Plant & Food RESEARCH
RANGAHAU AHUMĀRA KAI

www.plantandfood.com

Phosphorous acid for controlling *Phytophthora* taxon Agathis in Kauri

Horner IJ, Hough EG

February 2011

A progress report prepared for:

MAF Biosecurity

Horner IJ, Hough EG
Plant & Food Research, Havelock North

SPTS No. 5140

DISCLAIMER

This report has been prepared by The New Zealand Institute for Plant & Food Research Limited (Plant & Food Research), which has its Head Office at 120 Mt Albert Rd, Mt Albert, Auckland.

This report has been approved by:

Ian Horner
Scientist/Researcher, Horticultural Crop Diseases
Date: February 2011

Bob Fullerton
Science Group Leader, Pathology and Applied Mycology
Date: February 2011

Contents

1 Introduction

Phytophthora taxon Agathis (PTA) is a serious problem, killing kauri trees of all ages in forests in Auckland and Northland. Very few treatment options are available for infected or threatened trees.

Treatment with phosphite (phosphorous acid, PA) is a potential control for PTA. It has been used successfully for treating a wide range of *Phytophthora* diseases of many plant species. Its predominant use is horticulture and nurseries, with some use in forest systems.

Before testing PA on large kauri trees in the field, it is necessary to test the efficacy of phosphorous acid against PTA *in vitro* and on kauri seedlings. This will give an early indication of whether phosphorous acid is likely to be a useful field control, will help to predict minimum rates required for control, and will identify an upper concentration threshold to avoid phytotoxicity. The current work aims to:

- Determine in vitro sensitivity of PTA to phosphorous acid, and compare to other *Phytophthora* species commonly controlled by this product
- Determine phosphorous acid concentration thresholds for phytotoxicity in kauri
- Determine phosphorous acid concentrations required for PTA control
- Determine phosphorous acid efficacy against PTA infection in kauri seedling roots and trunks
- Test efficacy of phosphorous acid at preventing new infections and curing established infections.

Preliminary tests reported here assess the effect of phosphorous acid on growth of PTA mycelia in vitro, and compare these with effects on other *Phytophthora* species (*P. cactorum* and *P. cinnamomi*). Subsequent trials outlined here, and to be reported fully by June 2011, will test the efficacy of PA for controlling PTA in artificially infested kauri seedlings in the glasshouse.

2 *In vitro* response to phosphorous acid

2.1 Methods

Six isolates of *Phytophthora* taxon Agathis and two isolates each of *P. cactorum* and *P. cinnamomi* were used. All *Phytophthora* cultures were grown on V8 agar for 5 days, and then 5-mm diameter plugs from the margin of cultures were subbed to V8 agar amended with various concentrations of phosphorous acid. There were three replicate plates of each isolate for each PA concentration. PA concentrations tested were 0, 5, 15, 40, 100 and 250 ug/ml. The PA used was taken from a commercial preparation of Agrifos®600 (Key Industries), a solution of 60% phosphorous acid present as a mono- and di-potassium phosphonate. The PA was added to the agar after autoclaving, when it had cooled to about 70°C.

One day after subbing onto PA-amended media, the margins of all colonies emerging from the inoculum plug were marked using a fine marker pen on the bottom of the Petri plate. All subsequent measurements were taken from this reference point. Growth measurements were taken at three points on each plate, 4 and 7 days after subbing, and growth rates per day were calculated. Response curves were fitted to data averaged across each species, using a third order polynomial. From this, EC_{50} values were calculated.

Microscopic examinations of the cultures were made after 7 days, to assess potentially anomalous growth or sporulation on agar amended with PA.

2.2 Results and discussion

Summaries of *Phytophthora* colony growth on PA-amend media are given in Table 1 and Figures 1 and 2.

Mycelial growth of all three *Phytophthora* species was significantly inhibited by phosphorous acid. PTA was the most sensitive to phosphorous acid, with a 50% reduction in growth (EC_{50}) at 4.0 mg/L phosphorous acid, compared with EC_{50} values of 25.2 and 37.9 mg/L for *P. cinnamomi* and *P. cactorum* respectively.

In microscopic examinations, an increasing delay in sporulation of PTA was noted with increasing phosphorous acid concentration. In un-amended media, oogonial formation occurred on hyphae that were approximately 1.5 days old. At concentrations of 5 and 15 mg/L phosphorous acid, oogonial formation occurred on hyphae 2-3 and 3-6 days old, respectively. At 40 mg/L, two of the six PTA isolates had not produced oogonia within 8 days. The remaining four isolates had produced a small number of oogonia after 4-6 days, but these oogonia did not appear to mature normally, and in many cases were plasmolysed.

From these studies, we can conclude that PTA is highly sensitive to phosphorous acid, even more so than other *Phytophthora* species commonly controlled by this chemical in agricultural systems. Thus, further investigation of potential PTA control in kauri is warranted.

Table 1. Average colony growth (mm/day) of *Phytophthora* species on V8 agar media amended with various concentrations of phosphorous acid.

		Phosphorous acid concentration (mg/L)					
Species	Culture ID	0	5	15	40	100	250
PTA	HNM09003	5.00	1.93	1.06	0.52	0.08	0.00
PTA	H270	5.78	1.98	1.28	1.20	0.54	0.08
PTA	H263	4.56	2.07	1.57	1.04	0.59	0.24
PTA	H294	4.37	2.22	1.41	1.13	0.83	0.29
PTA	H303	5.11	2.87	1.59	1.37	0.50	0.00
PTA	H315	5.80	3.28	1.48	0.93	0.04	0.00
P. cactorum	H243	3.61	2.69	2.20	1.70	1.26	0.24
P. cactorum	H244	3.39	2.65	1.96	1.80	1.04	0.16
P. cinnamomi	H275	7.39	5.39	3.02	1.70	0.98	0.54
P. cinnamomi	H289	5.39	5.37	4.78	3.17	2.33	1.39

PTA = *Phytophthora* taxon Agathis

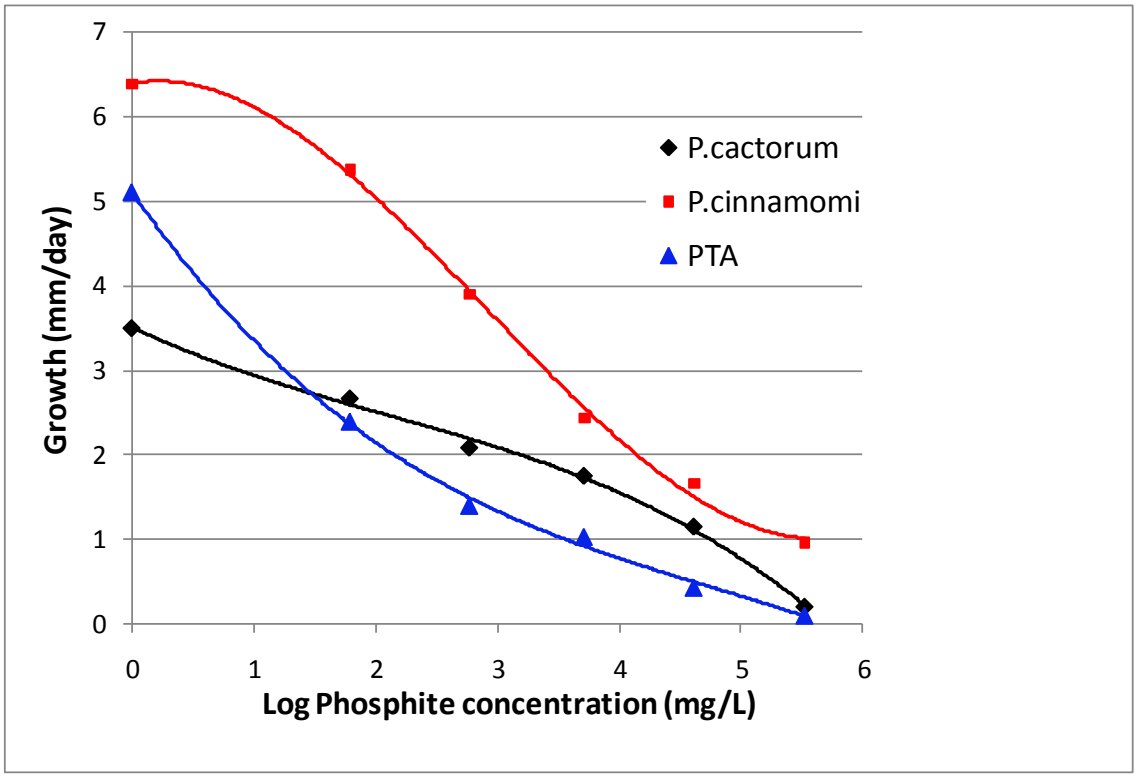
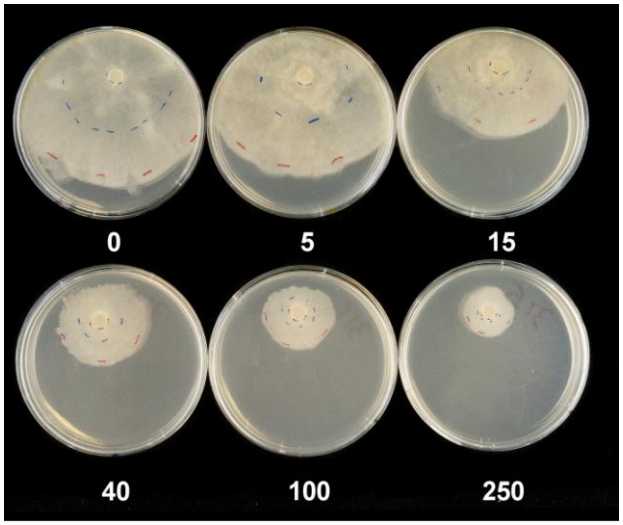
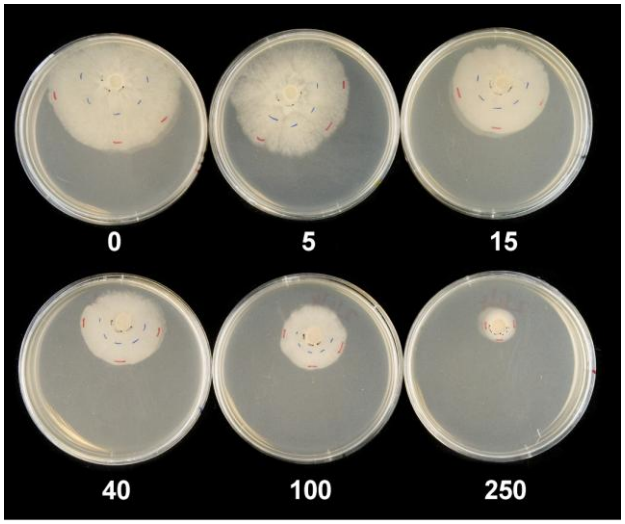


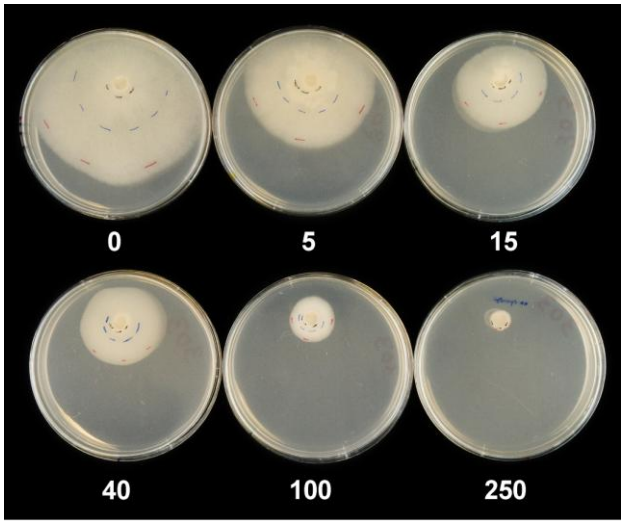
Figure 1. Growth of mycelial cultures of *Phytophthora cactorum*, *P. cinnamomi* and *Phytophthora* taxon Agathis (PTA), grown on V8-agar amended with various concentrations of phosphorous acid. Growth data are averaged for each species.



Phytophthora cinnamomi



Phytophthora cactorum



Phytophthora taxon Agathis

Figure 2. Cultures of *Phytophthora cinnamomi*, *P. cactorum* and *Phytophthora* taxon Agathis (PTA) grown for 8 days on V8-agar media amended with various concentrations of phosphorous acid. Numbers are phosphorous acid concentrations in mg/L. Black, blue and red marks in plates indicate colony margins after 1, 4 and 7 days, respectively.

3 Kauri seedling trials

The next step to determining potential of phosphorous acid for control of PTA in kauri is to test the product on seedlings. A trial has been established on kauri seedlings in glasshouse at Plant & Food Research in Havelock North. The trial will assess PTA disease progression/control in kauri seedlings inoculated on roots or stem, and treated either before or after inoculation with phosphorous acid applied to foliage, stem or soil. Plant material used was 2- or 3-year-old kauri seedlings in planter bags, sourced from Scion, Rotorua. The trial work, outlined briefly below, should be completed by June 2011 with a full report by July.

3.1 Methods

PTA inoculation methods:

- Trunk inoculation via wound
- Root inoculation via soil and flooding
- Un-inoculated controls.

Fungicide Treatments:

- Soil drench with phosphorous acid (two rates)
- Foliar spray with phosphorous acid (three rates)
- Trunk injection with phosphorous acid (two rates)
- Ridomil granules applied to soil
- Untreated controls.

Treatment application timing:

- 7 days before inoculating or
- 7 days after inoculating.

Assessments will include tree health, root health and lesion spread.

Progress to date: inoculation and treatments are completed (February 2011). Problems are being encountered with glasshouse temperatures approaching the upper limit for PTA survival. Because of delays in project approval, the work initially planned for mid winter is now being done in mid summer. The temperature problem is being managed as far as possible, but may have some impact on results.

For further information please contact:

The New Zealand Institute for Plant & Food Research Ltd
Plant & Food Research Havelock North (cnr Crosses and St George's Roads)
Private Bag 1401, Havelock North 4157.
Tel: +64-6-975 8880, Fax: +64-6-975 8881
Email: ian.horner@plantandfood.co.nz