Virulence, Sporulation, and Elicitin Production in Three Clonal Lineages of *Phytophthora ramorum*[1]

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Abstract

Phytophthora ramorum populations are clonal and consist of three clonal lineages: EU1 is the only lineage found in Europe with a few isolated nursery infections in the USA; NA1 is associated with natural infestations in California and Oregon as well as some nursery infections in North America, and NA2 has a limited distribution and has only been isolated from a few nurseries in the USA. Recent studies have shown that the clonal lineages may have varying degrees of aggressiveness on some host species, such as *Quercus rubra*. In this study, we examined virulence, sporulation, and elicitin production of five *P. ramorum* isolates from each of the three clonal lineages. Virulence (lesion size) and sporulation (sporangia production) were determined on inoculated detached leaves of rhododendron 'Nova Zembla'. Elicitin production was determined *in vitro*.

Lesion area differed between the clonal lineages ($p < 0.001$). Leaves inoculated with EU1 and NA2 isolates had significantly greater lesion area than NA1-inoculated leaves (approximately 4.2, 3.6, and 0.8 cm^2 respectively). Similarly, sporangia production was greatest in the EU1 and NA2 isolates compared to the NA1 lineage; however, considerable variation was noted between trials. In trial one, sporangia production was very low and there was no difference between clonal lineages ($p = 0.20$); however, there were significant differences among isolates ($p = 0.04$). Sporangia production was much greater in trial two than in trial one, likely because the leaves were misted more frequently in the 1 to 2 days before processing to increase sporulation. In trial two, there were significant differences among clonal lineages and isolates ($p = 0.0003$). Lineages EU1 and NA2 produced significantly more sporangia per leaf ($p < 0.001$) than did lineage NA1 (approximately 800, 1000, and 300 sporangia per leaf respectively).

Real-time PCR assays detected expression of both Class I elicitins (Ram-α1 and Ram-α2) in all 15 isolates. Of the two elicitins, only the Ram-α2 differed between lineage ($p = 0.000$) with nearly 2-fold higher levels of expression in the EU1 and NA2 lineages as compared to the NA1 lineage. Ram-α2 expression showed a positive linear relationship with isolate virulence or lesion size ($r^2 = 0.71$). The significant, positive, linear relationship was also observed between Ram-α2 expression and sporulation although it was not as strong ($r^2 = 0.21$).

In conclusion, isolates belonging to clonal lineages EU1 and NA2 are generally more virulent, produce more sporangia, and produce more Ram-α2 elicitin *in vitro* than do isolates belonging to lineage NA1. This suggests that Ram-α2 may contribute to the fitness of *P. ramorum*. Further studies are needed to determine what quantities of Ram-α2 are produced *in planta* and any additional factors that may directly influence its activity.

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