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RESEARCH NOTE

Sensitivity of the soil-borne pathogen *Phytophthora agathidicida*, the causal agent of kauri dieback, to the anti-oomycete fungicides ethaboxam, fluopicolide, mandipropamid, and oxathiapiprolin

Alana M. Thurston*[1], Lauren Waller[1], Leo Condron[2] and Amanda Black[1]

[1] *Bioprotection Aotearoa, Lincoln University, P.O. Box 85084, Lincoln, 7647, New Zealand*
[2] *Faculty of Agriculture and Life Sciences, Lincoln University, P.O. Box 85084, Lincoln, 7647, New Zealand*

**Corresponding author: alana.thurston@lincolnuni.ac.nz*

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Summary The oomycete *Phytophthora agathidicida* is the causal agent of kauri dieback, which threatens the survival of endemic kauri (*Agathis australis*) forests in Aotearoa|New Zealand. Current chemical control of *P. agathidicida* involves the application of either a mixture of halogenated tertiary amines or phosphite salts with some success, but neither treatment cures the disease. Recently, four anti-oomycete fungicides, all with different modes of action, have become commercially available. Here, we determined the inhibition potential of these fungicides on three *P. agathidicida* isolates, using agar dilution assays. The average concentration required to inhibit mycelial growth by 50% (EC_{50}) for ethaboxam, fluopicolide, and mandipropamid was 0.0916, 0.372, and 0.0196 µg/mL, respectively. Inhibition of *P. agathidicida* mycelia by oxathiapiprolin and its commercial formulation, Zorvec® Enicade®, was 0.000152 and 0.000309 µg/mL, respectively. Based on the EC_{50} values reported in this study, these fungicides are the most effective inhibitors of *P. agathidicida* mycelia when compared to previously screened fungicides, natural products, and plant extracts. Thus, their performance in this initial screening supports further research into their potential use as a kauri dieback management tool.

Keywords *Phytophthora agathidicida*, kauri (*Agathis australis*), kauri dieback, disease management, antimicrobial activity testing

INTRODUCTION

The survival of the Aotearoa|New Zealand kauri (*Agathis australis*), considered a taonga|treasure by Māori (Lambert et al. 2018), is presently threatened by the soil-borne, oomycete pathogen *Phytophthora agathidicida* (Horner & Hough 2014, Weir et al. 2015). Symptoms of this fatal disease include root and collar rot, excessive resin production, and both canopy discolouration and thinning (Beever et al. 2009). Kauri dieback has been documented across the natural growing range of kauri (Waipara et al. 2013, Bradshaw et al. 2019), and additional management options are needed to robustly control the disease.

Diseases caused by oomycetes are, in part, remedied with chemical treatments (Gisi & Sierotzki 2015). Unfortunately, oomycetes are largely unaffected by most commercial fungicides, as they lack pathways specific to true fungi (Hardham 2005). Current chemical control of kauri dieback is structured around managing the spread of *P. agathidicida* and the severity of disease symptoms. Disinfectant stations have been installed on boardwalks around kauri forests, with the aim of reducing disease spread by human vectors (Ministry for Primary Industries 2014). These stations disperse a mixture of halogenated tertiary amines (TriGene II Advance; MediChem International Ltd, UK), which suppresses *P. agathidicida* mycelial growth and zoospore activity, but does not eliminate the viability of long-lived, dormant oospores (Bellgard et al. 2010). Once infected, kauri can be treated with injections of phosphite (phosphonate) salts, a fungicide that significantly reduces lesion activity and spread, but does not cure the disease (Horner & Hough 2013, Bradshaw et al. 2019). There have been numerous additional screenings of synthetic chemicals and natural products for fungicidal activity against *P. agathidicida in vitro* (Lawrence et al. 2017, 2019, 2021), although these studies have not yet progressed to *in vivo* assessments.

New anti-oomycete fungicides with different modes of action to one another and to phosphonate fungicides have become commercially available over the last decade (Belisle et al. 2019). Ethaboxam is a thiazole carboxamide that functions as a β-tubulin inhibitor (Peng et al. 2019)

and consequently disrupts cellular division (Uchida et al. 2005). Fluopicolide (an acylpicolide) also impedes cellular division but does so by delocalising spectrin-like proteins (Jiang et al. 2015). Mandipropamid is a mandelic acid amide that targets the cellulose pathway and consequently hinders cell-wall biosynthesis in *Phytophthora* species (Blum et al. 2010, Gisi & Sierotzki 2015). Oxathiapiprolin inhibits a novel oomycete target, an oxysterol binding protein (OSBP) (Pasteris et al. 2016).

The objective of this study was to assess the potential of these four fungicides to manage kauri dieback. Thus, the baseline sensitivity of *P. agathidicida* mycelia was determined for three isolates and compared to previously screened chemical inhibitors.

MATERIALS AND METHODS

Isolates of *P. agathidicida*

Three isolates of *P. agathidicida* were used in this study. Isolate NZFS 3770 was isolated from the Coromandel Peninsula in 2006 (Studholme et al. 2016) and was acquired from the culture collection at Scion (Rotorua, New Zealand). Isolates ICMP 18969 and 18970 were isolated from Waipoua, Northland in 2011 (Manaaki Whenua Landcare Research 2013) and were provided by Manaaki Whenua Landcare Research (Lincoln, New Zealand).

Fungicides used

Ethaboxam (Chem Service, Inc., N-14143-50MG), fluopicolide (Merck, 41132), mandipropamid (Merck, 32805), and oxathiapiprolin (Chem Service, Inc., N-14266-10MG) were purchased as analytical standards. Zorvec® Enicade® (active ingredient 100 g/mL oxathiapiprolin) was provided by Corteva Agriscience™.

In vitro fungicide sensitivity of *P. agathidicida* isolates

The sensitivity of each *P. agathidicida* isolate to ethaboxam, fluopicolide, mandipropamid, and oxathiapiprolin was evaluated using an agar dilution assay. 20% clarified V8-juice (cV8) agar (Ferguson & Jeffers 1999) was amended with eight, two-fold dilutions of each fungicide, which had been dissolved in acetone, to yield a final concentration ranging from 1.56×10^{-5} to 4 µg/mL. Control plates were set up by amending 20% cV8 agar with acetone to yield a final concentration of 0.5%. Three biological replicates were established for each isolate and concentration of fungicide. A 3.8 mm agar plug containing active (i.e., leading edge) *P. agathidicida* mycelia was transferred to the centre of the growth media on a Petri dish. Petri dishes were sealed with Parafilm and stored in the dark at 22°C. After four days, the diameter of the mycelial growth was measured at four, equidistant, points using digital callipers (ROK DC-162MA) to two decimal places. Growth measurements were adjusted by subtracting the diameter of the initial agar inoculum. Percent inhibition was calculated using the equation: $(C_d - T_d)/ C_d \times 100$, where C_d refers to the average diameter of the control and T_d refers to the average diameter of the treatment. The concentration required to inhibit mycelial growth by 50% (EC_{50}) was estimated by log-transforming the concentrations and fitting the data with a non-linear regression using GraphPad Prism version 6.0 (Lawrence et al. 2017). The response of *P. agathidicida* isolates to different sources of oxathiapiprolin was analysed with a Two Sample t-test, which was performed on RStudio v1.1.463.

RESULTS

All isolates were highly sensitive to the anti-oomycete fungicides screened by this study (Table 1). The EC_{50} value ranges (average) were 0.0776–0.110 (0.0916), 0.318–0.412 (0.372), 0.0182–0.0206 (0.0196), and 0.000130–0.000169 (0.000152) µg/mL for ethaboxam, fluopicolide, mandipropamid, and oxathiapiprolin, respectively. The effect of fluopicolide on the growth of *P. agathidicida* mycelia at different concentrations is shown in Fig. 1. The EC_{50} value for the commercial formulation of oxathiapiprolin (Zorvec® Enicade®) differed from the analytical standard by a factor of approximately 2, with an average value of 0.000309 µg/mL.

Table 1 *In vitro* sensitivity average (95% confidence intervals) half maximal effective concentration (EC_{50}) values for inhibition of mycelial growth of three *Phytophthora agathidicida* isolates using the anti-oomycete fungicides ethaboxam, fluopicolide, mandipropamid, and oxathiapiprolin.

Fungicide	EC_{50} (µg/mL)		
	NZFS 3770	**ICMP 18969**	**ICMP 18970**
Ethaboxam	0.0776 (0.0715-0.0840)	0.0873 (0.0787-0.0967)	0.110 (0.0989-0.123)
Fluopicolide	0.318 (0.262-0.393)	0.412 (0.343-0.514)	0.387 (0.334-0.457)
Mandipropamid	0.0200 (0.0182-0.0220)	0.0182 (0.0160-0.0206)	0.0206 (0.0194-0.0219)
Oxathiapiprolin	0.000130 (0.000125-0.000135)	0.000158 (0.000147-0.000170)	0.000169 (0.000158-0.000180)
Zorvec® Enicade®	0.000314 (0.000290-0.000341)	0.000339 (0.000311-0.000370)	0.000273 (0.000244-0.000305)

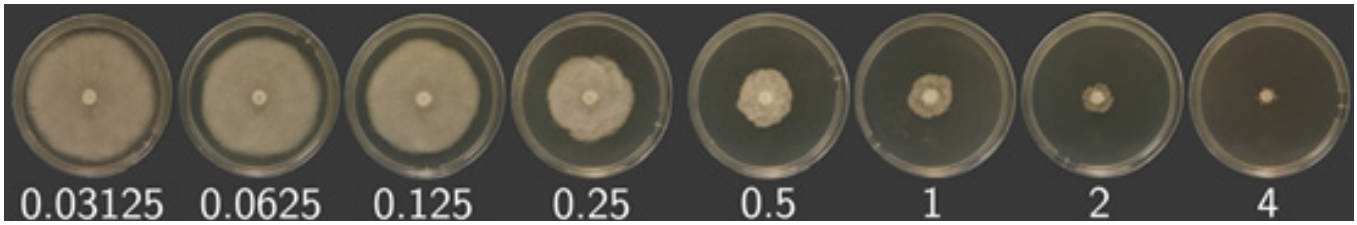

Figure 1 Mycelial growth of *Phytophthora agathidicida* in the presence of increasing concentrations (µg/mL) of fluopicolide in an agar dilution assay.

DISCUSSION

Kauri dieback is fatal to kauri of all ages and additional chemical management options are needed to reduce its spread and to control infection severity. This study sought to screen additional anti-oomycete fungicides, which have been successful in reducing disease incidence and severity in predominantly agricultural instances of *Phytophthora* spp. diseases (Zhang et al. 2005, Shin et al. 2010, Hao et al. 2019). The EC_{50} values reported here are consistent with those of other pathogenic *Phytophthora* species (Gray et al. 2018, Belisle et al. 2019). Furthermore, the EC_{50} value reported for oxathiapiprolin inhibition of *P. agathidicida* mycelial growth is in agreement with that from a recent study (Lacey et al. 2021).

The EC_{50} values for ethaboxam, fluopicolide, mandipropamid, and oxathiapiprolin inhibition of *P. agathidicida* mycelial growth were lower than all previously screened synthetic chemicals and natural products (Horner & Hough 2013, Lawrence et al. 2017, 2019, 2021). The narrow EC_{50} value ranges reported here suggest that the baseline sensitivity of *P. agathidicida* is not noticeably different between isolates.

As has been widely found, *Phytophthora* pathogens appear to be most sensitive to oxathiapiprolin when compared with ethaboxam, fluopicolide, and mandipropamid (Qu et al. 2016, Gray et al. 2018, Belisle et al. 2019, Hao et al. 2019). A recent report of oxathiapiprolin activity against *P. agathidicida* zoospores and oospores found that this fungicide was also highly toxic to other *P. agathidicida* life cycle stages (Lacey et al. 2021). Thus, it would be pertinent to screen ethaboxam, fluopicolide, and mandipropamid against *P. agathidicida* spores to characterise differences in fungicide sensitivity across life cycle stages of this pathogen, which could inform potential application methods.

This study found slight, but significant (P=0.002), differences between the toxicity of oxathiapiprolin from different sources (an analytical standard grade and a commercial formula) to *P. agathidicida* mycelia. This result highlights the need to screen fungicides as commercial formulations, which would be the form most likely applied in an environmental setting. Ethaboxam (INTEGO®), fluopicolide (Presidio®, Adorn®, and Infinito®), and mandipropamid (Revus®) are also available as commercial formulations, however, there is limited availability of these products in Aotearoa|New Zealand.

The results of this study suggest that ethaboxam, fluopicolide, mandipropamid, and oxathiapiprolin should be further considered as chemical control options for *P. agathidicida*. However, additional steps must be taken to assess their suitability including:

- conducting *in vivo* glasshouse studies;
- determining environmental toxicity and non-target effects on other microbes;
- assessing practical application methods for kauri;
- developing resistance-management schemes; and
- investigating potential synergism with currently applied chemical controls.

Additionally, the appropriateness of using these fungicides would need to be vetted by the indigenous communities who actively manage culturally significant kauri forests in accordance with their beliefs, values, and practices.

CONCLUSIONS

This screening experiment found that ethaboxam, fluopicolide, mandipropamid, and oxathiapiprolin are highly toxic to *P. agathidicida* mycelia. These preliminary results suggest that these fungicides warrant further evaluation as additional chemical control options for kauri dieback.

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