DIEBACK DISEASE – A CAUSE OF BORON DEFICIENCY IN FOREST TREES

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Abstract

The object of this study was to provide some basic information of the optimal growing conditions for *Acacia melanoxylon, Eucalyptus pauciflora, Eucalyptus viminalis* and *Pinus radiata* and to suggest ways and means of diagnosing the morphological growth abnormalities and deficiency symptoms caused by boron nutrient.

The importance of boron nutrition for *A. melanoxylon, E. pauciflora* and *E. viminalis* was confirmed. Absence of boron from mineral nutrition in Hoagland's nutrient solution in pot experiments resulted in "terminal shoot-dieback" in *A. melanoxylon*, and "apical bud-dieback" in *E. pauciflora* and *E. viminalis*. Injury due to insufficient boron in *P. radiata* developed slowly. Application of boron nutrient increased growth increment and foliar boron levels in these species.

1. Introduction

In view of the world wide occurrence of boron starvation in many economic forest plantations particularly in arid and tropical zones, and the increased stimulated interest of boron nutrition in tree growth, green-house pot experiments were conducted during the principal author's 15-month study course in the Department of Forestry, Australian National University, Canberra in the year 1976-77, under the supervision of Dr. B. N. Gardiner. *A. melanoxylon, E. pauciflora. E. viminalis* and *P. radiata* were grown and tested. This study outlines some of the observations made during this investigation.

2. Materials and Methods

The general method for studying the effect of a single nutrient element in plant growth usually involves the use of a nutrient solution in pot trials. To test the effect of boron nutrition on growth increment, visual morphological disorders and the Foliar mineral composition on *A. melanoxylon, E. pauciflora, E. viminalis* and *P. radiata*, Hoalgland's nutrient solution–2 was used and various levels of boron concentrations were maintained. These nutrient solutions contained the salts of all the macro as well as a small quantity of the micro-nutrient elements.

Humidity, temperature and ventilations of the green-house were controlled in an endeavour to reproduce conditions similar to those outside in the field where normal plantations are being carried-out. Supplementary illumination was also provided. Other nutrient solutions

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(basically modified Hoagland's nutrient solution–2) were held constant throughout the experiment except that of varying levels of boron nutrient. These solution were prepared using distilled water and purified chemicals (boron free) except as impurities in the reagent quality of the chemicals and were constantly aerated and re-newed. To avoid boron contaminations, distilled water for the nutrient solutions was made in plastic containers and low boron glasswares were used in all phases of the investigations.

2–i. Experiment No. 1.

Nine-month old, healthy and almost of uniform sized, seedlings of *E. viminalis* were transplanted in plastic pots (single plant per pot) on 28-5-1976. Mixed substrate of perlite and virmiculite were used as a media for plant growth. In order to leach out, if there were any traces of nutrient elements in the plant substrate, each pot was thoroughly washed with distilled water before the application of modified Hoagland's nutrient solutions. Initial seedlings height were recorded and to a base nutrient solutions (modified Hoagland's nutrient solution–2), boron concentrations were maintained in the order of :0,0.013, 0.026, 0.052 and 0.104 p.p.m. respectively throughout the investigation period.

Randomized complete block design was followed for the expt; and each treatment was replicated four-time. Sufficient amount of nutrient solutions were added daily according to the need of the plants for their sustenance. In order to avoid toxic accumulation of boron in the substrate, plants were washed thoroughly with distilled water once in a week. Plants were allowed to grow for a period of four-month and the experiment was wound-up on 28-9-1976. The temperature within the green-house under the studied period ranged from 29 to 30^{o}C and the relative humidity from 65 to 76% respectively. There was no coating on the glass-house to reduce the transmission of the radiant energy during the studied period. Foliar analysis was made using the standard method of Lambert (1976).

2–ii. Experiment No. 2.

In order to confirm these results further, seeds of *A. melanoxylon, E. pauciflora, E. viminalis* and P. radiata were germinated in pure sands in the growth cabinet. These when attained a reasonable height were transferred to the green-house, and transplanted single plant in each pot on 1-4-1977, suing pure sands as a substrate for plant growth. All the experimental procedure, such as the number of treatments-replications, determination of foliar boron levels; and the controlled environmental condition of the green-house were maintained in the same order as that of the experiment No. 1 and the experiment was wound up on 1-7-1977.

3. **Review of Literature**

Boron deficiency, a cause of dieback disease, has been reported in many countries in wide field plantations and these have been further confirmed by pot experiments conducted under the controlled environmental conditions. In tree species, boron deficiency has been reported in Acasia Wattle (Spurr 1952; Perkins and Aronoff 1956; Vail et al. 1957–61; Smith 1960; Elmer and Gosnell 1963; and Colling and Jones 1970); in *Acrocarpus fraxinifolius* (Savory

1962); in Apples (Russel 1957; Anon 1960; and Will et al. 1963); in *Betula verrucosa* (Ingested and Jacobson 1962); in Cherry (Bradford 1966; and Chapman 1966); in Citrus (Foroughi et al. 1974); in Coffee (Smith 1960; and Vail et al 1961); in Cox Orange Pippin (Hansen 1973–74); in Eucalyptus (Vail et al. 1957; Savory 1962; Cooling 1967-70; Steyn and Straker 1969; and Colling and Jones 1970); in *Gmelina arborea* (Savory 1962; Badanov 1964; and White 1964); in Grapes (Russel 1957; and Will et al. 1963); in *Grevillea robusta* (Vail et al. 1957; Smith 1960; Venkataramani 1963; Moore and Keraitis 1966; and Cooling and Jones 1970); in *Liquidambar styraciflua* (Hacskaylo 1960); in *Maesopsis eminii* and *Melia azedarach* (Savory 1962); in Peaches (Eaton 1944; and Childers 1954); in Pears (Russel 1957) and Persimon (Bradford 1966; and Chapman 1966); in Pines (Ludbrook 1940; and Smith 1943; Walker et al. 1955; Stone and Baird 1956; Vail et, al. 1957–61; Goslin 1959; Hacskaylo 1960; Savory 1962; Will et al. 1963; White 1964; Stone and Will 1965; Van Goor 1965; Appleton and Slow 1966; Lanuza de Marcos 1966; Proctor 1965–67; Raupach 1967; Tollenaar 1969; Cooling and Jones 1970; Gentle 1970; Snowdon 1971; Waring 1971; and Will 1971) in *Populus deltoides* (Hacskaylo and Vimmerstedt 1967); in Respberry (Will et at. 1963); and *Robinia pseudoacacia* (Haoskaylo 1960); in *Tecoma grandis* (Savory 1962); and in Walnut *Juglans regia* (Russel 1957; Vail et al. 1961; Bradford 1966; and Chapman 1966).

This study was undertaken in response to problems arising from the wide occurrence of boron Starvation in many economic tree plantations.

4. Results and Discussions

Fifteen-month study course at the Department of Forestry, Australian National University, Canberra during 1976-77 enabled the Principal author to search for a wide range of scientific literature for records of nutrient deficiencies causing symptoms resembling those of dieback disease. There are thirteen nutrient elements essential for the plant growth. Out of them some may be discounted as the cause of dieback disease, because their deficiencies are revealed by general stunting of growth, Chlorosis, or symptoms appearing first in the older leaves (Hambidge, ed. 1949). The nutrient elements whose deficiences are normally reflected by disorders of meristematic tissues are calcium (macro-nutrient), boron, copper, manganese and zinc (micro-nutrients). To test these assumptions, pot experiments were undertaken and the importance of boron nutrient for *A. melanoxylon, E. pauciflora, E. viminalis*, and *P. radiata* was studied under the controlled conditions of the green-house.

4–i. Morphological Growth Abnormalities in *A. melanoxylon*.

In *A. melanoxylon*, boron deficiency symptoms started just after one-month of the commencement of the application of nutrient solution. The visual morphological growth disorders became more and more pronounced both in character and in intensity when plants were deprived for a longer period from boron nutrition.

The apex of the main leading shoot first wilted and gradually lost its turgidity. Later on, it shrinked, bent, withered and finally the whole top of the plant died completely and dropped off. It is believed to be due to necrosis within the stem which reduced the normal movement of water to the appex (Savory 1962; Proctor 1965; Stone and Will 1965; van Goor 1965; and Snowdon 1971). This phenomena then extended downward on the lateral branches at the apex in a similar pattern. In similar study, likewise observations have been reported for Wattle (Spurr 1952; Perkins and Aronoff 1956; Vail et al. 1957–61; Smith 1960; Elmer and Gosnell 1963; and Cooling and Jones 1970). The main stem and those of the lateral branches became distorted with dark brown necrotic spots. Due to the repeated terminal shoot dieback, growth of the plant completely ceased both in the main leading shoot as well as in the lateral branches at the apex. The plants resulted in compressed, dwarfed and rosette type of vegetative growth and looked like a bush rather than normal plants. Likewise observations have been reported for other broad leaved trees (Eaton 1944; Childers 1954; Russel 1957; Anon 1960; Hacskaylo 1960; Smith 1960; Vail et al. 1961; Savory 1962; Ingestad and Jacobson 1963; Venkataramani 1963; Will et al. 1963; Badanov 1964; White 1964; Bradford 1966; Chapman 1966; Moore and Keraitis 1966; Hacskaylo and Vimmerstedt 1967; Cooling and Jones 1970; Forughi et al. 1974; and Hansen 1974).

Phylloids at the apex assumed light pale/yellow colouration at the tips which increased inward and downward. Later on this discolouration turned into orange red, purple and covered the whole surface area. It gradually became wilted at the tips and increased towards the base and ultimately lost its chlorophyll content and turgidity which shrinked, withered and shed at later stages. Resin exuded as a white colouring stuff from the auxil of the phylloids of the main leading shoot as well as from the lateral branches. No leaf formation occurred in boron deficient plants. Plants received more losses in terms of vigour and visual morphological abnormalities when starved for a longer period from boron nutrition. These results are in close accordance with the findings made by (Vail et al. 1957; Smith 1960; and Elmer and Gosnell 1963).

Same pattern of visual morphological growth setback also resulted in plants which received lower concentration of boron (0.013 and 0.026 p. p. m.B). However, losses were not so severe and acute, and the general growth was soon replaced by the development of newly healthy shoots from the lateral branches. Similar observation has been reported by Hansen (1973–74) for 'Cox Orange Pippin'. In contrast to this, plants which received higher concentration of boron (0.052 to 0.104 p.p.m.), were very vigorous, tall, healthy and developed a cluster of healthy multiside branches. The growth increment was 136.0 to 161.0 cm in 0.052 and 0.104 p.p.m. B as against 26.0 cm of the control plant which is highly significant Table–1). These results are in close conformity to Hansen (1973–74).

4–ii: **Morphological Growth Abnormaltites In *E. pauciflora* and *E. viminalis*:**

In *E. pauciflora* and *E. viminalis*, bud formed on the auxil of the leaf at the apex of the main leading shoot but it soon became brown, brittle, dry and then shed. Later on this disease spread to the lateral branches at the apex in a similar manner. Slowly and gradually the leaves at the apex became pale yellow in colour due to loss of chlorophyll contents. Discolouration started at the tips of the leaves and increased downward to the whole leaf area. Leaf lemana started bronzing and burning along the margins and tips and this extended inward to the midrib. Later on, swelling of the veins in small patches on both the main shoot as well as on the lateral branches started from the margins to the middle of lemana. Leaves became stiffed, reduced their size, developed chlorotic spots and curled inward and outward. Internodal spacing both on the main shoot as well as of the lateral branches was reduced. Main leading shoot and that of the lateral branches became weak, curled and hooked into J-shape and developed deformities of necrotic brown spots. Growth was completily ceased due to the repeated "apical bud-dieback" both on the main shoot as well as on the lateral branches. The whole plant developed into a rosette type of structure. Studying the effect of boron nutrition of various eucalyptus species, similar observations have been reported by some other investigators (Vail et al. 1957; Savory 1962; Cooling 1967; Steyn and Straker 1969; and Cooling and Jones 1970); The cause of the depressed growth, the distorted leaves and the death of the terminal bud is said to be the cause of an increase in starch and sugar contents of the leaves as a result of the disintegration of the phloem which prevents the translocation (Muckenhirn 1936).

The dieback diseased plants which were deprived from boron nutrition previously when supplied with higher concentration of boron (0.104 p. p. m), in nutrient solution (Expt. No.1), soon restored their normal vegetative growth (after 1–month of boron application) from the lateral branches with no apparent morphological growth abnormalities on the newly developed leaves and shoots. However, these plants did not continue to grow and did not recover entirely to catch-up the growth with those plants which received boron nutrient since the beginning of the investigation. With this serious growth check up they will obviously take a long time to recover and it is doubtful if they will catch-up with boron treated plants. These findings are in close accordance with (Steyn and Straker 1969).

The nature of attack was similar both in *E. pauciflora* and *E. viminalis* (both in Expt. 1 and 2). However, *E. viminalis* affected more severely than *E. pauciflora* in terms of general vigour, and visual morphological growth abnormalities. In contrast to this, plants which received regular supply of boron nutrition throughout the investigation period were vigorous and free from all sorts of morphological deformities. The growth rate of *E. pauciflora* was recorded as 103.0 cm in 0.104 p. p. m. B against 48.0 cm in control plants. Whereas, the growth increment in *E. viminalis* ranged from 145.0 and 154.0 cm against 37.0 and 54.0 cm of control plants (Expt. No. 1 and 2; Table-1). These figures are highly significant and are in close conformity with the findings of other investigators (Savory 1962; Cooling 1967; Steyn and Straker 1969; Cooling and Jones 1970; and Hansen 1973–74).

4–iii: Morphological Growth Abnormalities in *P. radiata*:

In *P. radiata*, there seemed to be no much differences between boron treated plants and those of the check plants Plants were uncharacterised by any visual morphological growth abnormalities and in general growth upto three-month of the investigation period. However, the most faint, peculiar and the pronounced character which was observed in this investigation was the development of leader type of side shoot just below the apex. Thsi is in conformity with the work done in likewise experiment (Gentle 1970; and Snowdon 1971). These results are also in accordance with Smith (1943), but at variance with Lanuza de Marcos (1966); and Snowdon (1971).

Studying the effect of micro-nutrients on growth of *P. radiata* seedlings, Smith found that boron deficient seedlings grown in Hoagland's nutrient culture were not appreciably inferior to those which received boron nutrition regularly upto the age of three monts. His investigation clearly shows that the advancement in general growth increment and visual morphological growth abnormalities started just after five-months of the application of boron nutrition.

Reporting on the incidence of dieback disease as the cause of boron deficiency, Lanuza de Marcos found that the application of low levels of boron in Hoagland's nutrient culture (0,0.0005 and 0.001 p. p. m. B) did not affect the growth increment of *P. radiata* seedlings. However, when boron was increased from 0.005 p. p. m, the growth height was significantly improved. Application of boron beyond this level did not change the growth, while more than 5 p. p. m boron in nutrient solution was reported as a toxic zone where severe toxicity symptoms were observed within twenty days of the application. Whereas, Snowdon observed boron deficiency symptoms in *P. radiata* grown in quarter strength modified Hoagland's solution with added boron within eighteen days. However, in this author's case the finance did not allow to continue this study for a longer period to confirm the findings of Ludbrook (1940); and Snowdon (1971).

5. **Effect of Boron Nutrition on Foliar Boron Level:**

Data presented in Table-2, indicate that, with increasing the boron supply in the nutrient solution, the boron concentration of the leaves/needles of *A. melanoxylon; E. pauciflora; E. viminalis;* and *P. radiata* increased against plants which received lower concentration of boron. It followed the order of : 5.2 to 12.1 p. p. m. B in *A. melanoxylon*; 5.4 to 14.4 and 5.3 to 20.9 p. p. m. B in *E. viminalis* (Expt. 1 and 2) respectively. In *E. pauciflora*, it was 9.1 to 19.7 p. p. m. B; whereas in *P. radiata*, it ranged from 12.0 to 17.5 p. p. m. B. These results are highly significant. Studying the effect of boron upon leaf development and growth of apple cultivar' Cox Orange Pippin' in nutrient solution, Hansen (1973–74), has likewise observed the foliar boron level with increasing boron concerntraion in the nutrient solution. In control plants, foliar analysis was not determined because of limited leaf samples.

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Table No. 1

EFFECT OF BORON NUTRITION IN HOAGLAND'S NUTRIENT SOLUTION OF GROWTH INCREMENT (CM) OF DIFFERENT FOREST TREE SPECIES

S.No.	Sp. studied	Expt. No.	Date of Transplanting	Date of Harvesting	Substrate used for plants growth	No. of plants per treatment	Expt. Treatments (p. p. m. B)				
							0	0.013	0.026	0.052	0.104
1.	*E. viminalis*	I	28-5-1976	28-9-1976	Mixed strate of perlite and vermiculite.	4	54	69	88	116	154
2.	*E. viminalis*	II	1-4-1977	1-7-1977	Pure sands	4	37	86	117	139	145
3.	*E. pauciflora*	"	"	"	"	4	48	69	87	98	103
4.	*A. melanoxylon*	"	"	"	"	4	26	65	97	136	161
5.	*P. radiata*	"	"	"	"	4	36	38	43	45	46

Table No. 2

EFFECT OF BORON NUTRITION IN HOAGLAND'S NUTRIENT SOLUTION ON FOLIAR BORON LEVELS (P.P.M.B) IN DIFFERENT FOREST TREE SPECIES

S.No.	Sp. studied	Expt. No.	Expt. period	No. of samples analysed	Part of samples analysed	Types of leaves needles analysed	Boron concentration in plant grown in Hoagland's Nutrient solution with indicated concentration of boron (p.p.m) Average values				
							0	0.013	0.026	0.052	0.104
1.	*E. viminalis*	I	4-month	4	Leaves	Healthy leaves	n.d*	5.4	11.3	11.6	14.4
2.	*E. viminalis*	II	3-month	4	"	"	n.d	5.3	9.5	17.0	20.9
3.	*E. pauciflora*	"	"	4	"	"	n.d	9.1	13.6	17.3	19.7
4.	*A. melanoxylon*	"	"	4	"	"	n.d	5.2	6.0	8.5	12.1
5.	*P. radiata*	"	"	4	Needles	Healthy needles	n.d	12.0	14.0	14.8	17.5

* = Not determined

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