Technique for Rapid Assessment of Tolerance of *Banksia* spp. to Root Rot Caused by *Phytophthora cinnamomi*

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ABSTRACT

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The technique of stem wound inoculation is described that provides a rapid, reliable method of assessing and calibrating tolerance of *Banksia* spp. to root rots caused by *Phytophthora cinnamomi*. Inoculated intact stems and excised stem-cuttings developed characteristic lesion lengths. Similar results for the relative susceptibility of a species were obtained using wound-inoculating and zoospore root-drenching procedures. Both techniques showed *Banksia ashbyi, B. verticillata, B. victoriae,* and *B. prionotes* susceptible and *B. baueri* tolerant to *P. cinnamomi*. Root lesions were difficult to measure because of secondary infection and irregularities in root morphology. Using intact, field-grown plants, stem lesion length was useful for assessing interspecific and intraspecific tolerance of *Banksia* spp.

Additional key word: Proteaceae

The genus *Banksia* L.f. (Proteaceae) comprises some 72 species, with all but one species (*B. dentata* L.f.) confined to Australia. Most species are found in the well-drained, improverished soils of the sand-plain (kwongan) areas of southwest Western Australia (6).

In recent years, many *Banksia* spp. have become important as horticultural subjects both in domestic plantings and large-scale commercial operations for cut-flower production. Root rots caused by *Phytophthora cinnamomi* Rands are the most serious threat to cultivation of *Banksia* spp., causing more than 20% loss in commercial *Banksia* plantings in Western Australia.

P. cinnamomi is widespread in the jarrah forests of Western Australia and has been isolated from dead and dying banksias in natural stands (10). Other *Phytophthora* spp. have been recorded on *Banksia* spp. These include *P. citrophthora* (Sm. & Sm.) Leon. on several species in Israel (P. Elphick, *personal communication*) and *P. cactorum* (Lebert & Cohn) Schröter and *P. nicotianae* var. *parasitica* (Dast.) Waterhouse on container-grown specimens in Western Australia (K. Dixon and K. Sivasithamparam, *unpublished*).

The symptoms of *P. cinnamomi* infection of banksias are wilting and death of new shoots and other unlignified tissues followed by marginal scorching of older leaves. Although these symptoms may be restricted to the top half of a mature plant, the disease eventually spreads, causing intense browning of vascular elements in the collar region of the stem, and finally, death of the plant (3,9).

Chemical control of root rot that has been effective with other tree crops such as avocado (14) does not appear to be as promising with *Banksia*, which like several other Proteaceous plants, is sensitive to fungicides such as metalaxyl (7,11). Moreover, by the time *P. cinnamomi* is diagnosed on this host, the disease is so well entrenched that fungicidal applications are futile.

To overcome this disease threat to *Banksia* floriculture in Western Australia, a research program has been initiated to select banksias for intraspecific and interspecific tolerance to *P. cinnamomi*. Similar studies with *Banksia* seedlings in Hawaii (3,4) indicate that variation exists among species.

This paper reports a wound-inoculation technique for rapid screening of mature *Banksia* plants for tolerance to *P. cinnamomi*. Intact and excised stems of container- and field-grown *Banksia* plants were used in the study. Comparisons were also made between root and stem lesion lengths with time on a range of *Banksia* material. Similar stem-inoculation techniques have been used in the selection of rootstocks in apples for resistance to stem canker caused by *P. cactorum* (1,5).

MATERIALS AND METHODS

Eighteen species were examined as stem cuttings, with replicates per species ranging from seven to 13 for inoculation treatments and five for uninoculated controls. Stems for inoculation were 18 mo to 2 yr old and of similar vigor and diameter (5–7 mm).

In root-inoculation studies, use of similar criteria for selection of inoculation points was hampered by the difficulty in extracting suitable roots without causing excessive damage to the plant. Hence root age and size were less homogeneous than stem material.

A wound-inoculation procedure modified from Tippett et al (12) was the most reliable. An oblique incision 5–7 mm long and 0.8–1.0 mm deep was made between alternate leaf insertions, or in the case of roots, at an easily accessible site free of major lateral connections. Agar plugs about 8 mm^3 were removed from the actively growing edge of a 3-day-old culture of *P. cinnamomi* growing on 10% V-8 juice agar (8) and inserted in the wound. To prevent desiccation and displacement of fungal inoculum from the wound, the inoculated area was securely bound with stretchable Parafilm and enclosed in aluminum foil. Controls comprised plugs of similarly aged V-8 juice agar inserted in the wounds and secured as described before.

Field inoculations consisted of 10 species and a varietal form with five stems per plant and five plants per species being inoculated with *P. cinnamomi*. Five stems of each species were used as controls. Studies using container-grown plants were conducted on nine species, with four used stems inoculated on two to six plants of each species. A similar number of roots and replicate plants was used for root inoculation. Controls for stem and root inoculations consisted of five replicates per species. Five species of *Banksia*, each with five replicates, were used in zoospore inoculations, with two replicates per species for control.

Zoospores produced using the technique of Byrt and Grant (2) were applied to premoistened soil of container-grown plants at the rate of 5,000 zoospores per plant. High moisture and humidity levels were maintained in the soil by enclosing the pot in a sealed plastic bag. The bag was removed after 48 hr and normal watering resumed.

All inoculated plants were harvested and examined 21 days after incubation. Temperatures ranged from 14 to 26 C for field trials and from 20 to 24 C for

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glasshouse trials, with inoculated cuttings being maintained in a humid clear plastic chamber at 20–23 C.

Serial sections (3 mm) of inoculated stem and root were plated onto a selective $P_{10}VP$ medium (13) to verify the presence of *P. cinnamomi*. The lesion severity index (LSI) in inoculated stems was assessed on a scale of 1–5 on the basis of lesion length, where 1 = 0–5, 2 = 5.1–10, 3 = 10.1–15, 4 = 15.1–20, and 5 = >20 cm (Table 1). Plants inoculated with zoospores were washed free of soil and the roots rated on a disease severity index (DSI) of 1–5, where 1 = no obvious disease symptoms and 5 = whole plant death.

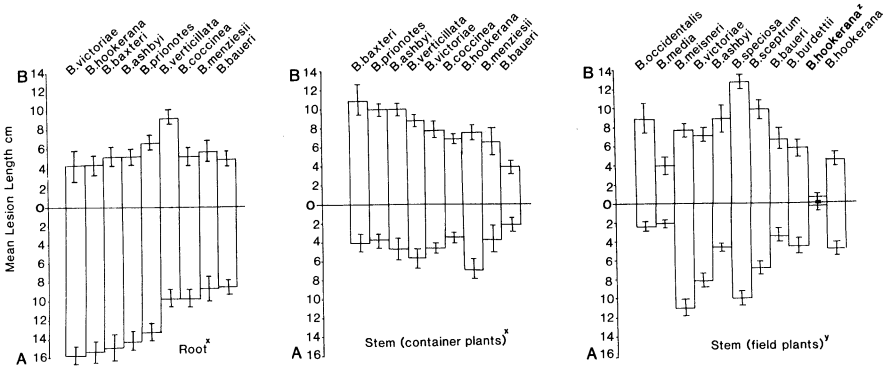

Fig. 1. Mean lesion length measured after 21 days and segregated for stem tissues into length from point of inoculation (B) toward or (A) away from stem apex. Root lesions were assessed similarly, with measurements taken (A) toward or (B) away from root apex. x = container-grown plants maintained under glasshouse conditions, y = field-grown plants, and z = presumably tolerant variety of *Banksia hookerana*.

RESULTS

Stem inoculations. Hyphal activity in tissues of all species examined were within 10 mm of the lesion front. Dark brown to black elliptical stem lesions were evident 6 days after inoculation with the pathogen. Lesion extension was usually greater toward the shoot apex (acropetal extension) (Fig. 1).

Nine species of container-grown *Banksia* showed a gradual and constant rate of lesion extension, although *B. ashbyi* E. G. Baker had accelerated fungal growth for a short period followed by retardation (Table 2). Similarly, *B. menziesii* R. Br. showed no appreciable lesion extension beyond 12 days after inoculation. The remaining species had achieved maximal lesion extension by 21 days. Necrosis was also observed in petioles and midribs of adjacent leaves, with resultant marginal scorching and death of the lamina. The range in stem lesion lengths for container-grown plants in terms of LSI is shown in Table 1.

Field-inoculated stems showed a wide range of lesion lengths, with LSI values ranging from 1 to 5 (Table 1). Significantly, the variant *B. hookerana* Meissner showed the greatest suppression of lesion extension of all species examined. Compared with the lesion length normally recorded for this species, lesion length in the *B. hookerana* variant was of the order of 40× less.

Consistent with lesion extension in intact stems of potted plants (Fig. 1), most field-inoculated stems showed enhanced acropetal rather than basipetal lesion growth (Fig. 1). Inoculated stem cuttings showed highly variable lesion lengths for the 17 species examined (Table 1). Comparing LSI values in Table 1, it is apparent that inoculations on excised stems generally overestimated a species response to *P. cinnamomi*.

Root inoculations. Root lesion extension is shown in Figure 1 and expressed as LSI values in Table 3. Measurements were complicated by irregularities in root morphology and also by the development of secondary infection sites. Variation in lesion shape and size was therefore greater for

Table 1. Lesion extension responses for inoculated stems either intact (field- and container-grown plants) or excised (cuttings of field-grown plants) for a range of *Banksia* spp.

	Stem lesion extension					
	Whole plants					
	Field		Container		Stem cuttings	
Species	Lesion length (cm)	LSI	Lesion length (cm)	LSI	Lesion length (cm)	LSI
B. speciosa R. Br.	22.8 a[w]	5.0 a[x]	...[y]	...	23.8 a	5.0 a
B. meisneri Lehm.	19.3 ab	4.3 ab	...	...	7.8 hi	2.3 i
B. sceptrum Meissner	16.2 bc	3.7 bc	...	...	16.0 def	3.7 de
B. victoriae Meissner	15.1 bc	3.6 bc	12.5 a	3.3 a	16.6 cde	3.8 cde
B. ashbyi E. G. Baker	13.4 cd	3.1 c	14.6 a	3.6 a	14.6 ef	3.5 ef
B. occidentalis R. Br.	10.8 d	2.6 d	...	...	19.5 b	4.5 b
B. burdettii E. G. Baker	10.5 cd	2.5 de	...	...	9.7 h	2.6 h
B. hookerana Meissner	10.4 de	2.4 de	14.5 a	3.7 a	17.0 cd	3.9 cd
B. hookerana[z]	0.2 f	1.0 f	...	...	...	...
B. baxteri R. Br.	10.1 de	2.5 de	14.9 a	3.7 a	16.4 de	3.7 de
B. media R. Br.	6.3 e	1.8 e	...	...	7.0 i	2.2 i
B. baueri R. Br.	...	...	5.8 b	1.9 b	...	...
B. prionotes Lindley	...	...	13.8 a	3.6 a	...	...
B. verticillata R. Br.	...	...	14.7 a	3.7 a	...	...
B. coccinea R. Br.	...	...	10.0 b	2.1 b	...	...
B. menziesii R. Br.	...	...	8.6 b	2.1 b	...	...
B. lemanniana Meissner	...	...	...	...	22.9 a	5.0 a
B. lindleyana Meissner	...	...	...	...	18.8 bc	4.2 bc
B. tricuspis Meissner	...	...	...	...	13.7 fg	3.3 fg
B. praemorsa Andrews	...	...	...	...	13.0 g	3.2 g
B. quercifolia R. Br.	...	...	...	...	6.8 i	2.1 j
B. caleyi R. Br.	...	...	...	...	6.5 i	2.1 j
B. elderana F. Muell. & Tate	...	...	...	...	3.0 j	1.5 k
B. pilostylis C. Gardner	...	...	...	...	...	...

[w] Numbers followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

[x] Mean lesion severity index (LSI) based on lesion length: 1 = 0–5, 2 = 5.1–10, 3 = 10.1–15, 4 = 15.1–20, and 5 = >20 cm.

[y] Plant material not available for testing.

[z] Variant of *B. hookerana*.

Table 2. Daily mean lesion extension (cm) by *Phytophthora cinnamomi* inoculated onto mature stems of a range of container-grown *Banksia* spp. maintained under glasshouse conditions

Day[a]	*B. hookerana*		*B. victoriae*		*B. baueri*		*B. prionotes*		*B. verticillata*		*B. coccinea*		*B. ashbyi*		*B. baxteri*		*B. menziesii*	
	$\bar{X}$[b]	SEM[c]	$\bar{X}$	SEM	$\bar{X}$	SEM	$\bar{X}$	SEM	$\bar{X}$	SEM	$\bar{X}$	SEM	$\bar{X}$	SEM	$\bar{X}$	SEM	$\bar{X}$	SEM
9	6.10	0.63	3.20	0.17	3.40	0.01	5.18	0.37	6.00	0.40	5.00	0.96	8.92	2.11	9.57	1.17	7.00	1.15
10	7.11	0.46	3.07	0.33	4.00	0.79	6.04	0.63	7.02	1.95	7.70	1.47	9.13	1.76	10.50	1.79	7.33	1.28
11	8.07	0.51	4.70	0.37	4.10	0.24	7.76	0.79	8.02	1.85	7.50	2.15	12.22	1.34	10.51	1.43	8.18	1.25
12	9.33	0.79	6.50	0.34	4.13	1.80	7.95	0.84	8.76	1.77	8.40	2.03	13.40	1.28	10.82	1.49	8.60	1.11
13	9.92	0.69	7.10	0.79	4.71	0.36	9.00	0.64	9.87	1.90	8.58	1.85	14.66	1.90	12.53	1.73	8.60	1.11
14	10.30	1.08	7.16	0.80	5.07	0.53	9.31	0.64	10.60	1.28	8.84	1.83	14.66	1.93	12.55	1.85	8.60	1.11
15	11.15	1.03	7.28	0.65	5.26	0.73	9.85	0.82	11.40	1.29	9.00	1.93	14.66	1.93	12.80	1.81	8.60	1.11
16	11.85	1.46	7.43	0.87	5.37	1.00	10.68	0.77	11.90	1.54	9.25	1.53	14.66	1.93	13.13	2.01	8.60	1.11
17	12.00	1.49	7.62	0.64	5.40	0.86	11.02	0.78	13.40	1.69	9.50	1.78	14.66	1.93	13.50	2.04	8.60	1.11
18	12.46	1.50	7.91	0.60	5.55	1.71	11.26	0.74	13.50	1.84	9.80	1.78	14.66	1.93	14.45	2.12	8.60	1.11
19	13.11	1.58	8.18	0.61	5.75	0.84	12.15	0.87	14.40	2.00	10.00	2.43	14.66	2.43	14.86	2.07	8.60	1.11
20	13.80	1.59	10.30	0.60	5.80	0.83	13.00	0.79	14.50	1.80	10.00	2.40	14.66	2.43	14.90	1.81	8.60	1.11
21	14.53	1.02	12.52	0.47	5.95	0.84	13.85	0.81	14.66	0.64	10.00	1.30	14.66	1.46	14.95	0.94	8.60	1.11

[a] Days since inoculation with *Phytophthora cinnamomi*.
[b] Mean lesion extension (cm).
[c] Standard error of the mean.

Table 3. Root and stem lesion extension values with correlation coefficient for container-grown *Banksia* spp. maintained under glasshouse conditions

Species	LSI		r[y]
	Root[x]	Intact stem[x]	
Susceptible			
B. baxteri	4.6 a[z]	3.7 a	0.58
B. verticillata	4.6 a	3.7 a	0.86
B. ashbyi	4.6 a	3.6 a	1.00
B. hookerana	4.4 a	3.6 a	0.63
B. prionotes	4.4 a	3.6 a	0.63
B. victoriae	4.3 a	3.3 a	0.56
Tolerant			
B. menziesii	3.2 b	2.1 b	0.87
B. coccinea	3.1 b	2.1 b	1.00
B. baueri	3.1 b	1.9 b	0.73

[x] Mean lesion severity index (LSI) for container-grown plants based on lesion length: 1 = 0–5, 2 = 5.1–10, 3 = 10.1–15, 4 = 15.1–20, and 5 = >20 cm.
[y] Coefficient of correlation between root LSI and stem LSI values.
[z] Numbers followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

Table 4. Response of container-grown *Banksia* spp. 21 days after drenching with zoospores[x]

Species	Mean disease severity index[y]
B. ashbyi	5.0 a[z]
B. verticillata	4.9 a
B. victoriae	4.8 a
B. prionotes	4.5 a
B. baueri	2.4 b

[x] Plants maintained under glasshouse conditions.
[y] Based on a scale of 1–5, from 1 = no obvious disease symptoms to 5 = whole-plant death.
[z] Numbers followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

inoculated roots than for stems. Fungal extension in the root was significantly greater from the point of inoculation toward, rather than away from, the growing apex of the root (Fig. 1). *B. verticillata* R. Br. and *B. menziesii* were the only species in which there was no significant difference in direction of lesion extension from the origin. Comparison between root and stem LSI values showed good to excellent correlation (Table 3).

Of the five container-grown species drenched with zoospores, only *B. baueri* R. Br. showed a significant degree of tolerance to *P. cinnamomi* (Table 4). The remaining four species showed advanced wilting and scorching of basal leaves 21 days after inoculation with 10-cm-long lesions that developed into the above-ground stems. The collar region in most of these susceptible plants was heavily infected and was often girdled.

Control inoculations. Lesion development was not observed in any control inoculations. Mild browning was evident around control incisions.

DISCUSSION

Root and stem lesions were highly correlated, particularly when LSI values are compared (Table 1). Furthermore, both techniques segregated the nine species tested in this study into two major tolerance groups. The outcome from zoospore drenching studies also showed a similar segregation of species. Both techniques showed *B. baueri* as possessing a degree of tolerance. This result demonstrates the potential use of stem inoculation for predicting tolerance of banksias to zoospore-initiated root infections. However, a larger number of species would need to be compared using both techniques before accepting stem inoculation as a reliable method to predict root tolerance.

In comparing the reproducibility of various stem inoculation procedures, similar lesion responses, when expressed as LSI values, were recorded for the four species common to all procedures (Table 1). LSI values for 10 species common to both field and cutting inoculations were again found to be similar. However, discrepancies in lesion extension occurred between procedures as in the instances of *B. meisneri* Lehm., *B. occidentalis* R. Br., *B. hookerana*, and *B. baxteri* R. Br. (Table 1). These irregularities may reflect physiological variability of samples and lack of control over the field environment rather than failure of technique. Indeed, under uniform conditions, the value of stem inoculation as a predictive technique is obvious as seen with the suppressed lesion extension recorded for the *B. hookerana* variant that had survived a natural infection by *P. cinnamomi*.

For ease of handling and containment of inoculum, cuttings are most convenient for screening purposes. Although lesion length was greater using excised stems than intact stems of either field- or container-grown plants (Table 1), the order of species based on lesion length was comparable between the techniques. Thus, it may be possible to calibrate variation in tolerance among these species or varieties by using lesion length of excised cuttings. Overall stem lesion length was more reliable than acropetal or basipetal lesion measurements for estimating species tolerance.

When extended to include all species and varietal forms of *Banksia*, a scale of tolerance to *P. cinnamomi* may be assembled. The stem-inoculation technique has the advantage of using mature stems, which presumably reflect the response of mature, intact plants. More traditional techniques for root-rot tolerance screening have used zoospore inoculation (3,4) on seedling banksias. Further refinement of the excised stem-inoculation procedure will facilitate widespread and rapid screening of wild *Banksia* stands for

varietal lines tolerant to *P. cinnamomi.*

Banksia spp. in pristine habitats may also be screened without danger of accidental introduction of *P. cinnamomi* and with the convenience of using stems of individual plants as genetically identical replicates.

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LITERATURE CITED

1. Borecki, Z., and Millikan, D. F. 1969. A rapid method for determining the pathogenicity and factors associated with pathogenicity of *Phytophthora cactorum*. Phytopathology 59:247-248.
2. Byrt, P., and Grant, B. R. 1979. Some conditions governing zoospore production in axenic cultures of *Phytophthora cinnamomi* Rands. Aust. J. Bot. 27:103-115.
3. Cho, J. J. 1981. Phytophthora root rot of *Banksia*: Host range and chemical control. Plant Dis. 65:830-833.
4. Cho, J. J. 1983. Variability in susceptibility of some *Banksia* species to *Phytophthora cinnamomi* and their distribution. Plant Dis. 67:869-871.
5. Dakwa, J. T., and Sewell, G. W. F. 1981. Influence of rootstock type and time of inoculation on the resistance of five apple scion cultivars to collar rot caused by *Phytophthora cactorum*. J. Hortic. Sci. 56:357-362.
6. George, A. S. 1981. The genus *Banksia* L.f. (Proteaceae). Nuytsia 3:239-473.
7. Greenhalgh, F. C. 1979. Effect of organic matter on phytotoxicity and efficacy of Ridomil for Phytophthora root rot control. Aust. Plant Pathol. 8:19-20.
8. Hwang, S. C., Ko, W. H., and Aragaki, M. 1975. A simplified method of sporangial production of *Phytophthora cinnamomi*. Mycologia 67:1233-1237.
9. Marks, G. C., Smith, I. W., and Kassaby, F. Y. 1981. Trunk infection of *Eucalyptus* species by *Phytophthora cinnamomi* Rands: A preliminary report. Aust. For. Res. 11:257-267.
10. Podger, R. D. 1972. *Phytophthora cinnamomi*, a cause of lethal disease of indigenous plant communities in Western Australia. Phytopathology 62:972-981.
11. Sivasithamparam, K. 1983. Cultivation of Australian plants—disease problems and their control. Aust. Hortic. 81:54-55.
12. Tippett, J. T., Shea, S. R., Hill, T. C., and Shearer, B. L. 1983. Development of lesions caused by *Phytophthora cinnamomi* in the secondary phloem of *Eucalyptus marginata*. Aust. J. Bot. 31:197-210.
13. Tsao, P. H., Tummakate, A., and Bhavakul, K. 1976. Recovery of *Phytophthora* species from old, badly decayed infected tissues of *Hevea brasiliensis*. Trans. Br. Mycol. Soc. 66:557-558.
14. Zentmyer, G. A. 1980. *Phytophthora cinnamomi* and the disease it causes. Monogr. 10. American Phytopathological Society, St. Paul, MN. 96 pp.