Survival of *Phytophthora cinnamomi* and *P. multivora* in Lime-amended Bioclay® (LaBC®) and LaBC® plus organic material

E. M. Davison and F. C. S. Tay

Department of Environment and Agriculture, Curtin University, GPO Box U1987, Perth WA 6845

February 2011

Abstract

Lime–amended BioClay® (LaBC®) is a high pH product developed as a soil amendment for use on Bassendean Sands of the Swan Coastal Plain. Composted mulch is used to clean equipment used in its production and lower the pH of the final product. If this composted mulch is contaminated with *Phytophthora cinnamomi* (the dieback fungus) this soil borne pathogen might be inadvertently spread to uninfested properties. In order to determine the likelihood of this occurring, pine plugs colonised by either *P. cinnamomi* or the similar pathogen *P. multivora*, were incubated in LaBC® or LaBC® + organic material for up to 21 days. *P. cinnamomi* survived for less no more than 6 days in both products, while *P. multivora* survived for no more than than 6 days in LaBC®, and for no more than than 14 days in LaBC® + organic material. This experiment shows that there is minimal risk of LaBC® or LaBC® + organic material being a source of these pathogens.

Background

Lime–amended BioClay® (LaBC®) is a product that has been developed by the Water Corporation of Western Australia (WA) for use as a soil amendment and for controlled release of nutrients for the Bassendean Sands of the Swan Coastal Plain. It comprises (volume to volume) 1 part lime biosolids from the Subiaco Waste Water Treatment Facility and 2.4 parts of virgin lateritic gravelly clay from the Eastern Metropolitan Regional Council (ERMC) Red Hill landfill site. The final product has a pH of about 12.5. It has been extensively tested for human pathogens, metals and metaloids, petroleum hydrocarbons and organochlorine pesticides, and has been found to be safe for humans, animals and the environment (Allen and Walton 2010, Humphries 2010).

Composted mulch from ERMC's accredited composting facility at Red Hill is used to clean the equipment used in the production of LaBC® and also to lower the pH of the final product. Concerns have been raised that the soil borne plant pathogen *Phytophthora cinnamomi* (the diebck fungus) might be able to survive in this composted woody plant material, and if it can also survive in the LaBC®, it could be inadvertently spread to agricultural properties. The following experiment was conducted to determine how long *P. cinnamomi* could survive in LaBC® and LaBC® + organic material. Another species, *P. multivora*, was also included in the experiment. *P.multivora* is widespread in WA and occurs in soils of higher pH than those where *P. cinnamomi* is found (Scott et al 2009).

Materials and Methods

Preparation of the *Phytophthora* inoculum: Young, actively growing pine branches 1-1.5 cm diameter with internode lengths of approximately 1 m were cut in spring from 5-9 year old *Pinus radiata* saplings. The bark was removed from the branches with a knife, and then cut into 2 cm lengths using a band saw. The pine plugs were frozen until required. Four 2 L Erlenmeyer flasks were each filled with about 200 plugs, these were soaked in deionised water for 24 hr, drained, then de-ionised water added to the depth of 1.5 cm in each flask, and the flasks stoppered with non-absorbent cotton wool wrapped in muslin and covered with aluminium foil. The flasks were autoclaved at 121°C for 30 mins on two successive days. After cooling the flasks were shaken to re-moisten the top plugs.

Four isolates of *P. cinnamomi* and three isolates of *P. multivora* were used. Isolation details are given in Table 1.

Table 1. *Phytophthora* isolates used in the survival experiment.

Isolate	Isolated from:	Date isolated	Comments
P. cinnamomi DP 4	*Banksia telmatia,* Eneabba, WA	1990	Provided by DEC
P. cinnamomi DP 51	*B. cuneata,* Popanyning, WA	1992	Provided by DEC
P. cinnamomi DP 55	*B. baxteri,* Fitzgerald River National Park, WA	1996	Provided by DEC
P. cinnamomi BTPC	Soil, Fitzgerald River National Park, WA	2007	Provided by DEC
P. multivora EB 3	*Xanthorrhoea preissii*, Enneabba, WA	2001	Identity confirmed by sequencing
P. multivora EB 13	Soil, location unknown	2001	Identity confirmed by sequencing
P. multivora EB 25	Soil and roots, Dandaragan, WA	2007	Identity confirmed by sequencing

The isolates were grown on pea agar (200 g frozen peas, macerated in a blender on high speed for 5 mins, 15 g agar, 1 L water) for 1 week at room temperature (approximately 22°C). Each flask was inoculated with either all of the four *P. cinnamomi* isolates or all of the three *P. multivora* isolates cut into about 10 mm squares. The flasks were shaken to distribute the inoculum between the pine plugs (Fig. 1). Flasks were incubated at room temperature and shaken weekly to ensure that the plugs did not clump together.

Figure 1. Pine plug inoculum

After 10 weeks two pine plugs were removed from each flask to check colonisation. Each plug was surface sterilised in 70 % ethanol for 30 sec., rinsed twice in sterile de-ionised water, dried, split in half using sterile secateurs, and then plated onto agar selective for *Phytophthora* (17 g corn meal agar, 100 mg ampicillin, 50 g hymexazol, 100 mg PCNB, 1 ml nystatin, 0.5 ml rifadin /L). Within 2 days *P. cinnamomi* or *P. multivora* grew vigorously from the plated pine plugs, showing complete colonisation.

Preparation of LaBC® and LaBC® + organic material: LaBC® and LaBC® + 10 % compost (used as a substitute for composted mulch) were prepared in the normal way by the Water Corporation and the Chemistry Centre. About 20 L of LaBC® were provided on 18 January 2011, and the first experiment was set up the following day. About 20 L of LaBC® + 10 % compost were provided on 25 January 2011, and the second experiment was set up on the same day.

Inoculation of LaBC® and LaBC® + organic material: Approximately 750 ml LaBC® was spread over the base of an aluminium foil container measuring 28 x 21 x 5 cm. Forty pine plugs colonised by either *P. cinnamomi* or *P. multivora* were spaced over this layer of LaBC® (Fig. 2), and then completely covered with an additional 1.5 L of LaBC® (Fig. 3). There were three replicate containers for each species. Each container was place in a large, sealed polythene bag to minimise drying, and incubated in a fume hood at room temperature (approximately 22° C).

After 6, 14 and 21 days 10 pine plugs were removed from each container, surface sterilised in 70 % ethanol for 30 sec., rinsed twice in sterile de-ionised water, dried, split in half using sterile secateurs, and then plated onto *Phytophthora*-selective agar. The agar plates were incubated at room temperature for 7 days and checked for the growth of *Phytophthora* spp. under a stereo microscope.

A similar protocol was used for LaBC® + compost. The control treatment was colonised pine plugs that had not been exposed to either LaBC® or LaBC® + compost.

Figure 2. Inoculum spread over the surface of LaBC®.

Figure 3. Inoculated LaBC® ready for incubation.

Results

Both *P. cinnamomi* and *P. multovora* were recovered from all of the colonised pine plugs that were not incubated in LaBC® or LaBC® + compost. However after 6 days exposure, *P. cinnamomi* and *P. multivora* were not recovered from LaBC® (Figs. 4 and 5). *P. cinnamomi* was not recovered from the LaBC® + compost, however *P. multivora* was recovered from a single pine plug (Table 2). No *P. cinnamomi* or *P. multivora* were recovered after 14 or 21 days exposure.

Table 2. Recovery of *Phytophthora* spp. from inoculated pine plugs incubated in LaBC® or LaBC® + compost. Each value is the mean percentage of three replicates of 10 pine plugs.

		Recovery (%) after different exposure times			
		0 days	6 days	14 days	21 days
LaBC®	*P. cinnamomi*	100	0	0	0
LaBC®	*P. multivora*	100	0	0	0
LaBC® + compost	*P. cinnamomi*	100	0	0	0
LaBC® + compost	*P. multivora*	100	3	0	0

Figure 4. Recovery of *P. cinnamomi* from pine plugs either not incubated in LaBC®(LHS), or incubated in LaBC® for 6 days (RHS).

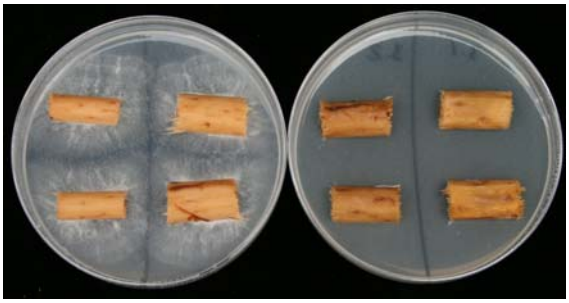

Figure 5. Recovery of *P. multivora* from pine plugs either not incubated in LaBC®(LHS), or incubated in LaBC® for 6 days (RHS).

Conclusions

The risk that either *P. cinnamomi* or *P. multivora* would survive in LaBC® or LaBC® + organic material is minimal because neither plant pathogen was recovered after exposure for 6 days in LaBC®, and after exposure for 14 days in LaBC® + organic material. It is very unlikely that either of these plant pathogens would be inadvertently introduced into agricultural properties in LaBC® or LaBC® + organic material.

Acknowledgements

We thank DEC for the provision of isolates of *P. cinnamomi*

References

Allen, D., Walton, K. (2010). *Evaluation of the effectiveness and environmental risks following the application of Lime-amended Biosolids to Bassendean sands. Part 3. Blends with clay. Report to the Water Corporation.* Chemistry Centre, Western Australia. http://www.watercorporation.com.au/_files/PublicationsRegister/10/LAB_Evaluation_Bassendean_Sands_Pt3.pdf

Humphries, R. 2010. Request of approval to classify Lime-amended BioClay® for unrestricted use (Based on the Victorian secondary beneficial reuse notification protocol). Water Corporation, unpublished document.

Scott, P. M., Burgess, T. I., Barber, P. A., Shearer, B. L., Stukely, M. J. C., Hardy, G. E. St. J., Jung, T. (2009). *Phytophthora multivora* sp. nov., a new species recovered from declining *Eucalyptus, Banksia, Agonis* and other plant species in Western Australia. *Persoonia*, **22**, 1-13.