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**Effector delivery and effector
characterisation in *Dothistroma* needle
blight of pines**

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Abstract

The filamentous fungus *Dothistroma septosporum* causes a serious foliar disease, Dothistroma needle blight (DNB), on *Pinus radiata* in New Zealand and on many pine species worldwide. Potentially correlated to changes in climate, this disease has been on the rise for 20 to 30 years, and current countermeasures often struggle to contain the damage it causes. A molecular approach to combat DNB could be promising. Effectors are small proteins secreted by pathogens to promote host colonisation, and have been a major focus of plant pathologists in recent years. However, effector biology in pathogens of gymnosperms has received little research attention. Here, candidate effectors (CEs) were selected using a series of computational prediction tools, as well as RNAseq data from a compatible *D. septosporum*–pine interaction. A shortlist of 55 highly *in planta* expressed CEs, predicted to be secreted to the apoplast, was characterised *in silico*. While almost half of them lacked a predicted function, none were exclusive to *D. septosporum*. Seventeen effector candidates of particular interest were taken forward for functional characterisation. Specifically, these proteins were screened for induction of plant defences in the form of cell death in the model plants *Nicotiana benthamiana* and *N. tabacum* using an *Agrobacterium* transient expression assay. Five CEs induced cell death in these plants, suggesting recognition by the plant defence machinery. Of those five, three are similar to previously described proteins. Effector screening methods are not available for pine, thus various approaches to achieve this were trialled. A high-throughput method to collect each protein in the apoplastic wash fluid of *N. benthamiana* was developed. This fluid was applied to *P. radiata* shoots raised from tissue culture to screen for a response, with promising results. Along with an array of *D. septosporum* CEs, these shoots may ultimately be used to screen for resistant pine genotypes. Selection of genotypes at this early stage could speed up DNB resistance screening for pine breeding and the protocol could be transferred to related pathosystems. This research also contributes to the molecular understanding of forest diseases and effectors that may be common among pathogens of distantly related hosts.

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Acronyms & units

aa	Amino acid	mg	Milligram(s)
ATTA	<i>Agrobacterium</i> transient transformation assay	ml	Millilitre(s)
Avr	Avirulence factor	mm	Millimetre(s)
AWF	Apoplastic wash fluid	mM	Millimolar
bp	Base pair(s)	MQ	Milli-Q
cDNA	Complementary DNA	ms	Millisecond(s)
CE	Candidate effector	min	Minute(s)
cm	Centimetre	ng	Nanogram(s)
d	Day(s)	n/a	Not applicable
dH ₂ O	Distilled water	NR	Neutral red
DNA	Deoxyribonucleic acid	nt	Nucleotides
DNB	Dothistroma needle blight	OD	Optical density
dNTP	Deoxynucleotide triphosphates	opm	Oscillations per minute
dpi	Day(s) post inoculation	pg	Picogram
Ecp	Extracellular protein	PAMP	Pathogen-associated molecular pattern
fmol	femtomol	PCR	Polymerase chain reaction
g	Gram(s)	PRR	Pattern recognition receptor
h	Hour(s)	qPCR	Quantitative PCR
IP	Invasion pattern	RNA	Ribonucleic acid
kDa	Kilo-Dalton	RPMK	Reads per million per kilobase
kV	Kilovolt	s	Second(s)
l	Litre(s)	SM	Secondary metabolite
M	Molar (1 M = 1 mol per litre)	v/v	Volume per volume (%)
μg	Microgram(s)	w/v	Weight per volume (%)
μl	Microlitre(s)		
μM	Micromolar		

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