

# AN EVALUATION OF STREAM MONITORING TECHNIQUES FOR SURVEYS FOR *PHYTOPHTHORA* SPECIES IN VICTORIA, AUSTRALIA.

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## INTRODUCTION:

In Australia, surveys for *Phytophthora* species generally rely on either soil or plant testing to confirm their presence in an area. Stream monitoring has become an important part of early detection surveillance systems for *Phytophthora ramorum* in the USA (Oak 2006). In the present study, water monitoring methods used for the detection of *Phytophthora* species in streams in the USA were evaluated in surveys in June 2006 and March 2007 at eight sites from four locations to the east of Melbourne, Australia. The methods included water filtration and stream baiting.

## SITE SELECTION:

The sites selected for the evaluation were on the basis they were in direct run-off zones from, or are located within, areas that had previously been recorded as positive for the presence of *Phytophthora cinnamomi*, Australia's most damaging *Phytophthora* species in native ecosystems and therefore a key target species for detection. Four locations were chosen on the eastern side of Melbourne in Warrandyte, Kilsyth, Kallista and Silvan (Figure 1). Two replicates at different points on the stream, were established at each location giving a total of 8 sites for testing.



Figure 1. Melbourne site locations for stream monitoring for *Phytophthora*: 1) Warrandyte, 2) Kilsyth, 3) Kallista, 4) Silvan.

## BAIT TYPE:

Leaves of four different species were trialled as baits to evaluate if this will provide the detection of a greater number of *Phytophthora* species. The bait types used were *Eucalyptus sieberi* cotyledons (often used in soil baiting in Victoria), undamaged *Rhododendron* leaves and mature *E. regnans* leaves. The 4<sup>th</sup> bait was herringbone cut *Rhododendron* leaves in the 2006 sampling, but this was substituted for uncut native *Pittosporum* leaves in 2007.

## STREAM BAITING METHOD:

Five leaves of each bait type were placed in separate fly wire mesh bags and floated in the stream as per the USA protocol (Oak 2006, Figure 2). The exposure period varied with the expected life of the bait. *Eucalyptus sieberi* cotyledons being the least robust bait were collected after one week and the other baits after two to three weeks depending upon the level of infection.

Change of colour of the underside of the *E. sieberi* cotyledons from purple to green (Figure 3C) and blotches on the other leaf baits (Figure 3A and 3B) were used as indicators of infection. Small pieces of the symptomatic tissue were plated onto PARPH agar, and following purification, cultures of different species produced were sequenced (rDNA ITS region).



Figure 2. Stream bait survey in Dandenong Creek where 5 bait leaves are placed in each bag and floated in the creek for 1-3 weeks.

## RESULTS:

*Phytophthora* species were isolated from all sites, bait types and filters used in the study. Species isolated in June 2006, included *Phytophthora gonapodyides*, *P. citricola*, *P. taxon Pgchlamydo*, *P. taxon* from Clade 8 and an unknown *P. taxon* from an unknown clade. Species isolated in March 2007 included, *Phytophthora cinnamomi*, *P. citricola*, *P. cryptogea*, *P. taxon* from Clade 2 related closely to *P. citrophthora*, *P. taxon* from Clade 8 related closely to *P. cryptogea* and an unknown *P. taxon* from an unknown clade (Tables 1 & 2). Species isolated varied with season, baits and method used. Further sequencing is currently being undertaken to identify the unknown species.

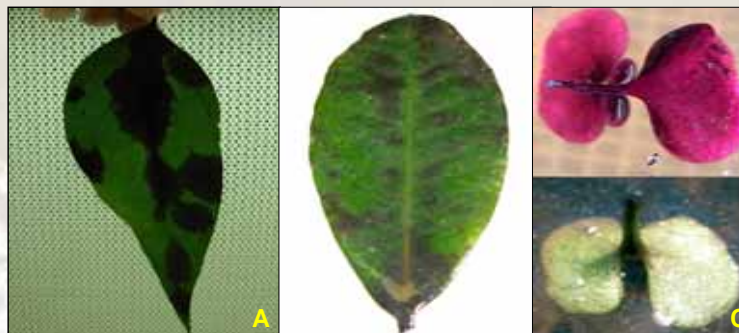


Figure 3. (A) *Eucalyptus regnans* and (B) *Rhododendron* leaf baits showing dark areas of infection. (C) *E. sieberi* cotyledons change colour when infected.

Table 1. *Phytophthora* species found in each location in the two seasons trialed.

Location	Winter Species	Summer Species
Kilsyth Dandenong Creek	<i>Phytophthora taxon Pgchlamydo</i>	Taxon related to <i>P. cryptogea</i>
	<i>Phytophthora taxon Clade 8</i>	<i>Phytophthora taxon</i> Unknown clade
Kallista Sassafras Creek	<i>Phytophthora gonapodyides</i>	<i>Phytophthora citricola</i>
	<i>Phytophthora taxon Pgchlamydo</i>	<i>Phytophthora cryptogea</i>
	<i>Phytophthora taxon Clade 8</i>	Taxon related to <i>P. cryptogea</i>
Warrandyte Yarra River	<i>Phytophthora citricola</i>	<i>Phytophthora citricola</i>
	<i>Phytophthora taxon Pgchlamydo</i>	<i>Phytophthora cinnamomi</i>
	<i>Phytophthora taxon Clade 8</i>	Taxon related to <i>P. cryptogea</i>
Silvan Woori Yallock Creek,	<i>Phytophthora citricola</i>	<i>Phytophthora cinnamomi</i>
	<i>Phytophthora taxon Pgchlamydo</i>	<i>Phytophthora cryptogea</i>
	<i>Phytophthora taxon Unknown Clade</i>	Taxon related to <i>P. citrophthora</i>
		Taxon related to <i>P. cryptogea</i>

Table 2. *Phytophthora* species found from each bait type/method in the two seasons trialed.

Bait Type	Winter Species	Summer Species
Cotyledons	<i>Phytophthora taxon Pgchlamydo</i>	Taxon related to <i>P. cryptogea</i>
	<i>Phytophthora taxon Clade 8</i>	
Damaged Rhododendron	<i>Phytophthora citricola</i>	-
	<i>Phytophthora taxon Pgchlamydo</i>	-
	<i>Phytophthora taxon Clade 8</i>	-
Mature Eucalypt	<i>Phytophthora taxon Pgchlamydo</i>	<i>Phytophthora citricola</i>
	<i>Phytophthora taxon Clade 8</i>	<i>Phytophthora cinnamomi</i>
	<i>Phytophthora taxon Unknown Clade</i>	<i>Phytophthora cryptogea</i>
		Taxon related to <i>P. cryptogea</i>
Undamaged Rhododendron	<i>Phytophthora taxon Pgchlamydo</i>	<i>Phytophthora citricola</i>
	<i>Phytophthora taxon Clade 8</i>	Taxon related to <i>P. cryptogea</i>
Pittosporum	-	<i>Phytophthora citricola</i>
	-	Taxon related to <i>P. cryptogea</i>
Filtration	<i>Phytophthora gonapodyides</i>	<i>Phytophthora cryptogea</i>
	<i>Phytophthora taxon Pgchlamydo</i>	Taxon related to <i>P. citrophthora</i>
		Taxon related to <i>P. cryptogea</i>
		<i>Phytophthora taxon Unknown clade</i>

## CONCLUSION:

Stream monitoring was shown to be an effective method for detecting *Phytophthora* species in Victoria, Australia. Using a combination of different baits and methods, the presence of *Phytophthora* species from different evolutionary clades were detected. Species of high quarantine importance (e.g. *P. ramorum* and *P. kernoviae*) were not detected in these tests. The implementation of these methods into routine surveillance activities in Australia to aid in early detection of exotic *Phytophthora* species is recommended.

## REFERENCES:

Oak, S (2006) *Outline of Protocol for 2006 Stream Sampling Pilot*, USDA Forest Service and Department of Entomology, Soils and Plant Science. Clemson University. USA

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