Phenotypic Variation in *Phytophthora ramorum*: Wild Type vs Non-Wild Type Isolates[1]

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Abstract

Phenotypic characteristics of four *Phytophthora 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, chlamydospore 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 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* causes 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 and maintained on 15 percent V8 agar. Phenotypic characters examined on original cultures were pathogenic aggressiveness; growth rate at maximum, optimum, and minimum temperatures; and chlamydospore production in vitro. Detached leaves of *Rhododendron* "Cunningham's White" were inoculated with each of the isolates and lesion size measured using APS ASSESS,

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and then *P. ramorum* was isolated from lesions onto PARP and transferred to 15 percent 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 chlamydospore production were measured on cultures from the original and first re-isolation for each isolate.

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. 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 chlamydospores in V8 agar than did *wt* isolates. 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. The optimum growth temperature was 20 °C for both *wt* and *nwt* isolates.

Non-wild type isolates were more variable than *wt* 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. *Wt* isolates performed better than *nwt* 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.

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