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R. Truman and J. Turner

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Forestry Commission of N.S.W.
G.P.O. Box 2667,
SYDNEY 2001 New South Wales,
Australia.

MINERAL DEFICIENCY SYMPTOMS IN EUCALYPTUS PILULARIS

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R. TRUMAN AND J. TURNER

SUMMARY

Deficiency symptoms due to lack of the major essential elements and iron were produced in *Eucalyptus pilularis* seedlings grown in sand culture. The symptoms shown are described, illustrated and discussed.

INTRODUCTION

At the present time the total area covered by eucalypt plantations in New South Wales approximates 23,500 acres. Over fifty per cent of this area has been established with *Eucalyptus pilularis* and a further eight percent with a mixture of *E. pilularis* and *E. grandis*.

Florence (1964) reported that the distribution of *E. pilularis* is limited by physical properties of the soil which restrict aeration, moisture permeability or penetration of the soils to depth, so that where plantations of this species have been established on non - *E.pilularis* sites, care has been taken to select deep, well drained soils of light texture or good structure(Anon. 1966).

Notwithstanding this, nutritional problems have been encountered and these are at present being investigated by means of pot and field fertilizer trials. The work reported here was intended to complement these trials by providing a means for the preliminary diagnosis of mineral deficiencies occurring in the nursery and during the first one or two years after planting out.

REVIEW OF THE LITERATURE

Nitrogen

Reports contained in the literature indicate that restricted height growth and branching, small and chlorotic leaves, and the premature shedding of the older leaves are symptoms common to nitrogen -deficient eucalypt seedlings. Chlorosis generally appears as a uniform pale yellow-green colour, although Kaul, Srivastava and Tandon(1970a) noted that the leaves of *E. globulus* showed yellow spots and interveinal chlorosis.

Advanced stages of deficiency are often characterized by the presence of anthocyanin in the leaves but the production of this pigment appears to vary both between and within species. The presence of anthocyanin was reported in the leaves of *E. alba* (Mello et al.1960),*E. camaldulensis* (Will,1961 a; Esparcia and Nuno,1964),*E.pilularis* (Will,1961a; Lacey,1964),*E. botryoides, E. gigantea* and *E.saligna*(Will, 1961a), and Eucalyptus "hybrid"(Hussain and Theagarajan,1966). Eucalyptus "hybrid" has been reported to be synonymous with *E.tereticornis* (Sinha,1970) and will be referred to as such throughout the remainder of this review.

Species for which no anthocyanin production was recorded are *E. gomphocephala* (Karshon,1963a) and *E.citriodora* (Kaul,Srivastava and Negi,1970 b).

Esparcia and Nuno(1964) reported a red colour in the leaves of *E.globulus* but although Kaul et al,(1970a) allowed deficiency in seedlings of this species to proceed to the stage when the older leaves were being shed, the only symptom they obtained was chlorosis.

Groves(1967) who investigated within-species variation in the growth and nutrition of *E.cladocalyx*, described nitrogen deficiency as being evidenced by chlorosis of the older leaves, often accompanied by the development of anthocyanin. In an experiment carried out in this laboratory no pigment was seen in the older leaves of this species but a red colour was evident on the stems, petioles and last formed leaves.

Phosphorus

As is the case with nitrogen deficiency, foliage symptoms due to lack of phosphorus generally appear first on the older leaves. Also,anthocyanin production is a feature of some species and not others.

Phosphorus deficiency in *E. cladocalyx* seedlings first appeared as purpling of the tips and margins, and as blotches of purple colour on the surface of the older leaves which were shed prematurely(Groves,1967). Purplish blotching on dark green leaves was recorded for *E.botryoides, E. camaldulensis, E.gigantea,E.pilularis* and *E.saligna*

(Will,1961a), and for *E.grandis* (Lacey et al.1966).

Mello et.al.(1960)described deficiency in *E.alba* as being first evidenced by numerous dark points on otherwise normal leaves. Later the points increased in size and the leaves developed an orange-yellow tint.

Necrosis was the only symptom recorded for *E.citriodora* (Kaul et al.,1970b) and *E.globulus* (Kaul et al., 1970a). In the former it appeared as spots, first on the older foliage and later on the younger leaves. In the latter it was evident as dry tips and margins of the lower leaves.

Hussain and Theagarajan(1966) reported that the leaves of *E.tereticornis* were small and malformed. Older leaves were dark bluish green while the younger leaves were pale green.

Sulphur

Attempts to produce sulphur deficiency symptoms in eucalypt seedlings have not always been successful. For example,Kaul et al.(1970a) failed to produce conclusive evidence of sulphur deficiency in *E. globulus* seedlings and a similar result was obtained by Kaul et al.(1970b) in the case of *E. citriodora*. In both cases acid-washed sand was used as the supporting medium so sufficient sulphur to supply the needs of the plants must have been present either as impurities in the salts used to prepare the nutrient solutions or possibly as sulphur dioxide in the atmosphere. In either case it would appear that the sulphur requirement of the two species is low.

Sulphur deficiency in *E. alba* was first evident in the younger leaves which showed uniform chlorosis and later a bronzelike colour. Branches showed a purplish tinge(Mello et al., 1960). Lacey(1964) reported that symptoms in *E.pilularis* first appeared as interveinal chlorosis on the latest formed leaves. This was followed by the development of a reddish colour at the base of the leaves. Even in severe cases the leaves did not redden completely, but the tips became yellow and then necrotic .Pronounced stunting of the seedlings occurred.

Restricted growth was also a feature of *E.tereticornis* (Hussain and Theagarajan,1966). Some leaves were malformed and the younger foliage was slightly pale in colour.

Magnesium

With the exception of *E.tereticornis*, the older leaves of which showed marginal or tip scorch with brown necrotic spots in the lamina(Hussain and Theagarajan,1966) the eucalypts which have been studied have shown chlorosis as the main symptom of magnesium deficiency.

Will(1961a) found that the lower leaves of *E.pilularis* became pale green and were often shed prematurely,leaving a long length of stem with only a tuft of leaves at the top.He obtained similar results with *E.gigantea*,*E.camaldulensis*, *E.botryoides* and *E.saligna*, although the last two species differed in that under conditions of mild deficiency they produced leaves which were larger than normal.

Mello et al.(1960) reported that the older leaves of *E.alba* seedlings first showed interveinal chlorosis localized alongside the mid-rib. The chlorotic areas then turned brown, at which stage necrosis was evident. The main veins continued to be separated from the affected regions by green tissue. Haag et al.(1962) described similar symptoms except that the chlorotic areas became grayish before the onset of necrosis.

Foliage of *E.globulus* showed interveinal chlorosis, the older leaves showing the most pronounced effects(Kaul et al. 1970a).Kaul et al.(1970b) reported that magnesium deficiency in *E.citriodora* appeared as chlorosis of the tips of the younger leaves and complete chlorosis of the older leaves. Strongly chlorotic leaves were shed prematurely.

Karschon (1963a,1963b) found that magnesium deficiency in *E.gomphocephala* in the field appeared first in the older foliage and then spread to the younger leaves.Deficiency was characterized by a fading of chlorophyll in local areas between the veins,either around the mid-rib or near the margins. Chlorosis spread until except for an area around the mid-rib the leaf blade was dull yellow. In the more advanced stages necrosis appeared at the lead tip and around the margins.

Calcium

Reports indicate that depending on the species involved, calcium deficiency symptoms appear first in either the terminal regions, the older leaves, or those situated near the middle of the crown. Also it is apparent that the calcium requirement of some species is relatively low.

Mello et al.(1960) reported that calcium deficiency symptoms in *E. alba* first appeared in the older leaves which had a pale green colour and showed reddish spots. As the shortage became more acute,necrosis appeared in the reddish areas after which the leaves withered and fell. Symptoms also appeared first in the older leaves of *E.citriodora* which were chlorotic with necrotic tips(Kaul et al., 1970b).

Will(1961b) who used perlite as the supporting medium found that *E. botryoides* and *E.saligna* seedlings receiving a solution lacking calcium grew well for two to three months and reached heights from six to twelve inches. Then the leaves near the middle of the crown curled and died. Symptoms then spread to the remaining leaves and the seedlings withered and died. Seedlings of *E.pilularis* grown under the same conditions showed somewhat different symptoms. These seedlings grew normally for four months, at the end of which time the terminal buds on the main stem and branches withered and died. This was followed by the production and subsequent death of shoots in the axils of the terminal leaves until as many as four dead shoots were contained in each axil. As these symptoms were developing, the terminal leaves curled and died back from the tips. This sequence of events was repeated basipetally as the seedlings died back along the main and lateral axes.

Lacey(1964) obtained no symptoms in *E. pilularis* in sand culture but in solution culture mottling appeared and was followed by the development of interveinal depression in the foliage. In severe cases the leaf tips of the last formed leaves were distorted. The sand used by Lacey contained 0.04% calcium expressed as CaO,some of which must have been available to the seedlings. Even so,the calcium requirement of *E.pilularis* appears to be low, this being also indicated to some extent by the delayed appearance of symptoms in the work carried out by Will(1961b) who used a solution containing no calcium.

Other species which may also have a low requirement for calcium are *E.globulus* (Kaul et al.,1970a) and *E.tereticornis* (Hussain and Theagarajan,1966) both of which failed to show conclusive symptoms when grown in acid-washed sand.

Potassium

Necrosis is the main symptom shown by potassium deficient eucalypts and is often associated with increased branching and/or malformed leaves.

Lacey(1964) found that in solution culture,axillary leaves of *E. pilularis* showed marginal necrosis. In sand culture leaf twisting occurred in the early stages but shortly disappeared. Axillary branches were well developed.No abnormal leaf colours were observed by Will(1961a,1961b) but in severe cases leaf margins became necrotic. Leaves were smaller than normal, often with buckled surfaces or margins. More branching occurred than normal,giving the plants a bushy round-topped appearance. Similar symptoms were found for *E.botryoides, E.saligna,E.gigantea* and *E.camaldulensis*.

In the case of *E. tereticornis*,branching occurred earlier than normal and the plants appeared somewhat bushy (Hussain and Theagarajan,1966). Many leaves were malformed with tip and/or marginal scorch. Necrotic spots were present in the lamina of the older leaves, some of which appeared slightly chlorotic.

Regular branching was noted in the case of potassium-deficient *E.globulus* seedlings(Kaul et al,1970a).In the early stages symptoms were confined to the presence of some brown spots on the leaves. Later however, the tips and margins of the older and then the younger leaves curled up giving them a cup-shaped appearance.

Complete lack of branching was reported for *E.citriodora* by Kaul et al.(1970b). Necrosis was the only foliage symptom observed and this was confined to the older leaves which were shed prematurely.

Iron

Accorsiet al.(1961) described iron deficiency in *E. tereticornis* growing in nutrient solution. Symptoms consisted of a yellowing of the blade of the younger leaves, the veins retaining their green colour. *E. camaldulensis* and *E. gomphocephala* growing on calcareous soil showed interveinal chlorosis (Karschon 1958,1963b). In acute cases, *E. gomphocephala* leaves were often reduced in size and were dull yellow or yellowish green-yellow.

Iron deficiency may be induced because of the uptake of toxic quantities of manganese. This was shown by Winterhalder (1963) who grew *E. gummifera* seedlings in pots containing surface soil taken from a gully rainforest community dominated by *Ceratopetalum apetalum*. The plants showed vigorous but unhealthy growth, the leaves being small, chlorotic and distorted in shape. Death of the terminal bud often occurred. Analysis of the chlorotic leaves showed much higher concentrations of manganese than those found in the leaves of *E. gummifera* seedlings grown in *E. saligna* forest soil. The chlorotic symptoms were corrected by spraying the leaves of affected seedlings with ferrous EDTA.

Trace elements other than iron

Accorsi et al.(1961) investigated deficiencies of molybdenum, manganese, copper, zinc and boron in *E. tereticornis* seedlings and found that leaf chlorosis was common to all.

Molybdenum deficiency showed out as chlorotic spots in the interveinal areas of the mature leaves, only narrow bands alongside the veins remaining green. A purplish colour due to anthocyanin appeared along the margins.

The younger leaves of manganese-deficient plants showed interveined chlorosis, the tissue near the veins remaining green. In acute cases the tips and margins withered and became a sandy colour. Eventually the entire leaf blade was affected.

Copper deficiency was evidenced by interveinal chlorosis of the younger leaves, the margins of which were irregular in appearance.

In the early stages of zinc deficiency, leaves showed mild interveinal chlorosis. The upper surface of the leaf blade showed purplish areas distributed between numerous discoloured punctuations. Near the margins there were also a few circular areas of lighter coloured tissue with brownish edges. Later, there was a marked shortening of the internodes leading to the formation of a rosette of small narrow yellowish leaves.

The younger leaves of plants deprived of boron showed interveinal chlorosis which first appeared near the margins and then extended towards the mid-rib. The tissue alongside the veins was green with a purplish tint.

Savory (1962) described boron deficiency symptoms in a number of eucalypts in Rhodesia,the main difference between species being in the severity of the disease. *E.saligna* and *E.resinifera* were only slightly affected, *E.grandis* was intermediate,and *E.citriodora*, *E.torelliana*, *E.alba* and *E.tereticornis* were badly affected.

The first symptom evident was crinkling and discolouration of the unfolding leaves of the apical bud. This symptom was repeated in the other buds on the upper part of the crown. The buds became brittle and died, and the mature leaves of the upper crown became discoloured and dropped off. Discolouration in *E.grandis* appeared as a reddish purple colour,at times preceded by yellow patches on the broad part of the leaves.The leaves of *E.citriodora* and *E.torelliana* showed yellowing but no purpling. Finally the bark of the main stem and the upper laterals became dark brown and necrotic,the necrosis starting at the buds and progressing down the stems.

MATERIALS AND METHODS

Seedlings of *E.pilularis* were grown in dune sand in six-inch diameter plastic pots equipped with fritted glass siphons.

The sand was purified by repeated soaking and leaching with 3% hydrochloric acid(Hewitt,1952) until the leachate gave a negative test for iron with ammonium thiocyanate,then by leaching with demineralised water until the leachate gave a negative test for chloride with silver nitrate,and finally by repeated soaking and leaching with the appropriate nutrient solution adjusted to pH5.5, until the pH of the leachate remained approximately the same.

After purification, six-inch diameter circles of one-quarter inch thick, black polyurethane foam plastic were placed on the sand. Seeds of *E. pilularis* were placed on the plastic and covered with a moist filter paper. Plastic bags were then placed over the pots and secured with rubber bands.

When seeds commenced to germinate, the plastic bags and filter papers were removed. It was found that the roots of the seedlings easily penetrated the foam plastic which in turn prevented the growth of algae.

The nutrient solutions used for all of the elements investigated were those of Hoagland and Arnon(1950), while in the nitrogen and magnesium series those of Walker et al.(1955) were also included.

Trace elements were added as 1 ml of the following solution to each litre of nutrient solution.

H_3BO_3	2.86 g
$MnCl_2 4H_2O$	1.81 g
$ZnCl_2$	0.104 g
$CuCl_2 2H_2O$	0.056 g
Na_2MoO4	0.021 g
Water to 1 litre	

Except for the iron deficient solution, iron was added in the form of NaFe -EDTA to give a concentration of 5 ppm Fe.

All pots were irrigated three times each week,sufficient solution being added to cause siphoning through the glass tube.

RESULTS AND DISCUSSION

Nitrogen

Seedlings which received the Hoagland and Arnon(1950) solution lacking nitrogen showed reduced height growth and short internodes. The leaves were smaller than normal.

A uniform pale green colour first appeared on the older leaves and then quickly spread to the younger foliage. As symptoms became more severe red blotches or spots of anthocyanin appeared on the older leaves and in some cases the veins also became red in colour. Leaves were shed from the base of the crown upwards(Plate 1).

In the "Low N" solution of Walker et al.(1955) which contains approximately 1 ppm N, stunting was not so marked and symptoms developed more slowly (Plate 2).

The foliage symptoms described above are similar to those obtained by Will(1961a) and Lacey (1964) and it appears that anthocyanin production is a regular feature of advanced stages of nitrogen deficiency in *E.pilularis*.

Phosphorus

Height growth of seedlings in this series remained normal for some time but eventually it became restricted and fell behind that of the controls. Regular branching occurred but branches were restricted in length.

At first, leaf symptoms were confined to the older foliage which appeared darker green than normal and showed a dark purple colour either as a continuous zone at the leaf tip or as irregular patches scattered over the lamina(Plate 3). Later the colour of these leaves faded, they became necrotic at the tips and were shed prematurely.

As symptoms extended upwards the purple colour became less pronounced, the main symptoms being dark green foliage, with fading and necrosis commencing at the tips of the leaves (Plate 4). As with nitrogen deficiency the above symptoms are in general agreement with those obtained by Will (1961a) and Lacey(1964).

Sulphur

The height growth of sulphur-deficient seedlings was not markedly less than that of seedlings receiving the complete solution.

Foliage symptoms were confined to the younger leaves which were pale green with pale yellow-green areas extending in from the margins. Later,bronzing followed by necrosis occurred at the tips and along the margins, and the leaves became reflexed(Plate 5).

These symptoms differ from those described by Lacey (1964) who obtained stunted plants with leaves which in the advanced stages of deficiency were red at the base and yellow at the tip. Lacey grew his plants in a growth chamber, while those used in the experiment described here were grown in a glasshouse and were therefore exposed to an atmosphere containing some sulphur dioxide. The symptoms reported by Lacey therefore possibly represent a sudden onset of acute deficiency, whereas those above are descriptive of the slow development of a relatively mild deficiency, and are probably more akin to symptoms found in the field.

Magnesium

Plants in the pots irrigated with the solution of Hoagland and Arnon(1950) were stunted with short internodes. Leaves were reduced in size.

Foliage symptoms first appeared as necrotic spots and blotches of anthocyanin on the older leaves. Then the leaves near the middle of the crown became reflexed and showed marginal chlorosis. These symptoms then extended to the youngest leaves(Plate 6).

As deficiency became more severe, the oldest leaves wilted and dropped. The chlorotic areas in the leaves near the middle of the crown extended inwards towards the mid-rib and in some cases brownish purple areas appeared near the margins(Plate 7). Finally, necrosis accompanied by anthocyanin progressed inwards from the margins,the leaves died and were shed(Plate 8). These symptoms were repeated acropetally until only a tuft of leaves remained at the top the stem.

Seedlings which received the "Low Mg" solution (0.24 ppm Mg) of Walker et al.(1955) were not markedly stunted and leaves were normal in size. No necrosis or anthocyanin production was noted in the lower leaves but as before, chlorosis appeared on the leaves near the middle of the crown and extended upwards to the youngest leaves(Plate 9).

As deficiency became more acute, chlorosis was followed by the stages mentioned above and leaves were shed from the base of the crown upwards.

These foliage symptoms differ from those described by Will (1961a) who obtained seedlings on which the lower leaves were pale green. Will did not state the concentration of magnesium he used but this was probably the same as mentioned in a later paper(Will 1961b), namely 1.2ppm. If so then the symptoms he described possibly represent a milder form of deficiency than that obtained in the experiment described here.

Calcium

Seedlings receiving the minus calcium solution continued to grow as tall as the controls for some time, but as symptoms became increasingly evident, height growth became restricted.

In the early stages of deficiency the lower leaves were somewhat leathery in texture and in some cases showed necrotic spots. As symptoms became more severe, the younger leaves became reflexed, with margins turned under and sometimes wrinkled. These leaves were pale green with irregular yellow-green areas, mainly at the tips and along the margins. Necrosis of the yellow-green areas followed, gradually extending inwards from the tips and margins until the leaves died(Plate 10).

While the above symptoms were developing,die-back of the terminal shoots occurred and this was followed by the production of up to four shoots in the axils of the younger leaves. In some cases these shoots died back (Plate 11) but in others the shoots produced leaves but remained stunted, giving the plants a bunchy-topped appearance. Later elongated axillary shoots with small pale green leaves were produced in the lower part of the crown(Plate 12).

Potassium

Almost from the outset plants deprived of potassium were stunted with short internodes, and had the appearance of a rosette.

Necrotic spots and patches of anthocyanin were evident on the upper surface of the leaves some of which were wrinkled and twisted. The lower surface showed marked anthocyanin production. Die-back of the seedlings commenced at the growing point (Plate 13).

Because of the sudden onset of deficiency the above symptoms can only be regarded as applicable to newly germinated seedlings growing in soils containing extremely low concentrations of available potassium. The symptoms described by Will (1961a, 1961b) who supplied his seedlings with a low concentration of potassium are probably more representative of deficiency in the field. Will found that the leaves were smaller than normal, often with buckled surfaces and margins. No abnormal foliage colours were apparent, except in severe cases when the leaf margins became necrotic. There was more branching than normal.

Iron

The growth of iron deficient plants was restricted. After their first appearance, foliage symptoms became more acute as each new pair of leaves were formed. Symptoms in increasing order of severity were mild interveinal chlorosis, marked interveinal chlorosis, leaves almost white, and finally leaves almost white with anthocyanin production along the veins and mid-rib. The leaves formed before the onset of symptoms remained normal in colour. (Plate 14).

No reports describing iron deficiency in *E. pilularis* were found in the literature, but the above symptoms are similar to those reported for other eucalypts, and in fact are characteristic of those found in most plants (Hewitt, 1963).

KEY TO IDENTIFICATION OF MINERAL DEFICIENCIES IN *EUCALYPTUS PILULARIS* SEEDLINGS

From a consideration of the deficiency symptoms obtained in the experiment reported here, and those described by Will (1961a,1961b) and Lacey (1964), the following tentative key is suggested as a means of identifying mineral deficiences due to lack of the major essential elements and iron.

I Leaves Chlorotic

A Chlorosis uniform over entire leaf area.

1. First the older leaves and then the younger leaves become pale green to yellow green. Later, spots or blotches of anthocyanin appear on the older leaves which are shed prematurely ....Nitrogen
2. Older leaves become pale green and are shed prematurely. Symptoms gradually extend upwards until only a few leaves remain at the top of the stem ....Magnesium
3. Younger leaves become pale green.Later, yellow-green areas extend inwards from the margins which become bronzed and then necrotic .. Sulphur

B Chlorosis interveinal

1 Young foliage shows interveinal chlorosis. As deficiency becomes more acute newly formed leaves become progressively more chlorotic until the last formed leaves are almost white.Iron
2. The latest formed leaves show interveinal chlorosis. Later a reddish colour developes at the base of the leaves extending towards the tip. In the advanced stages the tips of the leaves become yellow and then necrotic..Sulphur

C Leaf margins chlorotic

1. First the leaves near the middle of the crown and later the younger leaves become reflexed and chlorotic along the margins.Chlorosis accompanied at times by the appearance of brownish purple patches then extends inwards

towards the mid -rib. Finally the chlorotic areas gradually become necrotic with anthocyanin appearing along the dividing line between the chlorotic and necrotic zones. Older leaves may show blotches of anthocyanin and necrotic spots. Leaves are shed from the base of the crown upwards ....Magnesium

2. Younger leaves become reflexed and wrinkled with margins turned under.Irregular yellow-green patches appear along the margins and extend inwards towards the mid-rib.Necrosis then extends inwards from the margins until the leaves die.While these symptoms are developing the terminal shoots on the main stem and branches die back and this is followed by the production of a number of shoots in the axil of each terminal leaf. These axillary shoots may die or may produce leaves but remains stunted. Older leaves may be thicker than normal and show irregular necrotic spots........... Calcium

II Leaves not chlorotic

1, Foliage becomes a dark green colour, the oldest leaves also showing dark purple zones or patches. Later,fading and necrosis extends from the tip towards the base of the leaves which are shed from the base of the crown upwards .... Phosphorus

2. Small seedlings become stunted with short internodes. Leaves become twisted or wrinkled and show blotches of anthocyanin and necrotic spots. The leaves of older seedlings are smaller than normal and are often buckled. In severe cases leaf margins become necrotic. More branching occurs than normal,giving the plants a bushy-topped appearance ....Potassium.

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