

## Added phosphorus is associated with reduced severity of *Mycosphaerella cryptica* in *Eucalyptus globulus*

A.J. Carnegie<sup>1,2</sup> and P.K. Ades<sup>1</sup>

<sup>1</sup>Institute of Land and Food Resources, University of Melbourne, Parkville, Victoria, 3010, Australia

<sup>2</sup>Current address: Research and Development Division, State Forests of NSW, PO Box 100  
Beecroft, 2119, Australia

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### Summary

Leaf disease caused by *Mycosphaerella cryptica* was assessed on the adult foliage of a 6-year-old *Eucalyptus globulus* fertiliser trial in south-eastern Australia. The trial consisted of 16 treatments of various combinations of nitrogen, phosphorus, potassium and trace elements, originally established to determine the effects of these additions on tree growth. Disease was assessed as the proportion of the whole crown with leaf spot caused by *M. cryptica*. The trial had previously been measured for growth parameters (Bennett *et al.* 1997). The results from our study provide evidence that low levels of phosphorus were correlated with increased susceptibility of *E. globulus* to infection by *M. cryptica* compared with trees given high levels of phosphorus fertiliser. Treatments with no phosphorus added were significantly more diseased than those with up to 200 kg ha<sup>-1</sup> added phosphorus. Neither the addition of nitrogen, potassium nor a combination of trace elements had a significant effect on disease severity. Disease severity was negatively correlated with height and diameter, providing evidence that smaller trees had more disease than taller trees, but not necessarily proving 'cause and effect'. Various hypotheses are discussed to explain the results obtained from this study.

**Keywords:** phosphorus, phosphorus fertilizer, foliage, fungal diseases, *Mycosphaerella cryptica*, *Eucalyptus globulus*, Victoria

### Introduction

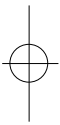
A major limiting factor in the profitability of eucalypt plantations in Australia is the low availability of nutrients in soils in areas suitable for large-scale planting (Attiwill and Adams 1993; Cromer 1996). The addition of various NPK formulations of fertilisers has become a routine practice in intensive plantation management in Australia. While this practice has increased growth rates (e.g. Cromer 1971; Cromer and Hansen 1972; Cromer *et al.* 1975; Schönau and Herbert 1989; Messina 1990, 1992; Cromer *et al.* 1991; Weston 1991; Birk and Turner 1992; Stone 1993; Judd *et al.* 1996; Bennett *et al.* 1997), there have been few studies of its effect on leaf diseases.

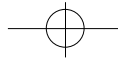
Fertilisers have been shown to affect disease severity in many crop plants. Nitrogen imbalance has frequently been found to increase the susceptibility of plants to pathogens (Yarwood 1959; Trolldenier 1969; Huber and Watson 1974; Lam and

Lewis 1982; Boquet and Johnson 1987; Agrios 1988) while amelioration of trace element deficiencies appears to reduce the incidence of diseases (Yarwood 1959; Huber 1980; Nichol *et al.* 1992). Increased susceptibility to fungal attack has also been demonstrated in potassium-deficient plants (Trolldenier 1969; Mengel and Kirkby 1987), but high levels of this element can also increase susceptibility of plants to various diseases (Agrios 1988). Application of phosphorus has been shown to decrease the severity of certain plant diseases (Trolldenier 1969; Boquet and Johnson 1987; Agrios 1988), but increase the severity of others (Cunfer *et al.* 1980; Agrios 1988).

Various effects of the addition of fertiliser on disease severity have also been reported for tree species. Adverse effects of fertilisers, such as an increase in susceptibility of seedlings to damping-off, have been reported in nurseries where nitrogen is applied (Hesterberg and Jurgensen 1972). Diller *et al.* (1946) observed that susceptibility to chestnut blight of old Japanese chestnut trees increased with the application of phosphorus and potassium. Many studies have shown that the addition of fertilisers increases the susceptibility of slash pine and loblolly pine to infection by fusiform rust (Boggess and Stahelin 1948; Gilmour and Livingstone 1958; Schmidt *et al.* 1972; Blair and Cowling 1974; Hollis *et al.* 1975; Rowan 1977; Rowan and Steinback 1977). Application of nitrogen has been shown to reduce little leaf disease caused by *Phytophthora cinnamomi* Rands in shortleaf pine (Roth *et al.* 1948; Roth and Copeland 1957), and to reduce the severity of *Verticillium* wilt of maple (Caroselli 1956). Increased susceptibility of trees to pathogens as a result of fertiliser (especially nitrogen) application has been attributed to increased succulence of the foliage, production of a complex nitrogen source that is required by the pathogen, or to the reduction of some metabolite that inhibits the pathogen (Hesterberg and Jurgensen 1972).

A potential obstacle to growing eucalypt plantations profitably in Australia and overseas is the damage caused by leaf pathogens. Species of *Mycosphaerella* have been the most common and destructive leaf pathogens of eucalypt plantations in Australia, New Zealand and South Africa. Leaf disease caused by species of *Mycosphaerella* results in a reduction in the photosynthetic area of surviving leaves and premature abscission of severely affected leaves, and can reduce the growth rate of trees (Lundquist and Purnell 1987; Carnegie *et al.* 1994; Carnegie 2000). One of the most important and damaging species in Australia and New Zealand, *Mycosphaerella cryptica* (Cooke) Hansf., has been found to infect both juvenile and adult foliage of over 50 species from the subgenera *Monocalyptus* and





*Symphyomyrtus* (Crous *et al.* 1995a) and has caused extensive damage in eucalypt plantations in Australia (Park and Keane 1982b; Carnegie *et al.* 1994, 1998; Dungey *et al.* 1997) and New Zealand (Beresford 1976; Cheah 1977; Dick 1982; Wilcox 1982a,b). One of the most susceptible hosts to leaf disease caused by *M. cryptica* and *M. nubilosa* (Cooke) Hansf. is *Eucalyptus globulus* Labill. (Carnegie *et al.* 1994, 1998) which is planted extensively in south-eastern and south-western Australia.

No information on the effects of fertiliser application on *Mycosphaerella* leaf diseases has been reported. This paper describes the assessment of a 6-year-old *E. globulus* fertiliser trial in south-eastern Australia to determine whether the application of fertilisers has an effect on severity of disease caused by *M. cryptica* in adult foliage.

**Methods**

**Description of trial**

A fertiliser trial of *E. globulus* ssp. *pseudoglobulus* (Nauden ex Maiden) Kirkpatr. (Jeeralang, Victoria provenance) was established in June and July 1989 by Amcor Plantations Pty Ltd at Glencoe, south-eastern Victoria, Australia, to determine the effects of various combinations of NPK and trace elements on survival and growth of eucalypts. A detailed site description has been published previously along with results on effects of nutrient addition on tree growth, including data on nutrient concentrations in foliage at age six (Judd *et al.* 1996; Bennett *et al.* 1997). The site had a deep, uniform, sandy soil, an annual rainfall of 620 mm and had previously carried two rotations of *Pinus radiata* D. Don. The available nitrogen, phosphorus and exchangeable potassium in the surface soil was relatively low. The experimental design was a complete 4 x 3 factorial of nitrogen and phosphorus with four additional treatments including two levels of potassium both with and without trace elements (Table 1) and there were three replicates. Nitrogen was applied at rates of 0, 200 and 400 kg ha<sup>-1</sup>, phosphorus at 0, 50, 100 and 200 kg ha<sup>-1</sup> and potassium at 100 and 200 kg ha<sup>-1</sup>. Each plot consisted of 64 trees spaced at 3.5 m x 2.9 m on a rectangular grid. One quarter of the total fertiliser was applied at each of 2, 9, 14 and 26 months post-planting.

**Assessment of *Mycosphaerella cryptica* on adult foliage**

In March 1995, 68 months after planting, the whole crown of each tree was assessed for severity of *M. cryptica*, with the aid of binoculars where necessary, by an observer walking along the rows of the trial and assessing the crown on a scale of 0 (no infection), 0.5 (<1%), 1 (1 to <5%), 2 (5 to <10%), 3 (10 to <15%), 4 (15 to <20%), 5 (20 to <25%) or 6 (>25%). This method was developed for *Mycosphaerella* leaf diseases in juvenile foliage of eucalypts; it was revised for use on adult foliage and has previously been used by Carnegie (2000) and Dungey *et al.* (1997). The eight internal trees of each plot were assessed allowing a buffer of 2-3 trees between treatments. Disease severity was compared with mean height and diameter at breast height of trees as measured by Bennett *et al.* (1997) 71 months after planting. Trees were sampled for foliar analysis 72 months after planting by Bennett *et al.* (1997).

**Table 1.** Fertiliser\* applied for the various treatments, severity of *Mycosphaerella cryptica* on adult foliage and mean height and diameter at breast height of trees in the *Eucalyptus globulus* trial at Glencoe

Treatment code	Nitrogen	Phosphorus	Potassium	Trace elements	Disease severity	Mean height (m)**	Mean diameter (cm)**
A	0	0	0	0	2.67	13.01	12.42
B	0	50	0	0	1.34	14.35	14.54
C	0	100	0	0	1.27	13.14	13.11
D	0	200	0	0	1.13	14.17	14.07
E	200	0	0	0	2.31	12.45	12.04
F	200	50	0	0	1.63	12.32	12.2
G	200	100	0	0	1.13	13.83	14.25
H	200	200	0	0	0.83	14.55	14.94
J	400	0	0	0	1.77	13.81	13.43
K	400	50	0	0	1.21	14.17	15.02
L	400	100	0	0	1.61	13.93	14.96
M	400	200	0	0	1.46	13.67	14.05
N	200	100	100	0	1.73	14.09	13.98
P	200	100	100	0.4	1.92	13.36	13.71
Q	400	200	200	0	1.06	15.08	15.74
R	400	200	200	0.8	1.55	14.56	14.88
Least Significant Difference (P= 0.05)					0.52	1.16	1.81

\*Nitrogen as urea (kg ha<sup>-1</sup>); phosphorus as single superphosphate (kg ha<sup>-1</sup>); potassium as muriate of potash (kg ha<sup>-1</sup>); trace elements as a mix of B (NaHO<sub>3</sub>), Cu (CuSO<sub>4</sub>), Mo (NaMoO<sub>4</sub>), Zn (ZnSO<sub>4</sub>), 6% each (kg ha<sup>-1</sup>).

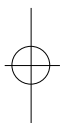
\*\*Data from Bennett *et al.* (1997), used with permission

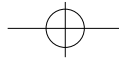
**Statistical analyses**

Analyses of variance were carried out for the severity scores to test for variation among treatments in susceptibility of adult foliage to disease. Product moment correlations were calculated to examine the relationship between disease and growth parameters. Analyses of variance were also carried out by pooling the effect of disease on height within each treatment (i.e. a pooled regression). A few obvious runts were removed from the data set and this reduced the error variance for height and diameter. All analyses were carried out using the GLM Procedure of SAS (SAS Institute 1992).

**Results**

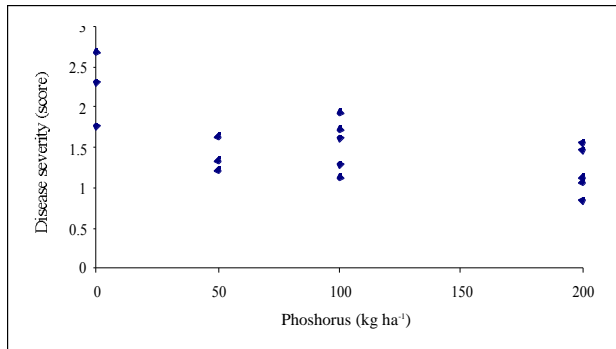
Severity of *M. cryptica* on the adult foliage of *E. globulus* at Glencoe varied significantly (*P* <0.001) over the 16 treatments at the time of assessment (Tables 1 and 2). Treatments A (Control) and E (N<sub>200</sub>) had the highest disease severity with mean disease severity scores of 2.67 and 2.31, respectively, while treatments H (N<sub>200</sub> P<sub>200</sub>) and Q (N<sub>400</sub> P<sub>200</sub> K<sub>200</sub>) had the lowest disease severity scores of 0.83 and 1.06, respectively. Phosphorus had a significant effect (*P* <0.001) on disease severity: treatments with no added phosphorus (A and E) were amongst the most severely affected by disease while those with high levels of added phosphorus (H and Q) were amongst the least affected (Table 1 and Fig. 1). While these were distinct





**Table 2.** Analysis of variance for disease severity of *Mycosphaerella cryptica* on adult foliage in the fertiliser trial at Glencoe

Source	df	Mean square	F value	Pr > F
Treatment	16	10.467	11.39	0.0001
Height	1	14.376	15.64	0.0001
Error	356	0.919		



**Figure 1.** Severity of disease caused by *Mycosphaerella cryptica* on *Eucalyptus globulus* at Glencoe plotted against phosphorus addition

trends, however, Table 1 and Figure 1 illustrate the variability in disease responses at each level of phosphorus addition. Neither the addition of nitrogen, potassium nor the combination of trace elements had a significant effect on disease severity. There was also no significant interaction between nitrogen and phosphorus. Foliar analysis at 72 months revealed that the addition of phosphorus significantly ( $P < 0.05$ ) increased the concentration of phosphorus in the leaves of sampled trees, but there was no significant increase at 72 months in concentrations of nitrogen or potassium due to addition of these elements (Bennett *et al.* 1997).

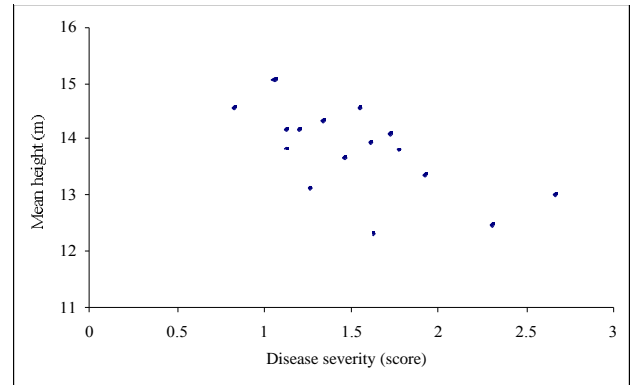
Disease severity was negatively correlated with the height ( $r = -0.27$ ,  $P < 0.001$ ) and diameter ( $r = -0.29$ ,  $P < 0.001$ ) measurements made by Bennett *et al.* (1997) (Figs 2-3). That is, trees with a lower average for these growth parameters, such as treatments A and E, had significantly ( $P < 0.05$ ) more disease than those with a higher growth average, such as H and Q (Table 1 and Figs 2-3). Treatments A and E were also significantly ( $P < 0.05$ ) smaller (height and diameter) than treatments H and Q. Treatment F (N<sub>50</sub> P<sub>50</sub>), which had the lowest average tree height and close to the lowest diameter in the trial, had significantly less disease than treatments A and E, which were not significantly different in height or diameter from F. Treatment F had significantly more disease and was significantly smaller than H and Q. In most cases, treatments with low averages for growth parameters, such as A and E, were surrounded by larger trees. There was also a significant negative association between disease severity and height within each treatment (Table 3). This suggests that disease was directly affecting tree growth, although this cannot be proved in this experiment.

### Discussion

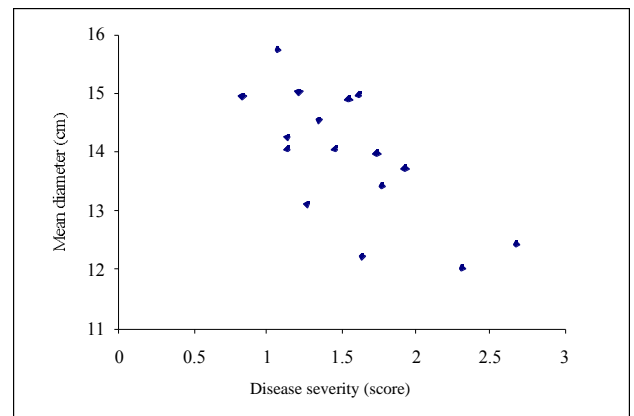
The method of disease assessment used here has previously proved useful for detecting significant differences in disease severity between *E. globulus* families (Dungey *et al.* 1997;

**Table 3.** Pooled analysis of variance for disease severity of *Mycosphaerella cryptica* on adult foliage in the fertiliser trial at Glencoe

Source	df	Mean square	F value	Pr > F
Treatment	16	5.969	6.71	0.0001
Height (Treatment)	16	2.398	2.70	0.0005
Error	341	0.889		

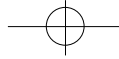


**Figure 2.** Severity of disease caused by *Mycosphaerella cryptica* plotted against mean height of *Eucalyptus globulus* at Glencoe



**Figure 3.** Severity of disease caused by *Mycosphaerella cryptica* plotted against mean diameter of *Eucalyptus globulus* at Glencoe

Carnegie 2000) and was able to detect a significant effect of phosphorus fertilisation on severity of disease caused by *M. cryptica* in this experiment. These results provide evidence that low levels of phosphorus increase the susceptibility of *E. globulus* to infection by *M. cryptica* compared with trees given high levels of phosphorus fertiliser. Treatments with no phosphorus added were significantly more diseased than those with up to 200 kg ha<sup>-1</sup> added phosphorus, although there was some variability in the phosphorus-disease relationship data. Phosphorus fertilisation may increase the resistance of plants to disease by accelerating the maturity of the foliage and thereby allowing the plant to escape attack by pathogens that invade younger foliage more readily (Trolldenier 1969; Agrios 1988). Since only newly emerged or recently expanded leaves are susceptible to infection by *M. cryptica* (Park 1988a), accelerating foliage maturity should reduce the opportunity for infection by this fungus. Perhaps the more important factor, however, is that fertilisation accelerates height growth and foliage maturity follows.



In this study, phosphorus content in leaves at 72 months was correlated with addition of phosphorus, which in turn was correlated with disease severity. However, there were no significant effects of nitrogen addition on foliar content at 72 months (Bennett *et al.* 1997) nor on disease severity (in the adult foliage), as has been reported for other tree diseases (e.g. Schmidt *et al.* 1972; Blair and Cowling 1974; Hollis *et al.* 1975; Rowan 1977; Rowan and Steinbeck 1977). In a 3-year-old *E. globulus* provenance trial in Gippsland, Victoria, there were no significant correlations between nitrogen content of leaves (fertilised at establishment) and severity of *Mycosphaerella* leaf diseases on the juvenile foliage (Carnegie, unpublished data). Assessments of disease on the juvenile foliage in the current trial would have been useful to determine the effects of the addition of the various elements on disease severity.

In contrast to the situation with *M. cryptica* reported here, fertilisation with phosphorus had no significant effects on incidence of *Phaeophleospora epicoccoides* (Cooke & Masee) Crous, F. A. Ferreira & B. Sutton (= *Phaeoseptoria eucalypti* Hansf.) in plantations of *E. grandis* Hill ex Maiden in South Africa (Nichol *et al.* 1992). In other experiments, phosphorus and nitrogen increased the severity of fusiform rust of slash and loblolly pine seedlings (Schmidt *et al.* 1972; Blair and Cowling 1974; Rowan 1977; Rowan and Steinbeck 1977) and 5-year-old trees (Hollis *et al.* 1975). In these experiments, it was concluded that increased rust severity was associated with increased host vigour, an hypothesis supported by others (Bogges and Stahelin 1948; Gilmore and Livingston 1958; May *et al.* 1973). Phosphorus deficiency has been shown to make *Populus* spp. more resistance to leaf rust caused by *Melampsora larici-populina* Kleb. (Suzuki 1973).

It is also possible that the differences in micro-climate between the canopies of the various treatments may account for the variation in disease severity among them. Disease severity caused by *M. cryptica* was negatively correlated with height and diameter in this trial, suggesting that *M. cryptica* was having a detrimental affect on growth of *E. globulus*. Reduced growth rates caused by *Mycosphaerella* spp. have been reported for *Eucalyptus* (Lundquist and Purnell 1987; Carnegie *et al.* 1994; Carnegie 2000), but care is needed when attributing causation in this study. In the present experiment, trees with no phosphorus added had higher levels of *M. cryptica* than those with 200 kg ha<sup>-1</sup> of phosphorus added. There is no proof from the data in this study that the increase in disease severity actually reduced tree height. The addition of nitrogen and phosphorus significantly increased growth of trees in this trial, and trees with no phosphorus added had a significantly smaller average height than those with 200 kg ha<sup>-1</sup> phosphorus added (Judd *et al.* 1996; Bennett *et al.* 1997). Thus it is evident that smaller trees had more disease than their neighbouring taller trees. The differences in micro-climate in the canopies of the smaller and taller trees may have affected disease development in the trees, and could account for the difference in disease severity between them.

Humidity is a major factor in the infection process of pathogens (Agrios 1988; Manion 1991). Reduced air movement in an *E. grandis* plantation in South Africa increased humidity, and hence, the incidence of the leaf pathogen *P. epicoccoides* (Nichol *et al.* 1992). Similarly, lower plant height due to low levels of phosphorus fertiliser has been reported to contribute to

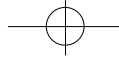
increased levels of *Septoria* glume blotch in wheat (Cunfer *et al.* 1980). High levels of humidity have also been shown to favour infection by *Mycosphaerella* spp. on *Eucalyptus* (Cheah and Hartill 1987; Park 1988a). The low phosphorus treatments may have directly affected tree growth and thus the smaller trees may have had less air movement and more shading than the taller trees in the high phosphorus treatments. This would have increased humidity and the duration of leaf wetness in the crowns of the smaller trees, thus favouring infection by *M. cryptica*. Shading of shorter plants by taller ones has been shown to affect symptom expression of *Septoria tritica* Desm. on various wheat cultivars (Benedict 1971), and increase dieback in Corsican pine (*P. nigra* var. *calabrica* (Loud.) Schn.) associated with the fungus *Brunchorstia pinea* (Karst.) v. Hohn. (Read 1968). Taller treatments may also have less turgor pressure in their leaves due to higher temperature and light intensity, and therefore, increased respiration which leads to closure of their stomata (Raven *et al.* 1982). Since *M. cryptica* penetrates leaves at least partly via stomata (Park 1988b) the closure of stomata could result in reduced disease severity in the taller treatments.

Another possibility is that treatments with more phosphorus may be able to replace foliage damaged or lost to disease more rapidly than those treatments with little or no phosphorus added. The addition of phosphorus has been shown to increase growth of many eucalypts (e.g. Weston 1991; Birk and Turner 1992; Judd *et al.* 1996; Bennett *et al.* 1997) and much of this increase has been attributed to an increase in the photosynthetic ability of trees due to the production of more leaves. A plant that has lost tissue due to disease may replace that tissue by initiating additional new growth (Kranz and Rotem 1988). For example, rubber plants produce successive leaf flushes after young leaves have been lost due to infection by powdery mildew (Populer 1972, in Kranz and Rotem 1988). The *E. globulus* trees in the current study with high phosphorus may have been able to replace more foliage at the time of assessment than those with no or little phosphorus, and hence, would have appeared to suffer less infection by *M. cryptica*, assessed as the proportion of the whole crown diseased.

Studies on the benefits of weed removal and fertiliser application in young *E. dumii* Maiden plantations in New South Wales have shown that a reduction in tree stress (reduced weed competition and increased fertiliser application) results in an increase in tolerance of trees to other stress-inducing factors, such as insects (Stone and Birk 2001). The present study showed that added phosphorus was associated with a reduction in disease severity in *E. globulus*, suggesting that a general increase in tree vigour results in greater health - in this case reduced disease severity. It is clear that more research is needed on the relative effects of various elements and especially phosphorus on disease of *E. globulus* caused by species of *Mycosphaerella*.

### Acknowledgements

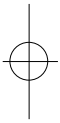
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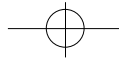


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