Cryptodiaporthe melanocraspeda Canker as a Threat to *Banksia coccinea* on the South Coast of Western Australia

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ABSTRACT

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In 1989, large numbers of *Banksia coccinea* in the south coast region of Western Australia were observed dying downward from the apical branches. Plant death in 15-year-old *B. coccinea* increased from 40 to 98% in a 2.7-year period. Complete death of the stand within a relatively short time was typical of many diseased stands of *B. coccinea* in the region. *Cryptodiaporthe melanocraspeda* (anamorph *Diplodina melanocraspeda*) was the most frequently isolated fungus from lesions from individual plants at four sites. Of 109 isolations from pooled lesion samples at 53 sites, 47% were *Botryosphaeria ribis,* 36% *C. melanocraspeda*, 8% *Microsphaeropsis* sp., 6% *Cytospora* sp., and 3% *Zythiostroma* sp. Pathogenicities of *Botryosphaeria ribis, C. melanocraspeda,* and the *Zythiostroma* sp. were compared in wound-inoculated stems of *Banksia baxteri* and *B. coccinea*. *Botryosphaeria ribis* formed small lesions, which did not girdle stems, and was considered a weak pathogen. Although the *Zythiostroma* sp. formed lesions that girdled stems, it was not considered a major cause of death due to infrequent isolation from *B. coccinea.* It was concluded that *C. melanocraspeda* infection was associated with death of *B. coccinea* because the fungus formed lesions that girdled stems and because it was frequently isolated from dying *B. coccinea* throughout the banksia's geographic range. Within the region, *C. melanocraspeda* was isolated also from other Proteaceae: *Banksia attenuata, B. baxteri, B. grandis, B. speciosa, Dryandra cuneata,* and *D. falcata.* This report is the first record of a *Zythiostroma* sp. on *Banksia* and extends the geographic and host range of *Cryptodiaporthe.*

Banksia coccinea R. Brown, or scarlet banksia, is a distinctive native plant species of Western Australia with no close relatives within the Proteaceae (8). It grows as a shrub or small tree up to 2 to 4 m high, mainly associated with tall shrub land, heath, or mallee-heath on deep white or gray sands along the south coast of Western Australia (17). Because of its unique scarlet flower, *B. coccinea* is frequently harvested commercially for the cut-flower industry (6).

B. coccinea is highly susceptible to the introduced soilborne pathogen *Phytophthora cinnamomi* Rands (12), and until recently *P. cinnamomi* was the only major pathogen recognized as threatening *B. coccinea* populations (17). However, in 1989 plants of *B. coccinea* were observed dying in large numbers in areas not affected by *P. cinnamomi*. Plants were dying downward from the apical branches, apparently through infection by canker fungi (15). We sought in this study to (i) survey diseased plants throughout the geographic range of *B. coccinea* to describe symptom expression, determine the distribution of canker-induced mortality of *B. coccinea,* and quantify isolation frequencies of suspected pathogens; (ii) measure mortality increase in one location; and (iii) evaluate the pathogenicity of fungi frequently isolated from diseased tissue.

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MATERIALS AND METHODS

Disease survey. Fifty-seven stands of all ages were sampled for symptoms of aerial canker throughout the geographic range of *B. coccinea* (Fig. 1). In stands at four sites (Table 1), isolations were made from one lesion per diseased plant (five to 100 plants per site). In another 53 sites, tissues from three to six lesions were pooled for each site before plating. Bark of the main stems and lateral branches of dying trees was removed, and the lesion margins were located. Tissue straddling the lesion margin was removed and surface-disinfested in 70% ethanol for 30 s, washed in distilled water, blotted dry, and plated on half-strength potato-dextrose agar (0.5 PDA) (19.5 g of Difco PDA and 7.5 g of Bacto agar in 1 L of distilled water). The plated tissue was incubated at room temperature under near-ultraviolet light for 1 to 2 weeks, and the fungi isolated were recorded. Isolates were stored on 0.5 PDA slants at 5°C or in sterile distilled water (4).

Mortality measurements. Mortality was measured in a diseased stand located about 1 km inland from the coast at Cheyne Beach (34° 53' 21" S, 118° 25' 18" E) 50 km east of Albany. The site was covered with 15-year-old *B. coccinea* scrub-heath up to 1 m high, growing on a gently sloping convex area of stabilized dunes. The soils were deep, infertile white sands over granite. Plant death over time was assessed from 26 September 1989 to 8 July 1992 in three 10 × 10 m plots positioned within the diseased area. The number of *B. coccinea* within the monitored plots ranged from 362 to 928, with a mean ± standard error of 661 ± 164 plants per plot.

Pathogenicity tests. The site for pathogenicity tests was adjacent to the mortality study and covered with *B. baxteri* R. Brown and *B. coccinea* scrub-heath near the crest of stabilized dunes. Host (*B. baxteri* and *B. coccinea*), assessment time (42 and 141 days after inoculation), and canker fungi and isolates (Table 2) were the independent variables, with longitudi-

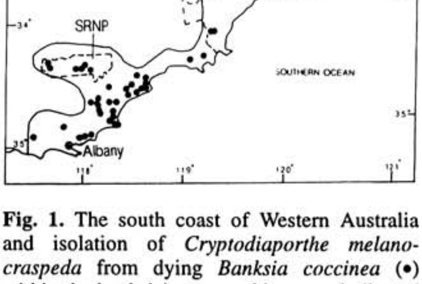

Fig. 1. The south coast of Western Australia and isolation of *Cryptodiaporthe melanocraspeda* from dying *Banksia coccinea* (•) within the banksia's geographic range indicated by a solid line (adapted from Taylor and Hopper [17]). Dashed lines indicate major National Parks: SRNP = Stirling Range National Park, and FRNP = Fitzgerald River National Park.

nal and tangential lesion development as the dependent variables. There were 10 replicates of each host–isolate–assessment time combination in a randomized block design.

Stems of *B. baxteri* and *B. coccinea* were wound-inoculated with test fungi in early February (midsummer) 1990 using methods described previously (16). An agar disk containing mycelium of the test fungus was bound to a fresh cut in the phloem. Control stems were inoculated in a similar manner with sterile agar disks.

Stems were removed from the field 42 and 141 days after inoculation. Lesion length above and below the inoculation point was measured after shaving off the outer bark, and tangential spread at the inoculation point was estimated. The presence of the test fungus was determined by plating 10 chips from the phloem tissue at the lesion margin of each inoculated stem on 0.5 PDA as described previously. Tissue from the control inoculations was plated as described for stems inoculated with fungi.

Data were examined for normality. Where appropriate, lesion measurements were log-transformed and percentage data arcsine-transformed to satisfy assumptions of normality. Analysis of variance (ANOVA) was computed using MGLH procedures of SYSTAT (18) with host, assessment time, and canker fungi and isolate as fixed variables and isolates nested within canker fungi. Outliers with Studentized residuals greater than 2.5 (18) were identified and removed from the data set. Significance of pairwise differences between means was determined using the Tukey-Kramer HSD procedure (18).

Table 1. Percent isolation of fungi from individual plants of diseased *Banksia coccinea* at four sites

	Site/No. plated				
Fungus isolated	**Bluff Creek (n = 8)**	**Bluff River (n = 27)**	**Cheyne Beach (n = 100)**	**Hunwick Road (n = 5)**	**Mean**
Botryosphaeria ribis	62	4	11	0	19
Cryptodiaporthe melanocraspeda	75	78	87	100	84
Zythiostroma sp.	0	0	0	20	5

Table 2. Isolates of *Botryosphaeria ribis*, *Cryptodiaporthe melanocraspeda*, and *Zythiostroma* sp. used in the pathogenicity test

Isolate no.	Fungus	IMI[a] no.	Host
DC24	*Botryosphaeria ribis*	336151	*Banksia baxteri*
DC37	*Botryosphaeria ribis*	336152	*B. coccinea*
DC41	*C. melanocraspeda*	335476	*B. coccinea*
DC42	*C. melanocraspeda*	335475	*B. coccinea*
DC27	*Zythiostroma* sp.	336153	*B. baxteri*
DC28	*Zythiostroma* sp.	336154	*B. baxteri*

[a] International Mycological Institute.

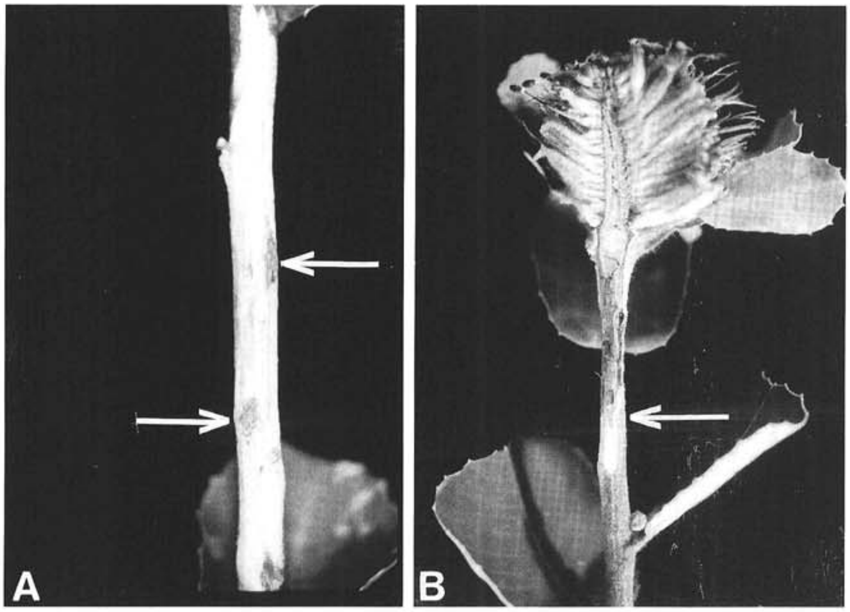

Fig. 2. *Banksia coccinea* showing symptoms of infection by *Cryptodiaporthe melanocraspeda*. **(A)** The outer bark removed showing brown necrotic lesions (arrows) expanding from leaf nodes. **(B)** Flower head and stem cut in half showing a lesion advancing down the stem from the flower head. Arrow indicates the lesion front between necrotic tissue and healthy tissue of the lower part of the stem.

RESULTS

Symptoms. Most affected plants exhibited dead leader and lateral branches, giving diseased stands a debilitated appearance. Initial symptoms were drying of leaves on shoot apices and branches. The leaves eventually became pale brown and were easily detached. Removal of the outer bark of stems shedding leaves revealed small, brown, necrotic lesions expanding from the leaf nodes (Fig. 2A). Lesions gradually enlarged, showing an orange to dark brown discoloration of the phloem. Lesion development from flower heads was common during flowering (Fig. 2B). The lesions progressively girdled lateral stems, causing branch dieback and eventual death of the plant. Lesion limits were not clearly defined on the stem surface, and callus formation was rarely seen. Resinosis did not occur. Small, black conidomata were produced beneath the outer bark in necrotic tissue. In moist weather during summer and autumn (December to May), conidomata produce pale white to pale pink spore tendrils on the surface of recently killed portions of stems. Small clusters of perithecia were produced beneath the bark in older cankers.

Mortality. Plant death increased from 40 to 98% in 2.7 years in the monitored area (Fig. 3). The mortality progress curve was similar to that associated with monomolecular diseases, with the greatest rate of mortality in summer months of December to February and least in winter months of June to August (Fig. 3). The complete death of the stand within a relatively short time was typical of other diseased stands of *B. coccinea*.

Isolations. *Cryptodiaporthe melanocraspeda* Bathgate, Barr, & Shearer

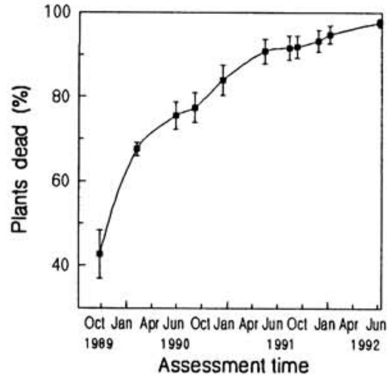

Fig. 3. Percentage (± standard error of the mean) of *Banksia coccinea* plants killed during a 2.7-year period in a stand infected with *Cryptodiaporthe melanocraspeda*.

(anamorph *Diplodina melanocraspeda*) (1), a previously undescribed fungus, was the most frequently isolated fungus from individual lesions at four sites (Table 1). Of 109 fungi isolated from pooled lesion samples at an additional 53 sites, 47% were *Botryosphaeria ribis* Gossenb. & Duggar, 36% *C. melanocraspeda*, 8% *Microsphaeropsis* sp., 6% *Cytospora* sp., and 3% *Zythiostroma* sp.

C. melanocraspeda was isolated from dying *B. coccinea* throughout the banksia's geographic range (Fig. 1). Within the region, *C. melanocraspeda* was also isolated from lesions in other Proteaceae: *Banksia baxteri* and *B. speciosa* R. Brown (three sites each), *B. grandis* Willd. and *Dryandra cuneata* R. Brown (two sites each), and *B. attenuata* R. Brown and *D. falcata* R. Brown (one site each).

Pathogenicity. Pathogenicities of *C. melanocraspeda* and *Botryosphaeria ribis* were tested because they were most frequently isolated from dying plants, and the *Zythiostroma* sp. was included because it occurred on *B. baxteri* within the test area. *B. baxteri* was used in the test because it often co-occurs in stands with *B. coccinea*.

All three fungi produced lesions in the two *Banksia* species (Fig. 4). Lesions in stems inoculated with test fungi were significantly longer than those in stems receiving control inoculations. Host, canker fungi, and isolate nested in canker fungi significantly affected lesion length (Table 3). Only *Zythiostroma* lesions significantly ($P \leq 0.05$) increased in length with time and were significantly longer in *B. baxteri* than in *B. coccinea* (Fig. 4). Lesions produced by *C. melanocraspeda* were significantly longer than those of *Botryosphaeria ribis* but significantly shorter than those produced by *Zythiostroma* in both hosts (Fig. 4). Only *Zythiostroma* lesions differed significantly in length between isolates.

Host, canker fungi, and isolate nested in canker fungi significantly affected tangential spread of lesions (Table 3). In both hosts, tangential spread of *C. melanocraspeda* was significantly greater than *Botryosphaeria ribis* but less than *Zythiostroma* (Fig. 5). Only *C. melanocraspeda* lesions differed significantly in tangential spread between isolates. There was a significant assessment time × host interaction (Table 3) as tangential spread of *C. melanocraspeda* decreased with time in *B. baxteri* and increased with time in *B. coccinea* (Fig. 5). A significant host × fungi interaction (Table 3) was due to greater tangential spread of *Botryosphaeria ribis* and *C. melanocraspeda* in *B. coccinea* than *B. baxteri* (Fig. 5).

C. melanocraspeda and *Zythiostroma* spp. girdled and killed stems, whereas *Botryosphaeria ribis* did not girdle any stem inoculated (Fig. 6). The amount of stem girdling by *Zythiostroma* sp. in both hosts and *C. melanocraspeda* in *B. coccinea* increased with time.

Forty-two days after inoculation, *Botryosphaeria ribis* was reisolated from *B. baxteri* and *B. coccinea* 76 ± 7% and 93 ± 4% of the time, respectively. The frequency of reisolation of *Botryosphaeria ribis* from *B. baxteri* did not significantly change with sampling time, but frequency decreased to 55 ± 8% for *B. coccinea* 141 days after inoculation. *Botryosphaeria ribis* was isolated also from 12 ± 6% of the *B. baxteri* control and at low levels (<5%) from the *C. melanocraspeda* and *Zythiostroma* sp. inoculation treatments. Reisolation of *C. melanocraspeda* from *B. baxteri* and *B. coccinea* stems inoculated with the fungus was 53 ± 10% for 66 ± 8% respectively at the first sampling time. Reisolation of *C. melanocraspeda* decreased significantly at the second sampling time to 5 ± 2% for *B. baxteri* and 9 ± 4% for *B. coccinea*. In *B. coccinea*, *C. melanocraspeda* was isolated also at low levels from the *Botryosphaeria ribis* and *Zythiostroma* sp. inoculation treatments. For both sampling times, reisolation of *Zythiostroma* sp. from inoculated stems was 100% for *B. baxteri* and 84 ± 5% for *B. coccinea*. In *B. baxteri*, *Zythiostroma* sp. was isolated also from the *Botryosphaeria ribis*, *C. melanocraspeda*, and control inoculation treatments (4, 6, and 12%, respectively) and the *Botryosphaeria ribis*–inoculated treatment of *B. coccinea* (2%).

DISCUSSION

Infection by *C. melanocraspeda* was associated mainly with death of *B. coccinea*. *C. melanocraspeda* was the most frequently isolated fungus from dying *B. coccinea* and demonstrated strong pathogenicity by forming relatively long lesions that girdled stems of *B. coccinea*. Although *Botryosphaeria ribis* was frequently isolated from dying plants, the fungus formed relatively small lesions that did not girdle stems, suggesting that it is a weak pathogen. *Botryosphaeria ribis* is a cosmopolitan fungus occurring on *Eucalyptus* spp. and *Banksia* spp. throughout southwestern Australia, and lesions were readily walled off in inoculations in *Eucalyptus* (14). The *Zythiostroma* sp. also demonstrated strong pathogenic ability, but it was isolated from *B. coccinea* much less frequently than *C. melanocraspeda*.

The decline of lesion length of *Botryosphaeria ribis* in both hosts and *C.*

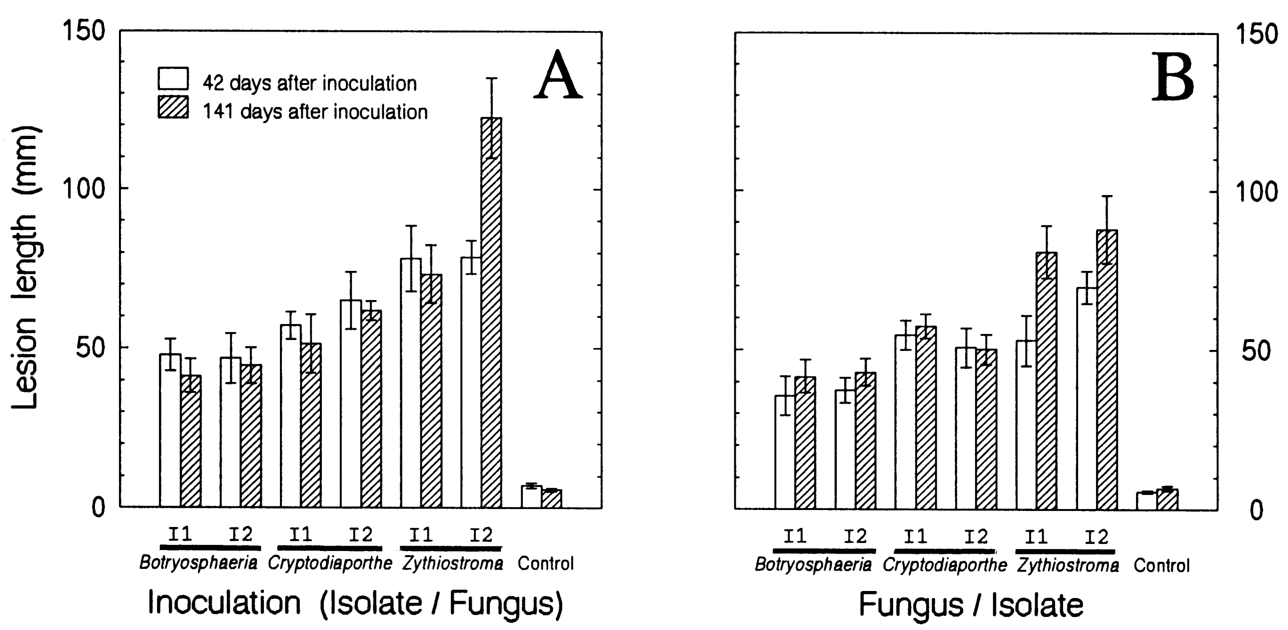

Fig. 4. Lesion lengths (± standard error of the mean) at two assessment times after field inoculation of stems of **(A)** *Banksia baxteri* and **(B)** *B. coccinea* with two isolates each (I1 and I2) of *Botryosphaeria ribis*, *Cryptodiaporthe melanocraspeda*, and *Zythiostroma* sp., and a control.

Table 3. Analysis of variance of lesion length and tangential lesion spread for a pathogenicity test of two isolates of *Botryosphaeria ribis*, *Cryptodiaporthe melanocraspeda*, and *Zythiostroma* sp. inoculated into *Banksia baxteri* and *B. coccinea* and assessed at two times

	Lesion length		Tangential spread	
Source of variation	**df**	**Mean square**	**df**	**Mean square**
Time	1	0.049*[a]	1	0.101
Host	1	0.025*	1	1.019**
Fungi	2	7.343**	2	12.901**
Isolate(Fungi)	3	0.390*	3	0.207*
Time × Host	1	0.455	1	0.126
Time × Fungi	2	0.355	2	0.221*
Host × Fungi	2	0.035	2	0.252*
Error[b]	193	0.138	207	0.057

[a] * = Significance at the 5% level; ** = significance at the 1% level.

[b] Error degrees of freedom differ due to deletion of outliers with Studentized residuals >2.5.

melanocraspeda in *B. baxteri* with assessment time suggests that host responses to infection confined lesions. This is supported by the facts that *Botryosphaeria ribis* did not girdle stems of either host, and that girdling of stems of *B. baxteri* by *C. melanocraspeda* was much less than that of *B. coccinea*.

The strong pathogenic ability demonstrated by the *Zythiostroma* sp. following inoculation of *B. coccinea* is surprising in view of the very low isolation frequency from naturally infected plants. The low isolation frequency may be due to unfavorable environmental conditions inhib-

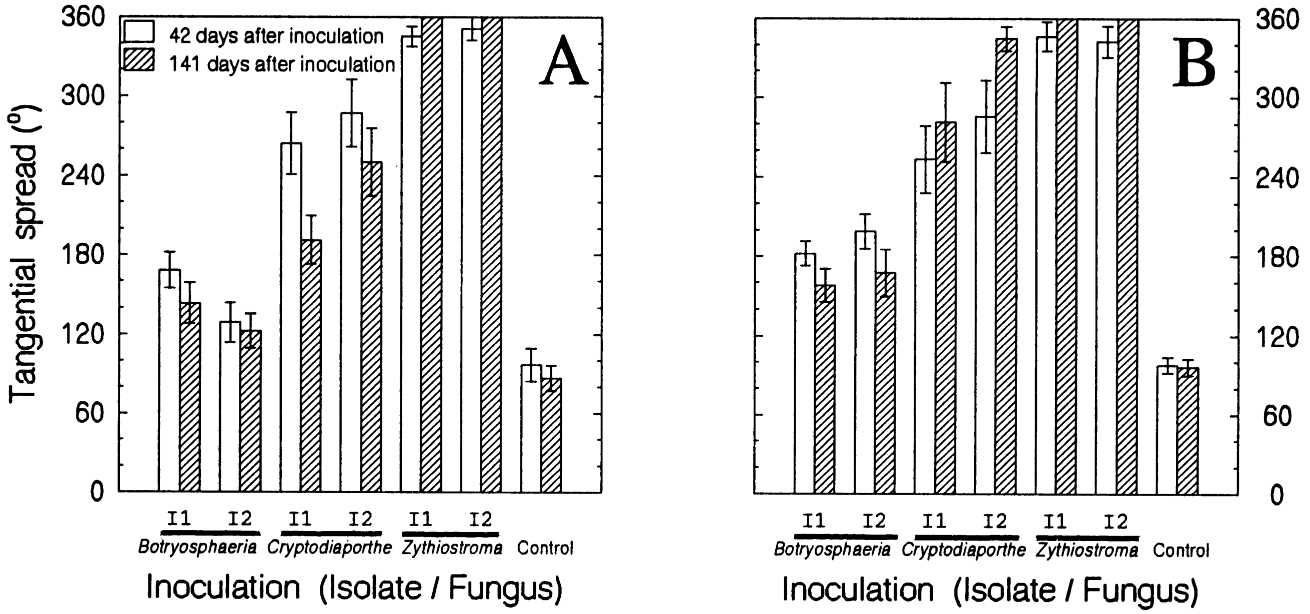

Fig. 5. Tangential spread (± standard error of the mean) at two assessment times after field inoculation of stems of **(A)** *Banksia baxteri* and **(B)** *B. coccinea* with two isolates each (I1 and I2) of *Botryosphaeria ribis*, *Cryptodiaporthe melanocraspeda*, and *Zythiostroma* sp., and a control.

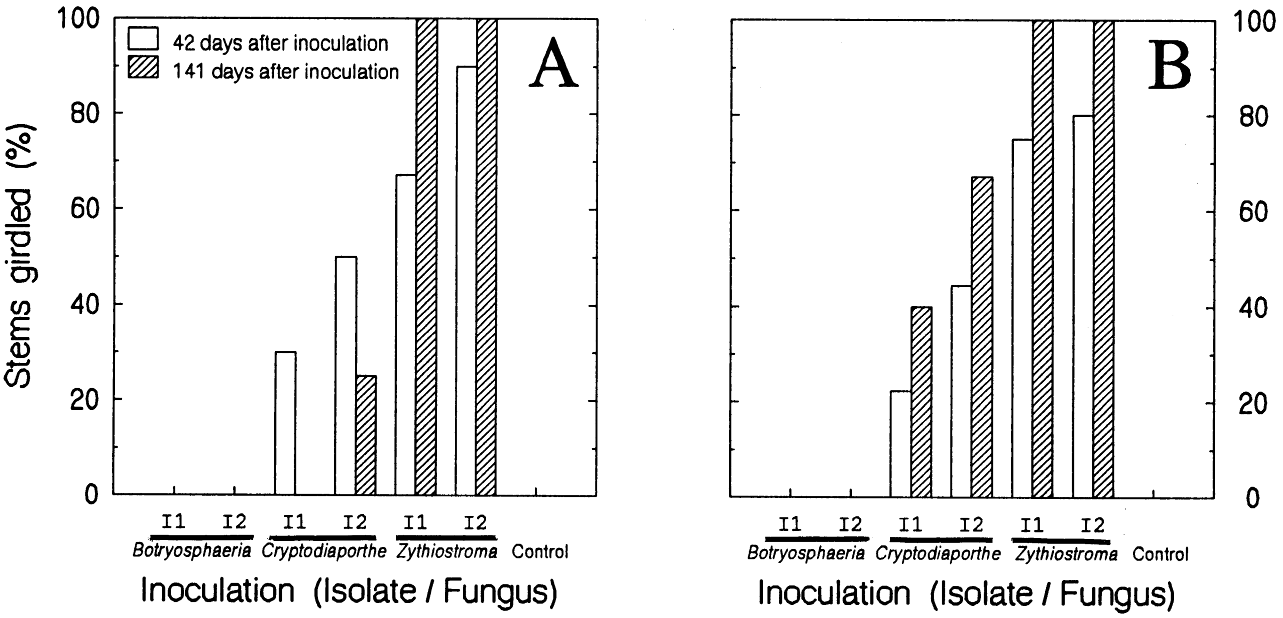

Fig. 6. Percentage of stems girdled at two assessment times after field inoculation of stems of **(A)** *Banksia baxteri* and **(B)** *B. coccinea* with two isolates each (I1 and I2) of *Botryosphaeria ribis*, *Cryptodiaporthe melanocraspeda*, and *Zythiostroma* sp., and a control.

iting infection of *B. coccinea* by *Zythiostroma* sp. Conditions affecting the infection process would be bypassed by the wound inoculation technique used in the pathogenicity test. The strong pathogenic ability of *Zythiostroma* suggests that long-term monitoring of its occurrence and the impact of this pathogen in *Banksia* communities is required.

This report extends the known host range of *Cryptodiaporthe* and *Diplodina*. Species of *Cryptodiaporthe* have been associated with canker of poplar (*Populus* spp.), willow (*Salix* spp.) (3,5,7,10), and pagoda dogwood (*Cornus alternifolia* L.) (13). Species of *Diplodina* have been associated with bark necrosis of sycamore (*Acer pseudoplatanus* L.) (9), twig blight of Asiatic chestnuts (*Castanea dentata* (Marsh.) Bork.) (2), and fruit rot of peaches (*Prunus persica* (L.) Batsch.) (11). In southwestern Australia, *C. melanocraspeda* has been isolated only from dying members of the Proteaceae: *B. attenuata*, *B. coccinea*, *B. baxteri*, *B. grandis*, *B. menziesii* R. Brown, *B. speciosa*, *D. cuneata*, *D. falcata*, and *D. sessilis* (Knight) Domin. (14). Because *C. melanocraspeda* differs from descriptions of other *Cryptodiaporthe* spp. (1) and is confined to a narrow range of hosts, the fungus is probably endemic to southwestern Australia. However, the devastating pathogenicity of *C. melanocraspeda* on *B. coccinea* is unexpected for an endemic species. In areas of southwestern Australia other than the South Coast Region, the pathogen is also killing *B. grandis* south of Perth and *D. sessilis* north of Perth (14). Information on the biology and ecology of *C. melanocraspeda* is needed to determine whether current impacts are short-term perturbations or part of long-term cycles in pathogen–community–environment interactions.

C. melanocraspeda canker has the ability to cause destructive losses in production and conservation values of *B. coccinea* communities. Because of the distinctive red flower, *B. coccinea* has considerable potential for selection and development for horticulture (8). Infection by *C. melanocraspeda* canker has constrained picking of flowers. Recognition of the disease resulted in the picking of flowers on Crown land being banned in 1991, forcing the supply of flowers to private land. Infection by canker reduced not only the number and quality of blooms but also the visual appeal of stands for the tourist industry. A stand in the Stirling Range National Park that was used as a stopping point for tourist buses during the flowering period is now practically destroyed by this disease. Ecologically, *B. coccinea* is a keystone species within the communities in which it occurs and is an important food source for birds (17). Priority must be given to the development and application of appropriate control strategies.

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