

# Phenotypic variation in *Phytophthora ramorum*: wild type vs non-wild type isolates

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## Abstract

Phenotypic characteristics of four *P. ramorum* isolates with atypical culture morphology (non-wild type, *nwt*) were compared with four "wild type" (*wt*) isolates using material from stock cultures and after re-isolation from lesions on inoculated Rhododendron leaves. Our preliminary results show that *nwt* isolates were more variable than *wt* isolates in all of the characters tested, and were generally lower in aggressiveness, chlamydo-spore production, and growth rate at all temperatures for both the original culture and when re-isolated from a host.

## Introduction

In earlier studies unusual culture morphology (Figure 1) and behavior were noticed among some NA1 isolates of *Phytophthora ramorum*. This "non-wild type" behavior was not observed in our collection of isolates from the EU1 or NA2 lineages, even though the isolates had been in culture for a similar amount of time. It has been suggested that subculturing *in vitro* cause culture instability and loss of virulence, and passage through the host can revive the isolate back to its original state. To study this, we compared four less virulent isolates (non-wild type; *nwt*) with four isolates of normal virulence (wild type; *wt*) in our culture collection. One objective of this study was to determine whether *wt* behavior could be restored to *nwt* isolates of *P. ramorum* by successive re-isolation from host material.

## Methods

Eight isolates of *P. ramorum* were selected (Table 1) and maintained on 15% V8 agar. Phenotypic characters examined on original cultures were pathogenic aggressiveness, growth rate at maximum, optimum, and minimum temperatures, and chlamydo-spore production *in vitro*.

Detached leaves of *Rhododendron* "Cunningham's White" were inoculated with each of the isolates and lesion size measured using APS ASSESS, and then *P. ramorum* was isolated from lesions onto PARP and transferred to 15% V8 agar. These re-isolates were inoculated onto rhododendron leaves and re-isolated two more times, for a total of three successive re-isolations.

Growth rate at maximum, optimum, and minimum temperatures, and chlamydo-spore production were measured on cultures from the original and first re-isolation for each isolate.



Figure 1. Isolates of *Phytophthora ramorum* grown on 15% V8 agar for two weeks. A. Representatives of the three clonal lineages NA1, NA2, and EU1. These have "wild type" morphology, with uniform growth rates and texture. B. NA1 isolates showing wild type (5046) and non-wild type morphology (5067 and 5061). Notice the differences in texture, growth rate, and sectoring in the *nwt* colonies when compared to *wt*. Non-wild type behavior was only observed in isolates from the NA1 lineage.

Table 1. Isolates of *P. ramorum* used in the study. Clonal lineage was determined by PCR-RFLP of the Cox1 mtDNA and *wt/nwt* was determined from colony morphology and lesion size on detached *Rhododendron* leaves in an earlier study.

Isolate	Strain number	Host	Type	Year isolated
5041	Pr-102	<i>Quercus agrifolia</i>	NA1 <i>nwt</i>	2004
5058	WSDA 4175	<i>Rhododendron</i> spp.	NA1 <i>nwt</i>	2004
5061	WSDA 1839	<i>Rhododendron</i> spp.	NA1 <i>nwt</i>	2003
5067	Pr 106	<i>Umbellularia californica</i>	NA1 <i>nwt</i>	?
5073	RHCC 23	<i>Rhododendron</i> spp.	NA2 <i>wt</i>	2005
5074	RHCC 4	<i>Rhododendron</i> spp.	NA2 <i>wt</i>	2005
5046	2339	<i>Lithocarpus densiflorus</i>	NA1 <i>wt</i>	2003
5039	03-74-D12-A	<i>Viburnum plicatum</i>	EU1 <i>wt</i>	2003

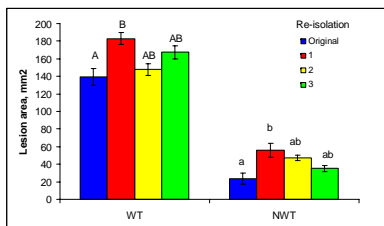


Figure 2. Differences in lesion size formed by *wt* and *nwt* isolates of *P. ramorum* on *Rhododendron* "Cunningham's White" leaves. Significant differences were found between *wt* and *nwt* isolates for all re-isolations, and between re-isolations within *wt* and *nwt* only for the original culture and the first re-isolation. Groups with the same letter are not significantly different (ANOVA, SNK multiple comparisons,  $p < 0.001$ ).

## Results

In both *wt* and *nwt* groups, there were significant differences in lesion size on detached rhododendron leaves between the original culture and the first re-isolation (Figure 2). Successive re-isolations were not different from the original culture and the first re-isolation. After re-isolation from the host, *nwt* isolates were still less aggressive than *wt* isolates. Along with lower aggressiveness on rhododendron leaves, *nwt* isolates produced fewer chlamydo-spores in V8 agar than did *wt* isolates (Figure 4). There was no difference in growth rate between the original culture and the first re-isolation for most isolates. However, *nwt* isolates were found to be more sensitive to temperatures below 2°C and above 28°C (Figures 5 and 6). The optimum growth temperature was 20°C for both *wt* and *nwt* isolates.

Non-wild type isolates were more variable than wild type in all characters tested. The greater variability suggests that these isolates are unstable or that slightly deleterious mutation(s) have accumulated in accordance with Muller's ratchet resulting in reduced fitness. Wild type isolates performed better than non-wild type isolates in all of the phenotypic characters examined. Why *nwt* survives and proliferates is still a mystery. To understand the cause of these phenotypic differences, the role of cytoplasmic elements and differences in mitochondrial and nuclear DNA are being examined. Further studies will also include examining sporulation of *wt* and *nwt* isolates on plant hosts.

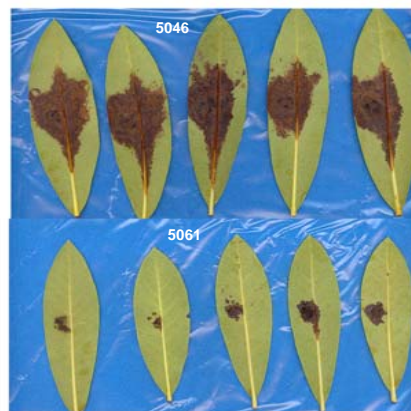


Figure 3. Lesions formed by wild type (5046, upper) and non-wild type (5061, lower) isolates of *P. ramorum* on *Rhododendron* "Cunningham's White" leaves after the third re-isolation.

## Acknowledgements

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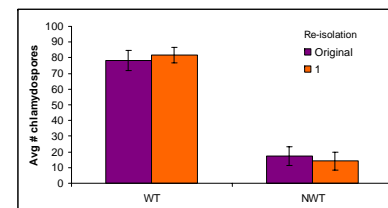


Figure 4. Chlamydo-spore production by *wt* and *nwt* isolates of *P. ramorum* in 15% V8 agar culture. There was no difference in number of chlamydo-spores produced between the original cultures and the first re-isolation from host material. However, *wt* and *nwt* were significantly different with *wt* cultures producing more chlamydo-spores than *nwt* cultures.  $P < 0.001$ , t-test.

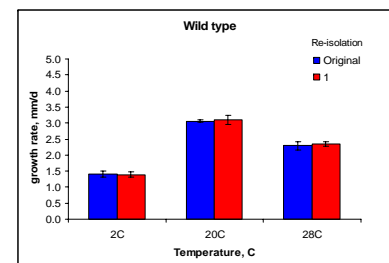


Figure 5. Growth rates for wild-type *P. ramorum* isolates at three temperatures. There was no difference in growth rate between the original cultures and the first re-isolation from host material.

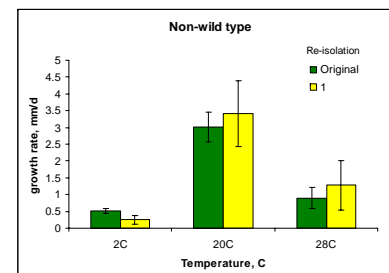


Figure 6. Growth rates for non-wild type *P. ramorum* isolates at three temperatures. As with *wt* isolates, there was no difference in growth rate between the original cultures and the first re-isolation. There was more variability among *nwt* isolates than among *wt* isolates and these isolates were more inhibited by temperature extremes than were *wt* isolates.