DEVELOPMENT OF
A NOVEL APPROACH OF ATTENUATING

FUNGAL DISEASE IN PINE TREES

FEATURE

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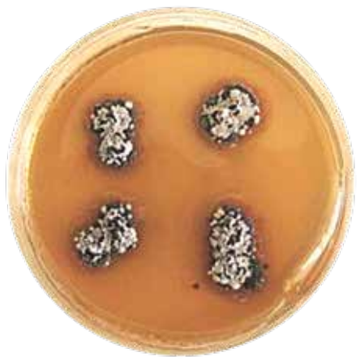
A devastating fungal disease of pine trees is causing major problems to the UK forestry industry. The disease, called Dothistroma needle blight (DNB) or red band needle blight, is caused by the fungus *Dothistroma septosporum* and was first described in Tanzania in the mid-20th century. Until the 1990s, DNB was mainly known for its devastating effects on the health of plantation pine forests in the southern hemisphere, particularly in New Zealand, Australia, and Africa (Bradshaw et al., 2019). Now severe DNB epidemics are a global phenomenon, with widespread damage and death of native and plantation pines, especially in Canada and Europe. In the UK, *D. septosporum* has been found on a range of conifer species and has emerged as a major threat to radiata pine, Corsican pine, lodgepole pine and Scots pine. Defoliation can continue annually and gradually weaken trees, significantly reducing timber yields and can result in tree death. In the UK, control of DNB relies on the use of pines resistant to the disease and good forest management such as thinning of trees to reduce humidity and therefore *D. septosporum* infection. To date, no chemical fungicides are approved for use in UK forests.



D. septosporum growing on 2% malt extract agar showing characteristic production of red dothistromin pigment (photograph Irene Barnes).

Mycoviruses naturally infect and spread within fungal populations, and they do not kill their host or cause harm but may reduce the virulence of their host – this phenomenon is termed hypovirulence. In the 1970s, a mycovirus called Cryphonectria hypovirus 1 was used to combat chestnut blight, another devastating tree disease caused by the fungus *Cryphonectria parasitica*, essentially rescuing the European chestnut forests. We believe that a similar approach can be utilised to combat DNB. The aim of our study was to identify for the first time mycoviruses causing hypovirulence in *D. septosporum*. For this investigation colleagues from Forest Research, Katherine Tubby and James Snowden, supplied us with a collection of UK *D. septosporum* isolates, originally assembled by Anna Brown of the Forestry Commission, and they facilitated the pathogenicity trials on saplings performed under controlled environmental conditions.

Screening *D. septosporum* isolates, we discovered that eight out of nine *D. septosporum* isolates contained double-stranded (ds) RNA, diagnostic for a mycovirus in an established viral family, the *Chrysoviridae* (Kotta-Loizou et al, 2020). The mycovirus infecting *D. septosporum* isolate D752.1 from West Argyll, Scotland, was cloned, sequenced, and nominated Dothistroma septosporum chrysovirus (DsCV)-1, the first mycovirus found in *D. septosporum* (Daudu et al, 2019). DsCV-1 was widespread in the Scottish *D. septosporum* population and present in exemplar isolates from Inverness, Ross and Skye, Moray and Aberdeenshire, and Cowal and Trossachs. Conversely, DsCV-1 was not detected in one *D. septosporum* isolates from Southern England. A DsCV-1-free D752.1 isolate was generated from the DsCV-1-infected, wild-type D752.1 isolate for comparisons of phenotype, growth, and virulence between the isogenic lines. This process ensured that any observed difference between the isolates is caused by DsCV-1.

Two independent pathogenicity trials were conducted using two-year old Corsican pine (*Pinus nigra* subsp. *laricio*) saplings. We compared the virulence and severity of DNB elicited on pine saplings by a panel of *D. septosporum* isolates including the DsCV-1-infected and DsCV-1-free isogenic lines of D752.1. Inoculated saplings were maintained under controlled environmental conditions of warmth, high humidity, and high leaf wetness for optimal fungal growth. Up until now similar investigations performed in a single pine species artificially inoculated with *D. septosporum* under controlled environmental conditions were limited. Virulence was measured using non-destructive image analysis and molecular assays, including quantitative polymerase chain reaction amplification of selected genes. For the latter, a qualitative diagnostics assay for the detection of *D. septosporum* infection in pine needles, already available and routinely used by Forest Research, was converted to a quantitative diagnostics assay.

The trials revealed that the DsCV-1-free D752.1 isolate tends to be more virulent as compared to the DsCV-1-infected D752.1 isolate, four other DsCV-1-infected *D. septosporum* isolates from Scotland and one DsCV-1-free *D. septosporum* isolate from Southern England. The DsCV-1-free D752.1 isolate causes significantly more extended needle damage in the form of red bands and has significantly higher fungal burden as compared to the DsCV-1-infected D752.1 isolate. DsCV-1 appears to decrease virulence, cause less extended needle damage, and possess lower fungal burden following interference with fungal host growth and gene expression.

The research team from left to right: Robert Coutts, Katherine Tubby, James Snowden, Ioly Kotta-Loizou and John Daudu.

Based on our findings in this investigation an alternative approach is proposed to mitigate the effects of DNB on pine trees by utilising a mycovirus as a biological control agent. DsCV-1-based biological control would be superior to other traditionally used methods since no chemical fungicides harmful to humans and ecosystems would be used, and there would be no risk of resistant pines suddenly becoming susceptible to the disease.

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