

PROJECT 4

MID-YEAR REPORT

THE CONTROL AND MANAGEMENT OF *PHYTOPHTHORA* *MEGASPERMA* IN THE NATIVE PLANT COMMUNITIES OF WESTERN AUSTRALIA

S. Bellgard, B. Shearer, C. Crane and B. Smith

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SUMMARY

Phytophthora megasperma is an active plant pathogen in National Parks directly to the north of Perth, extending up to Eneabba, and along the south coast in the Fitzgerald River National Park (FRNP). Where active, *P. megasperma* has high impact on structural species, e.g. *Banksia attenuata* in the northern sandplain and *B. speciosa* on the south coast. *In vitro* studies have demonstrated that phosphonate can inhibit mycelial growth and oospore production in *P. megasperma*. A field-trial to evaluate the efficacy of spray applications of phosphonate in retarding the spread of spot-infections caused by *P. cinnamomi*, *P. citricola*, and *P. megasperma* has been implemented in the northern sandplain. Annual assessments of the trial will commence October 1993. A similar spray-trial is planned for the vegetation at the foot of East Mt Barren in the FRNP, to be commenced October 1993. Morphometric analysis has identified 12 morphologically distinct groups within the *P. megasperma* species complex. Six of the groups have been described previously, while, the other six may prove to be endemic to WA. A study of the seasonal variation in oospore formation in *P. megasperma* has been commenced using a deliberate inoculation of surface field-soil with mycelial disks. The winter assessment has just been completed, and after one and two weeks in field soil, oospores were indeed formed on the mycelial mats.

INTRODUCTION AND AIM

Phytophthora megasperma is the most common *Phytophthora* species recovered from dieback affected vegetation of the northern sandplain (Hart *et al.* 1991). The infections extend over a range of approximately 160 km: commencing in Moore River National Park, and extending through areas adjacent to Badgingarra and Nambung National Park to Lake Logue Nature Reserve, just north of Eneabba (CALM 1990). *P. megasperma* has also been identified as an active pathogen in the area around East Mount Barren within the Fitzgerald River National Park (FRNP) and around the Hopetoun area to the east of the FRNP (CALM 1991). It has now become apparent that *P. megasperma* is active in the western side of the FRNP as well. In addition, *P. megasperma* has also been recovered from the Esperance area and within Cape Arid National Park. Because of its wide geographic distribution, and high impact where active, it is critical that work on this species continues to determine its' occurrence, behaviour and avenues of control.

Overall, this project aims to test a number of control options to retard the spread of the disease caused by the root-rot fungus *P. megasperma* and to provide a scientific basis for the management of native plant communities infested by *P. megasperma* to minimise future impacts.

1. CONTROL OF *P. MEGASPERMA*

1.1 Sensitivity of WA isolates of *P. megasperma* to phosphonate

To date, the most significant control measure proven to retard the activity of the dieback fungus *P. cinnamomi* is phosphonate. We have completed an *in vitro* study to assess the sensitivity of *P. megasperma* to phosphonate. We examined how phosphonate affected 1) radial growth, and 2) oospore production. The outcome of this part of the research was to assess whether or not phosphonate had any potential antifungal activity against *P. megasperma*.

Thirty fungal isolates from each of the six geographic regions from which *P. megasperma* has been retrieved from within WA were screened in this experiment (Table 1.1). Six millimetre diameter disks were taken from the margins of five day old cultures of the 30 test fungi and placed centrally on fresh agar plates containing modified Ribeiro's medium amended with either 0, 20 and/or 50 $\mu\text{g/ml}$ of *fos-ject* (neutralised form of phosphonate). Radial growth was assessed at four and eight days. Six millimetre diameter disks were cut from the margins of five day old cultures and placed onto fresh plates containing modified Ribeiro's chemically defined medium with β -sitosterol amended with either 0, 20, and/or 50 $\mu\text{g/ml}$ *fos-ject*. Cultures were incubated in the dark for ten days at 19°C. Oospores were counted within three fields at the 12, 9, and 6 o'clock positions or the nearest suitable point to them under 100 $\times$ magnification.

Overall, the radial growth rate at 0 $\mu\text{g/ml}$ was greater than that at 20 $\mu\text{g/ml}$, which was in turn greater than the growth rate at 50 $\mu\text{g/ml}$ (Table 1.2). The scatterplot of the overall mean radial growth rates of the 30 isolates at each of 0, 20, and 50 $\mu\text{g/ml}$ (Fig. 1.1), shows a gradual, but consistent decrease in radial growth rate with increasing *fos-ject* concentration. Thus, radial growth rate of isolates of *P. megasperma* are indeed sensitive to increasing concentrations of phosphonate.

The nonlinear regression carried out on the mycelial inhibition values generated a power function describing the relationship between % inhibition and *fos-ject* concentration (Fig. 1.2). Extrapolating from this curve, we derived the 50% inhibition (i.e. ED₅₀ value) concentration of phosphonate to be 482.52 $\mu\text{g/ml}$ for the 30 *P. megasperma* isolates tested.

A *post-hoc* comparison identified that overall, the number of oospores produced at 0 and 20 $\mu\text{g/ml}$ amended *fos-ject* were equivalent (Table 1.3). However, oospore numbers at 50 $\mu\text{g/ml}$ were significantly less than at either 0 and/or 20 $\mu\text{g/ml}$ amended *fos-ject* (Table 1.3). Thus, oospore formation is also sensitive to phosphonate. The outcomes from this research suggest that spray applications of phosphonate may indeed impair the spread and oospore formation of *P. megasperma*.

1.2 Efficacy of aerial applications of phosphonate to treat spot infections caused by *P. megasperma* in the northern sandplain

In March of 1993, we commenced a joint trial with TIWEST and Dr Ray Hart (Hart, Simpson & Associates) investigating the efficacy of spray applications of phosphonate in controlling spot infections caused by *P. megasperma*, *P. cinnamomi*, and *P. citricola* in sites adjoining national parks in the northern sandplain. The sites were chosen in conjunction with Dr Ray Hart (HSA), as he has been mapping the progress of a number of infections for the last five years. We have selected a total of six sites: these sites represent two *P. megasperma* infections, two *P. cinnamomi* infections, and two *P. citricola* infections. Within each site, the structural plant species were permanently tagged and their disease status assessed. The sites were sprayed between 1.6.93 and 7.7.93, with a 0.3% concentration of *Phosject*, using *synertrol* as a wetting agent. A follow-up spray was carried after six weeks. The sites will be monitored annually, commencing in October 1993 to document the health of the tagged plants and hence, the efficacy of the phosphonate spray application. The outcome of this part of the research is to see if aerial applications of phosphonate can retard the development of disease caused by *P. megasperma* in a field-based trial.

1.3 Proposed spray trial at East Mount Barren to confer resistance on susceptible seedlings

P. megasperma has had a high level of impact on structural species such as *Banksia speciosa* in the East Mt Barren area in the FRNP (K. Gillen, *pers. comm.*). An aerial spray with phosphonate of the infested sites is planned for October 1993. Of particular concern is the threat *P. megasperma* poses against seedlings of a number of key plant species regenerating after the wildfires of December 1989 (McCaw *et al.* 1992). It will be important to assess if phosphonate can confer any level of host-resistance to these regenerating seedlings. The outcome of this part of the research is to see if repeated low-level aerial applications of phosphonate can confer resistance to seedlings and impair the spread of *P. megasperma* in a field-based trial.

2. MANAGEMENT

2.1.2 The effect of site factors and season on oospore formation in *P. megasperma*

Management of national parks infested by *P. megasperma* requires an understanding of the seasonal variation in the activity of *P. megasperma*. We have documented the influence of temperature on radial growth, sporangium and oospore formation. More recently, we have just commenced two experiments examining: 1) the seasonal variation in oospore production and 2) the main environmental factors controlling oospore persistence and germination. The outcomes of this research will provide information relating to the type and abundance of propagules produced by *P. megasperma*, and when outbreaks of infestation are most likely to occur in infested areas.

Seasonal variation in oospore formation in *P. megasperma* has been undertaken using a deliberate inoculation of surface field-soil with mycelial disks harbouring *P. megasperma* (as per Shea *et al.* 1979). Colonised mycelial disks (enveloped in a 30 μ m nylon mesh bag) were placed into the soil at the infested site at Wongonderrah Nature Reserve in mid-winter. One and two weeks after emplacement, the mycelial disks were retrieved from

the field soil, stained, and the number of oospores produced assessed. At the time of each retrieval, soil temperature, air temperature, rainfall and soil moisture was also assessed.

We have just completed the winter emplacement of mycelial disks. After a week in the soil oospores were formed by all of the isolates tested (Table 2.1). It would also appear that after two weeks in the soil, oospore numbers were generally higher than after one week, however, this has not been tested statistically. This work represents the first of the four seasonal emplacement planned. And since we still have not as yet finished collating the soil data, no conclusions are possible at this stage. Significantly, we have demonstrated that *P. megasperma* can form oospores in soil, without a plant host. This may indeed prove to pose a challenging management issue.

2.2.1 Morphological differentiation of *P. megasperma* isolates retrieved from native plant communities of WA.

P. megasperma is a highly variable organism, and consequently, an understanding of the variability within the species is prerequisite to understanding/interpreting the behaviour of *P. megasperma* in the field. Several attempts have been made to define subgroups on the basis of morphology, pathogenicity, and host plant species. More recently, cytological and biochemical techniques have been employed to augment traditional morphological studies. It appears that the traditional morphospecies *P. megasperma* contains at least six distinct mainly host related biological species groups. Each of these groups has been shown to have a diagnostic isozyme pattern, but as yet, the process responsible for this observed genetic divergence within a single morphological taxon, has not been fully elucidated (Brasier and Hansen 1992). Our study aimed to quantify the morphological variation exhibited by WA isolates of *P. megasperma*, and compare the resulting classification to the six *isotypes* identified by Hansen *et al.* (1986).

The origins of the *P. megasperma* isolates studied are presented in Table 2.2. All isolates were maintained on CMA plates (12 ml) at 19°C in the dark. Colony diameter was measured on 4 day old plates grown at 25°C in the dark, and converted to radial growth rate (mm/day). Mean oogonium diameters were determined from measurements of 30 mature oogonia from 10 day old cultures growing on 10% V8 juice agar (uncleared) incubated at 19°C in the dark.

Mean oogonium diameter ranged between 23 and 47 µm for the 59 *P. megasperma* isolates examined (Table 2.2). The Scott-Knott procedure identified 12 morphologically distinct groups (Table 2.2). The six *isotypes* (as per Hansen *et al.* 1986) formed the foci of six morphologically groups (isolates with mean oogonium diameters followed by the letters 'a' to 'f'), around which a number of our WA isolates clustered (Table 2.2). In addition to the six groups formed by the *isotypes* provided by C. Brasier, were six other morphologically distinct groups (isolates with mean oogonium diameters followed by the letters 'g' to 'l' in Table 2.2). Isolates from groups 'g' to 'l' had mean oogonia diameters significantly smaller than those isolates belonging to groups 'a' to 'f' (Table 2.2). The outcomes of our research so far on oogonial characteristics, suggests that here in WA, we possess representatives of the six morpho-/isotypes identified by Hansen *et al.* (1986). Additionally however, we have identified another six morphologically distinct groups, which may prove to constitute additional *biological* species endemic to WA. We hope that isozyme analysis (to be carried out later this year) will provide a clearer insight into the taxonomic structure of *P. megasperma* here in WA.

3. The occurrence of *P. megasperma* in the native plant communities of Western Australia

To direct research and management, it is imperative that we have an up-to date map of the plant communities infested by *P. megasperma*. To this end, we will continue documenting the 1) host range, 2) the degree of impact, and 3) the geographic distribution of infection by *P. megasperma*. The outcomes of this research will be linked with the GIS-based project producing an up-to-date map of susceptible hosts and their distribution.

References

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Table 1.1 Host plants, location, and source of *P. megasperma* isolates used in the *in vitro* phosphonate sensitivity trial.

Isolate (spp.)	Host	Location	Source
DCE 177 (Pms)	<i>Pinus radiata</i>	Jarraewood	E. Davison
DCE 187 (Pms)	<i>Eucalyptus caesia</i>	Baldivis	E. Davison
DCE 440 (Pms)	<i>Conospermum triplinervum</i>	Eneabba	E. Davison
DCE 441 (Pmm)	<i>Banksia attenuata</i>	Minyolo Brook	E. Davison
DCE 442 (Pmm)	<i>B. attenuata</i>	Mullering Brook	E. Davison
HSA 1150 (Pmm)	<i>B. hookeriana</i>	Eneabba	R. Hart
HSA 1151 (Pmm)	<i>B. attenuata</i>	Eneabba	R. Hart
HSA 1152 (Pmm)	<i>B. attenuata</i>	Eneabba	R. Hart
HSA 1157 (Pmm)	<i>Hakea</i> sp.	Eneabba	R. Hart
HSA 1158 (Pmm)	<i>Hakea</i> sp.	Eneabba	R. Hart
*TH 2 (Pmm)	<i>B. ilicifolia</i>	Wongonderrah	T. Hill
*TH 3 (Pmm)	<i>B. ilicifolia</i>	Wongonderrah	T. Hill
TH 4 (Pmm)	<i>B. attenuata</i>	Wongonderrah	T. Hill
TH 8 (Pmm)	<i>B. attenuata</i>	Dandaragan	T. Hill
*DP17 (Pm?)	<i>B. attenuata</i>	Wongonderrah	B. Shearer
*DP18 (Pm?)	<i>B. attenuata</i>	Wongonderrah	B. Shearer
DP20 (Pmm)	<i>B. speciosa</i>	Cape Arid	B. Shearer
*DP21 (Pmm)	<i>Dryandra sessilis</i>	Cape Arid	B. Shearer
*SEB 108 (Pm?)	<i>Hypocalymma angustifolium</i>	Wongonderrah	B. Shearer
DC 3248 (Pmm)	Taken from soil	Cape Arid	M. Stukely
DC AHP1 (Pms)	<i>E. caesia</i>	Ardross	M. Stukely
*SEB 109 (Pmm)	<i>B. nutans</i>	FRNP	C. Wilkinson
SEB 110 (Pmm)	<i>B. speciosa</i>	FRNP	C. Wilkinson
*SEB 111 (Pmm)	<i>B. attenuata</i>	FRNP	C. Wilkinson
*SEB 116 (Pmm)	<i>B. attenuata</i>	FRNP	J. Webster
*SEB 118 (Pm?)	<i>B. nutans</i>	FRNP	J. Webster
P484 (Pm?)	Alfalfa	South Africa	C. Brasier
*P450 (Pm?)	Clover	Mississippi	C. Brasier
*P445 (Pm?)	Soybean	Wisconsin	C. Brasier
P439 (Pm?)	Douglas-fir	Oregon	C. Brasier

NB. Pmm = *P. megasperma* var. *megasperma*, Pms = *P. megasperma* var. *sojae*, Pm? = taxonomic affinity needs to be confirmed, FRNP = Fitzgerald River National Park, * = isolate used in oospore formation screening trial.

Table 1.2 Overall mean radial growth rates at each of 0, 20, and 50 $\mu\text{g/ml}$ of amended *fos-ject*

Data given are means ($\pm$ s.e.) of 150 replicates at each concentration.

<i>Fos-ject</i> concentration ($\mu\text{g/ml}$)	Mean radial growth rate (mm/day)
0	4.50 (0.12)a
20	4.21 (0.13)b
50	3.97 (0.13)c

NB. Values followed by dissimilar letters are significantly different at $p=0.05$ according to Duncan's New Multiple Range test.

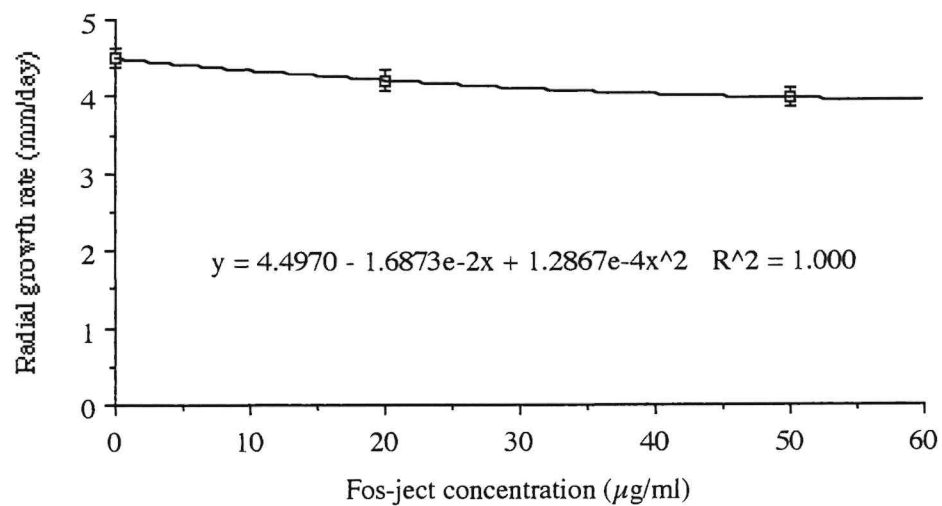


Fig. 1.1 Scatterplot of the overall mean radial growth rate for the 30 *P. megasperma* isolates tested at each of 0, 20, and 50 $\mu\text{g/ml}$ *fos-ject*. Data represent the mean of 150 replicates $\pm$ s.e.

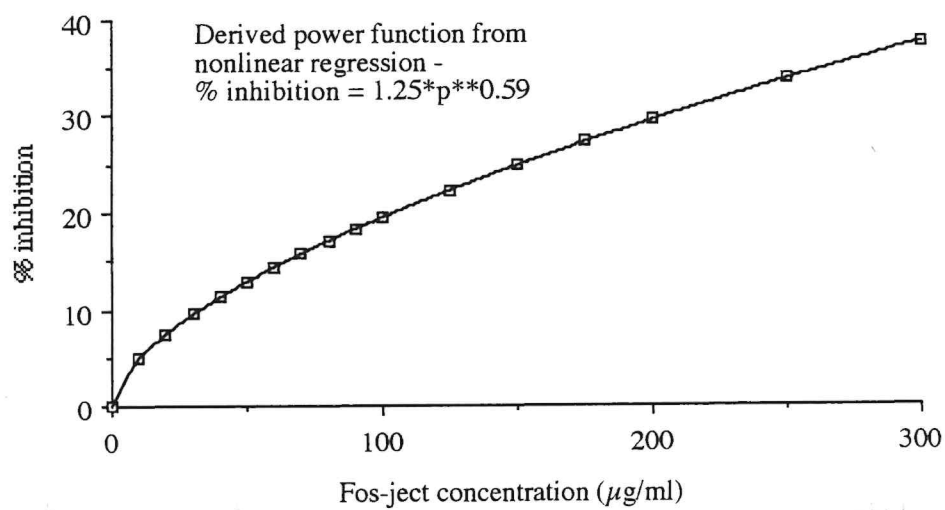


Fig. 1.2 Scatterplot of predicted mycelial inhibition values versus *fos-ject* concentration extrapolated from the derived power function.

Table 1.3 Overall oospore formation response at each of 0, 20, and 50 $\mu\text{g/ml}$ of amended *fos-ject*

Data given are means ($\pm$ s.e.) of 60 replicates at each concentration.

<i>Fos-ject</i> concentration ($\mu\text{g/ml}$)	Mean oospore formation
0	27.30 (0.79)a
20	26.77 (0.70)a
50	22.97 (0.69)b

NB. Values followed by dissimilar letters are significantly different at $p=0.05$ according to Duncan's New Multiple Range test.

Table 2.1 Oospores formed on mycelial mats emplaced in field-soil in winter 1993 at the Wongonderrah Swamp site

Data represent the mean ($\pm$ s.e.) of five replicates of each fungal isolate tested at each of five separate plots.

Season	Harvest	Isolate	Oospores formed
Winter	1 week	TH 2	2.3 (0.2)
		TH 3	1.0 (0.0)
		DP 17	0.3 (0.0)
		DP 18	2.2 (1.0)
		SEB 108	0.1 (0.0)
Winter	2 weeks	TH 2	3.5 (1.2)
		TH 3	1.4 (0.2)
		DP 17	1.3 (0.0)
		DP 18	1.3 (0.1)
		SEB 108	1.0 (0.2)

Table 2.2 Origin and characteristics of the *P. megasperma* isolates examined in morphometric study.

Isolate	Host/Isolated from....	Location	Growth rate (mm/day) ¹	Oogonium diam. ($\pm$ s.e.) (μ m) ²	Sporangium length ($\pm$ s.e.) (μ m) ³	Sporangium width ($\pm$ s.e.) (μ m) ⁴	Sporangium pore ($\pm$ s.e.) (μ m) ⁵
P452*	<i>Brassica</i> sp.	Great Britain	2.9	47.5 (0.7)a	60.0 (1.1)	39.3 (0.6)	8.7 (0.2)
TH 7	<i>Banksia prionotes</i>	N. Sandplain, WA	6.0	46.6 (0.6)a	39.4 (0.3)	25.4 (0.3)	10.2 (0.3)
DP17	<i>B. attenuata</i>	Wongonderrah, WA	3.5	45.9 (0.6)a	45.7 (1.2)	33.4 (0.7)	8.5 (0.2)
TH 1	?	Cape Arid, WA	3.5	45.8 (0.3)a	55.5 (0.7)	46.7 (0.5)	11.5 (0.3)
DC 3215	<i>B. occidentalis</i>	Black Point, WA	5.4	45.5 (0.8)a	44.7 (0.6)	32.4 (0.2)	9.1 (0.2)
PM 1738	<i>B. attenuata</i>	Namming, WA	1.9	45.1 (0.4)a	71.5 (1.4)	43.2 (0.9)	12.3 (0.4)
TH 6	<i>Leptospermum</i> sp.	Namming, WA	4.7	44.7 (0.5)a	55.2 (0.5)	36.2 (1.0)	11.7 (0.3)
DP25	<i>B. attenuata</i>	Wongonderrah, WA	3.8	43.9 (0.5)b	45.1 (0.6)	32.2 (0.4)	9.3 (0.2)
PM 3254	<i>Isopogon buxifolius</i>	Hassell H'way, WA	6.1	43.7 (0.6)b	42.1 (0.9)	31.3 (0.4)	9.9 (0.3)
P450*	Clover	Mississippi, USA	2.5	43.6 (0.6)b	46.1 (0.8)	28.5 (0.7)	8.6 (0.2)
DC27	<i>B. attenuata</i>	Cervantes Rd, WA	4.9	42.6 (0.6)c	42.5 (0.3)	32.0 (0.8)	8.9 (0.2)
HSA 1157	<i>Hakea</i> sp.	Eneabba, WA	5.1	42.5 (1.0)c	46.9 (1.4)	34.4 (1.0)	9.9 (0.3)
DP26	<i>B. attenuata</i>	Brand H'way, WA	4.8	42.4 (0.8)c	48.9 (0.7)	33.1 (0.9)	14.9 (0.3)
P471*	Apple	California, USA	5.1	42.1 (0.4)c	53.5 (2.4)	34.3 (1.1)	8.7 (0.2)
DC 1612	?	Hopetoun, WA	6.5	41.9 (0.9)c	38.7 (0.4)	25.2 (0.3)	9.5 (0.3)
DP28	<i>B. attenuata</i>	Bibby Rd, WA	3.3	41.3 (0.5)c	53.1 (1.3)	37.1 (0.7)	9.5 (0.2)
DCE 441	<i>B. attenuata</i>	Minyolo Brk., WA	3.0	41.2 (0.3)c	63.4 (2.8)	36.9 (1.0)	13.3 (0.3)
DP24	<i>H. prostrata</i>	Hedges, WA	4.9	40.7 (0.7)c	55.6 (1.2)	43.3 (0.6)	12.5 (0.4)
P439*	Douglas-fir	Oregon, USA	6.9	40.3 (0.3)d	53.0 (0.9)	36.8 (0.7)	10.1 (0.2)

DCE 439	<i>B. hookeriana</i>	Eneabba, WA	1.0	40.3 (0.4)d	34.3 (0.4)	33.7 (0.4)	8.9 (0.2)
TH 2	<i>B. ilicifolia</i>	Wongonderrah, WA	4.1	39.9 (1.0)d	48.1 (0.9)	37.3 (0.8)	12.7 (0.5)
SEB 109	<i>B. nutans</i>	FRNP, WA	5.1	39.1 (0.4)e	60.8 (1.6)	40.5 (0.8)	10.6 (0.3)
DC 3248	Soil	Cape Arid, WA	4.8	39.0 (0.5)e	50.2 (2.2)	41.7 (1.6)	14.4 (0.4)
SEB 111	<i>B. attenuata</i>	FRNP, WA	4.9	38.9 (0.7)e	50.3 (1.8)	38.9 (1.0)	12.1 (0.4)
P445*	Soybean	Wisconsin, USA	2.5	38.6 (0.3)e	33.9 (0.5)	24.5 (0.2)	7.7 (0.1)
TH 4	<i>B. attenuata</i>	Wongonderrah, WA	3.7	38.1 (0.5)e	39.9 (0.4)	27.0 (0.4)	9.9 (0.3)
SEB 118	<i>B. nutans</i>	FRNP, WA	3.3	38.1 (0.8)e	47.9 (1.2)	34.2 (1.0)	8.7 (0.2)
SEB 108	<i>Hypocalymma angustifolium</i>	Wongonderrah, WA	10.3	37.7 (0.5)f	25.1 (0.4)	23.6 (0.6)	8.8 (0.2)
TH 5	<i>B. attenuata</i>	Jurien Bay Rd, WA	3.8	37.5 (0.8)f	40.4 (0.9)	40.4 (0.9)	12.7 (0.4)
SEB 119	<i>Leucopogon</i> sp.	Boddington, WA	3.4	37.5 (0.6)f	47.5 (0.9)	33.9 (0.9)	8.9 (0.2)
SEB 113	<i>B. attenuata</i>	FRNP, WA	5.0	37.3 (1.0)f	41.3 (1.0)	40.4 (0.9)	12.4 (0.4)
P484*	Alfalfa	South Africa	2.4	36.6 (0.5)f	42.0 (0.5)	31.9 (0.3)	8.9 (0.2)
SEB 114	<i>B. baxteri</i>	FRNP, WA	4.8	36.6 (0.7)f	53.4 (1.6)	41.7 (1.3)	12.9 (0.4)
DP21	<i>Dryandra sessilis</i>	Cape Arid, WA	3.8	36.3 (0.8)f	45.5 (1.2)	36.9 (1.1)	10.9 (0.3)
SEB 116	<i>B. attenuata</i>	FRNP, WA	4.9	36.3 (0.7)f	50.5 (0.8)	36.7 (0.8)	9.1 (0.3)
SEB 115	<i>B. attenuata</i>	FRNP, WA	4.9	36.1 (0.8)f	51.8 (1.6)	39.7 (0.9)	15.9 (0.1)
HSA 1151	<i>B. attenuata</i>	Eneabba, WA	5.0	35.7 (1.1)f	43.9 (1.1)	34.1 (1.0)	8.7 (0.2)
SEB 1	<i>B. menziesii</i>	Yerramullah, WA	9.3	35.7 (0.6)f	33.6 (1.0)	33.6 (1.0)	8.4 (0.2)
TH 8	<i>B. attenuata</i>	Dandaragan, WA	5.9	35.5 (0.5)f	39.3 (0.3)	26.1 (0.5)	9.3 (0.3)
TH 3	<i>B. ilicifolia</i>	Wongonderrah, WA	4.4	35.5 (0.6)f	48.7 (0.2)	39.5 (0.2)	8.2 (0.1)
HSA 1152	<i>B. attenuata</i>	Eneabba, WA	3.6	34.9 (0.4)g	47.0 (1.6)	38.9 (0.4)	10.1 (0.3)
SEB 110	<i>B. speciosa</i>	FRNP, WA	4.5	33.3 (0.6)g	49.5 (1.8)	37.9 (1.0)	12.2 (0.4)
HSA 1150	<i>B. hookeriana</i>	Eneabba, WA	6.0	33.3 (0.9)g	55.1 (1.3)	37.8 (0.7)	10.3 (0.3)
HSA 1158	<i>Hakea</i> sp.	Eneabba, WA	4.2	32.9 (0.3)g	50.1 (1.7)	38.0 (0.6)	12.4 (0.3)

DC29	<i>B. speciosa</i>	FRNP, WA	10.1	32.1 (0.5)h	33.6 (0.6)	30.1 (0.6)	8.4 (0.2)
DP20	<i>B. speciosa</i>	Cape Arid, WA	4.6	31.9 (0.5)h	39.9 (0.5)	34.7 (0.4)	12.1 (0.3)
SEB 65	<i>H. victoria</i>	FRNP, WA	3.5	31.9 (0.6)h	49.7 (1.2)	41.8 (1.0)	13.3 (0.4)
SEB 112	<i>B. attenuata</i>	FRNP, WA	5.6	31.7 (0.4)h	50.0 (1.7)	38.0 (1.0)	15.9 (0.1)
DC 1321	Soil	Esperance, WA	5.4	31.5 (0.8)h	37.3 (0.6)	24.8 (0.5)	8.8 (0.2)
DCE 442	<i>B. attenuata</i>	Mullering Brk., WA	5.8	31.4 (1.0)h	51.6 (2.2)	32.9 (1.3)	14.2 (0.4)
DCE 177	<i>Pinus radiata</i>	Jarrahwood, WA	5.5	31.3 (0.6)h	45.9 (0.7)	31.7 (0.7)	11.9 (0.3)
R3-6 283	<i>P. radiata</i>	Jarrahwood, WA	5.2	29.8 (0.7)i	48.8 (1.2)	29.4 (0.7)	8.5 (0.2)
R1-2 283	<i>P. radiata</i>	Jarrahwood, WA	3.3	29.2 (0.8)i	51.7 (1.4)	30.9 (0.9)	8.7 (0.2)
DP18	<i>B. attenuata</i>	Wongonderrah, WA	2.8	28.6 (0.6)j	42.9 (1.0)	35.9 (1.0)	10.9 (0.3)
DC 480 3R	<i>P. radiata</i>	Jarrahwood, WA	5.3	26.2 (0.3)k	64.6 (1.3)	42.2 (0.7)	13.0 (0.4)
DC AHP 1	<i>Eucalyptus caesia</i>	Ardross, WA	4.9	25.9 (0.3)k	50.3 (1.1)	28.5 (0.6)	9.9 (0.3)
SEB 117	<i>H. ruscifolia</i>	Hedges, WA	4.2	25.5 (0.3)k	38.1 (1.6)	27.7 (0.7)	9.9 (0.2)
DCE 440	<i>Conospermum triplinervum</i>	Eneabba, WA	3.7	24.8 (0.3)l	57.2 (1.4)	38.5 (0.6)	9.1 (0.3)
DCE 187	<i>E. caesia</i>	Baldivis, WA	5.0	23.7 (0.5)l	38.8 (0.4)	25.1 (0.3)	9.5 (0.3)

NB: FRNP = Fitzgerald River National Park.

* = Isolate described in Hansen *et al.* (1986) and provided by C. Brasier (Forest Authority Research Division, U.K.).

1 = Fisher's LSD=0.3 (p=0.05).

2 = Entries followed by the same letter are not significantly different as indicated by the Scott-Knott procedure ($\lambda=500$, p=0.002).

3 = Fisher's LSD=1.2 (p=0.05).

4 = Fisher's LSD=0.8 (p=0.05).

5 = Fisher's LSD=0.3 (p=0.05).