SUSCEPTIBILITY OF THE JUVENILE LEAVES OF *EUCALYPTUS BICOSTATA* MAIDEN *ET AL.* TO INFECTION BY *PHAEOSEPTORIA EUCALYPTI* (HANSF.) WALKER

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Summary

The removal of the waxy coating from the surfaces of juvenile leaves of *Eucalyptus bicostata* Maiden *et al.* seedlings, by wiping lightly with cotton wool, greatly increased the infection of these leaves by water-dispersed spores of *Phaeoseptoria eucalypti* (Hansf.) Walker. The susceptibility of the juvenile leaves of *E. bicostata* seedlings to *Phaeoseptoria* leaf spot increased with physiological age but was apparently unaffected by the age of the seedling at the time the leaf was formed. Juvenile leaves of twin cuttings of *E. bicostata*, grown on a high-nutrient substrate, were more susceptible to *Phaeoseptoria* leaf spot than comparable leaves of twin cuttings grown on a low-nutrient medium.

I. Introduction

Phaeoseptoria eucalypti causes local necrotic lesions on the leaves of some eucalypt species (Walker 1962; Heather 1965). In native forest, infections seem to occur only on the subgenus *Macranthera* (sensu Pryor 1959), but in nurseries and glasshouses the leaves of seedlings of other eucalypt subgenera are attacked also (Heather 1965).

In glasshouses at Canberra natural infections of leaves of macrantherous eucalypt seedlings are most severe on species with green juvenile foliage and rare on those with glaucous leaves.

The significance of the glaucous layer of eucalypt leaves has been considered in relation to variation with altitude (Barber and Jackson 1957), photosynthesis (Cameron 1964; Heather 1965), and its possible role in resistance to insect and fungal attack (Barber and Jackson 1957). The fungistatic properties of apple leaf wax have been discussed in relation to infection by conidia of *Podosphaera leucotricha* (Ellis & Everh.) Salm. (Martin, Batt, and Burchill 1957).

Disease resistance in relation to leaf age has been studied with certain members of the genus *Septoria* (Scharen 1963; Green and Dickson 1957).

This investigation is concerned with the effects of glaucousness, nodal position of leaf, leaf age, and host nutrition on infection of *Eucalyptus bicostata* by *Phaeoseptoria eucalypti*.

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II. Materials and Methods

Seedlings for some experiments and the twin cuttings for the nutrition experiment were grown in a heated glasshouse. In all other instances the seedlings were grown from fumigated seed of *E. bicostata* under controlled conditions of day length and nutrition in the CSIRO phytotron at Canberra (Morse and Evans 1962) with day and night temperatures of 27°C and 22°C respectively. Uniform seedlings with 10–12 pairs of fully expanded, opposite, glaucous, juvenile leaves free of obvious damage were obtained in 6–8 weeks under these conditions.

The seedlings for inoculation were set on a rotating table and sprayed with a uniform concentration of spore suspension (7×10^4 spores/ml) from an automatic De Vilbiss atomizer. The inoculated plants were incubated at 25°C and at high humidity for 48 hr before being placed in a heated glasshouse with a minimum temperature of 18°C. Lesions developed on the seedling leaves 25–30 days from spraying and disease intensity was measured either as number of lesions per square centimetre of leaf area or percentage leaf area lesioned.

Table 1

PERCENTAGE LEAF AREA LESIONED BY *P. EUCALYPTI* ON LEAVES OF *E. BICOSTATA* SEEDLINGS IN RELATION TO WIPING TREATMENT AND NODAL POSITION

Leaf Position	Mean Percentage Total Leaf Area Lesioned		Percentage Difference between Means for Wiped and Unwiped Leaves	Significance of Difference*
	Wiped	Unwiped		
Lowest leaf pair	14·09	4·56	+208	$P < 0{\cdot}001$
Second lowest leaf pair	9·61	2·47	+298	$P < 0{\cdot}01$
Third lowest leaf pair	11·58	5·22	+121	Not significant

* From Student's *t*-test.

III. Results

(*a*) *Leaf Glaucousness and Nodal Position*

(i) *Experiment 1*

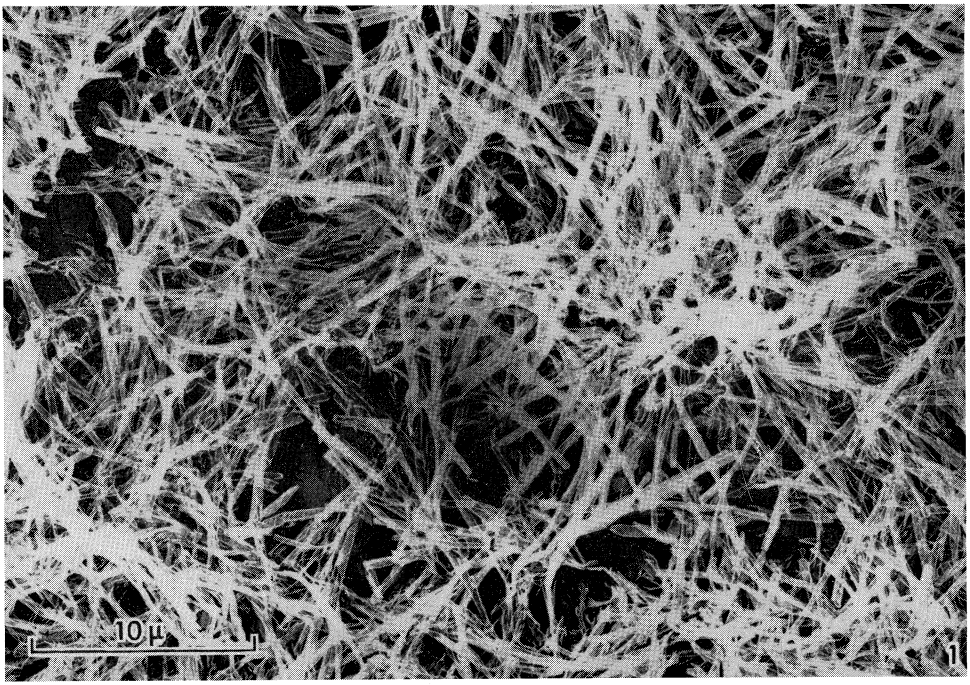
Preliminary experiments indicated that the glaucousness of leaves could be removed by wiping lightly with cotton wool without any obvious damage to the superficial cells of the leaf (Plate 1).

The foliage of each of 12 *E. bicostata* seedlings, raised outside the phytotron, was reduced to three pairs of opposite glaucous leaves. The glaucous coating was lightly wiped from both faces of one member of each leaf pair on each plant. The plants were inoculated, incubated, and percentage leaf area lesioned assessed for the wiping treatment and leaf nodal position (Table 1).

(ii) *Experiment 2*

The crowns of each of four *E. bicostata* plants, raised in the phytotron, were reduced to six pairs of opposite, fully expanded leaves (i.e. 48 leaves in all were

INFECTION OF *E. BICOSTATA* LEAVES BY *P. EUCALYPTI*



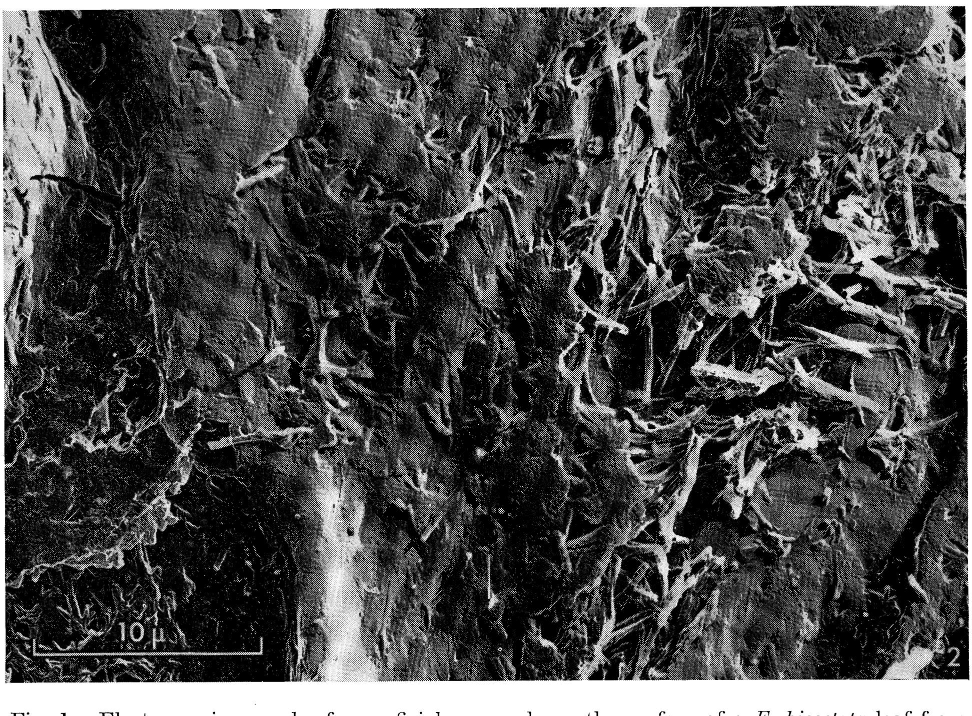


Fig. 1.—Electron micrograph of superficial wax rods on the surface of a *E. bicostata* leaf from plants raised on the 27°C day/22°C night temperature regime in the Ceres phytotron.

Fig. 2.—Electron micrograph of a comparable surface to that in Figure 1 after wiping lightly with cotton wool. Note the scattered debris from wax rods and the discontinuous plates overlying the cuticle.

used in the experiment). The crown on each plant was divided into two groups of three upper and three lower pairs of leaves. The glaucous coating was removed from one member of each leaf pair on each plant. The plants were inoculated, incubated, and lesion number per square centimetre of leaf surface assessed at 32 days. The effect of wiping, the influence of leaf level, and the interaction of these factors were analysed by an analysis of variance test (Table 2).

These two experiments showed that, irrespective of the conditions under which *E. bicostata* plants were grown, the glaucous coating on leaves significantly reduced the disease intensity of *Phaeoseptoria* leaf spot, and that lower leaves are more susceptible to disease than upper leaves on the same plant.

Table 2

ANALYSIS OF THE EFFECT OF REMOVAL OF GLAUCOUSNESS AND THE LEVEL OF LEAVES ON *E. BICOSTATA* SEEDLINGS ON THE PERCENTAGE LEAF AREA LESIONED BY *P. EUCALYPTI*

Analysis of variance

Source of Variation	Degrees of Freedom	Mean Sum of Squares	Variance Ratio	Increased Disease Incidence
Wiping (A)	1	0·487	37·045***	470%‡
Leaf levels† (B)	1	0·086	6·542*	81%§
Plants	3	0·017	1·315 (n.s.)	
A × B interaction	3	0·002	0·154 (n.s.)	
Error	39	0·013		
Total	47			

* $P < 0\cdot05$. *** $P < 0\cdot001$.

† The six leaf pairs were divided into two groups of three leaf pairs, upper and lower, on each plant.

‡ Wiped *v.* unwiped.

§ Lower level *v.* upper level.

(iii) *Experiment 3*

The shoots of seven *E. bicostata* plants, grown in the phytotron, were reduced to three corresponding leaf pairs and inoculated with *P. eucalypti*. The percentage leaf area lesioned at each nodal position on each plant was determined after 40 days. An analysis of variance test showed that leaf nodal position was significant in disease intensity at $P < 0\cdot001$. Disease intensity between adjacent leaf pairs, was compared by Student's *t*-test (Table 3).

(*b*) *Leaf Age—Significance of Ontogenetic and Physiological Age*

Groups of six plants of *E. bicostata* were selected at random at 3, 5, 9, and 15 weeks from planting. At the time of sampling the leaves on each plant were classified for age of the plant when leaf formed (ontogenetic age) and chronological age (physiological age) in the following way.

Plants selected at 3 weeks after establishment had three pairs of opposite leaves. These leaves were classified as ontogenetic age up to 3 weeks and physiological age up to 3 weeks. Plants selected at 5 weeks after establishment had six pairs of opposite leaves. Of these the lower three pairs were of up to 3 weeks ontogenetic age and now 3–5 weeks physiological age. The top three pairs of leaves were of 3–5 weeks ontogenetic age and up to 3 weeks physiological age.

Table 3

DIFFERENCES IN DISEASE INTENSITY, EXPRESSED AS PERCENTAGE OF LEAF AREA LESIONED, AT THREE SUCCESSIVE NODAL POSITIONS* ON PLANTS OF *E. BICOSTATA*

Nodal Position (above the cotyledons)	Difference in Disease Intensity (%)	Level of Significance
Node 5–node 6	193	$P < 0\cdot01$
Node 5–node 7	878	$P < 0\cdot001$
Node 6–node 7	235	$P < 0\cdot01$

* Leaves above and below those at the three nodal positions used in the experiment were removed prior to inoculation.

Table 4

MEAN NUMBER OF LESIONS PER SQUARE CENTIMETRE ON *E. BICOSTATA* JUVENILE LEAVES OF VARYING PHYSIOLOGICAL AND ONTOGENETIC AGE

All differences between readings in vertical columns, except that between the two readings marked †, are significant at $P < 0\cdot05$ or better. Differences between readings in a horizontal row are not significant at $P < 0\cdot05$

Physiological Age (weeks)	Ontogenetic Age (weeks)			
	Up to 3	3–5	5–9	9–15
Up to 3	0·31†	0·18	0·14	0·16
3–5	0·45†	0·42	0·39	
5–9	0·67	0·63		
9–15	0·77			

Following leaf classification each plant was inoculated, incubated, and lesion number per square centimetre of leaf assessed 40 days after inoculation (Table 4). These results indicate the significant increase in susceptibility of *E. bicostata* juvenile leaves with physiological aging of the leaf. By contrast there is no evidence of association of disease susceptibility with leaves produced at a particular stage of the apical bud's ontogeny.

(*c*) *Host Nutrition*

Twin cuttings were prepared from buds of identical onotgenetic age on five *E. bicostata* seedlings grown outside the phytotron. The rooted cuttings were potted in washed sand. One member of each pair was given a high-nutrient treatment (half strength Hoagland's solution once per day and tap water once per day), the other a low-nutrient treatment (half strength Hoagland's solution once per week and tap water twice per day). Two months after potting, each plant had five pairs of fully expanded, juvenile leaves of comparable ages. The plants were inoculated, incubated, and lesion number per square centimetre of leaf surface assessed at 40 days after inoculation (Table 5). These results show that, for the nutrient treatments used, juvenile leaves of *E. bicostata* plants grown on a high-nutrient substrate are more susceptible than those of genetically comparable plants grown on a lower-nutrient substrate.

Table 5

NUMBER OF LESIONS PER SQUARE CENTIMETRE ON *E. BICOSTATA* JUVENILE LEAVES OF TWIN CUTTINGS IN RELATION TO NUTRIENT LEVEL AT WHICH CUTTINGS WERE GROWN

Parent Plant No.	Cuttings Grown at High Nutrient Level (A)	Cuttings Grown at Low Nutrient Level (B)	Difference (C)	Ratio (A–B)/B
4	1·030	0·472	+0·558	1·18
7	1·631	0·544	+1·087	1·99
8	0·857	0·591	+0·266	·45
9	0·977	0·374	+0·603	1·61
10	0·589	0·271	+0·318	1·17
Mean	1·017	0·450	+0·567*	

* The difference between the means of the two nutrient treatments is significant at $P < 0 \cdot 05$.

IV. Discussion

The glaucous coating of *E. bicostata* leaves consists of a superficial layer of fine wax rods and an underlying layer of wax plates adjacent to the cuticle as occurs in certain other eucalypt species (Hallam 1964; Hall *et al.* 1965). Lightly wiping the leaf surface removes the superficial rods with little disturbance to the plate layer. The wax rod layer is obviously a very efficient barrier to the infection of *E. bicostata* leaves by water-dispersed conidia of *Phaeoseptoria eucalypti* and probably explains the comparatively low level of natural infection observed in this macrantherous species. Investigations, to be reported separately, have shown that the glaucous leaves of *E. bicostata* are very hydrophobic and in addition contain water-soluble materials inhibitory to the germination of conidia of *P. eucalypti*. Selected progeny of glaucous and green-leaved eucalypt hybrids are likely to show higher resistance to *P. eucalypti* leaf spot than the green, juvenile-leaved parent.

Increased disease resistance of successively younger leaves as reported for *E. bicostata* is in agreement with the pattern of resistance of tobacco leaves to *Perono-*

spora tabacina (Hill 1959), but differs from observations on certain diseases caused by the genus *Septoria* (Green and Dickson 1957; Scharen 1963). The positive correlation of increased disease incidence with physiological age of leaf (Table 4) distinguishes this disease complex from others showing variation in disease with ontogenetic age (Yarwood 1959).

The surfaces of upper leaves, compared with lower leaves, on *E. bicostata* seedlings, have 21% more ether-soluble materials (significant at $P<0\cdot05$) (Heather 1965). Acropetally increased glaucousness of leaves of *E. bicostata* plants may be one explanation of reduced disease intensity on upper leaves. Weathering of surfaces as the leaf ages might be expected to reduce the effectiveness of the glaucous barrier to infection. However, the mechanical effect of glaucousness may not be the only reason for increased disease susceptibility with leaf age. The lower leaves of *E. grandis*, a non-glaucous species, were 200% more susceptible (significant at $P<0\cdot05$) to *Phaeoseptoria eucalypti* leaf spot than upper leaves on the same plants (Heather 1965). While upper and lower leaves were equally suitable as substrate for the *in vitro* culture of *P. eucalypti* (Heather 1965), the *in vivo* situation could be quite different. A decrease in active resistance is a possible explanation of increased disease intensity with leaf age and it is noteworthy that pisatin production in pea pods has been found to decrease with aging (Cruickshank 1963).

The literature on the effect of host nutrition on disease susceptibilty is extensive and gives contradictory results (Stakman and Harrer 1957). Diseases caused by obligate parasites appear to increase in intensity on hosts grown on a high-nutrient substrate, while those caused by facultative parasites show reduced disease incidence following nitrogenous fertilization of the host (Stevens 1960). No general conclusions can be drawn from the results of the single experiment on host nutrition but the variability of the ratio (A–B)/B (Table 5) suggests a nutrient–genotype interaction and indicates a complex mechanism of disease intensity control. The low disease incidence of plant No. 10 (Table 5) on both high and low nutrient substrates may result from it being a genotype with higher than average resistance to *P. eucalypti* leaf spot.

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VI. References

Barber, H. N., and Jackson, W. D. (1957).—Natural selection in action in *Eucalyptus*. *Nature, Lond.* **179**, 1267–9.

Cameron, R. J. (1964).—Light intensity and the growth of *Eucalyptus* seedlings. Ph.D. Thesis, Australian National University.

Cruickshank, I. A. M., and Perrin, Dawn R. (1963).—Studies on phytoalexins. VI. Pisatin: the effect of some factors on its formation in *Pisum sativum* L. and the significance of pisatin in disease resistance. *Aust. J. biol. Sci.* **16**, 111–28.

GREEN, G. J., and DICKSON, J. G. (1957).—Pathological histology and varietal reactions in *Septoria* leaf blotch of barley. *Phytopathology* **47**, 73–9.

HALL, D. M., MATUS, A. I., LAMBERTON, J. A., and BARBER, H. N. (1965).—Infraspecific variation in wax on leaf surfaces. *Aust. J. biol. Sci.* **18**, 323–32.

HALLAM, N. D.—Sectioning and electron miscroscopy of eucalypt leaf waxes. *Aust. J. biol. Sci.* **17**, 587–90.

HEATHER, W. A. (1965).—Some aspects of the ecology and pathology of *Phaeoseptoria eucalypti* (Hansf.) Walker on some species of the genus *Eucalyptus*. Ph.D. Thesis, Australian National University.

HILL, A. V. (1959).—Occurrence, spread and severity of blue mould, *Peronospora tabacina* Adam, of tobacco in field plants. *J. Aust. Instit. agric. Sci.* **25**, 53–8.

MARTIN, J. T., BATT, R. F., and BURCHILL, R. T. (1957).—Fungistatic properties of apple leaf wax. *Nature, Lond.* **180**, 796–8.

MORSE, R. N., and EVANS, L. T. (1962).—Design and development of Ceres—an Australian phytotron. *J. agric. Engng Res.* **7**, 128–40.

PRYOR, L. D. (1959).—Species distribution and association in *Eucalyptus*. In "Biogeography and Ecology in Australia." (Eds. A. Keast, R. L. Crocker, and C. S. Christian.) (W. Junk: Den Haag.)

SCHAREN, A. L. (1963).—Effect of age of wheat tissues on susceptibility to *Septoria nodorum*. *Pl. Dis. Reptr.* **47**, 952–4.

STAKMAN, E. C., and HARRER, J. G. (1957).—"Principles of Plant Pathology." (Ronald Press: New York.)

STEVENS, P. B. (1960).—Cultural practices in disease control. In "Plant Pathology: An Advanced Treatise". Vol. III. (Eds. J. G. Horsfall and A. E. Dimond.) (Academic Press, Inc.: New York and London.)

WALKER, J. (1962).—Notes on plant parasitic fungi. I. *Proc. Linn. Soc. N.S.W.* **87**, 162–76.

YARWOOD, C. E. (1959).—Predisposition. In "Plant Pathology: An Advanced Treatise". Vol. 1. (Eds. J. G. Horsfall and A. E. Dimond.) (Academic Press, Inc.: New York and London.)