Needle disease strategy Research Programme

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Minimum *Phytophthora pluvialis* zoospore concentration for red needle cast infection *in planta*

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EXECUTIVE SUMMARY

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The problem

Artificial inoculation methods for infecting *Pinus radiata* needles with *Phytophthora pluvialis* have been developed. The minimum zoospore concentration needed to get reliable infection using a detached needle assay has been investigated but has not been tested *in planta.* Knowing this lower limit will allow researchers to substantiate findings from artificial inoculation assays, contribute to our understanding the minimum zoospore levels required for field infection to occur and the biology of the organism.

This project

The objective of this study was to determine the minimum *P. pluvialis* zoospore concentration required to get infection *in planta*. Needles from three different *Pinus radiata* clones were inoculated with four different zoospore concentrations plus a control, using 10 ramets per clone per treatment. Needles were scored for lesions after 11 days incubation. The average lesion length and average lesion number were analysed using a generalised nonlinear least-squares models and pair-wise comparisons between clones were performed.

Key Results

All three clones were able to be successfully infected with *P. pluvialis in planta*. An exponential correlation between disease and zoospore concentration was observed with high *P. pluvialis* zoospore concentration required to achieve high levels of infection. There was a significant difference in infection between clones.

For both lesion length and number there is a threshold concentration between 200 and 2000 zoospores per ml where infection became exponential. Based on these results it would not be recommended to undertake artificial inoculations with concentrations using 200 or fewer zoospores per ml. From a risk assessment perspective, these results show that while infection could occur at all zoospore concentrations, the impact of inoculation at concentrations of 200 zoospores per ml or below is minimal.

Implications of Results for Client

Research into the control of the disease red needle cast (RNC) caused by the foliar pathogen *P. pluvialis*, depends on artificial inoculation to test treatment efficacy because of the risk of relying on the vagaries of weather and natural inoculum for disease development. For artificial inoculations, knowing what zoospore concentrations can produce reliable results helps researchers validate findings and maximise operational screening of products being tested. This research is a first step towards determining a zoospore concentration threshold for *in planta* artificial inoculations.

The results are important for understanding RNC in the field and how disease severity could be reduced through control methods that target a reduction in inoculum production. As RNC lesions developed at very low zoospore concentrations it is unlikely any product would provide near total control. However, the significant difference in lesion length at concentrations below 200 zoospores per ml suggests inoculum density has an impact on disease severity and it is likely that reducing inoculum density would result in effective control.

Further Work

Results from this *in planta* assay and from previous zoospore concentration experiment using detached needle assays need to be analysed and compared to validate the detached needle assay results as well as the minimum zoospore concentrations required for infection.

This *in planta* assay has further reduced the threshold concentration where exponential infection occurs. In the detached needle assay the threshold was found to be between 5000 to 200 zoospores per ml and this has been reduced to between 2000 to 200 zoospores per ml. Further testing is required to further narrow this range, testing concentrations between 200 to 2000 zoospores per ml would be recommended.

Further testing and research into the physiological differences between host material of different ages and on different age needles is also required to elucidate any influence this has on disease progression and expression. Determining what mechanisms are responsible for the lesion formation changes observed between the different zoospore concentrations tested, and also for concentrations above 5000 zoospores per ml, would also be recommended.

Finally, understanding dose response to zoospore inoculation would also be recommended. In particular, comparing the effect of one high concentration inoculum exposure to multiple, low concentration inoculum exposures, to further understand the effect of zoospore concentration on disease expression,