# Monitoring macrofungi on DRF sites sprayed with Phosphite

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#### Summary

Aerial application of phosphite is critical for the protection of susceptible plant populations in the south coast region of Western Australia. In order to determine if phosphite has any effect on fungal communities, surveys were conducted at Gull Rock and Vancouver Peninsula in July 2004 and June 2007 where populations of *Banksia* have been sprayed at three year intervals since 1996. Sprayed and non-sprayed infested areas had similar fungal assemblages at both Gull Rock and Vancouver Peninsula, The Gull Rock site showed that the diversity of macrofungi is greater in healthy woodland than in woodland infested with *P. cinnamomi*, and that fungal communities in infested woodland (either sprayed or non-sprayed) are vastly different to that found in healthy woodland. It would appear from the findings that dieback has a far greater impact on fungal communities than that of phosphite. We also suggest that a more comprehensive monitoring effort is needed in order to assess whether phosphite has any effect on fungal communities. Additional monitoring also needs to be undertaken to determine the effects

that Phytophthora dieback disease has on fungal communities in the south coast region.

Cover Photos: *Main*: Disease front at Gull Rock; *Below from left*: *Banksia coccinea* flower, infested woodland at Vancouver Peninsula, *Psilocybe coprophila, Galerina* sp.

#### Introduction

*Phytophthora cinnamomi*, the cause of Phytophthora dieback disease, is a significant threatening process in the south west botanical province (Shearer *et al.* 2007) and is pushing some threatened flora species to the brink of extinction. Currently there is no known method of eradicating the disease once introduced. The use of the chemical phosphite to control *P. cinnamomi* is one of the most important tools available to land managers (McComb *et al.* 2008) in protecting threatened flora. Phosphite is systemically translocated through the plant in both the xylem and the phloem. At higher concentrations it has a direct fungicidal effect on the survival and growth of the pathogen (Smillie *et al.* 1989, Afek and Sztenjnberg 1989) while indirect effects involve those that boost the plants immune response to invasion by the pathogen thus enabling the plant to switch on plant defense mechanisms (Guest 1984, Guest and Grant 1991).

Aerial application of phosphite was developed for use in native plant communities in the early 1990's by the Department of Environment and Conservation (formally the Department of Conservation and Land Management). Ongoing research and monitoring of phosphite treated plant communities continues to show that regular application of phosphite reduces the rate of disease extension, and is effective in increasing plant survival rates and enhancing the health of threatened ecological communities in Western

Australia (Shearer *et al.* 2004, Tynan *et al.* 2001, Moore and Barrett 2007) and in Victoria (Ali and Guest 1998). Ongoing aerial application of low volume phosphite to threatened flora and threatened ecological communities is a critical part of the management actions required by the Department of Environment and Conservation to meet biodiversity protection objectives until effective alternative treatment options are developed.

Fungi comprise a major component of the biodiversity of Australian forest and woodland ecosystems (Bougher 1995, May and Simpson 1997) and they play important roles in the decomposition of organic matter, nutrient recycling and nutrient uptake into plants via mycorrhiza formation (Tommerup and Bougher 2000). These roles are especially important in ecosystems on nutrient poor soils such as those of southwestern Australia (O'Connell and Grove 1996). Fruit bodies of hypogeous (truffle-like) species also form a significant part of the diet of many species of native Australian mammals (Claridge *et al.* 1996) and decay fungi contribute to the development of suitable habitat for many species of native birds and animals (Perry *et al.* 1985, Simpson 1996).

While it is recognised that phosphite application is critical for the protection of susceptible plant populations, it is also important to monitor effects on other components of the biota. In order to determine if phosphite has any effect on fungal communities, surveys were conducted at Gull Rock and Vancouver Peninsula in July 2004 and June 2007 where populations of *Banksia* have been sprayed triennially since 1996. Monitoring is also necessary to satisfy requirements set out by the Australian Pesticides and Veterinary Medicines Authority for the renewal of the off-label permit for the aerial application of phosphite to native vegetation which includes "*Further monitoring of effects on non-pathogenic and beneficial fungi and secondary effects on organisms that depend on these fungi for food and nutrient cycling is required*". Although they are an important component of the fungal flora, hypogeous fungi were not included in the survey, as it would have necessitated major disturbance to the sites that would affect future monitoring.

## **Monitoring at Gull Rock**

#### Site and treatment

The Gull Rock location occurs in the priority ecological open low *Allocasuarina fraseriana* – *Eucalyptus staeri* woodland in association with *Banksia coccinea* thicket and is dominated by several species of *Banksia*, including *B. coccinea* and *B. attenuata* infested with *Phytophthora cinnamomi*. The monitoring site, located on Ledge Beach Road, is an infested area approx 1 ha in size, being approx. 0.5 ha on either side of an unnamed track. The area on the western side of the track (Fig. 1) has had two autumn applications (4-6 weeks apart) of phosphite in each of the years 1996, 1999, 2001, 2004 and 2006 at the rate of 40% phosphite at 30 L ha<sup>-1</sup>. In 2003 a single application of 60% phosphite at 20 L ha<sup>-1</sup> was applied. The eastern side of the road has not been sprayed (Fig. 1). Each area extends about 120 m north-south and about 30-40 m east-west on either side of the road.



Figure 1. *Banksia coccinea* and *B. attenuata* woodland at Gull Rock infested with *Phytophthora cinnamomi*. *Left*: the disease front on the area sprayed with phosphite and *right*: the disease front in the untreated area.

Healthy woodland dominated by *B. coccinea* (Fig. 2), approximately 200 m north of the infested (and sprayed) area, was used as a 'control' to determine the fungal community on an equivalent but un-infested *B. coccinea-B. attenuata* site.



Figure 2. Healthy Banksia coccinea and B. attenuata woodland at Gull Rock.

## Survey method

In each sprayed and unsprayed area and within the healthy woodland, all macrofungal species and the number of fruit bodies were recorded in each of two 100 m transects. The two transects were 10 m apart, and each was 4 m wide.

## Results

Thirty-six species of fungi were recorded at the Gull Rock site, 19 in 2004 and 26 in 2007. In total, 24 species were recorded in the healthy (non-infested) population,12 on the infested area sprayed with phosphite and 14 in the adjacent unsprayed infested area (Table 1). The total number of species recorded on the infested site (combined sprayed and unsprayed area) was 18, of which 8 species were recorded in both the sprayed and

unsprayed area. The macrofungal species assemblages in healthy and infested areas were vastly different. The majority of species recorded in the healthy population were not recorded in the infested population and *vice versa*, as only six species were recorded in both the healthy and infested (combined sprayed and unsprayed )areas. They included an unidentified *Cortinarius* and *Inocybe australiensis* which are mycorrhizal, *Coltricia oblectans* and *Pycnoporus coccineus* which are saprotrophic, a species of *Hypomyces* that was parasitic on *C. oblectans* and the lichenised *Lichenomphalia chromacea*.

Species	Life	Healthy			Pc + Phosphite			Pc - Phosphite		
	Mode <sup>1</sup>	2004	2007	Total	2004	2007	Total	2004	2007	Total
Amanita umbrinella	М	1		1						
Calocera sp.	S							42		42
Coltricia oblectans	S		5	5	9	23	32	45	16	61
Cortinarius fibrillosus	Μ		1	1						
Cortinarius sp. 'chestnut'	Μ					4	4			
<i>Cortinarius</i> sp. 'purple brown'	М	3		3						
Cortinarius sp. 'pointy cap'	М		1	1						
Cortinarius sp. (Telamonia)	М		41	41						
Cortinarius sp. 'yellow fibrillose cap, white stem'	М		3	3						
Cortinarius spp. (unidentified)	М	1	4	5	1	1	2			
Crepidotus nephrodes	S					2	2			
Discinella terrestris	S	35	4	39						
Galerina spp.	S								1	1
Gymnopilus allantopus	S								3	3
<i>Gymnopilus</i> sp. 'slender'	S					11	11		5	5
Hypomyces sp. on Coltricia oblectans	Р		1	1		2	2	2		2
Inocybea aff. australiensis	М	9		9		24	24		31	31
<i>Inocybe</i> sp. 'scaly cap'	М				11	6	17	4	21	25
Laccaria lateritia	М				3		3			
Laccaria masonii	М				448	610	1058	106	3097	3203
Lactarius sp. 'creamy yellow'	М		2	2						
Lepiota cristata	S	1		1						
Lepiota sp. 'creamy brown'	S		1	1						
Lichenomphalia chromacea	L	9		9		3	3	1	2	3
Lichenomphalia sp. 'orange'	L		3	3						
Marasmiellus sp.	S		53	53						
Mycena aff. maldea	М		2	2						
Mycena mijoi	М	3		3						
Mycena sp. 'tiny white, dec. gills, on soil'	S		2	2						
Mycena sp. 'orange striate, viscid'	S		3	3						
Mycena sp. 'tiny white umbrella'	S	7		7						
Omphalotus nidiformis	S							2		2

Table 1. Species (and abundance) of macrofungi recorded at Gull Rock in healthy and dieback-affected (Pc) woodland in 2004 and 2007.

Species		Healthy			Pc + Phosphite			Pc - Phosphite		
-	Mode <sup>1</sup>	2004	2007	Total	2004	2007	Total	2004	2007	Total
Psilocybe coprophila	S							1		1
Pycnoporus coccineus	S	4		4					2	2
Resupinatus cinerascens	S		8	8						
Thelephora aff. terrestris 'light brown'	М				2		2	6		6
# species (Total = 36)		10	16	24	6	10	12	9	9	14
# fruitbodies (Total = 4754)		73	134	207	474	686	1160	209	3178	3387
Number of mycorrhizal species (M)		4	6	9	5	5	7	3	3	4
Number of saprotrophic species (S)		5	8	12	1	3	3	3	5	7
Number of species with other life modes (C/P)		1	2	3	0	2	2	3	1	3

 $^{1}$  M = mycorrhizal, S = saprotrophic, P = parasitic and L = lichenised (symbiotic with algae)

There was a similar number of species recorded on sprayed and non-sprayed sites within the infested area, but two species, *C. oblectans* (Fig. 3) and *Laccaria masonii* (Fig. 3) were more abundant in the area not sprayed with phosphite (although this varied between the two monitoring years) and *L. masonii*, the most abundant species, was not recorded in the healthy area. Generally, there were even numbers of mycorrhizal and saprotrophic species in each treatment (Table 1).



Figure 3. The most common species found in the infested woodland at Gull Rock. *Left: Coltricia oblectans* (note *Hypomyces* sp. on the underside of the middle specimen) and *right: Laccaria masonii*.

There was little difference in the species composition between the sprayed and nonsprayed infested areas. The difference in species composition between the infested and healthy areas may be attributed to the lack of groundcover, and the obviously more open nature of the community in the infested area as a result of dieback. The ground cover in the healthy area was dominated by monocots, such as *Lomandra* spp., *Dasypogon bromeliifolius* and *Hypolaena exsulca*, which likely create a moister environment with little or no exposed ground. In contrast, the ground in the infested area was very exposed with little or no litter layer.

## Monitoring at Vancouver Peninsula

#### Site and treatment

The Vancouver Peninsula site is coastal woodland dominated by *Eucalyptus marginata*, *Banksia grandis* and the threatened *B. brownii* (Fig. 3). The sprayed area is approximately 1ha in size (100 x 100m) on a southwest facing slope directly above the shores of Princess Royal Harbour. The site has had two autumn applications (4-6 weeks apart) of phosphite in each of the years 1996, 1999, 2001, 2004 and 2006 at the rate of 40% phosphite at 30 L ha<sup>-1</sup>. In 2003 a single application of 60% phosphite at 20 L ha<sup>-1</sup> was applied. An unsprayed area, also 1 ha in size, situated approx. 200 m southeast of the sprayed area was also selected for survey.

## Survey method

In each sprayed and unsprayed area all macrofungal species and the number of fruit bodies were recorded on two 100m transects which ran along the contour of the slope. The two transects in each area were about 40 m apart and each was 4 m wide. No monitoring was undertaken on an uninfested area as disease expression at this location was not obvious and it could not be guaranteed that dieback was not present.



Figure 3. Banksia grandis - B. brownii woodland at Vancouver Peninsula. Left: sprayed with phosphite and right: untreated.

#### Results

In all, 61 species of macrofungi were recorded (Table 2), 35 in 2004 and 42 in 2007. Almost twice as many fruit bodies were recorded in 2007. The total number of species recorded in each area was very similar (43 in the area sprayed with phosphite and 39 in the untreated area). There were similar numbers of mycorrhizal and saprotrophic species recorded in each area (Table 2). Twenty one species occurred in both treatments, 22 were only recorded in the area sprayed with phosphite and 18 were only recorded in the unsprayed area, suggesting that species assemblages were different in each area. There were almost three times as many fruit bodies recorded in the area sprayed with phosphite than in the unsprayed area (Table 2).

Species	Life	+]	Phosph	nite	- Phosphite		
	Mode <sup>1</sup>	2004	2007	Total	2004	2007	Total
Amanita cf. bruneibulbosa	М		1	1	1		1
Amanita sp. (unidentified)	Μ	1		1	1		1
Amanita xanthocephala	Μ	2	2	4			
Armillaria luteobubalina	P/S	8		8		5	5
Anthracobia sp. 'orange'	<b>M</b> ?		100	100			
Coltricia oblectans	S	8	2	10			
Coprinus spp.	S		3	3			
<i>Cortinarius</i> sp. 'brown with purple stem'	Μ		6	6		2	2
Cortinarius sp. 'chestnut'	Μ		2	2	9	7	16
Cortinarius sp. 'pointy cap'	Μ		5	5		4	4
Cortinarius sp. 'purple-brown'	М		30	30	5		5
Cortinarius sp. (Telamonia)	М					10	10
Cortinarius sp. 'viscid orange'	М					1	1
Cortinarius sp. 'viscid pink-brown'	М				1		1
Cortinarius spp. (unidentified)	М		9	9	11		11
Discinella terrestris	S					100	100
Descolea maculata	Μ		13	13			
Entoloma sp. 'honey'	S	1	1	2			
Entoloma sp. 'tall grey-brown, grey stem'	S	2	2	4	1		1
Fistulinella mollis	S		1	1			
Galerina spp.	S	15	43	58	11	3	14
Grandinia sp. 'simple spines'	S					1	1
Gymnopilus allantopus	S					10	10
Gymnopilus sp. 'slender'	S				3		3
Hydnum repandum	S	44		44	2		2
Hygrocybe cantharellus	Μ		2	2			
Hygrocybe conica	Μ	1		1			
Inocybe australiensis	М		10	10			
<i>Inocybe</i> sp. 'scaly cap'	Μ	5	1	6	4		4
<i>Inocybe</i> sp. 'tan skirt'	Μ	1		1			
Laccaria lateritia	Μ	42	37	79	3		3
Laccaria masonii	Μ	59	22	81	1		1
Lactarius eucalypti	М					2	2
Lactarius sp. 'creamy yellow'	М				2		2
Marasmius sp. '223'	S					1	1

Table 2: Species (and abundance) of macrofungi recorded at Vancouver Peninsula in 2004 and 2007.

Species	Life	+ ]	Phosph	nite	- Phosphite		
	Mode <sup>1</sup>	2004	2007	Total	2004	2007	Total
Melanophyllum echinatum	S	2		2			
Mycena albidocapillaris	S		25	25		1	1
Mycena carmelliana	S		2	2			
Mycena mijoi	S		1	1			
Mycena sanguinolenta	S	1		1			
Mycena sp. 'buff umbrella'	S		5	5		5	5
Mycena sp. 'tiny white scaly cap'	S		6	6		1	1
Mycena sp. ' tan dome with v. long slender stem'	S	26	14	40			
Mycena spp. (unidentified)	S	6		6			
Paxillus sp.	Μ	2		2			
Peziza sp. 'hollow sphere'	S				1		1
Pisolithus sp.	Μ	1		1			
Pluteus lutescens "yellow'	S					1	1
Poronia erici	С					2	2
Psathyrella sp.	S	8		8	2		2
Psilocybe coprophila	С	4	25	29	3	4	7
Pycnoporus coccineus	S		1	1		3	3
Ramaria sp. 'grey-brown'	Μ				9		9
Rickenella fibula	S	32	63	95			
Russula clelandii	Μ			0		1	1
Russula floctonae	Μ			0	1		1
Russula neerimea	Μ	5		5	2		2
Russula sp. 'white-white'	Μ				4		4
Scleroderma cepa	S		1	1			
Stereum hirsutum	S				2		2
Stropharia semiglobata	С		2	2			
# species (Total = $61$ )	)	23	31	43	22	20	39
# fruitbodies (Total = 956)	)	276	437	713	79	164	243
Number of mycorrhizal species (M)		13	13	21	15	8	22
Number of saprotrophic species (S)		10	16	20	6	10	15
Number of species with other life modes (C/P)		0	2	2	1	2	2

 $^{1}$  M = mycorrhizal, S = saprotrophic, P = parasitic, and C = coprophilous

Only two taxa were recorded on both sprayed and non-sprayed areas in both 2004 and 2007. *Galerina* spp. (Fig. 4, a complex of 2-3 very similar and difficult to distinguish species) is saprotrophic and *Psilocybe coprophila* (Fig. 4) which fruits on kangaroo scats. The most common genera recorded were *Cortinarius* (Fig. 4) and *Mycena* with eight species each. Species of *Cortinarius* are mycorrhizal and all eight were recorded in the unsprayed area and five in the sprayed area. *Mycena* are saprotrophic and in contrast to

Cortinarius, all eight were recorded in the sprayed area and only three in the unsprayed area.



Figure 4. Common fungi recorded at Vancouver Peninsula. *Left: Galerina* sp.; *centre: Psilocybe coprophila*, and *right; Cortinarius* sp. 'purple brown'.

## Discussion

The Gull Rock site shows that the diversity of macrofungi is greater in healthy woodland than in woodland infested with *P. cinnamomi*, and that fungal communities differ in infested woodland compared to healthy woodland. In jarrah forest, dieback-free and infested sites were found to have significantly different macrofungal floras. Diversity was about 1.8 times greater and abundance 1.5 times greater on dieback-free sites and only 20 % of species occurred on both sites (Anderson 2007). Despite a paucity of species in infested woodland at Gull Rock, the abundance of *L. masonii* was exceptionally high. High abundance of a small number of species is common following disturbance, and has also been reported following fire (Robinson *et al.* 2008).

It is difficult to assess whether phosphite has any effect on fungal communities. It would appear from the findings that dieback has a far greater impact on fungal communities than that of phosphite, presumably through indirect impacts such as the loss of mycorrhizal host plants, a decreased litter layer (Anderson 2007), increased daytime soil temperatures and a decrease in soil moisture (Gochenaur 1981). Species richness was similar on sprayed and unsprayed areas. Species abundance was lower on the sprayed area at Gull Rock (same result if *L. masonii* is not taken into account), but was higher on the sprayed area at Vancouver Peninsula. L. masonii, the most dominant species, was more abundant in the unsprayed area at Gull Rock and in the sprayed area at Vancouver Peninsula. Species assemblages in the sprayed and unsprayed areas were different at the Vancouver Peninsula site, but similar at the Gull Rock site (but species diversity was very low at Gull Rock). As suggested in an earlier report (Robinson and Smith 2004), in order to assess whether phosphite has any effect on fungal communities a more comprehensive monitoring effort is needed to capture a larger portion of the fungal flora expected on phosphite treated, untreated and healthy non-infested sites. In addition, and perhaps more important, is the need to determine the effect that Phytophthora dieback disease has on fungal communities in the south coast region.

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