Characterization of European and American *Phytophthora ramorum* Isolates due to their Morphology and Mating Behavior *in vitro* with other heterothallic *Phytophthora* species

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Introduction

The heterothallic *Phytophthora ramorum* (Werres, de Cock, Man in´t Veld) is a severe pathogen on hardy ornamentals and trees in Europe and in North America. Since its first detection and description (Werres et al. 2001), many isolates have been collected and identified. Initial studies indicated that there was genetic and phenotypic variation between the European and North American *P. ramorum* populations (Brasier & Kirk 2004, Hansen et al. 2003, Ivors et al. 2004). To date, however, there have been not many detailed morphological studies with a wide range of isolates from both continents and none with regard to differences between the two mating groups. In the presented studies *P. ramorum* isolates were characterized by morphology and their mating behaviour *in vitro* with other heterothallic *Phytophthora* species.

Material and Methods

- 94 *P. ramorum* isolates, 67 from Europe and 27 from North America were examined. Isolates from Europe were from *Rhododendron* (48), from *Viburnum* sp. (18) and from *Quercus rubra* (1). The North American isolates came mainly from *Quercus* sp. (5), *Lithocarpus densiflora* (3), *Umbellularia californica* (3), *Arbutus menziesii* (2) and *Sequoia sempervirens* (1) but also from *Rhododendron* sp. (4 from Canada, four from the USA), from *Viburnum* sp. (1) and from *Vaccinium* sp. (2). One isolate originated from a soil sample.
- Colony pattern, vegetative growth rate, sporangium and chlamydospore size and mating behavior were measured and calculated according to Werres et al. (2001).
- Statistical calculation was with t-test and Mann-Whitney Rank Sum Test (p<=0.05).

Results

1 - Vegetative growth, sporangia and chlamydospores

- The A1 isolates showed no distinct **colony pattern** but the A2 isolates showed more compact colony morphology, a flattening colony edge, or grew with sectors (Fig. 1).
- **Vegetative growth** rate at optimum temperature was significantly higher for A1 than for A2 isolates (Fig. 2). The single European A2 isolate (BBA 26/02) grew as fast as the A1 isolates (3.4 mm d^{-1}).
- There was no significant difference between **sporangia size** and **L:B ratio** of A1 and A2 isolates.
- **Chlamydospore size** of A1 isolates was significantly higher than that of A2 isolates. Only the single A2 isolate from Europe produced chlamydospores as big as A1 isolates (mean 56.4 µm) (Fig. 2).

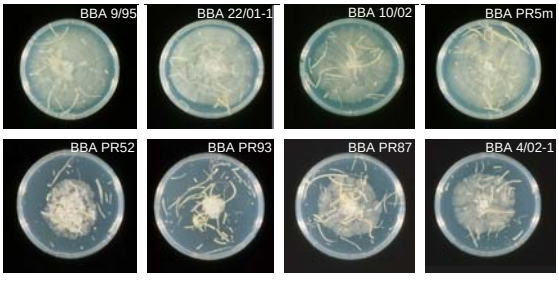
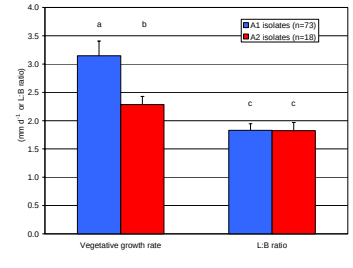


Fig.1. Example for colony pattern of A1 (upper row) and A2 isolates (lower row)



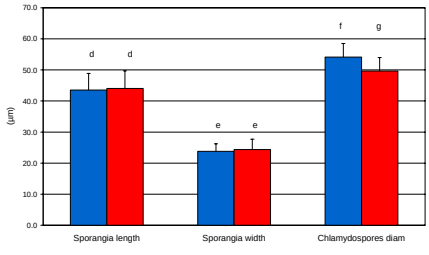


Fig.2.

Vegetative growth rate, sporangia and chlamydospore size (p<= 0.05)

2 – Mating on Carrot Piece Agar (20°C, incubation period 6 wk)

- All isolates could be paired successfully with the tester strains, except three isolates from the USA.
- All **European isolates** paired with heterothallic *Phytophthora* species of mating type A2, except the Belgian isolate BBA 26/02. Seven **North American isolates** (including the four Canadian isolates from nurseries) could be determined as mating type A1, 17 as mating type A2.
- The most **successful mating partner** was *P. cryptogea* (for 79.2 % of all isolates), followed by *P. cinnamomi* (50%), *P. drechsleri* (17.3 %) and *P. cambivora* (13.6 %).
- There was a clear **separation between isolates of mating type A1 and A2**: None of the A1 isolates could be paired with *P. cambivora* while 83.3 % of the 18 A2 isolates accepted this mating partner, also the **single European A2 isolate** (BBA 26/02) . On the other hand 68.5 % of the 73 A1 isolates accepted *P. cinnamomi* while only 27.8 % of the A2 isolates produced gametangia with this species.

Source	Isolate no./ No. of isolates	Mating partners (mating type A1)			
		P. cambivora (BBA21/95-KII)	*P. cinnamomi* (BBA69094)	*P. cryptogea* (BBA65909)	*P. drechsleri* (BBA65172)
Europe (Belgium)	BBA 26/02	X [a]	X	X	X
USA	17	14 [b]	4	15	7 [b]

Source	Isolate no./ No. of isolates	Mating partners (mating type A2)			
		P. cambivora (BBA20/95-2bIII)	*P. cinnamomi* (BBA62660)	*P. cryptogea* (BBA63651)	*P. drechsleri* (BBA62679)
Europe	66	0	46	66	9
Canada	4	0	3	3	1
USA	3	0	1	3 [a]	1

a) X = succesfull mating b) one isolate with only a single degenerated oospore

Summary

There were significant differences between *P. ramorum* isolates of mating type A1 and A2. The A1 isolates showed a homogenous colony pattern, grew faster and had bigger chlamydospores than the A2 isolates. Furthermore none of them produced oogonia with *P. cambivora*. The A2 isolates were much more heterogenic and most of them accepted *P. cambivora* as mating partner. The morphological character of the single A2 isolate from Europe (BBA 26/02) was similar to the A1 isolates, the mating behavior was similar to that of the A2 isolates.

Differences within the studied *P. ramorum* population seemed to be more influenced by the mating type than by the source (e.g. nursery, forest) of the isolate.

Literature:

Brasier C.M., S. Kirk (2004): Production of gametangia by *Phytopthora ramorum in vitro*. Mycol. Res. 108: 823-827.

Ivors K.L., Hayden K.J., Bonants P.J.M., Rizzo D.M., M. Garbelotto (2004): AFLP and phylogenetic analysis of North American and European populations of *Phytophthora ramorum*. Mycol. Res. 108(4): 378-392.

Hansen E.M., Reeser P.W., Sutton W., L.M. Winton (2003): First report of A1 mating type of *Phytophthora ramorum* in North America. Plant Disease 1267.

Werres S., R. Marwitz, W.A. Man in `t Veld, A.W.A.M. de Cock, P.J.M. Bonants, M. de Weerdt, K. Themann, E. Ilieva, R.P. Baayen, 2001: *Phytophthora ramorum* sp. nov., a new pathogen on Rhododendron and *Viburnum*. Mycl. Res. 105(10): 1155-1165.