SOIL BORON APPLICATION FOR THE ALLEVIATION OF BORON DEFICIENCY OF AVOCADO (*Persea americana* Mill.) IN THE KWAZULU-NATAL MIDLANDS

By

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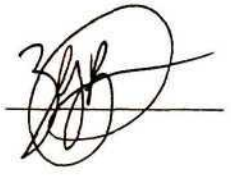
ABSTRACT

The avocado tree's requirement for additional boron in B deficient soils has traditionally been met solely by foliar sprays in South Africa. Since boron is regarded as poorly phloem translocated in most plants including avocado, foliar applications are unlikely to cater for the requirement of the entire tree. Foliar sprays are made prior to leaf analysis so that artificially high readings are likely. A survey of the boron status of four KwaZulu-Natal avocado orchards showed all soils to be in the deficient range, viz. <1 mg kg^{-1}. Leaf analysis records on these estates appeared inflated with more than occasional spurious results. Despite marginally adequate leaf boron concentrations, widespread deficiency symptoms were noted in all orchards. For foliar application, leaf analysis of spring flush leaves does not provide a true indication of orchard boron status. Soil applications of borax (11 % B) in the range 0 to 60 g m^{-2} (soil canopy area) $year^{-1}$ split into three applications, succeeded in increasing orchard B levels to above the recommended optimum of 40 mg kg^{-1} without any deleterious effects visible on the feeder roots or tree, except at the highest rates. Initial uptake of soil B was slow, particularly in older orchards and with standard rates as developed in Australia (typically between 5 and 20 g m^{-2} $year^{-1}$, split into at least 3 applications). Higher application rates (40 and 60 g m^{-2} $year^{-1}$) showed greater effectiveness at raising leaf boron concentrations, particularly in the second season. Toxicity occurred with 40 and 60 g m^{-2} $year^{-1}$ rates, 18 months after initial applications were made. High application rates indicated the tolerance of established avocado orchards to very high soil B concentrations. Soil applications increased fruit yield through increased fruit size in younger 'Hass' trees. Older, more deficient orchards did not show increased fruit size within the experimental timespan. Glasshouse trials supported findings in that soil B applications significantly increased leaf B concentrations ($P < 0.001$) proportional to soil application rate. Recently grafted young potted trees were extremely sensitive to soil boron applications which were not split, with toxicities occurring at low application rates. 'Edranol' seedling, a rootstock of Guatemalan origin was shown to be ca. 40 % more efficient in boron uptake than clonal 'Duke 7', the widely used rootstock in South Africa. Results indicate that boron deficiency is primarily the result of soil deficiency rather than poor rootstock uptake and translocation. On the Inanda soil type used and under the conditions of the experiments, it is suggested that application rates do not exceed 20 g borax m^{-2} $year^{-1}$ (split into

3 applications) in severely deficient trees (10-30 mg kg^{-1} B leaf analysis), and rates of ca. 10 g borax m^{-2} $year^{-1}$ would be adequate in marginally deficient trees.

DECLARATION

I hereby declare that the research work reported in this thesis is the result of my own investigations, except where acknowledged.

Signed 

(Zac Jon Bard)

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INTRODUCTION

The South African avocado industry has expanded rapidly and recently exceeded 12 000 hectares with plantings in the Northern Province, Mpumalanga and KwaZulu-Natal regions. The industry is largely export orientated and exported 8.8 million cartons (35 200 t) in 1994 having a value exceeding R 100 million. Drought, hail and other factors have in latter years reduced production below expected levels, however production is expected to more than double by 2000 if environmental factors are not limiting. The KwaZulu-Natal avocado industry accounts for ±10 % of the total plantings and dominates the 'late' export market and has a reputation of producing fruit of exceptionally high quality.

The industry is based largely on 'Fuerte' and 'Hass' cultivars, with smaller plantings of the dated 'Edranol' in addition to 'Ryan' and 'Pinkerton'. Popularity of 'Hass' has increased amongst growers because of its superior export prices and yield, the latter being higher than all cultivars other than 'Pinkerton'. 'Hass' has a tendency to bear a large number of small fruit (<200 g) particularly in the warmer production areas providing a more stressful environment.

The industry is largely based on the clonal 'Duke 7' rootstock after *Phytophthora cinnamomi* nearly destroyed the entire industry in the early 1980's. Although phosphonate injections have provided effective control against *Phytophthora*, most growers have resisted planting other cultivars which are regarded as more *Phytophthora* sensitive than clonal and seedling 'Duke 7' rootstocks.

The KwaZulu-Natal avocado growing areas have been established on the highly weathered, leached, well drained oxisols in the cooler mist belt zones. The environment is conducive to leaching of easily leached nutrients, including the uncharged boric acid molecule, and routine leaf analysis has shown the need for remedial measures. Researchers have for many years recommended time consuming and costly foliar applications to correct and alleviate B deficiency in avocado. Although they have often acknowledged that B is poorly phloem translocated in most plants, they have regarded soil applications as ineffective and extremely dangerous (Coetzer *et al.*, 1993, 1994). This widespread perception has arisen from occasional trials using soil applications showing poor uptake of soil applied B (Coetzer *et al.*, 1993; Robbertse & Coetzer,

1990; Robbertse *et al.*, 1992). It was reinforced by pot trials by Robbertse *et al.* (1992) in which young avocado plants died of "boron poisoning" without raising leaf B concentration into levels of toxicity or showing symptoms of toxicity in the leaf. It was common belief that foliar sprays were adequately controlling B deficiencies, as leaf analyses showed B levels close to or above the original lower level of 30 mg kg^{-1}. However, the pioneering work of Whiley and later Smith revealed the nature and common occurrence of a wide range of deficiency symptoms in Australia (Whiley *et al.*, 1996; Smith *et al.*, 1995, 1997). Subsequent studies showed that B was a severe problem in South African orchards with many of the deficiency symptoms having been unrecognised until very recently (Whiley *et al.*, 1996).

Foliar sprays are extremely time consuming and costly since until recently, due to fear of soil compaction, no mechanical implements have been used in avocado orchards in South Africa. Growers have relied on labour to spray trees with hand-held lances operated from tanks on tractors using strategically placed roads. Such spraying operations are slower than mechanised spray machines and farmers spend a great deal of time on spraying operations. Many growers have resorted to spraying B simultaneously with other sprays such as zinc, and preventative fungal copper sprays to control *Pseudocercospora purpurei*, a severe post harvest disease of avocado fruit. In this situation, B applications are generally not timed for correct phenological stages, viz. flowering or on emergence of the summer flush growth, in effect decreasing the efficiency of an already inefficient practise. In addition, farmers have been mixing sodium borate pentahydrate (Solubor®) with zinc oxide which is regarded as a non-compatible combination (Coetzee,1997[1]).

Furthermore, researchers have not identified possible external contamination of leaf samples by foliar B sprays. The recommended time of foliar applications is at 50 % flowering, at which time the developing spring flush (later used for leaf samples) is exposed to foliar applications. Whiley *et al.* (1996) raise the probability of much of the sprayed B being lodged in cuticular wax platelets.

Recent research in Australia has indicated that seedling Guatemalan rootstocks are more efficient at B uptake than are Mexican rootstocks (Whiley *et al.*, 1996). Since the South African avocado

[1] Coetzee J. Central Agricultural Laboratories, Phelindaba.

industry is based almost entirely on clonal 'Duke 7' (Mexican) rootstock, the industry has an additional obstacle to overcome in order to rectify the problem of widespread B deficiency.

Boron has implications for fruit postharvest quality in avocado (Smith *et al.*, 1997). Since the South African avocado industry relies largely on export markets more distant than its competitors such as Spain and Israel, postharvest quality and storage are essential for the long term survival of the industry. Although quality of fruit arriving overseas has dramatically improved through improved technology (Kotze, 1990), there is still considerable waste. More stringent minimum overseas standards resulting from increased worldwide production and the formation of the European Economic Union, demands almost perfect fruit for their markets. Although not all post harvest quality problems can be related to B deficiencies, it is possible that sound nutrition could have significant effects on internal postharvest quality.

Australian research has shown that soil applications of B fertilizers can be safely used to overcome the previously largely unrecognised B deficiency symptoms, and to raise leaf B levels to the now accepted optimum range of 40-70 mg kg^{-1} D.M. On the other hand, South African avocado growers, until the start of this study, relied exclusively on one or more foliar B sprays, early in spring and subsequently, to raise leaf B levels above 30 mg kg^{-1} and in more recent years 40 mg kg^{-1}. These foliar sprays were regarded as adequate, largely because the wide range of B deficiency symptoms were not recognised. Wolstenholme (1987) drew attention to the early Australian research, and posed the question "Are we catering for the avocado's boron needs?" A subsequent visit to Australia confirmed impressions of a widespread B deficiency in our orchards in spite of a long history of foliar sprays. The urgent need for local research on soil B applications became apparent.

The rationale for the research undertaken was summarised by Wolstenholme & Bard (1996) as follows :-

- The fact that B deficiency symptoms are still widespread in South African avocado orchards suggests that the "foliar sprays only" philosophy is inadequate to fully alleviate the problem.
- Foliar sprays probably only provide temporary relief from B deficiency, but can be helpful at the "critical period" of flowering and fruit set.

- There is uncertainty about how much of the sprayed B penetrates into leaves and flowers, and how much is intercepted by cuticular wax platelets. This may lead to "inflated" leaf analysis figures, in spite of normal leaf washing procedures (Whiley *et al.*, 1996).
- Boron is required by all dividing cells, with a primary structural role and secondary physiological roles. There is therefore a continuous need for B in living cells.
- Most movement of B is acropetal, following the transpiration stream. The extent of phloem movement in the avocado is unknown.
- Foliar B applications do not adequately cater for the needs *inter alia*, of the roots.
- Soil B applications can cater for the needs of the entire tree, and can be split to target critical phenological stages (Whiley *et al.*, 1996).
- The importance of healthy avocado feeder root growth has been highlighted by mulching studies (Moore-Gordon *et al.*, 1994, 1997). The beneficial responses may be explained, in part, by improved B nutrition.
- It is probable that B, like Ca, (Cutting & Bower, 1989) has a key role in fruit quality. Soil applications would have a better chance of ameliorating fruit physiological problems than foliar applications.

This study aimed at investigating a range of soil B application rates, including those which would induce toxicity in both orchard and pot trials over two growing seasons. The main objective was to narrow the range of application rates for typical KwaZulu-Natal avocado soils, to those which relieved deficiency symptoms, and improved tree performance and fruit quality. It was appreciated that a single two-season study would not provide all the answers. However, some 10 years research, mainly in S.E. Queensland under fairly similar environmental conditions, provided the main guidelines. An additional objective, therefore, was to evaluate whether Australian guidelines are applicable under local conditions.

1. LITERATURE REVIEW - THE ROLE BORON IN PLANTS WITH REFERENCE TO DEFICIENCY AND TOXICITY IN THE AVOCADO

Boron (B) has been recognised as an essential plant micronutrient for over 80 years (Agulhon, 1910), however its importance and value in crop production has only been realised relatively recently. B deficiency is recognised as one of the most common micronutrient problems in agriculture (Mortvedt & Woodruff, 1995) however range between sufficient and toxic concentrations is narrow (Gupta *et al.* 1985). Specifically, the highly weathered, leached and acidic oxisols in which avocados are grown in South Africa and the humid subtropics of Australia show inherently low levels of B. Availability is further decreased by chemical adsorption, while high pH, high soil calcium concentration and dry soil conditions can induce B deficiency (Gupta, 1979; Gupta & MacLeod, 1981). Soils are seldom able to meet the plant's B requirement and deficiencies become magnified as orchards age and soil B reserves are depleted.

B deficiency symptoms have been described for many crops grown in controlled environments. In South Africa, many of the field symptoms in avocado have only recently been recognized and are often of a chronic magnitude. Conventionally used foliar sprays are believed to be poorly absorbed and translocated, and leaf nutrient analyses may give artificially high readings due to absorption by cuticular wax. This leads to masking of the deficiency which could affect tree health, yield and fruit size (Whiley *et al.*, 1996), with symptoms becoming acute as the tree ages. Effects on fruit quality are suspected but remain to be determined. The problem has been compounded by the lack of recognition, until recently, of most of the wide range of deficiency symptoms in avocado trees.

Low soil B levels in avocado orchards are corrected using soil applications in New Zealand and Australia. In Chile, where B deficiency is associated with extremely weak branch strength, soil B applications are used to combat chronic B deficiencies on calcarious soils (Toerien, 1997[2]). B deficiency has recently been suspected in Israeli orchards (Whiley, 1995[3]). The South African avocado industry has relied solely on foliar B application, recommended at 50 % flowering. The

[2] Toerien, J.C. AvoData, Tzaneen.

[3] Whiley, A.W. Department of Primary Industries, Queensland.

major drawback of soil application is that applications have to be precise since there is a narrow range between B application causing deficiency and that resulting in toxicity (Keren & Bingham, 1985). Toxicity may cause severe tree damage and even death. Recent field trials in Australia have shown orchard avocado trees to be far more tolerant of soil applications than previously believed (Whiley, 1995[4]). Soil applications have been preferentially used in Australia for over 15 years, having longer lasting effects. Soil applications have increased fruit size of 'Hass' avocado by 15 % (Smith *et al.*, 1995). It is believed that foliar applications, only increase leaf B levels since mobility and redistribution of foliar applied B appears limited (Whiley *et al.*, 1996). Consequently, effects are confined to sprayed leaves, since B is not relocated to actively growing plant parts where it is most needed. It is believed that the small fruit problem of 'Hass' trees in South Africa may be partly attributed to a B deficiency. Foliar applications are thought to mask more obvious symptoms characteristically seen in leaves. Foliar sprays increase B levels in April leaf analysis, however the fraction of physiologically active B is far less than indicated because of contamination. Leaf analysis is therefore unlikely to give a true indication of orchard B status.

Little is known about plant B uptake from the soil. Studies in many plant species have shown uptake and translocation to be genetically determined. Research in Australia has shown Mexican rootstocks such as 'Duke 7' less efficient at B uptake than other selections (Smith *et al.*, 1995). Hence South Africa should be more prone to deficiency, having almost its entire industry based on clonal 'Duke 7' rootstock.

The literature review which follows is a summary of the main factors which appear to impact on the problem of boron deficiency in South African avocado orchards. An exhaustive review has not been attempted.

[4] Whiley, A.W. Department of Primary Industries. Queensland.

1.1 BORON IN SOILS

1.1.1 General properties

Boron has an atomic mass of 10.811 and a melting point of 2300 °C. Two stable isotopes are present in nature, ^{10}B (18.98 %) and ^{11}B(81.02 %). At room temperature, B is inert except to strong oxidising agents such as HNO_3. When fused with oxidising alkaline mixtures, it forms borates. The only important oxide is boric oxide (B_2O_3), which is acidic, soluble in water, and forms boric acid $B(OH)_3$, a very weak acid. In nature, B is fairly rare and occurs primarily as the borates of Ca and Na. B has a constant valency of III and never behaves as a cation (Adriano, 1986).

1.1.2 Natural occurrence

Readily available sodium and calcium borates are found naturally in most soils (Adriano, 1986). These are derived from dissolution of tourmaline, $[H_2MgNa_9Al_3(BO)_2Si_4O_{20}]$ a mineral found in soils derived from acid rocks and metamorphosed sediments. Since the dissolution of tourmaline occurs very slowly, it is not regarded as a good source of plant available B (Graham, 1957).

In nature, B is found as a constituent of borax ($Na_2B_4O_7 \cdot 10H_2O$), kernite ($Na_2B_4O_7 \cdot 4H_2O$), colemanite ($Ca_2B_6O_{11} \cdot 5H_2O$), ulexite ($NaCaB_5O_9 \cdot 8H_2O$), tourmaline ($H_2MgNa_9Al_3(BO)_2Si_4O_{20}$) and axinite ($Ca_2Al_2BSi_4O_{15}(OH)$). Borax has been used as both a herbicide and a fertiliser for hundreds of years (Winsor, 1952).

1.1.3 Types of soil boron and their extraction

Total soil B in normal soils ranges from 2 to 100 mg kg^{-1} (Swaine, 1955; Bergmann, 1992), with an average of about 30 mg kg^{-1}. Total B content depends largely on the parent material from which the soil was formed. High B can be expected in soils formed from marine shales (Bingham *et al.*, 1970) while low levels can be expected from soils formed from acid igneous rocks and fresh water sedimentary deposits. Soil texture often affects total soil B: since B is easily leached (Gupta *et al.*, 1985), sandy soils tend to have lower levels particularly when soil organic matter content

is low (Adriano, 1986).

Total soil B is an unreliable index of plant availability since usually only about 5 % of total B is available to plants (Berger & Truog, 1939; Adriano, 1986). Hence for diagnostic purposes, extractable B determines effectively available B present in soils. Extractable B can be determined by three different methods. The hot water extraction method remains the most accurate (Wear, 1965) since hot water soluble boron is regarded as the best index of plant available B. Extraction with $CaCl_2$ (Cartwright *et al.*, 1983) and mannitol, although considered more convenient, has been shown to be biased since it extracts less B from soils with low B content, and more B in soils with high B content.

Boron content can also be determined using soil saturation extract. This method gives lower values than hot water extraction, however variability of results and narrow range of sensitivity make interpretation problematic (Adriano, 1986).

1.1.4 Boron in the soil profile

In neutral to slightly acid soil conditions, the concentration of B in the soil profile is largely dependent on presence of organic matter and soil texture. Occurrence of B is often associated with organic matter. Since organic matter is most prevalent in the surface layer horizons, total B concentration is usually higher in the surface layers than in underlying layers (Adriano, 1986).

Soil texture has a strong effect on total B concentration. Total B can be expected to be on average twice as high in clayey samples as in sandy samples. Texture may in many cases be specific to each horizon as in the case of a duplex soil (Adriano, 1986).

Natural leaching can mobilise available B down the soil profile. This process can be especially rapid on deep well drained sandy soils (Winsor, 1952). In saline-alkaline soils, trends are somewhat contrasting. Since South African avocado production is overwhelmingly limited to acid soils, behaviour of B in alkaline soils will not be discussed.

Boron solubility decreases dramatically as soil pH increases. Leaching of B does not occur as readily as chloride, sulphate and nitrate salts and B may require two to three times as much water to be removed from the profile (Bingham *et al.*, 1972). It is nevertheless regarded as an easily leached nutrient.

1.1.5 Forms and speciation

Four main types of B occur in soils:

(i) Water soluble

(ii) Adsorbed

(iii) Organically bound

(iv) Fixed (in clays and mineral lattices)

Water soluble B is regarded as a plant response indicator. When determined using hot water extraction, it is designated as the "available" B. Adsorbed B represents the fraction that is precipitated or adsorbed onto surfaces of soil particles, maintaining equilibrium with soluble B. The fraction of total B in water soluble form depends largely on environmental conditions. Normally, 10 % of total B would be expected in this form (Vinogradov, 1959), however in regions of high salinity, this could rise as high as 80 %. High levels of adsorbed B are only found in soils that have abnormally high levels of B, usually found in arid and semi-arid areas. Alluvial soils have as much as 30 % of the total B in the adsorbed form. This form of B is readily leached from soil (Bingham *et al.*, 1970).

Boron is also found in the organic fraction of the soil. In general, soils high in organic matter are high in B (Fleming, 1980). Little is known about the complexing and availability of B in organic matter. B is known to exhibit an affinity for alpha-hydroxy aliphatic acids and ortho-dihydroxy derivatives of aromatic compounds. B is also known to react with sugars, notably the type produced by microbial degradation of soil polysaccharides (Adriano, 1986).

Boron may be entrapped in the clay lattices of borosilicate minerals, where it can substitute for Al^{3+} and Si^{4+} ions. Tourmaline and axinite are examples of this (Krauskopf, 1972).

1.1.6 Adsorption and fixation

There are several mechanisms of chemical combination of B with soils (Eaton & Wilcox, 1939; Hingston, 1964; Sims & Bingham, 1967, 1968):

(I) Anion exchange.

(ii) Precipitation of insoluble borates with sesquioxides.

(iii) Sorption of borate ions or molecular boric acid.

(iv) Formation of organic complexes.

(v) Fixation of B in the clay lattice.

The major inorganic adsorption sites are:

(i) Fe and Al-hydroxy compounds present as coatings on, or associated with clay minerals.

(ii) Fe or Al-oxides in the soils

(iii) Clay minerals, especially of the micaceous type

(iv) Mg-hydroxy coatings on the surface of ferromagnesian minerals (Ellis & Knezek, 1972).

1.1.7 Soil factors affecting plant available boron

As discussed in section 1.2, natural levels of B in the soil depends largely an parent material and the levels of B contained in the rock. Numerous additional factors influence availability of B.

1.1.7.1 Clay minerals and soil texture

The ability of clay minerals to adsorb B is a function of pH. In ranges commonly found in soils, the following pattern is expected amongst clays:

Illite > montmorillonite > kaolinite (Fleet, 1965).

Hence, the properties of these clays present in the soil influence the amount of sorption occurring. Maximum adsorption occurs in the alkaline range (Hingston, 1964).

Sandy soils are less able to hold B than are clayey soils (Kubota *et al.*, 1948) so that deficiencies

are more likely in sandy soils. In addition, sandy soils are generally prone to have a low organic matter content, decreasing total soil B content. Toxicities are less likely to occur in clayey soils since clay minerals reduce leaching by adsorbing B (Gupta, 1968).

1.1.7.2 pH

Soil pH can influence the mobility and phytoavailability of B. In general, B becomes less available to plants as pH increases. Liming therefore reduces the amount of plant available B and consequently reduces B concentrations in the leaves (Adriano, 1986). Liming may also increase B deficiency or reduce B toxicity. Sensitivity to pH changes in the soil varies greatly in plants. While some species show increased or decreased B in parallel with pH value, others only show marked differences after a critical pH is reached (Peterson & Newman, 1976; Gupta & Macleod, 1977).

Boron adsorption by soil and soil constituents is largely pH dependant, with adsorption maxima in the alkaline range. Plant mechanism of B uptake is also affected by pH (Adriano, 1986).

1.1.7.3 Oxides of iron and aluminium

Boron adsorption was found to be far greater for Fe- and Al-coated kaolinite or montmorillonite than for uncoated clays (Sims & Bingham, 1968). It follows that hydroxy Fe and Al compounds present in layer silicates or as impurities dominate over clay minerals *per se* in determining adsorption characteristics. Furthermore, B adsorption by certain soils is primarily due to their free Al oxide contents (Bingham *et al.*, 1971).

1.1.7.4 Organic matter

Boron sorption by organic matter is not fully understood. Its importance in soil B relations is however well recognised. Evidence shows that it positively affects soil B in many different and often unrelated ways. Initially, humus was found to exhibit chemical affinity for B, hence playing an important role in its chemical retention in soils (Parks & White, 1952; Gu & Lowe, 1990). The mechanism of this retention, although inadequately studied, is believed to be complex formation

between B and diols present in humus (Boeseken, 1949).

Organic matter is one of the main sources of B in acid soils (Gupta, 1979). Positive relationships have been found between soil organic matter content and available B (Gupta, 1968). Martens (1968) emphasised that organic matter is believed to contain significant reservoirs of B which become available after microbial degradation. In addition, sorption sites act as a pool from which B is supplied to the solution or where B is sorbed. Organic matter presence hence has a significant effect on B distribution between the solid and liquid phases in soils (Yermiyahu *et al.*, 1995). Boron sorption by organic matter plays an important role in buffering fluctuations in the B available in soil solution.

Sorption is also affected by soil pH (Yermiyahu *et al.*, 1995). Increasing pH (up to pH = 8.9) causes increased B sorption. This occurs because the soil B fraction which exists mainly as $B(OH)_3$ at lower pH, shows a greater tendency to form charged $B(OH)_4^-$. Since organic matter has a greater affinity for $B(OH)_4^-$, sorption increases.

1.1.7.5 Interactions with other elements

Since B deficiency is more prevalent in limed soil, it was originally suggested that antagonism existed between Ca (and possibly Mg) and B. Several attempts were made to use the Ca/B ratio in plants as a diagnostic criterion in B fertilization, with little success (Drake *et al.*, 1941; Gupta, 1972). More recently, Gupta & MacLeod (1981) concluded that the effect of lime in reducing B uptake was due to soil pH rather than the Ca^{2+} level in the soil. Controversy still surrounds this argument.

1.1.7.6 Plant factors

As with other micronutrients, B uptake and translocation appears to vary between avocado horticultural races. Whiley *et al.* (1996) have shown in Australia that Guatemalan rootstocks e.g. 'Velvick' are more efficient at B uptake than the (Mexican) 'Duke 7' rootstock, which is widely used in South Africa. Similarly, soil toxicity levels are likely to differ between races. Limits are at present still largely unknown, although research has shown the avocado to be far more tolerant

to B than was originally thought. In comparison to *Citrus* spp., the avocado can tolerate 10 times the boron level in soil application inducing toxicity (Whiley, 1995[5]).

1.1.7.7 Other factors

Environmental factors have been shown to influence boron nutrition of plants. Incidence of boron deficiency increases under drought conditions (Scott *et al.*, 1975). There is much circumstantial evidence in avocado orchards that deficiency symptoms are more evident after drought than in seasons adequate rainfall and/or irrigation. In the avocado, B nutrition is also affected by feeder root health. Thus greater uptake occurs during and immediately after root flushes, and decreases in parallel with feeder root deterioration since uptake becomes limited by root condition. Leaf B levels are particularly low in Australia during flowering which is accompanied by attrition of feeder roots, in addition to the high B demands during flowering, pollination and fruit set (Whiley & Schaffer, 1994).

Drought conditions can also alleviate toxicity symptoms in plants (Gupta *et al.*, 1976). Such conditions reduce uptake of B in plants, because mass flow in the rhizosphere is restricted.

1.1.8 Critical soil boron concentrations

Although no data exist specifically for avocado, indications are that safe and adequate soil B concentrations for a wide range of crops are in the range 0.3 - 0.8 mg kg^{-1} using hot water extraction method. Deficiencies generally appear below 0.3 mg kg^{-1}, and toxicity depends largely on the clay content of the soil. Bergmann (1990) developed current norms (TABLE 1) which are cited in Marschner's (1995) book on plant mineral nutrition.

Since most South African avocado growing soils have reasonably high (>30 %) clay content, soils would be expected to contain at least 0.35 mg kg^{-1} to successfully sustain avocado production. Further work is required to determine safe and toxic levels of soil B for avocado production.

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[5]Whiley, A.W. Department of Primary Industries, Queensland.

1.2 PLANT UPTAKE, DISTRIBUTION AND MOBILITY

Nett uptake of B in vascular plants is influenced by the rate of transpiration, however B distribution within the plant is not as simplistic (Raven, 1980). Mobility and redistribution of B is of major importance when evaluating foliar applications. The extent of B movement in phloem tissue, is of particular interest and still needs thorough explanation. While distribution has been shown to occur in some species, quantitative evidence is still lacking.

TABLE 1: Hot water soluble boron values used for interpretation of soil analysis (Bergmann, 1990).

Soil Type	Clay content (%)	Low (mg/kg)	Medium (mg/kg)	High (mg/kg)
Sand	7	<0.15	0.25-0.15	>0.25
Sandy loam	8-15	<0.20	0.30-0.20	>0.30
Heavy loamy sand	16-25	<0.25	0.40-0.20	>0.40
Loam, clay loam, clay	>26	<0.35	0.60-0.35	>0.60

1.2.1 Uptake and distribution

Research over many years has failed to conclusively ascertain whether B uptake in plants is passive or active. Tanaka (1967a) working with sunflower, suggested (based on a 1:1 stoichiometry between B uptake and H^+ release) that some of the B accumulated in excised roots was passively absorbed by formation of ß-polysaccharide complexes in free spaces. Tanaka (1967b) found absorption capacity in monocotyledons to be less than in dicotyledons. Later, research provided evidence for a non-metabolic uptake process based on observations of an equilibrium of internal and external B concentrations. Observations were unaffected by temperature of metabolic inhibitors. Furthermore, B uptake was noted to fall dramatically as external pH increased, this being closely related to shifting away from the undissociated $B(OH)_3$ form. Further indications are that the cell membranes are readily permeable to $B(OH)_3$ and much

less to $B(OH)_4^-$ (Oertli & Grgurevic, 1975).

Other studies have shown that about 90 % of total B recovered was reversibly accumulated, however the remaining 10 % was sensitive to temperature and metabolic inhibitors (Bowen & Nissen, 1976, 1977). This seems to indicate active transport, although no accumulation against a concentration gradient was found. In contrast, there is agreement amongst numerous authors (Bowen, 1968, 1969; Wildes & Neales, 1971; Nissen, 1974; Shu, 1988) that metabolism was in some way involved, rather than specifically active transport. Hence, there is more evidence to support passive rather than active uptake. Thellier *et al.* (1979) showed that total B accumulation in the cytoplasm reached levels much higher than were present in the external medium, which is not likely to occur by passive transport. This might be explained in terms of the behaviour of B *in vivo*. Formation of *cis*-diol complexes with B effectively depresses readily available B concentration in the cell and allows further uptake via passive uptake.

1.2.2 Distribution and mobility

Numerous reports show genetic variability within a crop species with respect to requirements (Brown & Jones, 1971; Shelp, 1993). B uptake appears to be controlled at root level. In some crops such as celery (*Apium graveolens* L.) (Pope & Munger, 1953) and tomato (*Lycopersicon esculentum* Mill.) (Wall & Andrus, 1962) uptake has been shown to be genetically controlled. Brown & Jones (1971) identified two strains of tomato differing in their susceptibility to B deficiency. Further investigation showed differences in B uptake efficiency between them. When B proved limiting for the "inefficient" strain, shoot tissue analysis showed substantially lower leaf B levels in the mentioned strain. However, root B concentrations were similar for both strains. This suggests that differences ascribed to rootstocks may be due to translocation, rather than differing in ability to absorb soil B. A similar situation has been found in avocados: Research has shown that Guatemalan rootstocks are far more B efficient than the widely used Mexican 'Duke 7' rootstock (Whiley *et al.*, 1996). It still remains to be shown whether this is controlled by root uptake or rootstock translocation to the scion. It is also possible that root vigour, as expressed by rooting volume or total feeder root length, may be definitive in explaining observed differences.

Once B is in the xylem, its distribution is related to loss of water from shoot organs (Bowen,

1972). B concentration is dependent on leaf age in species where phloem mobility is limited, relating to the amount of transpiration occurring. Steep gradients may be found within a single leaf (petioles & midribs < middle of lamina < margins and tips) (Oertli & Roth, 1969), but are normally only pronounced when B supply is excessive.

Boron has generally been regarded a as relatively immobile nutrient after being deposited in transpiring organs. Differences in phloem B concentration and source leaves are not as large as originally thought (Tammes & van Die, 1966). Furthermore, similarity between B concentrations in source leaves and phloem seem to indicate that B supply for younger tissues could originate from phloem tissue.

1.2.2.1 Phloem mobility

The relative phloem immobility of B (Oertli & Richardson, 1970) is based on the fact that phloem B concentrations are notably low. However, when re-evaluating evidence presented in the last two decades, it is possible that there are reasons for apparently low phloem B concentrations, and in some species B is more mobile than originally believed.

Phloem B levels are comparatively low because of high permeability of cell membranes to B which allows for rapid movement out of the phloem tissue. Moreover, counter-current extraction of B from phloem to xylem fluid (Oertli & Richardson, 1970) redirects B to the apical meristems where it is required for growth and development processes. Alternatively, low phloem B can be attributed to formation of stable boric acid esters typically found in cell wall material in leaf tissue. This reduces B concentrations that are available for phloem transport. In spite of this, it has been shown for many plants that boric acid esters only constitute a minor portion of the total B, hence their role in reducing phloem B concentrations is questionable. Rather, this illustrates the behaviour of B in plants; McIlrath (1965) showed that the soluble B fraction shows a dramatic decline when deprived of external B. Hence it seems that plants preferentially satisfy their mobile B fraction because of the high reactivity of B with *cis*-diols. This happens through the movement of B towards the apices and hence occurs at the expense of younger forthcoming growth. It is therefore necessary to satisfy the plant's immobile (or structural) B before it is possible to get B

to shoot meristems where it is most needed (Robbertse, 1995[6]). Plant B should ideally be measured in terms of soluble B since this is B fraction available for growth in addition to an indication of the B toxicity limits in the plant. It seems that foliar B sprays used in the South African Avocado industry only provide B enough to satisfy the structural B requirements, and raise B levels in annual leaf analyses. Hu & Brown (1994) note that when B is low to adequate, the majority of cellular B (>95 %) is associated with cell wall pectins necessary for cell wall expansion.

Shelp (1993) in his review claimed that results of unpublished data suggested that B is sufficiently mobile in phloem tissue thus able to meet the demands of developing organs in the presence of adequate B supply. Shelp (1987) concluded that B supply in broccoli was primarily dependant on phloem supply. When comparing phloem supply with a number of other nutrients, B had the second highest phloem supply dependence after zinc.

New ideas on the controversy surrounding B phloem mobility come from the work of Brown & Hu (1996, 1997) and Hu *et al.* (1997). The former authors noted that there is no considerable evidence that in most higher plant species, B is almost completely phloem immobile, e.g. squash and tomato, and such spp. B leaf concentrations decrease from old to young leaves, and B toxicity symptoms occur first at the margins of old leaves. However, they note that many Rosaceous spp. e.g. *Malus*, *Prunus*, and *Pyrus* do not accumulate high leaf B levels, and B toxicity symptoms do not usually occur in leaves but rather in young stems and fruit. The apparent phloem mobility in these species has been demonstrated using foliar applied ^{10}B by Hanson (1991) and Picchioni *et al.* (1995). All these species have sorbitol as a major translocation carbohydrate. Brown & Hu (1996) obtained evidence that in sorbitol-rich spp, B is freely mobile, while in sorbitol-poor spp it is largely immobile. They proposed that B mobility in apple, pear and almond is mediated by the formation of B-sorbitol complexes. Hu *et al.* (1997) have since shown that in celery, which accumulates mannitol and where B is also phloem mobile, phloem B is present as the mannitol-B-mannitol complex, while in peach B occurred as a mixture of sorbitol-B-sorbitol, fructose-B-fructose or sorbitol-B-fructose. They claim that these are the first successful isolations and characterisations of soluble B complexes from higher plants. They also provide a mechanistic

[6] Robbertse, P.J. Margaretha Mes Institute for Seed Research, University of Pretoria.

explanation for phloem mobility in sorbitol, mannitol and dulcitol accumulating spp.

These findings are relevant to avocado, where the 7 carbon sugar alcohol is known to be involved, along with sucrose, in translocation, but where leaf toxicity symptoms are typical of phloem immobile species.

1.2.2.2 Remobilisation

In attempting to elucidate the mobility of B, many researchers have performed experiments where the plant is firstly grown in conditions of B sufficiency and thereafter starved of B. In such experiments, B redistribution cannot be compared with B mobility because there is increasing evidence suggesting that B partitioning changes dramatically after B supply has been removed (Gupta, 1993). Nevertheless, such experiments are useful in determining what the plant is capable of even if though it may be under stressed conditions.

Decreasing B gradients between mature and young leaves have been found in grape (*Vitis vinifera* L.) (Scott & Schrader, 1947), broccoli (Shelp, 1988), as well as cotton and turnip (McIlrath, 1965). Oertli (1993) on the contrary, found little remobilisation in tomato (*Lycopersicon esculentum* Mill.), however what redistribution did occur was transported almost exclusively to the roots rather than emerging leaves.

In conclusion, it appears B uptake is largely genetically controlled. Roots can be efficient or inefficient at B translocation. B appears to be reasonably mobile within phloem tissue, however plants must have efficient roots wrt B uptake and supply of B must be adequate and continuous. In addition, it is imperative that the plant's structural B requirement is satisfied because of preferential adsorption. In perennial tree crops this is likely to require continuous and adequate B supply from an early tree age.

In avocado, remobilisation from mature leaves to developing inflorescences has been reported by Whiley & Schaffer (1994) who noted a significant decline in leaf B in mature leaves adjacent to developing inflorescences. Similarly, Coetzer *et al.* (1988) using ^{10}B showed remobilisation from mature leaves to developing flushes. While the former authors noted remobilisation when leaf B

concentration exceeded 40 mg kg^{-1}, the latter authors cited relocation only to occur above concentrations of 90 mg kg^{-1}. Whiley *et al.* (1996) is of the opinion that the latters results were clouded by contamination of foliar sprays and indicate the capacity of the avocado leaf to absorb B into the waxy cuticle so that it cannot be removed by normal sample preparation.

1.3 PHYSIOLOGICAL ROLE OF BORON IN PLANTS

The physiological role of boron in plant nutrition is still the least understood of all the mineral nutrients (Marschner, 1995). Research aimed at determining the role of B has focused on physiological events when B is withheld or resupplied after deficiency. Consequently, secondary effects resulting from of lack of or sufficiency are well documented rather then specific role of B. This poor knowledge may seem surprising since on a molar basis, the requirement for B, at least for dicotyledonous plants is higher than that of any other micronutrient. Difficulties involved in B research have impeded researcher's efforts to determine a definite physiological role. In short,over 60 years of research has failed to elucidate its specific role. More recently, evidence has been presented indicating that B has an association with pectin in cell walls (Hu & Brown, 1994; Hu *et al.*, 1996, 1997; Matoh *et al.*, 1996) as well as playing a definite role in cell membrane integrity (Marschner, 1995; Cakmak *et al.*, 1995). Brown & Hu (1997) suggest that the primary function of B is as a structural component of growing tissues. A recent summation or interpretation of the physiological role of B in higher plants is given in Fig. 1. More recently, Brown & Hu (1997) suggested that there is growing evidence that B plays only a structural role in growing tissues of higher plants.

1.3.1 Evolution of a boron requirement

Boron is an essential element for the normal growth of monocotyledons, dicotyledons, conifers, ferns and several diatom species, however is not essential for fungi and most algae (Gupta, 1993). Some members of the Graminae such as wheat and oats have a lower requirement for B than do other monocotyledons and dicotyledons. No definite proof exists to show that B is a nutritional requirement for bacteria or animals (Nielsen, 1988).

Lovatt (1985) proposed that plant B requirement evolved in parallel with the development of vascular tissue. A continually improved vascular system aided passive transport of B to the transpiration terminus, ie. the shoot apical meristem of a primitive plant. Thus, shoot apical meristems were first plant tissues exposed to significant levels of B. Subsequent B accumulation affected plant species differently. Adaptive strategies of some plants enabled them to incorporate B into normal metabolic events occurring in meristems, thereby preventing toxic levels of B

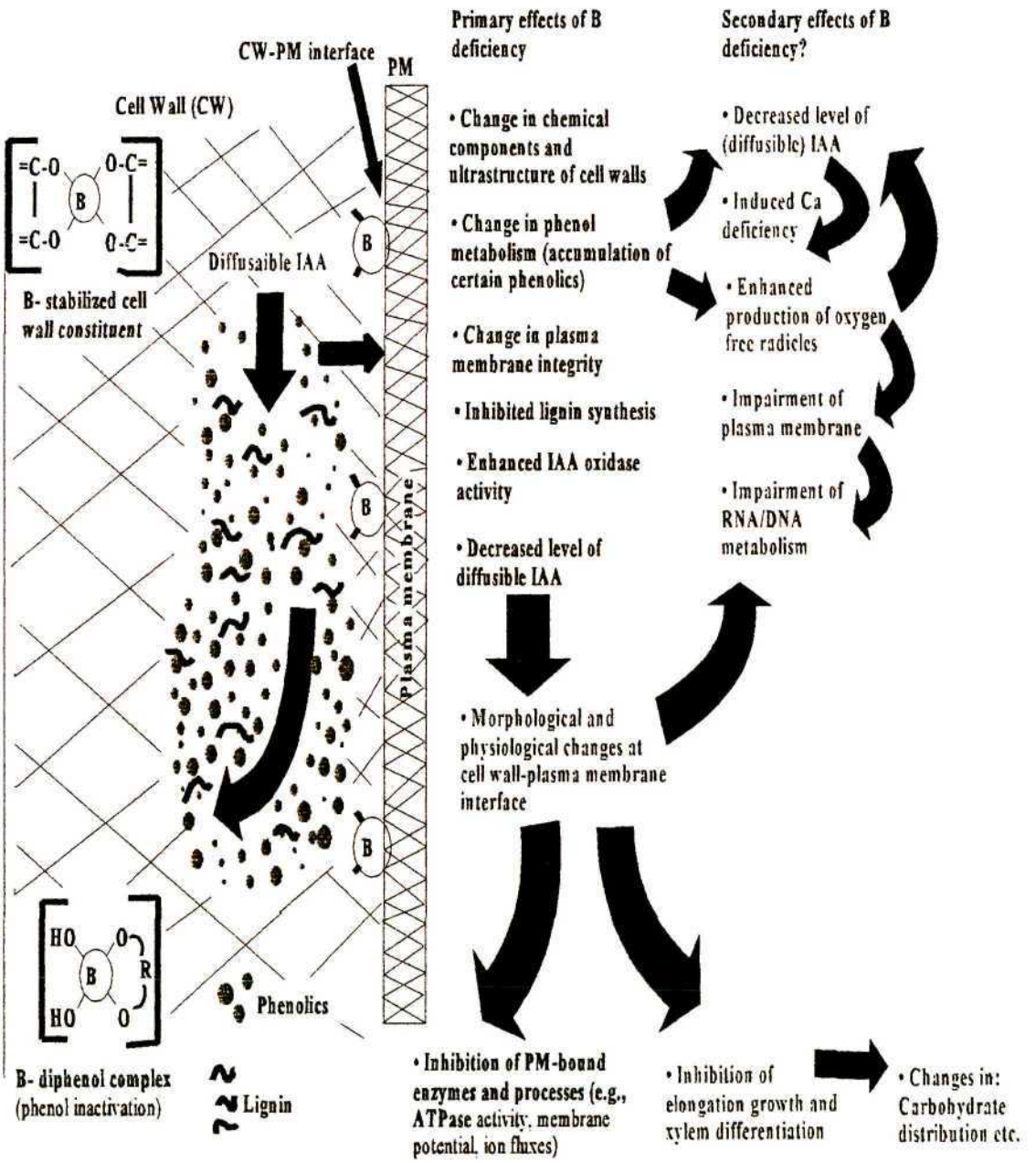

Figure 1. Physiological role of B in plants (Marschner, 1995).

accumulating. Less adapted plants, unable to perform this, perished.

1.3.2 Role of boron

Boron is neither an enzyme constituent, nor is there convincing evidence that it directly affects enzyme activities (Marschner, 1995). It is nevertheless incorporated into various organic entities, the significance of these still unknown. The list of possible roles is endless. Only pertinent and probable roles will be discussed here.

1.3.2.1 *Cis*-diol complexes

Boric acid has an outstanding capacity to complex with diols and polyols, particularly with *cis*-diols. Polyhydroxy compounds with an adjacent *cis*-diol configuration are required for formation of such complexes; compounds include a number of sugars and their derivatives (e.g. sugar alcohols and uronic acids), in particular mannitol, mannan, and polymannuronic acid. These compounds serve as constituents of the hemicellulose fraction of cell walls. In contrast, glucose, fructose and galactose and their derivatives (e.g., sucrose) do not have this *cis*-diol configuration and thus do not form stable borate complexes (Gupta, 1993). Some *o*-diphenolics, such as caffeic acid and hydroxyferulic acid, which are important precursors of lignin biosynthesis in dicotyledons (McClure, 1976), possess a *cis*-diol configuration and hence form stable borate complexes. The significance of these complexes is still poorly understood, however they must surely have some role in sugar transport, particularly across membranes. Most stable borate complexes are formed with *cis*-diols on a furanoid ring, namely the pentoses ribose and apiose, the latter being a universal component of the cell walls of vascular plants (Loomis & Durst, 1992). The high B requirement of gum-producing plants is most likely related to the function of B in forming cross links with the various polyhydroxy polymers such as galactomannan. In addition stable complexes are formed with ribose sugar (the principle component of RNA), and also NAD^+ (Loomis & Durst, 1991).

In higher plants, a substantial fraction of plant total B content is bound in complexes in *cis*-diol configuration in cell walls (Thellier *et al.*, 1979). The higher B requirement of dicotyledonous plants in particular, is believed to be related to higher proportions of compounds with *cis*-diol

configuration in cell walls, such as pectic substances and polygalacturonans (Loomis & Durst, 1992). This view was formulated by Tanaka (1967b) after showing vast differences in levels of highly complexed B between monocotyledonous and dicotyledonous species. Differences are believed to roughly reflect the boron requirement for optimal growth. As previously noted, >95 % of cellular B is associated with cell wall pectins where it may be critical for cell wall expansion (Hu & Brown, 1994).

1.3.2.2 Sugar transport

Sisler *et al.* (1956) postulated that B was involved in sugar transport across cell membranes. Aiding transport, B either complexed with sugar to form a sugar-boron complex or alternatively B was associated with the membranes. Reduction in sugar transport has been recognised in other plants where high carbohydrate levels are seen in leaves, however this is more likely to be as a result of vascular tissue breakdown (a secondary effect) rather than direct effect of B deficiency. The possible role of B in sugar transport has become less convincing since sucrose, the main transport sugar in most plants, complexes poorly with B (Shelp, 1993), hence role of B in transport across membranes in this manner seems unlikely. However, a role in sorbitol, mannitol, or dulcitol rich plants in the Rosaceae, Oleaceae, Rubiaceae, Umbelliferae and Celastraceae (Hu *et al.*, 1997) has much more support.

1.3.2.3 Cell elongation & cell division

Indications are that B is involved both in cell division and cell elongation (Bohnsack & Albert, 1977). Cell elongation relates to B in terms of auxin metabolism, whereas cell elongation seems to relate to the effect of B on overall tissue development. Both topics will be dealt with in further sections. The well known B deficiency symptom of death of shoot apical meristems is strongly supportive of a role in cell formation.

1.3.2.4 Respiration

B deficiency initially tends to increase respiration, however a decline is noted as deficiency symptoms become evident (Shelp, 1993). Evidence indicates a shift in substrate flux from

glycolysis to the pentose phosphate pathway (Dugger, 1983; Eichorn & Augsten, 1974). More profound effects seem evident in carbohydrate metabolism, and Shelp (1993) recommends further research in this field.

1.3.2.5 Cell wall biosynthesis

B deficiency is thought to inhibit activity of UDPG-pyrophosphorylase which is thought to result in a reduction in availability of UDPG for cellulose biosynthesis (Shelp, 1993).

1.3.2.6 Protein, amino acid and nitrate metabolism

It is unlikely that B is directly involved in nitrate metabolism. Although numerous papers have shown B to affect nitrate metabolism, they have failed to take into account organ age (Shelp, 1993).

1.3.2.7 Tissue differentiation, auxin and phenol metabolism

Interrelationship between B, differentiation, auxin and phenol metabolism is greatly disputed (Shelp, 1993). Jarvis *et al.* (1984) showed that while auxin was responsible for regeneration of roots on *Phaseolus vulgaris* cuttings, B was necessary for primordia to develop. Further research investigating RNA levels indicated that the relationship between B, root regeneration and auxin metabolism was unclear (Ali & Jarvis, 1988). Goldbach *et al.* (1990) proposed that B had an indirect effect on auxin via changes in membrane configuration thereby affecting auxin movement.

Phenol accumulation resulting in browning reactions has been observed in plants growing in conditions of long term B deficiency (Shkolnik, 1984). Where B is not limiting, it forms complexes with many phenolic compounds thereby reducing phenolic concentration (Cakmak *et al.*, 1995). Lewis (1980) proposed that B had a role in lignification, since phenolic buildup (in the absence of B) would affect lignin biosynthesis resulting in cell damage. To complicate matters further, phenol content is highly dependant on light intensity (Marschner, 1995) and phenolic build up is an effective inhibitor of IAA oxidase activity (Birnbaum *et al.*, 1977). In short, all pathways

are interlinked and make interpretation of direct effects almost impossible. However, the possible of B in several fruit browning disorders of avocado is worthy of research.

1.3.2.8 Membrane function

Cell walls are dramatically altered and show up at macroscopic (e.g. 'cracked stem''stem corkiness' etc; Bergmann, 1988, 1992; Shelp, 1988) and microscopic levels (Loomis & Durst, 1991,1992). Both cell wall thickness and the proportion of cell wall material in proportion to total dry matter are higher in B deficient tissues (Rajaratnam & Lowry, 1974; Hirsch & Torrey, 1980). Cell wall thickness in celery increases from 1 μm in sufficient plants to 4 μm in deficient plants (Spurr, 1957). Chemical composition of cell walls also changes markedly, and callose formation accumulates in sieve tubes of B deficient plants (Venter & Currier, 1977) which may impair phloem transport.

Boron is thought to have a structural role in cell walls. Substitution of germanium for B has been achieved in sunflower (McIlrath & Skok, 1966) and tomato (Brown & Jones, 1972). Development of visual deficiency symptoms could be delayed for a number of days since germanium is thought to bring about increased mobility of B in plants by preferential substitution in the cell wall. Substitution in the cell wall is thought to be non specific and indicates a structural role for B rather than a catalytic or regulatory role. Marschner (1995) proposes a definite cell wall structural role (Fig. 1).

1.3.2.9 Root elongation

Inhibition or cessation of root elongation is one of the most rapid responses to B deficiency. Bohnsack & Albert (1977) showed that root elongation inhibition in squash occurred as soon as 3 h after removal of B supply. Elongation ceased almost entirely after 24 h. This gives the roots a stubby and bushy appearance since B deficiency is associated with a change in the direction of cell division from normal longitudinal to radial and an increase in the maturation and differentiation of cells close to the root tip (Shelp, 1993). An approximately 3 h lag period between cessation of root elongation and IAA oxidase activity indicates change in its activity as a secondary event of B deficiency. Spiral thickenings in xylem elements occurred close to the root

tip, lateral roots appear near the apex, and the cell wall of the inner root cortex was thicker. Enhanced cell division in the radial direction with a proliferation of cambial cells and impaired xylem differentiation are also typical in the sub-apical tissue of B-deficient plants. It has been demonstrated that mechanical disruption of shoot meristem can cause similar morphological changes in B-sufficient plants, suggesting that impaired xylem differentiation may only be indirectly related to B nutrition (Krosing, 1978).

1.4 IMPORTANCE OF BORON IN AVOCADO

Boron deficiency was first documented in the avocado in 1943 (Haas), however further research was not initiated for another 3 decades. The full range of deficiency symptoms was first described by Whiley *et al.* (1996) in Australia. Miyasaka, *et al.*,(1992) noted identical symptoms in the avocado in Hawaii. Boron deficiency is widespread in South African avocado orchards (Wolstenholme & Bard 1996). The deficiency still remains largely uncontrolled despite routine boron foliar sprays over a period of many years. In 'Hass' it is believed that fruit size is significantly affected in addition to the misshapen fruit which are characteristic of the deficiency. The B deficiency problem is far worse in South African than Australian orchards, as deficiency symptoms were largely unrecognised in the former, and Australian growers have been routinely used soil rather than foliar applications.

Boron research in the avocado is not for the faint hearted. Borax applications have been previously used as "non-selective herbicides" on railway tracks (Winsor, 1952). Despite warnings of potential dangers of B toxicity, orchard avocados have been shown to be surprisingly tolerant of high levels of soil applied B. This can possibly be attributed to absence of root hairs in the avocado in addition to its high B requirement. A recent investigation showed no toxicity effects where the soil B level exceeded toxicity limits of less tolerant species by a factor of 10 (Whiley, 1995[7]). Toxicity symptoms are severe and can range from mild leaf necrosis to tree death, and there have been several cases of over-dosage in Australian orchards.

Although foliar leaf analyses are still considered to be the most useful tool in identifying B

[7] Whiley, A.W. Department of primary Industries, Queensland.

deficiency (Whiley *et al.*, 1996), continued use of foliar sprays in South Africa reduce the reliability of leaf analysis. Since symptoms are widespread and often close to chronic in magnitude, it is clear that foliar sprays have been insufficient to alleviate the problem in local orchards.

1.4.1 Effect of rootstock on boron uptake

Boron uptake and translocation appears to be controlled by the rootstock in the first instance. In other plant species, uptake of B can be low in spite of adequate soil B levels. This argument is supported by higher B levels in roots than in leaves. In such plants, transportation or translocation of B from roots or rootstock upwards is the limiting factor.

Scion cultivars show differing tolerance to deficiency. 'Sharwil', an Australian cultivar, has been shown to be most susceptible to deficiency, while 'Fuerte', 'Reed' and 'Hass' have reasonable tolerance (Whiley, *et al.*, 1996). In South Africa, 'Ryan' appears to be extremely sensitive to B deficiency (Bard, personal observations). Guatemalan rootstocks ('Edranol', 'Nabal', & 'Velvick') whether seedling or clonal are regarded as more efficient at B uptake than the widely used Mexican (clonal 'Duke 7', seedling 'Topa Topa', and 'Mexicola') rootstocks (Whiley, 1995[8]). Breeders will hopefully take this into consideration when evaluating prospective rootstock selections.

Soils in which avocados are grown in South Africa are typically low in B. Since avocados are grown in similar climates (cool subtropical, high rainfall) in New Zealand and Australia, the South African situation is somewhat analogous to these. Australia have the advantage that their industry is largely based on Guatemalan seedling rootstocks and hence have a range of rootstocks which are generally more efficient at B uptake and translocation. South Africa's preferred rootstock (clonal 'Duke 7') is less efficient when compared to Guatemalan selections (Smith *et al.*, 1995, 1997).

In Israel, soils in contrast are slightly to moderately alkaline and saline conditions would be

[8] Whiley, A.W. Department of Primary Industries, Queensland.

expected to supply adequate B, especially with the high B levels in poor quality irrigation water available. Leaf B concentrations are seldom, if ever measured. Rootstock development has enabled the Israelis to grow avocados in spite of soil adversities. Whiley *et al.* (1996) noticed widespread B deficiency at Kibbutz Giv'at-Haim Ihud. This possibly results from the alkaline soil conditions which renders B less soluble. In addition, the role of rootstocks tolerant to salinity possibly plays a role in B deficiency. Similarly, Californian growers and researchers visiting South Africa in 1996 suspected B deficiency in Californian orchards in sandy conditions, although in most orchards B toxicity is more likely.

The role played by the rootstocks in B uptake is still requires further research particularly in South Africa. Rootstocks which can tolerate saline conditions have been termed 'tolerant rootstocks.' This description however is not scientific. Rather, these should be termed 'poor translocators' since it is this characteristic that enables them to withstand these conditions (Ben-Ya'acov, 1995[9]). Thus salinity tolerance is the function of genetic translocation potential. Similarly, South African research should be aimed at identifying rootstocks that have a high B translocation potential to withstand conditions of B deficit. The available evidence suggests that Guatemalan rather than Mexican rootstocks should be investigated.

Extensive research involving B by Robbertse & Coetzer has been carried out over a number of years using trees grown in controlled environments. In concluding their studies, these authors suggested that roots were susceptible to B toxicity and recommended continued use of foliar sprays. It is possible that they used excessive rates for the restricted root systems of potted plants. Coetzer *et al.* (1993) suggested that mycorrhizae were susceptible to B toxicity, citing this as a detraction from soil applications. In contrast, Dixon *et al.*(1989) showed supplementary B application to increase colonisation of mycorrhizae in *Citrus jambhiri*, a genus more sensitive to B toxicity.

1.4.2 Flower and pollen development

Boron deficiency adversely affects the growing points of higher plants. This symptom has been

[9]Ben-Ya'acov, A. Volcani Institute, Israel.

noted in a wide range of crops. Often, B deficiency affects reproductive structures more than vegetative structures (Mozafar, 1995). Blamey (1975) reported large scale disruption of reproductive structures of sunflower (*Helianthus annuus* L.). There appears to be a greater requirement for B during flowering, probably because of the amount of cell division and elongation taking place in addition to high B requirement of pollen.

Hanqing (1995) found that B applications corrected the large scale disruption of flower development, particularly affecting the reproductive structures of oil seed rape when grown on low B soils. Deficiency results in the 'flowers but no seeds' syndrome, widely found in China.

B also has an effect of increasing pollen producing capacity of anthers, and pollen grain viability (Agarwala *et al.*, 1981). The latter effect is important when considering that pollen viability of avocado pollen is poor.

1.4.3 Pollen germination and pollen tube growth

The requirement of B for pollen germination is the best known role of B in plants. Research has shown pollen germination to be dependant on both internal B concentration, which would be supplied by parent plant, and external B concentration which is supplied either by female stigma or in nutrient solution (when germinating *in vitro*) or even a foliar spray. Poor germination resulting from insufficient B supply to parent plant may be compensated for by external application (Montgomery, 1951). Similarly in avocado, the B content of external solution is most important (Coetzer & Robbertse, 1988) and can compensate for deficiency of pollen donor. However, B in the pollen parent is important for producing sufficient numbers of pollen grains.

Robbertse *et al.* (1990) working in *Petunia* concluded that B has a chemotropic role in pollen tube growth, since pollen tubes were attracted towards higher B concentrations in the pistil.

1.4.4 Pollination and fruit set

Pollination of the avocado flower is regarded as a major yield limiting factor in Israel (Gazit,

1995[10]). In the southern hemisphere where avocados are mainly grown in cooler, wetter regions, pollination is regarded as the first major bottleneck which may affect fruit yield if severely limiting (Wolstenholme, 1995[11]). In these areas, poor yield in isolated cases may be partly attributed to lack of pollination, however, it is generally not believed to impose any major limitations on yield.

Flowers of B treated plants have a greater chance of having their flowers pollinated with a good source of pollen. This is because while self pollination (from pollen from the same flower) occurs to a very limited extent, chances are that adjacent flowers from the same tree have the greatest likelihood of being visited by honey bees (Ish-Am, 1995[12]). Furthermore, B plays a role in pollen tube growth and since the flower can only be fertilised within certain time constraints, B effectively increases the window in which flowers may be pollinated (Jagarnath & Lovatt, 1996) since pollen tube growth in the presence of B is more rapid. Thus the benefits of a higher plant B level are numerous, particularly at the critical period of flowering and fruit set. Foliar B sprays are consequently targeted at this period.

The effect of B on nectar composition has been widely described (Hasler & Maurizio, 1949; Smith & Johnson, 1969; Eriksson, 1979). Avocado flowers are unattractive to honey bees, the quality of the nectar produced being one of the many reasons (Ish-Amm, 1995[12]). Avocado nectar is unfavourable because it is composed of nearly 100 % sucrose, while bees have a preference for hexose sugars such as fructose and glucose. It is possible that B could make avocado flowers more attractive to honey bees by changing nectar composition and thereby increase pollination.

The South African avocado industry promotes foliar B sprays at 50 % flowering aiming at increasing pollination and subsequent fruit set. However, benefits of this are sometimes questionable in the light of the events prior to and during pollination. Avocado pollen is relatively small in size in comparison to other species, and furthermore it has a thin exine which makes it

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[10]Gazit, S. Faculty of Agriculture, Hebrew University of Jerusalem.

[11]Wolstenholme, B.N. Department of Horticultural Science, University of Natal.

[12]Ish-Am, G. Department of Botany. Tel Aviv University, Israel.

susceptible to desiccation. Extensive research and testing has shown that relative humidity is extremely important in maintaining avocado pollen viability (Loupassaki & Vasilakakis, 1995), and partly explains why desiccating berg winds are disastrous for fruit set. Avocado pollen is highly specific with respect to conditions which stimulate germination. In addition, it is regarded as showing problematic germination since it is one of few known species that cannot be germinated *in vitro* on solid agar without a nutrient solution (Sahar & Spiegel-Roy, 1984).

Once the anthers dehisce, pollen lifespan becomes determinate and may require a vector such as the honey bee for effective pollination. However, honey bees are not attracted to the avocado flower because of the nature of the sugar content produced by the nectaries. Pollen size is too small for bees to manage effectively (Ish-Am, 1995[13]). Once the pollen has reached the receptive female flower, it sticks to liquid exuded by the female flower. This liquid also serves as to suspend fragile pollen grains in aqueous solution, preventing mechanical damage and desiccation. In hot dry conditions (berg winds), this liquid is likely to be evaporated, or may be withdrawn by the plant (Mans, 1995[14]), in effect decreasing the chances of effective pollination.

Properly timed B sprays at flowering increases pollen germination and pollen tube growth. Benefits are only transient. Lovatt (1995[15]) suggests a pre-bloom spray when all inflorescences are in the 'cauliflower' stage rather than at 50 % flowering as used in South Africa. B applied in the pre-bloom stage is easily remobilised and moved to the growing inflorescence timeously. Similar results are reported by Coetzer *et al.*(1993) using radio isotopes. Whiley & Schaffer (1994) noted a significant decline of B concentrations of mature leaves adjacent to developing inflorescences.

1.4.5 Fruit and fruit growth

Harkness (1959) showed avocados with B deficiency produced 30 to 100 % fruit containing seeds

[13]Ish-Am, G. Department of Botany. Tel Aviv University, Israel.

[14]Mans, C. Haffenden Groves. Schagen, South Africa.

[15]Lovatt, C.J. Department of Botany, Plant Sciences. University of California, Riverside.

with brown necrotic areas. In addition, it was suggested that lack of B might have a role in alternate bearing in avocado trees, however no ensuing work has been documented.

Smith *et al.*, (1995) showed adequate B increased fruit size by up to 30 % over B deficient trees. Bohnsack & Albert (1977) suggest that B is involved in both cell division and cell elongation in squash root tips. Since the avocado fruit continues cell division throughout development to fruit maturity (Blumenfeld & Gazit, 1974), it is possible that the effect would be beneficial throughout the growth of the fruit. Since Schroeder (1953) indicated that an increase in cell size is responsible for early fruit growth and cell division responsible for fruit size increase in the latter stages of development, a constant B supply would be necessary throughout the development of the fruit.

1.4.6 Soil vs. foliar boron application

Research in South Africa over a number of years has focused on comparing foliar and soil application both in controlled environments and field studies (Robbertse *et al.*, 1989; Robbertse & Coetzer, 1990; Robbertse *et al.*, 1992; Coetzer *et al.*, 1993; Coetzer *et al.*, 1994). Results have been confined to effects of B on pollination and fruit set, in addition to uptake of B by the leaf when comparing foliar against soil applications. Trials have looked at short term effects using mostly single, low dosage soil B applications and uptake into leaves in following season. Views are that it takes a number of years before uptake of soil B can be detected in leaves particularly in older trees. Applications have been at relatively low dosages compared with Australian experimental application rates, although their pot trials used heavy rates leading to very high leaf B concentrations.

Trials have failed to recognise foliar uptake is limited and B is poorly phloem mobile, preventing significant remobilisation to other parts of the plant. Recent evidence suggests remobilisation is dependant on plant sorbitol concentration, which is dependant on plant species. In low sorbitol species, B remains largely immobile (Brown & Hu, 1996). Sorbitol content of avocado remains to be established, although the sugar alcohol persitol is known to be involved, along with sucrose, in carbohydrate translocation.

Australian researchers have acknowledged the benefit of properly timed foliar sprays, but have found such applications ineffective at correcting deficiencies of chronic magnitude (Whiley *et al.*, 1996).

Whiley *et al.* (1994) showed that B included in trunk injections increased leaf B concentration however failed to reach the accepted norm of 50 mg kg^{-1}.

1.4.7 Boron deficiency and toxicity symptoms in avocado

Smith *et al.* (1995) and Whiley *et al.* (1996) described B deficiency symptoms in avocado as:

- marginal necrosis of younger leaves
- crimped (corrugated) and bumpy regions between veins of younger leaves
- shotholes in younger leaves
- loss of apical dominance, often resulting in multiple shoot production
- prostrate or downwards growth of branches
- swelling of stem nodal regions (Chronic symptom)
- splitting of the midrib on the under side of younger leaves
- uneven lamina development of younger leaves - cell expansion stopped on one side of leaf followed by localised necrosis.

Misshapen fruit are also indicators of acute and chronic B deficiency, although only a small percentage of fruit are affected.

While collectively or singly, the above symptoms have serious repercussions on tree growth, health and architecture, it still remains to be proven whether these affect tree yield.

Toxicity symptoms occur on older leaves in mild cases (Smith *et al.*, 1995) however more severe toxicity doses can result in defoliation. Toxicity becomes evident as necrosis on tips of older leaves, gradually spreading across the leaf margin. Development of chlorotic areas between healthy and necrotic leaf tissue follows. The boundary between healthy and necrotic leaf tissue is sharp and diffuse between chlorotic and healthy tissue.

In addition to the above symptoms, the formation of stem cankers are associated with extremely low leaf B concentrations in Australia (Whiley *et al.*, 1988; Broadley *et al.*, 1991).

1.4.8 Effect of mulching on boron availability

Moore-Gordon *et al.* (1994) and Moore-Gordon *et al.* (1997) have shown mulching of 'Hass' with pinebark effective in increasing fruit size by up to 10 %. While it is likely that increase of fruit size by mulching results from many interacting factors, Whiley (1996) postulated that it was likely that one of the effects of pinebark mulch is increasing B concentration and availability of soil B. Mulching reduces soil water loss via evaporation, hence increased soil moisture leads to increased soil B availability (since B is maintained in the available aqueous form) and improved root scavenging ability. Results of Moore-Gordon *et al.* (1994) and Moore-Gordon *et al.* (1997) showed that, grass mulch, (a mulch having a low B content) did not increase fruit size. The difference between the 2 mulches occurs since acid leached soils have inherently low soil B concentrations, hence composted pinebark acts as a B source in addition to its effect of increasing availability. Wander & Gourley (1943) showed increased soil B concentration immediately below an old mulch in an apple orchard. Increased soil B levels are reflected in increased B levels in leaves. Mulching would therefore be particularly advantageous in avocados where surface feeder roots, responsible for most nutrient uptake, grow in this region where B levels are raised considerably. Increased root health as a result of mulching also effectively increases B availability since improved feeder root health and growth increases uptake.

1.4.9 Interaction with other nutrients

Jaime *et al.* (1992) noted an interaction between potassium (K) and B in sand cultured greenhouse trials with avocados. High levels of K tended to decrease B uptake and *vice versa.* Both effects were not statistically significant.

1.5 BORON FERTILIZERS AND BORON MEASUREMENT IN PLANTS

1.5.1 Boron fertilizers

Borax ($Na_2B_4O_7 \cdot 10H_2O$) has been used commercially for over 2000 years (Mortvedt & Woodruff, 1995). Standard borax contains 11.3 % B. Borax products containing fewer waters of hydration have become more popular since they contain a high percentage B. Anhydrous borax (21.5 % B) is sold as fertilizer but is not commonly used.

Solubor ($Na_2B_4O_7 \cdot 5H_2O$) (Foliarel) is a more refined form of borax. It contains 20.5 % B and has a much higher solubility than borax, particularly in cold water, and is thus suitable for foliar sprays. Its fine particle size makes it less suited for soil applications since it is easily wind blown and difficult to apply. Furthermore, its high solubility makes it more prone to leaching.

Boric acid is completely water soluble, but is seldom used because of the premium price per unit B. Leaching due to high solubility also detracts from its use. It has potential use for fertigation.

Colemanite ($Ca_2B_6O_{11} \cdot 5H_2O$) and ulexite ($NaCaB_5O_9 \cdot 8H_2O$) are less soluble B fertilizers and are used mainly for crops growing on sandy soils in high rainfall areas where leaching of B would be problematic. Ulexite, despite having potential because of low solubility (making it advantageous on sandy soils), price and physical properties, is not recommended since it contains 5 % Cl.

Fritted glass products (slow release source of B) are seldom used on their own since they contain variable amounts of B, but are used in manufacturing "boronated" fertilisers. In spite if this they have potential for correcting severe B deficiencies (Mortvedt & Woodruff, 1995) particularly on sandy soils where leaching is most rapid.

Principle B fertilizers are listed in TABLE 2.

In South Africa, and in KwaZulu-Natal in particular, choice of B fertiliser is largely determined by availability and cost.

More recently, B fertilizers having superior solubility and plant availability to Solubor been developed, and could increase efficiency of foliar sprays (Tredgold, 1997[16]) although they are unlikely to provide sufficient quantities of B utilized by the plant. It should however be emphasised that although use of such fertilizers might increase uptake of foliar applied B, redistribution of B through the phloem is unlikely to be increased.

1.5.2 Boron detection in plants

Two colorimetric methods are widely reported in the literature. The Carmine method (Hatcher & Wilcox, 1950) requires use of concentrated sulphuric acid. The Azomethane-H method (Shahina *et al.*, 1967) does not require sulphuric acid and is therefore preferred by many researchers. Both methods allow determination of B in concentrations from 0.5 to 10 mg kg^{-1} (Bingham, 1982). More recently, the curcumin colour method has been introduced (SAA, 1980). Reagents develop a red colour in the presence of oxalic acid, and absorbance is measured spectrophotometrically at 540 nm.

Borosilicate glass is best avoided in the above mentioned methods. Since borosilicate glass is common apparatus in laboratories, it can be used after acid soaking in 10 % HCl for at least 5 days (Bester, 1995[17]). Kaplan *et al.* (1990) as well as John *et al.* (1975) have reported successful use of acid washed borosilicate glass. Bester (1993) recommends sample duplication to detect erroneous results.

Alternatively, analysis can be done using Inductively Coupled Plasma (ICP-AES) Atomic Emission Spectroscopy at 249.77 nm. This method enables accurate B determination, but is extremely expensive.

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[16]Tredgold, L. Fertex Plant Nutrition, Nelspruit.

[17]Bester, H.C. Department of Soil Science, University of Natal.

TABLE 2. Principle boron fertilisers (after Tisdale *et al.*, 1995).

Source	Formula	% Boron
Borax	$Na_2B_4O_7 \cdot 10H_2O$	11
Boric acid	H_3BO_4	17
Colemanite (Portabor)	$Ca_2B_6O_{11} \cdot 5H_2O$	18
Sodium pentaborate	$Na_2B_{10}O_{16} \cdot 10H_2O$	18
Sodium tetraborate		
Fertiliser borate-48		
(Agribor, Tronobor)	$Na_2B_4O_7 \cdot 5H_2O$	15
Fertiliser borate-68	$Na_2B_4O_7 \cdot 5H_2O$	
	$+ Na_2B_{10}O_{16} \cdot 10H_2O$	21
Ulexite	$NaCaB_5O_9 \cdot 8H_2O$	10

2. A SURVEY OF THE BORON STATUS OF FOUR KWAZULU-NATAL ORCHARDS

2.1 INTRODUCTION

Boron deficiency has until recently remained a largely unrecognised problem in South African avocado orchards and is believed at present, to be a major limiting factor in avocado production. Leaf boron concentrations were seldom measured in the past. In recent years, when B has been measured, contamination from foliar sprays has probably lead to somewhat inflated values due to the lodging of some of the boron in cuticular wax platelets.

Soil B analysis has never been performed and is to date not measured commercially by any analytical laboratory in Natal.

The aim of this chapter is to provide a benchmark of the status of B in South African avocado orchards, when foliar B sprays have been used. Quantification of leaf B concentrations measured in the past can than be compared with visual symptoms of B deficiency and the degree to which leaf analyses have been inflated can be tentatively accessed. Soil analysis results indicate degree of possible deficiency in the soil, or alternatively indicate the gross feeding nature of the avocado with respect to B.

2.2 MATERIALS AND METHODS

2.2.1 Leaf analysis trends

2.2.1.1 Data collection

Farm records containing data containing results of annual leaf analysis samples and yields were obtained from 4 avocado growing estates in KwaZulu-Natal. Results were obtained from Cooling and St. Paul Estates at Bruyns Hill (both clonal rootstocks), Everdon Estate in Howick (clonal rootstock) and Baynesfield Estate near Richmond (seedling 'Edranol' rootstock) for the years 1985 to 1996 (TABLE 3). Full description of soil types are presented in Chapter 3. Annual

average leaf nutrient concentrations for all cultivars ('Fuerte', 'Hass', 'Edranol', 'Pinkerton', 'Ryan' and 'Rinton') for each estate were calculated.

TABLE 3: Data available for orchard leaf boron status survey.

Estate	Number of orchards	Data available	Predominant soil type
St. Paul	11	1985-1996	Inanda
Everdon	27	1992, 1995	Inanda/Hutton
Baynesfield	16	1993-1996	Inanda
Cooling	19	1985-1996	Hutton/Inanda

In addition to leaf B concentrations, soil samples were taken in existing orchards from Cooling, Everdon and Baynesfield Estates and soil B concentration was determined using the hot water extractable boron method (Wear, 1965).

2.2.2 Soil boron concentrations

Eight soil samples were taken from each of Baynesfield, Cooling and Everdon Estates. After drying and sieving through a 2 mm sieve, B was extracted using the hot water extraction method (Wear, 1965) at Northwest Laboratories, Lichtenburg.

2.2.3 Visual symptoms of boron deficiency

Symptoms associated with B deficiency in avocado were first documented by Smith *et al.* (1995) and which were described in Chapter 1, were assessed.

2.2.3.1 Methods

Field excursions to growing areas in the KwaZulu-Natal Midlands were undertaken. Since all orchards showed ubiquitous symptoms, no study was undertaken to determine which areas were most greatly affected by deficiency symptoms, however each region was characterised by differences in most notable deficiency symptoms.

2.3 RESULTS AND DISCUSSION

2.3.1 Leaf analysis norms

Long term averages all showed sub-optimal (<40 mg kg^{-1}) leaf concentrations (Fig. 2). These results clearly indicate the severe shortage of B in orchards. It must be emphasised that these results do not reflect true leaf B concentration in the tree since contamination from foliar B sprays are likely to have increased B values in annual leaf analysis.

Annual leaf B averages showed no clear trends, although leaf B concentrations at Baynesfield decreased to chronically low levels over four years (Fig. 3). However, the other estates showed inconsistent and occasional unusually high values in spite of chronic deficiency symptoms. Indications from these results are that foliar applications can lead to contamination of leaf analysis samples. Substantial residue resulting in spurious results indicates poor uptake by the leaf. Chronic and severe B deficiency symptoms indicate that translocation within the avocado does not satisfy the B requirement. Leaf B analyses results should always be sceptically reviewed when foliar B sprays have been applied.

It should nevertheless be emphasised that the salvation of South African avocado orchards has probably resulted from leaf applications applied during flowering or fruit set, providing at least a temporary relief at a critical time. Spray residues dripping onto the natural leaf mulch or soil will only provide a minimal input for subsequent root uptake, as will rain causing leaf leaching. Efficiency of foliar applications is substantiated by the fact that no symptoms of greater magnitude than chronic deficiency (blackening of the trunk cross sectional area) have to date, been recorded in the field.

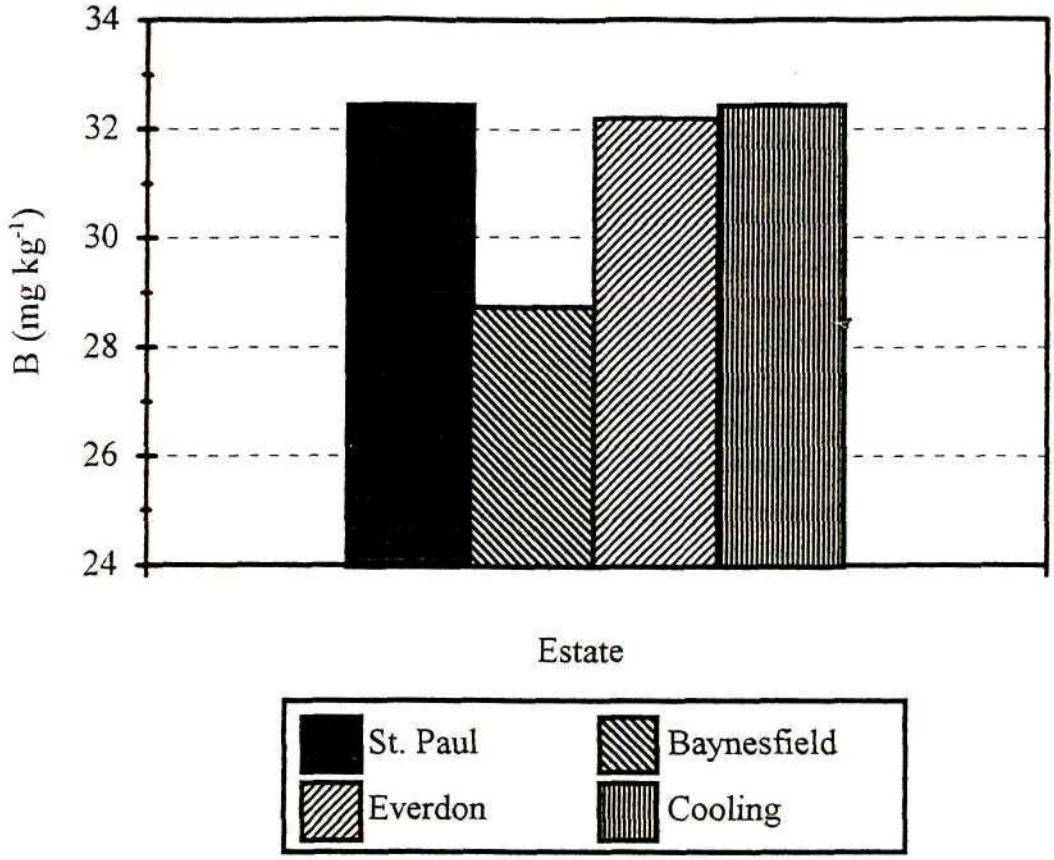

Figure 2: Average leaf boron concentration for 4 KwaZulu-Natal avocado orchards.

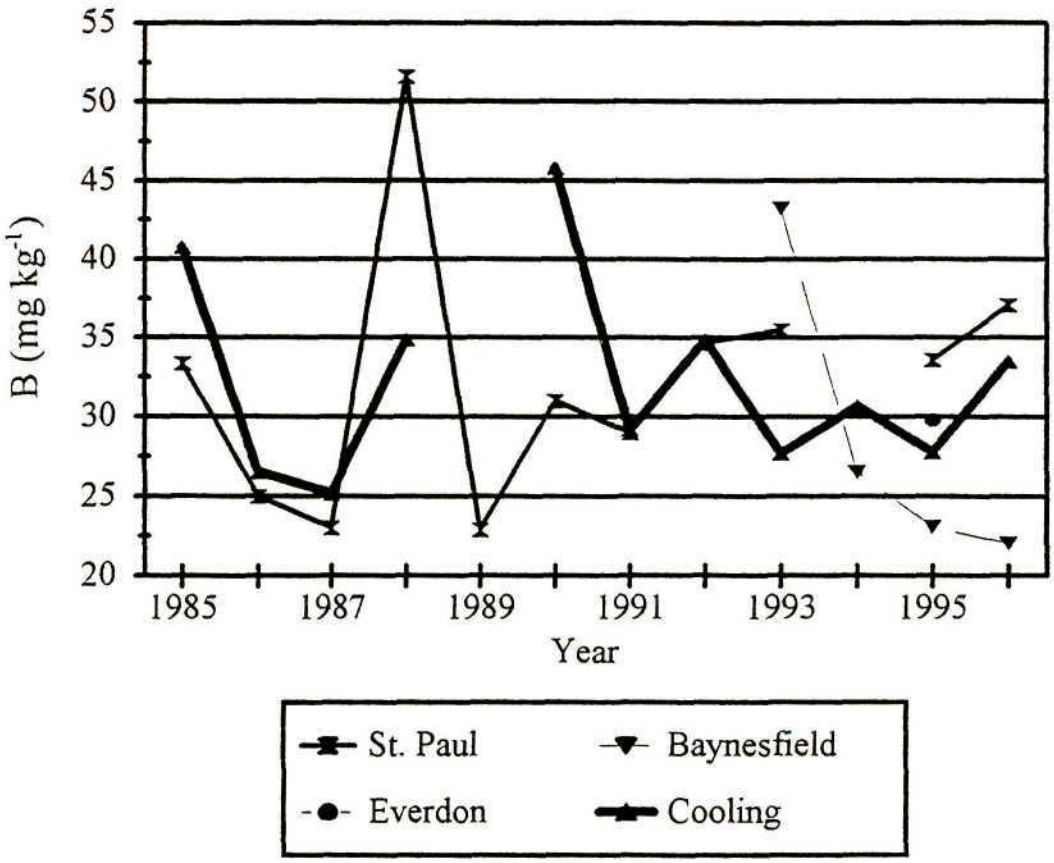

Figure 3: Leaf boron concentration in 4 KwaZulu-Natal avocado orchards.

2.3.2 Soil boron concentrations

Soil B concentrations showed all soils to be in the deficient (<1 mg kg^{-1}) range. Highest average concentration occurred at Baynesfield Estate (Fig. 4). Cooling Estate was marginally higher than Everdon Estate, even though the latter has soils with much higher clay fractions.

Differences can be explained by factors affecting soil B retention in the soil profile. Parent material is Table Mountain Sandstone for St. Paul and Cooling, Middle Ecca shale or dolerite for Everdon Estate, and Lower Ecca shale or dolerite for Baynesfield. All soils are highly weathered, leached and acid therefore having inherently low levels of B. Differences can be therefore attributed to effects of clay fraction and organic matter in the soil profile, that have minimised the effects of leaching. It should be noted that hot water B extraction results cannot be used to indicate levels of available B, particularly where concentrations are in the deficient range since many other soil factors influence availability.

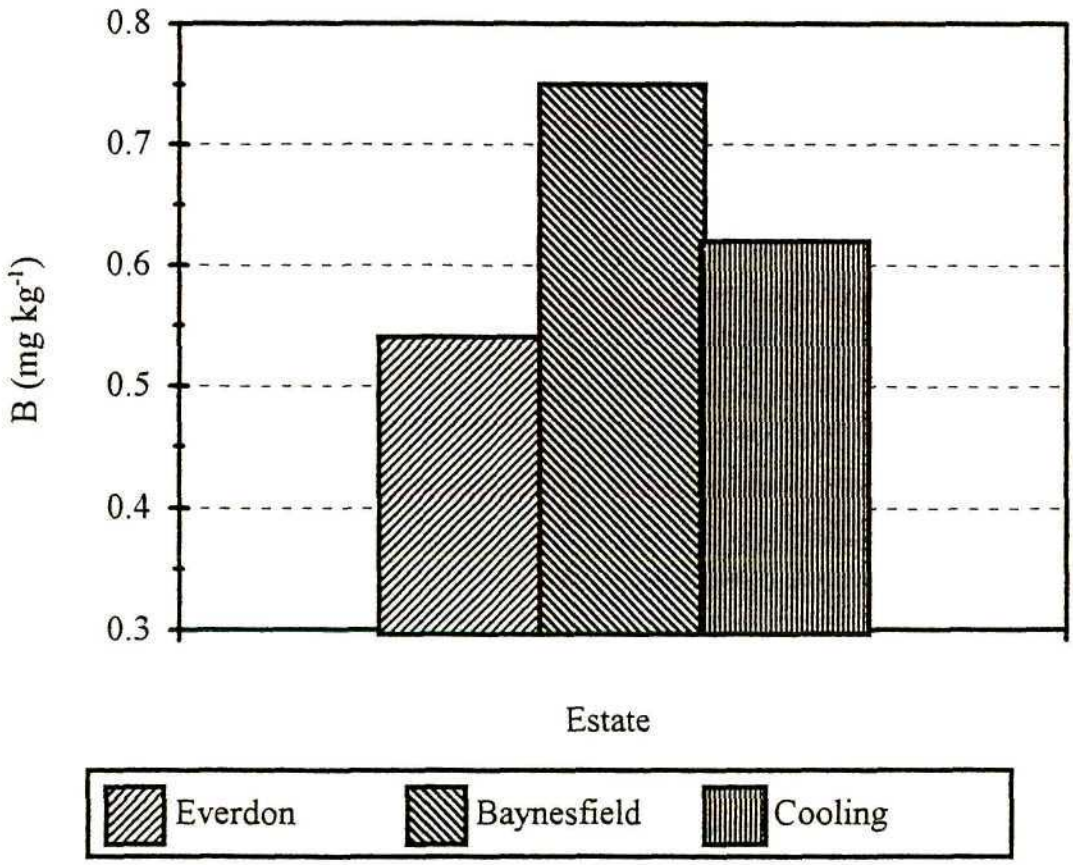

Figure 4: Soil boron concentrations of 3 KwaZulu-Natal avocado orchards.

2.3.3 Visual symptoms of boron deficiency

The following symptoms associated with B have been notably most pronounced throughout the survey:

Complete loss of apical dominance due to death of growing points and subsequent branching occurring from below the apical meristem ('Witches broom' syndrome) was most prevalent on 'Ryan' trees of Cooling Estate (Fig. 5). More chronic symptoms were found in non-commercial orchard trees growing in the Winterskloof area (Fig. 6). Malformation of 'Fuerte' fruit (Fig. 7) was most commonly seen on trees on Cooling Estate as well as, and to a lesser degree Everdon and Baynesfield Estates. Varying degrees of leaf "shotholing" were common in all orchards.

Nodal swelling was most prevalent in 'Fuerte' trees at Baynesfield, however chronic symptoms were found on 'Fuerte' trees at Winterskloof (Fig. 8). Shotholes in young leaves were found in 'Hass', 'Fuerte' at all estates and 'Pinkerton' at Cooling (Fig. 9), however this symptom has not been observed to date on 'Edranol' or 'Ryan'.

Of all cultivars, 'Fuerte' appeared most sensitive to B deficiency showing all deficiency symptoms particularly on Baynesfield Estate. 'Hass' showed all symptoms to a lesser degree.

Seedling 'Edranol' trees showed deficiency symptoms of chronic magnitude. Since 'Edranol' is believed to be more efficient at B translocation than 'Duke 7'(Whiley *et al.* 1996), this indicates that deficient soil conditions are responsible for widespread deficiency rather than inefficiency of 'Duke 7' rootstock.

Small fruit, associated with B deficiency, were noted on all Estates, but were notably less prevalent on Everdon Estate on soils with a high clay percentage. Sandier soils, often on steeper slopes however showed typical small fruit symptoms.

Loss of apical dominance was widespread (Figs. 10-13) in all cultivars.

Figure 5: Boron deficiency symptoms showing loss of apical dominance (witches broom syndrome)

Figure 6: Chronic and very severe boron deficiency in trees from Winterskloof showing total loss of apical dominance leading to a weeping growth pattern.

Figure 7: Boron deficiency shown in hook shaped fruit.

Figure 8: Chronic and very severe boron deficiency in 'Fuerte' trees at Winterskloof, showing nodal swellings

2.4 CONCLUSIONS

In conclusion, all soils examined showed soil B concentrations in the deficient range. All orchards showed deficiency symptoms bordering on chronic magnitude, in spite of leaf analysis indicating concentrations in the marginally deficient range. It can be concluded that leaf sprays are only partly effective, affect leaf analysis and falsely inflate results. To prevent contamination, growers should apply foliar B as a pre-bloom spray as suggested by Lovatt & Jagarnath (1996), since at this stage the spring flush is underdeveloped. Spray exposure will be limited to developing flowers and contamination of the spring flush, which is used for analysis in March will be negligible. Alternatively, summer flush leaves should be used for leaf analysis in May as is done in Australia. Finally, results indicate that practises currently used to prevent B deficiency in South Africa, have not been successful. Some of the severe symptoms illustrated in Figs. 6 and 8 would be associated with leaf B levels below 10 mg kg^{-1} in Australia, and probably with greatly reduced root growth and seriously impaired aerial growth and fruiting. The fact that such symptoms exist in commercial orchards is an indication of the extent of the problem, previously undiagnosed as acute B deficiency.

Figure 9: Boron deficiency shown in "shotholing" of leaves.

Figure 10: Boron deficiency shown by loss of apical dominance in young 'Pinkerton' trees.

Figure 11: Boron deficiency shown by loss of apical dominance in young 'Pinkerton' trees.

Figure 12: Chronic and severe boron deficiency shown by loss of apical dominance in mature 'Fuerte' trees at Winterskloof.

Figure 13: Boron deficiency shown by loss of apical dominance in mature 'Fuerte' trees at Cooling Estate.

3. SURVEY OF SOIL PARAMETERS AFFECTING SOIL BORON DYNAMICS

3.1 INTRODUCTION

Soil parameters affecting B plant uptake have been reviewed in Chapter 1. This chapter aims at establishing soil properties in deficient avocado growing soils. Boron had not been measured in any avocado growing soil in KwaZulu-Natal prior to this study, and initially there was uncertainty as to whether boron deficiency occurred because of low levels of soil B or whether the avocado has high B requirement. In addition, the avocado is known to be inefficient at B uptake relative to other orchard crops. Comparison of soil properties in different growing areas would aid development of recommendations in accordance with soil buffering capacities.

3.2 MATERIALS AND METHODS

3.2.1 Hot water extractable boron

Hot water extractable boron (Wear, 1965) was determined at Northwest Laboratory in Lichtenburg. This method of analysis was selected in preference to calcium chloride extraction, the method widely used in Australia, because no commercial laboratory had adopted the latter method in South Africa.

3.2.2 Clay mineralogy analysis of soils

3.2.2.1 Introduction

It is widely known that soil B is affected by soil clay mineralogy. Soils on which avocados are grown in Kwazulu-Natal midlands generally have a Humic A horizon (Bard, personal observations), which gives indications of well weathered, leached, acid soils. Soil conditions imply that these soils would all contain weathered 1:1 clays such as kaolinite rather than relatively less weathered 2:1 clays such as illite and gibbsite. The latter less weathered clay minerals have a greater potential for B sorption, and therefore soils containing greater quantities of these minerals

would be expected to have a higher B buffering capacity.

Individual soil minerals can be detected qualitatively using x-ray crystallography. Soil samples are irradiated with monochromatic K-α radiation and diffraction is measured with a scintillation counter. Individual minerals and clays can be identified by their interlayer spacings, smaller spacings resulting in a greater degree of diffraction.

3.2.2.2 Materials

An X-ray crystallography machine with a Phillips diffractometer was required to perform the experiment on selected soils from each area.

3.2.2.3 Procedure

Representative topsoil samples were selected from Cooling, Everdon and Baynesfield Estates, as well as from the Winterskloof area from which soil was used for potted experiments. Soils from each area are described:

i) Cooling

Humic/Orthic A horizon over a red apedal B horizon. Divisions between the orthic and humic areas are seldom clear. In the dated Binomial Classification System (MacVicar *et al.*, 1977) where a humic A horizon by definition is required to have more than 2 % organic matter, most of the areas would have fallen into the orthic category. In the recent Taxonomic System (MacVicar *et al.*, 1991) where a humic A horizons must have by definition more than 1.8 % organic matter, most of the area will classify as a humic A horizon. In general, highest lying areas have least organic matter which is generally < 1.8 % organic matter and therefore fall into the orthic A category. Notably, the adjacent farm, St. Paul, is of slightly lower altitude and has only humic topsoils. Hutton and Inanda are predominant soil forms.

ii) Everdon

Humic A horizons dominate the western side of the estate. On the south and south-eastern areas of the estate, only orthic A horizons are found. Red apedal B horizons dominate the subsoil horizons with the occasional yellow brown apedal B horizon occurring in lower lying areas usually reflecting less well drained profiles. Soil is in many places limited in depth (<2 m) where

underlying shale poses a barrier to root growth and draining of water. Clearly such soils are unsuited to avocado production according to current guidelines. All soils have extremely high organic matter content (>1.8 %) however some of them have high cation exchange capacity which prevents them from falling into the humic category. Hutton, Clovelly, Inanda and Magwa are dominant soil forms. Steeper slopes have Glenrosa soil form.

iii) Baynesfield

Humic A horizons over red/yellow brown apedal B horizons. Inanda and Magwa are predominant soil forms. Soil forms are uniform across most of the avocado growing areas of the estate. Depth is seldom limiting. Topsoil is characterised by extremely fine particle size.

iv) Winterskloof

Cool temperatures throughout the year in this south east facing valley are condusive to the preservation of organic matter. Humic A horizons are found overlying red apedal B horizons the latter having an extremely high (>60 %) clay fraction. Soils depth varies greatly, however frequent sandstone outcrops limit depth. Heavy nature of subsoil horizon provides ideal conditions for infection of *Phytophthora cinnamomi* which is a severe problem in the area.

After oven drying soil to constant mass at 50°C, soil was passed through a 2 mm sieve to remove organic matter. Sieved samples were ground gently using a soft plastic utensil following which they were subjected to X-ray crystallography analysis to determine mineralogy of the soil (Hughes, 1996[18]).

3.2.3 Soil organic matter content

3.2.3.1 Introduction, materials and methods

Organic carbon content of soils from St. Paul, Everdon and Baynesfield Estates were obtained from farm records where soil samples had been analysed for fertilisation recommendations. In addition, organic carbon was measured for soil from the Winterskloof area, because soil was used for potted experiments. Soil samples had originally been submitted to Cedara for analysis, and organic carbon was estimated by Near Infrared Spectroscopy (NIRS). Organic carbon content

[18]Hughes, J.C. Department of Agronomy, University of Natal, Pietermaritzburg.

was multiplied by the conversion factor of 1.8, to obtain value for organic matter content.

3.2.4 Clay percentage

Clay fraction analysis results presented were obtained from farm records. Since results were obtained using different methods, some crude in nature, comparison is not fully accurate. Figures however, give indications of differences in clay percentage between growing areas in KwaZulu Natal. At Everdon, and St. Paul Estates, clay percentage was determined using the pipette method in the Cedara Soil Laboratory. Results from Cooling were obtained using the hydrometer method and for Baynesfield, clay texture tests were performed in the field.

3.3 RESULTS AND DISCUSSION

3.3.1 Hot water extractable boron

Results were presented in Chapter 1.

3.3.2 Clay mineralogy

Full clay mineralogy analysis is presented in APPENDIX 1. Soil samples from Everdon and Baynesfield Estates contained the greatest proportion of kaolinite. Soil from Cooling showed slightly higher levels of gibbsite (TABLE 4). No notable differences were shown in proportions of illite and montmorillonite, which have the greatest ability for B adsorption. It should be emphasised that the method used serves as a guide and more sensitive analysis would have to be performed to determine quantitatively, exact differences existing. Nevertheless, while differences may exist between areas, indications show they are marginal and unlikely to play a significant role in soil B dynamics.

Results confirmed original suspicions that clay mineralogy was dominated by kaolinite. Since this clay mineral shows the smallest capacity for B adsorption, mineralogy in avocado growing soils in KwaZulu-Natal could be eliminated as a factor affecting B dynamics to any notable degree.

TABLE 4: Clay fraction analysis of soil from Everdon, Baynesfield and Cooling Estates.

Sample Site	Montmorillonite	Kaolinite	Illite	Gibbsite
	Counts			
Everdon	<500	1500	<500	<500
Baynesfield	<500	1500	<500	<500
Cooling	<500	800	<500	1000

3.3.3 Organic matter content

Cooling Estate showed lowest levels of organic carbon, followed closely by neighbouring St. Paul Estate (Fig.14). Differences between the two estates could possibly be explained by lower topographic position at St. Paul, resulting in marginally lower temperatures that repress organic material decomposition. The cooler areas, Baynesfield and Winterskloof showed highest organic carbon content, probably as a result of slower rates of decomposition in cooler climates.

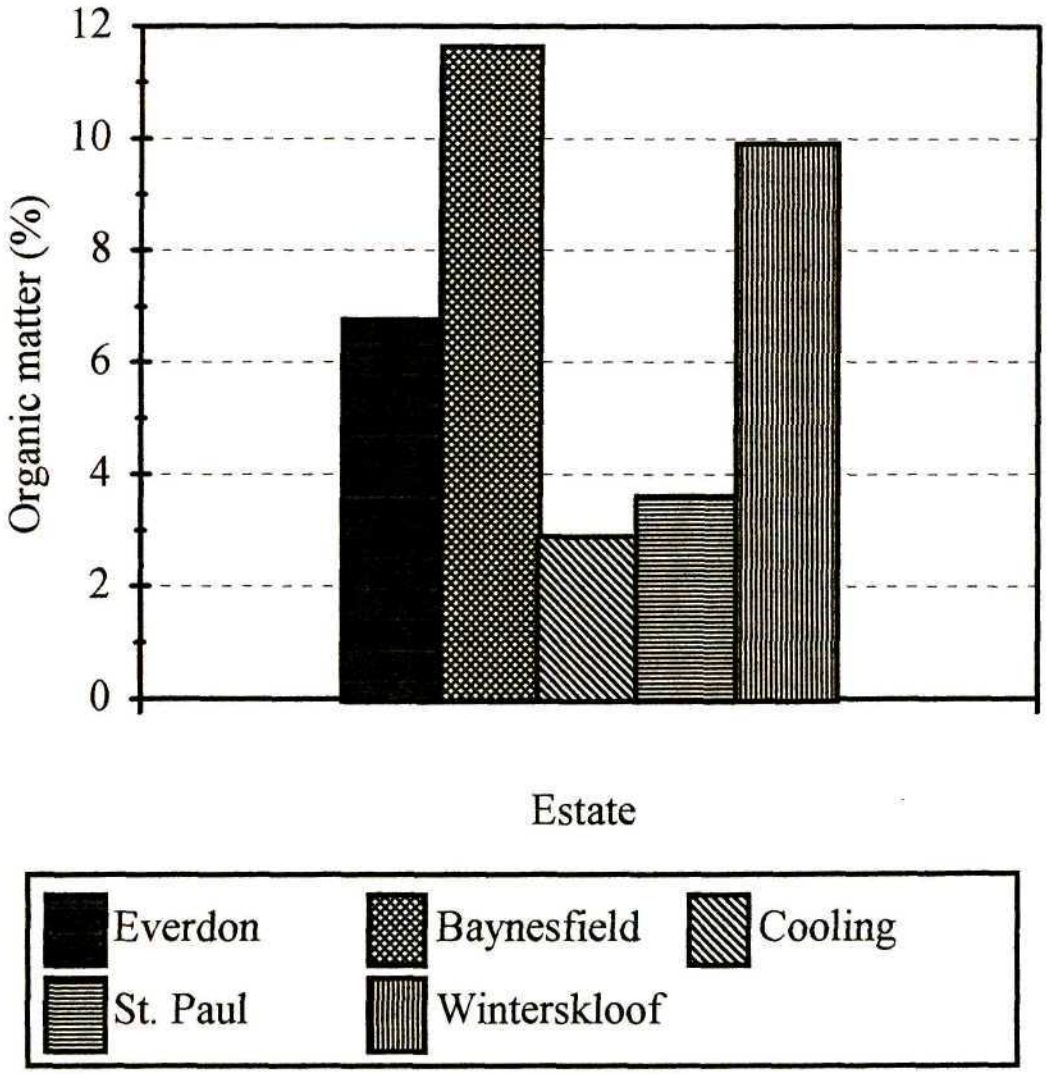

Figure 14: Organic matter content of 5 KwaZulu-Natal Estates.

3.3.4 Clay percentage

Everdon Estate showed highest clay percentage (Fig. 15), while soil from Baynesfield Estate showed marginally less. Cooling Estate showed slightly lower clay percentage than the neighbouring St. Paul Estate. This probably arises since orchards on Cooling are located on higher lying areas which are more likely to be of lighter texture than St. Paul Estate, where orchards are located in a valley microclimate. Samples from Winterskloof showed the lightest texture thus leaching could explain why deficiencies observed surpass chronic magnitude (Figs. 6 & 8).

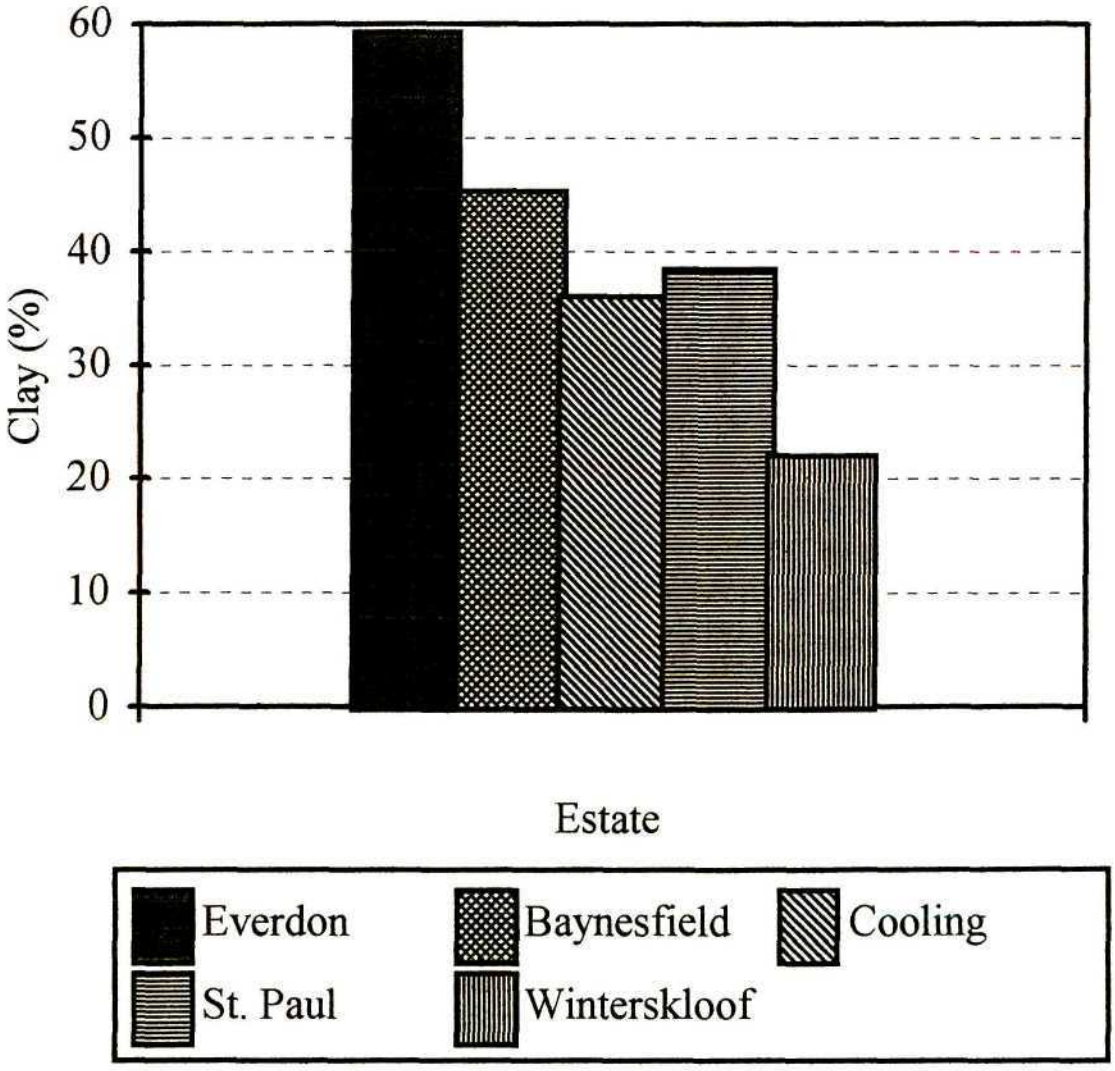

Figure 15: Soil clay percentage of 5 KwaZulu-Natal estates.

3.4 CONCLUSIONS

Results indicate considerable differences in organic matter content and soil clay percentage, while soil B analysis indicated all soils were in similar ranges. Since soil organic matter and clay percentage are responsible for the buffering capacity of the soil, preventing toxicity, each soil would have a different buffering capacity relating to the content of the aforementioned properties. Soils at Everdon Estate are expected to have a higher buffering capacity than soils from St. Paul and Cooling Estates since the latter have lower content of both properties. However, comparisons between Baynesfield and Everdon cannot be drawn since they have oppositely high and low values. Clay mineralogy is not considered a distinguishing property in evaluating a soils buffering capacity, since all soils tested appear to have similar properties.

In conclusion, although all soils are chronically deficient of B, corrective application rates for avocado will differ in accordance with physical properties. Further research is required to quantify buffering capacity of clay fraction and organic matter content.

4. EFFECT OF SOIL BORON APPLICATION ON ESTABLISHED ORCHARD TREES

4.1 INTRODUCTION

South African researchers and extension officers have for years promoted the use of foliar B application to remedy boron deficiency in the avocado, claiming that soil applications could easily induce toxicities at root level, may have a detrimental effect on feeder root health and were ineffective at increasing leaf B levels. In spite of this, there was still some doubt as to the effectiveness of foliar applied B, because of a number of factors;

I) The avocado leaf has a thick waxy cuticle and this poses a physical barrier to B entering the leaf particularly, on the upper leaf surface.

ii) The thick cuticle is believed to intercept some of the applied B, especially in mature leaves.

iii) B is known to be poorly phloem translocated in most plants, thus the effect of the sprays remains largely localised in the leaves since remobilisation to other parts of the tree is poor, particularly to the roots and developing fruit.

In spite of these apparent disadvantages, critics claimed that soil applications were potentially dangerous and less effective, older trees in particular taking years to respond (Robbertse, 1995[19]; Koekemoer, 1995[20]). It should be noted that contamination by foliar sprays was not considered, nor the possibility that sprays were giving poor indications of true orchard B status in South African orchards.

Visual deficiencies associated with B deficiency still remained unrecognised in South African orchards until the mid-1990's. The pioneering work of Queensland researchers, led by Whiley in the 1980's, and more recently by Smith *et al.* (1995, 1997) and Whiley *et al.* (1996) was brought to the attention of South African growers by Wolstenholme (1987), and later Whiley *et al.* (1996) and Wolstenholme & Bard (1996), when it became obvious that chronic B deficiencies were still

[19] Robbertse, P.J. Margaretha Mes Institute for Seed Research, University of Pretoria.

[20] Koekemoer, J. Omega Estate, Hazyview.

the norm in South Africa, in spite of many years of foliar sprays.

The experiments were undertaken to initiate trials on the effectiveness of soil boron application and its effect on avocado tree health and yield in established, commercial orchards. In addition, the experiment endeavoured to determine at what soil application rate toxicity would occur, thus giving indications of margins of safety on typical avocado soils in the midlands of KwaZulu-Natal.

4.2 MATERIALS AND METHODS

4.2.1 Experimental sites

Trials were established at 2 growing areas in the KwaZulu-Natal midlands. Initially a trial was established at Everdon Estate (lat. 29 °27'S, long. 30° 16'E), a Hans Merensky holding near Howick. Everdon lies on the elevated plateau on the edge of the Umgeni Valley and falls under Bioclimatic Group 3 (the mist belt) of Subcoastal Natal (Phillips, 1973). Dominant soil forms are Hutton and Clovelly with Inanda occurring on the northern side of the Estate. Orthic A horizons are typically moderately structured, with a clay percentage of ca. 46 %. The parent material is middle Ecca shale and dolerite. Soil depth varies greatly over the estate, from very deep (>3 m) to less than 1 m. The mean elevation is 1082 m above sea level, and the rainfall averaged 1007 mm for an 80 year period (Anon, 1992). Later in the year, a second trial site was established at Cooling Estate (lat. 29°27'S, long. 30°40'E) situated at Bruyns Hill near Wartburg. Cooling, also on the plateau overlooking the Umgeni Valley, is in Bioclimatic Group 2 of Subcoastal Natal (Phillips, 1973) and has a mean elevation of 950 m above sea level. Inanda soil form predominates, with ca. 35 % clay, derived from Table Mountain (Clarens) sandstone, with excellent physical properties and good depth (Macvicar *et al.*, 1977).

The project was initiated at Everdon in March 1995 on 'Hass' trees on clonal 'Duke 7' established in 1984, currently at a 7 x 7 spacing. This area is known as block A2 and is situated on the south, south-eastern boundary of the Estate on <1 % slope. At this time, soil boron applications were still considered to be risky and dangerous. Hence it was agreed that B treatments would be administered in rows, thus limiting possible damage and tree death, while leaching effects would have the greatest effect on neighbouring trees in a row. In addition, it was agreed that the soil

applications could only be applied up to 20 g borax m^{-2} soil canopy area $year^{-1}$. The trial combined the effects of soil applications with mulching, since it is known that mulching reduces evaporation from the soil thereby maintaining boron in a solubilised and readily available pool. Treatments were administered in rows (APPENDIX 2). Results are not presented since leaf analysis could not be performed because of limited funds.

In July 1995, Everdon Estate disclosed its intentions of using aerial boron and zinc sprays during flowering. Since no blocks or trees could be exempted from the treatment, the pre-requisite of the trial, ie. no foliar boron sprays was violated. This prompted a trial at a different locality and trials were initiated at Cooling in September/October 1995. Recordings were discontinued at Everdon once successful trial sites at Cooling had yielded results.

Two trial sites were established at Cooling. The first site involved 'Hass' on clonal 'Duke 7' planted in 1987. These trees showed chronic B deficiency symptoms when the trial was initiated. Treatments were applied in rows for similar reasons as the former site. Boron was applied in the form of borax (11 % B) at 5 rates: 5, 10, 20, 40 and 60 g borax m^{-2} canopy area $year^{-1}$ (APPENDIX 3), split into 3 equal applications in October, February and April. Although the site had a gradient of less than 1 %, the highest treatment was located on the lower lying area to minimise the risk of leaching, contamination and potential death of adjacent rows (APPENDIX 2). The trial was designed as a long term trial and there would still be 6 replications per treatment after thinning planned for 1997.

This trial was repeated in younger 'Hass' on clonal 'Duke 7' trees planted in 1992 (APPENDIX 2). This trial site was gently sloping (< 5 %) and showed excellent uniformity particularly wrt flowering and fruit set.

4.2.2 Standard management

No foliar B sprays were applied to any experimental trees or adjacent trees at Cooling (to prevent drift) for the duration of the trial. Irrigation was based on tensiometer readings on both estates and was applied through 2 microjets per tree, when readings dropped below -40 kPa. Clean cultivation was practised at Everdon, while interrow areas were maintained by mixed cover crops

at Cooling.

4.2.3 Data collection

Data collection spanned from March 1995 to February 1997. Since B at Everdon was applied in April, harvest in 1995 was not recorded. Similarly, at Cooling no harvest was measured since B was initially administered at harvest. Leaf samples of mature spring flush leaves were taken before 07h00 (while still wet), wiped with a cloth to remove any residue, before placing in a paper packet. Pooled leaf samples were taken for treatments on a monthly basis, however due to financial constraints were not all analysed. Six fruit of average uniform size were taken from each treatment on a monthly basis form January to July and after pooling radial sections, fresh samples were prepared for total phenolic quantification respectively. Fruit were harvested in July at Cooling and in November at Everdon. Fruit size was measured gravimetrically and fruit size distributions were recorded according to the following mass classes; count 24 and smaller (oil factory or reject) = ≤170 g; count 22 = 171 to 190 g; count 20 = 191 to 210 g; count 18 = 211 to 235 g; count 16 = 236 to 265 g; count 14 = 266 to 305 g; count 12 = 306 to 365 g. Twenty random fruit were taken from packhouse lines for each treatment and stored at 3.5 °C for 28 days. On removal from cold storage, fruit were subjected to the firmometer test (Swarts, 1981).

Phenological events, including root and shoot flushes as well as flowering were recorded from March 1995 to November 1996. Root flushes were measured by removing litter from under the south eastern side of the tree and evaluated according to the rating developed by Kaiser (1993).

4.2.4 *In vitro* pollen germination

4.2.4.1 Introduction

The role of B in pollen germination is widely recognised and research over many years by Robberstse, Coetzer and co-workers has shown the importance of B in pollen germination. In spite of this, the role of B in pollen germination has yet to be evaluated for the avocado *in vitro*. This experiment aimed at determining the effect of soil B applications on *in vitro* pollen germination. In addition, results could give indications of B uptake, since leaf analysis at

flowering seldom reflects availability of B at this time.

4.2.4.2 Materials

Pasteur pipettes and sterilised petri dishes were used to perform the experiment.
The following reagents were required: (1) 15 % sucrose solution, (2) 1 % agar solution, (3) calcium nitrate monohydrate - 1000 mg L^{-1}, (2) magnesium sulphate - 300 mg L^{-1}, (3) potassium nitrate - 100 mg L^{-1}, (4) boric acid - 100 mg L^{-1}.

4.2.4.3 Procedure

The method used for pollen germination (*in vitro*) was modified from Sahar & Spiegel-Roy (1984). The experiment was carried out in the field at Everdon in Spring, 1995. Avocado flowers were cut and anthers were immediately placed in 6 mL liquid medium containing 15 % sucrose and (in mg L^{-1}) $Ca(NO_3)\bullet H_2O$, 1000; $MgSO_4$, 300; KNO_3, 100 and H_3BO_3, 100. In each testtube, undehised anthers from 15 flowers were added, before maceration. The solution was gently stirred before placing 2 drops onto 1 % agar containing 15 % sucrose and minerals. Petri dishes were placed in an incubator at 26 °C for 3 h, whereafter the percentage pollen germination was counted at 75.6 magnification.

The procedure was repeated 3 times for B treated trees and 3 times for control trees. The experiment was however only performed once during the 1995 season, since foliar B applications had been proposed at 50 % flowering.

4.2.5 Leaf boron concentration

Leaf B concentration was determined using ICP-AES, since this was regarded as the most suitable method of analysis for the purpose of this study.

4.2.5.1 Materials

Apparatus in this technique included: (1) Janke & Kunkel® analytical mill, (2) Gallenkamp® muffle

furnace, (3) Fried® electric hotplate, (4) Labotec® forced draught oven, (5) 50 mL volumetric flasks, (6) Varian® Radial ICP-AES.

Reagents included (1) concentrated nitric acid, (2) 50 % hydrochloric acid, (3) 0.1 % Triton X-100, (4) N.I.S.T. 1573a tomato leaves reference material.

4.2.5.2 Procedure

Leaves were dried in a forced draught oven at 60 °C for 3 h and milled in a hammermill. Extraction technique for ICP analysis as described by Verbeek (1984) was used. A sample of 1.250 g of milled leaf material was weighed out in porcelain crucibles and placed a muffle furnace whereafter the temperature was increased to 450 °C over 4 to 6 hours. After 12 h at the prescribed temperature, samples were removed and allowed to cool before 2 mL concentrated nitric acid was added under a fume hood. Samples were heated gently on a hotplate until all the nitric acid had evaporated off. Samples were returned to the furnace for a further 4 h. After cooling, 2.5 mL 50 % hydrochloric acid was added and heated gently until the sample was fully digested. Liquid sample was filtered through Whatman® no. 542 filter paper into 50 mL volumetric flasks and 2.5 mL 50 % hydrochloric acid was added as well as 2.5 mL triton X100 before making up to the graduation mark. After vigourous shaking, samples were poured into 75 mL plastic containers until further analysis was performed.

For each batch of samples prepared, blanks were prepared in an identical manner. Similarly, 1.250 g tomato leaves obtained from the National Institute of Standards & Technology, U.S.A. were prepared to validate the preparation method.

Inductively coupled plasma atomic emission spectroscopy (ICP-AES) was used to determine the leaf nutrient concentration of B, Ca, Mg, Na, Cu, Zn & Mn. The radial ICP was used with technical assistance at the Umgeni Water Analytical Services Laboratory, Pietermaritzburg. Initially, the ICP was allowed to perform automatic self-calibration and stabilization before standards, and blanks were introduced into the instrument via a peristaltic pump. Computer programmes used linear regression to calculate the accuracy of the curve (straight line) and produced an r value. Calibration was failed if r was less than 0.99. Samples were poured out

onto trays that filled an autosampler and the instrument performed the analysis. Quality control was performed every 10 samples and samples were re-run if they fell out of limits. It should be noted that when analysing for B, samples were not left in borosilicate testubes for longer than 1 h before analysing.

Dilutions of 1/10 were used for all micronutrients while 1/20 dilutions were used for all macronutrients, excepting B which was analysed undiluted. When very high values were produced that were not within the straight line function, samples were diluted as seen fit.

Concentrations for B were produced in $\mu g\ L^{-1}$, therefore conversion to mg kg^{-1} was:

C ($\mu g\ L^{-1}$) x 1/1000 x 50/1.25

Values indicating concentrations of Ca, Mg, Na and K were produced in mg L^{-1}, therefore conversion to percent was;

C (mg L^{-1}) x 50 x 20 x 1/1.25 x $1/10^4$

Values indicating concentrations of Cu, Zn and Mn were produced in mg L^{-1}, therefore to convert to actual leaf mg kg^{-1}:

C (mg L^{-1}) x 500 x 1/1.25

4.2.6 Fruit total phenolic concentration

4.2.6.1 Extraction

4.2.6.1.1 Materials

Apparatus and materials for this technique included: (1) Polypropylene centrifuge tube, (2) Laboratory shaker, (3) Whatman no. 1 filter paper, (4) Hitachi® Himac centrifuge, (5) Hot water bath, (6) Beckman® DU-65 spectrophotometer. Reagents required were: (1) Gallic acid, (2) 100 % chloroform, (3) 100 % hexane, (4) 60 % methanol, (5) Folin-Ciocalteu reagent, (6) 20 % sodium carbonate.

4.2.6.1.2 Procedure

The procedure used was identical to that used by Donkin (1995) which was a modification of the method developed by Torres *et al.* (1987).

Pooled freeze-dried avocado samples were ground into a powder in liquid nitrogen using a mortar and pestle. A sample of 0.20 g was weighed out into a polypropylene centrifuge tube and 10 mL 100 % chloroform and 10 mL 100 % hexane added. After agitating in a laboratory shaker for 2 h, the solution was centrifuged at 5000 rpm (2510g) for 10 min and filtered through Whatman® no. 1 filter paper. The filtrate was discarded and the residue returned to the tube before 20 mL 60 % ethanol was added. The extract was filtered through Whatman® no. 1 filter paper and the residue discarded. Two replications of 0.1 mL of the sample were placed in 20 mL test tubes and 6 mL of water and 0.5 mL Folin-Ciocalteu added. After thorough vortexing, the solution was allowed to stand for 1 to 8 minutes when 1.5 mL 20 % sodium carbonate was added followed by 1.9 mL water, bringing the total volume to 10 mL. The solution was vortexed and incubated at 50 °C for 2 h. A standard curve was prepared using a dilution series of gallic acid between the range 2.5 μg mL^{-1} and 160 μg mL^{-1}. A blank was prepared using 0.1 mL water and was used to calibrate the spectrophotometer. Finally, absorbance was measured at 765 nm on a spectrophotometer.

4.2.7 Tree architecture

B is known to play an important role in maintaining apical dominance in avocado trees, with deficiency associated with death of apical meristems and loss of apical dominance, producing the 'witches broom' symptom. This leads to a modification of the architectural structure of the tree. This survey aimed at determining if B applications had altered the growth pattern of field avocado trees, as indicated by the nature of the summer flush growth.

4.2.7.1 Procedure

Degree of branching was measured on trees in the young trial at Cooling. Branching was measured on the 1995 summer flush using a scale of 0 to 3; 0 = unbranched; 1 = branched however still maintaining apical dominance; 2 = branched and showing evidence of loss of apical dominance; 3 = total loss of apical dominance with 'witches broom' symptom.

4.2.8 Postharvest fruit quality

Since B (and Ca) are associated with maintaining cell membrane integrity, it was suggested that B could play a role in maintaining postharvest fruit longevity. In addition, it could be possible that some postharvest physiological disorders might be associated with more severe cases of B deficiency. While most physiological disorders are associated with 'Fuerte' fruit, this experiment aimed at determining the effect of B on fruit postharvest characteristics in 'Hass'.

4.2.8.1 Materials

Apparatus required included: (1) Cold room, (2) Firmometer,

4.2.8.2 Procedure

Fruit were stored at 3.5 °C for 28 days after which the fruit were removed and firmometer readings were established. Fruit were allowed to ripen at 20 °C and ripening time and physiological disorders were recorded. Data were subjected to analysis of variance to determine

if there were any statistically significant differences between treatments.

4.3 RESULTS AND DISCUSSION

4.3.1 Pollen germination

Pollen germination was increased by 32.5 % in soil B treated trees (TABLE 5). No statistics are presented for this experiment, since this was a preliminary trial and was discontinued when foliar applications were applied at Everdon Estate. Results indicate that mulching further increased pollen viability, probably since mulching moderates soil water relations and increases root growth, thereby increasing availability of soil B. These preliminary findings need to be confirmed by more detailed studies.

TABLE 5: Effect of tree boron treatment on pollen germination.

Treatment	Germinating pollen	Non germinating pollen	% Germination
Control	137	772	15.0
B treated	353	390	47.5
B treated + Mulch	474	299	61.3

4.3.2 Leaf B concentration

Leaf B concentrations showed that soil B application was effective at raising B concentrations in leaf and fruit tissue (Figs. 16 & 17) to the accepted norm of 40-70 mg kg^{-1} (APPENDIX 5). Initial uptake occurred slowly. All applications were shown to increase leaf B concentrations proportionally to the application rate. Rate of uptake increased dramatically in December/January 1997, causing toxicity on the 60 g m^{-2} treatment, 15 months after initial application (Figs. 18, 19 & 20). Symptoms appearing as marginal interveinal necrotic areas, were initially visible at the

leaf apex, moving progressively towards the petiole end. Toxicity symptoms were often associated with chlorosis and abscission of older leaves (Figs. 21 & 22). Leaf B concentration showed cyclical variation throughout the year. Highest leaf concentrations occurring during January 1997 and June 1996 indicated that times of greatest uptake were November to February followed by April to June. This coincided with the time of greatest feeder root growth (Fig. 23). All soil applications raised leaf B concentrations higher than those of the control. The amount of B measured in leaf tissue was proportional to the application rate. Although leaf B concentrations were initially in the same range bordering on deficiency, final concentrations were different. Furthermore, it should be noted that control leaves showed the greatest decrease in leaf B

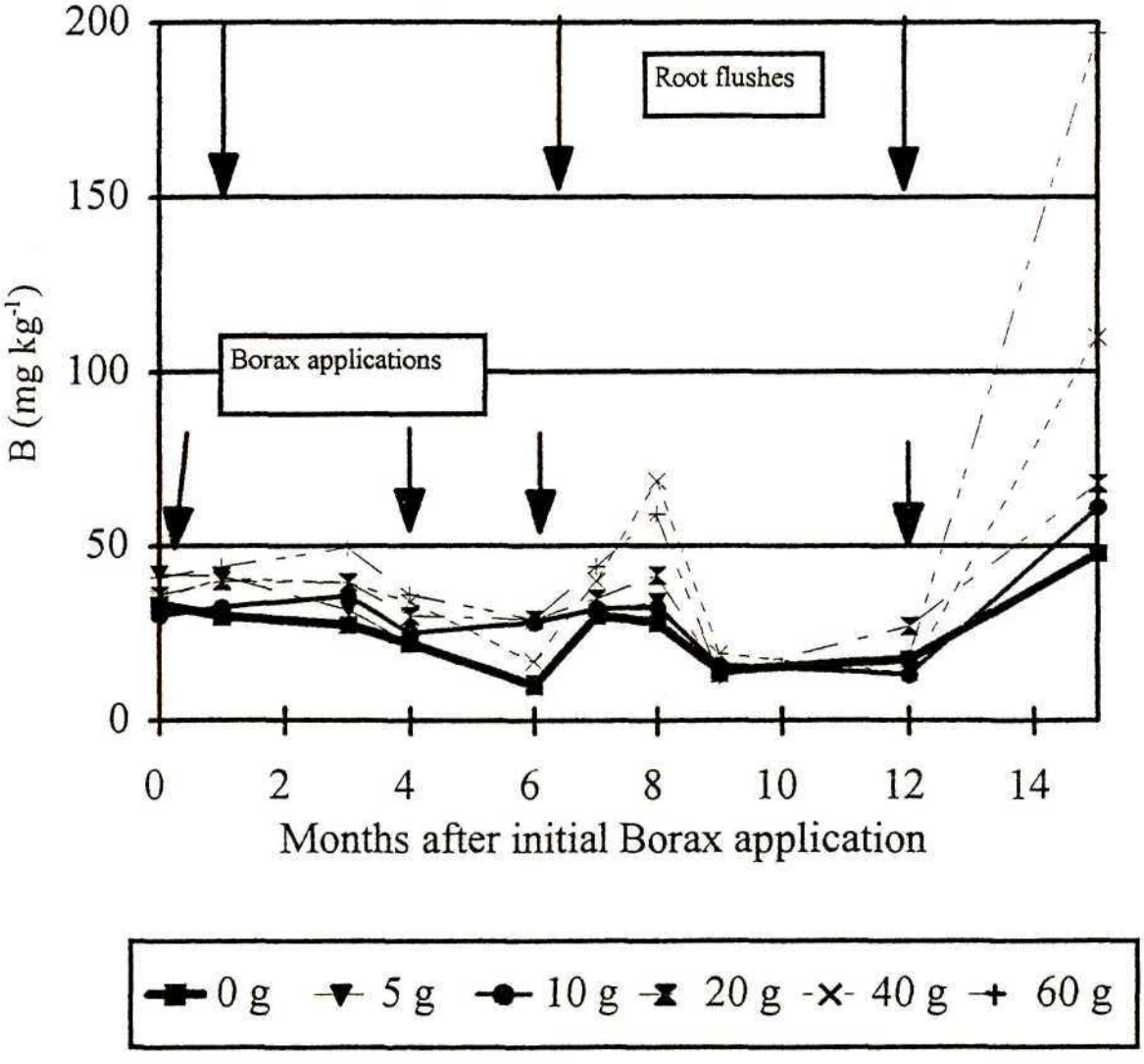

Figure 16: Effect of soil boron application on leaf B concentration of mature 'Hass' trees. Rates are g of borax m^{-2} canopy area $year^{-1}$, divided into three applications in October, February and April.

concentration between February and April 1996 for older trees, and between April and May for the younger trees. This was the time during which flushes were expanding and maturing and fruit growing, clearly a time when requirement for B is high.

Toxicities were initially suspected in mature trees during October 1996 in the 60 g m^{-2} $year^{-1}$ treatment and were confirmed when severe toxicity symptoms appeared at both 40 and 60 g m^{-2} $year^{-1}$ levels in January 1997 (Figs. 18, 19 & 20). In the mature tree trial, the two highest rates (40 and 60 g borax m^{-2} $year^{-1}$) both led to foliar B concentrations of >100 mg kg^{-1}, which is regarded

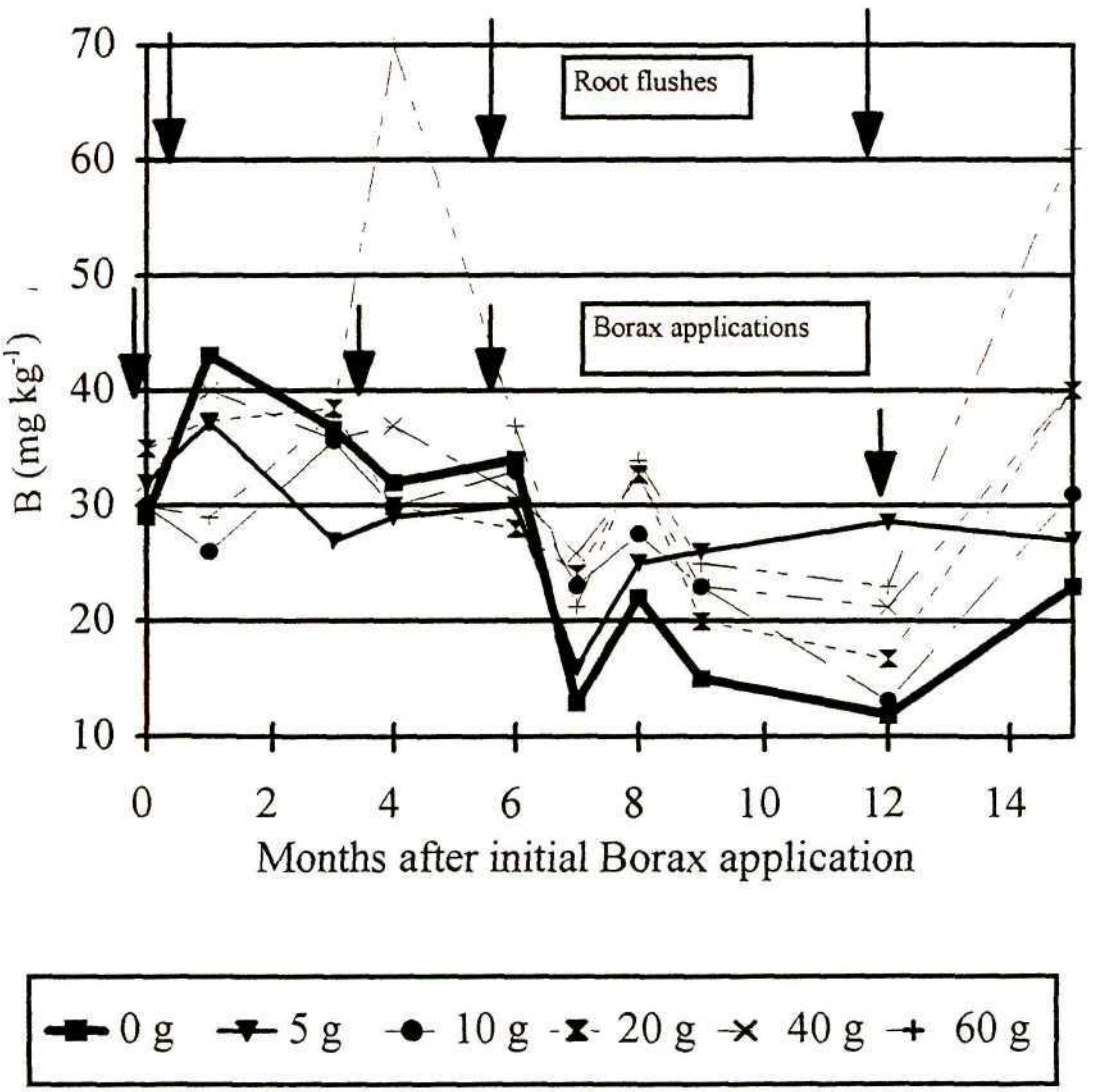

Figure 17: Effect of soil boron application on leaf B concentration of young 'Hass' trees. Rates are g of borax m^{-2} canopy area $year^{-1}$, divided into three applications in October, February and April.

as toxic in Australia. In younger trees however, the highest rate (60 g) resulted in a leaf B level within the desired range after 14 months.

Fruit mesocarp analysis showed that B was translocated to developing fruit, proportionally to the soil application rate (Fig. 24). B levels showed a decreasing trend from February to April, probably resulting from a dilution effect form the rapidly growing fruit. The sharp increase in fruit B concentration from April to June (from 30 to 70 mg kg^{-1} in the 40 g m^{-2} treatment) indicates effectiveness of soil B applications in supplying B to the maturing fruit. The control and lowest application rate (5 g m^{-2}) did not show as great an increase in fruit mesocarp B concentration. In these treatments, increase in fruit B concentration could have been supplemented by remobilisation from adjacent leaves. The increase in fruit B concentration between April and June would have been assisted by the root flush noted in late May (Fig. 23).

Soil B applications should be made so as to optimise B supply during peak uptake periods during the root flush. Applications should preferably be made 4 to 6 weeks before these periods to enable surface applications to dissolve with rainfall or irrigation. Since the peak uptake period from April to June occurs during autumn to winter, application during February would be preferable since rainfall is far more efficient at dissolving applied borax across the entire drip line area than is irrigation. Soil B application during summer wet months is particularly important in dryland orchards, since rainfall is essential to dissolve applied B. Where B is applied through the irrigation system, B application can be injected into irrigation water during peak uptake periods. Timing of application becomes less important once soil B reserves have increased to the adequate range. However, the sandier the soil, the more the annual application should be split, to reduce the danger of toxicity.

Results suggest that initially a moderately high application rate (20 g m^{-2}) would ameliorate deficiency within a shorter period, however would only be necessary for the first year whereafter a low maintenance dose (5 g m^{-2}) could be applied. Leaf analysis should be used as a tool to determine application rate. Sampling during February would also be advisable should toxicity be suspected, since leaf B concentrations are at a peak during this interval. Application rates in Australia are typically within this general range, depending mainly on soil texture and organic matter content.

Figure 18: Early symptoms of boron toxicity shown in mature 'Hass' trees on Cooling Estate.

Figures 19: Boron toxicity symptoms shown in mature 'Hass' trees on Cooling Estate.

Figure 20: Advanced boron toxicity symptoms seen in mature 'Hass' trees on Cooling Estate.

Figure 21: Leaf chlorosis associated with boron toxicity symptoms seen in mature 'Hass' trees on Cooling Estate.

Figure 22: Leaf chlorosis associated with boron toxicity symptoms shown in mature 'Hass' trees on Cooling Estate.

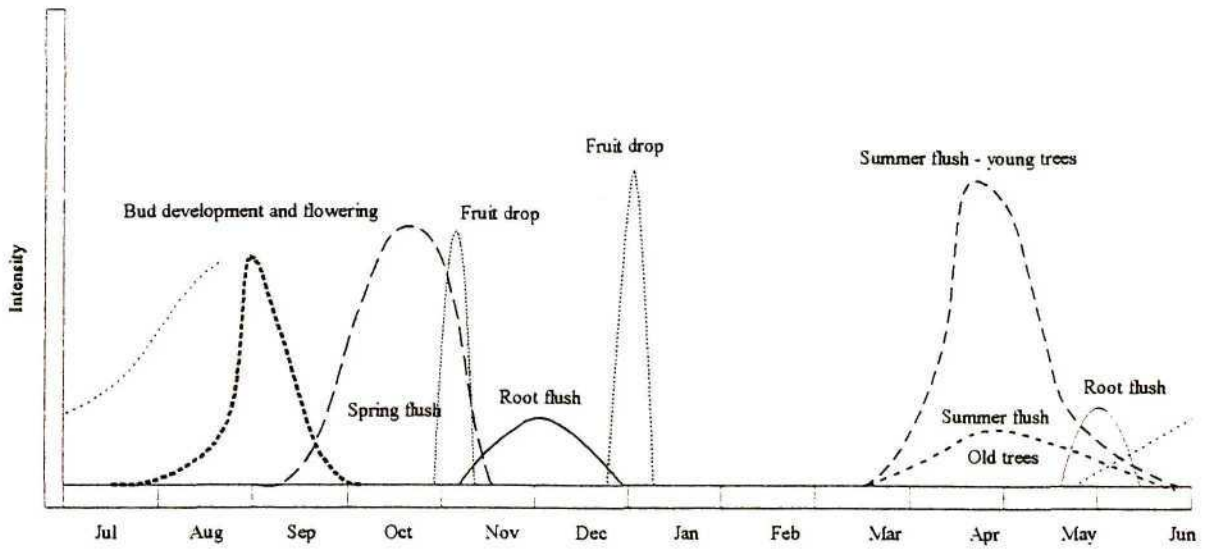

Figure 23: Phenological cycles at Cooling Estate for 1996/97 season.

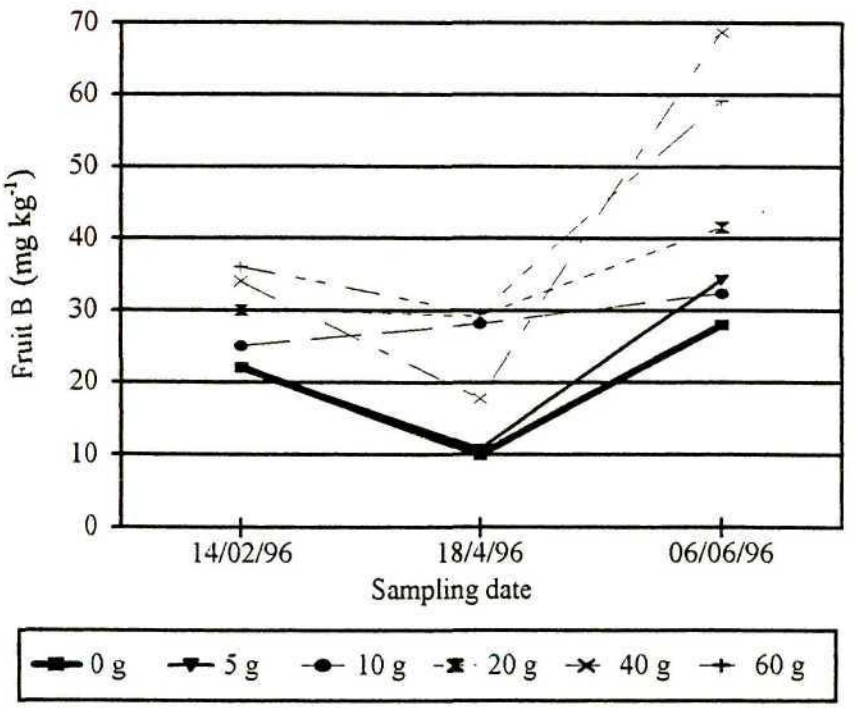

Figure 24: Effect of soil boron application on fruit mesocarp B concentration of mature 'Hass' trees. Rates are g borax m^{-2} soil canopy area $year^{-1}$, divided into three applications in October, February and April.

4.3.3 Leaf K concentration

Leaf potassium (K) concentration displayed considerable differences between treatments (Figs. 25 & 26) although all were in the accepted optimal range of 0.75-1.25 % (APPENDIX 5) at time of leaf sampling in March. Differences in leaf K are probably attributed to yield, since fruit are heavy sinks for K. K levels in young trees between 4 - 9 months after first B application (February to July), were in a similar range in younger trees, while differences in older trees are greater. This results from variation in tree yield amongst older trees, each having different requirements for K, proportional to crop load. After harvest, K concentration rose dramatically. In young trees, increase in leaf K concentration is greatest in control and lowest (0 and 5 g m^{-2} respectively) treatments while higher dose rates that had beared higher yield in the previous 1996 season showed lowest increase. This is possibly attributed to the K deficit of higher yielding B treated trees, K deficit developing as a result of heavier crop loads.

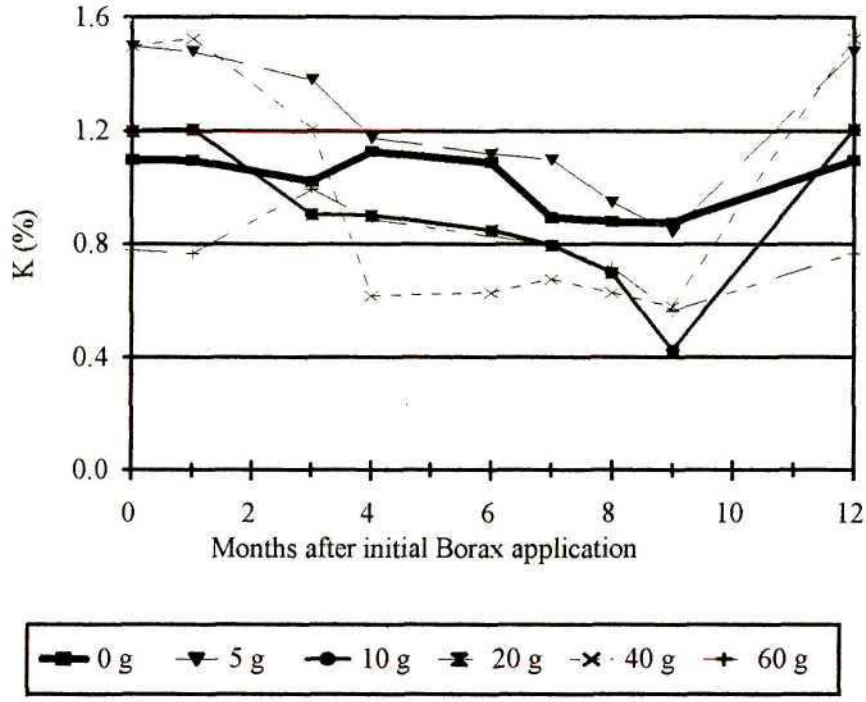

Figure 25: Effect of soil boron application on leaf potassium concentration of mature 'Hass' trees. B rates are g borax m^{-2} soil canopy area $year^{-1}$, divided into 3 applications in October, February and April.

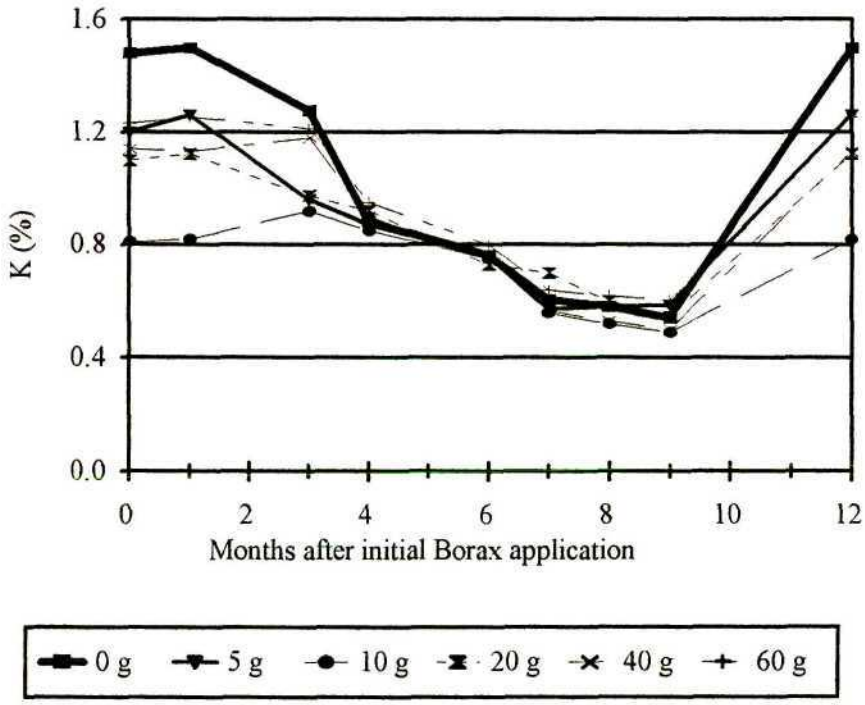

Figure 26: Effect of soil boron application on leaf potassium concentration of young 'Hass' trees. B rates are g borax m^{-2} soil canopy area $year^{-1}$, divided into 3 applications in October, February and April.

4.3.4 Leaf Ca concentration

Leaf calcium (Ca) concentrations were all marginally below the lower limit of the optimal range of 1.00 % (APPENDIX 5). This reflects low soil Ca status and the Estate owners' conservative approach to application of lime. No striking trends were seen (Figs. 27 & 28). Differences between treatments were greater in older trees than younger trees, resulting from high tree yield variation within the orchard block. Calcium levels do not appear to fluctuate greatly throughout the season, indicating that calcium is an immobile nutrient having a structural role. It is also noteworthy that leaf calcium levels in young trees were greatest in the control and 5 g m^{-2} treatments indicating possible antagonism between Ca and B at higher B application rates (Fig. 28). Further research is required on the calcium/boron relationship in the avocado, particularly since lime is in general either not applied or over applied in Natal avocado growing soils.

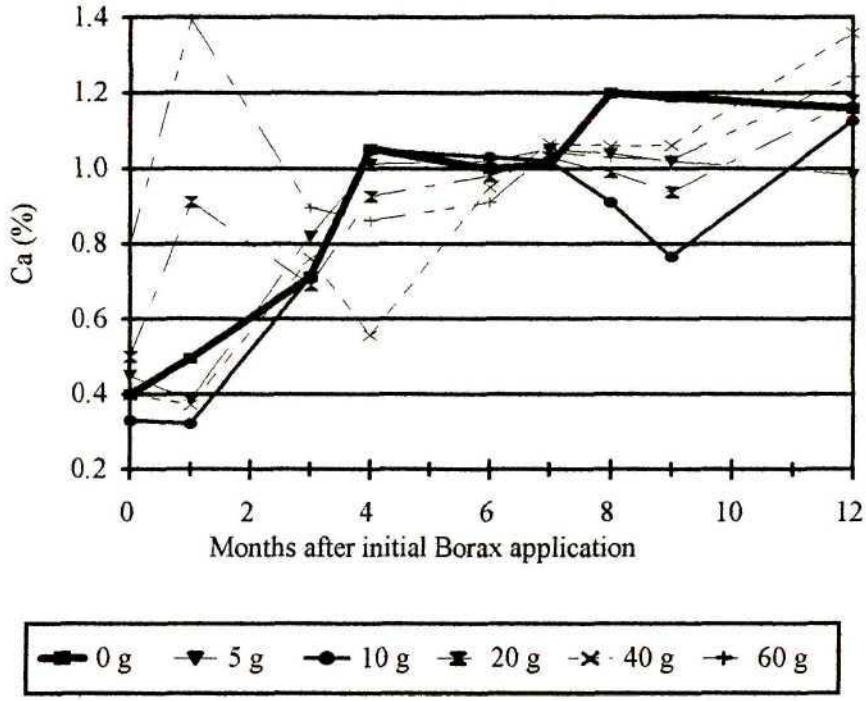

Figure 27: Effect of soil boron application on leaf calcium concentration of mature 'Hass' trees. B rates are g borax m^{-2} soil canopy area $year^{-1}$, divided into 3 applications in October, February and April.

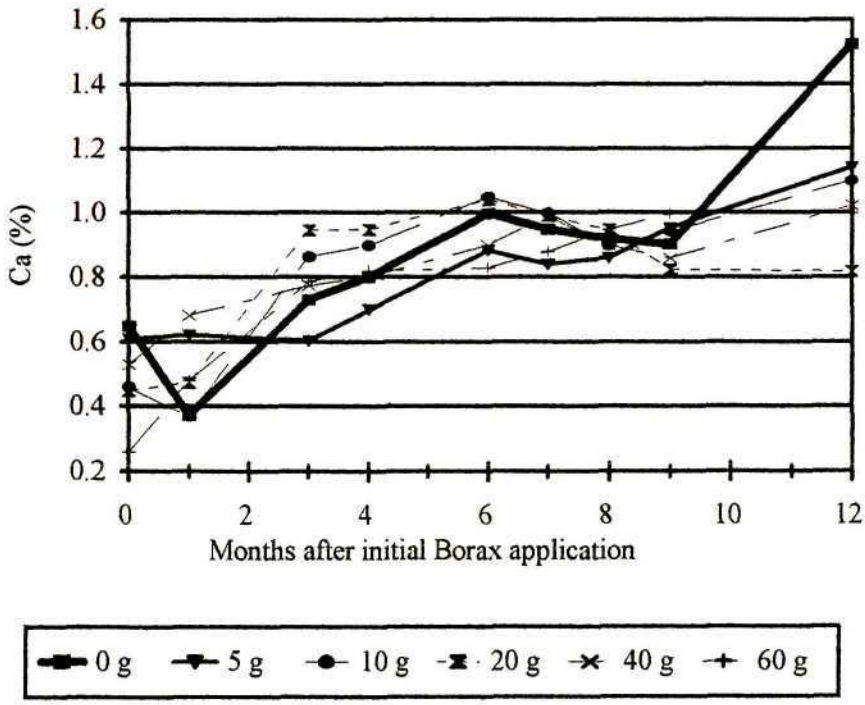

Figure 28: Effect of soil boron application on leaf calcium concentration of young 'Hass' trees. B rates are g borax m^{-2} soil canopy area $year^{-1}$, divided into 3 applications in October, February and April.

4.3.5 Leaf Mg concentration

Magnesium (Mg) concentrations all fell within the optimal range of 0.4-0.8 % (APPENDIX 5). Similar trends to Ca were shown (Figs. 29 & 30). Fluctuations were small throughout the season indicating the immobile nature of Mg, having a similar role to Ca, in the plants structural framework.

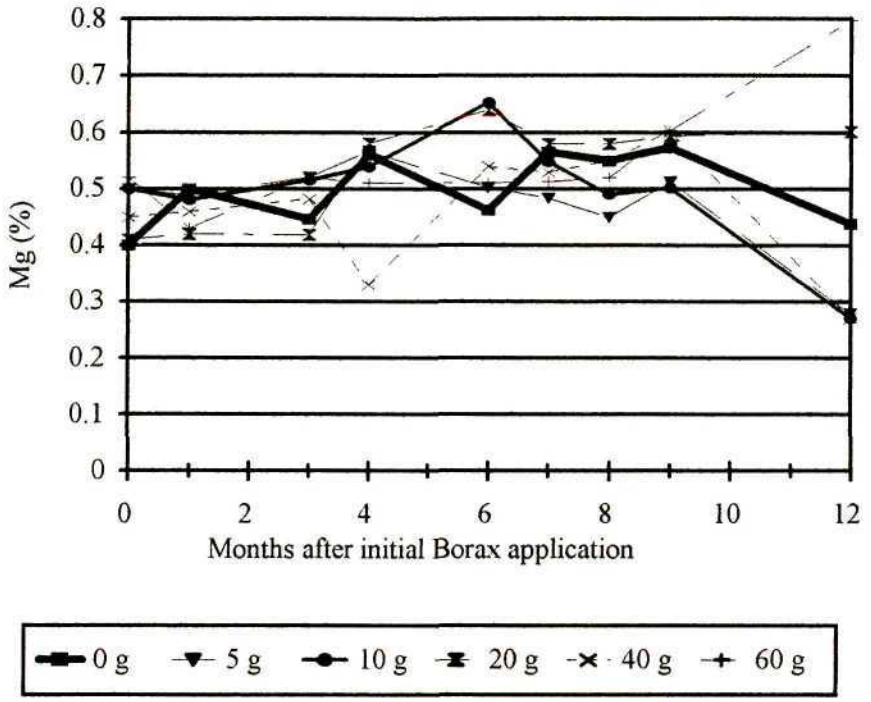

Figure 29: Effect of soil boron concentration on leaf magnesium concentration of mature 'Hass' trees. B rates are g borax m^{-2} soil canopy area $year^{-1}$, divided into 3 applications in October, February and April.

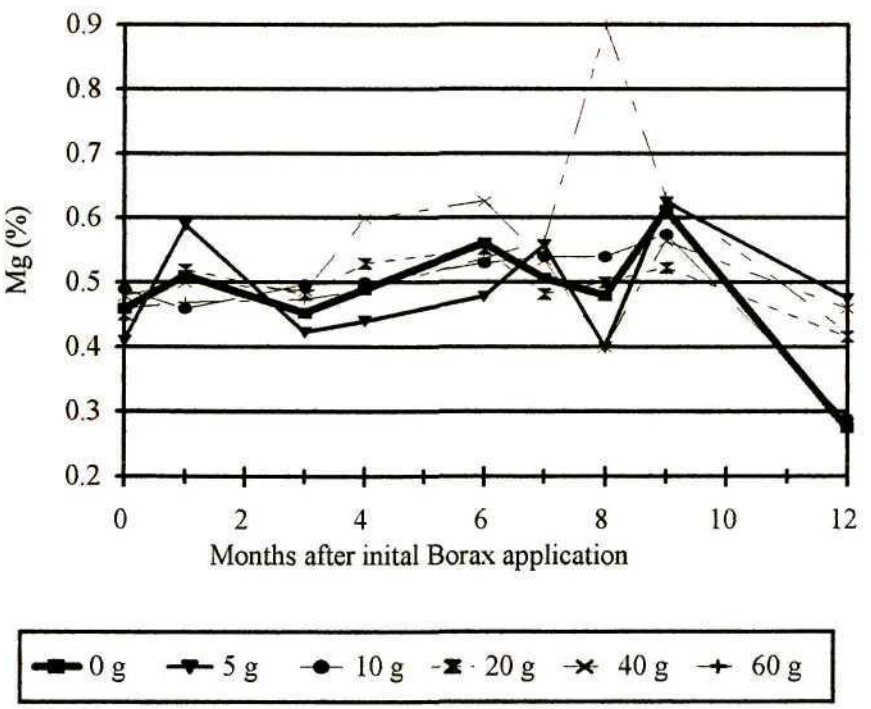

Figure 30: Effect of soil boron concentration on leaf magnesium concentration of young 'Hass' trees. B rates are g borax m^{-2} soil canopy area $year^{-1}$, divided into 3 applications in October, February and April.

4.3.6 Leaf Na concentration

Sodium (Na) showed above normal (0.06 %) leaf concentrations in all treatments. Maximum concentration was noted between 7 - 8 months after initial application (May - June) (Figs. 31 & 32). Peak concentration during this time probably results from decrease in rainfall during this time, hence less leaching occurs, and dryer soil profiles effectively increase soil Na concentration. Apart from the high value obtained for the 60 g m^{-2}, 7 months after the initial application, in the young trees (Fig. 32), differences between control and B treated trees are marginal. This indicates that B application in the form of sodium borate (borax) will not have any effect on leaf Na concentration. Furthermore, B toxicity cannot be detected by measuring leaf Na concentration. Effect of Na is more likely to be observed in the rootstock, since Na is not readily translocated to the scion.

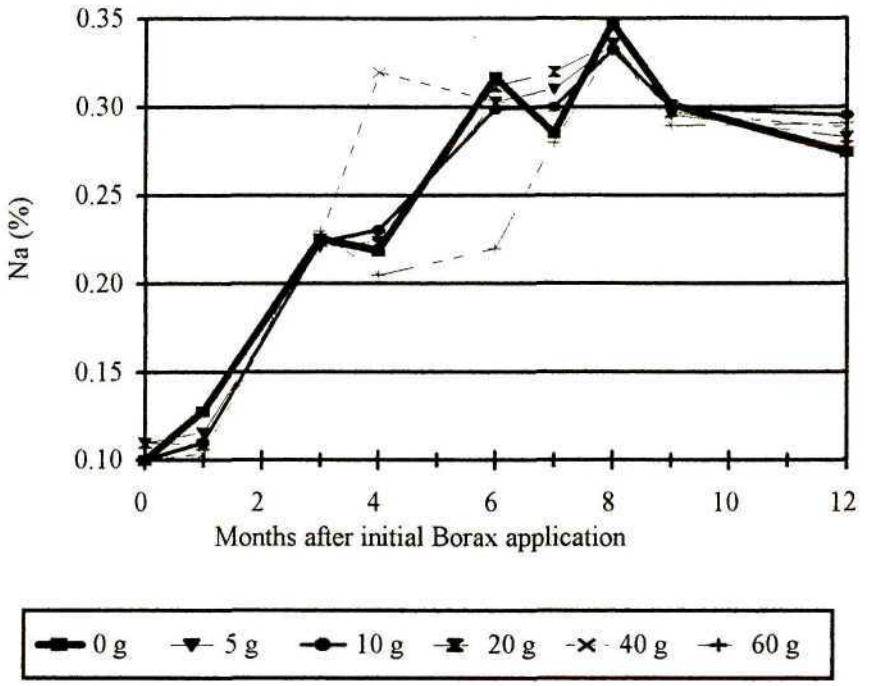

Figure 31: Effect of soil boron concentration on leaf sodium concentration of mature 'Hass' trees. B rates are g borax m^{-2} soil canopy area $year^{-1}$, divided into 3 applications in October, February and April.

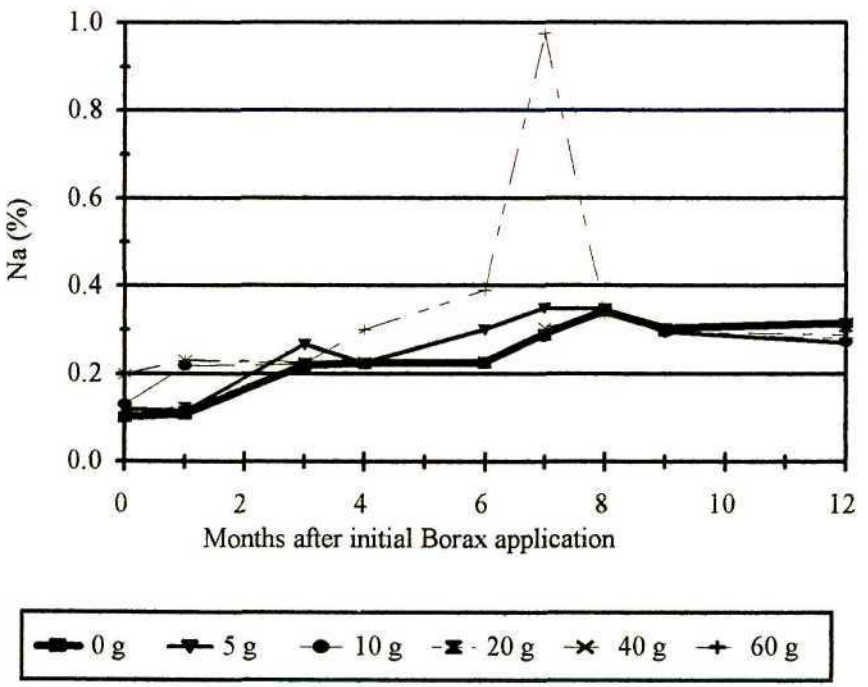

Figure 32: Effect of soil boron concentration on leaf sodium concentration of young 'Hass' trees. B rates are g borax m^{-2} soil canopy area $year^{-1}$, divided into 3 applications in October, February and April.

4.3.7 Leaf Cu concentration

Copper (Cu) leaf concentrations rose dramatically between 2 - 8 months after initial application (December to June) (Figs. 33 & 34) while during ensuing months, leaf concentrations did not exceed 25 mg kg^{-1}. This period of high leaf Cu occurs as a result of numerous foliar copper oxychloride sprays applied during this time to counter fungal attack. Although sprays are often discontinued as early as March, decrease in leaf concentration only occurred in July, when rains removed spray residues. Notably, the 1995/1996 season was characterised by heavy winter (July) rainfall which undoubtedly affected leaf values. All values during this time grossly exceeded the norm 5-15 mg kg^{-1} (APPENDIX 5) giving clear indications of contamination..

Time of leaf sampling occurs during the time of foliar copper application, hence results indicate that leaf analysis during this time do not reflect true leaf concentrations because of contamination. Indications are that leaf concentrations are otherwise generally low, hence copper sprays were not effective at raising leaf Cu concentration.

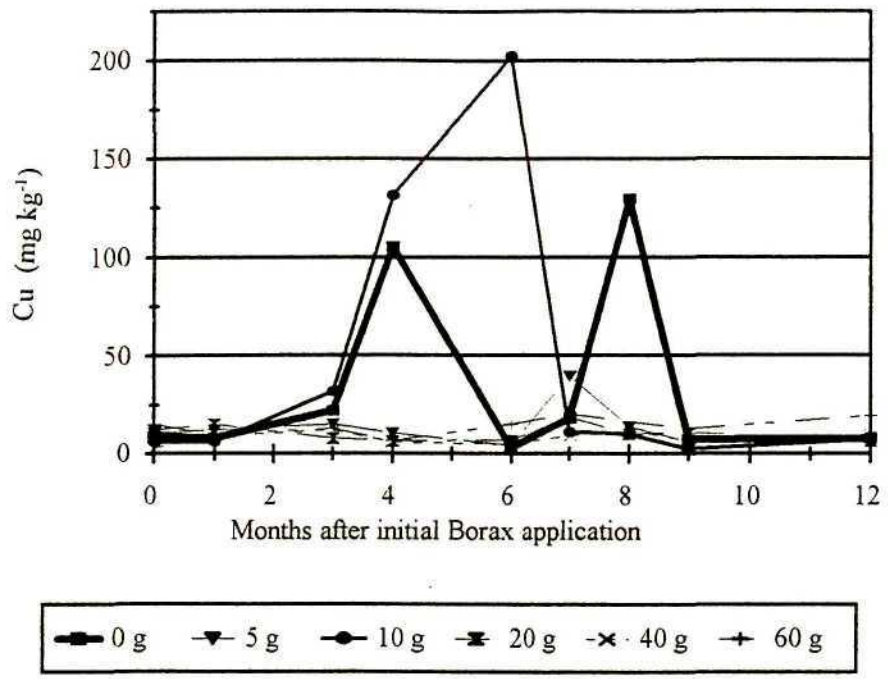

Figure 33: Effect of soil boron concentration on leaf copper concentration of mature 'Hass' trees. B rates are g borax m^{-2} soil canopy area $year^{-1}$, divided into 3 applications in October, February and April.

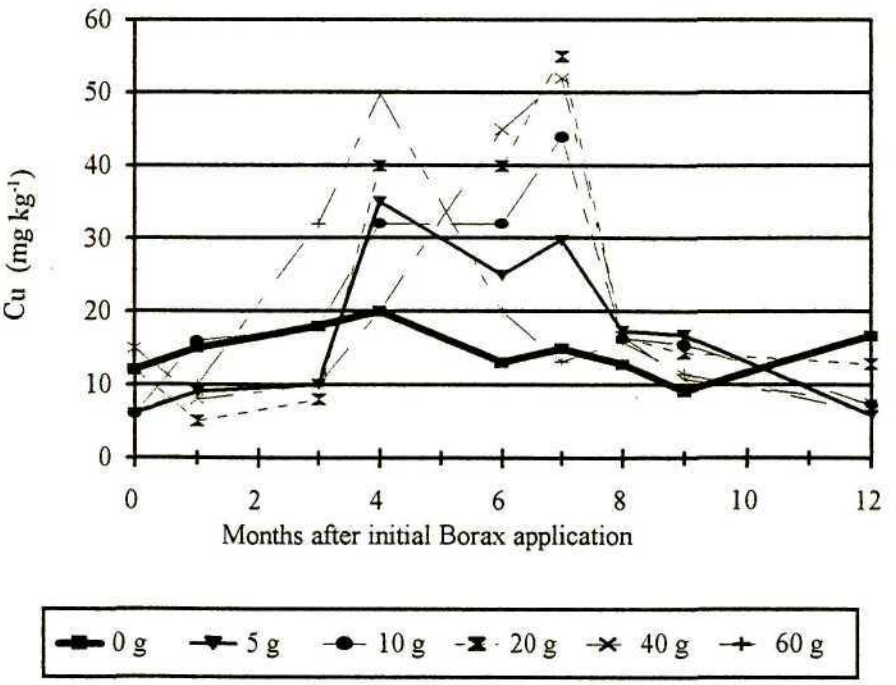

Figure 34: Effect of soil boron concentration on leaf copper concentration of young 'Hass' trees. B rates are g borax m^{-2} soil canopy area $year^{-1}$, divided into 3 applications in October, February and April.

4.3.8 Leaf Mn concentration

Leaf manganese (Mn) concentrations displayed unclear trends (Figs. 35 & 36). Extremely high leaf Mn concentration (> 250, APPENDIX 5) indicate possibility of contamination from foliar sprays (from Mn based fungicides). Variation throughout the year could result from high rainfall or over irrigation during times when feeder root health is good. Wet soil profiles in acidic soils provide conditions for increased availability of Mn, which is taken up by the plant. Results indicate that there is no shortage of Mn, and excessive quantities are more likely to be of a problem.

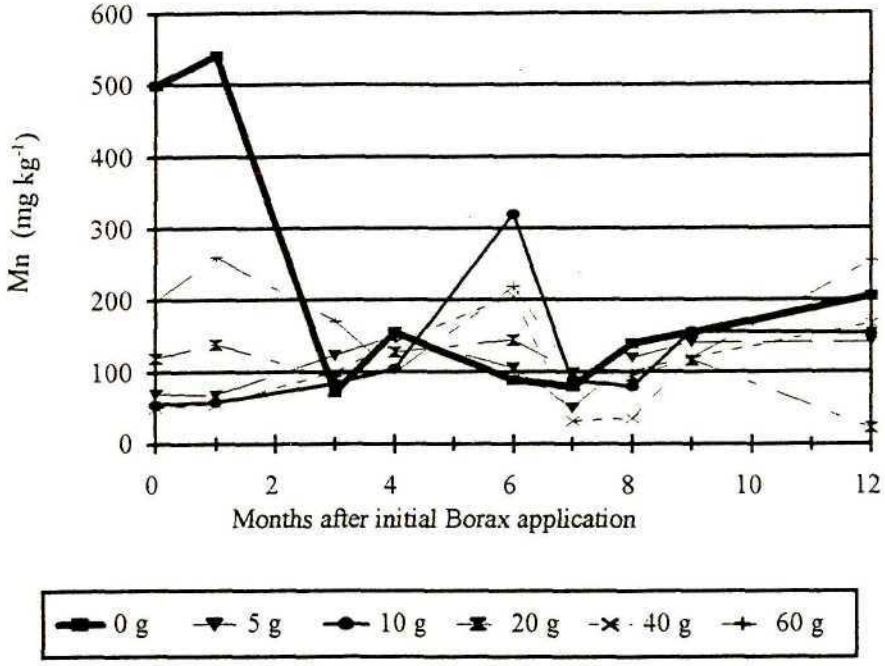

Figure 35: Effect of soil boron concentration on leaf manganese concentration of mature 'Hass' trees. B rates are g borax m^{-2} soil canopy area $year^{-1}$, divided into 3 applications in October, February and April.

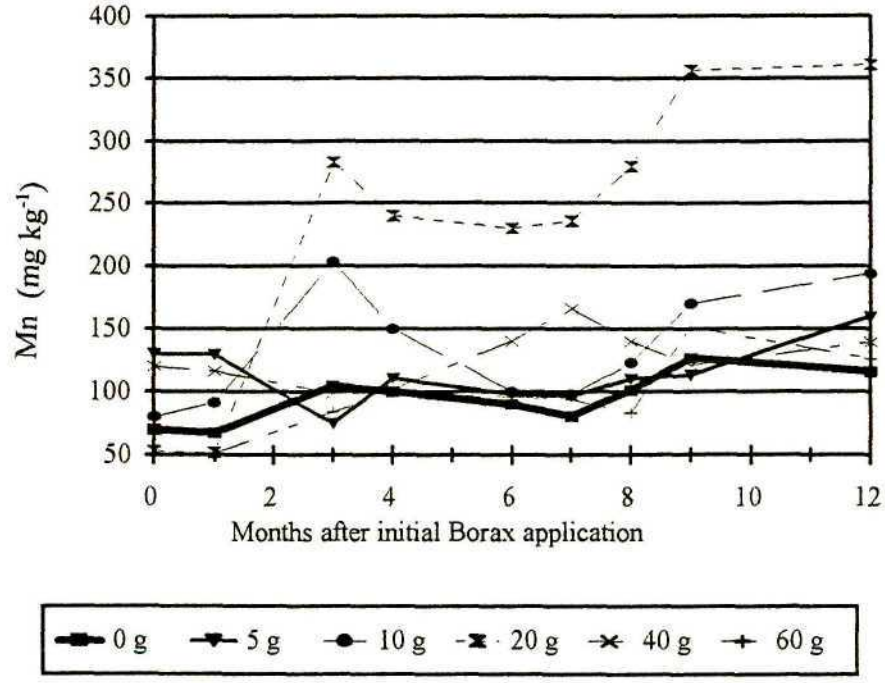

Figure 36: Effect of soil boron concentration on leaf manganese concentration of young 'Hass' trees. B rates are g borax m^{-2} soil canopy area $year^{-1}$, divided into 3 applications in October, February and April.

4.3.9 Leaf Zn concentration

Zinc (Zn) displayed similar trends to Cu, indicating possible contamination during times of Cu sprays (Figs. 37 & 38). All treatments show sufficient leaf Zn (25-100 mg kg^{-1}, APPENDIX 5). Sprays however do not appear to have had a lasting effect on raising leaf Zn concentration throughout the season. Soil applications would probably be more beneficial.

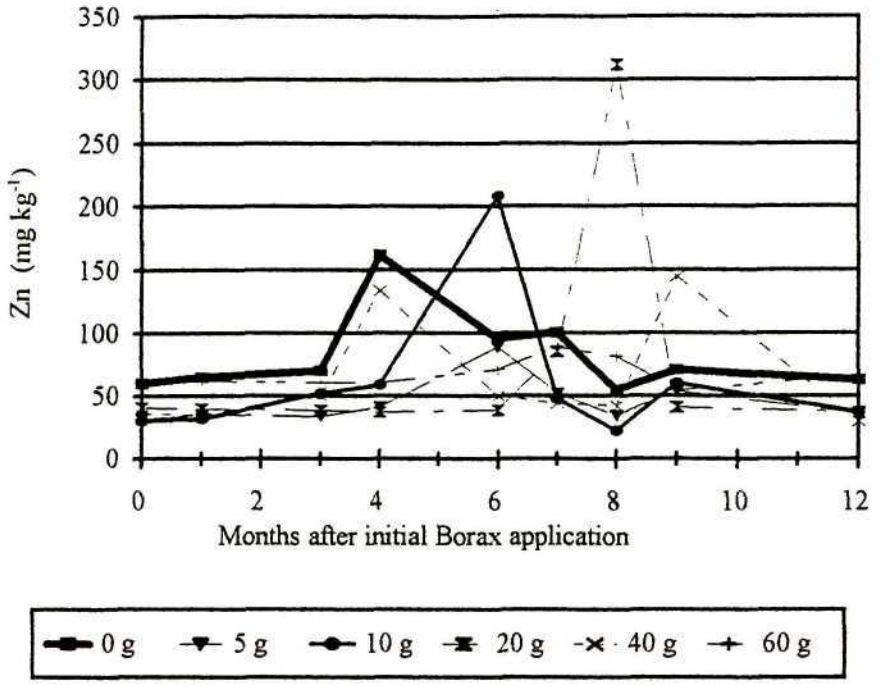

Figure 37: Effect of soil boron concentration on leaf zinc concentration of mature 'Hass' trees. B rates are g borax m^{-2} soil canopy area $year^{-1}$, divided into 3 applications in October, February and April.

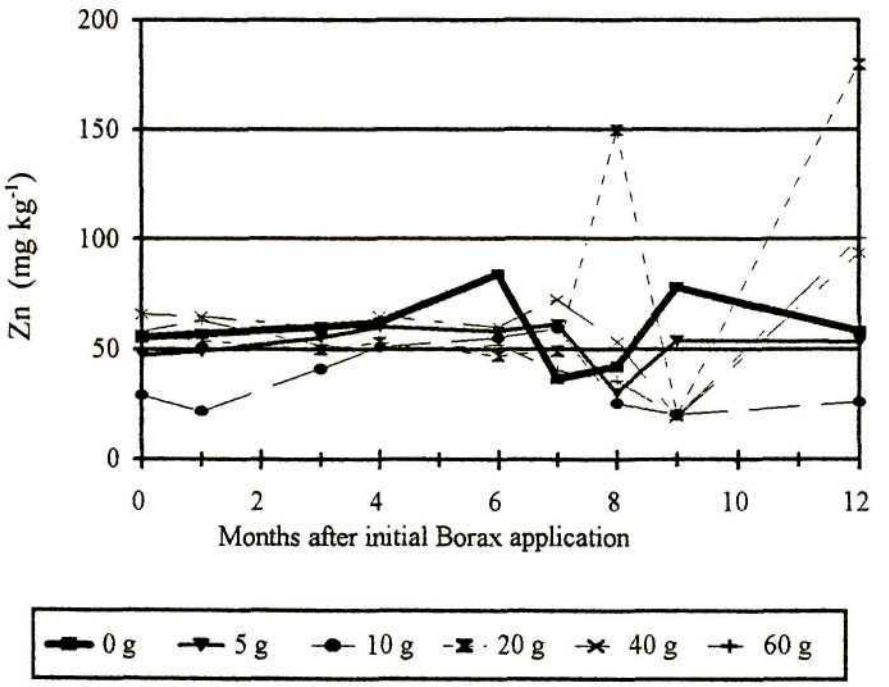

Figure 38: Effect of soil boron concentration on leaf zinc concentration of young 'Hass' trees. B rates are g borax m^{-2} soil canopy area $year^{-1}$, divided into 3 applications in October, February and April.

4.3.10 Fruit total phenolics

No consistent differences in trends were noted in fruit phenolic concentration from February to June 1996 (Figs. 39 & 40). Although deficiency could possibly increase phenolic concentration, results did not show this, since there are many interacting factors that would affect phenolic concentration. It is also possible that the role of B in avocado phenolic dynamics is involved in complexing (or inactivation) rather than affecting phenolic biosynthesis. It is likely that fruit sample size was not large enough, since only fruit growing in direct vertical alignment with a B source would receive adequate B supplies. Achieving an even spread of applied B is problematic, particularly at lower dose rates where it is impossible to uniformly distribute small amounts of borax over the entire drip line area.

Fruit total phenolic concentration was extremely high when comparing with results obtained by Donkin (1995) determining total phenolic concentration of avocados form Everdon Estate during the 1994 harvest season. Donkin noted total phenolic concentration to be in the region of 2 μg g^{-1} dry mass with little variation throughout the season. In contrast, results showed phenolic concentration in the range of 7 - 35 μg g^{-1} dry mass (Figs. 39 & 40) showed considerable fluctuation throughout the season. Differences between results obtained in this experiment and results of Donkin (1995) could have resulted from differences between the sites and growing season. While the 1994 season was regarded as a dry season associated with excellent fruit quality, the wetter 1996 season could have caused increased fruit phenolic concentration and could at least in part have contributed to the poorer quality of fruit in this season.

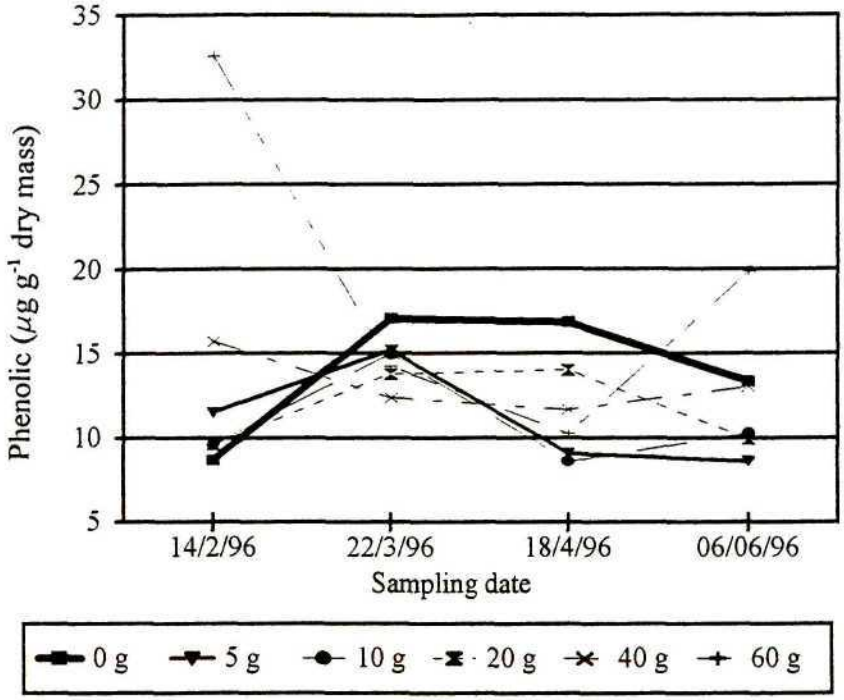

Figure 39: Effect of soil boron application on total fruit mesocarp phenolics of mature 'Hass' trees. B rates are g borax m^{-2} soil canopy area $year^{-1}$ divided into 3 applications in October, February and April.

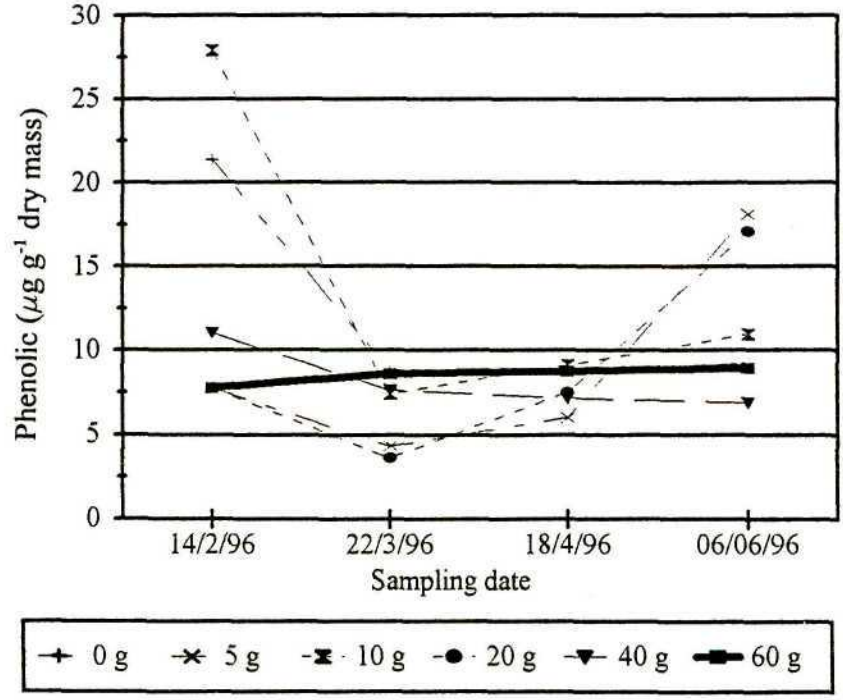

Figure 40: Effect of soil boron application on total fruit mesocarp phenolics of young 'Hass' trees. B rates are g borax m^{-2} soil canopy area $year^{-1}$ divided into 3 applications in October, February and April.

4.3.11 Tree Architecture

Tree architecture was shown to be modified by B application (Fig. 41). Moderate branching predominated in 0 g m^{-2} and 10 g m^{-2} applications, slight branching in 20 g m^{-2} and 40 g m^{-2} applications, while unbranched stems predominated in the 60 g m^{-2} treatment. An abnormality existed in that the 5 g m^{-2} treatment was largely unbranched or exhibited moderate branching. This could be as a result of leaching from the adjacent 40 g m^{-2} treatment (APPENDIX 2c), since the growing season was characterised by heavy rainfall.

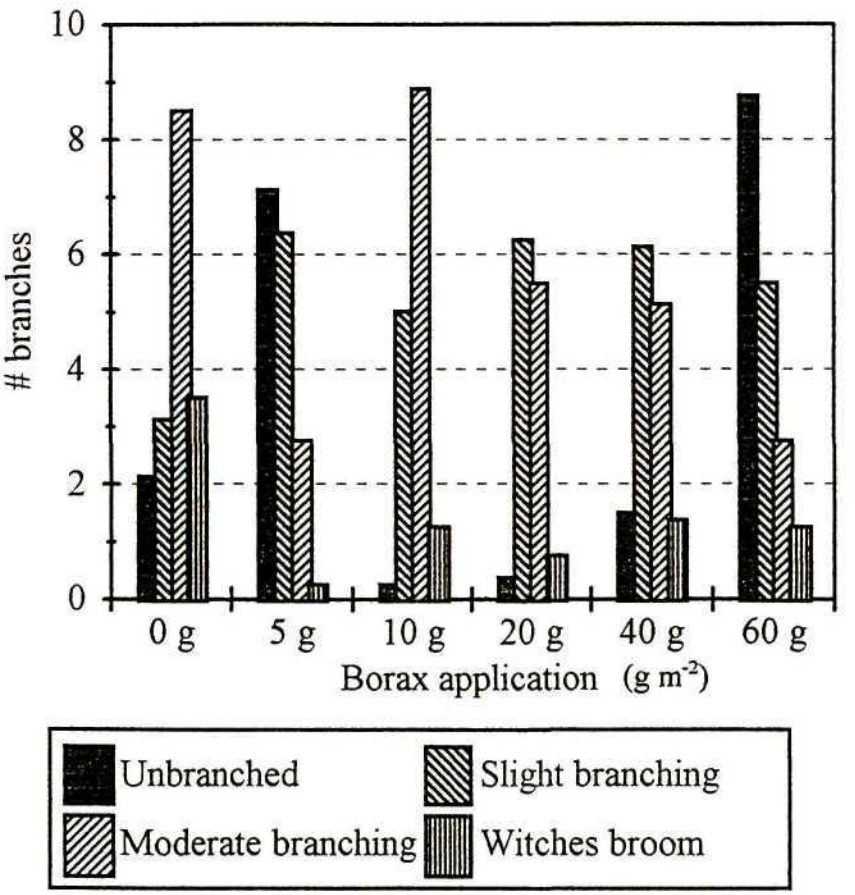

Figure 41: Effect of soil boron application on branching of young trees.

4.3.12 Postharvest quality

Soil B applications showed significant differences fruit firmness (after 28 days in cold storage) from mature trees while percentage mass loss in cold storage showed no significant effect or trend (Figs. 42 & 43) in mature trees. In the former, there was no trend with increasing B application, hence other factors are probably also largely responsible. Soil B application did not have any affect on firmness or mass loss on fruit harvested from younger trees. The possible positive effect of B can would be attributed to the role of B in maintaining membrane stability. Increased firmness would result from water retained, adding turgor pressure and stability to the cell. More severe deficiency in older trees may explain the more positive effect of soil application, whereas younger less deficient trees, were able to supply adequate B to developing fruit. Clearer results might have been attained if a larger sample size had been used, however this was not possible since supervision of time consuming harvesting operations are the priority during this time. Smith *et al.* (1997) found that soil B application significantly increased postharvest storage life. Further research is required in this area under South African conditions.

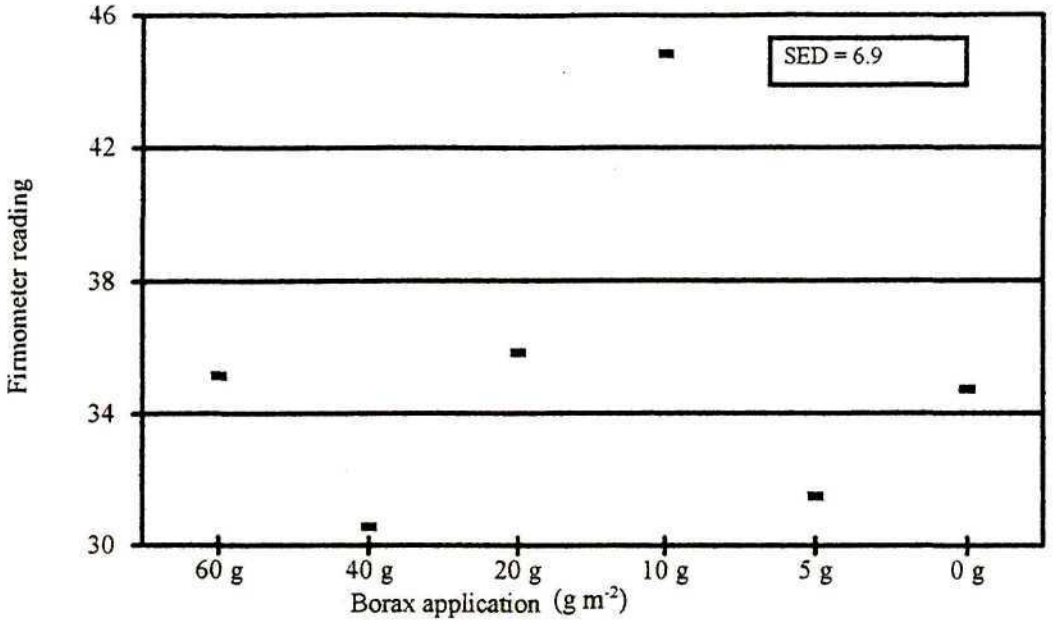

Figure 42: Effect of soil boron application on fruit firmness of fruit from older trees after 28 days cold storage.

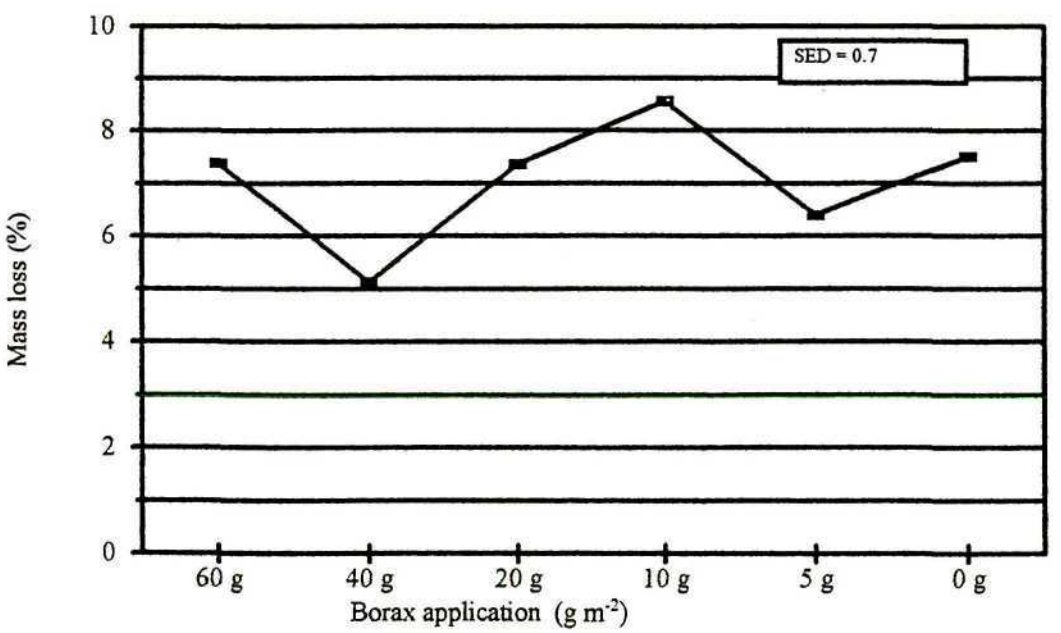

Figure 43: Effect of soil boron application on fruit percentage mass loss of fruit from older trees after 28 days cold storage.

4.3.13 Fruit size

Soil B applications increased fruit yield per tree in younger trees proportionally to application rate by up to 5 % (Fig. 44). Increase was not significant. Applications increased the number of fruit per tree proportionally to application rate up to 15 % in the 60 g m^{-2} treatment (Fig. 45) however, effect was not significant. Average fruit size was increased proportionally to application rate upto 4 % in the 60 g m^{-2} treatment (Fig. 46). Effect was not significant. Fruit of larger count sizes were produced by trees treated with B (Fig. 47).

Variation in older trees masked the effect of B and no trends were noted (Fig. 48). Older trees would be expected to take a number of seasons to recover from chronic B deficiency, and in particular to regenerate an efficient root system. Older trees produced more fruit of smaller count sizes than younger trees (Fig. 48), a well known response in 'Hass' (Whiley & Schaffer, 1994; Moore-Gordon *et al.*, 1997).

In younger trees, an increase in tree yield proportional to application rate (Fig. 44) resulted from a change in the fruit size distribution. Increased fruit number and therefore mass, particularly in the larger counts (14 and 16), contributed to larger mean fruit mass (Fig. 47). Increased number of fruit per tree would have also contributed to increased tree yield. The difference between the control (27 kg $tree^{-1}$) and 60 g m^{-2} $year^{-1}$ (32 kg $tree^{-1}$) at a spacing of 6 m x 6 m (277 trees per hectare) amounts to an increased yield of 1385 kg ha^{-1} or 2000 kg ha^{-1} on a 5 m x 5 m spacing. Since this was a young orchard with wide tree spacing, benefits would increase in proportion to tree canopy area. The experiment should be performed on a statistically designed experimental plot and results monitored for a number of seasons before any definite conclusions can be drawn. Results are summarised in TABLE 6.

TABLE 6: Summary of effects of B application of fruit yield.

Parameter	Control	60 g m^{-2} Borax	% difference
Average no fruit per tree	119.6	138.1	+15
Average fruit mass	222.8	232.0	+4
Average fruit yield per tree	26.98	32.00	+5

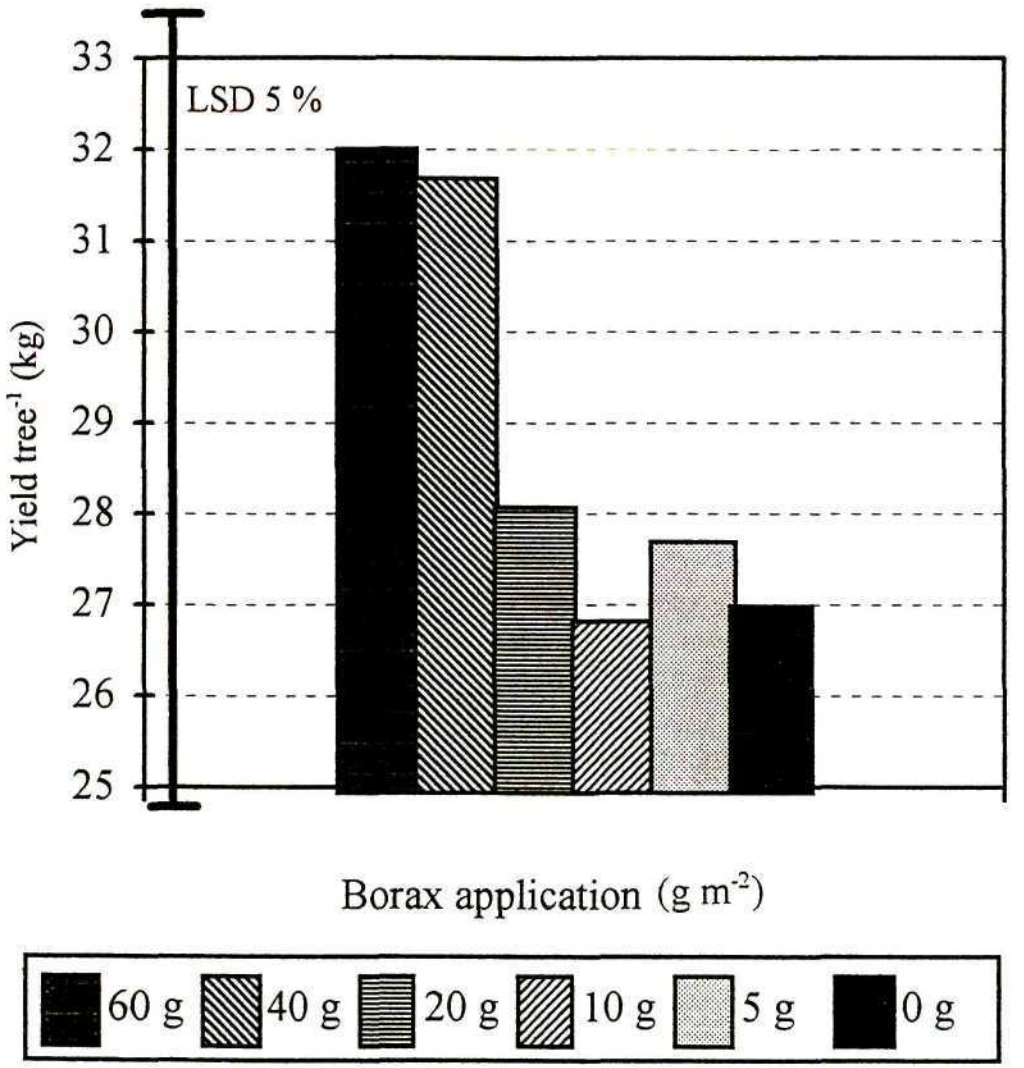

Figure 44: Effect of soil boron application on tree yield of young trees. Application rates are g of borax m^{-2} canopy soil area $year^{-1}$, divided into three applications.

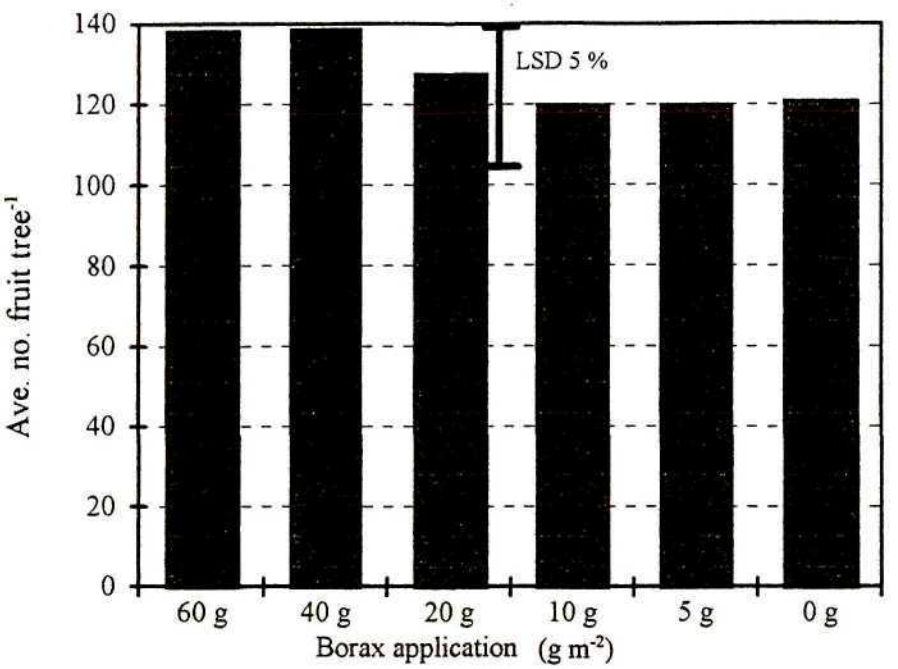

Figure 45 Effect of soil boron application on average number of fruit per tree. Application rates are g of borax m^{-2} canopy soil area $year^{-1}$, divided into three applications.

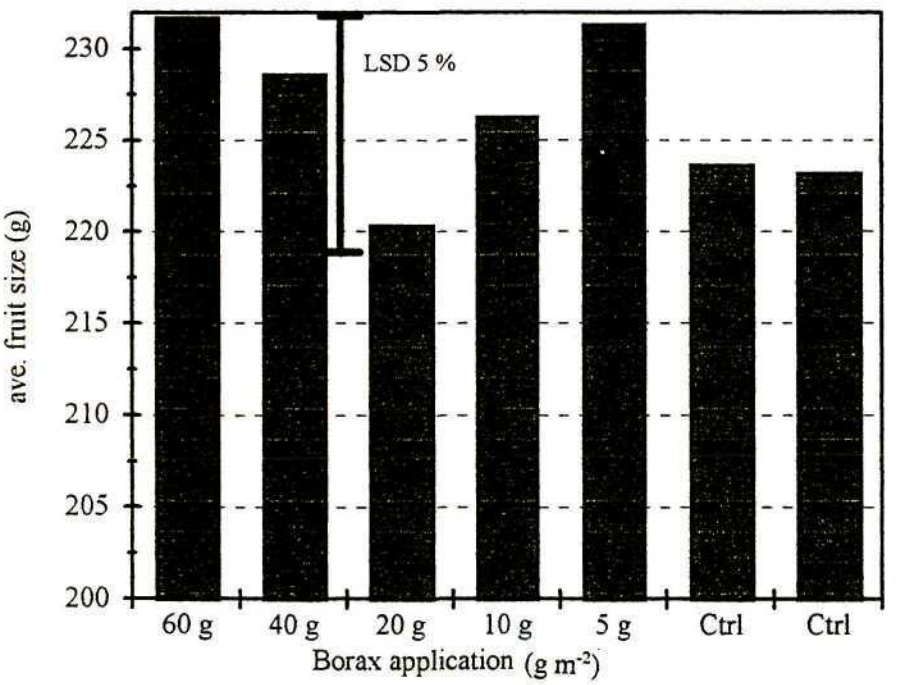

Figure 46: Effect of soil boron application on average fruit size of young trees. Application rates are g of borax m^{-2} canopy soil area $year^{-1}$, divided into three applications.

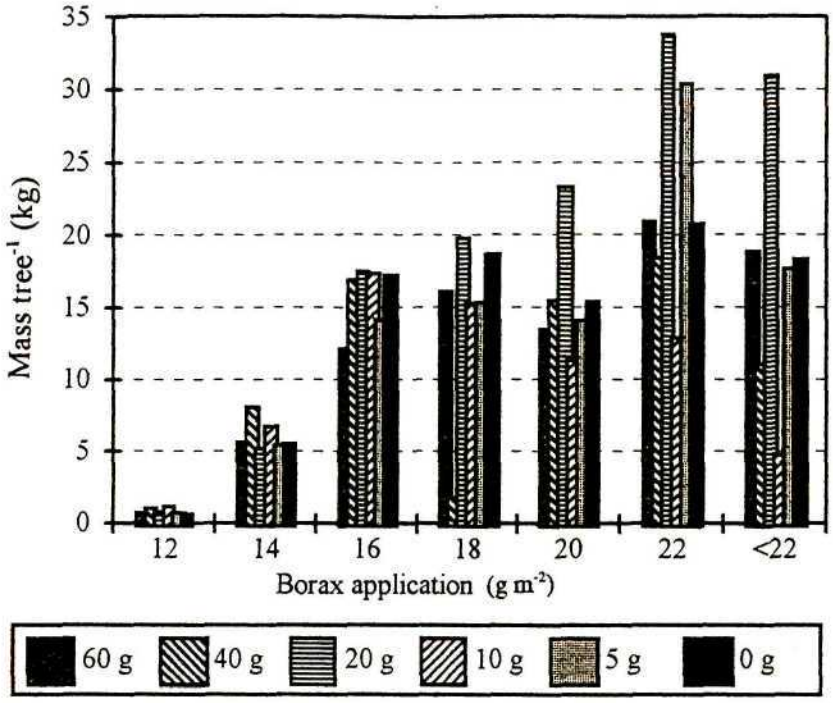

Figure 47: Effect of soil boron application on fruit mass per count in mature 'Hass' trees. B application rates are g borax m^{-2} soil canopy area $year^{-1}$ divided into 3 applications in October, February and April.

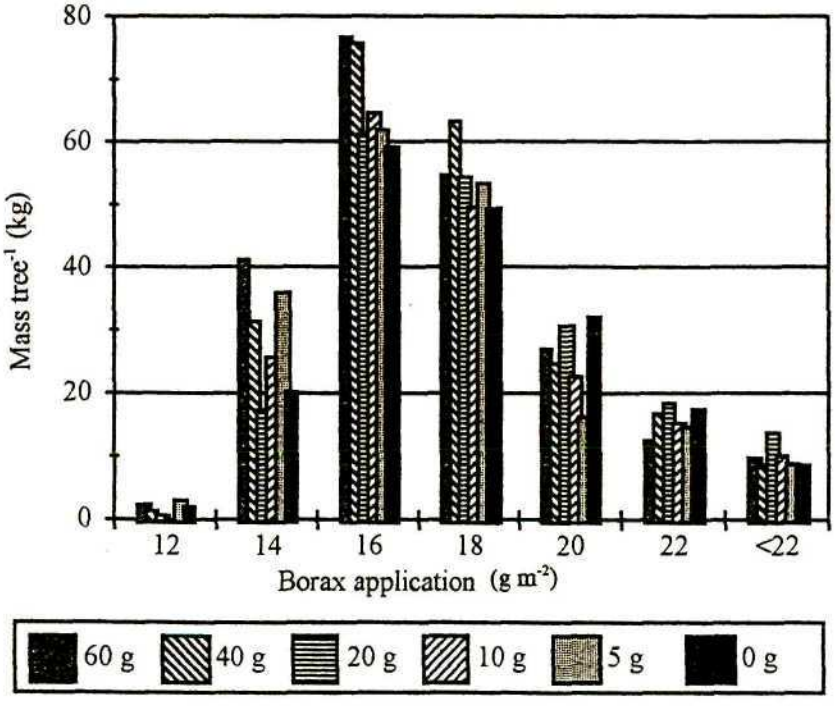

Figure 48: Effect of soil boron application on fruit mass per count in younger trees. B application rates are g borax m^{-2} soil canopy area $year^{-1}$ divided into 3 applications in October, February and April.

Increase in total fruit mass in smaller counts is clearly evident for young trees (Fig. 38), however no trends or differences were evident on yield of mature trees (Fig. 39).

4.4 DISCUSSION AND CONCLUSIONS

Preceding chapters have indicated that B deficiency is widespread in South African avocado orchards. This chapter indicates some of the effects that these deficiencies are causing in South African orchards, highlighting perceived benefits. Effects on tree production are numerous.

B affects pollen viability as seen in increased germination rate in treated trees. While pollination is not in itself a major problem in South African production, researchers have often hypothesised that the small fruit problem in 'Hass' could at least in part be related to pollen parent. The cultivar 'Ettinger' has been identified as a potent pollen parent (Conradie, 1997[21]) for 'Hass' in

Israel, however the physiological reason for this has not been investigated. The possibility exists that better pollinators are more effective utilizers and translocators of B. Since B plays a vital role in pollen function, it could play a significant role in this regard.

Results show that soil B applications are a relatively safe and efficient method of increasing avocado leaf B concentration. Results are in agreement with Miyasaka *et al.* (1992) who found a non significant increase in average fruit mass. While initial uptake during the first season after application was slow, rapid uptake occurred during the second season. Slow initial uptake in older trees can be explained by the severe nature of the deficiency, and probably the relatively poor root growth and probable *Phytophthora cinnamomi* infection in such trees. Furthermore, much of the B taken up may initially be tied up in the structural framework (roots and stem) before leaf concentration rises. Rate of uptake was greatest during times of feeder root growth, therefore applications should be made timeously to maximise efficiency of applications. Whiley & Schaffer (1994) similarly noted that leaf B concentration progressively declined in parallel with feeder root condition between the period of root flushing and flowering, the latter period when feeder root condition is particularly poor and leaf B concentration is at its lowest. During

[21]Conradie, W. Westfalia Estate, Duiwelskloof.

flowering and fruit set, demand for B is critical despite low feeder root health. Such findings should be taken into consideration when developing fertigation programmes, to facilitate uptake when feeder root condition is at its best. In addition, this demonstrates the benefit of a properly timed foliar B spray when feeder roots are unable to meet the trees high B requirements during flowering.

The very high rates (40 and 60 g m^{-2}) caused severe toxicity during the second season after application, appearing first in the older leaves, similar to other species that show poor phloem mobility. The similar nature of the toxicity symptom to other species where B is phloem immobile (Brown & Hu, 1996) is evidence for the poor phloem mobility of B in the avocado. In contrast, Whiley & Schaffer (1994) found significant decline of B in mature leaves adjacent to developing inflorescences. Similarly Coetzer *et al.* (1993) found that ^{10}B was remobilised from mature leaves to developing inflorescences. Indications are that the avocado is able to remobilise a certain (although probably limited) amount of B during this time of high B requirement. It is possible however that amounts of B remobilised are insufficient to meet the demand for B during this time and mobilization should be seen as the avocado trees method of coping with high B requirement and low B availability (due to poor feeder root health) during flowering and fruit set.

In applications of 5, 10 and 20 g m^{-2} borax, trees showed no toxic effects throughout the duration of the experiment. The lowest doses can only be regarded as maintenance doses, and higher applications upto 20 g m^{-2} borax are recommended (with caution) to rectify cases of severe deficiency within a single season. Results show that soil applications are able to raise leaf concentrations to the accepted optimum of 40 to 70 mg kg^{-1} without any foliar applications. Leaf samples analysed with the total absence of foliar sprays are considered to give true indications of plant B status. Indications are that the avocado is more tolerant of carefully chosen levels of soil B than the perception of local researchers and advisers, and in general these preliminary results are in agreement with rates used in Australia (Whiley *et al.*, 1996; Smith *et al.*, 1995). It is important that rates be continually adjusted on the basis of leaf analysis, and the wide range of factors which impact on B nutrition.

Soil applications successfully increased fruit size in younger trees, proportionally to the application rate. It should be emphasised that reasons for this were that the highest doses raised

leaf concentrations to the optimal leaf B range within the timespan of the experiment. Determination of the effect of B on fruit size was not possible in older trees because of high tree to tree variation within the orchard, the more severe nature of the deficiency and numerous other interacting factors affecting fruit size. There is evidence from Australia that adequate B nutrition improves 'Hass' fruit size (Smith *et al.*, 1997).

Results obtained can only be used as guidelines for B application to different soil types. Although soils at Cooling Estate are generally of lower clay percentage than most avocado growing soils in South Africa, the high rainfall experienced and relatively high topsoil organic matter content (a characteristic of the relatively cooler climate when compared with other production areas) are likely to have provided significant buffering against toxicity. Growers in warmer production areas (e.g. Levubu, Letaba and Kiepersol areas) where soils are less leached and borehole water, which can contain high levels of B, is used, should be more cautious with soil B applications even though the deficiency symptoms experienced in the field may be more severe than those seen in the KwaZulu-Natal midlands. Growers in sandier soils such as the Nelspruit, Barberton and Schagen production areas should also be more careful since sandier soils (derived from granite parent material) with extremely low organic matter content, are more prone to B toxicity. In such conditions, annual applications should be split into more than 3 applications and rates should be more conservative (5-10 g borax m^{-2} $year^{-1}$, according to leaf analysis).

A further limitation of the research, is that of the mesic climate in the KwaZulu-Natal midlands. This "mist belt" production area is less prone to severe droughts than production areas in the Northern Province and Mpumalanga areas. Nightly mist in some KwaZulu-Natal production areas, and particularly the Bruyns Hill production area, can amount to several millimetres of 'rain' per night (Seele, 1997[22]). Soil B applications still need to be tested during and following drought conditions, since rewetting of soil profiles that have considerable amounts of unused B could cause toxicity from the sudden increase in available soil B. Hence, in these warmer areas fertigation using small amounts of B per application would probably be more suited, since small quantities applied when needed (rather than in advance as in standard soil applications) could be easily leached if drought conditions are expected.

[22] Seele, W.P. Cooling Estate, Wartburg.

In hindsight, the experiment could have been performed on a completely randomised block or other statistically valid design. Initially, the trial was designed at the growers request, to keep all highest (and potentially most dangerous) applications in close proximity, the reason being to prevent movement of high concentrations of B to adjacent trees. In addition, the prime objective of the trial was to determine the effectiveness of soil B applications in raising leaf B concentrations without causing toxicity, rather than investigating the effect of B on fruit size. Research has shown that the avocado is reasonably tolerant of the application rates used. In addition possible effects of leaching between adjacent trees or rows did not appear to affect the trial in any way. A statistically valid design could have increased accuracy of measured yield increases. A further improvement would have been replication of leaf samples, however this was prevented by high cost of B analyses and a limited budget.

Indications are that soil borax applications did not cause any risk of leaf Na toxicity, although this requires further analysis in root samples.

Spurious results observed in some analyses of Mn, Cu and Zn indicate that analysis for these elements during this time gives poor indications of tree nutrient status, possibly due to contamination.

It should be noted that since only two seasons (in part) of results are available, the experiment should ideally be continued for at least another two seasons to reduce error. The two highest borax rates should be discontinued, since they are unlikely to be recommended in the field, and were in fact purposely chosen to induce toxicity.

5. EFFECT OF SOIL BORON APPLICATION ON RECENTLY GRAFTED TREES IN CONTROLLED ENVIRONMENTS

5.1 INTRODUCTION

As field experiments have shown, chronic B deficiencies can be effectively overcome. However this can take some time if root systems are degraded. There was much speculation *a priori* in the local avocado industry as to whether 'Duke 7' rootstock was capable of soil B uptake in sufficient quantities. Australian researchers had suggested that other rootstocks, particularly those of Guatemalan origin such as 'Velvick' and 'Edranol' seedlings are approximately 20 % more efficient in taking up B than the clonal 'Duke 7' rootstock (Smith *et al.*, 1995). This still needed to be proven in South Africa, on local avocado growing soils, which are somewhat different to those in Australia. This experiment therefore originally aimed at comparing the effectiveness of two different rootstocks (Mexican clonal 'Duke 7' vs. Guatemalan seedling 'Edranol') in two different soils. Since the 'Duke 7' rootstock in this trial had the advantage of being transplanted under more mesic autumn conditions, and the 'Edranol' started later and hardened off in colder winter conditions, comparisons of the amounts of boron translocated were difficult. The emphasis of the experiment therefore shifted to what soil B levels would induce toxicity for the two rootstocks, and the associated leaf concentrations of B in leaf tissue. Rootstock comparisons were however possible since differences were fairly substantial despite differences in time of planting.

The experiment also aimed at probing the lime/calcium:boron relationship, which is surrounded by substantial controversy in the South African avocado industry.

5.2 MATERIALS AND METHODS

Fifty 'Hass' nursery plants on clonal 'Duke 7' rootstock were obtained from Westfalia Nursery, Duiwelskloof after the first flush following grafting had matured, ie. relatively immature nursery trees with few leaves. Plants were transplanted into 8 L white plastic drained containers containing Inanda soil forms from the Winterskloof area (Soil 1), or Cooling Estate, Bruyns Hill (Soil 2). The former site, a cool south-west facing valley near Hilton, was selected because avocado trees in this area showed chronic B deficiency symptoms, in addition to its sandier nature

(parent material Middle Ecca sandstone) which one would expect to produce toxicity symptoms under relatively low application rates. The Bruyns Hill soil was derived from Clarens sandstone and had a higher clay content. Before transplanting, special care was taken to remove the composted pinebark growing medium used in the nursery, from the roots, as this was presumably higher in B content than soils used. The experiment was designed as a 4 x 2 factorial, with 4 levels of B (2, 4 and 8 g borax m^{-2} pot surface area) applied to the two physically and chemically contrasting soils. The fourth treatment was 8 g borax m^{-2} applied in combination with 40 g calcitic lime and 40 g gypsum per pot, and aimed at investigating the calcium/boron relationship. Lime was mixed into the profile to a depth of 30 cm, while gypsum was applied to the soil surface. The experiment was repeated using very young 'Hass' nursery trees grafted on seedling 'Edranol' rootstock, which were received in May 1996. The entire experiment was arranged as a completely randomised block design in Glasshouse 2 in the Controlled Environment Research Unit at the University of Natal, Pietermaritzburg. Growing conditions were affected by an unusually warm autumn, extremely cold winter and a prematurely hot spring. Night temperatures however were maintained when possible above 7 °C by a 2 kW fan heater in cold weather and between 18° C (night) and 28 °C (day) by fans and evaporative cooling through a wet wall.

Although a 100 % transplant success rate was obtained for the 'Duke 7', 10 plants on 'Edranol' rootstock died during dry conditions causing high evaporative demand after transplanting. Fortunately, this occurred before B treatments had been applied, hence the trial could be modified so as to lose one replication for each treatment. These trees were also considerably less mature (post-grafting) than the trees on 'Duke 7', and therefore did not endure transportation from the Duiwelskloof nursery as well.

Pots were raised on bricks to minimise the risk of *Phytophthora cinnamomi* infection. In addition, white pots were specifically used to keep soil temperatures as low as possible, a further preventative measure to minimise chances of disease infection. A copper sulphate foot dip was positioned at the glasshouse door for treatment of shoes when entering the trial area. Plants were individually irrigated by hand to field capacity (determined by the glistening point method) on a daily basis to minimise leaching.

5.2.1 Soil analyses

5.2.1.1 Soil Fertility

The two soils were analysed for P, K, Ca, Mg, and Zn in addition to soil organic carbon, pH(KCl), acid saturation and cation exchange capacity at Cedara Feed Laboratory. Soil B analysis was performed at Noordwes Laboratory, Lichtenburg using the hot water extraction method (Wear, 1965).

5.2.1.2 Soil texture

Soil texture was measured by separating into clay, silt and sand components. After mixing with calgon solution, the sample was agitated in a measuring cylinder. Three minutes was allowed for settling of sand and silt fractions, whereafter the density of the solution was determined using a hydrometer. Following this, silt and clay fractions were removed from the cylinder to determine the sand fraction. The difference between the sand and calculated clay fraction provided the silt fraction value.

5.2.1.3 Soil water holding capacity

Soil water holding capacity was determined using the glistening point method developed in the Department of Agronomy, University of Natal (Anon, 1993). A volume of 10 ml oven dried soil was placed into a 30 ml beaker and weighed. Distilled water was added by slow single drops until glistening point was reached, whereupon the soil plus beaker mass was noted. Water holding capacity was calculated as grams water held per gram of soil.

5.2.2 Growth rates

Stem diameter was measured at a height of 2 cm above the soil surface initially and at the termination of the trial. Fresh and dry mass of shoot and root system were determined at the termination of the trial, and the root:shoot ratio was calculated. Differences in tree branching patterns were also noted.

5.2.3 Leaf nutrient concentration

Leaf boron concentration was measured 2 weeks prior to the termination of the trial. At this time, 3 to 5 leaves were taken from each plant, dried at 60 °C before ashing and analysing for K, Ca, Mg, Na, B, Zn, Cu and Mn as described in section 4.2.2. Due to the very young and immature nursery trees received from Westfalia Nursery, with few and very young scion leaves, it was not possible to sacrifice leaves for a complete leaf analysis at the start of the trial. Results of B analysis were subjected to regression analysis to determine the linear effect of increasing soil B application.

5.3 RESULTS AND DISCUSSION

5.3.1 Soil analyses

5.3.1.1 Soil chemical composition

Results reflect typical avocado soil fertility; both soils being relatively acidic, and extremely deficient in P (TABLE 7). Soil 1, originating from a midslope position in Winterskloof is slightly less acidic and somewhat more fertile. In addition, this soil has considerably more organic carbon and B (TABLE 8). Both soils can be classed in the low B range.

TABLE 7 : Soil analyses for soils used

	P ($mg\ L^{-1}$)	K ($mg\ L^{-1}$)	Ca ($mg\ L^{-1}$)	Mg ($mg\ L^{-1}$)	Total cations	Acid saturation	pH (KCl)	Zn ($mg\ L^{-1}$)	Organic carbon (%)
Soil 1	3	509	2188	603	17.22	0	5.4	3.4	5.0
Soil 2	5	229	1382	245	9.67	2	4.5	1.3	2.8

TABLE 8 : Soil B concentrations of soils used

Soil origin	Boron concentration (mg kg^{-1})
Soil 1 - Winterskloof	0.20
Soil 2 - Wartburg	0.08

Chemically, both soils are very similar, showing low levels of soil P, normal to above normal levels of K, Ca and Mg (APPENDIX 5). The high CEC of the Winterskloof soil (Soil 1) can be attributed to the high organic carbon content. Both soils show low acid saturation, soil 1 having a pH in the ideal range, however soil 2 showing a more acidic nature. Zinc concentrations are low for both soils, typical of soil characteristics in the KwaZulu-Natal midlands. Soil 1 has an extremely high organic carbon content, while soil 2 has sufficient organic carbon to classify it as a humic A topsoil. It should be noted that the sample of soil 2 was taken from Cooling Estate, which was discussed in chapters 2 and 3, however higher soil organic carbon levels of this sample can be explained by the cooler microclimate of the area where the sample was taken, which is located some distance from the estate's avocado section. Soil from this area was used to ensure *Phytophthora* free conditions, which could have affected results, particularly since 'Edranol' rootstock is far more *Phytophthora* sensitive than 'Duke 7' rootstock.

5.3.1.2 Soil texture

Soil 1 proved to be exceptionally sandy for a KwaZulu-Natal avocado growing soil and was classed as a loam using the soil texture triangle. Soil 2, considered a light textured avocado growing soil in comparison to other avocado growing estates, was classed as a sandy clay (Fig.49).

Thus both soils were relatively light textured soils on which B toxicity is more likely to occur than on more representative avocado growing soils, which have typically 35-55 % clay. Theory would suggest that toxicity would occur on the sandier soil 1 at lower application rates than it would on soil 2.

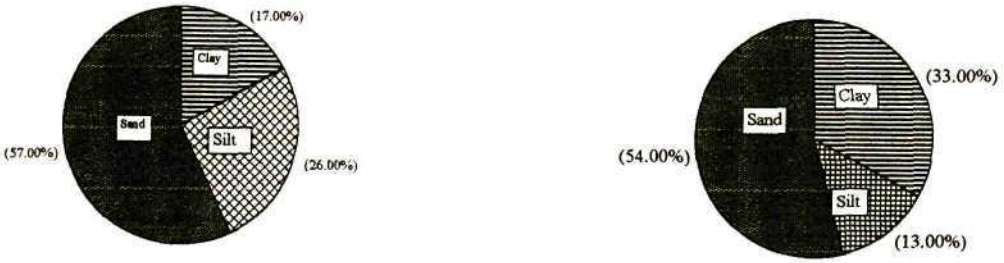

Figure 49: Soil particle analysis fractions for soil 1 and soil 2.

5.3.1.3 Water holding capacity

Water holding capacity of soil 1 was higher than soil 2 (Fig. 50) despite sandier texture. This can possibly be explained by the high organic matter content of soil 1, enabling it to hold greater volumes of water.

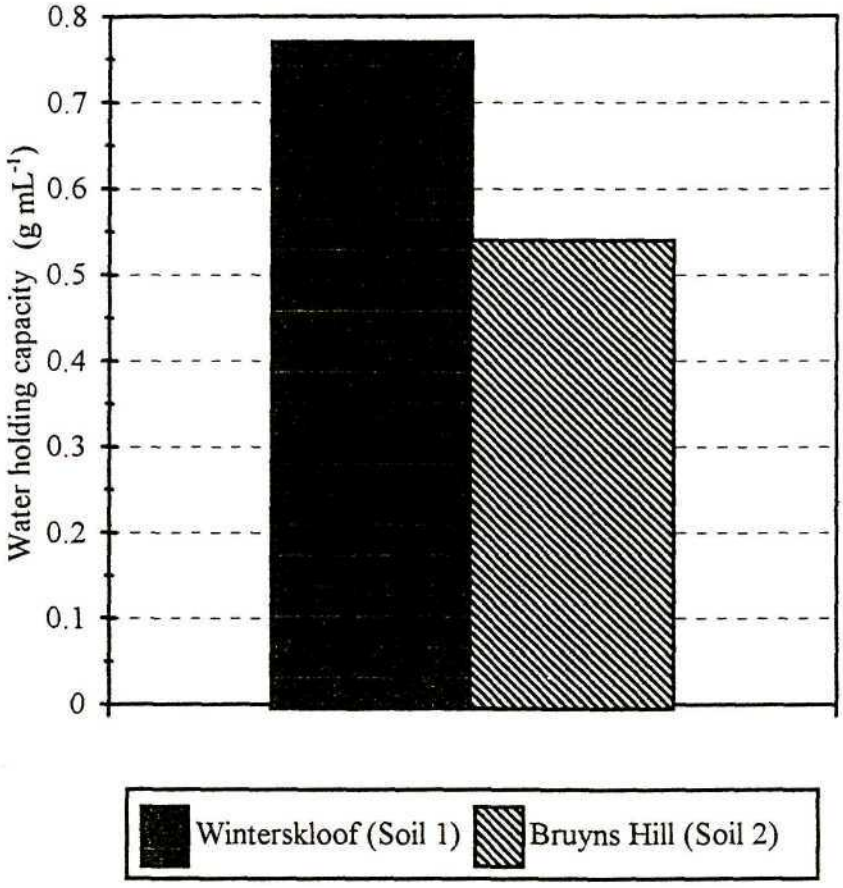

Figure 50: Soil water holding capacity from Wintershoof and Bruyns Hill determined using the glistening point method.

5.3.2 Growth Rates

5.3.2.1 Shoot flushing

No marked differences resulting from boron treatments were evident between soil type for 'Duke 7' rootstock (Figs. 51 & 52). It is evident that one full shoot flush was completed, peaking between two and three months after the start of the trial. Shoot growth then declined markedly, to be followed by a second flush which peaked when the trial was terminated after 5 months. Growth in 'Edranol' began slowly (Figs. 53 & 54), since trees were planted 2 months after 'Duke 7'. 'Duke 7' growth appeared to be greatest in the 12 g m^{-2} treatment in soil 1. This difference is probably not attributable to the effect of the B. It appears that apart from this abnormality, soil type and soil B application have no effect on shoot flushing.

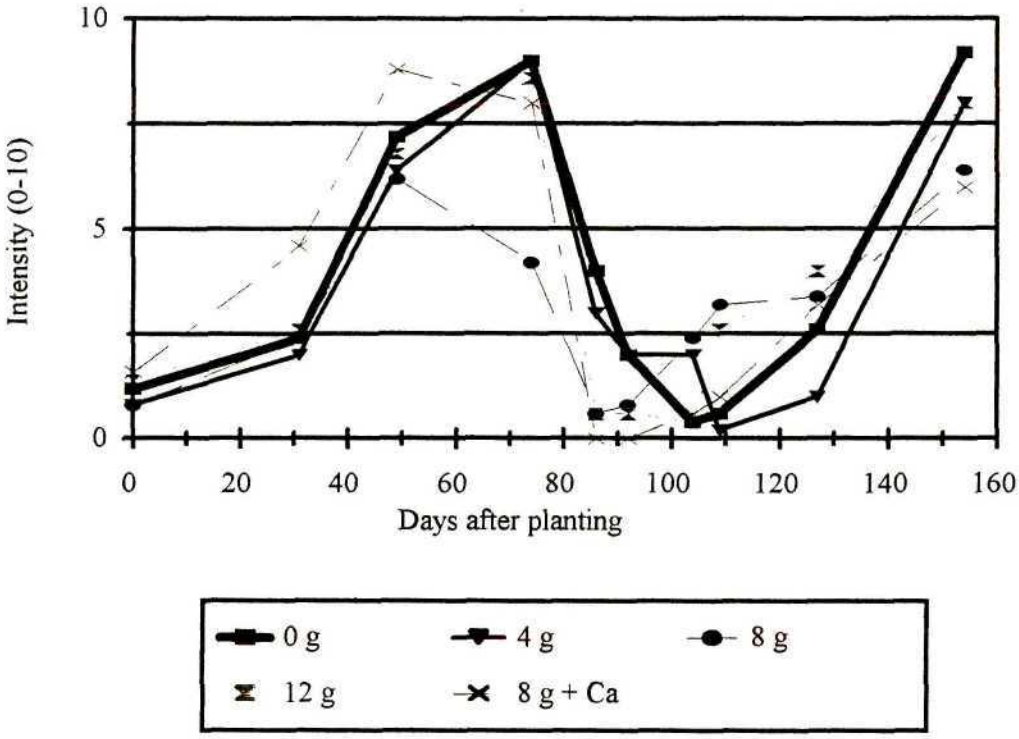

Figure 51: Shoot flushing of 'Hass' on 'Duke 7' rootstock growing in soil 1. Applications are g borax m^{-2} pot surface area applied as one single application after planting. Ca indicates application of 40 g calcitic lime and 40 g gypsum per replication.

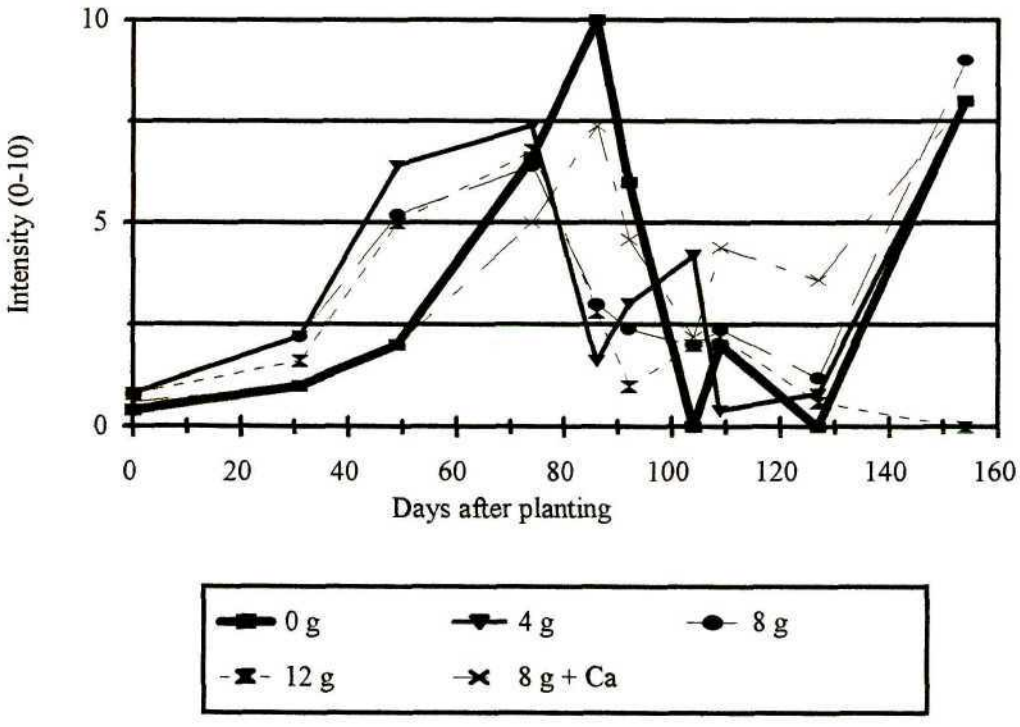

Figure 52: Shoot flushing of 'Hass' on 'Edranol' rootstock growing in soil 1. Applications are g borax m^{-2} pot surface area applied as one single application after planting. Ca indicates application of 40 g calcitic lime and 40 g gypsum per replication.

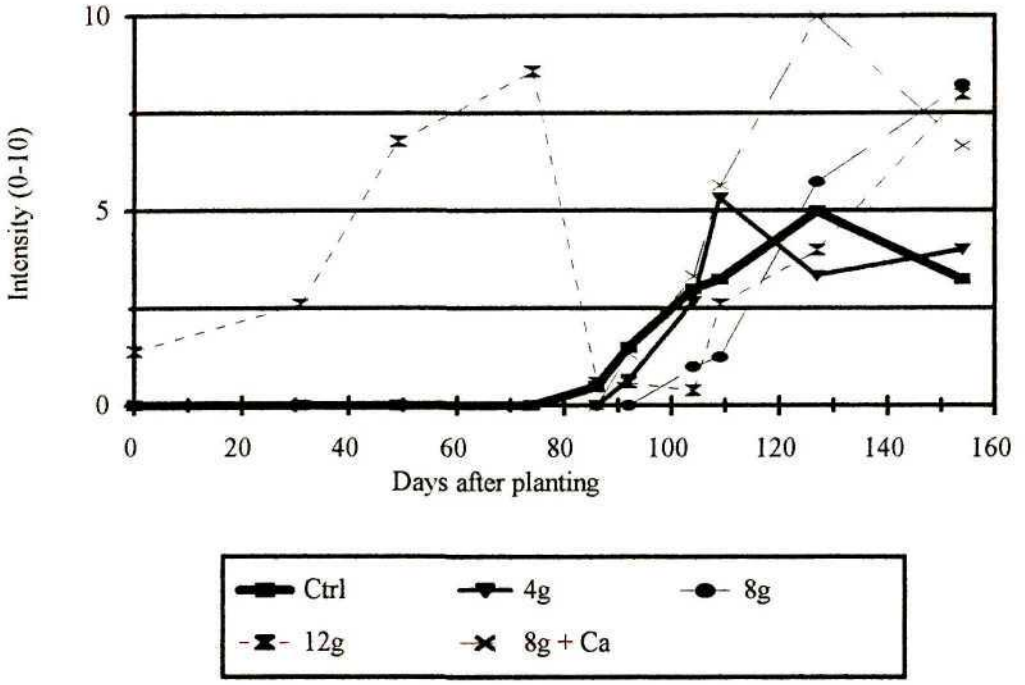

Figure 53: Shoot flushing of 'Hass' on 'Duke 7' rootstock growing in soil 2. Applications are g borax m^{-2} pot surface area applied as one single application after planting. Ca indicates application of 40 g calcitic lime and 40 g gypsum per replication.

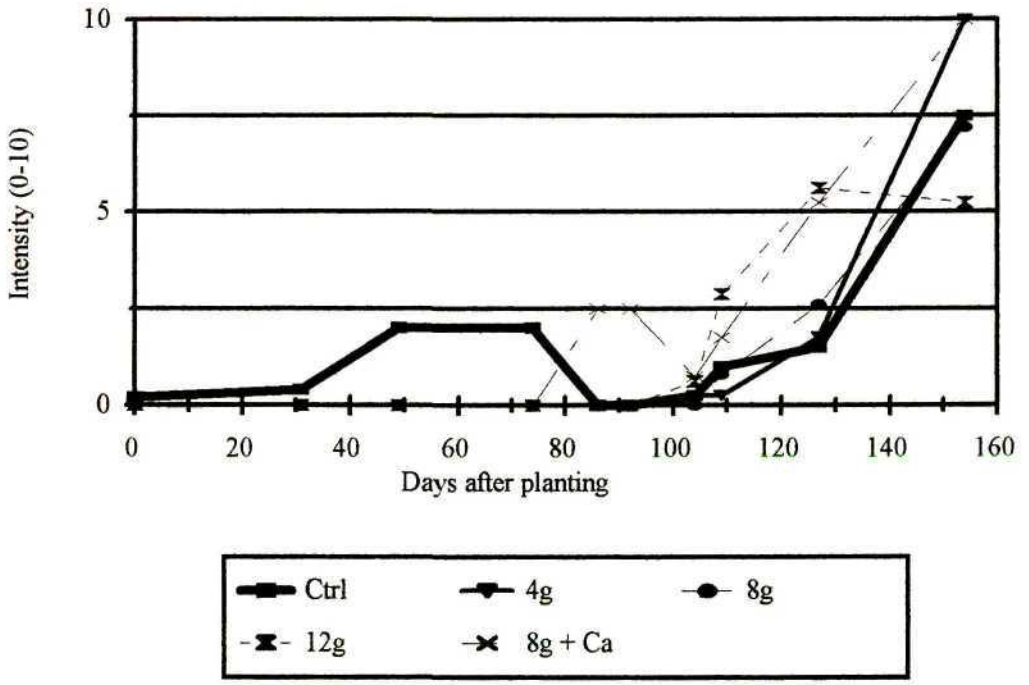

Figure 54: Shoot flushing of 'Hass' on 'Edranol' rootstock growing in soil 2. Applications are g borax m^{-2} pot surface area applied as one single application after planting. Ca indicates application of 40 g calcitic lime and 40 g gypsum per replication.

5.3.2.2 Stem diameter

Effect of B application rate and soil type on stem diameter were highly significant ($P< 0.001$) in 'Duke 7'. Stem diameter decreased linearly with increasing B application (TABLE 9). Negative effects of B appeared to be reversed in both rootstocks by Ca application (Figs. 55 & 56).

TABLE 9: Effect of soil B application on stem diameter of 'Duke 7' and 'Edranol' rootstocks. Applications are g borax m^{-2} pot surface area applied as a single application after planting.

Treatment ($g\ m^{-2}$)	'Duke 7' diameter (mm)		'Edranol' diameter (mm)	
	Soil 1	Soil 2	Soil 1	Soil 2
0	12.0	11.1	11.5	10.4
4	11.0	9.8	12.2	9.5
8	11.0	8.7	9.0	8.9
12	9.0	9.0	6.4	7.9
8 + Ca	12.7	10.0	10.2	9.7
Mean	11.2	9.7	9.9	9.3
SED	0.63		0.58	

5.3.2.3 Root fresh and dry mass (overleaf)

Increasing soil B applications resulted a linear decrease in root fresh dry mass in both 'Duke 7' and 'Edranol' rootstocks (Figs. 55-58). Liming reversed the effects of B applications. Effect of soil type on root fresh mass was highly significant ($P< 0.001$) in 'Duke 7' plants (Fig. 55). Soil 1, having a lower clay percentage, showed an increase in root fresh mass of 173 g (± 27.4) over soil 2. This sandier soil resulted in a more developed root system, probably because more pronounced dry periods warranted a greater root system for water uptake. Soil 2 showed linear decrease in root fresh mass with increasing soil B application up to 8 g. This resulted from toxic levels of B held in the soil which slowed growth considerably. Effect was not significant. No consistent decrease in root mass was noted in soil 1, probably as a result of B leaching, hence toxicity effects resulting from highest application rates were marginal. 'Edranol' showed highly significant ($P<0.01$) effects of both soil type and B application (Fig. 56). Fresh mass decreased linearly with increased B application in both soils.

Similar trends were seen in root dry mass results (Figs. 57 & 58), reflecting root fresh mass results. Effect of soil B application was significant in the case of 'Duke 7' ($P< 0.05$), where increasing B application decreased dry mass. Results obtained in 'Edranol' were similar, however level of significance was higher ($P<0.01$). In 'Edranol', effects of B application and soil type were both highly significant for root fresh and dry mass ($P< 0.001$). Mass decreased linearly with increasing B application (Figs. 56 & 58). This resulted since 'Edranol' is more efficient at B uptake than 'Duke 7', hence 'Edranol' root growth would have been affected at lower application rates, toxicities occurring at lower doses.

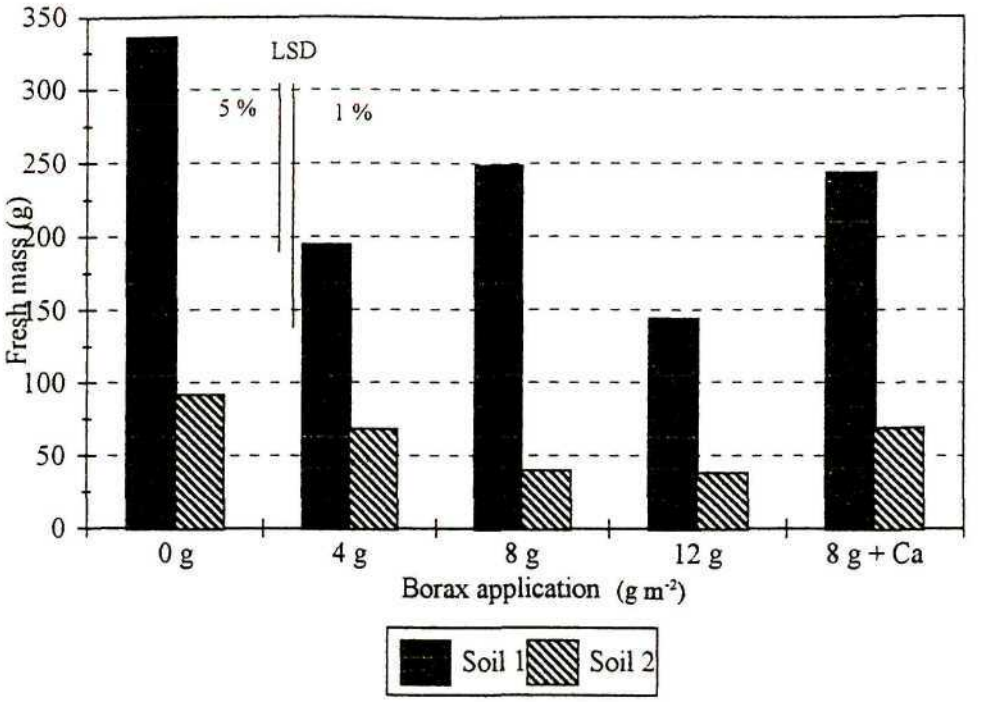

Figure 55: Effect of soil boron application on root fresh mass of plants growing on 'Duke 7' rootstock. B rates are g borax m^{-2} pot surface area applied in 1 single application after planting. Ca indicates application of 40 g calcitic lime and 40 g gypsum per replication.

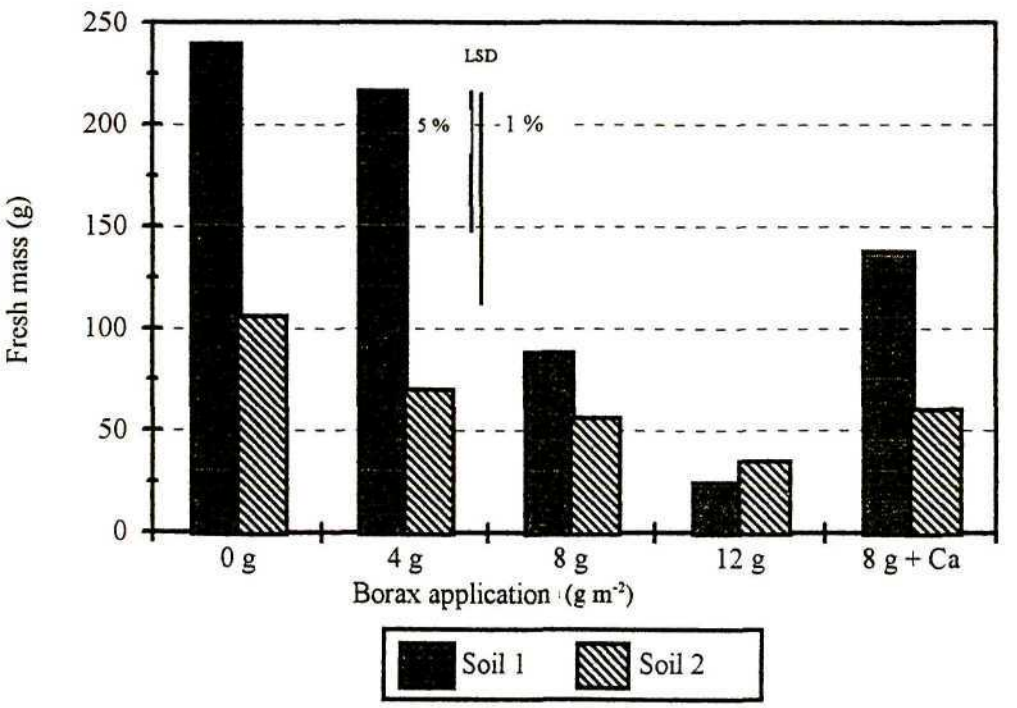

Figure 56: Effect of soil boron application on root fresh mass of plants growing on 'Edranol' rootstock. B rates are g borax m^{-2} pot surface area applied in 1 single application after planting. Ca indicates application of 40 g calcitic lime and 40 g gypsum per replication.

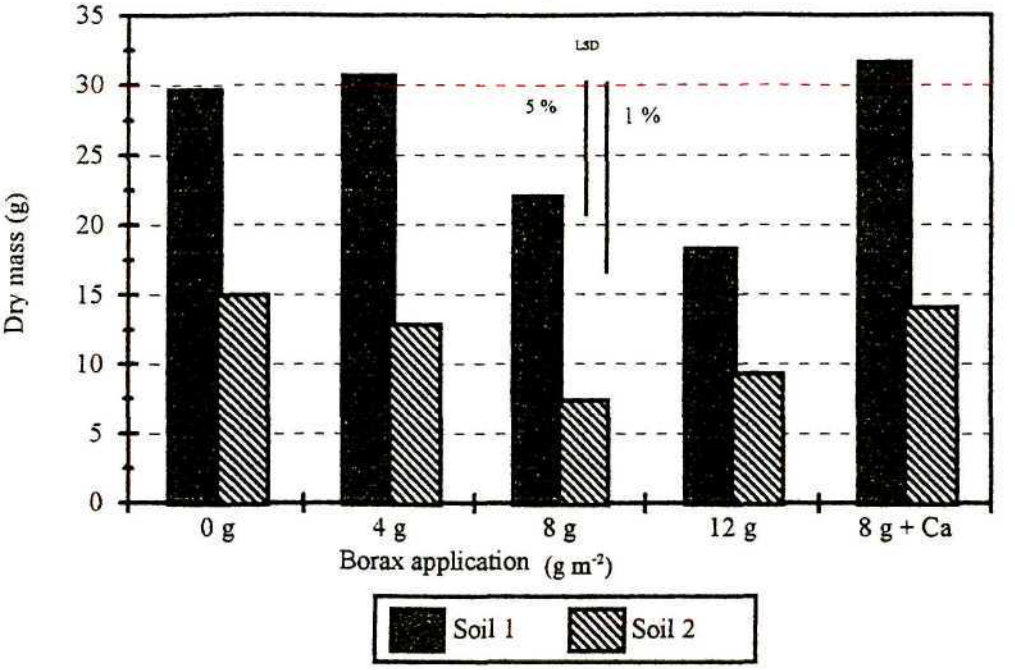

Figure 57: Effect of soil boron application on root dry mass of plants growing on 'Duke 7' rootstock. B rates are g borax m^{-2} pot surface area applied in 1 single application after planting. Ca indicates application of 40 g calcitic lime and 40 g gypsum per replication.

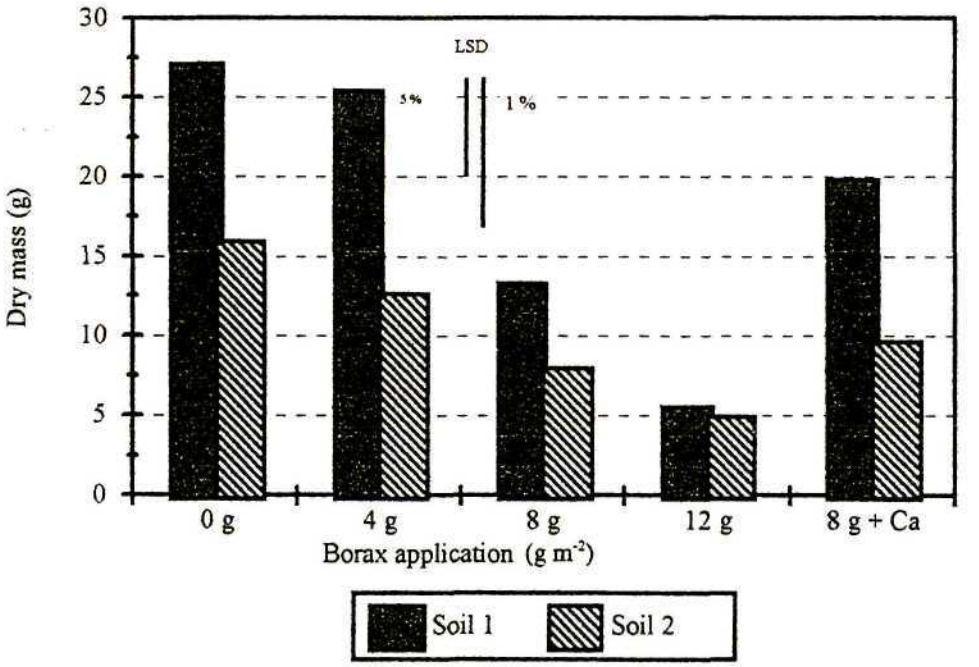

Figure 58: Effect of soil boron application on root dry mass of plants growing on 'Edranol' rootstock. B rates are g borax m^{-2} pot surface area applied in 1 single application after planting. Ca indicates application of 40 g calcitic lime and 40 g gypsum per replication.

5.3.2.4 Shoot fresh and dry mass (overleaf)

Increasing soil B applications resulted in a linear decrease in shoot fresh and dry mass (Figs. 59-62). Lime reversed the effect of B in 'Edranol' but not 'Duke 7'. Results exhibited similar trends for shoot system fresh and dry mass (Figs. 59 - 62). The effect of soil B application on shoot fresh mass was not significant in 'Duke 7', however, effect of soil type was highly significant ($P< 0.001$) (TABLE 10). Soil 1 showed on average 173 g (±27.4) higher dry mass (Fig. 61). This can be attributed to larger root systems in soil 1, enabling sustenance of a larger shoot system. There was a trend for shoot mass to decrease linearly with increasing soil B application in both soils. Decreases can be attributed to increasing B application, affecting root system size thereby affecting shoot systems. At highest application rates, toxicity resulted in defoliation, resulting in dramatic decreases in shoot system mass.

Soil B application up to 12 g m^{-2} and soil type resulted in highly significant ($P< 0.001$) differences in fresh mass of 'Edranol'. Shoot fresh mass decreased linearly in soil 2 with increasing application, however, decreases were only noted in soil 1 above 4 g m^{-2} (Fig. 60). The significant effect of soil application can be attributed to higher efficiency of B uptake in 'Edranol', causing toxic effects at lower doses. 'Duke 7' shoot dry mass was significantly affected by B application ($P< 0.05$) while the effect of soil type was highly significant ($P< 0.001$). Dry matter decreased linearly with increasing B in soil 2, however decreases were only noted in soil 1 at doses above 4 g m^{-2} (Fig. 62). In contrast, root dry mass increased linearly ($P< 0.01$) (Fig. 57), while effect of soil type was not significant (TABLE 10). In addition, the limed soil treatment significantly decreased dry mass.

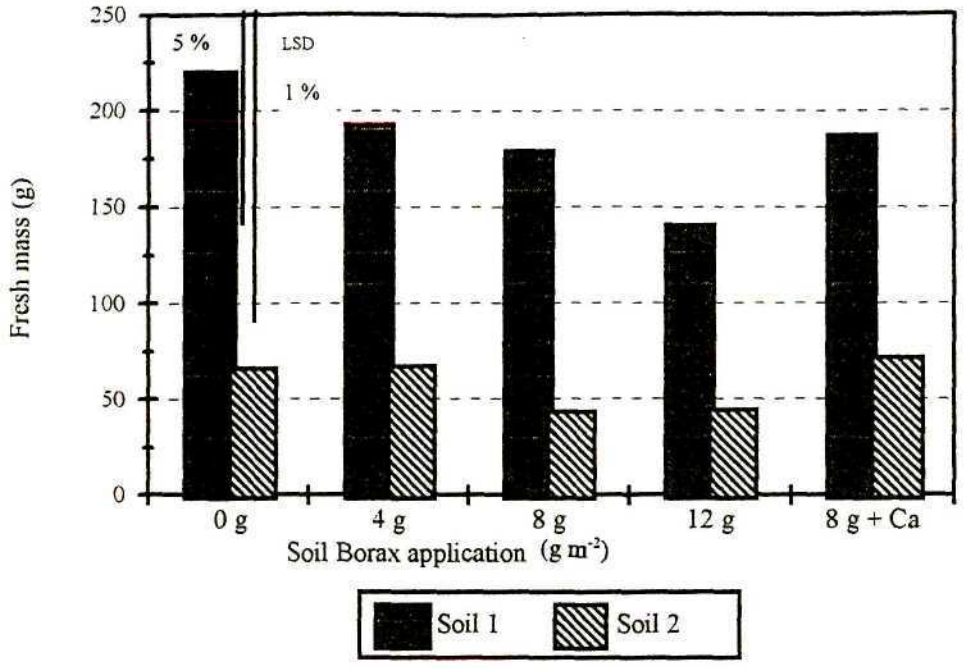

Figure 59: Effect of soil boron application on shoot fresh mass of plants growing on 'Duke 7' rootstock. B rates are g borax m^{-2} pot surface area applied in 1 single application after planting. Ca indicates application of 60 g calcitic lime and 60 g gypsum per replication.

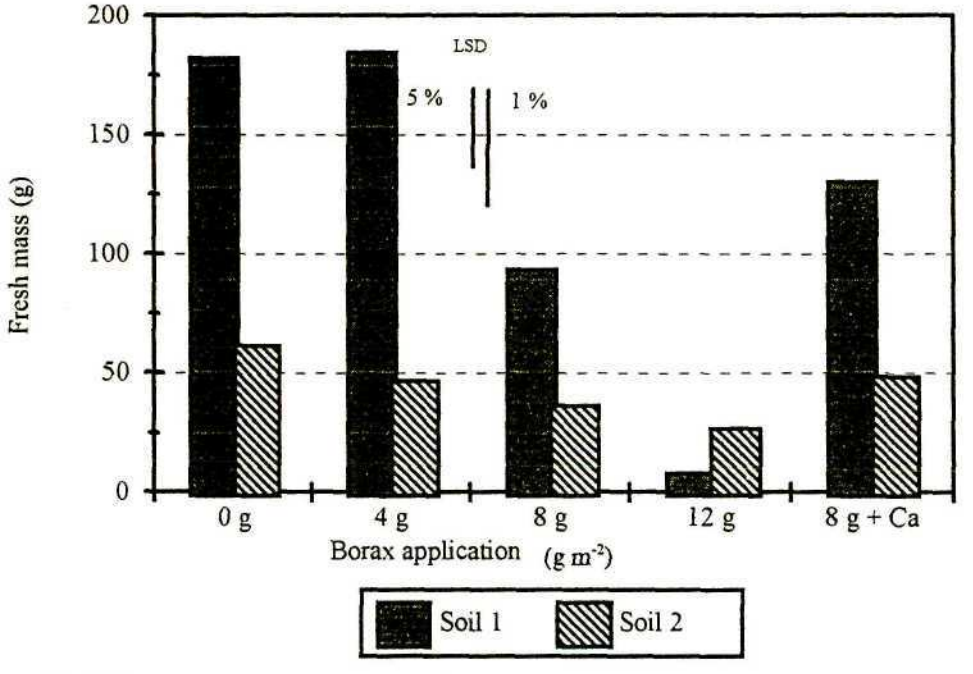

Figure 60: Effect of soil boron application on shoot fresh mass of plants growing on 'Edranol' rootstock. B rates are g borax m^{-2} pot surface area applied in 1 single application after planting. Ca indicates application of 40 g calcitic lime and 40 g gypsum per replication.

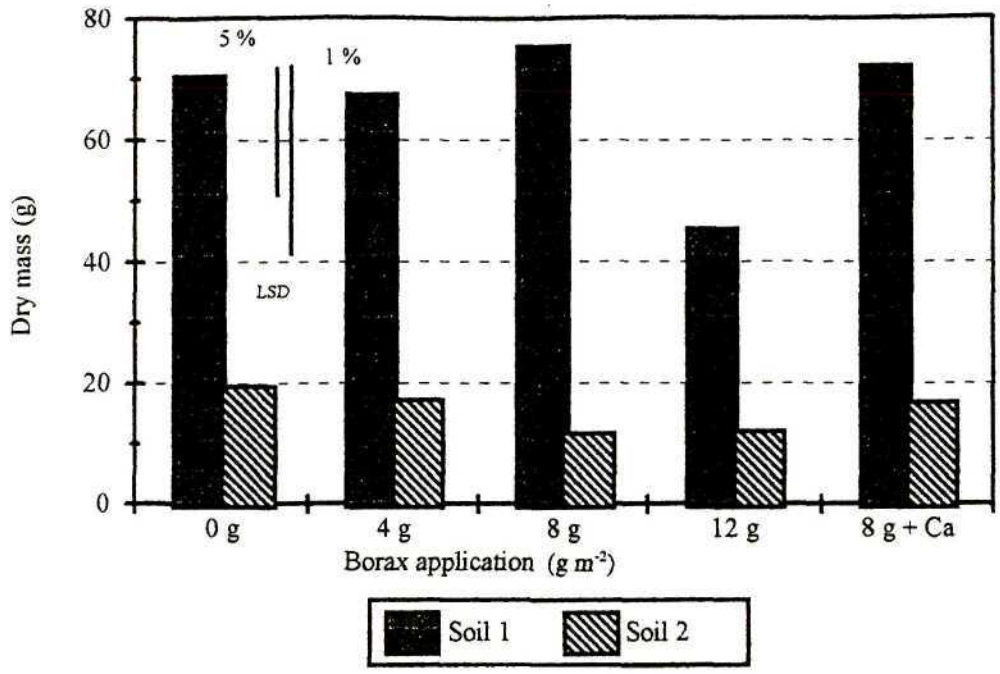

Figure 61: Effect of soil boron application on shoot dry mass of plants growing on 'Duke 7' rootstock. B rates are g borax m^{-2} pot surface area applied in 1 single application after planting. Ca indicates application of 40 g calcitic lime and 40 g gypsum per replication.

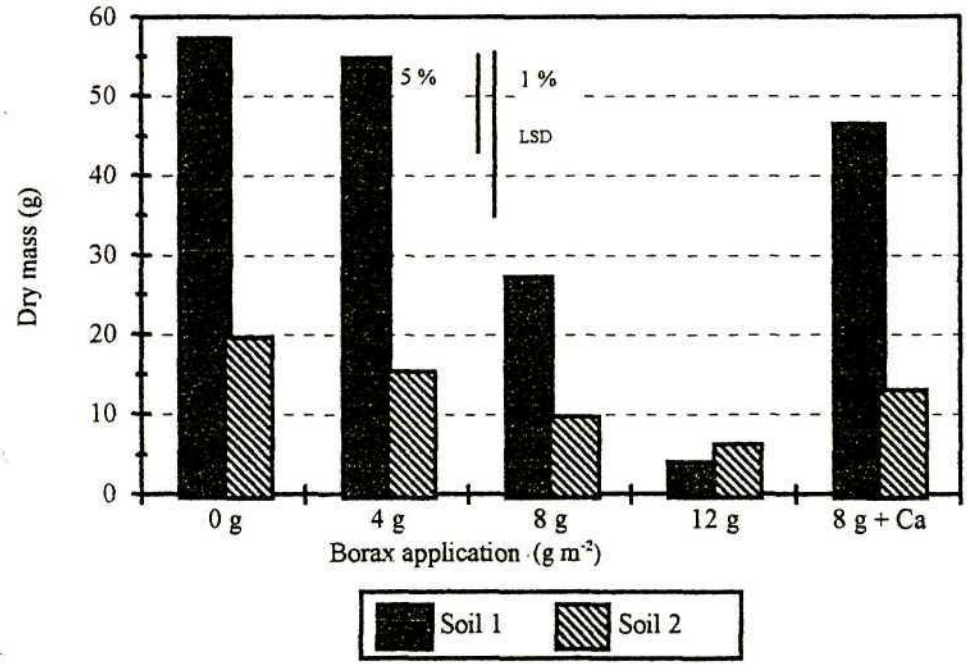

Figure 62: Effect of soil boron application on shoot dry mass of plants growing on 'Edranol' rootstock. B rates are g borax m^{-2} pot surface area applied in 1 single application after planting. Ca indicates application of 40 g calciti lime and 40 g gypsum per replication.

5.3.2.5 Root:shoot ratio

No significant effects were noted on root:shoot ratio (Figs. 63 & 64).

In summary, increasing soil B application rate, resulted in lower root mass, which in turn resulted in lower shoot mass. It appears that under the particular conditions of this trial, in which soil B applications were not split (as would be done in the field), and leaching was purposely kept to a minimum, conditions approaching soil B toxicity developed more quickly than would have been the case under field conditions.

TABLE 10: Statistical significance of the effects of soil B application on growth parameters.

Rootstock	'Duke 7'		'Edranol'	
Parameter	Soil type	B application	Soil type	B application
Stem diameter (mm)	★★	★★	NS	★★
Root fresh mass (g)	★★	NS	★★	★★
Root dry mass (g)	★★	★	★★	★★
Shoot fresh mass (g)	★★	NS	★★	★★
Shoot dry mass (g)	★★	★	NS	★★
Root:shoot ratio	★★	NS	NS	★★

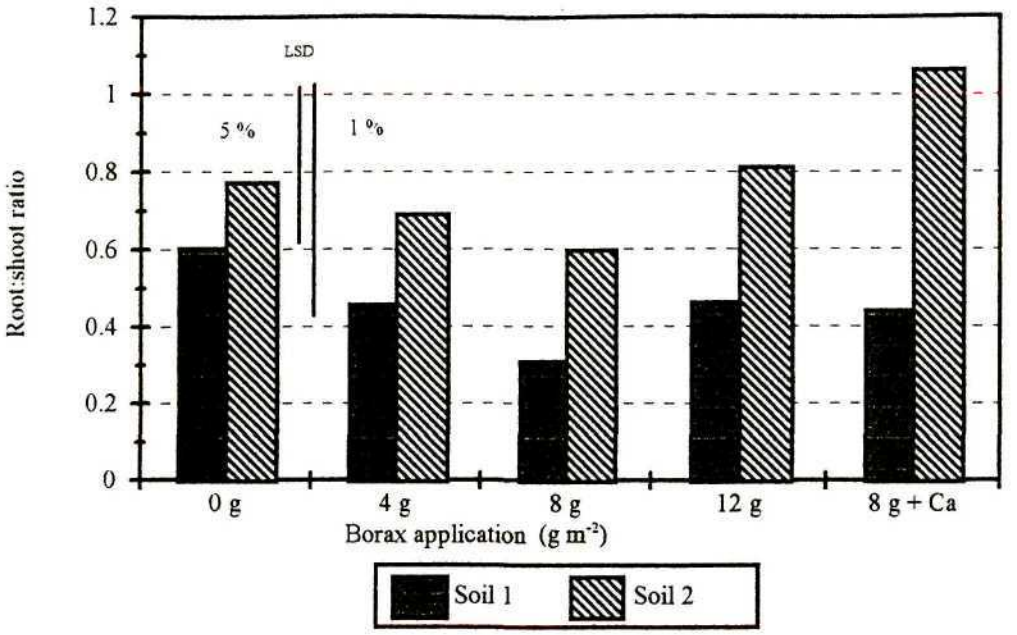

Figure 63: Effect of soil boron application on root:shoot ratio of plants growing on 'Duke 7' rootstock. B rates are g borax m^{-2} pot surface area applied in 1 single application after planting. Ca indicates application of 40 g calcitic lime and 40 g gypsum per replication.

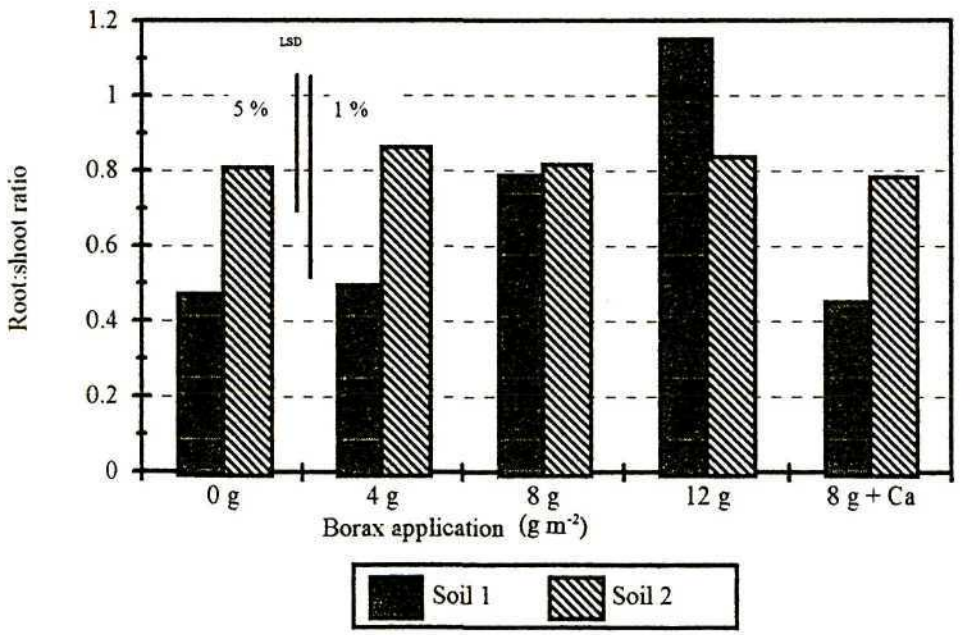

Figure 64: Effect of soil boron application on root:shoot ratio of plants growing on 'Duke 7' rootstock. B rates are g borax m^{-2} pot surface area applied in 1 single application after planting. Ca indicates application of 40 g calcitic lime and 40 g gypsum per replication.

5.3.3 Leaf nutrient concentration

5.3.3.1 Boron

Control plants showed leaf B concentrations of below the norm of 40 mg kg^{-1} (APPENDIX 5) although 'Edranol' rootstocks showed marginally higher concentrations than 'Duke 7' rootstock (Fig. 65). The effect of soil B application on leaf B concentration was highly significant ($p < 0.001$) for both 'Duke 7' and 'Edranol' rootstocks, while the effect of soil type was only

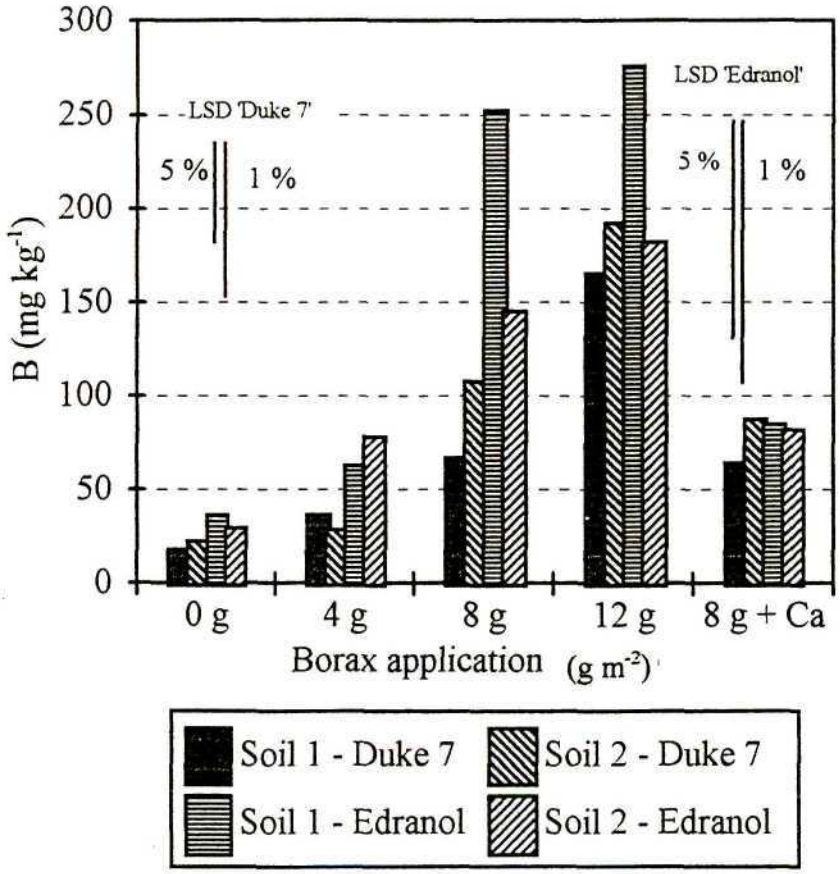

Figure 65: Effect of soil type and boron application on leaf boron concentration of 'Hass' plants growing on 'Duke 7' and 'Edranol' rootstock. B rates are g borax m^{-2} pot surface area applied after planting. Ca indicates application of 40 g calcitic lime and 40 g gypsum per replication.

significant ($p < 0.05$) in 'Edranol'. 'Edranol' was significantly more efficient at soil B uptake ($p < 0.01$), since mean B scion leaf concentration for both soils was 121 mg kg^{-1} as opposed to 86

mg kg^{-1} in 'Duke 7' (TABLE 11). This suggests that 'Edranol' was ca. 41% more efficient at B uptake than was 'Duke 7' even though the latter had an advantage from the earlier planting. Similarly, Smith *et al.* (1997) showed seedling 'Velvick', a rootstock of Guatemalan origin ca. 20 % more efficient at B uptake than clonal 'Duke 7' rootstock. In addition, only the highest application (12 g m^{-2}) raised leaf B 'Duke 7' above 100 mg kg^{-1} in 'Duke 7', while 8 g m^{-2} proved sufficient to achieve this in 'Edranol'. The untreated control plants indicated availability of soil B was less in soil 1 than in soil 2. This is anomalous since analysis indicated more B in soil 1 than soil 2. The effect of soil type on mean leaf B concentration in 'Edranol' was not statistically significant. Regression equations for all 4 rootstock-scion combinations are:

'Duke 7' - Soil 1	$y = 12.24x + 10.47$	$r^2 = 0.643$
'Duke 7' - Soil 2	$y = 12.10x + 4.97$	$r^2 = 0.895$
'Edranol' - Soil 1	$y = 24.46x + 11.06$	$r^2 = 0.561$
'Edranol' - Soil 2	$y = 14.00 + 25.25$	$r^2 = 0.470$

When comparing rootstocks in the same soils, greater y intercepts and steeper gradients for 'Edranol' mathematically show increased capability of this rootstock for B uptake.

In both rootstocks, leaf B concentration increased linearly with soil application for 'Duke 7' in soil and 'Edranol' in soil 2. However leaf B concentration increased exponentially with soil application for 'Duke 7' in soil 2 and 'Edranol' in soil 1.

TABLE 11: Effect of boron application and soil type on 'Hass' leaf B concentration on clonal 'Duke 7' and 'Edranol' seedling rootstocks.

Combination	Mean (mg kg^{-1})	Lowest (mg kg^{-1})	Highest (mg kg^{-1})	Average (mg kg^{-1})
'Duke 7' - Soil 1	72	18	166	86
'Duke 7' - Soil 2	100	41	216	
'Edranol' - Soil 1	142	36	276	121
'Edranol' - Soil 2	100	29	82	

Toxicity symptoms appeared at the two highest application rates (8 and 12 g borax m^{-2} soil

surface area) for both rootstocks. It is hypothesised that toxicity symptoms occurred after leaf B concentrations exceeded 120 mg kg^{-1} (Figs. 59 & 60). This is in line with the work of Smith *et al.* (1995, 1997) in Australia. Toxicity symptoms were initially noted in older leaves. Symptoms became more severe after the growth of successive flushes, young leaves seldom reaching full size before necrosis resulted in leaf abscission. Tree death began from the shoot tips moving gradually towards the rootstock. Since the rootstock death was preceded by scion death, indications are that avocado roots are extremely tolerant of soil B despite severe toxicity in the shoot system. This supports the accepted dogma that B moves mainly via the xylem stream to actively growing transpiring shoots. The fact that toxicity symptoms first appeared in older leaves, suggests that unlike sorbitol-translocating deciduous fruit trees (Hanson, 1991; Picchioni *et al.*, 1995; Brown & Hu, 1996), B is not easily phloem mobile in avocado.

Limed treatments were significant for 'Edranol' rootstock, where liming decreased 'Hass' leaf B concentration by 167 and 63 mg kg^{-1} (± 20.9) for soils 1 and 2 respectively. This result indicated the potential use of lime in reducing the danger of toxicity. It also indicated why liming is part of Whiley's (1997[23]) three point plan to combat B toxicity. These results indicate the antagonism between lime and B and illustrates the possibility of lime (or possibly gypsum) induced B deficiency. More research is required in this area in avocado, particularly since it still remains to be determined whether the antagonism results from competition between Ca and B (Gupta, 1972) or whether it arises from decreased solubility of B resulting from raised soil pH (Gupta & MacLeod, 1981). Overliming could be at least part of the reason for chronic and very severe B deficiency symptoms observed by the author in commercial orchards in the Northern Transvaal production area. This region is characterised by soils which are better endowed with Ca and which are far less acidic in nature than soils in the KwaZulu-Natal production areas. A long term study investigating liming rates and liming interaction with B is overdue.

Results indicate clearly that 'Edranol' seedling rootstock is far more efficient at B uptake and translocation than is clonal 'Duke 7' rootstock. However, clonal 'Duke 7' rootstock is still able to translocate sufficient B to the scion, since toxicity symptoms were produced.

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[23]Whiley, A.W. Department of Primary Industries. Queensland.

Figure 66: Leaf margin necrosis in old leaves due to boron toxicity in glasshouse trials.

Figure 67: Severe boron toxicity in young leaves in glasshouse trials.

Figure 68: Effect of soil boron application on tree growth habit (Increasing B application left to right).

Hot water extractable B is not a good indicator of soil B availability, since soil 1 had higher hot water soluble B content. However deficient leaf B concentrations in plants on 'Duke 7' rootstock were more common in this soil. Plant response to B application indicated that 'Duke 7' rootstock was more responsive to increasing soil B applications in soil 2 than in soil 1. The converse was apparent in plants on 'Edranol' rootstock, where plants showed a faster response to B applications in the sandier soil 1. This is likely to have occurred as a result of leaching. While experimental conditions attempted to prevent any leaching out of pots from occurring, leaching may have taken place in the soil profile. While the more clayey soil 2 maintained a source of B near the soil surface, the sandier soil 1 may have allowed mobilization of B to lower parts of the soil profile. Since avocado feeder roots have highest activity in upper zone of the profile, B would have been far more available in soil 2 than soil 1 over the long term. In the case of 'Edranol' which showed faster and more efficient B uptake, uptake from both soils occurred at a faster rate than in 'Duke 7', raising leaf B concentrations before leaching removed prominent soil B reserves. It is thus possible that this effect described (viz. when comparing the uptake of B from soil 1 for both rootstocks) could have resulted from the effect of leaching rather than from differences in B uptake and translocation between 'Edranol' and 'Duke 7'.

Results indicate that B uptake was sensitive to liming in the case of 'Edranol' seedling rootstock, although less so for clonal 'Duke 7'. A further effect of soil B application was noted in soil 1. Increasing soil B application produced more upright plant growth habit (Fig. 61). This is likely as a result of the effect of B on apical dominance. Deficient conditions result in the loss of apical dominance (Marschner, 1995; Whiley *et al.*, 1996).

Results indicate the importance of rootstock in B uptake similar to results of Smith *et al.* (1995, 1997). An applications of 4 g m^{-2} was sufficient to get leaf concentrations on 'Edranol' above 50 mg kg^{-1}, higher applications resulting in toxicity symptoms. Applications of 4 g m^{-2} to 'Duke 7' rootstock did not raise leaf B concentrations above 50 mg kg^{-1}, and toxicity was only induced in the 16 g m^{-2} treatment. In addition, toxicity symptoms only become visible in leaves when B concentration exceed 100 mg kg^{-1}, again in agreement with the results of Smith *et al.* (1995).

It must be emphasised that B was applied in one single application to a very limited soil volume rather than in three split applications, hence the sensitivity to the applications. In theory,

applications should have been split into 3 equal applications, where toxicity would not have been induced as easily. This emphasises the reason for split applications to reduce danger of toxicity.

5.3.3.2 Potassium

All treatments showed potassium (K) levels above the lower limit of the norm of 0.75 % K (APPENDIX 5), however, some of the 8 and 12 g m^{-2} treatments showed above normal (>1.25 %, APPENDIX 5) K levels. The effect of different soil B applications on the leaf K content of the 'Hass' scion was unclear although there was a non-significant trend for increasing leaf K with increasing leaf B level on seedling 'Edranol' rootstock (TABLE 12). 'Duke 7' in soil 1 showed no clear trend, while soil 2 exhibited a curvilinear trend, since K concentration decreased after the 8 g m^{-2} application (TABLE 12). The possible reason for this is the severe B toxicity symptoms developed in the 12 g m^{-2} treatment, resulting in defoliation. Large necrotic areas developed on the leaf blade. Since potassium is involved in the cell cytosol, necrotic regions would not have this K requirement, hence decreasing the leaf K content.

Lime appeared to have no effect on leaf K concentration. This is surprising in view of the well known K-Ca antagonism in plants.

TABLE 12: Effect of soil B application on leaf K concentration in 'Duke 7' and 'Edranol'.

Treatment	0 g m^{-2} (%)	4 g m^{-2} (%)	8 g m^{-2} (%)	12 g m^{-2} (%)	8 g m^{-2} + Ca (%)	Mean (%)
'Duke 7'- Soil 1	1.11	1.09	0.91	0.99	1.02	1.02
'Duke 7'- Soil 2	0.94	0.81	1.57	1.46	1.15	1.19
'Edranol'- Soil 1	0.92	0.80	1.05	1.69	1.07	1.11
'Edranol'- Soil 2	0.87	0.99	1.17	1.60	1.21	1.17

5.3.3.3 Calcium

All treatments showed no shortage (>0.5 %) of calcium (Ca) (Fig. 69). The effect of soil B application on leaf Ca concentration was significant on 'Duke 7' ($p < 0.01$) but not in 'Edranol' rootstock. 'Hass' leaves on 'Duke 7' rootstock had highest leaf Ca concentration at the 8 g m^{-2} borax level in both soils (Fig. 63). No clear trends were found on 'Edranol' rootstock, possibly because they were harvested at a younger stage of growth than were clonal 'Duke 7' rootstock. No effect was evident resulting from the 8 g m^{-2} + Ca treatment.

Results indicate the possibility of synergy between B and Ca in 'Duke 7' up to 8 g m^{-2}, whereafter possible antagonism occurred. Although the literature generally indicates antagonism between Ca and B, agronomists moot at the possibility of a synergistic relationship between the two within a certain range.

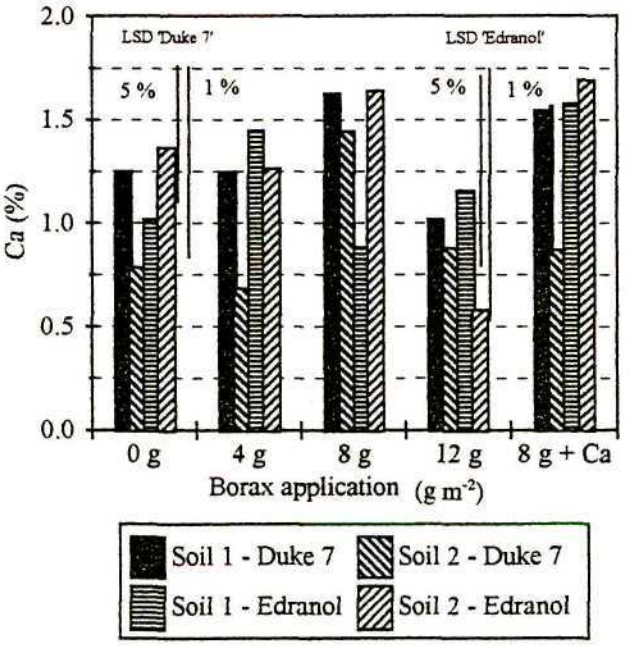

Figure 69: Effect of soil type and soil boron application on leaf calcium concentration of 'Hass' plants growing on 'Duke 7' and 'Edranol' rootstock. B rates are g borax m^{-2} pot surface area applied after planting. Ca indicates application of 40 g calcitic lime and 40 g gypsum per r

5.3.3.4 Magnesium

All treatments showed no shortage (<0.25 mg kg^{-2}) of magnesium (Mg), however some treatments, notably the 8 g m^{-2} + Ca in Soil 2 showed excessive Mg leaf concentrations. Magnesium, similar to Ca, exhibited maximum uptake in the 8 g m^{-2} treatment in 'Duke 7' ($p < 0.05$) in both soils while 'Edranol' showed no clear trend (Fig. 70). Limed treatments did not have any significant effect on leaf Mg concentration.

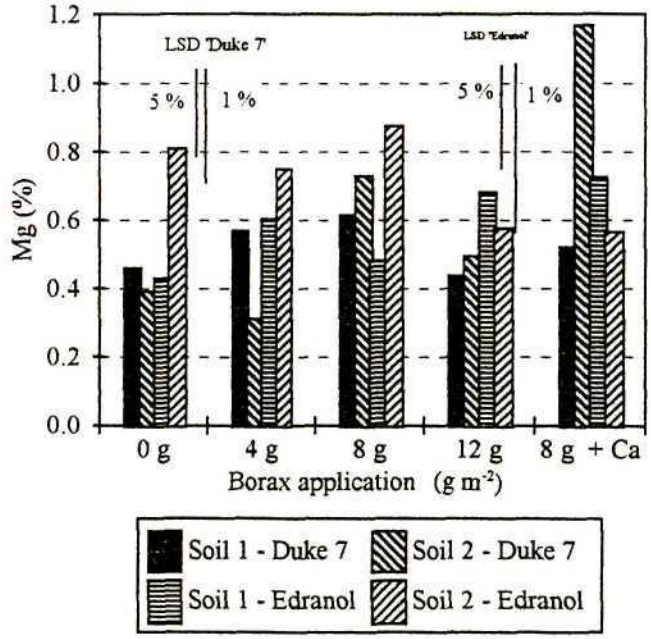

Figure 70: Effect of soil type and soil boron application on leaf magnesium concentration of 'Hass' plants growing on 'Duke 7' and 'Edranol' rootstock. B rates are g borax m^{-2} pot surface area applied after planting. Ca indicates application of 40 g calcitic lime and 40 g gypsum per replication.

5.3.3.5 Sodium

All sodium (Na) concentrations were in the excessive (> 0.25 %, APPENDIX 5) range (Fig. 71). Results showed that leaf sodium (Na) concentrations in 'Duke 7' were significantly higher ($p < 0.001$) in soil 2 (TABLE 13). This probably results from higher clay fraction in soil 2, which prevents leaching of Na applied in the form of borax. In 'Edranol', soil 2 showed marginally higher concentrations, which were not statistically significant (Fig. 71).

TABLE 13: Differences in leaf sodium concentration for 'Duke 7' resulting from soil type.

Mean 'Duke 7' (%)	Mean - Soil 1 (%)	Mean - Soil 2 (%)	SED (%)
0.367	0.310	0.424	0.0283

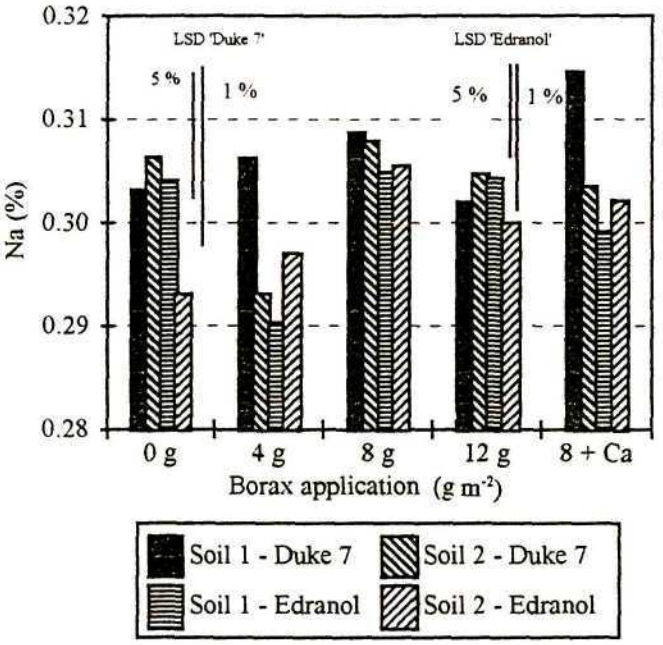

Figure 71: Effect of soil type and soil boron application on leaf sodium concentration of 'Hass' plants growing on 'Duke 7' and 'Edranol' rootstock. B rates are g borax m^{-2} pot surface area applied after planting. Ca indicates application of 40 g calcitic lime and 40 g gypsum per replication.

5.3.3.6 Copper

All copper (Cu) concentrations were in the excessive (>25 mg kg^{-1}, APPENDIX 5) range. Results show that soil type in 'Duke 7' had a significant influence on leaf Cu concentration (TABLE 14). Soil 2 showed significantly higher ($p < 0.05$) copper in soil 2 than in soil 1. This probably results from higher naturally occurring copper levels in soil 2 in addition to its higher clay percentage, reducing the effect of leaching. Soil 2 showed decreasing leaf Cu concentration in 'Duke 7' (Fig. 72), although this trend was not significant.

TABLE 14: Effect of soil type on leaf copper concentration in 'Duke 7'.

Mean 'Duke 7' (mg kg^{-1})	Mean - Soil 1 (mg kg^{-1})	Mean - Soil 2 (mg kg^{-1})	SED (mg kg^{-1})
101	65	138	29

No significant effects or trends were visible in 'Edranol' (Fig. 72). This was probably because trees were harvested at an age when trees were too young for differences to become apparent.

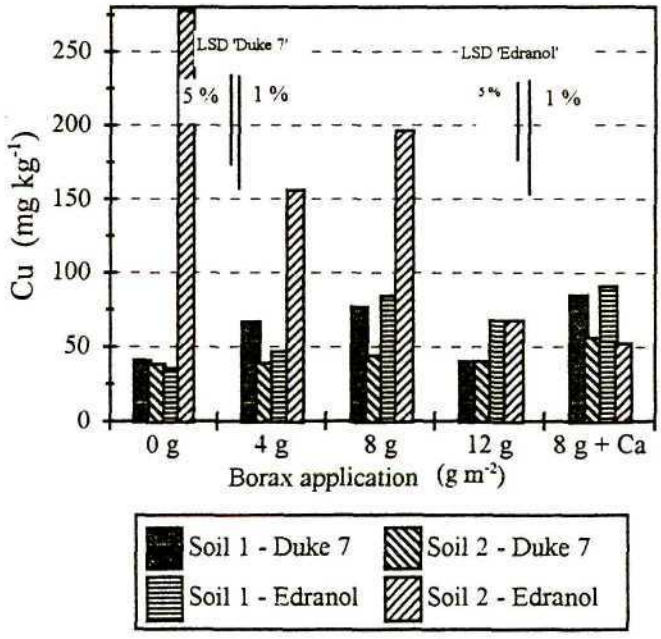

Figure 72: Effect of soil type and soil boron application on leaf copper concentration of 'Hass' plants growing on 'Duke 7' and 'Edranol' rootstock. B rates are g borax m^{-2} pot surface area applied after planting. Ca indicates application of 40 g calcitic lime and 40 g gypsum per replication.

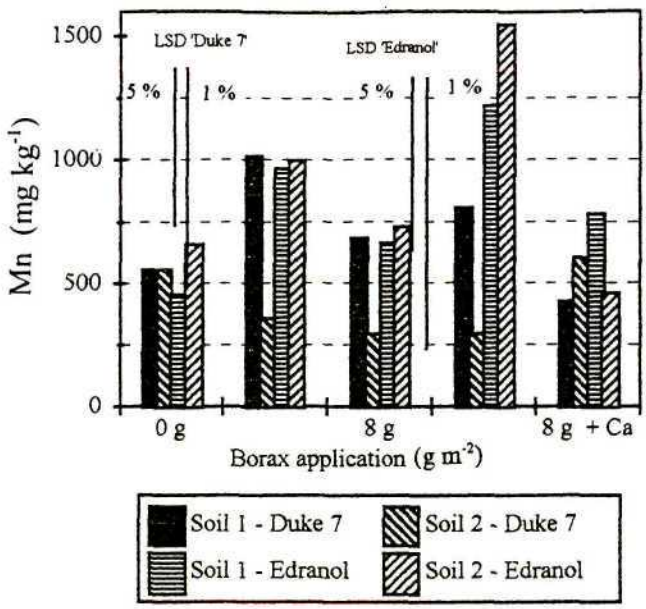

Figure 73: Effect of soil type and soil boron application on leaf manganese concentration of 'Hass' plants growing on 'Duke 7' and 'Edranol' rootstock. B rates are g borax m^{-2} pot surface area applied after planting. Ca indicates application of 40 g calcitic lime and 40 g gypsum per replication.

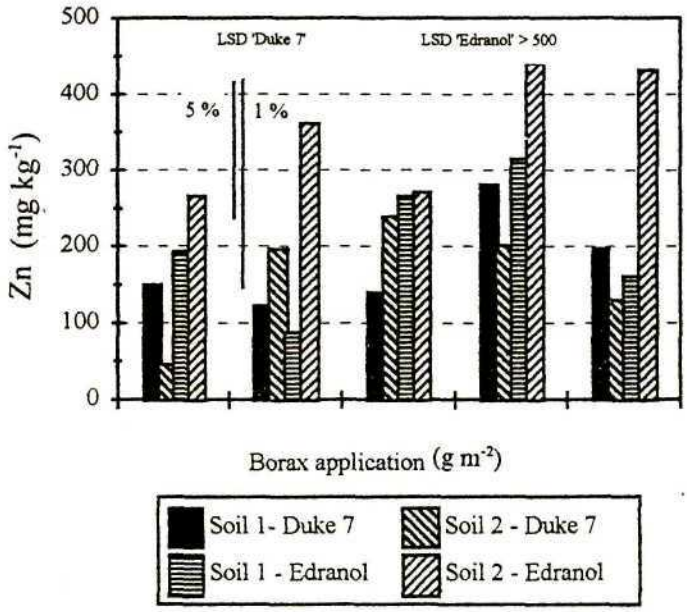

Figure 74: Effect of soil type and soil boron application on leaf zinc concentration of 'Hass' plants growing on 'Duke 7' and 'Edranol' rootstock. B rates are g borax m^{-2} pot surface area applied after planting. Ca indicates application of 40 g calcitic lime and 40 g gypsum per replication.

5.3.3.7 Manganese

All leaf manganese (Mn) concentrations were above the normal (>250 mg kg^{-1}, APPENDIX 5) range (Fig. 73). Increasing B application significantly increased leaf Mn concentrations in Edranol ($p < 0.05$). Leaf Mn levels were raised to a greater degree with increasing B application in soil 2 (TABLE 15). This probably resulted since higher B applications resulted in decreased shoot mass. Decreased transpiration resulted in soil profiles that were substantially wetter than control trees, having a larger shoot mass and requiring greater amounts of water. It is suggested that wetter soil profiles allowed for dissolution of soil Mn resulting in increased availability to the plant. This is a well known effect in wet orchard soils (Spencer, 1995[24]).

No significant effects were noted in 'Edranol', probably because plants were harvested at a younger stage of growth than 'Duke 7'.

TABLE 15: Effect of soil boron application on leaf manganese concentration in 'Duke 7'.Ca represents application of 40 g calcitic lime and 40 g gypsum per replication.

Treatment	0 g m^{-2} (mg kg^{-1})	4 g m^{-2} (mg kg^{-1})	8 g m^{-2} (mg kg^{-1})	12 g m^{-2} (mg kg^{-1})	8 g m^{-2} + Ca (mg kg^{-1})	Mean (mg kg^{-1})
'Edranol'- Soil 1	453	966	662	1221	780	816
'Edranol'- Soil 2	610	999	728	1547	461	869

The effect of lime on 'Hass' leaf Mn was not significant. Since liming did not significantly decrease leaf Mn concentrations, it can be concluded that soil pH was not raised into a range which decreases Mn availability.

5.3.3.8 Zinc

All treatments were in or above the normal zinc (Zn) range (>50 mg kg^{-1}, APPENDIX 5).No significant effects were noted (Fig. 74). Soil B application appears to have no effect on uptake of Zinc, with or without combination of lime.

[24]Spencer, J. Spencer & Holley Agronomic Services, Pietermaritzburg.

GENERAL DISCUSSION AND CONCLUSIONS

South African avocado orchards clearly have a severe B deficiency problem that has remained unrecognised until recently. Previously recommended foliar applications have not been able to meet orchard requirements, even when copious amounts of water are used to apply sprays. Indications are that avocado growing soils in KwaZulu-Natal are very deficient in B, thus resulting in B deficiency. Since deficiency symptoms were noted in trees on seedling 'Edranol' rootstock, implications are that low soil B is responsible for deficiency symptoms rather than inefficiency of the widely used 'Duke 7' rootstock. Relatively high amounts of organic matter and moderately heavy soil texture, imply that soil buffering capacity will reduce chances of toxicity, as will mesic climatic conditions with relatively high and reliable rainfall.

Field trials have shown soil applications to be effective at raising leaf B concentrations in the complete absence of foliar sprays. Moreover, indications are that the avocado is fairly tolerant of high soil B concentrations. Probable contamination of foliar analyses, and ignorance about deficiency symptoms, has resulted in the rampant deficiency remaining undiagnosed for decades. Although more efficient rootstocks might enable more effective B uptake, applications will still be necessary to correct low levels of available soil B.

Pot trials have indicated that small plants in restricted rooting volumes are extremely efficient at B uptake. Thus additional supplementation of nursery tree B requirements (in addition to standard micro-element supplementation) is probably not necessary. Field observations suggest that adequate soil supply sustains tree requirement initially, however as the root systems age and become less efficient despite greater canopy area, deficiencies become magnified. Infection of roots with *Phytophthora cinnamomi* root rot in older orchards further reduces B uptake.

Deficiencies have developed over many years, and it is the author's opinion that such situations cannot be remedied overnight. Correction should rather be performed gradually, with *Phytophthora* control, leaf analysis and soil analysis being key tools to successful rehabilitation. Applications should be substantial enough to raise soil availability within a feasible timespan. Where deficiencies are severe, a minimum application of 10 g borax m^{-2} $year^{-1}$ is recommended. Leaf applications, as recommended by Whiley *et al.* (1996) are unlikely to rectify more severe

deficiency symptoms. Sufficient evidence exists indicating the limitations of foliar B applications: Although remobilisation has been shown to occur to an unquantified but probably limed extent, it only occurs once B reserves have been raised to the norm of ca. 40 mg kg^{-1}. Hence implications are that foliar applications require sufficient leaf B concentrations (likely to be supplied by soil applications) to render them most effective. Furthermore, many years of research have neglected to quantify to amount of foliar applied B taken up by avocado leaves. Researchers assumed that substantial uptake occurred since remobilisation of foliar applied B was noted to a limited extent. Further research is required to determine the amount of foliar applied B that is taken up through the cuticle without becoming trapped in the waxy cuticle platelets on the avocado leaf.

It should also be noted that foliar applications should not be totally disregarded or ignored since they improve pollination and fruit set. Foliar applications will remain useful until soil applications have led to buildup of substantial reserves of B within the plant and corrected deficiencies of more a severe nature. The author wishes to emphasise that current practises have not been successful, mainly because of the waxy nature of the avocados leaves and relatively low solubility of foliar applied B products. Use of B products with a higher solubility, use of solubor with agents facilitating uptake (e.g. urea) may increase the efficiency of foliar sprays. In addition, it is recommended that where foliar applications are applied during the development of the spring flush, leaf analysis samples should be taken from the summer flush growth as is done in Australia.

Slower recovery of older trees used for field trials probably resulted from weak and *Phytophthora* infected root systems. In this regard, Australian growers are advised to control root rot before applying B deficiency corrective measures.

Standardisation of analytical techniques used for leaf and soil analysis would also aid safe B fertilization recommendation. Currently, different laboratories use different methods for determination of leaf and soil B. In the case of soil B determination, it generally accepted that the calcium chloride extraction method has replaced the outdated hot water extraction method which is used in Australia. Leaf analysis is best performed by wet digestion in porcelain or soda ash glass rather than borosilicate glass. The former glass does however have its limitations since it is not as heat tolerant as borosilicate glass. Indications are that B determination is best performed by ICP rather than colourimetric methods. Cost of ICP equipment probably prevents most

laboratories from using this specialised instrument.

In the future, it is likely that xylem sap analysis techniques which will probably be performed *in situ* are likely to replace time consuming leaf analysis.

The inconclusive nature of the effect of B on fruit phenolic concentration probably reflects inadequate sample size. In this respect, the work of Brown & Hu (1997) suggesting a primary structural role of B in higher plants is noteworthy. Postharvest research requires further investigation, particularly in cultivars notorious for postharvest problems. Secondary B effects may well (as for Ca) be implicated in physiological disorders.

This research has shown that soil B applications are an effective and relatively safe (provided they are carefully used) method of raising leaf B concentration above the accepted norm of 40 mg kg^{-1} without the use of any foliar sprays. Since soil B applications have been shown to be of great importance to the South African avocado industry, it has opened the door for important research of a more physiological nature, viz. the relative phloem mobility of B, sorbitol (or perseitol) content of the avocado, and the precise physiological role of B in the avocado. An understanding of these topics will enable the industry to develop an effective integrated and safe B management programme where soil B applications can be supplemented by correctly timed foliar sprays. However the sophistication of the Australian B recommendation, as implemented in the computer driven AVOMAN package taking into account numerous variables, warns against over-generalisation of a highly complex topic.

Further research is required to bridge the gap between nursery plants, that usually show no deficiency symptoms and extreme sensitivity to soil B applications, and grossly deficient orchard trees that show chronic deficiency symptoms and relatively high tolerance of soil B applications. Research should aim at determining at what age the transition between B sufficiency:deficiency occurs, which will prevent B deficiency developing at a young age.

Further research is also required to investigate the susceptibility and tolerance of other cultivars ('Fuerte', 'Ryan', 'Edranol' (scion) and 'Pinkerton') in addition to the numerous scion/rootstock combinations. It is also possible that many internal fruit quality problems (or "physiological

disorders") in 'Fuerte' and possibly 'Pinkerton' (South Africa's problematic cultivars wrt. internal fruit quality) could at least in part be related to B deficiency. The temptation, however to attribute all non-resolved problems to a single micro-element, even if it is an important limiting factor, should be resisted.

The South African avocado industry should investigate the possibility of using 'Velvick' seedling rootstock since it is a proven rootstock for the Australian avocado industry. It also stands to reason that future research on avocado rootstocks, which in the past has been dominated by the need for tolerance to *Phytophthora cinnamomi* root rot, must also take horticultural needs into account. These include efficient nutrient uptake, not least B on the leached acid soils supporting the South African avocado industry.

SUMMARY

All avocado growing soils tested in the KwaZulu-Natal midlands showed deficient levels of B (<1 mg kg^{-1}). Long term averages of leaf B concentration in all areas were less than 33 mg kg^{-1}, which falls below the accepted optimum of 40 - 70 mg kg^{-1} in spite of regular spraying to control the deficiency. Yearly leaf B concentration averages occasionally showed excessively high values followed by lower values the following year, indicating that contamination is inflating results. Deficiency symptoms were widespread in spite of routine regular sprays attempting to control the problem.

Typical avocado soils are extremely weathered, acid and infertile. Soils do not have significant levels of gibbsite (the clay mineral having the greatest capacity for adsorption), therefore soil buffering capacity which decreases risk of toxicity, is largely dependant on the two remaining factors, soil clay percentage and soil organic matter content. All topsoils were either humic or possessed characteristics very similar to humic and had relatively high topsoil organic matter content (≥3 %). All soils showed relatively high (>30 %) clay fractions with the exception of soil from the Winterskloof area, which was lighter texture (20 % clay). These soils are expected to have moderate to high buffering capacities resulting from these physical and chemical characteristics.

Soil B applications successfully increased leaf concentrations to the optimum range of 40 to 70 mg kg^{-1} within the timespan of the experiment. Highest rates (40 and 60 g borax m^{-2} $year^{-1}$) resulted in toxicity 15 months after initial application. Toxicity symptoms, viz. marginal leaf necrosis were identical to the symptoms described by Smith *et al.* (1995) and Whiley *et al.* (1996). Fruit B concentrations showed rapid B concentration increase during the maturation phase of the fruit. Increases in leaf B concentration were noted after the occurrence of a root flush. The avocado is reasonably tolerant of soil B applications. Indications are that when applied in judicious amounts (typically 5-20 g borax m^{-2} an^{-1}) least 3 applications per year, soil B applications are a safe and efficient means of controlling and correcting B deficiency in the avocado. However the actual rate is affected by many factors, and also depends on accurate annual leaf analysis.

Increased yield resulting from increased fruit size was noted in young 'Hass' trees, however larger more deficient trees did not show any increase in fruit size during the 2-year lifespan of the trial. *Phytophthora* root rot could have been a complicating factor.

Glasshouse trials indicated that young recently grafted trees were extremely sensitive to soil B application at similar rates to the orchard trial, but applied in a single dose. Highest application resulted in almost immediate toxicity symptoms identical to those noted in mature orchards trees. 'Edranol' seedling rootstock of Guatemalan origin showed ca. 41 % higher leaf B concentrations (over the range 0 to 12 g borax m^{-2}, deficient to toxic symptoms produced respectively) than clonal 'Duke 7' of Mexican origin. B applications had highly significant effects on both shoot and root fresh and dry mass.

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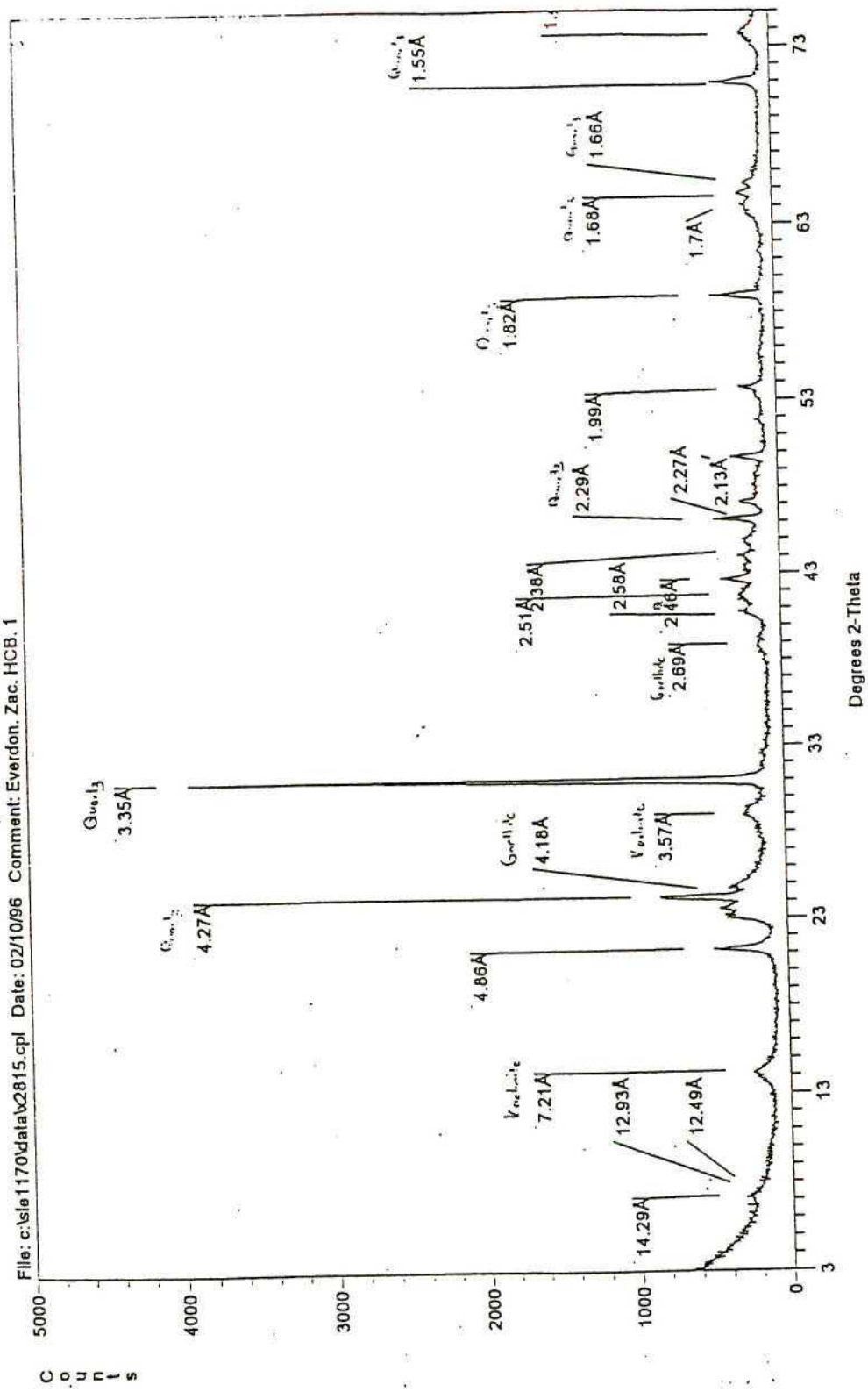
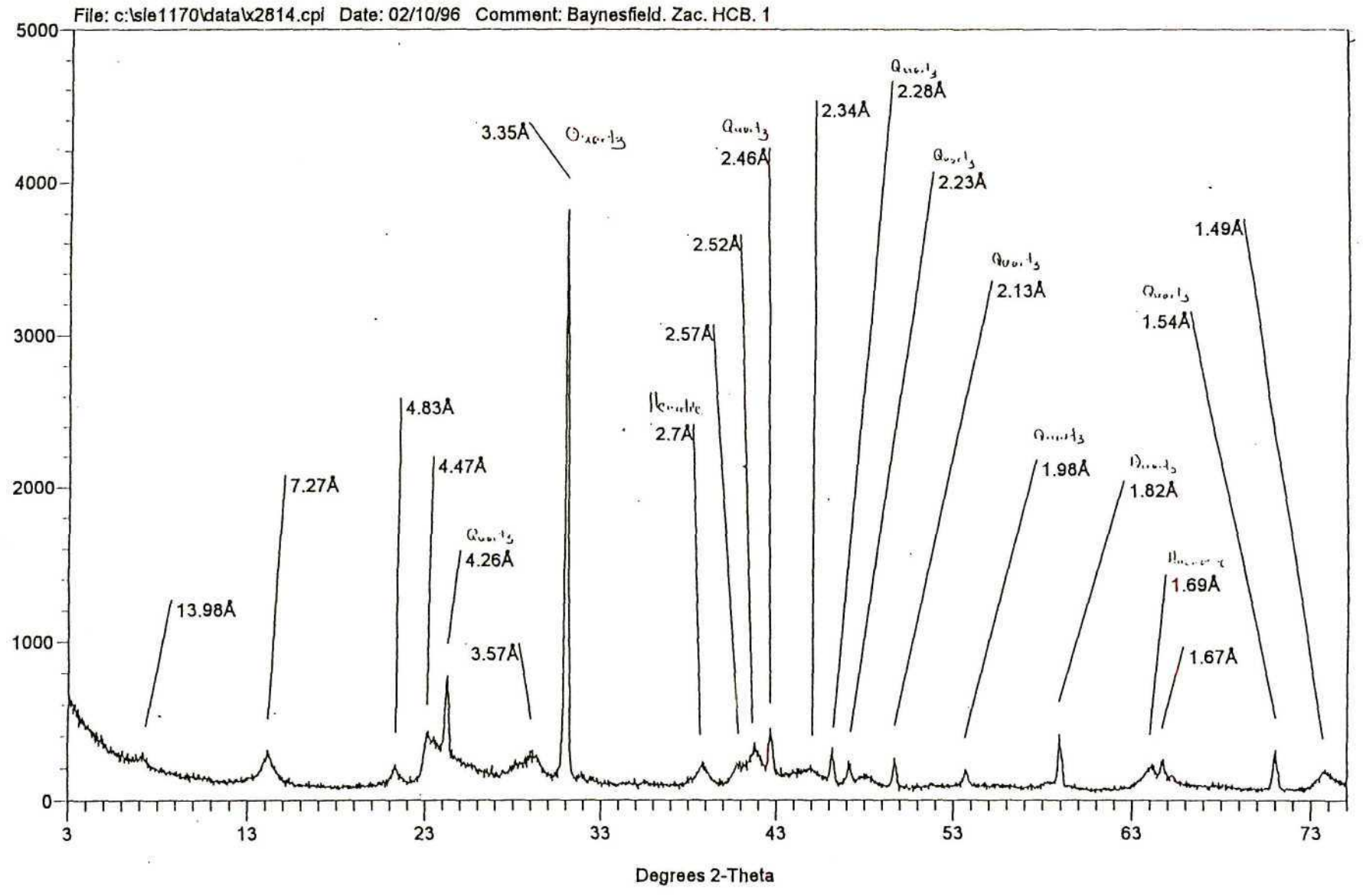
APPENDICES

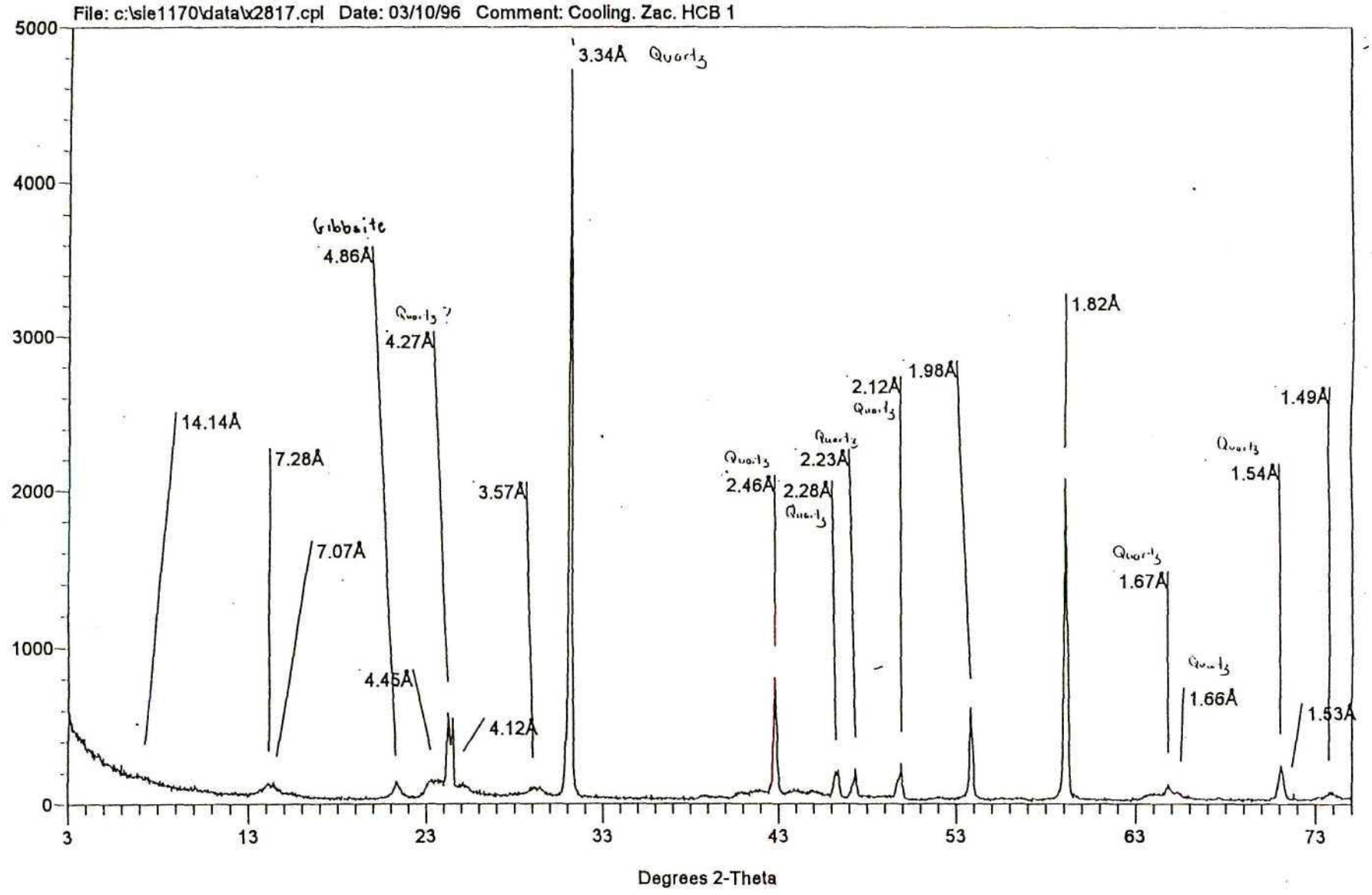
APPENDIX 1: Clay mineralogy analysis



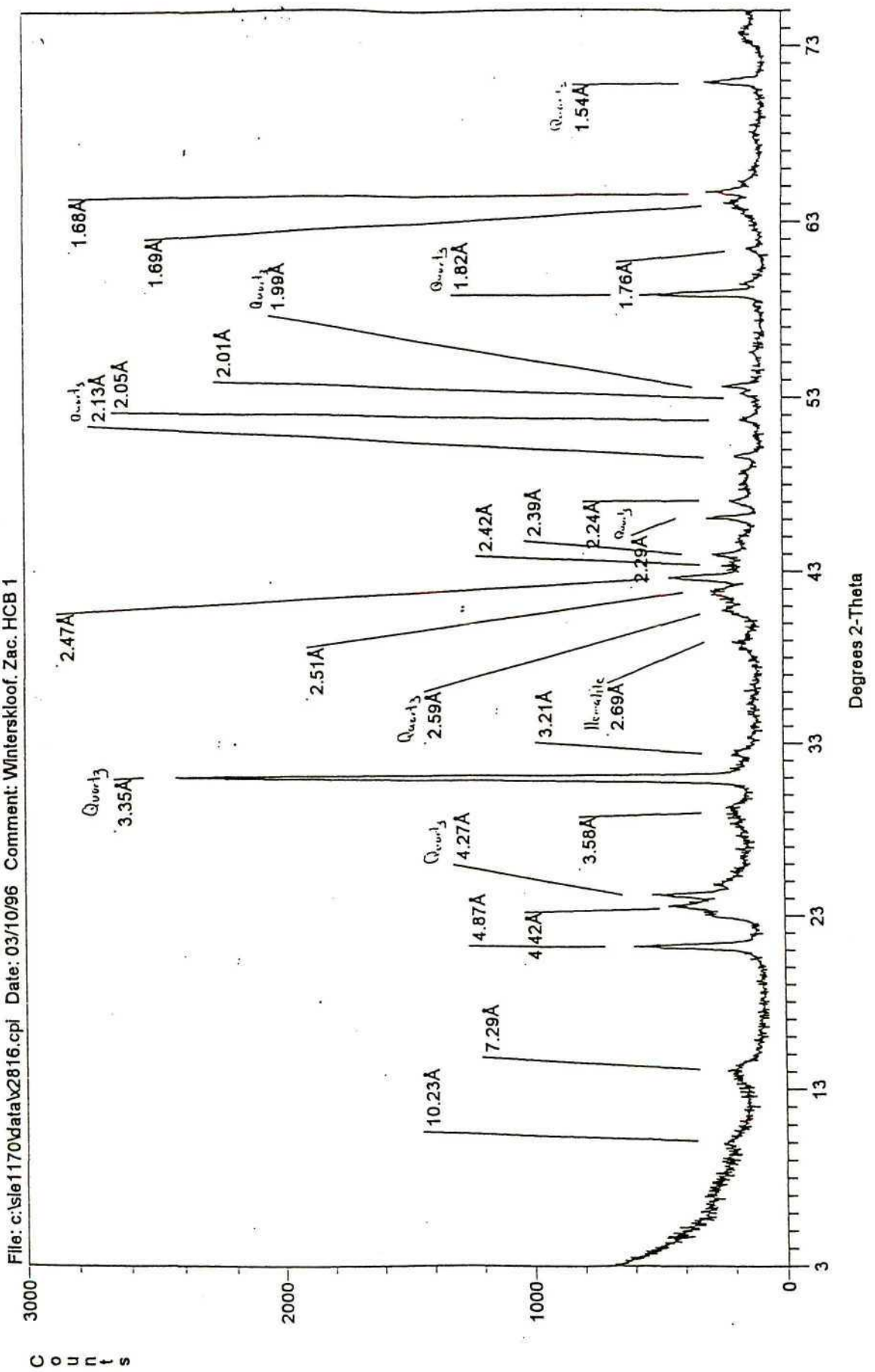
Appendix 1a: Clay mineralogy analysis from Everdon Estate.



Appendix 1b: Clay mineralogy analysis from Baynesfield Estate.



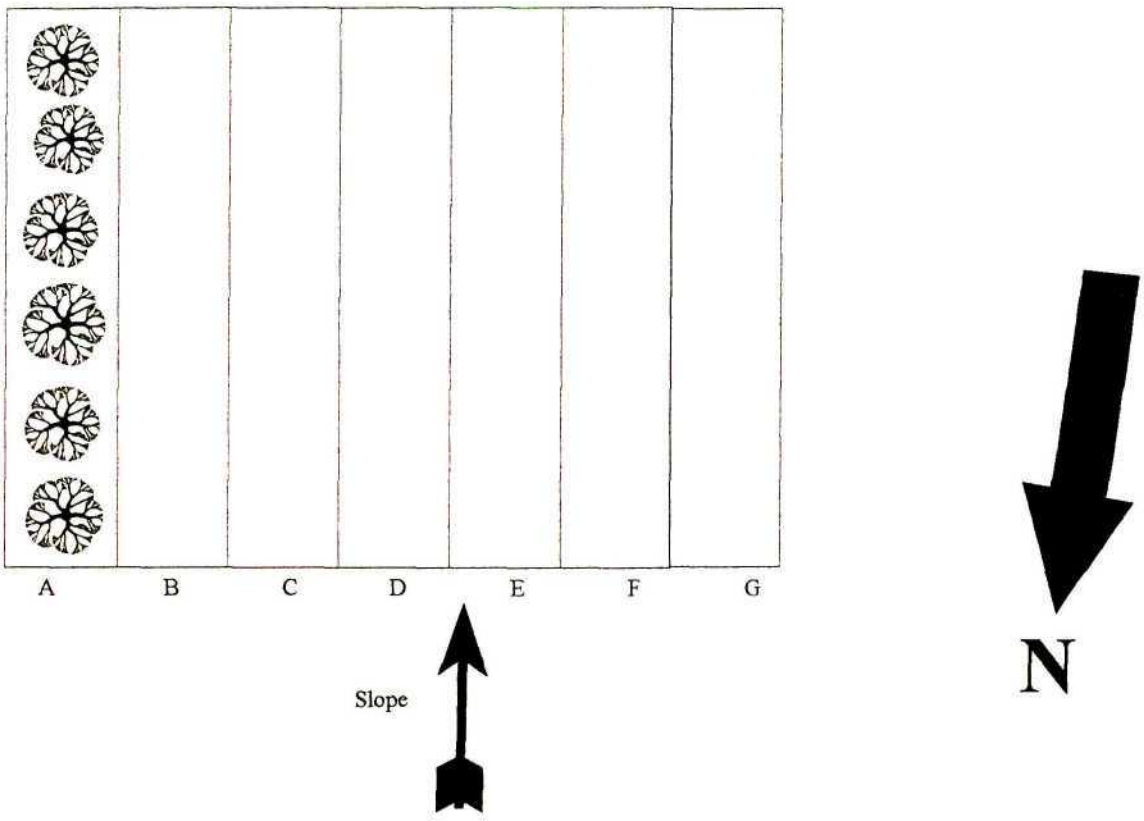
Appendix 1c: Clay mineralogy analysis from Cooling Estate.



Appendix 1d: Clay mineralogy analysis from Winterskloof Estate.

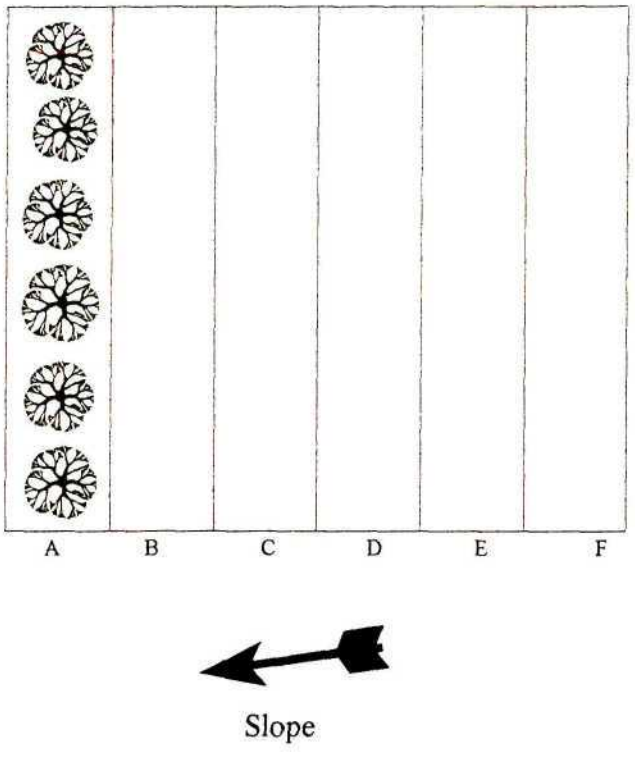
APPENDIX 2: Field layout of experimental orchards, on the Estates Everdon and Cooling respectively.

Appendix 2a: Layout of field trial at Everdon Estate

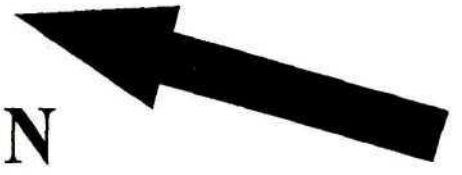


A 20 g Borax/m^2/year
B 0 g Borax/m^2/year
C 0 g Borax/m^2/year
D 20 g Borax/m^2/year
E 20 g Borax/m^2/year
F 0 g Borax/m^2/year
G 0 g Borax/m^2/year

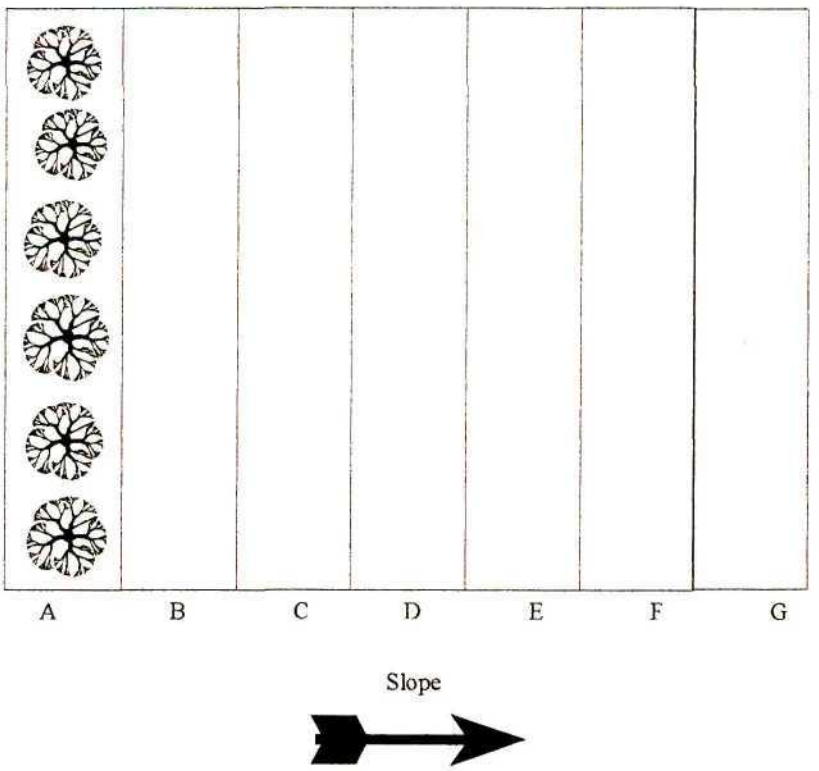
Appendix 2b: Layout of field trial at Cooling Estate using older trees



A 60 g Borax/m^2/year
B 20 g Borax/m^2/year
C 40 g Borax/m^2/year
D 10 g Borax/m^2/year
E 5 g Borax/m^2/year
F 0 g Borax/m^2/year



Appendix 2c: Layout of field trial at Cooling Estate using young trees



A Control
B 20 g Borax/m^2/year
C 10 g Borax/m^2/year
D 40 g Borax/m^2/year
E 5 g Borax/m^2/year
F 0 g Borax/m^2/year
G 60 g Borax/m^2/year

APPENDIX 3: Borax application according to tree size.

Recomended Borax application repeated 3 times yearly					
	Dose (g/m^2)				
Tree diam	5	10	20	40	60
½	0.7	1.3	2.6	5.2	7.9
1	2.6	5.2	10.5	21.0	31.4
2	10.5	21.0	41.9	83.8	125.7
3	23.6	47.1	94.3	188.6	282.9
4	41.9	83.8	167.6	335.2	502.9
5	65.5	131.0	261.9	523.8	785.7
6	94.3	188.6	377.1	754.3	1131.4
7	128.3	256.7	513.3	1026.7	1540.0
8	167.6	335.2	670.5	1341.0	2011.4

APPENDIX 4: Determined leaf nutrient concentrations of certified leaf standard

Certified Mass Fractions

Macroelements	Certified	Measured
Element	Mass fraction (%)	
Calcium	5.05 ± 0.09	5.10
Nitrogen	3.03 ± 0.15	
Phosphorus	0.216 ± 0.004	
Potassium	2.70 ± 0.05	2.68
Microelements		
Element	Mass fraction (mg/kg)	
Boron	33.3 ± 0.7	33.1
Copper	4.7 ± 0.14	4.6
Manganese	246 ± 8.0	250
Sodium	136 ± 4.0	138
Zinc	30.9 ± 0.7	40.2

APPENDIX 5: Leaf and soil analysis norms for avocado.

LEAF

Element	Shortage	Below normal	Normal	Above normal	Excess
N	1.4	1.41-2.19	2.20-2.40	2.41-2.69	2.7
P	0.05	0.06-0.07	0.08-0.15	0.16-0.24	0.25
K	0.35	0.36-0.74	0.75-1.25	1.26-2.24	2.25
Ca	0.50	0.51-0.99	1.00-2.00	2.01-2.99	3.00
Mg	0.25	0.26-0.39	0.40-0.80	0.81-0.99	1.00
Na			0.01-0.06	0.06-0.24	0.25
Cu	3	4	5-15	16-24	25
Mn	19	20-49	50-250	251-749	750
Zn	20	21-24	25-100	101-299	300
B	14	15-49	40-70	71-99	>100

SOIL

Element	Shortage	Below normal	Normal	Above normal	Excess
P	2	3-7	8-29	28-45	46
K	100	101-149	150-250	251-499	500
Ca	250	251-749	750-1000		
Mg	50	51-99	100-300		
$pH_{(H_20)}$	4.5	4.6-5.4	5.5-6.5	6.6-7.5	7.6
Ca:Mg Ratio			2.5-5.0		
(Ca + Mg):K Ratio			5-10		