F.), in* Taxus *and* Rhododendron. Kingston, RI, USA: University of Rhode Island, MSc thesis.

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* (in press).

Werres S, Marwitz R, 1997. Unbekannte *Phytophthora*. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz* **108**, 113–20.

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.

Widmer TL, 2009. Infective potential of sporangia and zoospores of *Phytophthora ramorum*. *Plant Disease* **93**, 30–5.

Yewhalaw D, Legesse W, Van Bortel W *et al.*, 2009. Malaria and water resource development: the case of Gilgel-Gibe hydroelectric dam in Ethiopia. *Malaria Journal* **8**, 21.