Botryosphaeria ribis Infection Associated with Death of *Eucalyptus radiata* in Species Selection Trials

B. L. SHEARER, J. T. TIPPETT, and J. R. BARTLE, Research Station, Department of Conservation and Land Management, Dwellingup, Western Australia 6213

ABSTRACT

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Dark brown discoloration of the phloem and light purplish brown discoloration of the xylem were associated with death of *Eucalyptus radiata* in species selection trials. Xylem discoloration and extensive kino veins often formed ahead of phloem necrosis. Of the 22 trees examined, *Botryosphaeria ribis* was isolated from lesion margins in 13 trees and *Cytospora eucalypticola*, from lesion margins in 12 trees. In glasshouse and field pathogenicity tests, *E. radiata* was the only *Eucalyptus* species of the four tested where there was both considerable longitudinal and tangential extension of *B. ribis* in the phloem. The fungus was confined in *E. calophylla* and *E. cladocalyx*, and callus formed at the lesion margins. *B. ribis* became established in the phloem of *E. marginata* but was contained by the formation of necrophylactic periderms. *C. eucalypticola* failed to establish in *E. cladocalyx* and was contained in *E. marginata* and *E. radiata* phloem. The environment of trial sites in the low-rainfall zone of the *E. marginata* forest may be marginal for *E. radiata*, and environmental stress may have been a predisposing factor affecting resistance to infection.

A series of five species selection trials were established in the late 1970s to select tree species suitable for reforesting areas of the *Eucalyptus marginata* Donn. ex Sm. forest altered by *Phytophthora cinnamomi* Rands infection, bauxite mining, and agricultural development (2). These trials are distributed across the rainfall and topographic gradients of the northern and central regions of the *E. marginata* forest and contain, on average, two seed lots of 70 species in plots of 0.5 ha per seed lot. *Eucalyptus* species predominate and were selected on the basis of adaptation to semiarid and saline environments, fire resistance, performance in other plantings, timber production potential, and aesthetic qualities.

In two trials in the low-rainfall forest zone (900 mm annual precipitation) located adjacent to each other in upper and lower topographic positions, *E. radiata* Sieb. ex DC. subsp. *radiata* were dying in large numbers 2–3 yr after planting. The deaths were strikingly specific to *E. radiata*, affecting four plots dispersed over an area of about 200 ha. We sought in this study to describe symptom progression and determine the rate and cause(s) of *E. radiata* mortality.

MATERIALS AND METHODS

Site. The study area is situated at 116°28′ E longitude, 32° 53′ S latitude, in the low-rainfall zone of the *E. marginata* forest. *E. radiata* was planted in two areas: a ridge-top plot (elevation 350 m) was established in 1979 on typically lateritic soil profiles of sandy gravel over duricrust, and a midslope plot (elevation 270 m) was established in 1980 at the head of an ill-defined drainage line on sandy gravel over clay soils. Vegetation before clearing was an open *E. marginata* and *E. calophylla* R. Br. forest with an admixture of *Banksia grandis* Willd.

Isolation. The main stems and lateral branches of dying trees were cut longitudinally and transversely and symptoms described. Tissue was removed from the necrotic, healthy tissue interface at lesion margins, surface-sterilized in 70% ethanol for 30 sec, washed in sterile distilled water, and plated onto half-strength potato-dextrose agar (0.5 PDA)

Table 1. Percentage of *Eucalyptus radiata* trees in each decline rating class assessed in four consecutive years

Assessment date	Years after planting[a]	Decline rating[b]			
		Minimum	Moderate	Severe	Dead
24 June 1982	2.02	47	22	12	19
8 April 1983	2.81	17	11	17	56
18 May 1984	3.93	9	10	10	71
2 May 1985	4.88	6	10	11	73

[a]Trees planted mid-June 1980.
[b]Minimum = <10% of leaves necrotic, moderate = 10–50% of leaves necrotic, and severe = >50–100% of leaves necrotic.

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(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.

Inoculation. Pathogenicity of fungi isolated was tested in 1-yr-old stems (diameter 4–6 mm) in the glasshouse and in coppice stems of *E. marginata* (diameter 29–60 mm) and stems of 3-yr-old trees of *E. cladocalyx* F. Muell. and *E. radiata* (diameter 17–69 mm) at the trial site. Stems were inoculated in the field in mid-February (midsummer). An oblique incision was made with a scalpel blade through the outer bark and into the phloem. An agar plug 2 mm in diameter for glasshouse inoculation and 7 mm in diameter for field inoculation was cut from the actively growing edge of a colony and inserted into the incision to contact the freshly cut phloem. The incision was closed, covered with moist sterile cotton wool, and bound with plastic tape to prevent desiccation. Ten

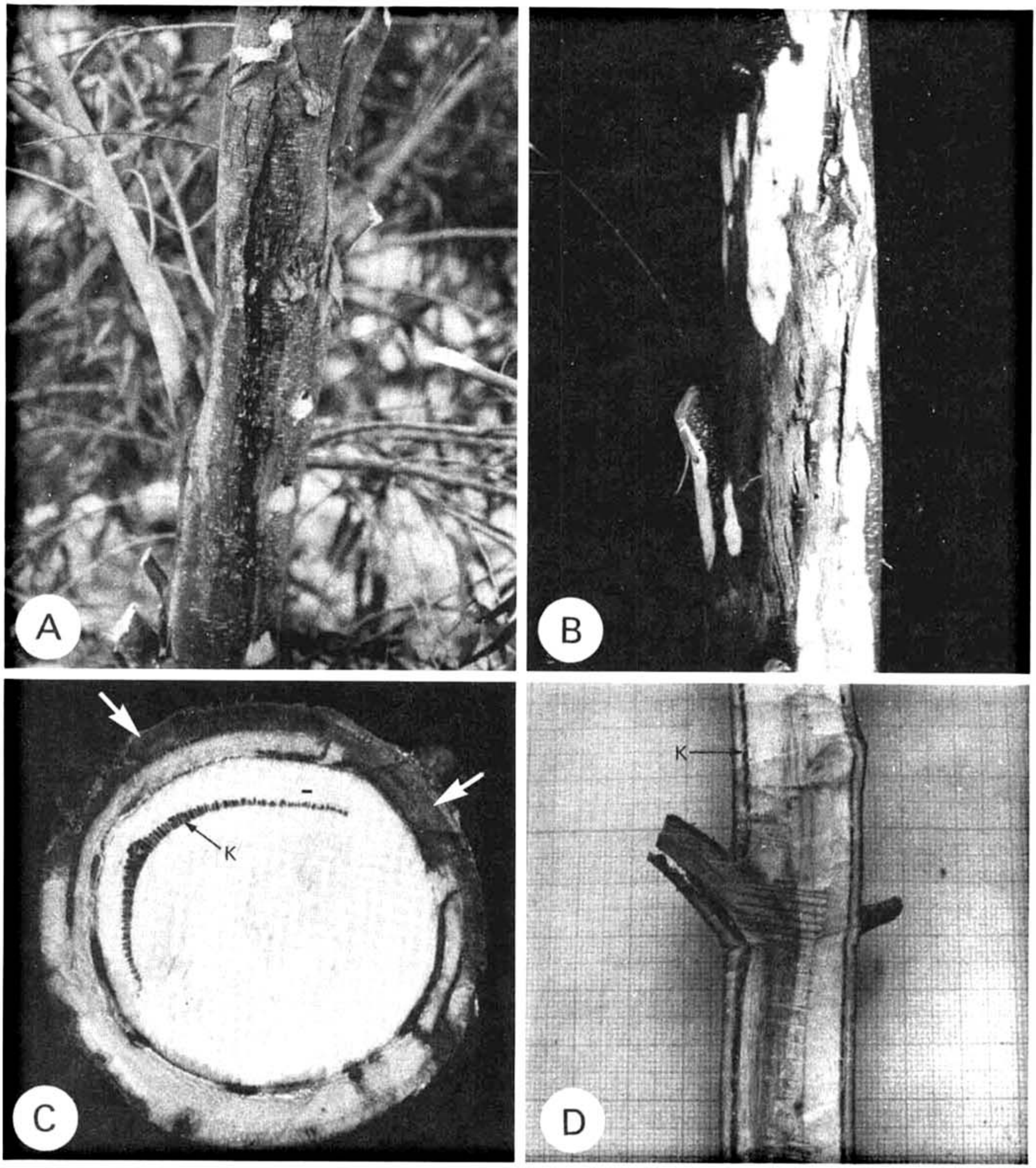

Fig. 1. Symptoms associated with decline and mortality of *Eucalyptus radiata* in species selection trials. **(A)** Stem (5 cm in diameter) with canker. Extensive bark cracking and kino exudation most obvious symptoms. **(B)** Outer bark stripped from stem to show brown discoloration of affected phloem. **(C)** Lesion in transverse section. Little xylem discoloration but dark lesion in bark (arrows). A kino vein (K) formed some months prior to harvest included in the xylem. **(D)** Pattern of xylem discoloration at branch junction. Phloem necrosis limited to small lateral branch but xylem discoloration extending into main branch. The larger branch had reacted with formation of a kino vein (K).

trees were inoculated per treatment. In addition to stems, twigs (8 ± 1 mm in diameter) of *E. cladocalyx* and *E. radiata* were inoculated with *B. ribis*. Control stems were inoculated in a similar manner with sterile agar.

Assessment. In the midslope plot, crown health was assessed each year from 1982 to 1985 (Table 1).

Thirty-three days after inoculation in the glasshouse and 2–15 mo after inoculation in the field, stems were removed from the trees and longitudinal and transverse cuts made through the point of inoculation. The cut surfaces were cleaned with sharp Stanley blades and lesion margins in the phloem determined. The presence of the test fungus was verified by plating tissue onto 0.5 PDA.

Samples from lesions were fixed in 5% formaldehyde (5) and sectioned by hand. Toluidine blue O (0.1% aqueous) and Sudan black B (in saturated solution of 70% ethanol) were used (5) to determine if new layers of periderm had formed at lesion boundaries.

RESULTS

Symptoms. Death of *E. radiata* trees was first noted in 1982. Two years after planting of the midslope plot, 19% of the trees had died, and 3 yr after planting of the ridge plot, 34% of the trees had died. Seed from two sources were planted in each plot, but there was no difference in the number of deaths between sources. By 1982 in the upland plot, 35% of trees of seed lot 11983 had died compared with 33% of seed lot 12631. From 1982 to 1984, there was a nearly linear increase in deaths in the midslope plot followed by a leveling-off between 1984 and 1985 (Table 1).

Rate of symptom expression varied from gradual decline resulting from death of twigs and lateral branches to rapid death of the leader. Often, the rapid death of trees was associated with lesions in laterals reaching and girdling the main stem. Trees growing in ash beds tended to survive the longest.

Cankers were evident in the stems of declining trees as sunken cracked areas. In some cases, the outer bark and phloem were cracked extensively, exposing the xylem, and kino exuded from cracked areas (Fig. 1A). Cankers were associated with chocolate brown to light brown discoloration of the phloem (Fig. 1B–D) and light purplish brown discoloration of the xylem. Often, xylem discoloration was observed ahead of phloem necrosis (Fig. 1D). Extensive kino veins formed ahead of phloem discoloration (Fig. 1C,D).

Isolation. Of the 22 trees examined, *Botryosphaeria ribis* Gossenb. & Dugg. was isolated from the lesion margins in 13 trees and *Cytospora eucalypticola* van der Westhuizen from lesion margins in 12 trees. Both fungi were isolated from the same lesions in four trees. Pathogenicity tests were conducted in the glasshouse and field with both *B. ribis* and *C. eucalypticola*.

Pathogenicity tests: glasshouse. *B. ribis* established lesions in all four *Eucalyptus* species inoculated, but there were considerable differences between hosts in the pattern of invasion 33 days after inoculation (Fig. 2). *E. calophylla* contained longitudinal and tangential extension by the fungus (Fig. 2) and containment of lesions was associated with rapid callus formation (Fig. 3A). Lesions in *E. cladocalyx* stems were long and narrow (Fig. 2). Necrophylactic periderms formed the boundaries of the lesions (Fig. 3B), which ceased to extend tangentially. Lesion development in *E. marginata* was the reverse to that in *E. cladocalyx*; while longitudinal extension was limited, tangential extension was not. Only in *E. radiata* did *B. ribis* lesions extend both longitudinally and tangentially (Fig. 2).

E. radiata was the only host inoculated with *C. eucalypticola* in the glasshouse. In all cases, lesions were contained. Mean longitudinal lesion extension of *C. eucalypticola* in *E. radiata* was significantly less than that for *B. ribis* (5 ± 1 mm for *C. eucalypticola* compared with 47 ± 8 mm for *B. ribis*) and did not extend tangentially from the inoculation point.

Pathogenicity tests: field. *B. ribis* established in the three *Eucalyptus* species inoculated, but the pattern of invasion determined 2 and 15 mo after inoculation differed considerably between hosts (Fig. 4). In *E. cladocalyx*, initial lesion extension was contained and callus formed (Fig. 5A). For *E. cladocalyx*, longitudinal lesion lengths were the same at both assessment times and recovery resulted in decreasing tangential measurements as lesioned tissue sloughed-off with time (Figs. 4 and 5B). In field-inoculated *E. marginata*, tangential growth of the fungus was greater than longitudinal growth. At the first assessment, all *B. ribis* lesions in *E. marginata* were contained by necrophylactic periderms (Figs. 4 and 5C). As observed in *E. cladocalyx*, recovery resulted in decreasing tangential measurements as lesioned tissue sloughed-off with time (Fig. 4). *E. radiata* was the only host in which *B. ribis* developed extensive lesions that remained active (Fig. 4).

C. eucalypticola failed to establish in stems of *E. cladocalyx* (Fig. 4). The fungus established in *E. marginata* stems, but the lesions were confined and sloughed-off with time. In *E. radiata, C. eucalypticola* formed significantly smaller lesions than *B. ribis* (Fig. 4), and they

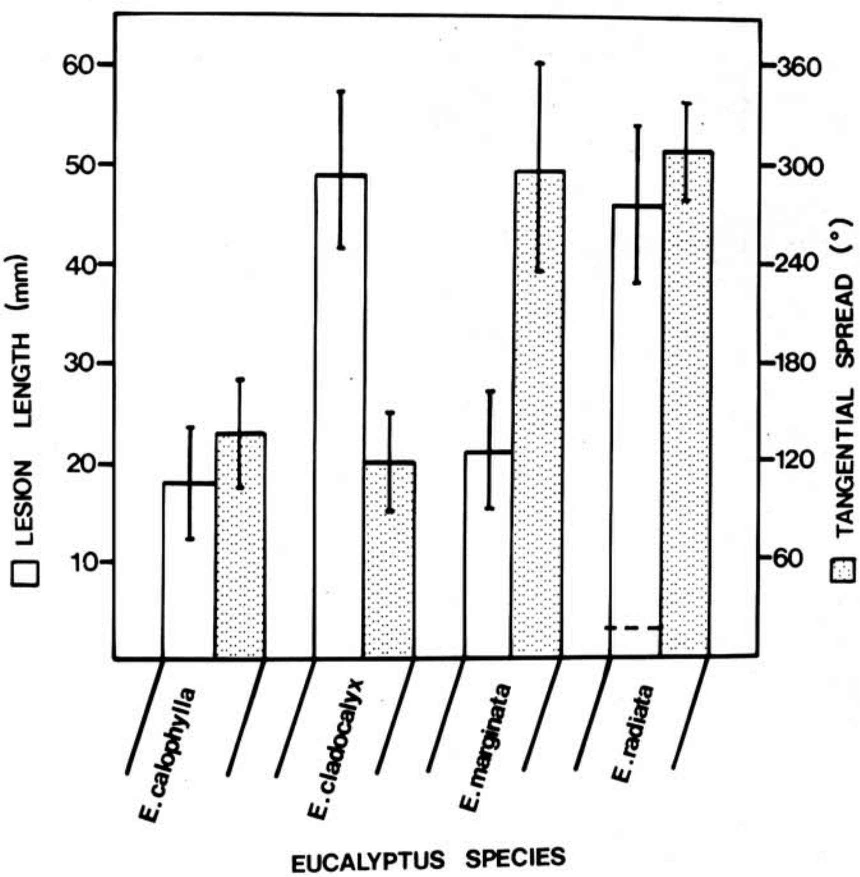

Fig. 2. Lesion lengths and tangential spread (± standard error of the mean) of *Botryosphaeria ribis* in stems of four *Eucalyptus* species in a glasshouse 33 days after inoculation. --- = Size of control inoculations. No evidence of the control cut could be found in transverse section.

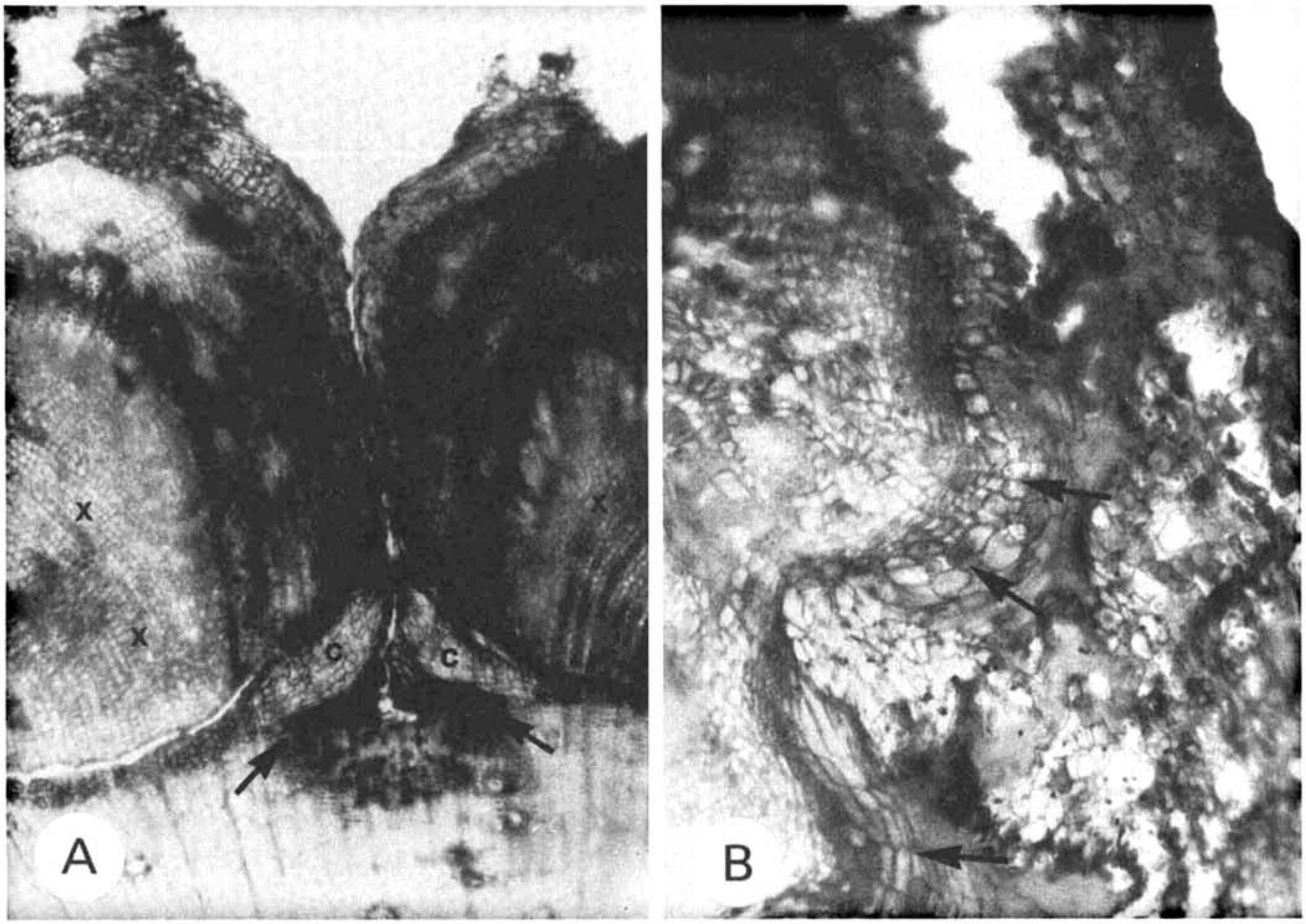

Fig. 3. Transverse sections through *Eucalyptus* stems inoculated with *Botryosphaeria ribis* in a glasshouse. **(A)** Inoculated *E. calophylla* with a small area of cambium killed (arrows) but new wood (X) and callus (C) occluded the limited area of necrosis. **(B)** Boundary between healthy and necrotic tissue in inoculated *E. cladocalyx* stem. Sudan black was taken up by the suberized walls of cells constituting a necrophylactic periderm (arrows).

formed in the outer phloem.

Pathogenicity tests: twigs versus stems in the field. *B. ribis* lesion length in *E. cladocalyx* twigs was not significantly different from that in stems. Lesion length in *E. radiata* twigs was significantly less than in stems: 21 ± 7 mm in twigs compared with 182 ± 66 mm in stems.

DISCUSSION

Symptoms observed and results of pathogenicity tests suggested that death of *E. radiata* was associated with infection by *B. ribis*. The observed sunken, cracked areas, brownish discoloration of the phloem, production of kino, and purplish staining of the xylem were consistent with symptoms of *B. ribis* infection reported previously (6,7,12,13). *B. ribis* establishment was similar in both glasshouse and field inoculated material, despite differences in tissue age and environment. There were characteristic differences in longitudinal and tangential lesion development in the *Eucalyptus* species. The long but narrow lesions found in inoculated *E. cladocalyx* stems illustrate the need to determine tangential spread when assessing resistance to *B. ribis* infection. Callus development along the lateral margins of lesions in *E.*

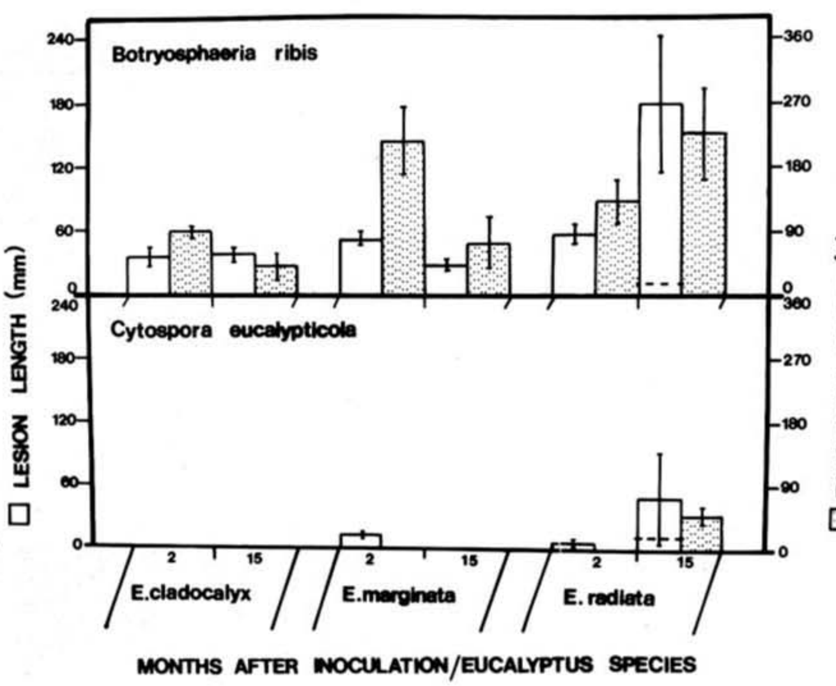

Fig. 4. Lesion lengths and tangential spread (± standard error of the mean) at two assessment times after field inoculation of stems of three *Eucalyptus* species with *Botryosphaeria ribis* and *Cytospora eucalypticola*. --- = Size of control inoculations in *E. radiata*. No evidence of the control cut could be found in transverse section. Control inoculations in *E. cladocalyx* and *E. marginata* had completely healed 15 mo after inoculation.

calophylla and *E. cladocalyx* was similar to the reaction described for *E. viminalis* in response to *B. ribis* (12).

Both *C. eucalypticola* and *B. ribis* were isolated in about equal frequency from lesion margins in dying *E. radiata*. However, *C. eucalypticola* did not establish in *E. cladocalyx* and lesions were confined to small areas in *E. marginata* and *E. radiata*. Davison and Tay (4) also observed limited invasion of *E. marginata* by *C. eucalypticola*. The limited lesion development and high isolation frequency suggest that *C. eucalypticola* is a nonagressive facultative parasite. Presence of the fungus could not be considered a contributing factor to the decline and death of *E. radiata* observed.

The symptoms in *E. radiata* suggested that *B. ribis* invasion of the main stems could have originated from infected lateral branches. Toole (11) found *Liquidambar styraciflua* L. twigs less than 5 mm in diameter were more likely to be infected by *B. ribis* than twigs greater than 8 mm in diameter. Our inoculation studies did not suggest that lateral branches (mean diameter of 8 ± 1 mm) were more susceptible to invasion by *B. ribis* than stems (mean diameter of 44 ± 6 mm). Entry of *B. ribis* into a host may be via wounds and lenticels (7,9), but Davison and Tay (4) found no association between isolation of *B. ribis* from cankers in *E. marginata* and insect damage. How the pathogen infects *E. radiata* needs to be determined.

The natural distribution of *E. radiata* is in central and eastern Victoria and New South Wales, with best development in moist forests on moderately deep soils (8). The seeds planted in the trials were thought to have originated from a few trees growing above 700-m elevation in the Victorian Alps (3). The climate at *E. radiata*'s site of origin would have been cooler with a more even annual distribution of rain throughout the year than that experienced in the eastern *E. marginata* forest where the trials are located. This suggests that the *E. radiata* planted in the trials may have experienced environmental stress, with an associated decline in resistance to infection.

Stress can be an important factor affecting host susceptibility to infection

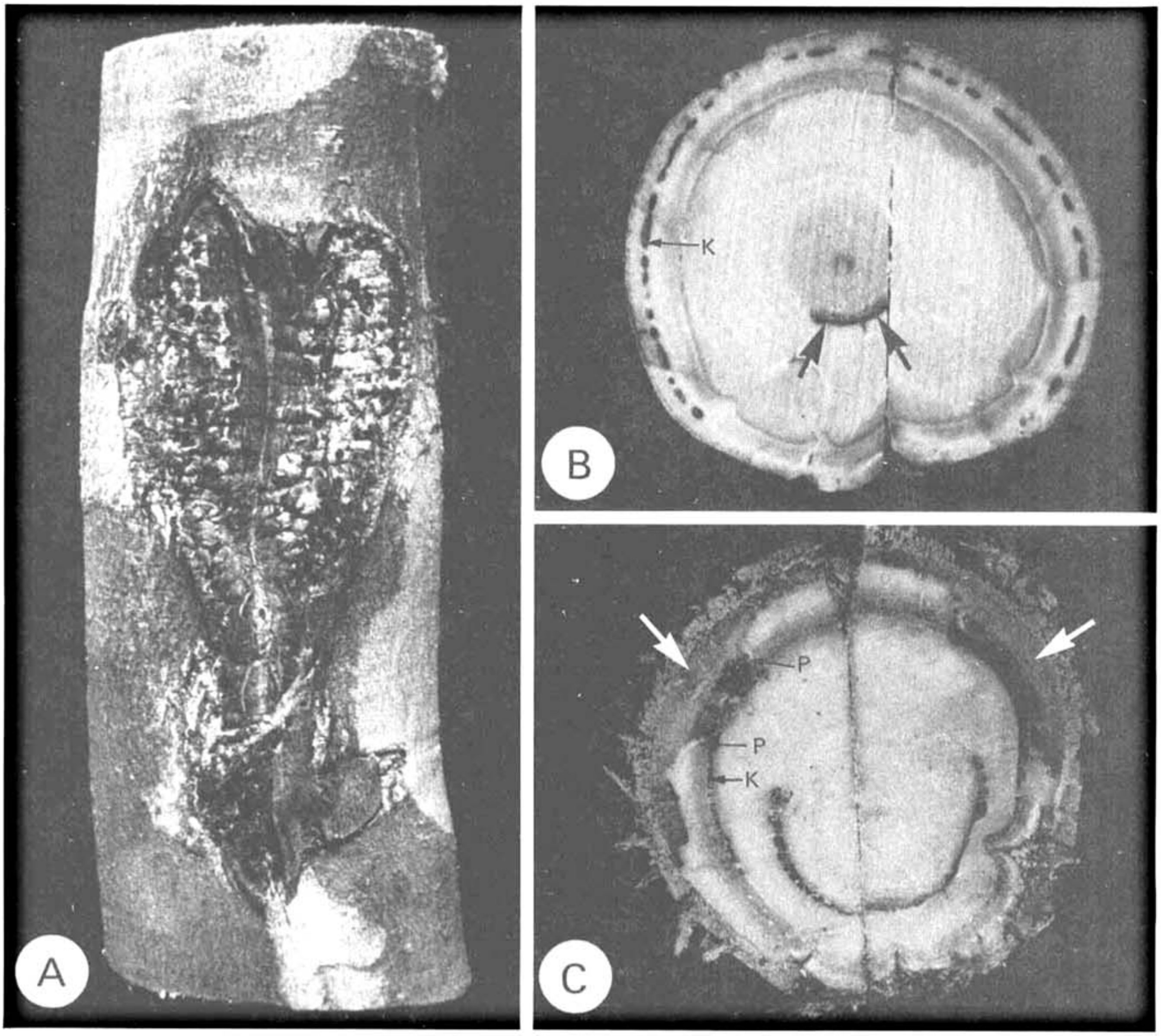

Fig. 5. *Eucalyptus* stems harvested 15 mo after inoculation with *Botryosphaeria ribis* in the field. **(A)** Surface view of *E. cladocalyx* stem showing callus and new wood closing gap where cambium had been killed. **(B)** Transverse section of *E. cladocalyx* stem. Kino (K) in outer phloem and xylem discoloration in wood reflecting past infection (arrows). Only slight distortion of tissues reflect that a lesion had formed. **(C)** Transverse section of *E. marginata* stem. Phloem discoloration (arrows) associated with kino veins (K). Necrophylactic periderms had formed around confined areas of necrosis (P).

by pathogens (10). Drought and nutrition have been implicated as factors favoring *B. ribis* infection of apple (1), macadamia (6), and pine (7). Because *E. radiata* survived longer on ash beds, nutrition may have influenced the outcome of the interactions studied.

In a recent survey of the selection trials, there was considerable variation in tree mortality between species within and between trials (3). Although mortality can be due to a wide range of factors such as quality of nursery stock, establishment practice, site, and species, the results from this study illustrate the need to assess the effects of pathogens on the performance of tree species used to rehabilitate disturbed areas.

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