The Genetics of Boron Tolerance in Barley

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Chapter 1. Literature Review (cont.)

ABSTRACT

The genetics of tolerance to boron (B) of a number of barley genotypes was investigated, with a view to applying this knowledge to the development of efficient strategies for breeding and selection in segregating populations. Field trials were conducted at high B sites using F_2 derived lines, segregating for B tolerance. It was determined that tolerance to high levels of soil B is largely genetically determined and that the growing of intolerant genotypes under high B conditions may lead to yield losses close to 20% compared to tolerant lines. A hydroponic system is described for screening seedlings for tolerance to high B. Genetic studies primarily involved three barley lines: Sahara 3771 (highly tolerant); CM 72 (moderately tolerant); and Stirling (intolerant). These lines were crossed in all combinations and F_1, F_2 and F_2-derived F_3 populations investigated for tolerance to B. Tolerance was found to be partially dominant, and controlled by allelic loci in the two tolerant lines. Though B tolerance is a quantitative trait, in that a continuity in response to high B is observed, it was determined that there are at least three major genes determining B tolerance in Sahara 3771 and two in CM 72. Preliminary results from mapping studied using restriction fragment length polymorphisms (RFLPs) suggest that these genes may be located on chromosome arms 2L and 7S. In addition to these genetic studies, physiological studies were undertaken which indicated that the cell wall may be implicated in determining tolerance to boron, and although B tolerance may be expressed in pollen, application of high levels of B to parent plants does not change the genetic distribution of a segregating population in the following generation. The implications of the findings of the genetic and physiological studies were discussed in relation to breeding strategies, and knowledge about the genetic and physiological control of B tolerance in barley.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any University and the author consents to the thesis being made available for photocopying and loan if applicable, if accepted for the award of the degree.

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Erratum

As pointed out by one examiner the word 'content' was used in several places in this thesis when 'concentration' was intended. Content has been misunderstood in many literature citations because of its technical meaning is units per plant/plant part (total amount) compared to concentration meaning units per unit tissue.

INTRODUCTION

Barley (*Hordeum vulgare* L.) is Australia's second largest grain crop. In 1991 over 2.5 million hectares were sown, producing more than 4.1 million tonnes of grain; about 80% of which was exported (ABS, 1992a). In 1990-91 the total Australian barley crop was valued at A$568.3m (ABS, 1992b). The exported crop represented about 12% of the world trade of barley (NFF, 1991).

Boron (B) toxicity in barley was first recognised in Australia in 1983 (Cartwright *et al.*, 1984). At the Gladstone site, the affected areas were estimated to incur a yield loss of 17%. Since then surveys of grain boron content (Ralph, 1991) and of leaf symptoms (Hirsch and Manton, 1989) as well as anecdotal reports (Khan *et al.*, 1985) indicate that high boron soils are widespread in the southern Australian cereal belt. Thus, it is likely that boron toxicity is, in some years at least, causing losses in the order of millions of dollars to the barley industry of Australia.

Amelioration of B toxicity is impractical in southern Australia, through increasing pH, since soils are already alkaline, or leaching since water resources limited. It has been necessary to seek a solution to the problem through the development of cereal varieties that tolerate B toxicity (Rathjen *et al.*, 1987). An understanding of the nature of the inheritance and physiology of boron tolerance in plants allows breeders to make more appropriate decisions about effective breeding methodology and techniques. Knowledge about the physiology of tolerance to boron toxicity would help design efficient, reliable and routine screening methods, suitable for incorporation into breeding programmes. In the future this may include novel methods such as pollen selection, single cell selection using tissue culture, or use of RFLP (restriction fragment length polymorphisms) markers. Production of boron tolerant

barley cultivars will increase yields on land presently affected by boron toxicity, and may allow exploitation of land previously unsuitable for barley production.

It is useful to establish when undertaking genetic studies:

1. To what extent the character is controlled by heritable factors and how these factors interact with the environment
2. The degree of genetic variability available in the gene pool
3. Whether the character is inherited through nuclear or cytoplasmic transmission
4. The dominance and epistasis relationships acting
5. How many genes are involved in determining the level of tolerance and
6. The location within the genome of these genes.

In more general terms, soil mineral stresses are increasingly becoming growth-limiting factors for crop plants in many parts of the world. Until now, most mineral stresses have been dealt with by fertilizer or agronomic practices.

> Scientists "*...have successfully modified the soil environment with fertilizers to suit the plants that breeders have produced...*" (Graham, 1984).

Changing soils to meet plant needs may not be the most practical or economical solution to all mineral deficiency and toxicity problems in soils (Clark and Duncan, 1991). Geneticallly modifying plants to grow on soils with mineral problems without loss of yield or quality has great merit. Lower input requirements and reduced pollution and environmental problems could be some of the benefits obtained by having more tolerant plants to grow on soils with low or toxic nutrient availability. Indeed, breeding plants for adaptation to mineral stresses must form an important part of the strategy for attaining a sustainable global food production system. Projections of future needs for world cereal production must take into account the traditional 10 to 15 years required to produce new barley cultivars (Cartwright *et al.*, 1987).

Chapter 1

LITERATURE REVIEW

BORON TOXICITY

Boron (B) is an essential plant micronutrient, but it is also phytotoxic if present in excess. The range between deficient and toxic levels of B is narrower than for any other nutrient element (Reisenauer *et al.*, 1973).

History

The earliest report of B toxicity of barley in the field was by Christensen (1934) soon after B was recognised as an essential nutrient. He described a range of symptoms, including "non parasitic leaf spots", associated with certain kinds of soils in Minnesota, USA. He was able to reproduce these symptoms by the application of B under controlled conditions.

Penman and McAlpin (1949) and Sauer (1957) were the first in Australia to recognise crop damage due to high levels of boron. They reported damage due to B toxicity of citrus and vine grown under irrigation on sodic calcareous earths at Mildura, in northern Victoria. It was not until 1983 that B toxicity was recognised in South Australia in barley grown under dryland conditions (Cartwright *et al.*, 1984). It has since been shown that B toxicity occurs at many locations across the cereal belt of South Australia (Cartwright *et al.*, 1986). Hirsch and Manton (1988) estimated that the total area of the South Australian barley growing area showing B toxicity symptoms in 1988 was 289,200 ha, over 41% of the total area sown that year.

Distribution

The levels of B in grain of barley crop affected by B toxicity have been found to reflect the concentration of soluble B in soil (Cartwright *et al.*, 1984). Based on this observation, a survey of nutrient concentrations in barley grain sampled throughout South Australia in two years has

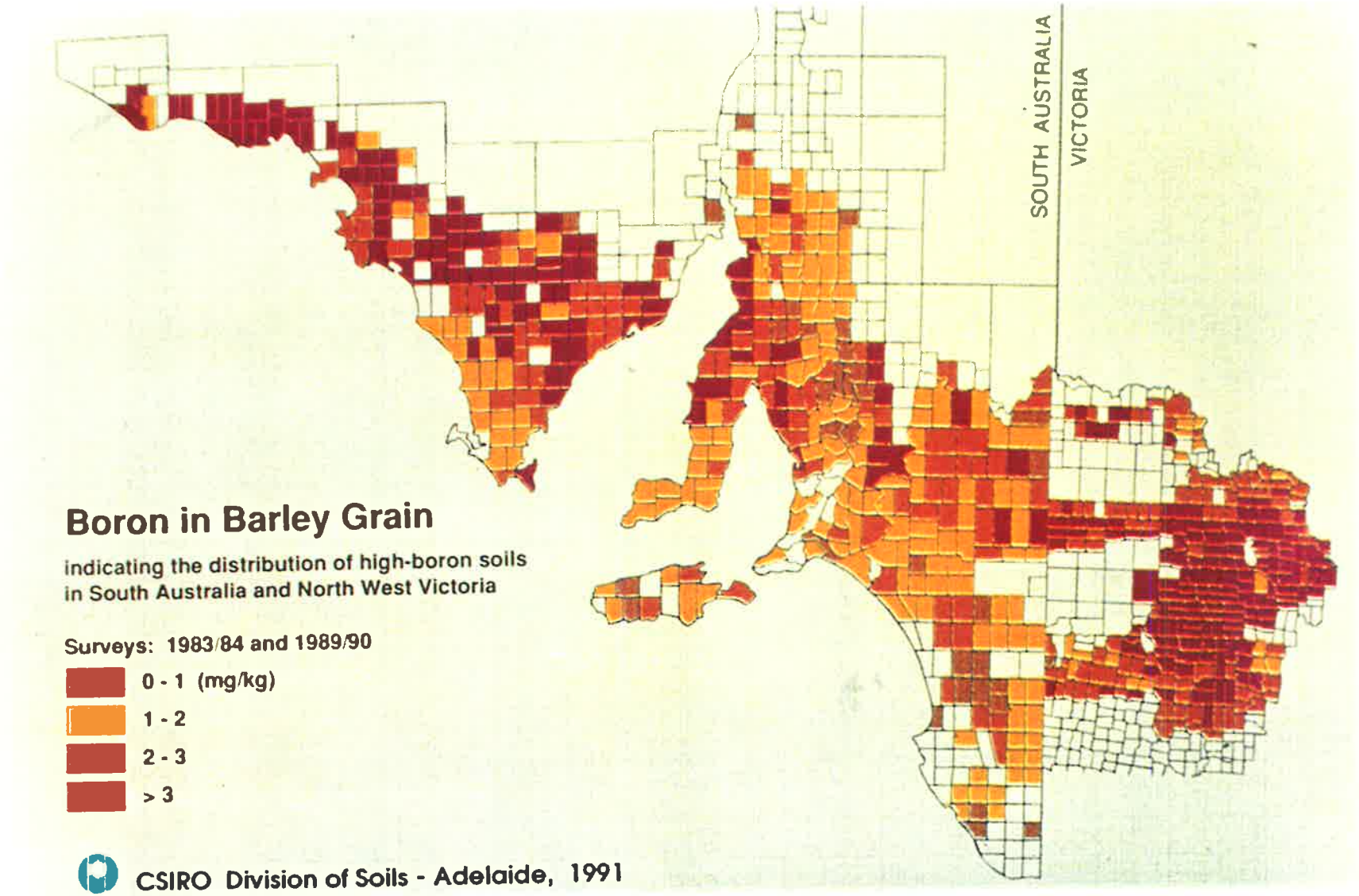


Figure 1.1. Boron concentration of barley grain samples collected from South Australia and Victoria in 1984 and 1990. Greater than 2 mg/kg indicates a risk of boron toxicity.

provided evidence for the distribution of potentially toxic levels of B in the soils of upper Eyre Peninsula, upper Yorke Peninsula and parts of the Murray Mallee (Cartwright *et al.*, 1986). The problem has also been recognised in parts of the cereal belt of Western Australia (Khan *et al.*, 1985) following the release of the barley cultivar, Stirling, which is very sensitive to B toxicity. Ralph (1986, 1991) published maps constructed by the CSIRO Division of Soils based on B levels of barley grain produced in South Australia and Western Victoria (Figure 1.1.). It is probably significant that these are areas in which the wheat cultivar Halberd is grown most extensively (Rathjen and Pederson, 1986). This distribution and the superior performance of Halberd during drier seasons and in districts with low average rainfall (Rathjen, 1977), provided initial evidence for the boron-tolerance of Halberd (Cartwright *et al.*, 1987). The variation in tolerance to B that exists in wheat (and barley) is potentially useful for improvement of yields on soils with high levels of soluble boron.

Symptoms

Boron toxicity in cereals is difficult to diagnose under field conditions. Only barley has specific symptoms (Eaton, 1944), and even these may be confused with those of fungal disorders (Rathjen *et al.*, 1987) such as Arno Bay Blotch (*Pyrenophora hordei*) or Spot Blotch (*Bipolaris sorokiniana*) (Wallwork, 1992) (Figure 1.2.). Cartwright *et al.* (1986) described symptoms in barley as a:

> *"condition characterized by chlorosis and necrosis extending from leaf tips, and with brown lesions forming initially at the margins, and later over the distal half or more of the laminae. Oldest leaves are affected first, and successive leaves become affected in sequence. In severe cases brown lesions are present on leaf sheaths, stems, ears and awns."*

No simple field test to confirm a diagnosis based on leaf damage symptoms is yet available and so confirmation of the problem is dependent upon plant and soil analysis.

Diagnosis

The prediction of the severity of the effects which B toxicity will have on a crop on the basis of a soil test is a difficult problem (Cartwright *et al.*, 1984). Gupta, *et al.* (1985) discussed in their

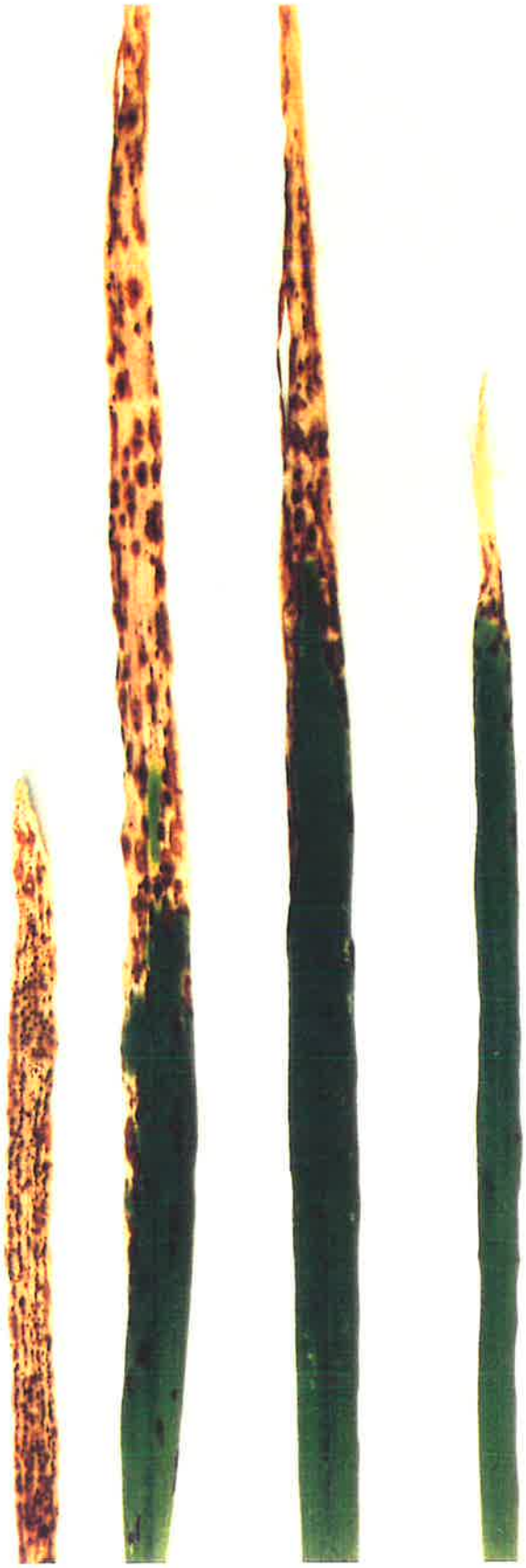


Figure 1.2. Leaf symptoms typical of B toxicity in barley.

review, soil and environmental factors influencing B toxicity and deficiency in plants. Accumulation of B in plants is often said to be a function of the concentration of B in soil solution (Hatcher *et al.*, 1959) and the amount of water transpired by the crop (Oertli, 1962), since B uptake is generally considered to occur via mass flow. However, Cartwright *et al.* (1984) pointed out that under field conditions, the final concentration of B accumulated will also depend upon the distribution of soluble B and roots with depth in the soil. Their results suggested that it was the B in the deeper parts of the subsoil that most strongly influenced the patterns of symptoms and B concentrations found in the plants. However, in the absence of measurements of root density, they could not say what proportion of the root system actually grew into the 70-80 cm depth interval and encountered high concentrations of soluble B. Rainfall may also affect the use of foliar analysis for diagnosing B toxicity (Nable and Moody, 1992).

Affected soils

A wide range of soils in the southern Australian cereal belt are high in soluble B, including; red brown earths, calcareous earths and heavy grey clays (Cartwright *et al.*, 1986). Soils usually contain less than 4 mg kg^{-1} easily extractable boron, but in toxic soils extractable B is frequently above 20 mg kg^{-1}, and may occasionally exceed 100 mg kg^{-1}. These soils are generally alkaline throughout or have an alkaline reaction trend with depth. The soluble B concentration is usually highest in the subsoil, often rising to a well-defined maximum within 100 cm of the soil surface. Rathjen *et al.* (1987) suggested that this form of profile is probably related to the average depth of penetration of the wetting front due to precipitation. Boron toxicity is often associated with arid and semi-arid regions (Manyowa and Miller, 1991). High B contents are not necessarily related to high salt contents, but rather occur most commonly in non-saline, sodic soils (Rathjen *et al.*, 1987).

Other Sources

Boron toxicity can occur under 3 main conditions.

(1) in soils inherently high in B or in which B has naturally accumulated (Eaton, 1944; Wilcox, 1960; Chauhan and Powar, 1978; Severson and Gough, 1983);

(2) through the use of irrigation waters high in B leading to B accumulation and concentration in soil (Wilcox, 1960; Branson, 1976); and

(3) as a result of over fertilization with minerals high in B (MacKay *et al.*, 1962; Gupta *et al.*, 1976).

In some places the problem of B toxicity has been made worse by the use of irrigation water with a high B content derived from wells or springs located near geothermal areas or geothermal faults (Manyowa and Miller, 1991). High levels of B in irrigation waters was described in California, USA as early as 1931 (Scofield and Wilcox, 1931). Acidification of some neutral soils or alkaline soils increases availability of B and in some cases to toxic levels (Aldrich *et al.*, 1955). Boron is usually present in sewage sludges and effluents due to the use of borates and perborates in detergents. Hence, the uncontrolled application of municipal composts, sewerage sludges and effluents on agricultural land has in some cases resulted in B toxicity (Sopper and Kardos, 1973; Neary *et al.*, 1975). One of the main constraints of land utilization and/or disposal of fly ash is the B content due to the B-enriched status of some of the combustion residues of coal, such as fly ash (Adriano *et al.*, 1980). Boron toxicity has been reported in lowland rice at IRRI in the Philippines (Ponnamperuma and Yuan, 1966; Ponnamperuma *et al.*, 1979) and in volcanic areas (Krauskopf, 1972). Some high B regions have been reported in Israel (Aubert and Pinta, 1977) which may have implications about potential sources of tolerant germplasm.

Other Toxicities

Boron is only one of a number of elements to cause phytotoxicity under natural conditions. Toxic metals for example, occur naturally in soils since parent rocks and minerals contain the metals (Foy *et al.*, 1978). However, only a few metals are reported to cause phytotoxicity in soils. This is probably due to the fact that most toxic metals occur as inorganic compounds, or are bound to organic matter or clay fractions (Hodgson, 1963; Allaway, 1968; Jenne, 1968). As result only Al, B, Cu, Fe, Mn, Ni and Zn toxicities have been reported frequently (Manyowa and Miller, 1991). Toxicities of such metals as Pb, Co, Be, As and Cd occur only under very unusual conditions (Foy *et al.*, 1978), and Cr, Ag, Sn, Ga, and Ge are toxic in solution cultures, but are not phytotoxic in soils.

Summary

Boron toxicity is a problem in many areas of the southern Australian cereal belt, and may occur in a range of soil types. The narrow range between deficiency and toxicity makes the management of soils high or low in B difficult (Manyowa and Miller, 1991). Crop species differ widely in their tolerance to excess B; some plants are benefited by concentrations of B high enough to injure more sensitive plants (Brown *et al.*, 1972). Thus, selecting plant species tolerant to excess B seems more practical than changing the soil to fit the plant. Dvorák *et al.* (1991) stated that:

> *"understanding of the genetic and physiological mechanisms by which plants cope with adverse soil conditions is critical for the development of efficient strategies for breeding stress tolerant cultivars."*

THE CHEMISTRY OF BORON IN PLANTS AND SOILS

The unique chemical properties of boron (B) dictate its special behaviour in soils and plants. These characteristics have formed the basis for the many hypotheses attempting to predict the mode of action of B as a nutrient essential to the metabolism of vascular plants.

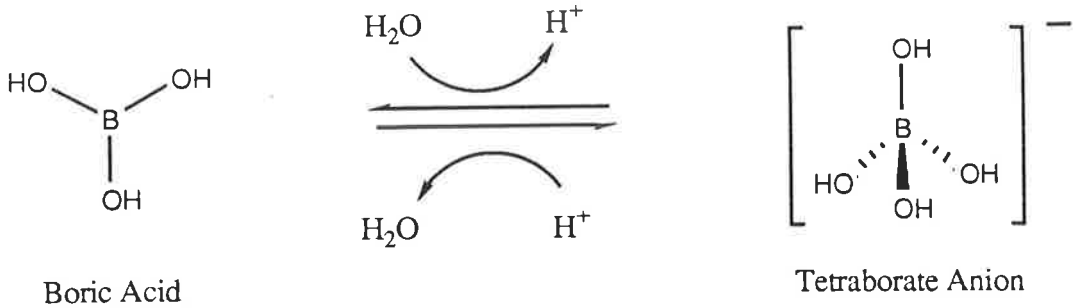
Boron is a nonmetal with an atomic number of five and a constant valence of 3^+. It is a member of Group IIIA of the periodic table, a family otherwise comprised of active metals. Boron is a "metalloid" and exhibits bonding and structural characteristics intermediate between metals and non metals. Like carbon (atomic number six), B has a tendency to form double bonds and macromolecules (Lovatt and Dugger, 1984). There are several features almost unique to B and the IIIA group of elements. These include the ability to form electron-deficient molecules (such as B trifluoride) and bridge bonds (such as those in diborane, B_2H_6) (Lovatt and Dugger, 1984).

Forms

The way B behaves in soil solutions is unlike the other trace elements. Boron has a maximum coordination number of four due to the small size of the atom (Loomis and Durst, 1991) (Figure 1.3). Boron occurs in aqueous solutions, at pH values less than 7.0 as boric acid. The planar $B(OH)_3$ is a weak monobasic acid that acts as an electron acceptor, that is as a Lewis acid. The

tetrahedral borate anions predominate in solutions of pH higher than 7.0 according to the reaction below:

$$B(OH)_3 + H_2O \rightleftharpoons B(OH)_4^- + H^+ \qquad pKa = 9.25 \ (25°C;\ Dean, 1979)$$



Boric Acid

Tetraborate Anion

Figure 1.3. The molecular structure of boric acid and the borate anion.

The undissociated boric acid is the preferred form taken up by plant roots (Marschner, 1986). Due to a number of similarities in physicochemical properties between $B(OH)_3$ and $Si(OH)_4$ comparisons are sometimes drawn between their biological properties (Raven, 1983; Nable *et al.*, 1990b).

Boron occurs chiefly as an oxyanion due to its small size (ionic radius = 0.23 Å) and its tendency to form covalent bonds (Evans and Sparks, 1983). These oxyanions are moderately to highly soluble (a saturated solution at 20°C is about 0.75M $B(OH)_3$ (Raven, 1980)). Boron then as the borate ion $H_2BO_3^-$ is readily leached in soils (Knight, 1991).

If B occurs in solution at concentrations above 0.1M, polyborate species are formed by the addition of one OH^- ion per B atom present. The cyclic trimeric anion is the polyborate formed under most conditions.

Complexes

Within plants the ability of B to form complexes with certain organic compounds is important. It is often stated that the ability of B to complex with compounds is dependent on their having adjacent OH- groups in the *cis* position (Lovatt and Dugger, 1984). It was suggested by Raven (1980) that "glucose, fructose and galactose and their immediate derivatives do not have the relevant *cis* diol configuration" to form B complexes. This is not the case, as is demonstrated by

the electrophoretic mobilities of these molecules (Frahn and Mills, 1959). In fact it is the distance between the two O-atoms of the diol which is important in determining the degree of ester formation, and the accompanying acidification (Loomis and Durst, 1991). Thus, sugars, sugar alcohols, uronic acids and other compounds in plants having an appropriate configuration of hydroxyls to displace the hydroxyls on the tetraborate anion, that is those with an OH at both the C_6 and C_4 position, can form complexes with B. Compounds able to form complexes include mannitol, mannan, and polymannuronic acid. These molecules serve, for example, as constituents of the hemicellulose fraction of cell walls (Marschner, 1986) in many plants, though they only occur at low levels in grasses. Some o-diphenolics, such as caffeic acid and hydroxy ferulic acid, which are important precursors of lignin biosynthesis in dicotyledons (McClure, 1976) and grasses, also form stable borate complexes. Of those polysaccharide components which dominate in barley (Fincher, 1992), cellulose and xylans do not have appropriate configurations to bind with B, but 1-3 β-glucan units in miced linked β–glucans may.

The type of complexes formed between B and polyhydroxyl compounds depends on the ratio of borate to the polyol and the pH. In plants with the normal physiological level of B, it has been proposed for diols that the type of complex formed when the diol to borate ratio is high is the BD2 type, while the form that exists under low diol to borate ratio is the BD type (Lovatt and Duggar, 1984). In general, with increase in pH, compounds of type BD and BD2 increase at the expense of type A (Zittle, 1951) (Figure. 1.4.).

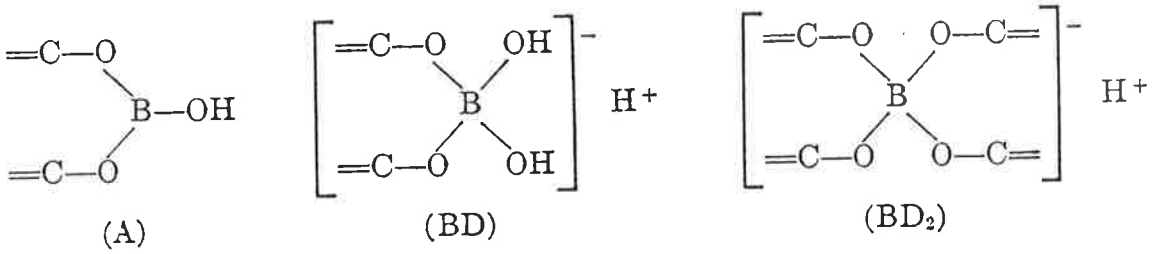


Figure. 1.4. Compounds formed by sugars and sugar alcohols in the presence of tetraborates (Zittle, 1951).

Compounds with more than two hydroxyl groups react more strongly, and the intensity of the reaction increases with increase in the number of the appropriately oriented hydroxyl groups (Zittle, 1951). *Cis*-inositol, with three hydroxyls in the right conformation to form borate complexes, forms the strongest known borate complexes. This however is a synthetic molecule. The naturally occurring seylloinositol forms a *bis* tridentate when seyllitol is heated in borate solutions. There are four types of known polyolborate complexes: (1) uncharged triesters; (2) bidentate tetrahedral anions; (3) tridentate tetrahedral anions; (4) tetradentate (bis bidentate) tetrahedral anions. The fourth of these tie chains of polyol molecules together at about 90° to each other, and hence can form a gel network with considerable physical strength. This may explain why B is essential for germination of pollen, where a rigid polymeric structure is required. Candidates for molecules likely to produce this cross-linking in plants include β-1-3-glucans and β-1-2-glucans with participation of the C_6 hydroxymethyl and C_4 hydroxyls. β-1-4 linked structures such as cellulose cannot form these tetradentate structures involving the C_6 and C_4 hydroxyls. Tetrahedral borate complexes of polyols are anionic and move in an electric field. Borate-diol complexes have a much lower pKa than the free boric acid (Raven, 1980).

Summary

The unique chemical properties of B means that it occurs in soil solution in a number of forms depending on its concentration and the pH, and under most conditions is highly soluble. The ability of B to complex with a large number of biologically important substances may alter the involvement of those substances in the metabolic reactions of plants (Lovatt and Dugger, 1984).

BORON IN SOILS

Boron (B) is present in soils in various minerals, in organic matter, and in the soil solution, in equilibrium with B adsorbed on surfaces of soil particles (Bingham, 1973). It is the soluble proportion which is available to plants (Hatcher *et al.*, 1959). Many properties of the soil influence the total amount of B present and the proportion of this available to plants.

> *"The physical, mineralogical, chemical, and biological properties of soils govern the concentration of an element in the soil solution that, along with the rate at which the element can be replenished, governs the element's bioavailibility."*(Page *et al.*, 1990)

The bulk of B originates from soil minerals, hence the B content of the soil tends to reflect the B content of the parental material (Whetstone *et. al.*, 1942). Boron is universally distributed in soils (Eaton and Wilcox, 1939) and is present in the earth's crust at about 10 mg kg^{-1} (Evans and Sparks, 1983). Soils on average though have a higher content of B than rocks (Norrish, 1975). Sedimentary rocks, particularly marine shales are generally richer in B than igneous rocks. The abundance of B in shales suggests that dissolved borates have been predominant in sea water throughout the earth's history (Krauskopf, 1972; Evans and Sparks, 1983), though Lovatt (1985) suggests that since the oldest life forms do not have a B requirement, the oceans have not always been high in B. In nature, B is found as a constituent of axenite, tourmaline, ulexite, colemenite, and kermite (Evans and Sparks, 1983) as well as borax. The B in rock, however, is not very available to plants and most of the plant-available B comes from B adsorbed and precipitated onto the surfaces of soils particles and from the decomposition of soil organic matter (Russell, 1973; Bingham, 1973; Bowen, 1977). In general the total B content of soils is around 20 to 200 mg kg^{-1} (Mengel and Kirkby, 1982), but only about 0.4 to 5 mg kg^{-1} is available to plants (Gupta, 1979). That is, less than 5% of the total soil B is available for crop uptake (Gupta, 1968).

Adsorbed B

Boron precipitated and adsorbed on surfaces of soil particles is probably of greatest importance to plant growth because an equilibria exists between adsorbed and soluble B (Eaton and Wilcox, 1939; Russell, 1973; Bingham, 1973; Gupta *et al.*, 1985). Retention of borate by soils probably involves the reaction of borate ions with surface hydroxyls meeting the steric requirements of the diol-borate complex as suggested by Sims and Bingham (1968). Plants respond primarily to the soil solution B independently of the amount of B adsorbed by soil and the adsorbed pool of B acts as a buffer against sudden changes in solution B (Hatcher *et al.*, 1959). Consequently, conditions affecting equilibia between adsorbed and soluble B are important when considering plant nutrition and diagnostic procedures for soils and irrigation waters (Bingham, 1973).

Availability

The availability of soil B depends on soil texture, pH, and soil moisture content (Evans and Sparks, 1983; Hatcher *et al.*, 1959; Bingham *et al.*, 1970; Stinson, 1953; Wear and Patterson,

1962). In general, B concentrations are usually higher in clay and loam soils than in sandy soils (Gupta, 1968) and increases in the pH of the soil system in the alkaline range results in increased adsorption. The major consequence of this is that B can be more easily leached from sandy (Wilson *et al.*, 1951) or acid soils (Aubert and Pinta, 1977). Deficiencies are prevalent in humid regions due to leaching, but can also be induced by drought. In acid conditions much of the total B in soils is held by organic matter and is released by microbial action. However, under drought conditions microbial activity is decreased and thus B remains complexed and unavailable to the plant (Berger, 1949). A more detailed knowledge about how B uptake is affected by these factors would improve our assessment of B deficiency and toxicity under different conditions (Gupta *et al.*, 1985).

In general, both total and plant-available B can be high in arid or semi-arid areas where leaching is limited. Generally, soils that have developed in humid regions have low amounts of plant-available B because of leaching, and B deficiency is widespread. Boron like sodium and chlorine, is soluble and will accumulate where salts accumulate. In semiarid areas, B in the subsoil often exceeds that in the surface soil (Whitestone et. al., 1942; Berger, 1949; Werkhoven, 1964; Hutchinson and Viets, 1969; Il'in and Anikina, 1974). Often B in such soils exists as sodium and calcium salts (Berger, 1949) and is usually found at toxic levels in saline and sodic soils (Hutchinson and Viets, 1969; Il'in and Anikina, 1974; Gupta *et al.*, 1985) or in areas with a shallow water table (Wilcox, 1960).

Irrigation in many parts of the world is frequently a source of high soil B (Eaton, 1935). Though the levels in the water is rarely high enough to cause injury directly, B may accumulate over a number of seasons to high levels in soils (Eaton, 1935). B in irrigation waters may be natural, from pollutants (Kelley and Brown, 1928) or where evaporation is high, may concentrate in reservoirs. Boron toxicity may arise from human activities, most commonly overuse of fertilizers high in B (Gupta *et al.*, 1985).

Amelioration

Leaching from the soil is a method most often suggested to ameliorate B toxicity problems (Eaton and Wilcox, 1939; Prather, 1977). Bingham *et. al.* (1970) found that irrigation rates and

depths of water applied had a significant effect on the extent of B leaching. Though leaching may reduce plant available B in soils initially, soluble B concentrations increase again with time following reclamation. This is likely due primarily to release of B from the adsorbed fraction and to redistribution of B from bypassed to leachable portions of reclaimed soil (Bingham and Rhoades, 1986).

Boron as boric acid can be removed from water with strong base anion-exchange resins during deionization, although all other ionic species are also removed, rendering the operation uneconomical if B is the only objectionable constituent. Kunin (1973) has developed a B-selective ion-exchange resin called Amberlite XE-143. These methods however are expensive and impractical on a commercial scale. Another proposed method of reducing the B available to plants is by liming. In some soils by producing an increase in pH this may increase B fixation, thus reducing availability (Midgley and Dunklee, 1940; Wolf, 1940). This however is not practical in already highly alkaline soils.

Summary

The behavior of B in soils is complex, and may be influenced by many properties of the soil and the environment. Since B concentration in soils often varies with depth and determining that fraction of the B which is available to plants is difficult, soil testing alone is not a suitable method for prediction of B toxicity. It is important when dealing with the affects of high soil B on plants to consider the different forms that B can take in soils and the factors influencing the equilibrium between these forms.

BORON AND OTHER ELEMENTS

For over sixty years, since it was first found to be essential to plant life, there has been considerable speculation about the relationship between boron (B) and other nutrients, both in the soil and in plants. The most commonly mentioned relationships are with calcium and pH, but others include potassium (K), zinc (Zn), phosphate (P), nitrogen (N), magnesium (Mg), sulphur (S), sodium (Na) and aluminium (Al). Gupta (1979) in one of his reviews discussed a number of these relationships at length.

Ca in Soils

Many studies suggest that Ca uptake is related to B uptake and to the appearance of B deficiency symptoms in plants. However these studies have yet to provide a complete understanding of Ca-B interactions. Reeve and Shive (1944) reported that in tomato plants B deficiency symptoms became more pronounced and toxicity symptoms became less pronounced as the Ca concentration of the medium was increased. Analyses of plant tissues, however, often failed to define a clear relationship between B and Ca (Morris, 1938; and Talibli, 1935). Tanaka (1967) also found Ca may inhibit B uptake. Other researchers (Brennen and Shive, 1948; Gilbert, and Robbins, 1950; McIlrath and de Bruyn, 1955; Reeve and Shive, 1944) have reported that increased Ca levels generally resulted in lower B content of the plant, when soil B levels were high. Minarik and Shive (1939) concluded that these inconsistencies between the results of the field observations and chemical analyses of plant tissue arose from varying effects of each element on plant species and differences in experimental conditions.

pH

In general, B becomes less available to plants with increasing soil pH, but findings are not always consistent (Gupta, 1979). Midgley and Dunklee (1940) found that symptoms attributed to overliming injury were in fact due to B deficiency caused by fixation of B in the soil as a result of increased pH. Gupta and MacLeod (1977) found that the sources of Ca and Mg were important, and that observed affects on B nutrition were mostly the result of pH effects. pH effects have also been observed by Barber (1971), Gupta and Cutcliffe (1972), Wear and Patterson (1962), Barber (1971), Gupta (1972b) and Gupta and MacLeod (1977)

Cook and Millar (1939) and Wolf (1940) have shown that when the pH value and Ca or Mg concentration are raised simultaneously there is a much greater reduction in B absorption than when either factor is increased separately. Fox (1968), puzzled by an apparent tolerance to high soil B by cotton and alfalfa in Peru, found that in cotton at least, that high pH and Ca together had a big effect on B uptake, but had little effect separately.

Ca in the Plant

Brenchley and Warrington (1927) were among the first to suggest that a functional relationship exists between B and Ca in the plant. They suggested that even with an adequate supply of Ca at the roots, this element cannot be effectively utilized in the absence of B. Marsh and Shive (1941) noticed a similarity between Ca and B deficiency symptoms in many plants. They suggested that the soluble, metabolically effective Ca in active tissue is correlated with the supply of B in this tissue.

Leaf tissue Ca/B ratios have been considered as indicators of the B status of crops (Gupta, 1979). Gupta (1972b) suggested that the Ca/B ratios of greater than 1370 in barley boot stage tissue appeared to be indicators of B deficiency and that values of 10-45 are indicative of severe B toxicity, while 180 is about optimal. He pointed out that the use of the Ca/B ratio in assessing the B status of plants should be viewed in relation to the sufficiency of other nutrients in the growing medium and in the plant. Cartwright, Zarcinas and Spouncer (1986) found at a high B site at Gladstone, South Australia, mean ratios were 31 and 213 for toxic and normal samples respectively, and other nutrients measured fell within a normal range (Schulz and French, 1976). The results of experiments conducted on alkaline soils by Manchanda and Yadav (1978) suggested that Ca/B ratio in barley straw was not a reliable index for determining the magnitude of B problem in the soil.

Zinc

Recently, a relationship between B and Zn had been postulated. Graham (1984) pointed out that B toxicity is frequently associated with alkaline soils and salinity in semi-arid environments (Bradford, 1966; Cartwright *et al.*, 1984), and since many of these soils types may also be low in Zn (Lindsay, 1972), Zn deficiency could be a predisposing factor in B toxicity. Zinc fertilizers, added to the topsoil, may not cure the problem since the Zn is required in the environment of those roots where the B concentration is high, usually the subsoil roots. He suggested that genetic Zn efficiency, that is an ability to tolerate low soil Zn, may contribute to the control of B toxicity under these conditions. Under low light conditions Leece (1978) found B prevented inactivation of Zn and increased plant growth in maize. This effect was not repeated under higher light. He concluded that it is unlikely that Zn inactivation under normal field conditions

is caused by marginally deficient B levels. Graham, *et al.* (1987) tested the hypothesis that Zn deficiency enhances the accumulation of B in barley. At low Zn they found B uptake was enhanced compared with those supplied with adequate Zn. They suggested then that the effects of Zn on B uptake, supported the concept that Zn performs a protective role at the external surfaces of, or in, root-cell membranes.

Singh, Dahiya and Narwal (1990) found that Zn deficiency in wheat may enhance B absorption and transport to such an extent that B may accumulate to toxic levels in plant tops. They found that the application of B increased the tissue concentration of B in wheat plants more in the absence than in the presence of Zn. They suggested that Zn application appeared to have created a protective mechanism in the root cell environment against excessive uptake of B.

Other Elements

Gupta (1979) believes that among the macronutrients, N is of utmost importance in affecting B uptake by plants. Chapman and Vanslow (1955) found that liberal N applications are sometimes beneficial in controlling excess B in citrus. Nitrogen was also found beneficial under high B conditions by Jones *et al.* (1963) and Gupta *et al.* (1973) and under glasshouse conditions but not in the field by Gupta *et al.* (1976). Gupta (1979) suggested this may indicate that application of N is helpful in alleviating B toxicity only on soils low in available N content.

The effects of P, K, and S are less clear than those of N on the availability of B to plants (Gupta, 1979). Wolf (1940) found Mg had a greater effect of B reduction in plants than did Ca, Na, or K, but the differences between Ca and Mg effects were small. Reeve and Shive (1944) suggested that B deficiency is exacerbated by excess K. Nasbaum (1947) observed increasing severity of B deficiency symptoms with decreasing application of P, or increasing application of either N or K. Tanaka (1967) also suggested that the concentration of K in the growth medium strongly influences the accumulation of B in plant tissues. Other studies include those by Yamaguchi *et al.* (1958), Bubdine and Guzman (1969) Stoyanov (1971) and Kar and Motiramani (1976). More recently, Bingham *et al.* (1987) found no interaction between B toxicity and salinity. LeNoble *et al.*(1991) suggested B may alleviate to some extent problems caused by high aluminium to alfalfa roots, but this was not supported for wheat by Taylor and

Macfie (1991). Nable (1988b) showed that an excess B supply had no detectable effect on the concentrations of other nutrients (P, K, Mg, Ca, Cu, Zn, Mn, Fe) in several genotypes of barley and wheat. Gupta (1979) conceded that it is possible that various crops behave differently.

Summary

Though consistent relationships between B and other nutrients are difficult to prove, it is clear from the above studies that when considering plant stresses, whether they be nutritional or environmental, it is important to take into account a range of factors. No factor acts in isolation, and interactions under many circumstances will be important.

ANALYSIS FOR BORON IN PLANTS AND SOIL

Plant tissue or soil extracts can be assayed to diagnose whether a plant is experiencing deficiencies and toxicities of trace elements. Generally, these data are compared with criteria that define ranges of concentrations believed desirable. A plant's response to trace elements is affected by many factors specific to the species and soil. These factors complicate the diagnosis of trace element deficiencies and toxicities (Page *et al.*, 1990).

Plant Analysis

The nutritional deficiencies and toxicities of plants can be diagnosed according to visual symptoms and plant tissue analysis. Visual symptoms caused by the deficiency or toxicity of one element often are similar to those of another element. Therefore, an elemental analysis of the leaf tissue is needed to confirm the cause. Early recommendations on boron tolerance of plants were largely based on visual symptoms. Francois (1984) however, believed that visual symptoms of boron (B) toxicity do not, at least in the case of tomatoes, necessarily correlate with the yield of marketable product.

Use of plant analysis as a diagnostic tool has a history dating back to studies on plant ash content in the early 1800s. Chemists working on the composition of plant ash recognised that relationships existed between yield and nutrient concentration in plant tissue. Quantitative methods for interpreting these relationships in a manner that could be used for assessing plant

nutrient status arose from the work of Macy (1936). Since then much effort has been directed toward refining plant analysis as a diagnostic tool (Smith, 1986). Advances in capabilities of instruments have made available more analytical techniques and more simplified procedures (Smith, 1986).

Critical Nutrient Concentrations in Plants

The concept of critical nutrient concentrations forms the basis of most methods for using plant analysis to assess plant nutrient status. In general, sensitive plants accumulate more B than tolerant (Eaton, 1935). Smith (1986) defined the "critical concentration for the specified plant part as that concentration of the single nutrient at which growth or production is found experimentally to be at a predetermined proportion of maximum eg 90%". This definition applies both to toxicity and deficiency. Critical value should be a range rather than a single value and should be defined experimentally. Empiricism is necessitated by a lack of knowledge regarding the functions of elements and lack of a means of measuring the effective concentration of nutrients at sites of reaction within plant cells (Smith, 1986).

The critical value for a particular nutrient is affected by numerous physical, environmental and biological factors. As early as 1935 Eaton recognised that responses to high levels of boron varied with soil type, climatic conditions and genotype and that boron is not uniformly distributed in plants spatially or temporally (Eaton, 1935). Chapman (1966) comprehensively reviewed the diagnostic criteria for major and trace elements.

Paull *et al.* (1990a) tried to establish in wheat which plant parameters and growth stage was optimal for rating genotypes with respect to tolerance to boron toxicity. They found that genotypic differences with respect to tissue boron concentration were greatest at harvests taken before maturity. In contrast to field results, boron concentration in grain from pot experiments was not an indicator of B concentration in shoots. Total dry matter production and height rankings were consistent between pot and field experiments and between growth stages.

Nable *et al.* (1990) discussed a range of problems in the establishment of critical values and the use of foliar analysis for diagnosing boron toxicity in cereals. These included, firstly, the close

relationship between boron accumulation and transpiration rates (Raven, 1980) and secondly, the potential for rain to leach boron from leaves (Oertli, 1969). These problems may account for conflicting reports of critical values and discrepancies between results from glasshouse- and field-cultured plants (Gupta *et al.*, 1976; Gupta, 1977; Paul *et al.*, 1988). Published critical values for boron toxicity in cereals range from 15 to 125 mg B kg^{-1} dry matter (Davis *et al.*, 1978; Gupta, 1977; Gupta *et al.*, 1976; Kluge and Podlesak, 1985; Riley, 1987). Reuter (1986) lists toxic concentration ranges for boron in barley. In whole shoots the level considered toxic ranges from 40 to 125 mg kg^{-1} depending on growth conditions and sampling age, generally based on 90% maximum grain yield. Other values listed were youngest emerged blade (YEB) at Feekes Stage 7-8 (see Large, 1954), 120 mg kg^{-1}, YEB+1 Feekes 7-8, 228 mg kg^{-1} and 308 mg kg^{-1} and mature grain greater than 3 mg kg^{-1}.

Page *et al.* (1990) stated that *"the elemental content of the tissue usually depends solely on the availability of the element in the soil"*. With respect to boron this is clearly not often the case. Doubt then is raised on the usefulness of applying critical values for boron toxicity measured in leaves when the environmental conditions of the plants being tested vary from those under with the critical levels were originally derived (Nable *et al.*, 1990d).

Other Approaches to Plant Analysis

Smith (1986) discussed some concepts other than critical concentration which may, in some circumstances, be more appropriate. Where a physiological basis for a nutrient ratio can be established, a ratio can be used to interpret plant analysis. Surveys of nutrient concentrations in 'deficient' and 'adequate' plants have been used to establish standard nutrient concentrations in some species. Other methods mentioned by Smith (1986) which at present are not relevant to boron studies include crop logging, spot tests, biochemical techniques and physiological techniques.

Soil Analysis

In general total B content of soils ranges from 7 to 80 mg B kg^{-1} (Bingham, 1982). The total boron content of soils however, has little bearing on the status of boron available to plants (Bingham, 1982). Eaton (1935) recognised that an equilibrium exists between the undissolved B

in the soil and the available B in the soil solution and a need for suitable determination methods for total and available boron.

Hot-water-soluble (HWS) boron is used most frequently to assess available levels of boron in soils (Page *et al.*, 1990). Berger and Truog (1939) first developed the method, which involved refluxing a soil solution in water for 5 minutes. Baker and Mortensen (1966) modified this procedure to use an aqueous solution of 0.1% $CaCl_2.2H_2O$. Keren and Bingham (1985) listed the minimum concentration of HWS B associated with optimum yield of barley as 0.1mg B kg^{-1}. Maas (1984) rated the relative tolerance of crops to boron in soil solutions at saturated water content. Barley was listed in the moderately tolerant, 2.0 mg l^{-1} class (Page *et al.*, 1990). These results must be viewed with caution. Physical and chemical properties of the soil influence an extraction result, so the deficiency and phytotoxicity threshold of an element may vary widely in different soils (Smith, 1986).

Cartwright *et al.* (1983) suggested that a $CaCl_2$-mannitol extraction method was a more convenient soil test for plant-available boron than the standard hot water soluble method, and was as good in predicting the response in boron uptake by plants. They suggested that the lack of information on boron in Australian soils may be in part due to the difficulty in making soil boron determinations on a routine basis.

Ideally, the soil extractant used to indicated the availability of the trace element would account for contributions of all applicable physical, chemical and biological processes in the soil related to the uptake of trace elements. Actually, no static extraction process can simulate these dynamic processes. The use of trace element extractants to evaluate the soil's deficiency and toxicity potential is derived from observations on amounts of an element extracted and the corresponding plant responses. Calibration of the criteria for conditions specific to the site and the crop is advisable (Page *et al.*, 1990).

Critical Nutrient Concentration in Soils

Ayres and Westcot (1976) classified the relative tolerance of a wide variety of crops to concentrations of B in irrigation water. This study was based on work by Eaton (1944) and on

visual symptoms of plants grown in sand cultures irrigated with nutrient solutions containing various concentrations of B. Sensitive crops show reduced yield or symptoms of injury when boron concentration in soil was greater then 0.3 mg l^{-1}, while tolerant crops withstood B concentrations in soil solution of 4 mg l^{-1} without showing any symptoms. Barley rated as semitolerant, showing symptoms of B toxicity between 1 and 2 mg B l^{-1}. Other workers to present information on the relative tolerance of crops to boron include Maas (1984), Ayers and Westcot (1976), Keren and Bingham (1984) and Gupta *et al.* (1985)

In the toxicity range, plants respond to boron in the soil solution rather than boron adsorbed on soil particles. Under field conditions soils are variable from place to place and in different horizons (Eaton, 1935) Hence, solution and sand culture data are generally used to evaluate the response of plants to boron.

Methods of Determination

A method for determination of boron should be precise, rapid and applicable to samples having a relatively wide concentration range (Bingham, 1982). Berger and Truog (1944) listed four main classes of methods for determination of boron in soils and plants. They are the quinalizarin method, titration, the curcumin method and spectroscopic methods.

Bingham (1982) put forward the azomethine-H method as preferred for determining the B content of water, soils and plant materials. Due to improvement in technical abilities elements may now be determined by various spectroscopic techniques including atomic absorption (AAS), X-ray fluorescence (XRF), flame emission and arc/spark emission spectroscopies, spectrophotometry and specific ion electrodes (Zarcinas *et al.*, 1987). Inductively coupled plasma - optical emission spectrometry (ICP-OES) has become an established analytical tool since it was first introduced by Greenfield *et al.* (1964) and Wendt *et al.* (1965) (Zarcinas, 1984).

Zarcinas and Cartwright (1983) pointed out the advantages of ICP analysis over such techniques as spectrophotometry for analysis of soil and plant material. The advantages are greater sensitivity; simultaneous multi-element analysis with direct readout of results; greatly reduced

interelement and matrix interference effects; and a wide dynamic range. They listed the detection limit for boron (wavelength 2497.7 Å) as 1 $\mu g\ l^{-1}$.

In some situations inductively coupled plasma - mass spectroscopy (ICP-MS) may be more appropriate. Sharp (1991) listed the advantages of ICP-MS as its high sensitivity for most elements (the detection limit for B is 0.1pg l^{-1}); an ability to carry out isotypic and isotope ratio determinations and to use isotope dilution internal standardization for high accuracy determinations; a greatly simplified spectra compared with the optical techniques; and the ability to carry out rapid semiquantitative analyses for the majority of elements.

ICP-OES and ICP-MS generally require sample presentation as a liquid. This involves the destruction of organic material by wet or dry-oxidation. Different methods exist for this, depending on the elements of interest (Zarcinas, 1984). Zarcinas and Cartwright (1983) found that for plant tissue analysis, the use of nitric acid only for digestion was advantageous.

Problems for Collecting, Handling and Analysing Plant Materials

Much larger errors are likely to occur at the phase involving sample collection and handling of the nutrient assessment process than during the actual analysis. One should be aware of sources of error and take steps to minimise these.

Collecting representative samples is most important. Sampling strategy must fit the aim of the study. Sampling should be done at a similar time and developmental stage for comparisons and be taken from appropriate plant parts (Reuter *et al.*, 1986). Care must be taken to avoid sample contamination, especially when dealing with trace elements, during handling and sample preparation, including transport, any decontamination procedure, drying, grinding and storage. Where boron is concerned one must be particularly aware of fragile leaf tips which are likely to contain the highest levels of boron, and if a washing step is to be undertaken one must be aware of the possibility of some of the boron being leached from leaves (Smith, 1986).

Summary

Plant tissue and soil analysis should be undertaken and interpreted with their limitations in mind. Though useful indicators of boron toxicity in some circumstances, many other factors may be involved in determining the ultimate response of the plant to this stress. The most reliable assessment of plant nutrient status will be obtained by pooling information from as many sources as possible including, soil tests, field and glasshouse experiments, foliar symptoms, plant analysis, biochemical tests and physiological tests (Smith, 1986).

THE ESSENTIALITY OF BORON FOR HIGHER PLANTS

Boron (B) has been used as a fertilizer for more than 400 years, but it was not until this century that it was shown to be an essential element (Mengel and Kirkby, 1982). Warington (1923) was the first to firmly establish a borate requirement for higher plants. Agulhon (1910) had earlier found that addition of boric acid may be beneficial to plant growth including wheat and oats, but did not demonstrate a clear requirement. Mazé (1914) postulated a B requirement for maize, but his study was limited. Warington (1923) showed a clear B requirement for broad beans, and beneficial effects on several other leguminous dicots, but could not at that time demonstrate a requirement by gramineous monocots. Sommer and Lipman (1926) later extended this to other higher plants, including barley.

All three criteria for essentiality proposed by Arnon and Stout (1939) are satisfied, since (1) no higher plant is known that can complete all its growth requirements without B, (2) no other element can replace the requirement for B (germanium will only substitute for a short time), and (3) B is directly involved in the nutrition of the plant (though the exact nature of this role is not clear) (Skok, 1958).

The classes of organisms requiring B may give some clues about its functional role. Diatoms and certain similar organisms and vascular plants, require B, while most bacteria, fungi, green algae, and animals apparently do not (Gauch and Dugger, 1954; Hewitt, 1963; Lewis, 1980; Lovatt, 1985; Lovatt and Dugger, 1984; and Parr and Loughman, 1983). The range for optimal growth in higher plants is [illegible] between about 0.01 and 4.0 mg B l^{-1} (Wilcox, 1960).

Within the Angiospermae monocots have a much lower requirement for B than do dicots (Pilbeam and Kirkby, 1983). Loomis and Durst (1991) pointed out that the unifying factor seems to be that all of the life forms that require B have cell walls, cell wall matrices, or cell envelopes, that are rich in carbohydrates.

FUNCTION OF BORON IN PLANTS

The primary role of boron (B) in plants is an area of considerable controversy. In contrast to other trace elements, B has not been demonstrated to be a component or activator of any enzyme system (Chapman and Jackson, 1974). Many have looked for the first morphological or physiological effect of B deficiency or toxicity to clarify B's role in plant metabolism (Dugger, 1973; Lovatt and Dugger, 1986). The kinds of organisms for which it is essential and the ability of B to form complexes with many carbohydrates are generally considered clues to the function of B.

Gauch and Dugger (1954) reviewed many of the postulated roles of B. Lewin (1980) reviewed a range of experimental evidence. Other reviews on the subject include; Skok, 1958; Hewitt, 1963; Shkol'nik, 1952, 1967, 1970, 1974, 1984; Gupta, 1979; Dugger, 1983; Lovatt and Dugger, 1984 and Römheld and Marschner, 1991. Vasil (1987) discusses the role of B with particular reference to pollen germination and experiments using pollen. Parr and Loughman (1983) cited a list of ten postulated roles:

1. Sugar transport
2. Cell wall synthesis
3. Lignification
4. Cell wall structure
5. Carbohydrate metabolism
6. RNA metabolism
7. Respiration
8. IAA metabolism
9. Phenol metabolism
10. Membranes

It is not intended to review all of these theories in detail. Boron probably plays a role, either direct or indirect in all of these processes and many of the theories overlap considerably.

Deficiency symptoms

General deficiency symptoms observed in angiosperms include inhibition of root growth, breakdown of root and shoot apical meristems and malfunction of reproductive systems (Parr and Loughman, 1983). These responses to the absence of B are rapid, occurring within a matter of hours. The longer term effects are characterised by breakdown of cell walls and the presence of brown or black slime. Sucrose translocation is considerably reduced in B deficient plants before morphological symptoms are evident (Gauch and Dugger, 1953). It has also been reported that the degree of hydration of cells is regulated by B (Schmucker, 1933; Minarik and Shive, 1939; and Baker *et al.*, 1956) Some attention has also been given to changes in tissue development and cell wall thickness in a number of tissue types (Palser and McIlrath, 1956; Gauch and Dugger, 1954; and Stiles, 1946). The observations of Reed (1947) and Skok (1957b) indicate that cell division can proceed in the absence of B but cellular maturation and differentiation is prevented. Some of these effects are manifest as poor grain set (Rerkasem and Jamjod, 1989) and foliar symptoms of B deficiency of barley. Leaf blades of B deficient barley plants have been described as *"wrinkled, mishapen, irregularly chlorotic and occasionally had split margins (i.e. 'saw-tooth' edges) and longitudinal splits"* (Nable *et al.*, 1990a).

Borate complexes

Skok (1958) suggested a relationship may exist between the physiological action of B in the plant and the capacity of the borate ion to complex with various polyhydroxy and related compounds, including several of the common sugars. Isbell *et al.* (1948) have discussed this complexing reaction and reviewed much of the early literature pertaining to it. Torssell (1957 a,c,d) gave further attention to this subject and has extended it to various B compounds. Zittle (1951) and Weser (1967) reviewed and discussed the complexing reaction of borate with various substances of biological interest. Schmucker (1934), Winfield (1945a), and Hoagland (1948) were among the first to call attention to the possible relationship between the complexing property of borate and the function of B in plants.

Skok (1957b) substituted other complexing substances for B in plant growth, to see if the complexing ability is important. If either Al, Sr, or Ge is added with minute amounts of B, the plants develop B deficiency symptoms later, and are less stunted, than plants receiving the element or B alone. Tchakirian (1943) has shown that mannitol and several sugars, as well as other polyhydric alcohols, form complexes with germanic acid. This suggests that B and some of the other complexing substances may function in certain common ways to some extent even though none of the latter can replace B entirely. The temporary alleviation of B deficiency symptoms by complexing elements Sr, Al and particularly by Ge, strongly suggests that the physiological role of B is in part related to the complexing property of the borate ion, but the basis of this relationship is not clear. The fact that B is not reutilizable, that is, that an available supply is required at all time, suggests that this complexing reaction may be related to the formation of a structural unit or "building block" rather than to a metabolic step reaction.

Winfield's (1945a) attempts to isolate a boric acid complex in its native state from plant material however were unsuccessful. An explanation for this may lie in the chemistry of these complexes. He hypothesised that when plant material is treated with water, during attempted separations of its constituents, the dilution will result in hydrolysis of the borate complex or complexes. Even the pH changes accompanying killing of tissue and expression of sap will influence the equilibrium.

Problems

Parr and Loughman (1983) discussed some of the problems in pinpointing the primary role of B in plants. These include the minute amounts of B involved, in the order of micrograms per gram of tissue; the wide range of biochemical and physiological symptoms associated with deficiency over time, such that it difficult to distinguish primary from secondary effects; and difficulties in comparison of normal and deficient tissues due to differences in physiological age. They presented in an excellent table a summary of the major experimental approaches used to elucidate the primary function of B.

Lovatt and Dugger (1984) cited two significant factors contributing to the difficulty of elucidating B's mode of action. The first is the lack of a radioactive or heavy isotope of B that

would facilitate localisation and transport studies. Without a radioisotope of B, all metabolic investigations of a role for B in plant metabolism have been comparative studies employing B-sufficient and B-deficient tissues. Some studies however have made use of a (n,α) nuclear reaction with the stable isotope of B - ^{10}B (Martini and Thellier, 1980). The second is the difficulty of establishing a zero B concentration for deficiency studies, with problems from contamination from glass, chemicals, water and dust. Since the range between deficiency and toxicity is very narrow, an accurate knowledge of the amount of B available is required.

Sugar transport

Gauch and Dugger (1953, 1954) suggested a role for B in carbohydrate translocation. Carbohydrate content of plants is affected by B and leaves of B deficient plants are often high in sugar. Gauch and Dugger (1953) suggest that subsequent B deficiency symptoms are simply the expression of carbohydrate deficiency resulting form an impaired translocation system. They suggested that a negatively charged sugar-B complex can more easily traverse cell membranes than non-borated sugar molecules or the B might be a constituent of the membrane site across which the sugar moves. Sisler *et al.* (1956) and Mitchell *et al.* (1953) supported this idea.

The sugar transport theory fell out of favour because borate reacts weakly with sucrose, the major form of sugar translocated within higher plants, and that the concentration of B is particularly low in the phloem, the main conduction pathway for sugar transport (Pilbeam and Kirkby, 1983). Sisler *et al.* (1956), McIlrath and Palser (1956), and Skok (1957a) applied sugars and citric acid to plants but generally did not find alleviation of B deficiency symptoms or increased growth.

Weiser *et al.* (1964) distinguished between leaf uptake and translocation of sugars in plants. From an examination on a number of feeding experiments (Gauch and Dugger, 1953; Nelson and Gorham, 1957; and Tunowshka-Starck, 1960) they concluded that B does not enhance sugar translocation but does enhance the uptake of foliar applied sugar. Skok (1957a) found that sugar translocation into the top portions was significantly reduced in +B plants with excised growing tips. These results are not consistent with the hypothesis that B functions primarily in the translocation of sugar. Many agree that it appears quite probable that some relationship between

B and sugar translocation does exist. This relationship, however, may be indirect and related to cellular activity and growth or phloem breakdown (Skok 1957a; 1957b; 1958).

Lignification

Numerous investigators have reported that lignification of xylem elements in B-deficient plants is invariably poor, and that B is generally abundant in bark, wood, and the lignified tissues (Skok, 1958; Dutta and McIlrath, 1964). Reed (1947) reported that tissues of B-deficient plants are high in phenolic compounds. Siegel (1953, 1954) has shown that various phenolic compounds act as lignin precursors but that they are converted to lignin in plant tissues only if hydrogen peroxide is added. In this connection it is interesting to note that Alexander (1942) has shown that B-deficient plants are exceptionally high in catalase activity. This may deplete the hydrogen peroxide level sufficiently to reduce or prevent lignin formation, with resultant accumulation of the phenolic compounds. These observations add interest to the claim of Shkol'nik and Steklova (cited in Gauch and Dugger, 1954) that addition of hydrogen peroxide to the substrate improved the growth of B-deficient plants.

Lewis (1980) developed a hypothesis for the role of B in lignin formation from an evolutionary point of view. He proposed that the development of an essential role for B was a prerequisite for the evolution of vascular from prevascular plants, particularly for xylem development. He suggested that the origin of this role for B depended on the selection of sucrose in the green algae, the ancestor of higher plants, as a mobile and storage carbohydrate since, compared with the acyclic sugar alcohols accumulated in other algal groups, sucrose forms only a very weak complex with borate. He argued that only when borate was not sequestered by complexing with other carbohydrates did the way become clear for it to acquire an essential role. This acquisition catalysed the evolutionary dichotomy between non-lignified and lignified photosynthetic land plants. Boron subsequently become involved in two other requirements for success on land - the exploitation of soil for anchorage, water and minerals; and the emancipation of fertilization from external water - since this element is required for development of adventitious roots and, in angiosperms, for germination of pollen.

Cell wall synthesis and Cell wall structure

Loomis and Durst (1991) present a strong case for a close relationship between B and primary cell walls. They site Lewis' (1980) arguments as support for a role of B in synthesis or structure of the kind of primary cell wall that is found in plant groups that produce lignin, that is vascular plants. They argue that the symptoms of B deficiency appear at a stage of cell development before lignification, and also appear in cells that do not lignify, and include abnormalities of the primary cell wall during its early stages. The symptoms characteristic of B deficiency can be explained by disruption to normal plant function due to inadequate cell wall structure. They proposed then a model, with supporting evidence, for borate cell wall cross-links.

The proposal of a role for B in plant cell wall structure is not new. Schmucker (1933) showed the benefit of B in producing pollen germination. Schmuker (1935) suggested that compounds of B and sugar may play some part in the formation of the pectin in the pollen tube wall. Burström (1942) proposed that the physiological action of B was connected with its property of forming complexes with carbohydrates, and its primary influence was supposed to be exerted on the elongation of the cell wall. In discussing the role of B in the various parts of the cell, Smith (1944) assigned special importance to the wall. Torssell (1956) believed that B is involved in the regulation of cell wall formation, in that complexes between boric acid and carbohydrates control the deposition of oriented cellulose micelles. He attributed the stiffness of B-deficient tissues and cell walls to improper deposition of the cellulose micelles, which results in the prevention of further stretching. Spurr (1957) described effects of B on the morphogenesis of plant cell walls and believed that B is related to some phase of carbohydrate nutrition involving cell formation. Skok (1958) suggested that it appears that B functions in some manner in maturation and differentiation of plant cells rather than to cell division and is built into some structural unit where it is then unavailable for movement to the reutilization on another site. He suggests that B may be involved in the formation of some cellular structure or structures such as the cell wall, cell wall constituents, or other cellular entities. Slack and Whittington (1964) investigated the effects of differentiation and deficiency on radicle metabolism using ^{14}C. They found two early symptoms of B deficiency, an increase in incorporation of activity into pectic substances and an increased acid resistance. Their evidence suggested that B is not required for a specific enzyme involved in cell-wall synthesis but that borate ions act as bonding agents

between cell-wall polysaccharides. Some other early workers to advance a similar view were Winfield (1945b), Bobko (1949), Whittington (1959) and Starck (1963).

Odhnoff (1961) supported the idea of a role for B in stabilising cell walls. He cites as evidence the influence of B concentration on the kind of complexes formed with polyols and therefore the kind of bonds in cell walls. The BD and BD2 complexes Figure 1.4) are formed in different proportions depending on the relative concentrations (Isbell *et al.*, 1948, Deuel and Neukom 1949; Krejci *et al.*, 1949; Zittle, 1951). If B is scarce compared to carbohydrate, the complex would be preferably of the BD2 type. With richer B supply in relation to carbohydrate, proportionally more BD complexes would form. BD2 bonds ought to give a more rigid coupling of the carbohydrate chain than BD bond which may be supposed to slip more easily over each other increasing the elasticity of the tissue. In B-deficient plants the very small amount of B present should be bound as almost all BD2. In normal plants both BD and BD2 bonds may be expected. As long as the B atoms are relatively close in the cell wall, BD complexes will dominate and the elasticity is high. When the distance between the B atoms increases, relatively more BD2 complexes are formed and the elasticity decreases. Spurr (1957) remarked that celery grown at low levels of B was more flexible than normal. Boron deficient plants have stubby, brittle roots which suggests a role of B in rigidity of cell walls (Preston and Hepton, 1960).

There is considerable circumstantial evidence to support a role for B in cell walls. Large amounts of B are present in cell walls (Mayevskaya *et al.*, 1970). Neales (1960) pointed out that a supply of B is required throughout the growth and development of the plant and B may therefore have a structural role in which binding with diols is involved. Phenylboronic acid also complexes with diols and can substitute for boric acid in the diatom *Cylindrotheca fusformis*, whereas derivatives such as tetraphenyl borate do not substitute (Neales, 1967). While borate ions complex with two sugar molecules, phenylboric acid can complex with only one; this suggests that its stimulating effect on root growth (Torssell, 1956; Odhnoff, 1961) may result from the fact that it can link to only one polysaccharide chain. Ginzburg (1961) showed that pre-treatment with borate buffer prevented the separation of root-tip cells when treated with ethylene diamine tetraacetic acid (EDTA). This effect was not noticed with other buffers. Furthermore, several workers have reported an interaction between calcium and boron (Reeve and Shive,

1944) which suggests that borate as well as calcium ions act as bonding agents in cell walls. Alterations of cell wall development appear well before the symptoms of boron deficiency appear (Lee and Aronoff, 1966; Alekseyeva, 1971). Kouchi and Kumazawa (1975, 1976) reported an increase in the pectin and hemicellulose fractions but a decrease in the cellulose fraction under boron deficiency. Boron deficiency has different effects on the thickness of cell walls, depending on the type of tissue (Spurr, 1957). In B-deficient tissues the parenchyma and other thin-walled cells disintegrate; fragility of the stem is also frequently observed (Winfield, 1945b; Mayevskaya *et al.*, 1970). Other workers (Torsell, 1956; Starck, 1963) believe that B influences the elasticity of cell walls, and more specifically the arrangement of microfibrils, which largely determine this elasticity. Dugger (1983) observed disorganised microfibrillar structure of the cell walls of B-deficient sunflower plants.

Marschner (1986) reported that a substantial proportion of the total B content of higher plants seems to be complexed as stable cis-borate esters in the cell walls as demonstrated by Thellier *et al.*, 1979. The fact that the B requirement of dicotyledons is greater than that of monocotyledons Marschner (1986) presumed was related to the higher proportions of compounds with an appropriate diol configuration in the cell walls, mainly in the hemicellulose fraction and in lignin precursors as shown by Lewis, 1980a. It was reported by Tanaka (1967) that the content of strongly complexed B in the root cell walls is 3-5 mg kg^{-1} dry wt in monocotyledons (eg. wheat) and up to 30 mg kg^{-1} dry wt in certain dicotyledons (eg. sunflower). These differences, he suggested, roughly reflected the differences between the species in the B requirement for optimal growth. He postulated that the functions of this apoplastic B are somewhat similar to those of calcium in both regulating synthesis and stabilizing certain cell wall constituents, including the plasma membrane.

Gramineae

Loomis and Durst (1991) discussed the uniqueness of the Gramineae, with respect to correlations of B with other nutrients and of cell wall chemistry. In general, gramineous monocots have a low requirements for B and calcium, high silicon content and low content of pectic polymers. Carpita (1987) presented contrasting models of primary cell walls of dicots and gramineous monocots. The gramineous primary wall contains little pectic material.

Shkol'nick (1984) supported a role for B in dicots in phenol metabolism. He suggested that the relatively low sensitivity to B deficiency of wheat and barley, and the fact that they do not suffer many of the metabolic disruptions observed in dicots particularly, suggests a different primary role for B in these plants.

Membranes

A number of workers have suggested a role for B in membrane structure or function. They suggest that many symptoms of B deficiency in plants are secondary effects caused by changes in membrane permeability. Tanada (1978) suggested that B is required to stabilise a positive electrostatic charge in the plasma membrane. Tanada (1983) found that, on a protein basis, a major part of the B in protoplasts is localised in the membranes. Recently, several lines of evidence have appeared which suggest that B is necessary for some kind of membrane function (Hirsch and Torrey, 1980; Parr and Loughman, 1983; Pilbeam and Kirkby, 1983; Pollard *et al.*, 1977; Rothbejerano and Itai, 1981; Smythe and Dugger ,1980; Torchia and Hirsch, 1982).

Metabolism, hormones and nucleic acid

A vast array of more specific metabolic functions have been suggested as the primary function of B. These include involvement with DNA synthesis (Cohen and Albert, 1974; Krueger *et al.*, 1987, Lovatt, 1985 and Lovatt and Dugger, 1986) and RNA synthesis and metabolism (Shkol'nik and Soloviya, 1960; Sherstnev and Kurilenok, 1962; Shkol'nik and Kositsyn, 1962; Sherestnev and Razumova, 1965; Chapman and Jackson, 1974; and Jackson and Chapman, 1975). Shive (1936) suggested a role for B in protein synthesis.

Rajaratnam *et al.* (1971), Rajaratnam and Lowry (1974) and Artés *et al.* (1984) associated B with flavonoid synthesis. Loughmen (1976) suggested B may be involved in phosphate transport. Dugger and Humphreys (1960) suggested a role involving UDPG, as did Birnbaum *et al.* (1977).

A number of investigators have considered B to be related to the formation of pectic substances (Gauch and Dugger, 1954; Johnson and Dore, 1929; Marsh and Shive, 1941; Hoagland, 1948). Baker *et al.* (1956) on the other hand found leaves of B deficient plants to have a higher

concentration of pentosans and pectic substances than leaves of normal plants. Winfield (1945b) pointed out that *Aspergillus niger* and *Penicillium glaucum*, require neither B nor Ca, and are incapable of synthesising true pectic compounds. This theory was not supported by the experiments of Skok (1958).

Coke and Whittington (1967) stated that B-deficient tissue suffers from excess auxin either because the element is necessary for some growth process, such as cell wall formation or nucleic acid synthesis, which when impaired, results in the accumulation of auxin, or because the IAA-oxidation system is affected by phenolic inhibitors which B normally inactivates by complex formation. Shkol'nick (1984) supported a role for B in phenol metabolism of dicots. Jarvis *et al.* (1984) based on the interaction between auxin and B in adventitious root development, postulated a possible role for B in controlling endogenous auxin concentration via an effect on IAA oxidase.

Attempts have been made to compile the many, often contradictory results on B deficiency-induced metabolic changes in higher plants and to develop a more unified model of the action of B (Marschner, 1986). All these models are based on the capacity of B to form reversible or irreversible diol-borate complexes of different stabilities with substrates, enzymes, and/or membranes and in this way to affect enzyme activities and metabolic pathways. A primary function, proposed by Lee and Aronoff (1967), is based on the capacity of B to form 6-P-gluconate-borate complexes and thus restrict both the flux of substrate into the pentose phosphate pathway and the synthesis of phenols. As a result , glycolysis and the synthesis, for example, of hemicellulose and related cell wall material increase. Sound evidence exists for a shift toward an increase in the substrate flux into the pentose-phosphate pathway under B deficiency (Eichhorn and Augsten, 1974; Birnbaum *et al.*, 1977), which is also reflected in an accumulation of phenolic substances in B-deficient plants (Perkins and Aronoff, 1956).

Shkol'nik (1970) by collating the various effects of B deficiency on plant processes formulated a unifying theory. He proposed that the primary alteration in metabolic processes when B is withheld from the growing plant involves membrane breakdown. This breakdown, in turn, results in a release of RNAase from the bound, inactive form, which is followed by an alteration

in nucleic acid and protein synthesis. He further built on this theory in later publications (Shkol'nik, 1974,1984). Dugger (1973) suggested that these changes are related to the observed reduction in cellular phospholipids, membrane degeneration, increase in RNAase activity, and a possible shift in the catabolism of carbohydrates, with a larger fraction being oxidised via the pentose phosphate pathway. This in turn results in both an increased level of phenolic compounds and the inhibition of IAA oxidase. Consequently, the IAA level will be higher in B deficient plants. Thus, as outlined in Figure 1.5, under B deficient conditions, starch and glucose-6-P are produced from glucose-1-P, at the expense of UDPG, leading to increased production of sucrose and ribulose-5-P. To what extent the reverse is true under B toxic conditions is yet to be investigated.

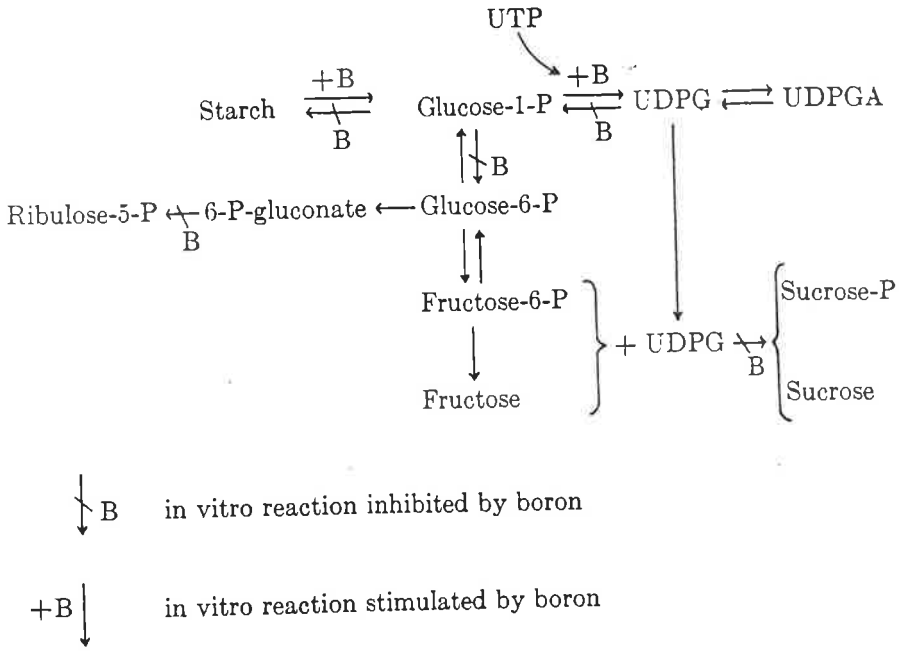


Figure 1.5. Proposed roles for boron in plant metabolism (from Dugger, 1973).

Lovatt and Dugger (1984) reviewed the role of B in plants from a biochemical perspective and concluded that B plays a regulatory role on a number of metabolic pathways or a cascade effect. They propose that the regulation by B occurs because of the ability of this element to complex with the large number of OH-rich compounds in plants and not because the element is involved directly in a specific metabolic reaction (Augsten and Eichhorn, 1976; Dugger, 1983).

Summary

Sidhu and Malik (1986) assigned the following putative major roles for B: 1, at the whole plant level (control of growth and differentiation); 2. at the physiological level (regulation of membrane permeability, absorption and translocation of sugar), and 3. at the biochemical level (control of enzymes concerning metabolism of carbohydrates, polyphenols and lignin, auxin and nucleic acid biosynthesis). The suggestion that B nutrition affects cell walls may have special relevance to malting quality in barley.

Robertson and Loughman (1974) said:

> *"the quest for a primary role of boron in higher plants has been confused by reports of a number of* ad hoc *responses associated with the onset of boron deficiency. The interpretation of these responses has been inconclusive as it was difficult to separate primary effects from secondary effects related to changes in growth and differentiation".*

It may be helpful, in this light, to study the effects of boron toxicity, as well as deficiency with the aim of elucidating further the function of B in plants. Though it is clear that B plays a role in a range of plant functions and structures, a more coordinated, multidisciplinary approach needs to be applied before the primary role(s) of B will be elucidated.

BORON AND POLLEN

Stimulation of pollen tube growth by boron (B) was first reported by Schmucker (1932a,b, 1933, 1935) with Nymphea pollen germinated *in vitro*. Many studies of the effect of boric acid or sodium borate on pollen germination have since been published. These compounds usually stimulate growth at concentrations of 10 p.p.m. to 100 p.p.m., depending upon the species and growing conditions with respect to B availability to the plant (Visser, 1955; Gauch and Dugger, 1954). The usefulness of different forms of B may depend on variable rates of B absorption from the nutrient medium, their relative effectiveness in metabolism, and the different ways in which the sugars complex with borate ions (Isbell *et al.*, 1948; Stanley and Lichtenberg, 1963). Studies into the forms of B most suited to stimulate pollen germination may cast some light on

the role of B in higher plants and its uptake, and how B may be applied to pollen as a means of selection for B tolerance.

Cereals

It has been known for many years that B is an essential ingredient in media for pollen germination of most species (Vasil, 1964), but pollen from some cereal species fails to germinate on a simple solution of only sucrose and H_3BO_3. The discovery that calcium must also be present to achieve maximum *in vitro* germination for pollen sown in low density (Brewbaker and Kwack, 1963) stimulated the development of an appropriate medium for rye (Pfahler, 1965) and maize (Cook and Walden, 1967; Pfahler, 1967, Pfahler *et al.*, 1982). Kariya (1989) reported successful germination of rice pollen and Furusho, *et al.* (1988) reported an artificial germination test of pollen of a barley cultivar and *Hordeum bulbosum*. Recently Cheng and McComb (1992) reported that wheat pollen gave up to 81.7% germination when collected from newly dehisced anthers and cultured on a 0.7% agar medium containing 100 mg l^{-1} H_3BO_3, 300 mg l^{-1} $CaCl_2.2H_2O$ and 0.75 M raffinose.

In cereal crops the most distinctive symptom of B deficiency is the reduction or elimination of grain set due to male-sterility (Rerkasem, 1989; Rerkasem, *et al.*, 1990). The symptoms may be relieved by B application, to increase the B in the flag leaf to about 12-13 mg B kg^{-1} (Rerkasem, 1989). Previously, Bergmann (1983) has suggested that B concentrations in the range of 5-10 mg B kg^{-1} were adequate for growth of wheat plants. However, B requirement for seed production is usually higher than that needed for vegetative growth (Marschner, 1986). Poor seed set due to B deficiency has been recorded widely in China (Li *et al.*, 1978), Brazil (da Silva and da Andrade, 1983), Thailand (Rerkasem, 1989) and Nepal (Khatri-Chhetri and Ghimire, 1992). It has been suggested that B deficiency affects pollen development during the pollen mother cell stage (Li *et al.*, 1978). Atrophy of anthers is common in B-deficient plants, but the embryo sac and the surrounding tissues remain unaffected (Löhnis, 1937, 1940; Whittington, 1957). Anthers appear to be especially sensitive to B deficiency (Heslop-Harrison, 1986) Different responses to soil B have been observed among genotypes (Rerkasem and Jamjod, 1989).

In vivo Requirement

Heslop-Harrison (1986) pointed out that despite the well-proven requirement of the grass pollen tube for B and calcium when cultured *in vitro*, the amounts actually available in the stigma in solution form are remarkably low. Vasil (1987) on the other hand, reported that flowers, especially the tissues of the stigma, style, and the ovary, often contain high concentrations of B (Bertrand and Silbersteirn, 1938; Bobko and Zerling, 1938; Gärtel, 1952; Thomas, 1952; Gauch and Duggar, 1954), which is said to play an important role in fertilization. Perhaps these two views can be reconciled, in that most of the B found in the stigma may be complexed. Pollen grains of many species are deficient in B (O'Kelley, 1957; Linskens and Kroh, 1970) but in nature this deficiency is often met by the high levels of B in the stigmatic and stylar tissues (Bertrand and Silberstein, 1938; Bobke and Zerling, 1938; Gärtel, 1952). Boron occurs in pollen at about 0.7 $\mu g\ mg^{-1}$ dry weight, while the stigma may contain 10 times that level of B (Stanley, 1971). The amount of B in pollen can be increased by the use of B-rich fertilizers, or by irrigation with B-rich water (Bobke and Zerling, 1938; Antles, 1951; Visser, 1955). Boron has been reported to be toxic to intact plants even at such low concentrations as 5-10 ppm; however, pollen grains can tolerate concentrations up to 1200 ppm, although optimum stimulation of germination and tube growth is obtained at concentrations of 10 -150 ppm, depending on the species, growing conditions, endogenous levels of B and B availability to the plant (Visser, 1955; Vasil, 1964).

Role of B and Pollen

A number of investigators have used pollen as a tool to gain knowledge about the role B plays in the life of higher plants. Vasil (1987) presented an excellent review of the role of B with reference to pollen. Pollen grains are isolated cells, generally low in B concentration. Hence studying pollen eliminates some of the complications which arise from working with whole plants.

Stanley and Lichtenberg (1963) suggested that to evaluate further the role of B in plants, one could determine if different organic and inorganic compounds of B, which have a different chelation affinity, or cell absorption capacity, yield different growth responses.

O'Kelley (1957) investigated sugar absorption by trumpet vine pollen grains. He concluded that B has a specific role to play in growth of the pollen tube, which is not closely related to any effect of B on either sugar absorption or respiration.

Sidhu and Malik (1986) studied the metabolic role of B in germinating pollen and growing pollen tubes. They pointed out that since pollen does not contain lignin it is unlikely the primary role of B involves lignin biosynthesis. Their experimental data do not support the thesis of Lewis (1980b), but indicate that B has a primary role in metabolic events concerning shifts in carbohydrate oxidation.

Gametophytic Selection

The topic of pollen selection was recently reviewed by Hormaza and Herrero (1992). Singh and Knox (1985) discussed the physiological genetics of pollen in the context of potential for gametophytic selection. Genetic selection for desirable agricultural characteristics generally takes place in the sporophytic phase of the life cycle of the crop. Plants alternate between the diploid sporophytic flowering phase, and the haploid gametophytic (ie. gamete-bearing) phase of their life cycles. Since the sporophytic phase is physically larger, of longer duration and directly associated with crop yield, it has traditionally been the object of breeding research. It has been shown, however, that genetic selection can also occur at the gametophytic phase, especially among pollen. In the haploid pollen, genes are directly expressed without the complication of dominance, and therefore both dominant and recessive variants may be picked up with appropriate screening tests. Because of large population sizes and haploid genotypes, pollen might provide an effective tool to screen for sporophytic characteristics.

Singh and Knox (1985) list several promising approaches to gametophytic selection:

(1) *In vitro* selection among cultured pollen tubes, with selection pressure applied via an artificial medium.

(2) *In vivo* selection during pollination, with selection pressure applied to pollen-stigma interactions, by increasing pollen competition or by introducing an environmental stress at the stigma surface.

(3) *In vitro* selection among pollen embryoids.

The latter type, including anther culture methods, will not be discussed here.

Mulcahy and Mulcahy (1983) discussed the use of pollen selection as a method for defining the genotype of its sporophytic parent, or for use in altering the genetic frequencies in the next generation. The success of haploid selection depends on genes being expressed postmeiotically in the pollen and these same genes being expressed in the sporophyte.

A number of reports suggest that many genes expressed in the sporophyte are expressed in pollen. Tanksley, *et al.* (1981) demonstrated that, in *Lycopersicon esculentum*, 60% of structural genes which are expressed in the sporophyte are expressed also in the pollen. Mulcahy and Mulcahy (1983) emphasized that this is not the transfer of gene products from the sporophyte to the gametophyte but rather, it is the postmeiotic translation of genes which are translated also in the sporophyte. A similar percentage was calculated by Sari Gorla *et al.* (1986) also using isozymes, for *Zea mays*. The results of (Mascarenhas *et al.*, 1985) indicate that 85% of corn pollen mRNAs are similar to those from roots and shoots, and about 15% of the mRNAs in pollen are unique to pollen and not found in roots and shoots. Thus since the majority of genes expressed in the sporophyte also appear to be expressed in pollen, then the way is open for selection for a variety of sporophytic traits to be applied at this gametophytic stage.

Bino and Stephenson (1988) reported tolerances and sensitivities for a wide range of agents are thought to be similarly expressed in the sporophyte and the gametophyte: eg., tolerances for zinc and copper toxicities (Searcy and Mulcahy, 1985a), salinity (Eisikowitch and Woodell, 1975; Sacher *et al.*, 1983), herbicides (Smith and Moser, 1985), antibiotics (Bino *et al.*, 1987), sensitivities for ozone (Feder, 1986), acidity and trace element toxicities (Cox, 1985). Zamir *et al.* (1981, 1982, 1983) demonstrated that pollen selection can be used to identify cold resistant sporophytes, and McKenna *et al.* (1983) reported that pollen selection can be used to select for increased competitive ability. Searcy and Mulcahy (1990) demonstrated that aluminium tolerance appears to be expressed in both pollen and sporophyte of tomato (*Lycopersicon esculentum* Mill.). In at least one case (Zamir *et al.* 1982), temperature sensitivity appears to be the result of the same genes being expressed in both pollen and sporophyte. These similar responses indicate a general overlap in genes expressed in the sporophyte and the gametophyte.

As a test system for its parental sporophytic qualities, pollen from a single plant can be tested against a wide range of stress conditions and the sporophytic source if still available for testing with other pathogens or other stresses. In *Zea mays*, for example, sporophytic susceptibility or resistance to *Helminthosporium maydis* can be predicted by measuring the response of pollen to the phytotoxin produced by that pathogen. When pollen is able to germinate in the presence of the toxin, the sporophyte that produced that pollen is also resistant to the toxin (Laughnan and Gabay, 1973). Mulcahy and Mulcahy (1983) suggest that pollen testing may be used to predict sporophytic sensitivity to Fusarium phytotoxins, and to toxicities of B and heavy metals.

The other system of selection is based upon the haploid genotypes of the individual pollen grains, where pollen is selected to influence expression of a character in the next generation. Portions of the sporophytic genome can thus be exposed to the uniquely intense selection which characterized the gametophytic portion of the life cycles. Pfahler (1983) demonstrated a clear theoretical advantage of pollen genotype selection when compared with conventional methods. Indeed, sporophytic gene expression is modified by chilling stress during pollen development and *in vivo* pollen tube growth in *Lycopersicon species* (Zamir *et al.*, 1982), or by subjecting the pollen to detrimental conditions, eg. prolonged storage (Linskens and Pfahler, 1973) prior to pollination.

An appropriate example using trace element toxicity is provided Searcy and Mulcahy (1985b). By raising plants in the presence of zinc and copper they introduced potentially toxic amounts of these elements into flowers of heterozygous tolerant plants of *Silene dioica* and *Mimulus guttatus*. During microsporogenesis, this selection pressure favourably induced development of zinc and copper tolerant gametophytes. During pollination, the amounts of heavy metals in the pistil reduced the chance of fertilization by nontolerant pollen (Searcy and Mulcahy, 1985b). In pollen selection for cold tolerance in tomatoes, competition in the style was found to be more effective than selection during pollen development (Zamir and Vallejos, 1982). A third site of selection indicated in the data of Searcy and Mulcahy (1985b), that seed abortion can also be affected by toxic metals in the pistil and the pollen genotype and could be an important selective factor in the development and maintenance of populations tolerant to heavy metals. Other researchers selected for increases in sporophyte vigour by inducing pollen competition (eg.

Mulcahy and Mulcahy, 1975) and obtained more vigorous progenies. Mulcahy *et al.* (1978) showed that increased vigour associated with pollen competition extends to the F_2 progeny in Petunia, indicating that gametophytic selection can modify the genetic composition of subsequent generations.

In vivo pollen selection may have many important agricultural implications (eg., Zamir and Vallejos, 1982; Davis *et al.*, 1987; Schlichting *et al.*, 1987). However, *in vivo* techniques also have limitations, especially because it is often difficult to induce a specific selection pressure during pollen formation or pollen functioning (Bino and Stephenson, 1988). For overcoming this problem, Simon and Sanford (1986) developed a technique for applying the challenging agent *in situ*. Injecting different concentrations of fusaric acid in the style of *Nicotiana* species, however, did not result in a positive selection. *In vitro* methods are not very appropriate to plant breeding especially with gramineae, but Bino and Stephenson (1988) discuss other applications such as in direct gene transfer technology. Anther culture is increasingly being used as an integral part of barley breeding programmes (Logue et al., 1993) and thus may be an appropriate time at which to apply a selection pressure, such as high B.

Summary

If suitable methods were found the value of success in gametophytic selection is likely to be high in commercial terms, since the procedure is capable of bringing selection one half generation forward in time, and the resources required for selection among large populations of pollen grains are very small compared with those required for selection among corresponding numbers of sporophyte plants (Singh and Knox, 1985).

BORON AND DISEASE

There have been a number of reports that the boron (B) status of plants may play a role in determining the susceptibility of plants to a range of diseases and pests. Table 1.1 lists some of the many organisms whose pathogenicity is enhanced on B impoverished hosts. This wide range suggests that general mechanisms of resistance may be involved (Graham, 1983), though the workings of those mechanisms are unknown. Graham's 1983 comprehensive review of trace

Table 1.1. Some species-pathogen combinations reported to be affected by B nutrition in the plant.

PLANT SPECIES	PATHOGEN	REFERENCE
barley	ergot (*Claviceps purpurea*)	Tainio (1961), Simojoki(1969)
barley	powdery mildew	Yarwood (1938), Eaton (1930)
barley	spot blotch (*Helminthosporium sativum*), powdery mildew (*Erysiphe graminis*)	Eaton (1930)
bean	*Fusarium solani*	Guerra & Anderson (1985)
bean	tobacco mosaic virus	Shimomura (9182)
Brassica	club root	Utkina *et al.* (1980), Antonova *et al.* (1974), Rohde (1952)
Brassica	*Plasmodiophora brassicae* (club root)	Dixon and Webster (1988), Dixon & Wilson (1983, 1984, 1985)
cabbage	club root	Antonova (1969)
cotton	*Verticillium albo-atrum*	Savov (1986)
flax (*Linum usitatissimum*)	wilt (*Fusarium ozysporum*)	Keane & Sackston (1970)
flax	Melampsora	Heggeness (1942)
groundnut	*Rhizoctonia bataticola*	Murugesan &Mahadevan (1987)
legumes	*Rhizoctonia solani*	Kataria (1982)
oil palm seedlings (*Elaeis guineensis*)	red spider mite (*Tetranychus pieroei*)	Rajaratnam & Hock (1972a, 1975)
potatoes	*Synchytrium endobioticum*	Hampton (1980)
sugarbeet	*Sclerotium rolfsii*	Edgington & Walker (1958)
sunflower	Powdery mildew (*Erysiphe cochoracearum*)	Butler & Jones (1955)
sunflower	mildew	Yarwood (1938)
swedes	clubroot *(Plasmodiophora brassicae)*	Vladimirskaya *et al.* (1982)
tomato	*Verticillium* wilt	Dutta and Bremner (1981)
tomato	*Fusarium* wilt	Edgington and Walker (1958)
tomato	tomato yellow leaf curl virus	Zaher (1985)
Vigna sp.	Rhizoctonia solani	Kataria and Grover (1987)
vine	gall mites	Gartel (1971)
wheat	powdery mildew	Schutte (1967)
wheat	mite (*Petrobia latens)*	Singh (1986)
wheat	rusts	Dennis & O'Brien (1937, 1938), Ismailov (1954)

elements and disease resistance and tolerance in plants has recently been updated (Graham and Webb, 1991).

Little is known about how B interacts with hosts and pathogens. Graham (1983) describes some possible ways that B nutrition may influence susceptibility to disease by looking at the biochemistry of B deficiency. Graham (1983) discussed how a hypothesis that plane of nutrition acts as a predisposing factor in disease by depressing phenol synthesis and cannot be extended easily to include the effects of B. Boron deficient plants are predisposed to infection yet accumulate large amounts of phenols, and furthermore, appear to have high polyphenol oxidase activity (Dear and Aronoff, 1965; Lee and Aronoff, 1967; Shkol'nik, 1974).

Other Elements

Some of the other trace-elements play an important role in disease resistance and in regulating the physiology of fungi (Dutta and Bremner, 1981) . For example, application of manganese either singly or in combination with iron has been found to be beneficial in controlling *Fusarium* wilt disease (Sarojini, 1951) and Askorova (1963) and Joham (1971) observed that copper and zinc controlled *Verticillium* wilt of cotton. Fungi, the major pathogens, however apparently do not require B at all (Bowen and Gauch, 1978). Fungi therefore may have a simple advantage over their hosts in B deficient environments (Graham, 1983).

Leaf Disease

Schmucker (1935) observed that the germination of pollen was enhanced in a B-containing solution. Oertli (1962) suggested that guttation fluid from leaves presents favourable conditions for the development of plant pathogens and it may be that B in the guttation liquid enhances the germination of spores of pathogens when the level of B in the host is toxic. This enhancement of germination need not necessarily contradict the findings of Bowen and Gauch (1978) that B is not essential for most fungi.

Rajaratnam and Hock (1975) when considering mite resistance suggested that it is unlikely that B has a direct effect *per se*, since it has not been shown that B has any effect on metabolic

pathways in animals, though it may be toxic at high doses. They suggest that is seems highly probable that the relative concentration of proanthocyanidin in the leaf of high B plants was responsible for conferring resistance to red spider mite attack.

Root Disease

Boron nutrition also influences infection by root diseases. Though B had little effect on the proportion of roots infected it was very effective in the suppression of sporogenesis of clubroot (Dixon and Webster, 1988). Boron effectively inhibited both the root hair and cortical stages of pathogen development. Dixon and Wilson (1983, 1984, 1985) achieved significant reductions in disease index with sodium tetraborate applied to the soil. Whether this control is due to direct fungitoxicity or to a more complex interaction is far from clear.

Dutta and Bremner (1981) evaluated trace elements for the control of *Verticillium* wilt of tomato. They found that B, when applied by root-dipping before inoculation, reduced the severity of the disease, but evidence indicated that there is no direct relation between its fungitoxicity and its chemotherapeutic potency. Boron was found to be stimulatory to the pathogen in liquid culture but gave very good control of the disease, which shows that increase of host resistance due to the application of nonfungitoxic trace elements may be caused by altered host metabolism. Consistent with this idea, Keane and Sackston (1970) found that flax exposed to B deficiency before inoculation developed more severe wilt than those moved to B-deficient solutions after inoculation.

Negative Results

Graham (1983) points out that the beneficial effects from applications of B are not universally recorded even where the element is deficient; examples are cited by Yarwood (1938), Cherewick (1944), Williams (1961), Wood (1967), Keane and Sackston (1970) and Pobegailo *et al.* (1980). Christensen (1934) found that toxic levels of B did not increase susceptibility of barley to spot blotch (*Helminthosporium sativum*) nor render the plants immune from attack of powdery mildew (*Erysiphe graminis*), as suggested by Eaton (1930). Boron had little or no effect on *Leptosphaeria maculans* in oil-seed rape (*Brassica napus* var. *oleifera*) (Krüger, 1982). Yarwood (1959) pointed out that his 1938 work and that of Heggeness (1942) lacked

repeatability owing to the delicate balances in the chemistry of this element and between host and pathogen. The genotype of both is also important in how B will affect the disease.

Summary

Interactions between B status and plant disease is likely to be complex, and the specific mechanisms may vary between plant and pathogen species. It is important to consider, however, that nutritional disorders in plants may have effects other than a direct one upon plant health.

UPTAKE AND TRANSLOCATION OF BORON IN PLANTS

Considerable disagreement still persists concerning the mode by which boron (B) is taken up into the plant from the soil and once inside the plant, how it is distributed between different parts of the plant. An up to date review of short and long-distance transport of boron and other micronutrients has been written by Kochian (1991). The simplest and still widely held view of the behaviour of B in plants is as follows. In vascular plants, B is carried passively in the transpiration stream and accumulates where the transpiration stream ends (Kohl and Oertli, 1961). Because B is relatively immobile in the phloem, very little of the accumulating B moves out of these tissues (Oertli and Richardson, 1970; Raven, 1980). Since the uptake of B is passive through the transpiration stream, B intoxication is a function of the concentration of B to which the plant is exposed, the length of exposure, and the rate of transpiration (Lovatt and Dugger, 1984). Though these statements appear to be largely true, some serious anomalies exist. Of particular interest in this review, is the fact that different plant species and genotypes within species show different levels of tolerance to high or low levels of B in the source media. These differences as a rule cannot be explained purely by variation in transpiration rate. Some other mechanism or mechanisms must be acting.

Nable (1988) found that some barley cultivars accumulated considerably more B than others, but whether limited accumulation resulted from restricted absorption, active exclusion or active efflux is unclear. The absorption of B by roots was thought to be predominantly a passive process with a small metabolic component and greatly influenced by transpiration rates (for a review see Raven, 1980). But no simple relationship was found between transpiration rates and

whole plant B uptake. If it is assumed that B is passively absorbed and entirely under the control of transpiration rates, then water use efficiency calculated on the basis of total plant B content would be approximately 7-fold greater in B resistant than sensitive cultivars (Nable, 1988). Such a range of water use efficiencies is much greater than ranges reported amongst cereal cultivars. Thus he concluded, it appeared that differences in tolerance to B toxicity could not be explained simply by control over transpiration rates. Other factors he suggested may differ between cultivars and substantially influence passive absorption of B include: a) surface area of the roots; B) composition of the root cell membranes and effects on permeability to B; and c) concentration of B adsorption sites in the free space, in particular the cis-diol content. He stated that no information was available on how these characteristics may vary between cultivars.

Variation between and within species

Boron uptake and distribution vary between species and within species. In some B-tolerant species, B fails to accumulate in the leaves, or does so at a reduced rate (Lovatt and Dugger, 1984). A similar level of B in lemon and carnation is required for injury, even though carnation is considered very tolerant (Lunt *et al.*, 1956) and lemon considered very sensitive (US Dept of Agric., 1954). Thus, sensitivity toward excessive B supply is usually related more closely to uptake rates than to differences in tolerance of tissues (Oertli *et al* , 1960). The mechanism is unknown. El-Sheikh *et al.* (1971) on the other hand, reported that in some B-tolerant species, a high concentration of B accumulates, but the leaves do not exhibit the symptoms associated with B toxicity.

Loneragan (1968) cautioned about the use of the concept of a "nutrient requirement". It must be made clear whether one is referring to a requirement in solution or in plant. Some plants with a high tissue requirement may have low solution requirement. Brown and Jones (1971) found that the internal (physiological) B requirement appeared to be the same for two tomato cultivars tolerant and susceptible to B deficiency. In wheat, pot and field experiments demonstrated a large range in the tolerance to high concentrations of B, and tolerant genotypes contained low concentrations in shoots and grain (Cartwright *et al.*, 1987; Rathjen *et al.*, 1987; Paull *et al.*, 1988a).

Active or passive

One of the major controversies centres around whether absorption of B by plants is active or passive. Dugger (1983) in his review summarised many of the investigations, to that date, into uptake and transport of B. Tanaka (1967a,b) presented evidence that B was passively absorbed by excised sunflower roots by means of borate complex formation with polysaccharides in the free space. Support for this conclusion was the observed stoichiometry of H^+ released and B uptake. On the other hand, Bingham *et al.* (1970a), under their experimental conditions, found that B absorption by excised barley roots was rapid, not accumulative, and was also a non-metabolic process but that absorption was more directly related to the concentration of $B(OH)_3$ than that of $B(OH)^-_4$. The entire plant was thought to be free space for B, thus supporting an earlier observation of intact barley seedlings by Oertli (1963).

In contrast, in his series of studies on B uptake by sugar cane leaf tissue, meristematic tissue and excised roots, Bowen (1968, 1969, 1972) reported that a fraction of B uptake was regulated metabolically, correlated with the concentration of the singly charged species $B(OH)^-_4$, and had the characteristics of a carrier-mediated absorption process, although B translocation from roots to shoot occurred passively in the transpiration stream. Active uptake was not apparent at 2°C (Bowen and Nissen, 1976). The three postulated compartments of free space B were: (1) a surface film of B; (2) water free space B; and (3) B bound in the cell wall. They suggested that the stoichiometric release of H^+ from roots after the 2°C uptake period indicated that B complexed with polysaccharides in the cell walls. The combination of active and passive processes proposed by Bowen (1972) could account for the lack of equivalence between B absorption and water movement noted in the Oertli's (1963) study. Oertli and Grgurevic (1975) studied the effects of pH on B absorption by excised barley roots. They found that relative B uptake decreased with an increase in pH (pH 6.0 = 100% uptake); this was similar to the decrease of the fraction of undissociated H_3BO_3.

Wildes and Neales (1971) proposed a model for B uptake, incorporating the active transport of the $B(OH)_4^-$ ion and the passive diffusion of $B(OH)_3$. They studied the uptake and desorption of B by carrot and beet disks. The maintenance of an internal concentration of diffusible B greater than that in the external solution was apparently dependent on the metabolic activity of the

tissue. They postulated then that both passive and active processes were involved in B uptake. Nissen (1974) contends that active B transport predominates at low external B concentration, and probably involves $B(OH)_3$ rather than $B(OH)_4^-$, while passive B transport may predominate at higher external concentrations.

Thellier *et al.* (1979) used enriched stable isotopic B and (n,α) nuclear reaction to measure unidirectional fluxes of borate between the cellular system of *Lemna minor* L. and the external medium. Their findings indicated that *L. minor* is able to accumulate B actively (Thellier and Le Guiel, 1967; Thellier and Ayadi, 1967; Thellier *et al.*, 1967). They postulated four B-containing compartments: (1) free space including easily dissociated borate monoesters; (2) cytoplasm; (3) vacuole; and (4) stable borate diesters in the cell walls.

Seresinhe and Oertli (1991) investigated the effects of B on growth of tomato cell suspensions. With increasing B levels in the medium, the B concentrations of cells were in near equilibrium with the media B, in their view indicating passive uptake. This relationship did not continue with B levels of 1.85 mM or higher. They attributed this observation to increasing toxicity altering the membrane properties of the cell.

The results of Nable's (1988) experiments on uptake kinetics of barley indicated that control of B uptake is a process unaffected by temperature and is non-metabolic. It has been postulated that it is probable that tolerance to B is related to the composition/structure of either the cell wall or cell membranes (Paull *et al.*, 1991a).

Nissen (1974) suggested that some of the differing viewpoints with regards the active or passive uptake of B can partly be ascribed to differences in methodology. Bowen and Nissen (1977) for example, unlike earlier studies (Bingham *et al.*, 1970; Oertli and Grgurevic, 1975) modified their procedure to include a minimum 30-min rinse which they believe is necessary to remove reversibly-accumulated B from the free space (Bowen and Nissen, 1976). In addition, these studies were conducted on a range of species and genetic lines within species. Mechanisms for either uptake or transport of B need not be identical for all plants. It can be inferred from

Bowen's results for example, that, unlike the cells of barley leaves, the cells of sugarcane leaves are impermeable to $B(OH)_3$ (Wildes and Neales, 1971).

B and water flow

There is considerable evidence to suggest that though influenced by transpiration, B uptake and transport is not directly proportional to water flow. Nable *et al.* (1990) examined the effects of evapotranspiration conditions on the distribution of B in leaves, and on shoot critical values for B toxicity, in solution culture experiments with barley. They found that increased water use resulted in increased B accumulation by plants and B was concentrated in the leaf tips. The relationship between shoot dry matter production and shoot B concentrations was markedly affected by evapotranspiration conditions, but the effect could be removed by not analysing leaf tips. Excluding the leaf tips also decreased the shoot B concentration at which shoot dry matter production was depressed. Spraying plants with water removed considerable B from leaves without affecting dry matter production. Their results indicated problems may exist in the establishment of critical values and the use of foliar analysis for diagnosing B toxicity. These problems may account for conflicting reports of critical values and discrepancies between results from glasshouse- and field-cultured plants.

Kohl and Oertli (1961) concluded that when B is supplied to roots in adequate or excess amounts, the flow of this element into the leaves is controlled by the transpiration stream. Oertli and Kohl (1961) suggested the possibility that water, carrying B with it, continues to move into leaf areas which have become necrotic, thus this "sink effect" may help reduce damage. The data of Husa and McIlrath (1965) do not necessarily contradict this conclusion, but they do indicate that the transpiration stream alone is not the determining factor in B deposition in leaves of the sunflower plant. Husa and McIlrath (1965) concluded that it appeared that one of the prime factors controlling the distribution of B in sunflower plants is the extent and location of the metabolic requirement for the element. Only when B is supplied to plants in excess of the requirements of the B metabolic sinks does it accumulate in the older leaves. When the supply is limited,they found that B does not tend to concentrate in the older leaves, even though these organs may still be the prime water sinks of the plant.

Metabolic sinks

The sink concept has also been mooted by others. Skok (1958) suggested that toxicity symptoms may be related to the fact that the actively growing younger tissues utilize B and remove it from the available pool while the older tissues no longer utilize B and permit it to accumulate. More recently Halbrooks *et al.* (1986) proposed that B translocation to shoots is controlled mechanistically by rates of dry matter accumulation during stages of rapid growth, ie sink effect, since they found B uptake directly related to transpiration rate, but upward movement of B in the xylem to shoots was not affected by the rate of transpiration.

Other factors

Factors other than transpiration may affect uptake and transport of B. Seasonal fluctuations have been observed in the appearance of B deficiency in a number of crops (*Vicia faba* and *Phaseolus multiflorus*; Warington, 1933; radish, Skok, 1941; soybean, MacVicar *et al.* , 1946; and barley, Williams and Vlamis, 1957,). Tanaka (1966) found with *Lemna pausicostata* that response to B varies with the intensity of the illumination though the reason was not clear. Yläranta *et al.* (1979) noted seasonal variations in micronutrient contents of wheat. In marine vascular plants, the B content is partially under photo-control (Pulich, 1978).

Eaton and Blair (1935) put forward two possible causes for differences in B absorption: (1) differences in cells that affect equilibria between external and internal B concentrations, and (2) differences between the non-mobile and mobile balance. Raven (1980) proposed that the regulation of B distribution in the cell is a function of the level of total intracellular B rather than free boric acid; the distribution within cells is dependent on passive permeation, active transport and cis-diol formation.

Site of control

The site of control of uptake appears to vary between species and genotypes. Brown and Ambler (1973) reported that roots controlled B movement from tomato roots to other organs by restricting transport of B from roots to shoots. Brown and Jones (1971) found in tomato roots with similar levels of accumulated B, the rate of B translocation into the shoot differed among genotypes by a factor of up to 5. Eaton and Blair (1935) studied the accumulation of B by

reciprocally grafted plants. They reported that the root stock was important for B transport and that the difference in transpiration alone could not account for differences in B uptake.

Nable (1988) on the other hand found in barley that tolerance to B toxicity was controlled by reduced uptake by the roots. He found B concentrations were much lower in roots than shoots in all lines. Tolerant cultivars were found to have a lower tissue B concentration, even when exposed to subtoxic B levels. If this feature proved to be universal, he suggested that this may have implications for screening methodology and breeding. Screening may be carried out at subtoxic levels, and breeding for tolerance to B toxicity may also be breeding for intolerance to B deficiency.

Huang and Graham (1990) reported that the difference in B uptake between wheat genotypes reported from field experiments is also expressed as differential growth at high B in callus, that is, at the cellular level. They asserted that their results suggested that differences among genotypes in resistance to toxic B concentrations may be related to cell membrane permeability to B. It has also been suggested that the major pathway for water transport across the root in wheat was through symplasm (Jones *et al.*, 1983). This suggestion implied that B influx also might be through the symplasm, a view consistent with differences in B accumulation among genotypes being located at cellular level, that is, in cell membranes rather than in the structure of endodermis or other differentiated root features. The distinct and consistent differences among genotypes in response to B toxicity both at the organ level and at the cellular level they suggested could serve as a basis for precise and efficient selection in a breeding program.

Huang and Graham (pers. comm.) investigated further the mechanism of B uptake, measured the effect of borate complexes with mannitol, fructose and caffeic acid and determined changes in membrane permeability on B uptake in rape and wheat genotypes. They considered three possibilities concerning the mechanism by which root cells of different genotypes govern B uptake. First, they suggested there may be differences between genotypes in root cell exudates with which boric acid can form complexes. Second, there may be differences in the concentration of B-binding sites in the root cell walls, as suggested by Nable (1988). Third, composition of the cell membrane may restrict B influx from the free space (Nable, 1988; Huang

and Graham, 1990). They presented evidence supporting the latter. They postulated that a difference in configuration of membrane lipids may result in the difference in B uptake between genotypes.

The Use of Protoplasts in Nutrient Uptake Studies

Plant protoplasts can be useful tools for the investigation of short-distance uptake and transport of nutrients. Though isolated protoplasts have been produced from some other species for some years, it is only comparatively recently that reliable methods have been devised for the preparation of protoplasts derived from grasses (Vasil, 1983).

Davey and Kumar (1983) discussed the use of plant protoplasts in physiological studies. They said that advantage of using isolated protoplasts is that they provide a homogeneous cell population, with organelle relationships typical of intact cells being maintained within each individual. They warned though, that fundamental to the use of protoplasts in physiological studies is whether or not isolated protoplasts can be considered as fully functional plant cells lacking their walls. Coutts (1982) has stressed that caution must be exercised when extrapolating results obtained with protoplasts to the whole plant level.

Leurs *et al.* (1982) have summarized research using protoplasts to study membrane transport in plants, and concluded that most investigations have centred upon the uptake of amino acids and sugars, with studies of ion uptake being relatively few and of recent origin. Gronwald and Leonard (1982) reported ion transport in protoplasts of *Zea mays* root cortical cells to be similar to that of intact root tissues. Seresinhe and Oertli (1991) investigated the effects of B on growth of tomato cell suspensions.

Phloem mobility

Mobility of B in the phloem is also in dispute. An early symptom of B deficiency is distorted apical growth and many investigators have also observed that plants can accumulate B to a toxicity level in their leaves and still show symptoms of B deficiency within a very short time after B is withheld from the substrate. These observations led to the conclusion that B is highly

immobile in plants (Gauch and Dugger, 1954) and that there is little or no re-utilization of B from older to younger plant parts similar to Ca (Loneragan, 1976).

Most evidence suggests that plants do not translocate B in their phloem (Campbell *et al.*, 1975). Lack of retranslocation means that plants need a continuous supply of these elements throughout their growth and that they quickly develop deficiency symptoms in growing tissues if the supply is interrupted (Albert and Wilson, 1961; Neales, 1960; Pollard *et al.*, 1977). Thus the growth and metabolism of meristems can be influenced rapidly by a cessation of the Ca and B supply to the plant (Albert and Wilson, 1961; Burström, 1968; and Cohen and Lepper, 1977). In B-deficient plants, growth of roots ceases and apical meristems atrophy (bean, Warington, 1923, 1926; tomato, Albert and Wilson, 1961, Brown and Jones, 1971; tobacco, Scholz, 1960; soybean, Brown and Ambler, 1969). In germinating bean seeds, relatively little of the B moves from cotyledons to the radicles which cease growing very quickly unless supplied with B in solution (Neales, 1960). Boron also accumulates in the old leaves of plants and generally stays there even when B deficiency occurs in the root or shoot apex (Eaton, 1944; Myers and Brunstetter, 1946; Vlamis and Williams, 1970; Brown and Jones, 1971). Nor does much B move laterally in leaves. Scholz (1960) using a split root technique, observed no lateral movement of B in a leaf of tobacco. In view of these facts, Epstein (1973) has hypothesized that B is excluded from the phloem and for this reason is not translocated.

Husa and McIlrath (1965) presented evidence suggesting that when high concentrations of B were supplied to the split root system some lateral movement of B could occur in sunflower leaves to fill metabolic requirements in other parts of the plant. At extremely high concentration of B, it is probable some B moves in tissues other than the xylem (Husa and McIlrath, 1965; Oertli and Richardson, 1970). However, Campbell *et al.* (1975) suggested that such movement may result from diffusion rather than from translocation in the phloem.

On the other hand, some evidence indicates that B is mobile in the phloem. Immobility of B was questioned for turnip as early as 1940 by Furguson and Wright (1940). Since then it has been questioned for several other species of Brassica (Chandler, 1941; Benson *et al.*, 1961; Shelp and Shattuck, 1987a,b,c) as well as for the stone fruits (Eaton, 1944), grape (Scott and Schrader,

1947), and cotton (McIlrath, 1965) when plants were subjected to B deficiency. Campbell, *et al.* (1975) found that for the development of their fruits, peanut and subterranean clover translocate B in their phloem. In apples, van Goor and van Lune (1980) found that during the growth period there was a linear accumulation of B even when the phloem was presumably supplying most of the nutrients to the fruits. Hanson and Breen (1985) using calculated transpiration rates and concentrations in xylem exudate, estimated that only 26% of the B entering prune flower buds was supplied by the xylem. Shelp and Shattuck (1987c) suggested that B-deficiency restricts the movement of sugars by preventing the loading of sugars onto the phloem for translocation to the root. Tammes and Van Die (1966) found by direct analysis 10 ppm B in the dried phloem exudate of *Yucca flaccida.*

Immobility

In sunflowers and mung bean, it was found about 50% of the B content was in the supernatant (soluble) fraction after tissue homogenization and centrifugation (Skok and McIlrath, 1957;Skok,1958). Under conditions of B deficiency, a proportionally large decrease in B occurred in the supernatant fraction's dialyzable (unbound) portion. This they said suggested that B is not reutilizable and that the relatively small amount of plant B available for further growth and development is the unbound or dialyzable portion of the supernatant fraction. Shive (1941) pointed out that monocots contain a higher percentage of their B in the soluble fraction than do dicots. This, Skok (1958) suggested, may be the reason why monocots usually take longer to show pronounced deficiency symptoms. Martini and Thellier (1980) found that when B was foliar applied, >98% remained at the point of application and less than 2% was mobile and useful to growth. They suggested that the immobilization was probably due to boro-ester bond formation between boric acid and the alcoholic groups of the cell walls of the leaf cells.

Though predominantly immobile, B in leaves is water-soluble and is readily lost by leaching or by guttation in barley (cf. Kohl and Oertli 1961; Oertli 1962, 1969). Over a short period a large fraction of B may be removed by the guttation drop, indicating according to Oertli (1962), that there are not been strong bonds fixing this element in plants. He, and later Nable and Moody (1990) in wheat, also showed B may be leached from leaves through rain water, and this he suggested may be a contributing reason why B toxicity is more prevalent in arid climates and B

deficiency more prevalent in humid climates. According to Oertli and Grgurevic (1975) the pKa of boric acid may be the reason why B is readily leached from tissues (Oertli, 1969). That is at physiological pH values, B will be mostly in a soluble form. Thus according to Bowen (1968) a small tightly bound fraction is explained through some chemical fixation rather than through an active transport into a compartment from which leaching was prevented by a special membrane system.

Chamel *et al.* (1981) employed mass spectrometry and utilized ^{10}B boric acid to show that foliar-applied B penetrates the epidermis and is translocated to other parts of radish plants. However, the majority of the applied B remained in the treated leaf. The distribution within the treated leaf was homogeneous, leading the authors to suggest that the slow transport of B out of the treated leaf was because it was complexed with polysaccharides.

Kohl and Oertli (1961) concluded from their studied into the distributuion of B in Easter lilies: 1. B moves passively in the transpiration stream; 2. B does not move out of leaves, probably because it is not transported by the phloem; 3. leaf injury by excess B is local and is a primary effect because of continual water loss from leaf tips and margins; and 4. guttation from leaves may result in a reduction of leaf B content below accumulated toxic levels.

Oertli and Richardson (1970) postulated a mechanism to explain the apparent immobility of B in plants. They showed that B readily enters bark and is translocated within bark under high boron conditons. They reasoned that since B remains water-soluble in plants, the immobility cannot be explained through a chemical fixation, lack of entry into phloem, or absence of phloem transport. They suggest that B readily penetrates and is translocated within the phloem, but re-enters the xylem of the leaf or petiole and then moves back into the leaf via the transpiration stream. A high local mobility of B, together with the essentially unidirectional flow of the transpiration stream, thus cause a cyclic movement of B so preventing the efflux of this nutrient. This, they say, explains the immobility over long distances. Ziegler (1975) questions "how the deleterious effect of B on the sieve-tube structure can be avoided" if the element is translocated in the phloem.

Summary

Raven (1980) proposed that : (1) the net uptake of boric acid by higher plants is influenced by the transpiration rate; (2) transport in the xylem is probably directly proportional to the rate of transpiration; (3) the redistribution of B in the phloem is very limited. Thus, it is most likely that the difference between barley genotypes with respect to B tolerance involves regulation of uptake at the cellular level. This knowledge has implications about the kinds of screening methods which may be suitable for selection for tolerance within a breeding programme.

THE GENETICS OF BORON TOLERANCE

History

A relationship between plant nutrition and genetics has long been recognised (Brown *et al.*, 1972). Harvey (1939) presented an early review of hereditary variation in plant nutrition. There is abundant literature on comparisons between species for susceptibility to B toxicity and deficiency (eg. Bradford, 1971; Eaton, 1944; Gupta *et al.*, 1985), such comparisons are based upon the assumption that one or a few genotypes can typify a species. For barley (Nable *et al.*, 1990) and many other species (Table 1.2) this is not true. Genetic control of B nutrition in plants has until recently been studied only in relation to deficiency. Pope and Munger (1953b) showed that sensitivity to deficiency was dependent upon a single gene in celery, as did Tehrani *et al.* (1971) in red beet. A single recessive gene controlled susceptibility to brittleness of stems (characteristic of B deficiency) in a mutant of tomato grown with low-B (Wall and Andrus, 1962). In contrast, the inheritance of susceptibility to B deficiency in table beets was shown by Kelly and Gabelman (1960) to be complex. An inherited difference in the response to B deficiency has also been reported for sunflower (Blamey *et al.*, 1980, 1984). Variation in tolerance of high B in cereals has been observed in wheat (Mehrotra *et al.*, 1980; Chatterjee *et al.*, 1980) and rice (Cayton, 1985). Sayre (1955) reported differences in element concentration between leaves of inbred lines of corn for B. Using modified diallel analysis methods, and parent-offspring regression Gorsline *et al.* (1961, 1964a, 1964b) attempted to estimate to what extent plant nutrition may be controlled by genetic factors, and therefore how profitable selection for these characters might be, in corn (*Zea mays* L.). They estimated heritability of B response from 0 to 63%. Gorsline *et al.* (1968) looked at P_1, P_2, F_1, F_2, B_1 and B_2 generations

derived from four inbred lines. Their experiments led them to hypothesise three genes to explain variation in response to B. Vose (1984) concluded, that in view of the range of possible mechanisms that can be responsible for genotypic variation in nutrition it is not surprising that different genetic systems may function for the same element in different plant species.

Until Cartwright *et al.* (1984) identified the occurrence of B toxicity in barley in South Australia, systematic investigations into the genetic control of tolerance to high B in cereals had received little attention. An assessment of B tolerance of various crop species by Wilcox (1960) revealed that barley and wheat were in general semi-tolerant to excess B. A number of obsevations suggested that selection for tolerance to B toxicity might be beneficial. Paull *et al.* (1986) noted that cultivars of similar ancestry respond alike. Cartwright *et al.* (1987) observed marked differences in relative yield between cultivars for wheat and barley when grown simultaneously in field trials at sites with 'high' and 'normal' concentrations of soil B. However, it is recognised that differences in the ranking of cultivars in such trials may be due not only to different responses to soil B, but also to other environmental factors varying between sites. In a field trial of 150 locally adapted barley cultivars and breeding lines grown at a high B site, the concentration of B in grain of high yielding lines was significantly less than for the lower yielding lines (Cartwright *et al.* 1987). Rathjen *et al.* (1987) reported a highly significant correlation between concentrations of B in shoots and grain yield over a diverse group of Australian wheat cultivars. Paull *et al.* (1988a) performed pot experiments and in general found good correlation with field results.

Genetic screening

For both breeding and genetic studies it is important to investigate the breadth of variation for the character of interest present in the available germplasm. Boyd *et al.* (1988) screened over 1500 genotypes of barley for differential responses to excess B in sand culture. Genotypes showed similar patterns of symptom development but considerable variation in time and severity of symptoms, and growth effects which reflected B accumulation in leaves. Australian cultivars in general showed little tolerance to high B stress. A significant yield reduction due to high levels of B in soil and plants has been reported for barley (Cartwright *et al.*, 1984) and the

Table 1.2. Some examples of observations of genetic variation within species in reaction to B.

SPECIES	OBSERVATION	REFERENCE
annual medics (*Medicago* spp.) and field peas (*Pisum sativum*)	Variation in response to high B concentrations	Paull *et al.* (1991b)
barley	Range of tolerance >1500 genotypes screened	Boyd *et al.* (1988)
barley	genetic differences in B accumulation	Nable *et al.* (1990)
celery	single-gene control of efficiency	Pope and Munger (1953b)
citrus	variation in B controlled with rootstock	Haas (1945)
citrus	response to high and low B	Chapman and Vanselow (1955)
corn (*Zea mays*)	variation in response	Gorsline (1961, 1964 a,b, 1969)
corn	differences in leaf conc.	Sayre (1955)
garden beet	varieties differ in susceptibility to deficiency	Walker (1945)
grain legumes	genetic variation in response to deficiency and toxicity	Rathjen (1977)
grapevine	varietal difference in B requirement	Scott (1941. 1944)
pea (*Pisum sativum*)	genetic variation in response to high B	Bagheri *et al.* (1992)
pecan (*Carya illinoensis*)	variation in tolerance to high B	Picchioni and Miyamotoa (1991)
red beet (*Beta vulgaris*)	variation in tolerance to B deficiency	Kelly and Gabelman (1960)
red beet	single gene control of symptom response to B deficiency	Tehrani *et al.* (1971)
rice	varietal differences to excess B	IRRI (1979, 1985), Cayton (1975)
S. cereale, Th. bedssarabicum, Ag. elongatum and *Ae. sharonensis*,	Tolerance to high B	Manyowa (1989)
sunflower, Jerusalem artichoke, walnut, citrus	concentration and accumulation dependent on rootstock	Eaton and Blair (1935)
sunflower (*Helianthus annus)*	variation within cultivars and inbred lines in leaf B composition	Blamey *et al.* (1980)
tomato	B inefficiency recessive, single gene	Andrus (1955), Wall and Andrus (1962)
tomato	variation in B transport	Brown and Jones (1971)
tomato	genetic control of B uptake	Brown and Ambler (1973)
tomato	genetics of B transport	Wann and Hills (1973)
Wheat (*Triticum aestivum*), tall wheat grass (*Ag. elongatum*) and barley (*Hordeum vulgare*)	variation in tolerance to excess B	Schuman (1969), Duke (1982), Paull *et al.* (1988a; 1988b)
wheat	variation in response	Chatterjee *et al.* (1980)
wheat	variation in reaction to B	Mehrotra *et al.* (1980)
wheat	>1500 lines tested	Moody *et al.* (1988)
wheat	variation in B tolerance at germination	Chhipa and Lal (1990)

widespread distribution of high levels of B in plant samples (Cartwright *et al.*, 1986) indicate that B toxicity is a major factor affecting yield in southern Australia.

Moody *et al.* (1988) tested over 1500 wheat lines in high B soil in the glasshouse. In contrast to the situation with barley however he only found only approximately 7% of these lines were more tolerant than Halberd, the most tolerant current Australian wheat cultivar . They found that those wheats which have been most widely grown in south-eastern Australia for most of the twentieth century were among the most tolerant of all Australian cultivars. They concluded that the dominance of tolerant cultivars in specific regions indicated that high concentrations of B had exerted a significant selection pressure and influenced the distribution of wheat cultivars. Interestingly, Moody *et al.* (1988) were able to associate the relative degree of B tolerance of several wheat cultivars with their source of origin; those from the USA, Canada, Egypt and NW Europe were mostly sensitive, those from Argentina, Australia, Turkey and Iraq had variable sensitivity, while those from Afghanistan, India and Japan were predominantly tolerant.

Other nutrients

There is considerable evidence for single gene control of many micronutrient efficiency factors (Epstein, 1972; Cartwright *et al.*, 1987). This was first demonstrated by Weiss (1943) who found that an iron-inefficient mutant of soybeans was controlled at a single locus with efficiency being dominant. Single-gene control of magnesium, as well as B, efficiency in celery was established from a study of recessive mutants (Pope and Munger, 1953a,b). Copper efficiency in rye was consistent with a single dominant gene controlling this character (Graham, 1984). The genetics of manganese efficiency in barley also appears to be under relatively simple genetic control (Graham *et al.*, 1983; Sparrow *et al.*, 1983).

Specificity and evidence of single-gene control of effects at the toxic end of the nutritional spectrum have been demonstrated in a number of different plant species (Antonovics *et al.*, 1971; Woolhouse and Walker, 1981; Macnair, 1981). Macnair (1981), for example, has shown that *Mimulus guttatus* controlled uptake of copper (Cu) at a single major-gene locus to confer protection against copper toxicity. A study on the genetic control of aluminium stress tolerance in barley by Reid (1970) suggested that aluminium (Al) tolerance is dominant. Cultivar

differences in tolerance to excess Manganese (Mn) have been reported for barley (Vlamis and Williams, 1967; Reid, 1970, 1976; Macfie *et al.*, 1989) and other members of the triticeae. Two Indian barley cultivars tolerated both deficiency and excess of Mn (Duke, 1982). In some barley cultivars, Al and Mn tolerances have been reported to coincide (Foy, 1977). The uptake of silicon (Si) was found to differ greatly amongst genotypes (Walker and Lance, 1991) and also reflected the relative susceptibility to B toxicity and B uptake (Nable *et al.*, 1990). Manyowa and Miller (1991) from their review of the literature made an interesting observation. They mooted the idea of mineral stress multitolerance and the possibility of tolerance to a range of stresses being controlled by alleles or clusters of the 'same' gene(s), predominantly on the homeologous group 5 chromosomes of members of the tribe *Triticeae*.

Chromosomal Location

The genetic control of tolerance to B was studied for wheat to allow the adoption of appropriate strategies for breeding tolerant cultivars. Areas of investigation included: (1) whether tolerance is under the control of major genes, (2) whether tolerance is expressed as a dominant character and (3) the chromosomal location of genes conferring tolerance to high concentrations of B (Rathjen *et al.*, 1987). The results of first two aspects would determine the breeding strategy, for instance if major genes are identified, tolerance could be transferred to locally adapted sensitive cultivars by backcrossing. Identifying chromosomes controlling B tolerance would assist in establishing linkage maps thereby allowing the identification of a closely linked marker which could be used for selection.

Paull *et al.* (1988b, 1991b) found B tolerance to be non-maternal and partially dominant, by looking at dry matter production and tissue B concentration in F_1, F_2 and F_3 generations produced from five genotypes varying in tolerance. The level of dominance expressed depended upon the level of B to which the line was exposed (Paull *et al.*, 1991a). They identified three combinations of lines which each segregate at a single, but different, locus with respect to tolerance to B, and further combinations which segregate to two or more genes. The major genes acted in an additive manner and were named *Bo1*, *Bo2* and *Bo3* (Paull *et al.*, 1991a). Transgressive segregation occurred in one combination (Paull *et al.*, 1991a).

A number of chromosomes of wheat have been implicated in the control of tolerance to B. Paull *et al.* (1988b) analysed Chinese Spring/Kenya Farmer substitution lines, and suggested that wheat chromosome 4A has a major effect in controlling the response to excess B, but that modifying genes appeared to be present on other chromosomes. Chromosome 7Eβ from *Lophopyron elongatum* (syn. *Agropyron elongatum*) was also implicated in the control of tolerance of B (Paull *et al.*, 1990b). Moody *et al.* (1990) showed that wheat lines with the genotype *Bo1 Bo2 Bo3* produced significantly higher yields than *bo1 Bo2 Bo3* lines when grown under high B conditions at a number of sites in South Australia. These studies have since led to the release of a B tolerant wheat cultivar, adapted to South Australian growing conditions; BT Schomburgk. This cultivar was produced using predominantly backcrossing methods (Paull *et al.*, 1992).

Manyowa and Miller (1991) reviewed the genetics of tolerance to high mineral concentrations in the tribe *Triticeae*. Although results by Paull *et al.* (1988b) suggest that useful variation in B tolerance exists among wheat cultivars, studies by Manyowa (1989) revealed even more potent sources of tolerance among other members of the tribe *Triticeae*. These included *S. cereale* cultivars, King II and Imperial, *Th. bedssarabicum*, *Ag. elongatum* and *Ae. sharonensis*, with the level of tolerance being highest in Imperial rye and *Ag. elongatum*. Manyowa (1989) undertook Chinese Spring/'alien' addition line studies to assign excess B tolerance genes to specific chromosomes of Imperial rye (*S. cereale*), *Ag. elongatum* and *Ae. sharonensis* . None of the CS/*Ag. elongatum* addition lines expressed a level of B tolerance significantly higher than CS, even though the lines showed significant genotypic differences in tolerance. Imperial rye and *Ae. sharonensis* addition lines for chromosomes 2R, 3R, 5R, 3S, 5S and 7S, consistently expressed a level of tolerance higher than that of CS. The homeologous group 5 additions were comparatively the most tolerant, implicating this chromosome may be involved in the control of tolerance to excess B. The group 3 chromosomes (3R and 3S), although not having similar major effects, appeared to show a similar type of allelic control.

Mapping Methods

A range of methods have been employed to elucidate the number of loci and chromosomal location of genes controlling various characters in barley. Tsuchiya (1976) and Tsuchiya and Huas (1973)

described allelism testing in barley, which distinguishes between different loci and varying alleles at one locus. Genes have been mapped using test lines carrying reciprocal translocations (Ramage, 1964; Jensen, 1971), trisomic chromosomes (Tsuchiya, 1963; Kaiser and Friedt, 1989; Tsuchiya, 1991), and telotrisomics (Shahla and Tsuchiya, 1990). Ditelosomic (Islam, 1987) and disomic (Islam *et al.*, 1981) wheat-barley addition lines have recently been utilised to assign barley genes to chromosomes and chromosome arms (Cannell *et al.*, 1992).

Multiple marker stocks can be used to map gene(s) controlling a character to one or more chromosomes in barley (Franckowiak, 1987). Wolfe (1983, 1984) developed both multiple dominant and recessive genetic marker stocks, as well as multiple recessive stocks for each of the seven barley chromosomes. These lines carry genes for easily identified, genetically recessive, morphological characters of known chromosomal location (Wolfe and Franckowiak, 1990). These stocks may be crossed with a line expressing the character to be mapped, for example tolerance to boron toxicity. In the F_2 generation plants are scored for the segregating characters and each character is tested for independent segregation, with respect to boron tolerance. Any non-independent segregation would imply linkage, and genes controlling boron tolerance could be assigned to one or more chromosomes.

Similarly, isozymes (Brown, 1983), structural proteins (Nielsen and Hejgaard, 1987) and restriction fragment length polymorphisms (RFLPs) (Gebhardt and Salamini, 1992) can be used as genetic markers to assign genes to chromosome locations. The advantages of these methods over morphological markers include; being able to screen plants early in development; generally, a lack of expression of dominance at this level; and a considerably greater level of polymorphism between barley genotypes. Recently a genome map has been constructed incorporating RFLP, polymerase chain reaction (PCR), isozyme and morphological marker loci, using Wolfe's marker stocks (Shin *et al.*, 1990). Barley doubled haploid lines, derived from either the *Hordeum bulbosum* or anther culture technique, are useful genetic material for the application of these mapping technologies, particularly RFLPs (Heun *et al.*, 1991; Graner *et al.*, 1991). These lines reflect the genotype of the F_1 gametes, and are homozygous at all loci.

Genetic potential for breeding

> *"Selective ion transport is under genetic control, and thus should be amenable to investigation by the disciplines of physiological and biochemical genetics, and to manipulation by plant breeders."* (Epstein, 1963)

Plants can be improved for mineral nutrition traits through breeding (Clark and Duncan, 1991). Techniques and methods are available to show that mineral nutritional traits in plants can be manipulated genetically. Vose (1984) said that to make use of any nutritional character in a crop improvement programme requires initially an adequate range of variation of the character, particularly in the direction in which improvement will be sought, to make selection appear promising. It is necessary to confirm to what degree the character is heritable, and the mode of gene action. Whether for example it is dominant or recessive, simple or multigenic, additive or non-additive. An understanding of the nature of genetic variances (additive, dominance, epistatic) and their interaction with nonheritable factors allows breeders to make more accurate decisions about effective breeding methodology and techniques (Clark and Duncan, 1991).

The genetic potential for improvement in tolerance to B in wheat and barley depends upon the source of unselected material and upon the range of the yield response curve to increasing levels of the toxic element in the environment, that is how far the upper critical limit of the range can be extended (Rathjen, *et al.*, 1987). The form of response curves between deficiency and toxicity has been demonstrated for B and other nutrients in cassava by Howeler *et al.* (1982). The options for improvement in tolerance to sub-optimal conditions are to select genotypes whose yield optimum falls in the appropriate portion of the range of the environmental factor in question, or to select genotypes with greater physiological plasticity, that is, adapted to a wider range of the environmental factor (Kuiper, 1984). A consequence of improvement in tolerance without increase in flexibility of adaptation must be an increase in susceptibility to deficiency, as noted for B (Loneragan, in Wright, 1977). On the other hand, if phenotypic plasticity can be increased, an important consideration in the case of commercial crops is whether productivity can be maintained over the extended range (Rathjen *et al.*, 1987; Nable *et al.*, 1990).

Boron nutrition is more problematical in terms of lack of flexibility than other mineral nutrients from which plants may suffer either deficiency or toxicity for two reasons (Nable *et al.*, 1990).

Firstly, both the difference between deficient and toxic levels of B in plant tissues, and between deficient and toxic levels of B supply are unusually narrow compared to the range for other nutrients (Eaton, 1944; Reisenauer *et al.*, 1973). Thus, small changes in the capacity to accumulate B can have marked effects on B status. Secondly, because both B deficient and toxic soils can sometimes occur in close proximity the choice of appropriate fertilizer practices and cereal cultivars can be confounded. For example, the mallee lands of southern Australia contain sandy dunes with intervening swales of heavier textured soils. Boron-rich soils in these landscapes are confined to swales, while adjacent sand ridges may have low available B. In the vertical plane B concentration varies considerably with soil depth (Cartwright *et al.*, 1984). It is possible then that below topsoils adequate or marginally deficient in B may lay B toxic soils.

Soil variability may also cause difficulty in designing effective trials to screen early generations of selections when the degree of replication is limited by seed supply (Rathjen *et al.*, 1987). The most efficient design with regard to plot size and replication may differ from one location to another; various corrective measures, such as moving means, nearest neighbour designs, and check plots, may also vary in efficiency from site to site. At the later stages of selection programs, where there is less restriction on replication, the major problem is in the choice of representative locations.

Selection strategies

Rathjen, *et al.* (1987) reported that the efficiency of selecting under stress has been questioned (Mederski and Jeffers, 1973; Rosielle and Hamblin, 1981). Rathjen, *et al.* (1987) asserted that there is no justification for undertaking a breeding program without an actual change in rank order of genotypes, with respect to yield in the target land area. Rank order among wheat selections has been found to vary in response to soil B levels (Cartwright *et al.*, 1987). Further, they stated that a breeding program is also unwarranted unless selection for tolerance produces an increase in mean performance over both stress and non-stress environments. In practice, the ability of farmers to take advantage of a tolerant cultivar, they suggested, may depend upon the spatial pattern of occurrence of the stress, that is in large homogeneous tracts, or in a patchy and sporadic distribution. Richards (1983) shown that most of the yield of wheat on salted paddocks

was derived from the least saline land, and therefore, argued that greater progress could be achieved by selecting for grain yield on non-saline soils.

Selection for yield under conditions of high soil B appears to result in the selection of B tolerant cultivars in most instances (Paull *et al.*, 1986). This may not always be the case, however, as other factors, such as the depth of the root system and time of maturity may also influence the response of a cultivar. A shallow rooted cultivar may be able to avoid the high B zone, while an early maturing cultivar may complete a significant part of its growth cycle before end of season moisture stress encourages exploration of deeper subsoil moisture reserves high in B. Both types of cultivars may appear B tolerant, on the basis of grain yield, during high rainfall seasons, however their B sensitivity would become apparent during a dry season.

Three general systems of breeding are most used for cereals in Australia (Rathjen *et al.*, 1987). The pedigree system is most applicable where the character under selection is easily recognised in the early segregating generations on the basis of the phenotype of spaced plants. The progeny method is widely practised in southern Australia and it is most applicable in selecting for increased grain yield where the advantageous genotypes are not identifiable on the basis of the phenotype. The backcross method require both a readily identifiable character and genetic control by one, or a few major genes.

Genetic engineering offers potential for improving genetic adaptation of plants to conditions where mineral elements are limiting or toxic, and for mineral nutrient-use efficiency, through the introduction of appropriate genes to desirable backgrounds (Clark and Duncan, 1991). Tissue culture, anther culture, and microspore culture are methods which can be allied with traditional breeding techniques (Foroughi-Wehr and Wenzel, 1990), for production of doubled haploids or as a method of sporophytic selection. Restriction fragment length polymorphisms (RFLPs) are being used to map localized genes into linkage groups, using linkage analysis, and may also be used as markers for screening. Gene isolation, identification, localization, and reproduction in the laboratory, however, are essential for success in the introduction of genes or DNA carrying desired nutritional traits. Conventional and population breeding approaches have been successful and should continue to be important.

Screening techniques

Development of a simple, rapid, and reliable screening method is needed to provide breeders with a tool for selecting plants with improved response to mineral stress (Stavarek and Rains, 1984). The general principles involved in screening, and the precautions to be observed when undertaking a screening program have been described by Foy and Wright (1977), Gerloff and Gabelman (1983), and Munns and Scott (1987). An understanding of the basis of tolerance is an aid to devising screening techniques, and to determining the mode of inheritance, so that efficient breeding strategies can be formulated (Rathjen *et al.*, 1987). The more closely a screening technique is based on the primary mechanism of tolerance, the less likely it is that environmental interactions can introduce error when selecting for tolerance. The solution culture approach has been used extensively by Gabelman and Gerloff (1982). The effectiveness of this technique depends on mechanism for tolerance (Cartwright *et al.*, 1987).

In the case of B tolerance in wheat, dominance varied with the level of B applied (Paull *et al.*, 1988b). The nature of inheritance of tolerance to B will influence the level of the treatments selected for screening in a breeding programme aiming at enhanced levels of tolerance. Paull *et al.* (1988b) proposed that the level should be chosen to be toxic to the homozygous sensitives rather than the heterozygotes for a backcrossing programme, whereas the level should be toxic to the heterozygotes but not the homozygous tolerant genotypes when screening an F_2 or other segregating generation. The optimal B treatment will also vary for different parental combinations.

Manyowa and Miller (1991) discussed some of the problems involved in screening for tolerance to toxicities. They stated that the main problem of a single concentration test is the choice of the test concentration. A complete genetic analysis requires a quantitative tolerance measure with a sufficient degree of resolution throughout the entire range of variation in tolerance, which is present among the organisms to be tested. If such a measure is inferred from a single concentration test, then a concentration should be chosen which is toxic to all the genotypes but which allows at least some growth of the most sensitive one. In some cases such a concentration does not exist. Even in cases where it does exist, it may be doubtful whether the sensitivity of the index will be sufficiently high throughout the entire range of variation in tolerance present.

The type of single concentration test applied by Macnair (1983) and Manyowa and Miller (1991) implies a basically qualitative criterion for tolerance, which has at least the advantage of a rapid and simple screening procedure. It is easy to conceive, however, that this type of test will only produce interpretable results, if the test concentration is chosen at such a level that the test effectively identifies one of the extreme genotypes (eg. the completely non-tolerant homozygote). The chance that this requirement can be met is expected to increase as the difference between the extreme genotype and tthe next most tolerant group becomes larger. If the difference is small, which may be expected in the case of polygenic control, then the range of suitable test concentrations will be narrow, especially if innate variation in root growth unrelated to tolerance is present. They proposed a sequential test, but some problems may arise with this kind of exposure.

Cartwright *et al.* (1987) presented a case for selecting the minimum pressure to distinguish efficient/inefficient types and compared two-level and single-level assessment. When single level comparison of genotypes is required they suggested the use of check plots. The results can be analyzed by running-mean techniques and interpreted by comparing test plot yield to the check genotype yield surface for the site generated from the array of check plot yields. Cartwright *et al.* (1987) have found the check plot yield array highly efficient at defining site variability and fertility trends. They propose then that efficient assessment then can be made with a minimum of replication.

Cell culture techniques can provide an alternative method of screening and selecting plants which are tolerant to mineral stress (Stavarek and Rains, 1984). Clark and Duncan (1991) cited an example where potassium chlorate stress during the embryogenic callus-induction phase was used to improve nitrate-reductase efficiency. The environment and nutrient conditions can be controlled uniformly and precisely, and a large number of cells can be screened rapidly in a relatively small area. The relatively undifferentiated nature of the cultured cells reduces the complications of differences in morphology and stages of development. However, the media must mimic field conditions accurately. The interaction of different ions as well as the pH of the medium may influence the selection results. There are several potential problems with cell culture systems that also should be considered. After selection, plants must be regenerated from

the selected cells. The capacity for regeneration decreases rapidly with time in most cell culture systems, however. This decrease may be a technical problem which can be overcome by a better understanding of improved media sequences. The expression of desirable traits in the regenerated plants as well as the heritability is important. The regenerated plants would maintain the characteristics of the cultivar and incorporate the new genetic traits that were selected in the cells without incorporating any deleterious genes. This method, however, will only be valid for mechanisms that occur at the cellular level and are not dependent upon whole plant structure (eg., root-shoot interactions) or whole plant functions (eg., photosynthesis, specialised xylem transfer cells). An understanding of the cellular mechanisms involved in stress tolerance will provide information to improve selection criteria (Stavarek and Rains, 1984).

Summary

Thus, in barley, and many other plant species, tolerance to toxic levels of B is largely under genetic control. Tolerance to B then is a character amenable to selection and breeding. Choice of the most effective selection strategies for barley will depend upon the source of tolerance genes, the mode of inheritance of B tolerance, the physiology of B tolerance, and the targeted environment.

Chapter 2

CONTRIBUTION OF GENETIC FACTORS TO BORON TOLERANCE IN THE FIELD

INTRODUCTION

The ability to identify and select barleys (*Hordeum vulgare*) tolerant to toxic levels of boron (B) in soils is important for improving yields and profitability of the barley crop in much of the cereal growing region of southern Australia (Cartwright *et al.*, 1984). Graham (1984) suggested that to argue that we should breed for nutritional characters, it is necessary to show (1) a need as pressing as other objectives, (2) there exists genetic potential to be exploited, and (3) it is agronomically, economically and ecologically feasible. Thus, when making decisions about breeding strategies, it is useful to be able to predict the magnitude of potential gains to be made through selection, and thus make a more informed decision about the strategies most appropriate to manipulating a particular trait. It is important then to gain some understanding of the degree to which expression of a trait is controlled by genetic, environmental and interactive factors.

Described below are the results of two field studies, using lines of barley that originated from selfing individual F_2 plants, over a number of generations, derived from the cross (1) Sahara 3771 x WI 2723 and from the cross (2) CM 72 x Stirling. The purpose of this study was to obtain an estimate of additive genetic components of phenotypic variances and thus to estimate the proportion of total variance attributable to genetic factors, in these populations.

A number of factors complicate the estimation of these genetic components: barley is an inbreeding crop; the experiment was restricted to one trial site, over three years; and natural spatial patchiness in occurrence of B in soils introduces a large amount of noise into field data.

MATERIAL AND METHODS

Plant Material

For Experiment 1, two homozygous lines of barley (*Hordeum vulgare* L.), Sahara 3771, a highly B tolerant six row land race, and WI 2723, a B intolerant two row breeding line were used as parents. In general, Sahara 3771 has a shorter growth habit, later anthesis date and longer but lighter grain, compared with WI 2723.

For Experiment 2, two homozygous lines of barley (*Hordeum vulgare* L.), Stirling, a highly B intolerant two rowed malting cultivar and CM 72, a moderately tolerant six row feed cultivar, were used as parents. In general CM 72 has a later anthesis date and smaller grain, compared with Stirling.

Both experiments used the intolerant, but otherwise locally adapted barley cultivar, Schooner as a grid of check plots. In Experiment 1 1989, a grid of the cultivar Schooner was not grown, and the WI 2723 plots failed to establish.

Field Trial Site

The lines in this study were grown at the Sharpe Brothers' farm, about three kilometres north of Two Wells, South Australia (34° 36'S, 138° 31'E, 11.0 m elevation) in the winters of 1989, 1990 and 1991. The trial sites were within two kilometres of each other. The predominant land use in the area is cereal production and sheep grazing. The terrain is a flat coastal plain with naturally high subsoil B concentrations (Cartwright *et al.*, 1987b). The soil type description for the USDA system is a fine, mixed, thermic, vertic, natric, xeralf and under the Northcote system falls into a DR 2.23 classification. The depth of topsoil varies from 0 to 30 cm and under the topsoil lies a clayey "B Horizon" then a carbonate layer. The maximum concentration of B generally occurs at the top of the calcium carbonate layer, where it is commonly at 20 to 30 $mgkg^{-1}$ at a depth of 30 to 40 cm. The soil is sodic and alkaline, with pH ranging from 5.8 to 9.0 in the topsoil, mostly between 7.0 and 9.0 in the subsoil and increasing up to 9.5 at a depth of 100 cm (B.A. Zarcinas, pers. comm.).

The climate is of a Mediterranean type with an average rainfall of 398 mm. The average annual rainfall for these three years was 361 mm (Table 2.1). Fertilizer and herbicides were applied according to farmer practices (Table 2.2). The previous cropping history on the three sites was faba beans, pasture and fallow respectively.

Table 2.1. Monthly and yearly rainfall in millimetres at Two Wells (Source: Bureau of Meteorology, S.A.).

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
1989	<.4	3	6	30	76	57	63	51	31	20	37	10	384
1990	18	4	<.4	5	9	56	74	73	24	18	3	48	332
1991	13	0	6	29	7	90	60	53	38	6	39	0	368

Field Trial Design

Experiment 1

In 1989, twenty two plots of each parental line and eighty eight F_2 derived F_4 families were grown in two replicates of a randomised block design. On three sides the replicate blocks were bordered by the barley cultivar Schooner, and along the common border by plots of unreplicated families. Plots were 4.2 m long and two rows wide, with rows 15 cm apart.

In 1990, fifteen plots of each parental line and one hundred and ten F_2 derived F_5 families were grown in each of three replicates of a randomised block design, through which ran a grid of thirty plots of the cultivar Schooner, giving a total of one hundred and seventy plots per replicate. Borders were of Schooner and plots were 4.2 m long and four rows wide, with rows 15 cm apart. In 1991, the experiment was designed in the same way as 1990, except families were F_2 derived F_6s.

Experiment 2

In 1990, fifteen plots of each parental line, and sixty two F_2 derived F_3 families were grown in each of two replicates of a randomised block design. In addition, in each replicate there was one plot each of Clipper, Galleon, Schooner, O'Connor, Skiff, WI 2645, WI 2728 and Sahara 3771 and twenty three plots of a control grid of WI 2737 (now cultivar Chebec). One plot of

Weeah was grown to balance the design. Plots were 4.2 m long and two rows wide, with rows 15 cm apart.

In 1991, fifteen plots of each parental line, and sixty two F_2 derived F_4 families were grown in each of three replicates of a randomised block design. In addition, in each replicate there was one plot each of Clipper, Galleon, Schooner, O'Connor, Skiff, WI 2645, WI 2728 and Sahara 3771 and twenty plots of a control grid of WI 2737.

Sample Collection and Analysis

Variables investigated were grain yield, shoot damage due to B, shoot B concentration, B concentration in grain, shoot dry weight, head type and tillers per plant. Some of these will be discussed in Chapter 3. Not all replicates of each experiment were sampled and analysed for all of these variables.

A plot was given an overall score of shoot damage due to B on a scale of 0 to 90, with 0 representing no visible damage and 90 total coverage by symptomatic spots. Whole plant tops were sampled for shoot B concentration. A number of plants from each plot was collected around the time of anthesis, dried, ground and subsampled. Analysis for B concentration in this material and grain was carried out using nitric acid digestion and ICP techniques described by Zarcinas *et al.* (1987). Shoot dry weight and number of tillers per plant were calculated from a sample of five to ten plants from each plot. Head type was classified as six row, two row or segregating, though a number of head type characters were seen to be segregating both within and between families.

Statistical Analysis

Shoot damage scores and yields were adjusted to compensate for variations within the trial area, presumably due to variation in soil B, using the computer programme MATLAB and a method described by Cullis *et. al.* (1989) for analysis of early generation variety trials. Frequency distributions of shoot damage scores for F_2 derived lines and controls, were constructed using DeltaGraph™, a Delta Point computer software package.

Table 2.2. Description of fertilizers and herbicides used at field trial sites in 1989, 1990 and 1991.

Year	Sowing Date	Fertilizer	Rate & Analysis	Herbicide	Analysis
1989	5 May	Top Phos™	100kg/ha @ sowing [P available 16.7%, S 4.4%]	Hoegrass™	1.5 l/ha @ 6wks [375g/l diclofopmethyl]
				Ally™	7g/ha @ 8wks [600g/kg metasulfuronmethyl]
				MCPA	100ml/ha @ 8wks [500g/l MCPA sodium]
1990	29 June	Top Phos™	100kg/ha @ sowing [P available 16.7%, S 4.4%]	Ally™	7g/ha @ 8 weeks
				Bromoxynil + MCPA	1.4l/ha @ 8 weeks [200g/l bromoxynil, 200g/l MCPA ester]
1991	13 June	Top Phos™ with Zn	100kg/ha @ sowing [P availabe 15.0, S 3.7%, Zn 2.5%]	Ally™	5g/ha @ 3wks
				Dicamba +MCPA	1.01 l/ha @ 11wks [300-340 g/l MCPA, 80g/l dicamba]

RESULTS

Experiment 1

The means and variances for overall plot yields and shoot damage scores of the parental and F_2 derived lines are given in Table 2.3. The "adjusted values" were calculated using the MATLAB package, based on the Schooner check plots, to adjust data for spatial variation in shoot damage symptoms within each experiment. Note that in Experiment 1, since a grid of the cultivar Schooner was not grown in 1989, and the WI 2723 plots failed to establish, the Sahara 3771 line was used as the correction grid for the MATLAB analysis. The environmental variance for this year then cannot be truly estimated. Shoot damage scores for F_2 derived families fell between the parental means. The adjusted data showed that the variance of the F_2 derived families exceeded that of the parents in each year. With regard to yield, the adjusted mean of the F_2 derived families was smaller than both parents in 1990 and fell between them in 1991. Variance for yield ranged over several orders of magnitude between years. F_2 derived family variances always exceeded parental variances.

The frequency distributions for shoot damage scores for each year, both in the raw form and adjusted using MATLAB are shown in Figures 2.2 and 2.3. The frequency distributions for each year for adjusted scores show different patterns. In both 1990 and 1991, the range of the F_2 derived families exceeds that of the parents, and the means of the families fall between the parents. The difference between the parental lines differs between years, as does the overall severity of symptoms. Similar trends can be seen in the raw data. Without the removal of the variation due to the within site environmental variation, the variance is much larger.

Experiment 2

Means and variances for shoot damage score and yield for parents and offspring based on overall family scores for Experiment 2 are given for 1990 and 1991 in Table 2.4. In both years the adjusted mean score for the families falls between that of the parents, and variances exceed that of each parent. With respect to yield, the mean of the F_2 derived families was less than that of either parent in 1990, but fell between them in 1991. Variances were an order of magnitude greater in 1991 than in 1990. In 1990 the variance of CM 72 exceeded that of the F_2 derived

families. Figure 2.4 describes the frequency distribution for raw shoot damage scores in Experiment 2, in 1990 and 1991 Figure 2.5 describes the frequency distribution based on data adjusted by MATLAB using the Schooner check grid. Like Experiment 1, after the removal of the variation due to the within site environmental variation, the variance is much reduced. The distribution pattern with regard to both means and variances differs considerably between the two years. In 1990 ranges were large, with the F_2 derived families spanning the range of both parents. The difference between the two parental means was 43. A dip occurs in the frequency distribution of CM 72 at score forty. In contrast, in 1991, distributions were uniformly normal, and the three genotypes closely grouped, between scores of sixty and ninety. The range of the F_2 derived families only slightly exceeded that of the parents.

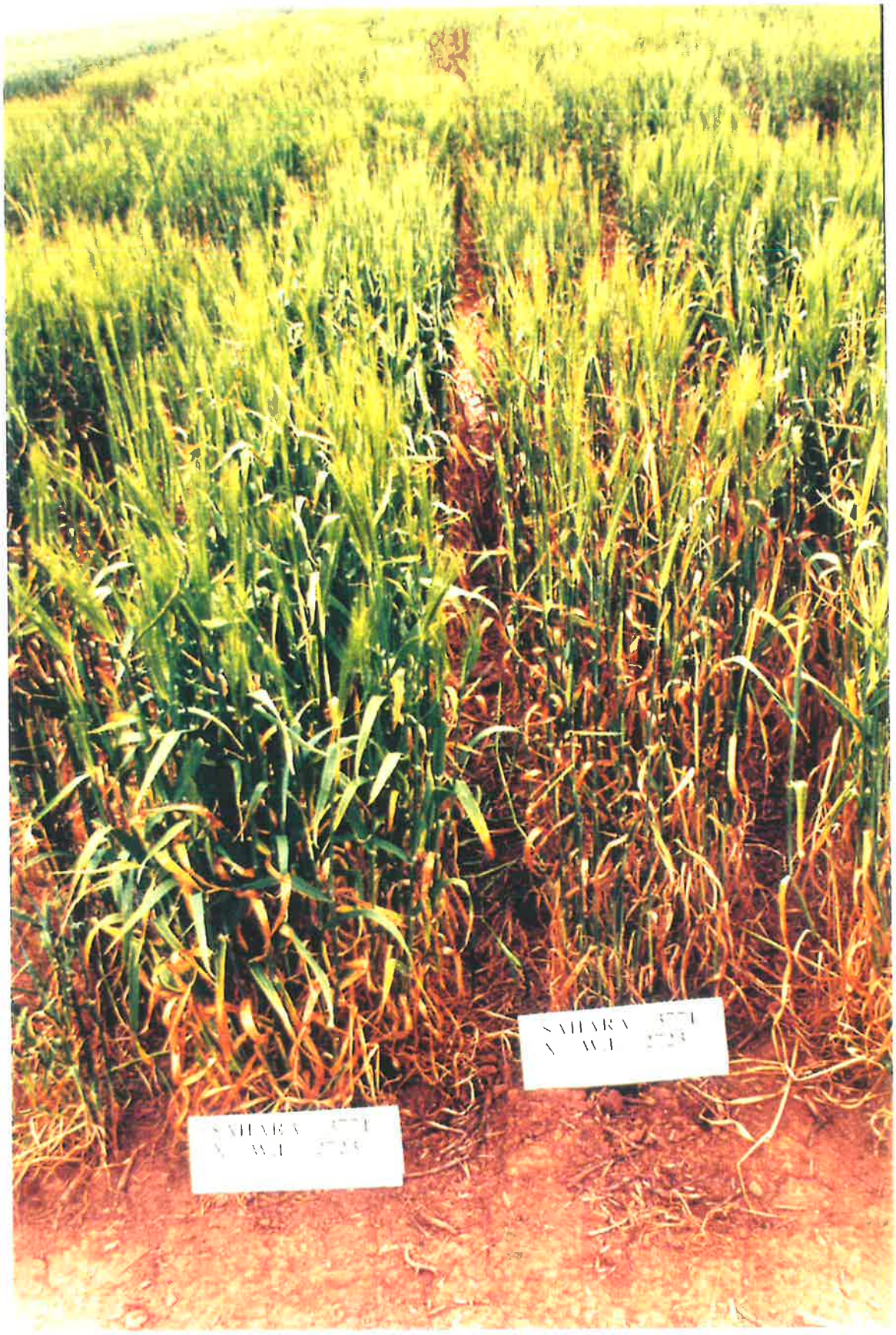


Figure 2.1. Variation in B toxicity symptoms between two F_2 derived lines, in Experiment 1 in 1989.

Table 2.3. Means and variances for shoot damage score and grain yield for parents and offspring for Experiment 1 in 1989, 1990 and 1991. Shoot damage score is assessed on a scale of 0 to 90 based on overall family scores. For an explanation of "Adjusted" values refer to the text. * represents missing values. #In 1989 yields were multiplied by 2 to compensate for the fact that only double row plots were sown rather than the standard four row.

			Raw shoot damage score		Adjusted shoot damage score		Raw grain yield (g/plot)#		Adjusted grain yield (g/plot)	
Year	Line	N	Mean	Variance	Mean	Variance	Mean	Variance	Mean	Variance
1989	Sahara 3771	44	66.0	70.8	66.0	0	1067	31759	1108	0.0
	WI 2723*	44	*	*	*	*	*	*	*	*
	F4 Families	176	71.9	189.4	72.0	39.7	712	71938	713	13140
1990	Sahara 3771	45	20.2	2.3	34.4	4.7	338	7057	310	1.6
	WI 2723	45	48.3	179.5	41.0	4.9	405	12211	311	2.0
	F5 Families	330	39.4	318.5	38.9	11.4	292	7970	309	2.3
1991	Sahara 3771	45	32.2	185.8	23.5	39.2	666	7814	735	1093
	WI 2723	45	62.9	507.4	60.7	21.7	997	18388	836	1588
	F6 Families	330	55.6	390.7	54.5	54.6	747	14549	760	1921

Table 2.4. Means and variances for shoot damage score and grain yield for parents and offspring based on overall family scores for Experiment 2 in 1990 and 1991. For an explantation of "Adjusted" see the text. #Yields from 1990 when double row plots were sown have been multiplied by 2.

			Raw shoot damage score		Adjusted shoot damage score		Raw grain yield (g/plot)#		Adjusted grain yield (g/plot)	
Year	**Line**	**N**	**Mean**	**Variance**	**Mean**	**Variance**	**Mean**	**Variance**	**Mean**	**Variance**
1990	Stirling	30	89.7	3.3	79.8	30.2	476	4043	472	294
	CM 72	30	13.7	24.0	36.7	27.0	496	18404	496	585
	F3 Families	124	70.6	440.2	68.0	149.5	445	8243	462.7	557
1991	Stirling	45	74.8	427.1	76.7	10.1	760	22592	813	2591
	CM 72	45	59.3	183.6	65.5	6.0	1103	8300	983	2362
	F4 Families	186	74.8	307.3	73.4	12.0	829	16362	848	2798

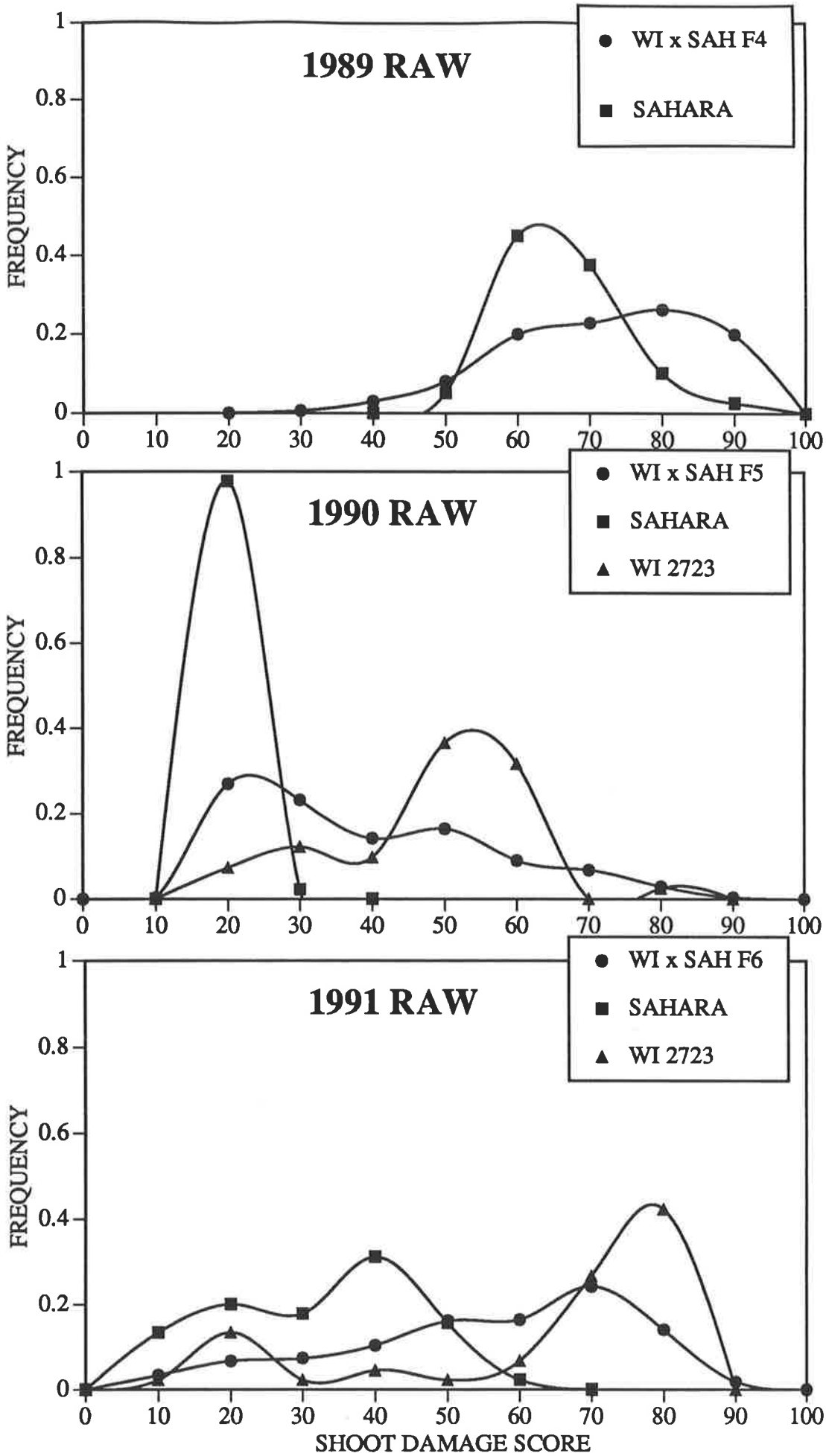


Figure 2.2. Frequency distribution of shoot damage scores for Experiment 1 based on raw data.

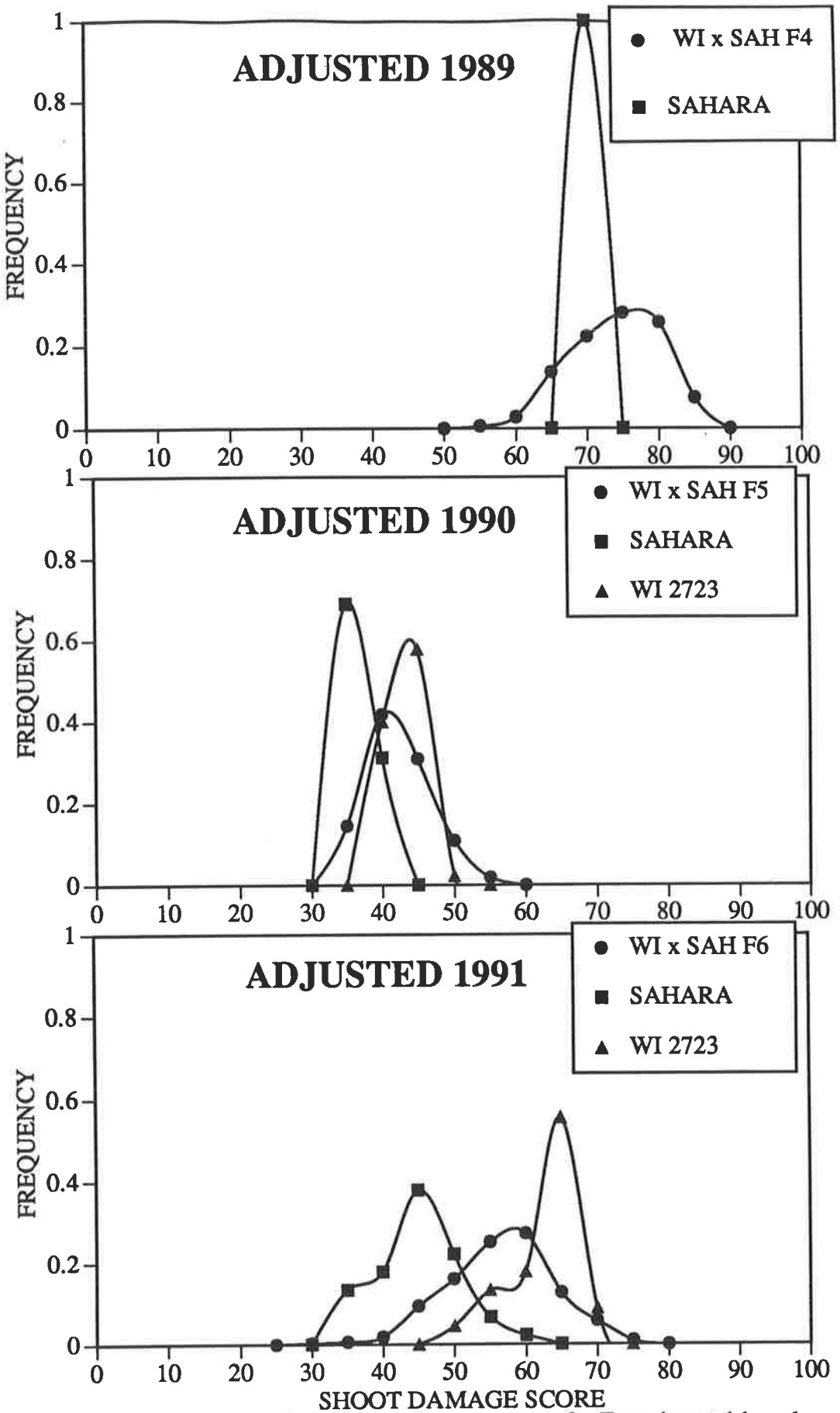


Figure 2.3. Frequency distribution of shoot damage scores for Experiment 1 based on data corrected by MATLAB (see text) using Sahara 3771 as a check line in 1989 and using the Schooner check grid in 1990 and 1991.

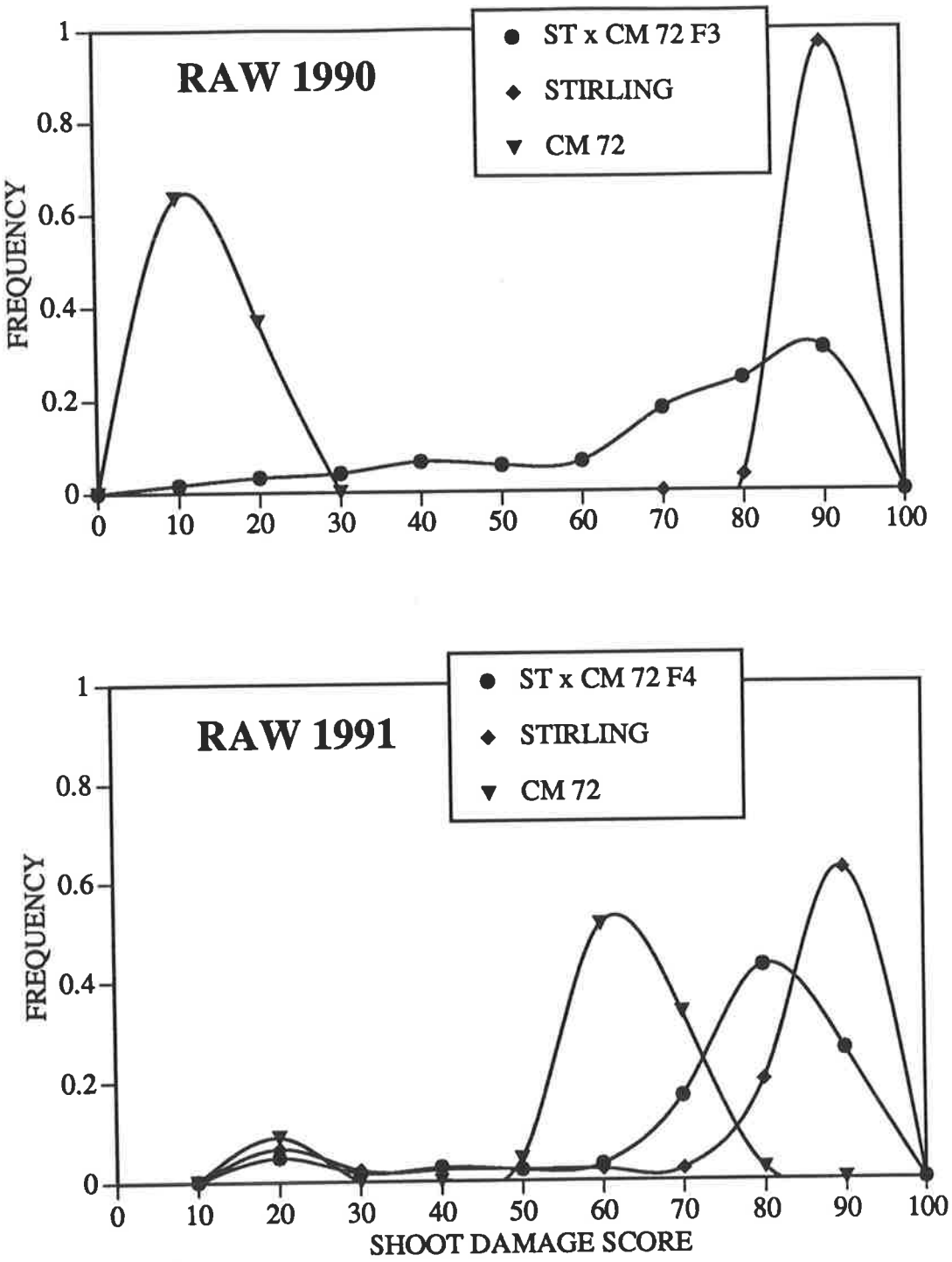


Figure 2.4. Frequency distribution of shoot damage scores for Experiment 2 in 1990 and 1991 based on raw data.

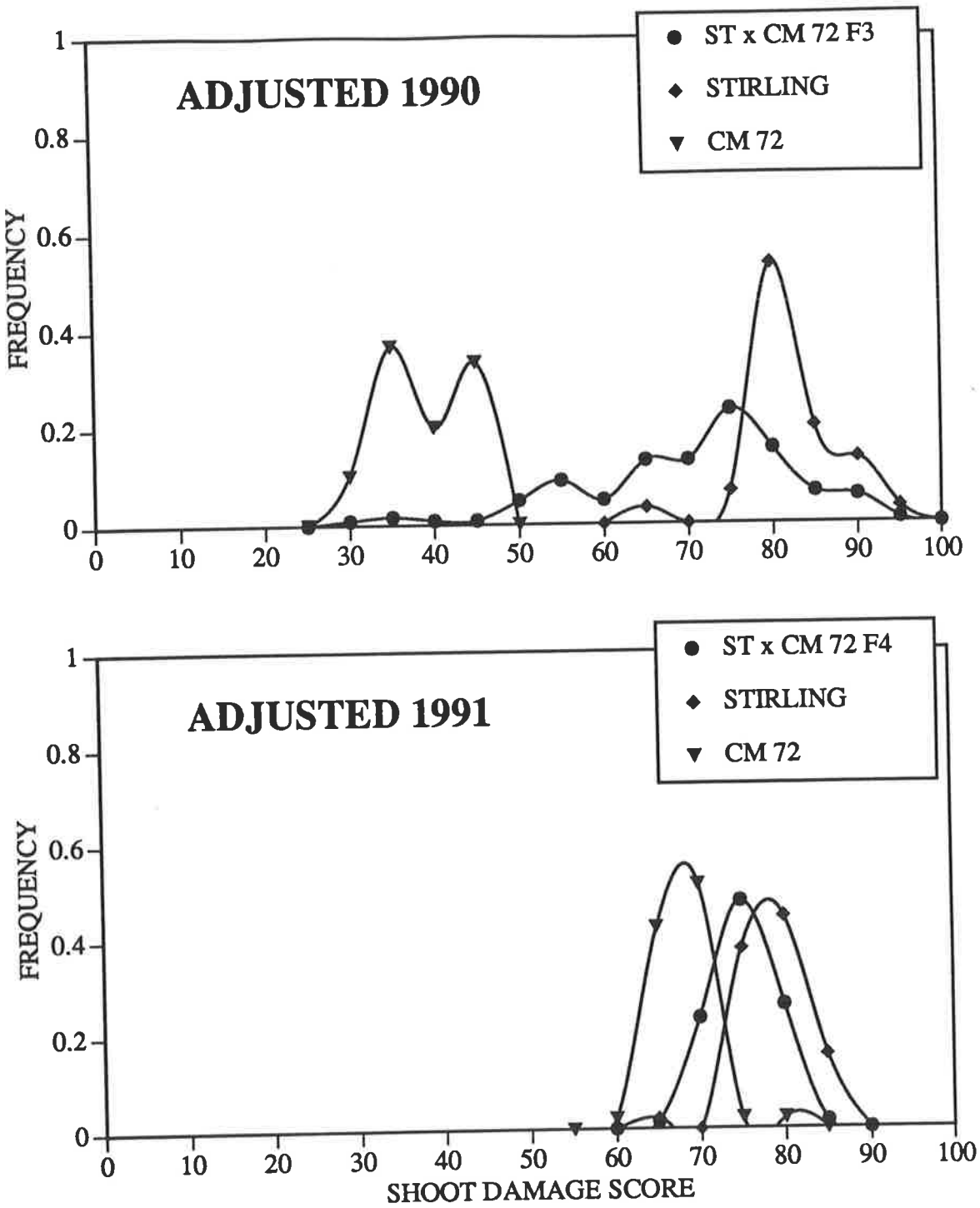


Figure 2.5. Frequency distribution of shoot damage scores for Experiment 2 in 1990 and 1991 based on data corrected by MATLAB (see text) using the Schooner check grid.

CONCLUSIONS

Total phenotypic variance is made up of a genetic, an environmental and an interaction component.

$$V_p = V_g + V_e + V_{g*e}$$

The interaction components in both Experiment 1 and 2 become evident when the difference between means of the two parental populations are compared for 1990 and 1991. If there was no genotype by environment interaction the difference between the two parental lines would have been the same each year. Since the parental lines are genetically uniform, for each year the variance within each parental line estimates the environmental variance. The genetic component of variance is that remaining, when the environmental and interaction variances are subtracted from the total phenotypic variance.

The genetic component can be further divided into effects due to dominance and due to additive genetic effects, as described by Mather and Jinks (1971). Since the effect of dominance decreases rapidly with progressing generations, by the F_6 generation, this component could be considered negligible. In terms of heritabilities then, the broad and narrow sense heritabilities will be very similar for Experiment 1 (where broad sense heritability is genotypic variance divided by phenotypic variance, and narrow sense heritability is additive genotypic variance divided by phenotypic variance).

A conventional parent-offspring regression (that is simply regressing the data from one year against the next) is unsuited to these data sets. Not only does this model assume no genotype by environment interaction, but it relies on there being no year to year environmental effects. These criteria are so clearly violated in both of these experiments, that the resulting estimates of heritability would be unreliable, and the interpretation of limited value. Instead a more empiric approach was taken, where a broad sense heritablity was estimated simply by subtracting environmental variance from total observed variance.

Experiment 1

The difference in severity of shoot damage symptoms, in both parental and F_2 derived lines, from year to year is clear (Figure 2.3). This is probably due largely to the variation in the length of the growing seasons. Symptoms were most severe when the season was long, as in 1989 when the sowing date was the 5th of May. When the season was short, as in 1990 when the sowing date was the 29th of June, the symptoms were much less severe. The year of intermediate season length, 1991 with sowing date the 13th of June, was intermediate in severity of symptoms. Since it is assumed that symptoms reflect B accumulation (Oertli and Kohl, 1961), and B is a largely phloem immobile element (Gauch and Dugger, 1954), a longer season would allow more B accumulation. The driest year was 1990 with only 248 mm of rain falling during the growing season, between June and November (Table 2.1). The effect of the dry conditions is not obvious on shoot damage symptoms but yields were reduced significantly. Lower soil moisture has been reported to reduce the level of available B in soil (Fleming, 1980), while others have suggested that in drier years roots would be expected to extend further and deeper in search of water, and thus increase the uptake of B by mass flow (Paull *et. al.*, 1986). Thus, the means of shoot damage score show significant year to year environmental effects.

Parental variances differed from year to year. The variance of the two parental lines were smaller in 1990 than in 1991, as were those of the F_2 derived lines (Fig 2.4). This suggests more uniform environmental conditions in that year, that is a smaller contribution overall by environmental variance. Drought conditions were experienced in this year, thus tolerance to water stress was the prime limitation to yield, not shoot damage score. It is surprising that the variance of the F_2 derived lines in 1989 exceeded that of 1990, since the experiment of 1989 was spatially a smaller experiment. This result may have been caused by the less precise MATLAB adjustment, since Sahara 3771 was used as the check cultivar that year. Sahara 3771 is less variable in its response to soil B than Schooner due to its high degree of tolerance. Only a relatively smaller number of plots was able to be used as the check cultivar and these plots were randomly distributed, rather than evenly spaced across the experimental area.

The term "heritability" in a general way refers to the fraction of the observed variance which was caused by the differences in heredity (Lush, 1941). This term has been used extensively in animal breeding, when dealing with random-mating populations. A number of complicating factors arise when dealing with plants (see Nyquist, 1991). The term "heritability" in this analysis, is used in its broadest sense. When a population is derived from crossing two inbreeding plant populations, the heritability is relevant only to the properties and dimensions of that particular experiment, and is largely a function of the difference between the parents with respect to the character being studied. In this study heritability will be considered to be the phenotypic variance minus the environmental variance (as described by the mean of the parental variances), divided by the phenotypic variance, thus:

$$h^2 = (V_p - V_e) / V_p.$$

So, estimates for broad sense heritability of shoot damage scores are 0.58 for 1990 and 0.45 for 1991, for the same character in the same population. The estimate for 1989 is 1.00 since environmental variance has been artificially adjusted to 0. These numbers estimate heritability in its broadest sense only and in an approximate way at that, but are sufficiently large to give evidence that in the crosses employed, B tolerance is controlled by a considerable genetic component.

In a selection programme the aim would be to select from those F_2 derived families which show a low B damage score, in the range of that of the tolerant parent, but with an increased yield, in the range of the locally adapted intolerant parent. For example, three F_6 lines showed damage scores of less than forty in 1991 in at least one replicate. They were lines twenty four, twenty six and thirty four. In other replicates the damage scores of these three lines were less than the overall family mean. Their average yields were 749 g, 775 g, and 815 g respectively, with line thirty four yielding 842 g in replicate three. The mean yield of WI 2723 in this year was 836.4 g. Line thirty four then is particularly promising with regard to combining B tolerance and yield. This example demonstrates the advances that may be made by selecting families from this cross. Similarly for the other years, these and other high yielding families with relatively low B damage, can be identified. In addition, F_2 progeny scores are a mean of a mixture of related genotypes within a family. Lines may also be selected within families, about that mean.

Experiment 2

It is evident from Figure 2.5 that this experiment has also been influenced by year to year environmental effects and genotype by environment interactions. The trend however differs from that shown in Experiment 1. In this case the larger variances and wider divergence between parents occurs in 1990 as compared to 1991. The influence of rainfall then is not a straightforward one, and may be affected by the genotypes of the plants upon which it is acting. One explanation involves an interaction between the short growing season of 1990 and the genetic predisposition of CM 72 to flower later than Stirling. In 1991 the time over which CM 72 was actively taking up water and nutrients exceeded that of Stirling, and thus this line may have accumulated a large amount of B in total. In 1990 the brevity of the season may have nullified this difference, and given an equal time for uptake, CM 72 accumulated less B than Stirling. To overcome this scores would need to be recorded based on ontogenic stage over a period of time, rather than on one day. This effect however was not evident in Experiment 1, though Sahara 3771 also flowers later than WI 2723.

Though two years data are insufficient to make firm conclusions about genetic determination, it would be expected that the dominance component of genetic variance would decrease progressively through early generations. Indeed the mean of the F_3 families most closely resembles that of the intolerant parent, with the mean of the F_4 families tending more toward the midparent value. If genetic factors alone were involved in this relationship one might conclude that tolerance to high levels of soil B was a recessive character. Experiments conducted under controlled conditions with early generation material, however found B tolerance to be a largely dominant character (see Chapter 4). Dominance relationships have been found to change with environmental conditions in wheat (Paull *et al.*, 1991a). The year by year environmental variation, genotype by environment interactions and/or epistatic effects may influence the shoot damage scores sufficiently to produce results difficult to interpret. For example, if lateness were a largely dominant character being inherited from the CM 72 parent, such that a proportion of progeny carried both lateness and intolerance, this may tend to produce symptoms more severe than would be expected were lines compared earlier in the season. Such an interaction may under some conditions move the distribution of symptoms expressed in families toward the intolerant parent. To obtain a better knowledge of the dominance characters of a trait, early

generation material must be studied and environmental effects minimised. This can be achieved in small scale experiments, under controlled-environment conditions. On the other hand, in a selection programme, it may be desirable to avoid dominance effects. This is generallly achieved by selecting at relatively late generations, eg F_5 or F_6; alternatively, it may be achieved by the production of double haploids from F_1 plants.

In 1990 the mean yield of both parental lines exceeded that of the F_3 families. This was probably the result of epistasis, that is non-additivity. Some genetic combinations may produce a synergistic effect on yield. When these combinations are disrupted by recombination, yield will fall. This yield reduction did not occur in the F_4 families in 1991, suggesting that this effect occurs only under certrain environmental conditions. In this case, water stress may have exerted a limiting effect on yield, which overshadowed any effects due to tolerance to B.

These complicating factors, though making it difficult to draw conclusions about the underlying genetic mechanisms of B tolerance, need not lead to the belief that selection in this cross under these conditions will be ineffective. In 1990, of the eight families scoring less than 50 as a shoot damage score in at least one replicate, four families showed yields more than the overall mean of the families, and approaching those of the parents. In 1991, these lines continued to perform well, with two lines showing below average B damage scores in all replicates and at least two replicates exceeding average yield. Thus elite lines show a degree of consistency from year to year.

In general, heritability estimates are of limited use. Broad sense heritabilities can be estimated using the formula described above: for shoot damage scores there were 0.99 for 1990 and 0.83 for 1991. These figures, though limited by the constraints discussed, indicate that in this cross under these conditions shoot damage scores are determined to a large extent by heritable components.

To sum up, though accurate estimates of heritability are limited by the inbreeding nature of barley, the year to year variation and genotype by environment interactions observed in these

experiments, it appears that tolerance to B toxicity is largely genetically determined. Thus, it is likely that significant gains can be made with respect to this trait through selection and breeding.

Chapter 3

BORON TOXICITY AND YIELD

INTRODUCTION

Boron (B) toxicity in barley was first described in South Australia in 1984 by Cartwright *et al.*. In that instance, by comparing patches in the paddock expressing symptoms with symptom free areas, it was estimated that B toxicity was responsible for a yield loss of 17% (Cartwright *et al.*, 1984). By comparing lines of known parentage in replicated field trials over a number of years a more accurate assessment of the effect of B toxicity on yield could be made.

The relationships between shoot damage score, tillering, herbage production, grain yield, shoot B concentration and grain B concentration were investigated. In order to obtain as unbiased a picture of the true relationships as possible, correlations were performed using data from individual plots and from line averages over replicates. A predictive model was applied in order to investigate to what extent the combined effects of genotype (line number) and shoot damage score could predict yield under high soil B conditions in the field. This study provides information about the degree of yield loss which can result when intolerant barley genotypes are grown on high B soils, and to what extent this yield loss may be reduced by growing tolerant cultivars.

MATERIALS AND METHODS

Genetic materials used and experimental conditions are described in Chapter 2. Experiment 1 was conducted in 1989, 1990 and 1991. The parental material was Sahara 3771, and WI 2723, and the F_2 derived families studied in the F_4, F_5 and F_6 generations. Experiment 2 was conducted in 1990, and the parental material was Stirling and CM 72. The families studied were F_2 derived F_3s and F_4s. Experiments were carried out at high soil boron sites near Two

Wells, South Australia. Parental and other controls, and F_2 derived lines were set out in a randomised block design. In 1990 and 1991 a grid of Schooner barley was sown throughout the trial plots. These plots were used to adjust the raw data using the MATLAB computer programme, according to the method described by Cullis *et al.*(1989). This adjustment was conducted to compensate for underlying environmental variation, presumably predominated by soil boron content. Plots were scored visually for shoot damage typical of damage due to boron, on a scale of 0 for no damage to 90, where spotting was seen over the entire plant. Contour plots of shoot damage scores and grain yield from Schooner check plots for Experiment 1 in 1990 and 1991 were constructed using DeltaGraph™, a Delta Point computer software package. Statistical analysis was aided using the Analysis of Covariance and Specify Model subroutines of the statistical software package JMP™. Plots were constructed using Deltagraph™ software on Apple MacIntosh.

RESULTS

The responses of grain yield, dry herbage weight and tiller number per plant in relation to shoot damage scores, B concentration in shoots and B concentration in grain were investigated. The control grid reflected considerable environmental variation within the experimental plots. Yield estimates here have been scaled for Experiment 1 1989 and Experiment 2 1990, when sufficient seed was available to sow only double-row plots, rather than the standard four-row plots. The contrast between shoot damage symptoms of Sahara 3771 and the Schooner border in 1989 can be seen in Figure 3.1.

Variation Within Experimental Areas for Shoot Damage Score and Grain Yield

The contour plots of shoot damage score and grain yield for Experiment 1 for 1990 and 1991 are shown in Figures 3.2 and 3.3. These were constructed using the raw data derived from the grid of Schooner check plots. Considerable variation for both variables is apparent in both years. Though some similarity can be seen in the patterns for shoot damage score and yield in each year, particularly 1991,they do not correspond well.

Figure 3.1. Contrast in B toxicity symptoms between Sahara 3771 and Schooner, in Experiment 1 in 1989.

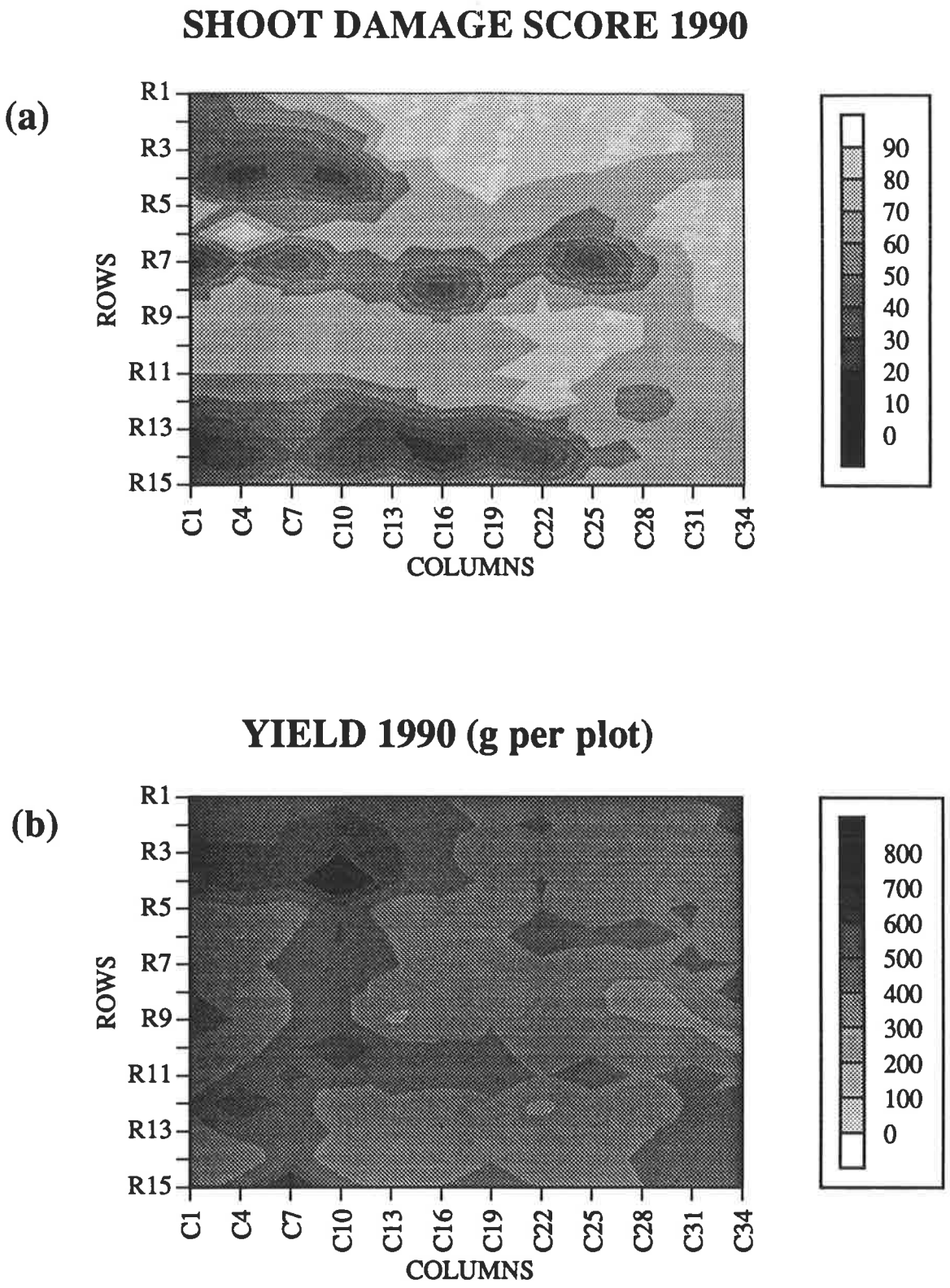


Figure 3.2. Contour plots describing shoot damage scores and yield in Experiment 1 in 1990, based on the raw data derived from the grid of Schooner check plots. It is suggested that these represent the underlying environmental variability within the experimental plot.

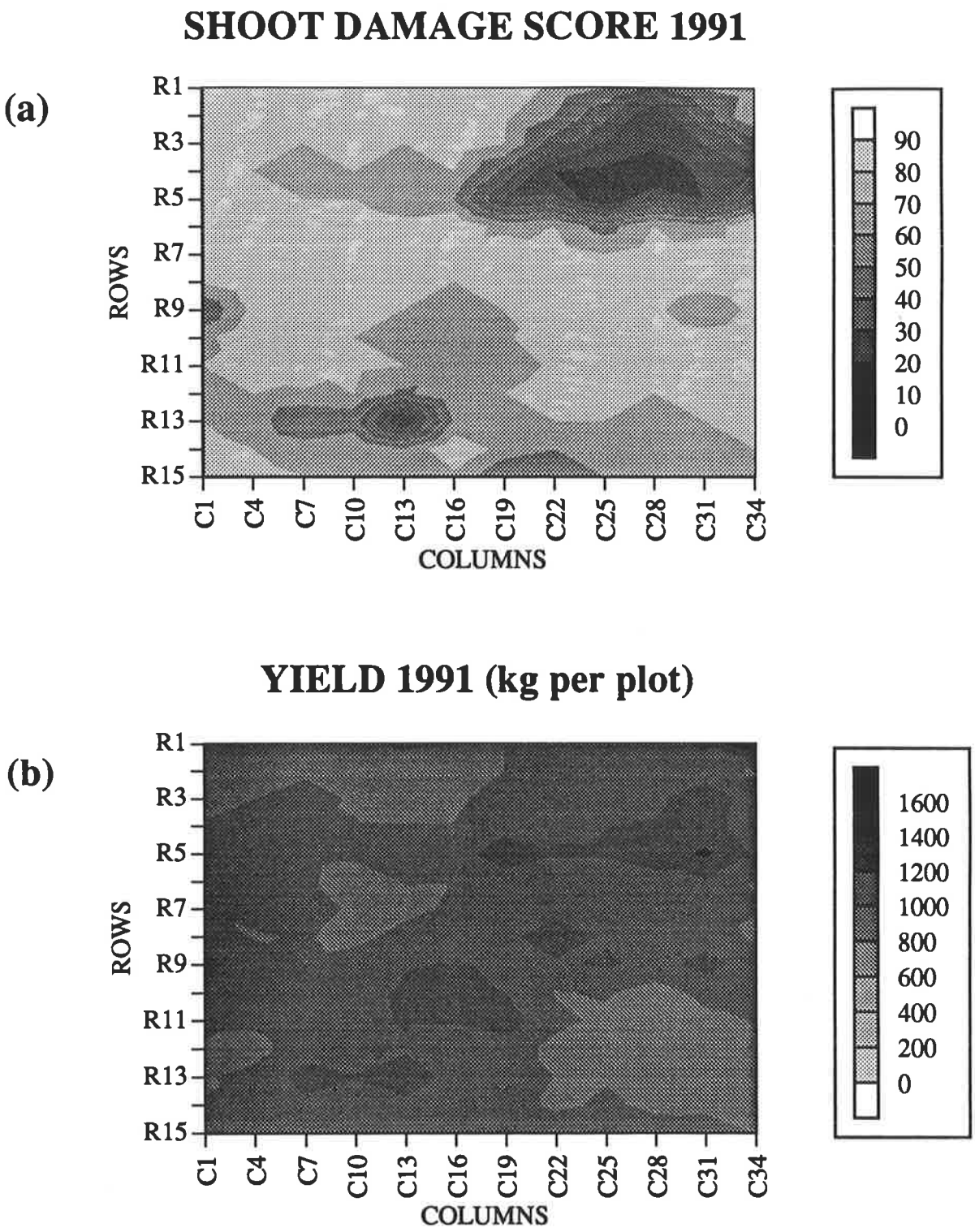


Figure 3.3. Contour plots describing shoot damage scores and yield in Experiment 1 in 1991, based on the raw data derived from the grid of Schooner check plots. It is suggested these reflect the underlying environmental variability.

Relationship Between Mean Shoot Damage Score and Mean Tillering and Herbage Production

Means were calculated from data from replicates of F_2 derived lines. Mean shoot damage scores were compared with mean tiller number per plant and mean dry weight of herbage per plant (Table 3.1). Owing to limited resources for sampling and analysis, not all replicates, nor each experiment was sampled every year. No significant correlation was observed between mean shoot damage score and either mean tiller number per plant nor mean dry weight per plant.

Table 3.1. Relationship between mean shoot damage score, tillers per plant and herbage dry weight for Experiment 1 in 1989 and 1991 and Experiment 2 in 1990.

Experiment and Year	n	Correlation Coefficients (r^2)	
		Mean Shoot Damage v Mean Tillers/Plant	Mean Shoot Damage v Mean Dry Wt/Plant
Experiment 1			
1989	88	0.006 n.s.	0.004 n.s.
1991	110	0.000 n.s.	0.018 n.s.
Experiment 2			
1990	62	0.011 n.s.	0.009 n.s.

Relationship Between Individual Shoot Damage Scores and Grain Yields

Shoot damage scores for each F_2 derived line from each replicate were plotted against grain yields (plots not shown). That is, each point represented an individual plot, rather than the mean of the replicates. This analysis was performed for each of the two experiments, in each year they were conducted.

For Experiment 1 in 1989 shoot damage score and grain yield were significantly correlated. The slope of the line indicates that for each increase in shoot damage score of one point, on average 6.5 g of grain yield per plot was lost. No significant correlation was seen between shoot damage score and grain yield in Experiment 1 in 1990. The range of grain yield in this year was reduced by two orders of magnitude and mean grain yield was less than 50% that of

the other years (Table 2.3). Under the conditions of 1991, increased shoot damage scores correlated with a small reduction in grain yield in Experiment 1.

Table 3.2. Relationship between individual shoot damage scores and grain yield for F_2-derived families from Experiment 1 in 1989, 1990 and 1991 and Experiment 2 in 1990 and 1991.

Experiment and Year	n	Correlation coefficient (r^2)	Slope	Range grain yield (g/plot)
Experiment 1				
1989	176	0.13**	-6.52	614
1990	330	0.01 n.s.	0.03	7
1991	330	0.02*	-0.87	300
Experiment 2				
1990	124	0.01 n.s.	-0.09	60
1991	186	0.10**	-4.92	250

No significant correlation was observed in Experiment 2 between shoot damage scores and grain yield in 1990. Mean grain yield was reduced by almost 75% compared to 1991 (Table 2.4) and the range was significantly reduced (Table 3.2). In 1991 a significant correlation was observed between shoot damage score and yield with a yield loss on average of 4.9 g per plot for each point increase in shoot damage score.

Relationship of Mean Shoot Damage Score , Mean Shoot Boron Concentration and Mean Grain Boron Concentration with Mean Grain Yield

Means were calculated from adjusted values from replicates of the same F_2-derived lines. Mean shoot damage scores, mean shoot boron concentrations and mean grain boron concentrations were correlated with mean grain yield (Table 3.3). Owing to limited resources for sampling and analysis, not all replicates, nor each experiment was sampled every year. Graphical representations of these results are presented in Figures 3.4, 3.5, 3.6, 3.7 and 3.8.

Table 3.3. Relationship between mean shoot damage score, mean shoot boron concentration and mean grain boron concentration with mean grain yield. #This analysis used raw data, not adjusted data, since shoot B concentrations were not available for Sahara 3771 control plots in 1989.

Experiment and Year	n	Mean shoot damage score vs mean grain yield (g/plot)		Mean shoot [B] (mg/kg) vs mean grain yield (g/plot)		Mean grain [B] (mg/kg) vs mean grain yield (g/plot)	
		Correlation coefficient (r^2)	Slope	Correlation coefficient (r^2)	Slope	Correlation coefficient (r^2)	Slope
Experiment 1							
1989	62	0.20**	-7.67	0.09**#	-5.21	*	*
1990	110	0.00 n.s.	0.01	*	*	*	*
1991	110	0.01 n.s.	-0.73	0.00 n.s.	-0.66	0.00 n.s.	3.91
Experiment 2							
1990	62	0.04 n.s.	-0.15	*	*	*	*
1991	62	0.21**	-7.12	*	*	*	*

Experiment 1 in 1989 showed a significant correlation between mean shoot damage score and mean grain yield (Figure 3.4a). The slope indicates that for each increase of one shoot damage score, on average a loss of 7.7 g of grain per plot occurs. A significant correlation was also observed between mean shoot B concentration and mean grain yield (Figure 3.4 b). Each increase of 1 mg/kg of B in shoot tissue corresponds on average with a loss of 5.2 g of grain per plot. The two measures of tolerance to B, mean shoot damage score and mean shoot B concentration (neither MATLAB adjusted) were significantly correlated ($r^2 = 0.11^{**}$, n = 62) (graph not presented).

In 1990 the correlation between mean shoot damage score and mean grain yield was not significant (Figure 3.5).

EXPERIMENT 1 1989

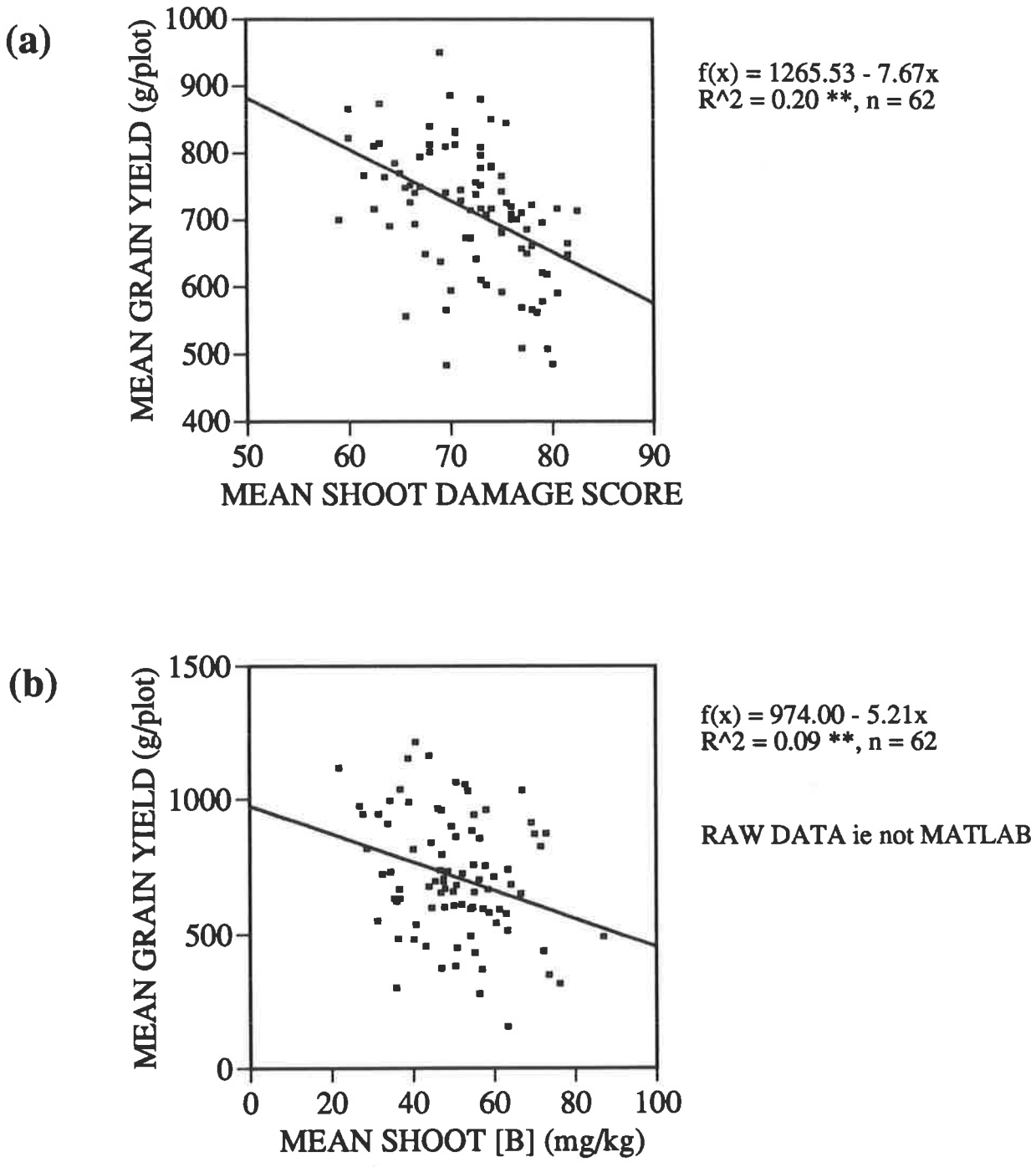


Figure 3.4. Relationships between (a) mean shoot damage scores and (b) mean shoot boron concentration (mg/kg) with mean grain yields (g/plot) for F_2-derived families from Experiment 1 in 1989. Plot (a) was produced using adjusted data. Plot (b) was produced from unadjusted shoot [B] and unadjusted grain yield data.

EXPERIMENT 1 1990

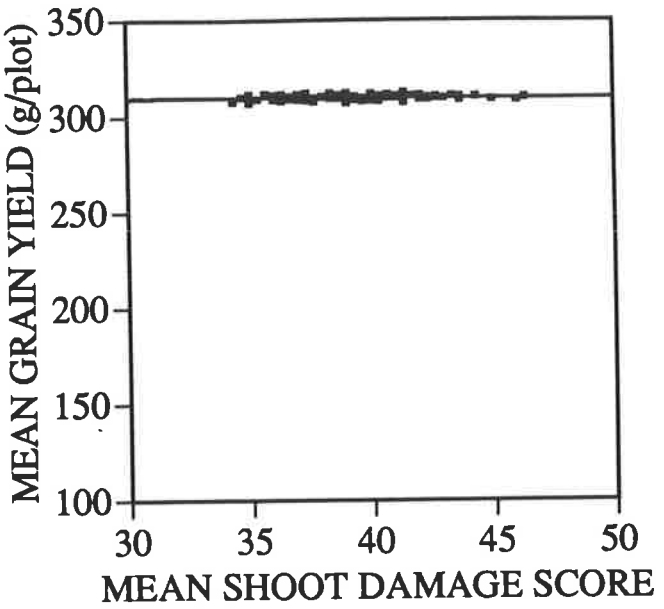


Figure 3.5. Relationships between mean adjusted shoot damage scores with mean adjusted grain yields (g/plot) for F_2-derived families from Experiment 1 in 1990.

EXPERIMENT 1 1991

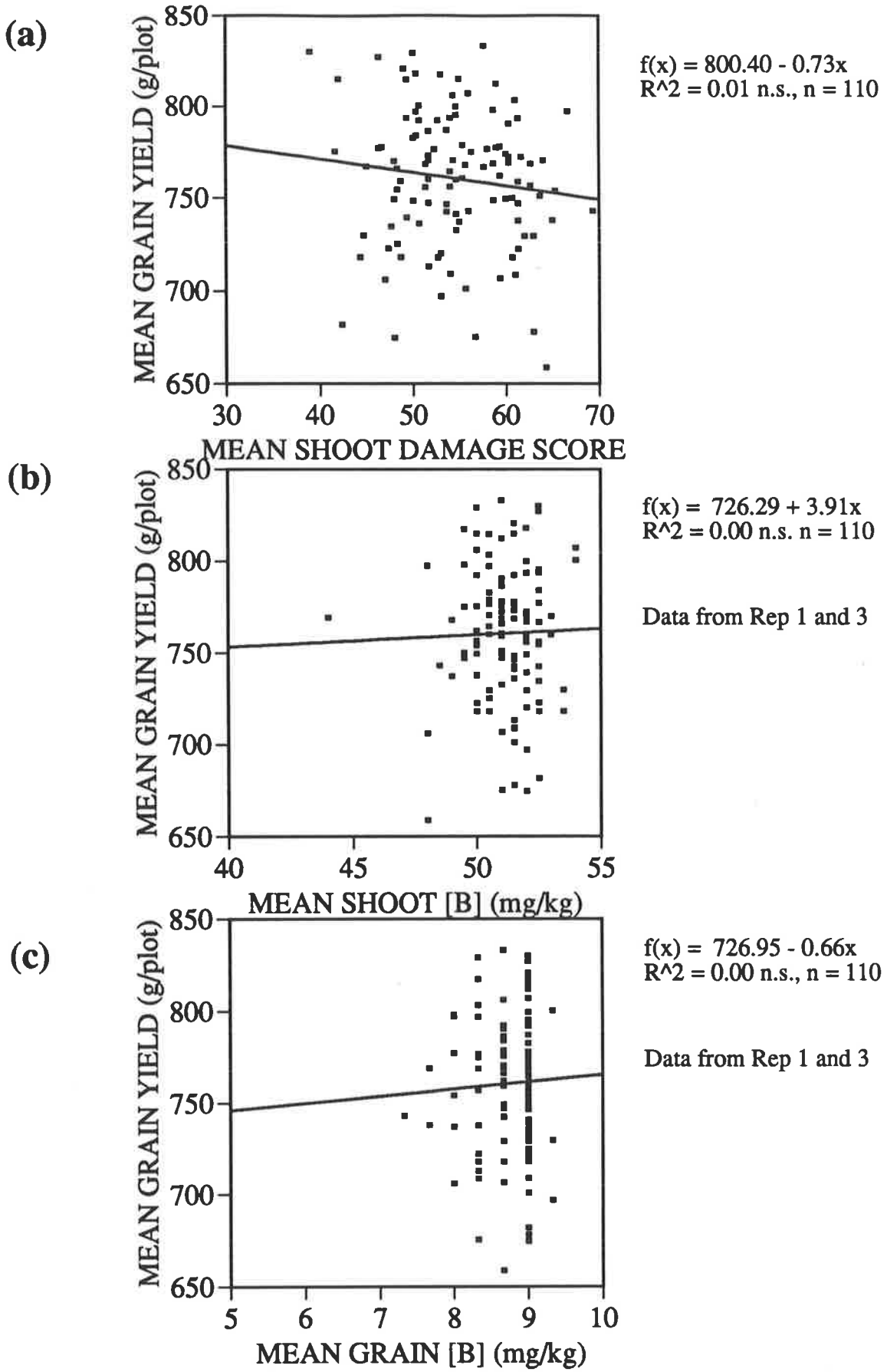


Figure 3.6. Realtionship between (a) mean adjusted shoot damage scores, (b) mean adjusted shoot boron concentrations (mg/kg), and (c) mean adjusted grain boron concentrations (mg/kg)with mean adjusted grain yields (g/plot) for F_2-derived families from Experiment 1 in 1991.

EXPERIMENT 2 1990

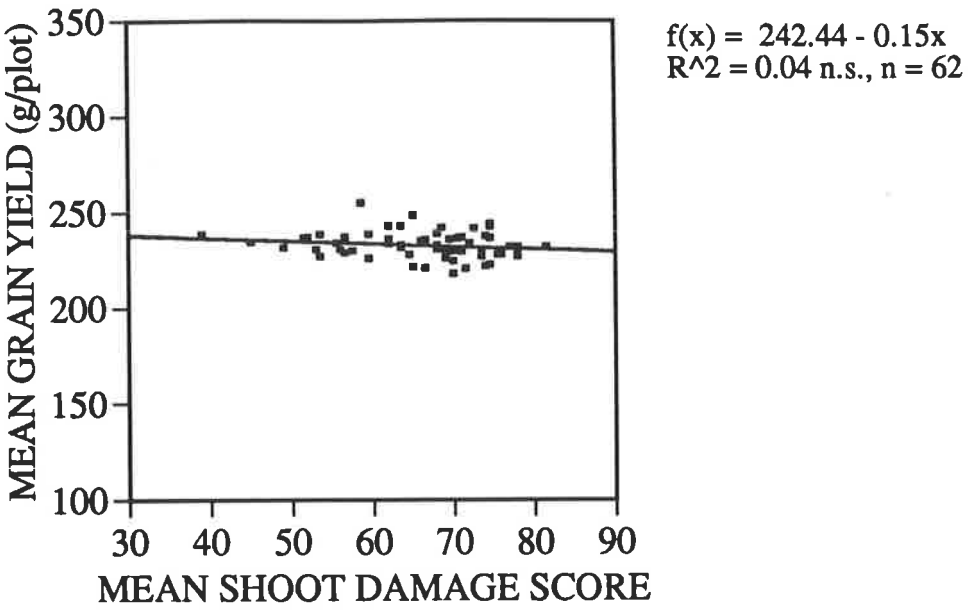


Figure 3.7. Relationship between mean adjusted shoot damage scores and mean adjusted grain yields (g/plot) of F_2-derived lines for Experiment 2 in 1990.

EXPERIMENT 2 1991

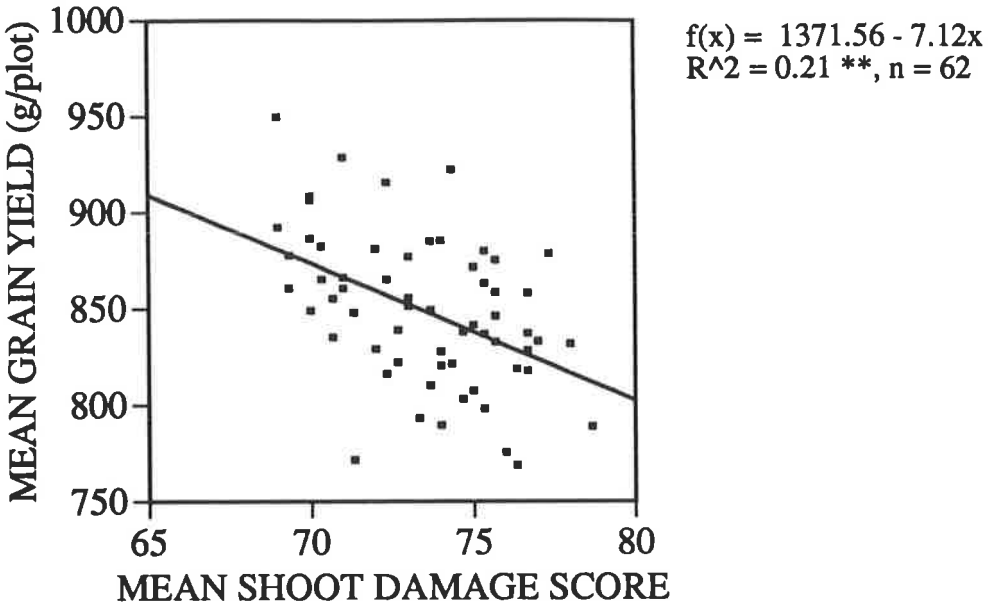


Figure 3.8. Relationship between mean adjusted shoot damage scores and mean adjusted grain yields (g/plot) for F_2-derived families from Experiment 2 in 1991.

In 1991 Experiment 1, mean shoot damage scores (Figure 3.6a), mean shoot B concentration (Figure 3.6b) and mean grain B concentration (Figure 3.6c) were all plotted against mean grain yield. None was significant. These three independent variables were however highly correlated with each other: shoot B concentration vs grain B concentration ($r^2 = 0.44^{**}$, n = 110), shoot damage score vs shoot B concentration ($r^2 = 0.18^{**}$, n = 110) and shoot damage score vs grain B concentration ($r^2 = 0.20^{**}$, n = 110) (graphs not presented).

In Experiment 2, the correlation between mean shoot damage score and mean grain yield was not significant in 1990 (Figure 3.7).

In 1991, however, the correlation between mean shoot damage score and mean grain yield was highly significant (Figure 3.8). The slope indicated that on average for each increase of ten points in mean shoot damage score a reduction of 71 g of grain yield per plot is observed. That is, from mean shoot damage score 70 to 80 a yield loss of more than 8% is experienced.

Prediction of Yield Using Shoot Damage Score and Line

Predictive models were devised statistically to investigate to what extent line number (F_2 derived family) and shoot damage score predicted yield. A model was created for each experiment for each year based on an Analysis of Covariance. The programme devised models to explain the response, Yield, using the two sources of variation, Line (a nominal variable) and Shoot Damage Score (an interval variable). In no case was the Replicate term significant, so it was not included as a separate term in the analyses and therefore was included in the error term. The model takes the following form:

$$y_i = b_0 + b_1x_{1i} + b_2x_{2i} + e_i$$

where: y_i is the mean Yield for the i th line
b_0 is the intercept
b_1x_{1i} is the effect of Line mean
b_2x_{2i} is the effect of mean Shoot Damage Score and
e_i is the error term.

This model was compared in its accuracy of prediction with that of the sample mean. In a

Whole-Model plot, observed values were plotted against predicted values. An Analysis of Variance table was produced where the Prob>F value indicated the probability of obtaining a greater F value by chance alone if the specified model fitted no better than the overall response mean.

Individual leverage plots were drawn for the regressors, Line and Shoot Damage Score. Each plot illustrated the residuals as they were and as they would have been if the regressor was removed from the model. The null hypothesis is that either the effect of Line mean is zero or the effect of mean Shoot Damage Score is zero. The 5% level of significance was used for acceptance or rejection of the null hypothesis (Tables 3.4). Significance could also be judged by applying 95% confidence intervals to the plots. If the confidence curves crossed the horizontal line of the mean, the effect for that factor was significant.

An r^2 value was produced for the Whole-Model for each experiment for each year (Table 3.4). The r^2 value estimates the proportion of the variation in the response around the mean that could be attributed to terms in the model rather than random error. It is also the correlation between the actual and predicted response.

Table 3.4. Description of the influence of line and shoot damage score on yield in Experiments 1 and 2, and the r^2 of the predictive model. Degrees of freedom are indicated in brackets. * signifies significance at the 10% significance level, ** signifies significance at the 5% level.

Expt and Year	Source of Variation (Prob. >F)		r^2 (Whole Model)
	Line	Damage Score	
Experiment 1			
1989	0.00** (87)	0.43 n.s. (1)	0.71**
1990	0.00** (109)	0.03** (1)	0.57**
1991	0.00** (109)	0.08* (1)	0.72**
Experiment 2			
1990	0.04** (61)	0.03** (1)	0.61**
1991	0.00** (61)	0.00** (1)	0.64**

CONCLUSIONS

Variation Within Experimental Areas for Shoot Damage Score and Grain Yield

The response surfaces for Experiment 1 demonstrate the large variation in soil B over the trial sites (Figures 3.2 and 3.3). They were constructed using the raw data derived from the grid of Schooner check plots for 1990 and 1991. Shoot damage scores are expected to reflect soil B to a large degree, because the plant material is genetically uniform and most other environmental factors, perhaps with the exception of other soil parameters, are expected to be relatively uniform when the trial area is small. Yield to some extent reflects response to B, but it is clear that other factors are limiting yield. It was because of this within experimental area, environmental variation that the data adjustments using MATLAB were applied.

Relationship Between Mean Shoot Damage Score , and Mean Tillering and Herbage Production

Increased shoot damage score did not correlate with reduced shoot dry weight or tiller number per plant in either experiment. In contrast Riley (1987) found that the main effect of B toxicity on barley appeared to be on vegetative growth. He conducted his experiments in a glasshouse using the barley cultivar Stirling and shoot dry weights were measured at booting stage. Within one intolerant cultivar exposure to increasing levels of B dry matter production was reduced. Paull *et al.* (1988) also conducted experiments in the glasshouse but used a number of wheat and barley lines. They found dry matter production reduced with increasing soil B application, but found in some genotypes, that grain yield and dry matter production began to be affected at different levels of soil B. In contrast, a series of F_2 derived genetic lines was used in these experiments. These lines are segregating not only for levels of tolerance to B toxicity but also for other genetic factors which may be influencing shoot dry weight and number of tillers per plant. Examples of genetic factors other than tolerance to B toxicity which may influence these characters include plant height, rooting habit, early vigour and days to flowering. In a field situation sampling methods introduce errors into estimates of tillers per plant and shoot dry weight. Plants were sampled at random, but the small sample size (five plants per plot) probably introduced a considerable degree of random error. An alternative to increasing sample size may be to conduct comparative experiments under controlled conditions in a glassshouse. Many agronomic characters however are not accurately predicted under

artificial conditions, particularly those influenced strongly by rooting pattern.

The genetic variability within F_2 lines may be significant when compared with variability between lines, further reducing accuracy in assessing the performance of lines. If lines were selected at a more advanced generation, genetic variability within lines would be reduced, and variability between lines increased. A double haploid population would represent the extreme of this situation, where there is large genetic variability between lines and no genetic variability within lines. If such a population was available for the kind of investigation presented here, interpretation of results would be simplified considerably. Alternately, isogenic lines, varying for tolerance to B but in an identical genetic background, would be useful in assessing the effect of B tolerance genes on yield of barley under high soil B conditions. Moody *et al.* (1990) reported that BC_3F_4 wheat lines, derived from parents which differ by one major B tolerance gene, had a 7% yield advantage over the intolerant parent when grown over twelve South Australian sites used by the Department of Agriculture for selection (ie not all sites were necessarily high B).

Relationship Between Individual Shoot Damage Scores and Grain Yields

In 1989 Experiment 1 grain yield reduction with increase in shoot damage score is clear. This relationship however was not observed in either experiment in 1990. In this year very little rainfall fell during the growing season (Table 2.1). Thus, it is likely that drought stress was an overriding factor in determining yield, and tolerance to boron did not significantly contribute to determining grain yield under those conditions.

In 1991, the correlation between shoot damage score and yield for Experiment 1 was significant, but the effect of damage score on yield was small. Experiment 2 on the other hand shows a high degree of correlation and a slope indicative of considerable grain yield loss correlated with increase in shoot damage score. An average reduction in yield of 16% between shoot damage scores of 60 and 90 is indicated.

A clearer picture of the effect of B tolerance on yield may be obtained by using line means instead of individual plot values. Using line means eliminates any block effects remaining after

the application of the MATLAB programme for removing trends in the data.

Relationship Between Mean Shoot Damage Score , Mean Shoot Boron Concentration and Mean Grain Boron Concentration , and Mean Grain Yield Using Line Means

In Experiment 1 1989 the correlation between shoot damage score and grain yield was improved by comparing the mean of the lines for each measurement (Figure 3.4a). On average there was a reduction in yield of 19% of grain per plot when shoot damage score increased from 60 to 80. There are lines which yielded poorly despite a relatively low mean shoot damage score (lines which appear below the fitted line), as well as lines which produced yields greater than would be expected for a relatively high mean shoot damage score (lines appearing above the fitted line).

Two explanations come to mind for lines producing a greater yield than predicted by damage score. Firstly, though it is thought that tolerance to B is controlled largely by an exclusion mechanism (Nable, 1988), there may be some degree of internal tolerance being expressed. That is, a plant may express a high degree of shoot damage, but still produce an acceptable yield. The most likely method for achieving this is by physiological compensation. Riley (1987) suggested that the barley cultivar Stirling may compensate for loss of photosynthetically active area by increasing the height of the primary tiller and area of the flag leaf. If yield was suppressed by B through other factors than reduction in photosynthetic area some lines, though sustaining shoot damage, may later rid themselves of excessive B by guttation (Oertli, 1962) or by increasing the percentage of B kept in the most soluble form, thus allowing leaching by any precipitation which may fall (Nable *et al.*, 1990b ; Nable and Moody, 1992). If this proved to be true, from a breeding point of view, it would be useful to combine these two kinds of tolerance, or to consider breeding for one form or the other for special purposes. For example if grain boron levels were considered important, exclusion mechanisms would lead to low B grain, whereas presumably internal tolerance mechanisms would lead to higher grain levels.

A second explanation could be that some lines due to factors other than tolerance to high levels of B will possess a greater yield potential than others. In each of the experiments the primary parents differed in many agronomic characters. It would be expected then that apart from

tolerance to B, many other genetic factors will be segregating between (and within) F_2 derived lines. One such factor seen to influence symptom development was days to flowering (refer to Chapter 2 discussion).

Mean shoot B concentration also correlated significantly with mean grain yield (Figure 3.3b). On average an increase in mean shoot B concentration from 20 to 80 mg/kg, led to a 37% reduction in mean grain yield. Under these conditions then it may be concluded that damage score was the better predictor of grain yield, since the r^2 value for mean shoot damage score v mean grain yield was 0.2 and for mean shoot [B] v mean grain yield was 0.09. Though the two measures of tolerance to B were highly correlated the correlation was not perfect. This suggests that at a given shoot B concentration some lines showed fewer symptoms than others; thus in some cases a mechanism other than exclusion of B may be playing a role in tolerance. This result could be achieved if plants stored B at a site within the plant where it cannot cause damage, for example in vacuoles or cell walls, or by an ability to chemically detoxify the B. Regardless of the mechanisms involved, it may be worthwhile from a breeding point of view to investigate both kinds of elite lines, that is those with a high yield and low shoot damage score and those with a relatively high yield at a high or moderate shoot damage score. If there proves to be an underlying independent genetic basis for these different responses, then intercrossing such lines could lead to lines more tolerant to high levels of B, that is maintaining high grain yield despite high levels of B in the soil, which combine more than one tolerance mechanism.

In 1990 neither Experiment 1 (Figure 3.5) not Experiment 2 (Figure 3.7) expressed significant correlation between mean shoot damage score and mean grain yield. In this year drought conditions were experienced during the latter part of the growing season (Table 2.1). Under such conditions water stress would be by far the overriding limiting factor in determining grain yield, with tolerance to B toxicity exerting little influence on final grain weight. The effect of drought is seen both on the low grain yields and narrow range of yield.

In 1991 Experiment 1 the relationships of mean shoot damage score, mean shoot B concentration and mean grain B concentration were investigated with mean grain yield per plot (Figure 3.6). None of the correlations was significant. The three independent variables are,

however, significantly correlated with each other, particularly mean shoot and grain B concentrations ($r^2 = 0.44^{**}$, n = 110). It is likely that in this case the lack of significant correlation with yield is a result of genetic and interaction effects rather than environment alone. The parents of these F_2 derived lines were Sahara 3771 and WI 2723. Since these parents are adapted to very different environments and differ significantly at many loci, as demonstrated by differences in many morphological characters, segregating genetic factors other than tolerance to B toxicity are more strongly influencing mean grain yield. It was therefore useful to consider a model for prediction of grain yield taking into account both shoot damage scores and the effect of line.

In Experiment 2 in 1991 mean shoot damage score was significantly correlated with mean grain yield (Figure 3.8). On average about 8% grain yield loss was observed when mean shoot damage score increased from 70 to 80. As from experiment 1 in 1989, elite lines are present, both those which yield well at low shoot damage scores and those which produce a grain yield greater than the mean for a high shoot damage score. Since the original parents of this cross, CM 72 and Stirling are agronomically better adapted to growing conditions experienced at this location than Sahara 3771, line, as a variable, though a significant factor, is not expected to play an overriding role in predicting yield.

Prediction of Yield Using Mean Shoot Damage Score and Line

The accuracy with which yield is predicted is considerably improved when the effect of both line and shoot damage score are taken into account (Table 3.4). The aim of including the effect of line was to take into account the genetic variability which influences yield but is not involved in determining tolerance to B toxicity. Significant r^2s were obtained for each experiment in each year. For each experiment in each year line contributed significantly as a source of variation to the model for predicting grain yield. Only in the case of Experiment 1, 1989 did damage score not contribute significantly to the predictive model. This suggests that in this year it may not have been shoot damage score, or the tolerance to B toxicity it reflects, which affected grain yield, but some other line effect, probably associated with shoot damage score. This association may be a morphological one, for example low shoot damage scores may correlate with an early flowering date, a character which may be advantageous in a short

growing season. Alternately, there may be genetic linkages between B tolerance (reflected in shoot damage score) and a character which was advantageous under the environmental conditions experienced in this year.

In general in Experiment 1, shoot damage scores and line effects together explain between 57 and 72% of the observed variation in grain yield. The 57% estimate was obtained in 1990 when drought conditions existed during the growing season, and water stress would be expected to be the main limiting factor in determining yield. Under conditions when tolerance to B toxicity is the primary limiting factor 71% of the variation in yield can be predicted by line and shoot damage scores. In Experiment 2, shoot damage scores and line effects predicted between 61 and 64% of the observed variation in yield.

Thus, it can be concluded that under some environmental conditions at least, tolerance to boron toxicity, whether it be measured in terms of shoot damage score or shoot B concentration, can have a significant influence on determining grain yield in barley. The data also suggest that there may be some lines able to obtain a high grain yield despite either a high shoot damage score or shoot B concentration. Line effects and shoot damage score together may explain the majority of variation in yield under high boron conditions in some years.

Chapter 4

DETERMINATION OF GENETIC CONTROL

INTRODUCTION

Boron (B) toxicity occurs when susceptible plants are exposed to excess soil B. Leaching of excess B from soils is impractical on a broad scale, so the only solution to the problem is to select or breed crop cultivars which can tolerate high levels of soil B. An understanding of the genetics, physiology and biochemistry of tolerance is needed to help introduce tolerance into locally adapted cultivars.

Two sources of B tolerance in barley have been identified. The Sahara lines express a high level of tolerance and CM 72 a moderate level of tolerance relative to Stirling, an intolerant Australian cultuvar (Boyd *et al.*, 1988). Genetic studies have been undertaken to characterise the inheritance of these two tolerances.

Occurrence of B toxicity is patchy, even within one paddock (Figures 3.2 and 3.3), so to determine the genetic mechanism of tolerance to B, environmental variation must be minimised. Experiments were established to determine the mode of inheritance of boron tolerance, including dominance relationships, gene interactions, whether transgressive segregation occurs, and the number of major genes involved in determining response to high levels of soil B. These were conducted under relatively controlled conditions using a hydroponic system in a glasshouse.

MATERIALS AND METHODS

Genetic Material

The genetic control of tolerance to B was studied utilizing three barley (*Hordeum vulgare* L.) genotypes known to differ in response to high levels of B (Table 4.1). Sahara 3771 is a land race line. The exact location of the original collection is unknown, but is believed to be the Saharan edge of the Algerian steppe. It was probably grown under a subsistence situation where reliability of yield is more important than high yield. CM 72 is a composite of two hundred and forty F_6 generation lines selected from the cross 'California Mariout'*4/CI 1179//2*California Mariout*?'Club Mariout'/3/'CM 67' (Schaller, *et. al.*, 1977). It is a six rowed cultivar grown for animal feed. Stirling is a modern, Australian cultivar, selected predominantly for its short growing season and high malting quality. It is adapted to the Western Australian cereal belt and is intolerant to high levels of soil boron.

Original seed of CM 72 and Stirling were kindly provided by Dr R. Boyd of the University of Western Australia. Sahara 3771/1 was derived from a selection from the Waite Agricultural Research Institute barley collection.

Table 4.1. Response to high concentrations of B for the three parent genotypes used in genetic studies (Boyd *et. al.*, 1988).

Genotype	Origin	Response
Sahara 3771	Land race, probably N. African or Algerian origin.	Highly tolerant
CM 72	Californian cultivar with Egyptian ancestry	Tolerant
Stirling	Cultivar from Western Australia	Intolerant

Screening

The three genotypes were crossed in all combinations, including reciprocals, to produce F_1 hybrid seed, both for testing for response to B and for the production of F_2 seed. F_2 seed was

obtained from F_1 hybrids grown in potting mixture without B applied. F_3 seed was obtained by selfing F_2 plants which had been screened in high-B hydroponic culture and then transplanted to potting mix. The seed derived from each F_2 plant was harvested separately, and hence became F_2-derived F_3 families.

Boron tolerance was tested in a hydroponic system under glasshouse conditions. Seed was imbibed in petri dishes on moist filter paper at 4°C for 2 days then transferred to 20°C for two to three days before planting. Germinated seeds were planted in a medium of coarse (<0.5 cm diameter) washed river sand. The hydroponics system consisted of eight nursery punnets with 4 x 2 cells fitted into each punnet tray. Two such trays fitted in plastic confectionery trays measuring 70 x 45 x 8 cm. Each confectionery tray was connected to a 40 or 60 litre reservoir with a submersible pump. Pumps operated for 30 minutes every 6 hours, when nutrient solution was pumped to a depth of 4 cm then allowed to drain back into the reservoir (Figure 4.1 and 4.2).

Nutrient solution initial concentration was: 1/4 strength "Top Hydroponic Solution™" (which includes trace levels of B) plus 142 mgl^{-1} KNO_3 and 0.05 mll^{-1} 100 mM stock Fe as HEEDTA, pH adjusted to 6.5 with NaOH (Table 4.2). Boron as boric acid was added to the nutrient solution 2 days after planting. Preliminary tests showed that the pH of the nutrient solution did not usually vary outside an acceptable range of between 6 and 8 in the 3 week experimental period, so pH was not adjusted further. Water lost through transpiration and evaporation was replaced periodically with reverse osmosis (R.O.) water.

F_1 and F_2

F_1s were tested at 0, 20 and 40 mgl^{-1} added B (B0, B20 and B40). F_2s and F_3s were screened at 20 mg l^{-1}, since differences in leaf damage scores were maximised at this level. Numbers of individuals tested per cross, and number of parental controls tested is shown in Table 4.3. F_1 seedlings parents were arranged in a randomised block design for testing at each of the three B levels.

Figure 4.1. Hydroponic system for screening barley seedlings for tolerance to B toxicity.

Figure 4.2. Symptoms expressed by seedlings subjected to 20 mg l^{-1} B for two weeks.

Table 4.2.
Composition of nutrient solution used in hydroponic screening.

ELEMENT	microM
N as potassium nitrate	1320.8
N as calcium nitrate	2213.2
total nitrate	3533.9
N added as ammonium	303.4
N total	3837.4
P added as water soluble	290.6
P total	290.6
Ca added as calcium nitrate	1047.9
K added as potassium nitrate	1406.6
Mg added as magnesium sulphate	411.3
Zn added as zinc sulphate	0.2
Cu added as copper sulphate	0.3
Mn added as manganese sulphate	3.2
Fe added as iron sulphate	17.9
Mo added as sodium molybdate	0.0
B added as sodium borate	4.6
S added as sulphates	421.0
PLUS	
KNO3	1404.5
Fe as HEEDTA	5.0
pH to 6.5	
Additional B as boric acid (20mg/l)	1849.97

F_2-derived F_3s

From each F_2 individual 12 progeny were tested together with 2 seedlings of each parent (Table 4.3). Seedlings were scored three weeks after adding the B, and rated from zero to twelve based on area of leaf damage on a scale of zero to four for each of the three oldest leaves. The F_2-derived F_3 families from the cross CM 72 x Sahara 3771 were an exception, and were scored after six weeks with the B treatment increased to 40 mgl^{-1} in the final week. Other variables measured were shoot dry weight and shoot B concentration in the case of the F_1s and plant height and tiller number for all generations. The concentration of B in whole shoots was determined by inductively coupled plasma spectrometry (ICP), following digestion in nitric acid according to the method of Zarcinas *et. al.* (1987).

Table 4.3. Boron concentration, number of seedlings tested and number of parental controls used for screening barley populations for genetic studies.

Gen'n	Cross	Date	[B] Tested	N	N each parent
F1	Sahara 3771 x Stirling	Aug	B0, B20, B40	12	10
	CM 72 x Stirling	Oct	B0, B20, B40	12	10
	CM 72 x Sahara 3771	Oct	B0, B20, B40	12	10
recip.	Stirling x Sahara 3771	Aug	B0, B20, B40	12	10
	Stirling x CM 72	Oct	B0, B20, B40	12	10
	Sahara 3771 x CM 72	Oct	B0, B20, B40	12	10
F2	Sahara 3771 x Stirling	Sept	B20	179	38
	CM 72 x Stirling	Nov	B20	184	36
	CM 72 x Sahara 3771	Dec	B20	184	32 (+8 Stirl)
F3	Sahara 3771 x Stirling	Sep-Feb	B20	175 families (12 plants each)	350
	CM 72 x Stirling	Feb-May	B20	183 families (12 plants each)	366
	CM 72 x Sahara 3771	Aug-Oct	B20-B40	44 families (12 plants each)	88

Statistical analysis

Graphs describing the relationship between B treatment and means of measured variables for parental lines and their F_1 progeny were constructed using the DeltaGraph™ computer software package. Curves were fitted using a spline function and error bars represent standard errors. Significant differences between variables based on F values were calculated using the SAS Institute JMP™ statistical computer software programme. Two way analyses of variance for genotype, B treatment and genotype by treatment interaction were performed. F_1 reciprocals were compared for each cross using a Student's t-test. Frequency distributions were constructed using DeltaGraph™. Chi-square values were calculated comparing F_3 family types with various expected ratios predicted by genetic models. If only one degree of freedom was present, Yates' correction (Yates, 1935) was applied. Leaf damage scores of F_2 plants were plotted against mean leaf damage scores within its F_3 progeny family and a straight line fitted using DeltaGraph™.

RESULTS

Treatment vs F_1 and Parental Means

The relationships between B treatment and five measured variables in parental lines and their F_1 progeny are described in Figures 4.3, 4.4 and 4.5. Standard errors, shown on the graphs as error bars, are small, since these F_1 plants are genetically uniform, and variation is a reflection of environmental variation upon the expression of any particular character. Significant differences based on two way analyses of variance F values and t values are set out in Table 4.4.

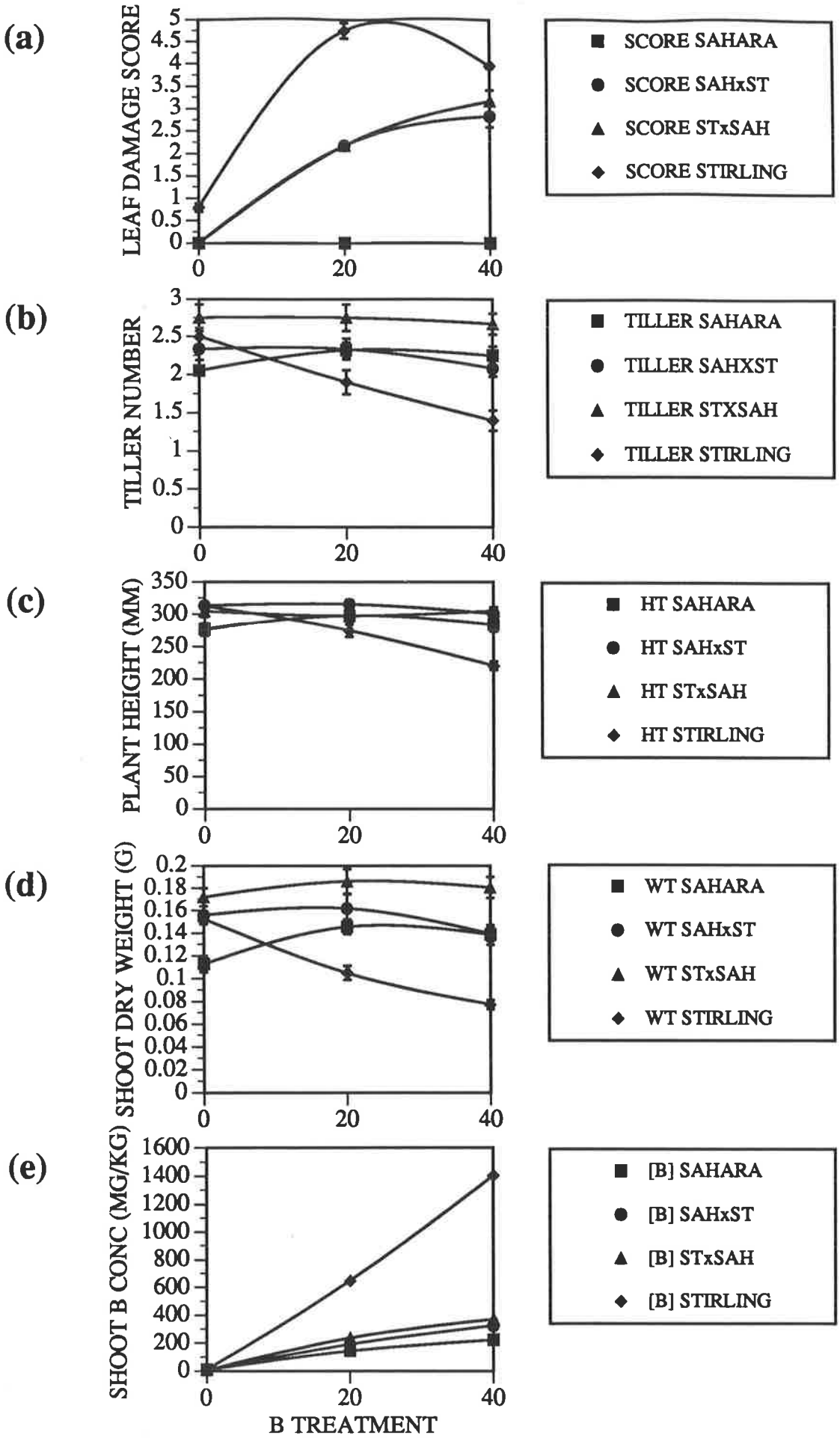


Figure 4.3. Boron treatments vs leaf damage score, tiller number, plant height (mm), shoot dry weight (g) and shoot B concentration (mg/kg) for Sahara 3771, Stirling and the F1 progeny.

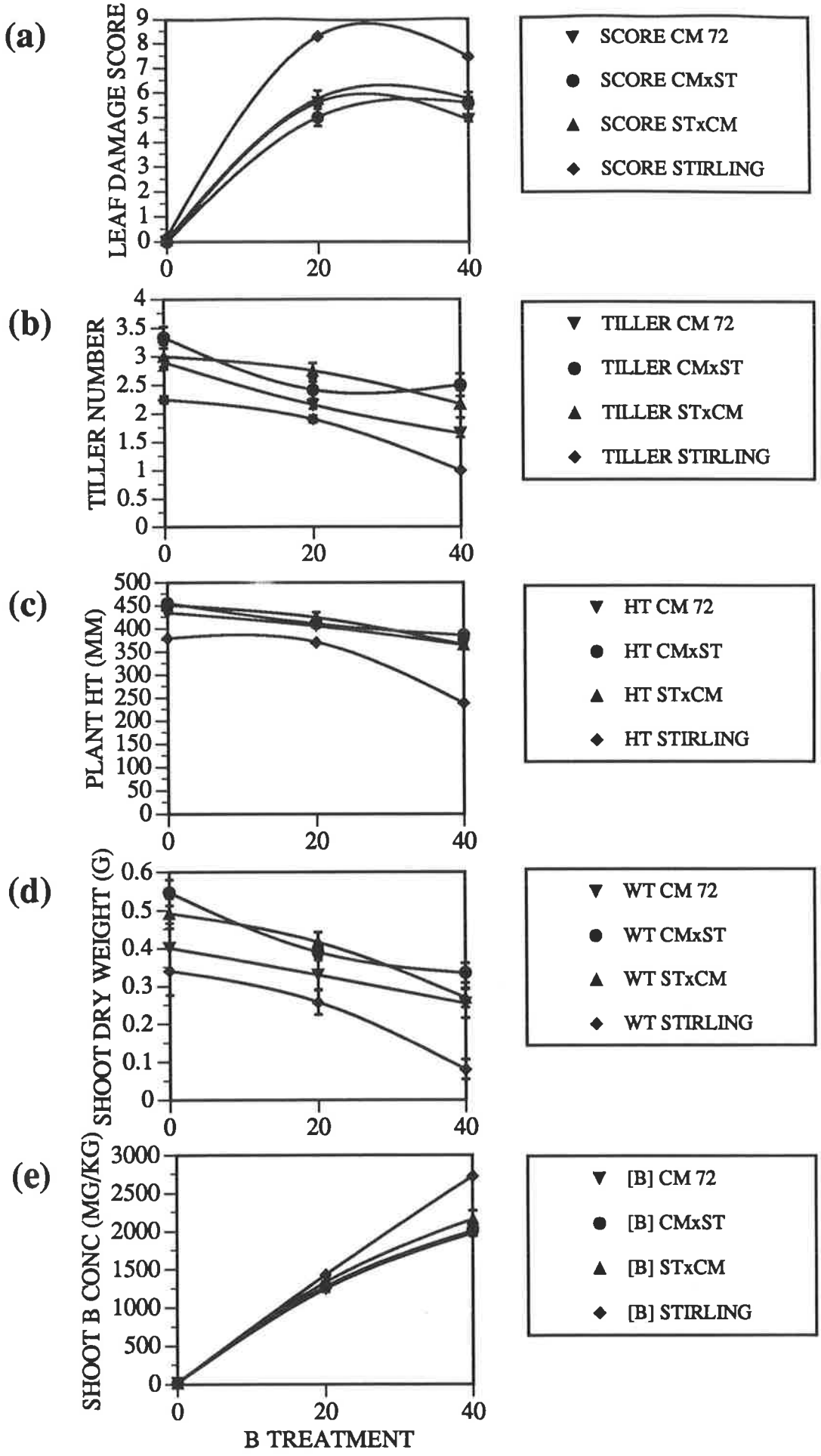


Figure 4.2. Boron treatments vs leaf damage score, tiller number, plant height (mm), shoot dry weight (g) and shoot B concentration (mg/kg) for CM 72, Stirling and the F1 progeny.

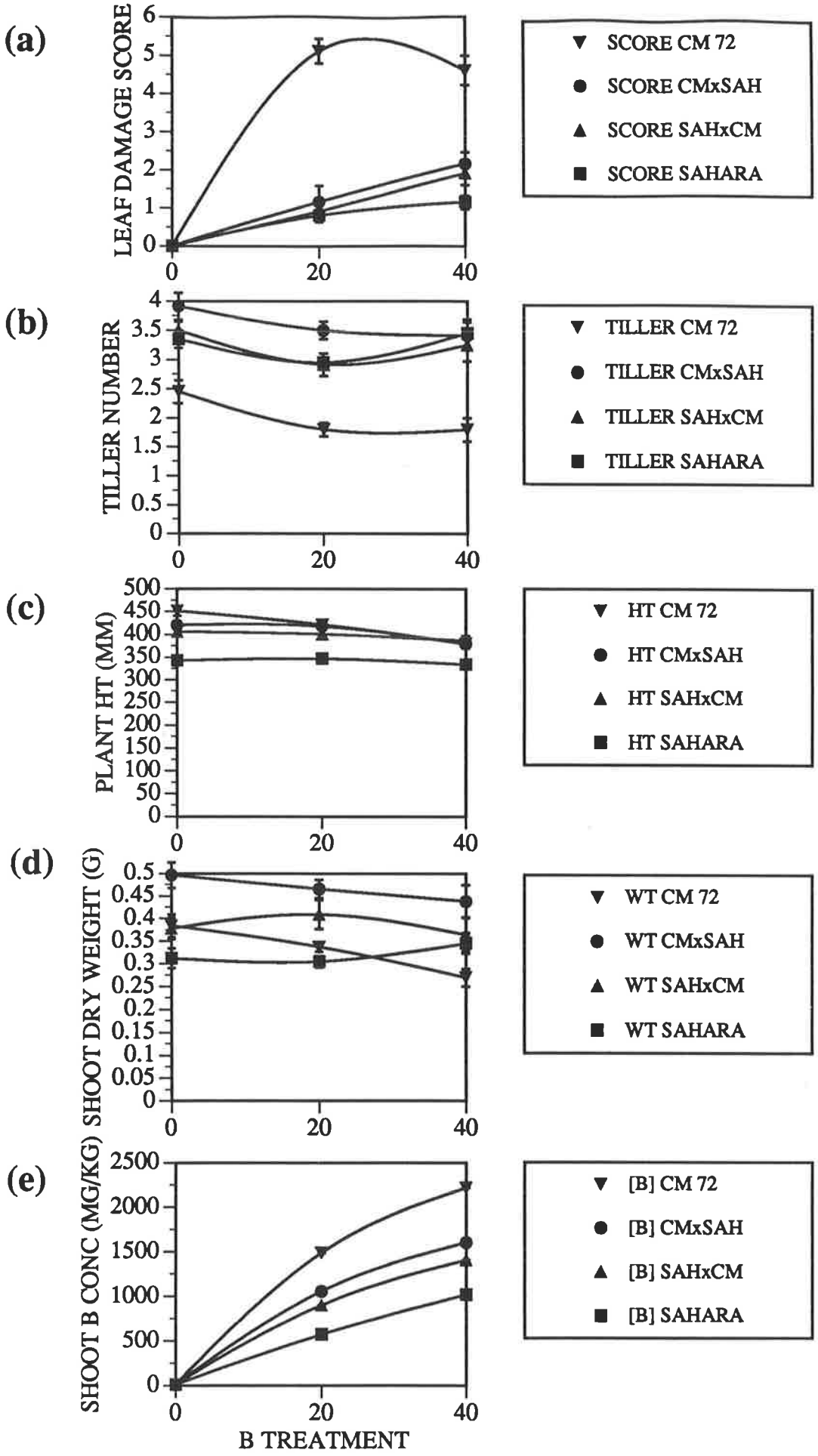


Figure 4.5. Boron treatments vs leaf damage score, tiller number, plant height (mm), shoot dry weight (g) and shoot B concentration (mg/kg) for CM 72, Stirling and the F1 progeny.

Table 4.4. Significance found for sources of variation for the measured parameters, based on analysis of variance and F statistics. Significant differences between the reciprocal F_1s in each case are based on the t statistic. Significance levels are represented: n.s., >.05 not significant; *, significant at the 0.05 level; ** significant at the 0.01 level; and *** significant at the 0.001 level.

Sahara 3771 x Stirling

Source of variation	Significant differences between parameter					
	Score	Tiller	Ht.	Wt.	[B]	[B]log
Genotype (F)	***	***	***	***	***	***
Reciprocal F_1s (t)	n.s.	***	n.s.	***	n.s.	n.s.
Boron Treatment (F)	***	**	***	**	***	***
Geno * Treat Int. (F)	***	***	***	***	***	***

CM 72 x Stirling

Source of variation	Significant differences between parameter					
	Score	Tiller	Ht.	Wt.	[B]	[B]log
Genotype (F)	***	***	***	***	***	***
Reciprocal F_1s (t)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Boron Treatment (F)	***	***	***	***	***	***
Geno * Treat Int. (F)	***	n.s.	***	***	*	**

Sahara 3771 x CM 72

Source of variation	Significant differences between parameter					
	Score	Tiller	Ht.	Wt.	[B]	[B]log
Genotype (F)	***	***	***	***	***	***
Reciprocal F_1s (t)	n.s.	*	n.s.	***	*	n.s.
Boron Treatment (F)	***	***	***	n.s.	***	***
Geno * Treat Int. (F)	***	n.s.	**	*	***	***

Sahara 3771 x Stirling

For the cross Sahara 3771 x Stirling (Figure 4.3a), leaf damage scores for the F_1s and the reciprocals did not differ. For both +B treatments the F_1s are intermediate between the parents; the B20 treatment more closely resembling Sahara 3771, the B40 more closely resembling Stirling. Leaf damage score decreases for Stirling from B20 to B40. This may be due to a slowing of transpiration rate and reduced growth, as indicated by reduced tiller number (Figure 4.3b), plant height (Figure 4.3c) and shoot dry weight (Figure 4.3d). A significant difference was seen between F_1s and reciprocals for both tiller number (Figure 4.3b) and shoot dry weight (Figure 4.3d), indicating a maternal component may be involved in determining these characters in this cross. For each of these variables the mean value in one or both of the F_1 progeny classes exceeded that of both parents, suggesting a possible heterosis effect. Stirling was the only line where tiller number (Figure 4.3b) and plant height (Figure 4.3c) decreased with increasing B application. Mean shoot dry weight (Figure 4.3d) decreased from 0.15 g to 0.08 g for Stirling between B0 and B40. For Sahara 3771 shoot dry weight actually increased from B0 to B20. Mean shoot B concentration (Figure 4.3e) increased in Stirling from 8.8 $mgkg^{-1}$ at B0 to 1403 $mgkg^{-1}$ at B40. The F_1 means did not differ significantly from Sahara 3771 at either B20 or B40 and increased only from between 7 and 9 $mgkg^{-1}$ at B0 to between 220 and 370 $mgkg^{-1}$ at B40. This general relationship between shoot B concentration and treatment held true if data was transformed with $\log_{10}$. A significant genotype by treatment interaction occurred for each character measured. Thus, neither the magnitude nor necessarily the direction in which any genotype reacts to a particular treatment in respect to any of the characters measured is the same as that of the other genotypes.

CM 72 x Stirling

The F_1s and the reciprocals showed no significant differences in any of the measured parameters for the cross CM 72 x Stirling (Figure 4.4 and Table 4.4). Leaf damage scores for F_1 plants resemble CM 72 in reaction to B treatments (Figure 4.4a). The reduction in damage score from B20 to B40 is observed in both parents in this case. In general, tiller number (Figure 4.4b), plant height (Figure 4.4c) and shoot dry weight (Figure 4.4d) declined with higher B treatments though to a greater degree for Stirling than the other genotypes. The F_1 mean exceeded both parents under both treatments in tiller number (Figure 4.4b) and shoot dry

weight (Figure 4.4d) which again suggests that some degree of heterosis may be occurring. Shoot B concentration (Figure 4.4e) in the F_1 progeny showed no significant difference with that of CM 72, but was significantly less than that of Stirling at the B40 treatment. All genotypes resembled each other closely for this character at the B20 treatment.

Sahara 3771 x CM 72

Progeny from the reciprocal crosses between Sahara 3771 and CM 72 (Figure 4.5 and Table 4.4) showed significant differences for shoot dry weight (Figure 4.5d), for tiller number (Figure 4.5b) and shoot B concentration (Figure 4.5e), which may indicate some maternal influences on these characters. The F_1s and reciprocals showed no significant difference for the other characters measured. CM 72 showed a high leaf damage score (Figure 4.5a) relative to Sahara 3771 and the F_1s. Leaf damage score was lower at B40 than at B20, similar to that observed for Stirling in the other crosses. At B20 the F_1s do not differ from Sahara 3771, but a difference is discernible at B40. CM 72 showed a lower mean tiller number (Figure 4.5b) than the other genotypes at B0, and this also declined significantly from B20 to B40, where the other lines maintained between three and four tillers on average. Sahara 3771 was shorter (Figure 4.5c) than the other genotypes at all treatments, but showed no real decline with increasing B. The other genotypes declined in height with increasing B, most significantly, CM 72. No overall significant difference was detected between treatments with respect to shoot dry weight (Figure 4.5d), though a general decline in mean weight can be seen for CM 72 and an increase for Sahara 3771, from treatment B20 to B40. As seen for both of the other crosses, at least one F_1 combination exceeded both parents at one or both treatments with respect to tiller number (Figure 4.5b) and shoot dry weight (Figure 4.5d). The shoot B concentration of F_1 plants and the reciprocals fall midway between the parental lines at B20, and closer to Sahara 3771 at the B40 treatment (Figure 4.5e).

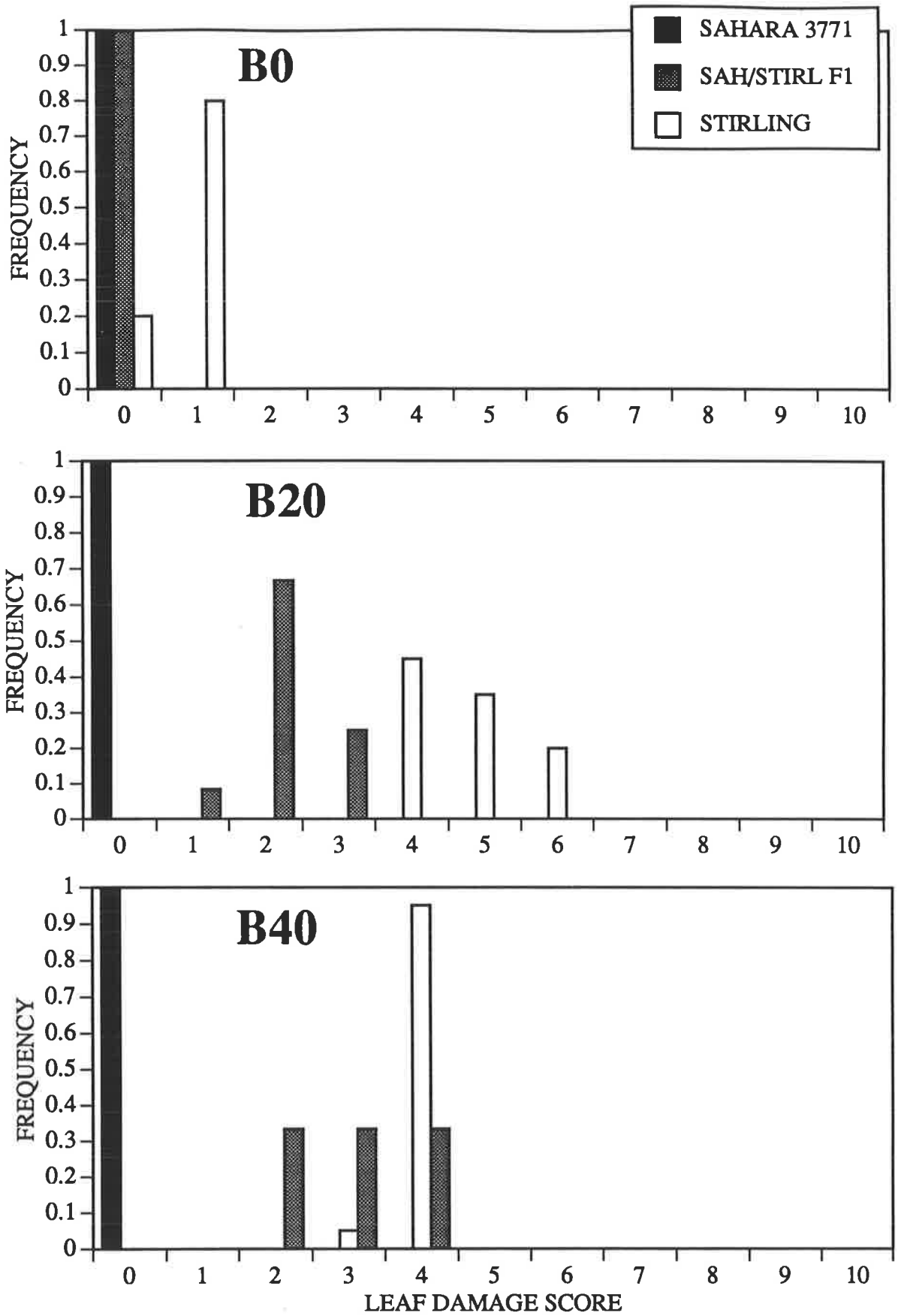


Figure 4.6. Frequency distribution of plants scored for leaf damage for Sahara 3771, Stirling and the F1 population at treatments B0, B20 and B40.

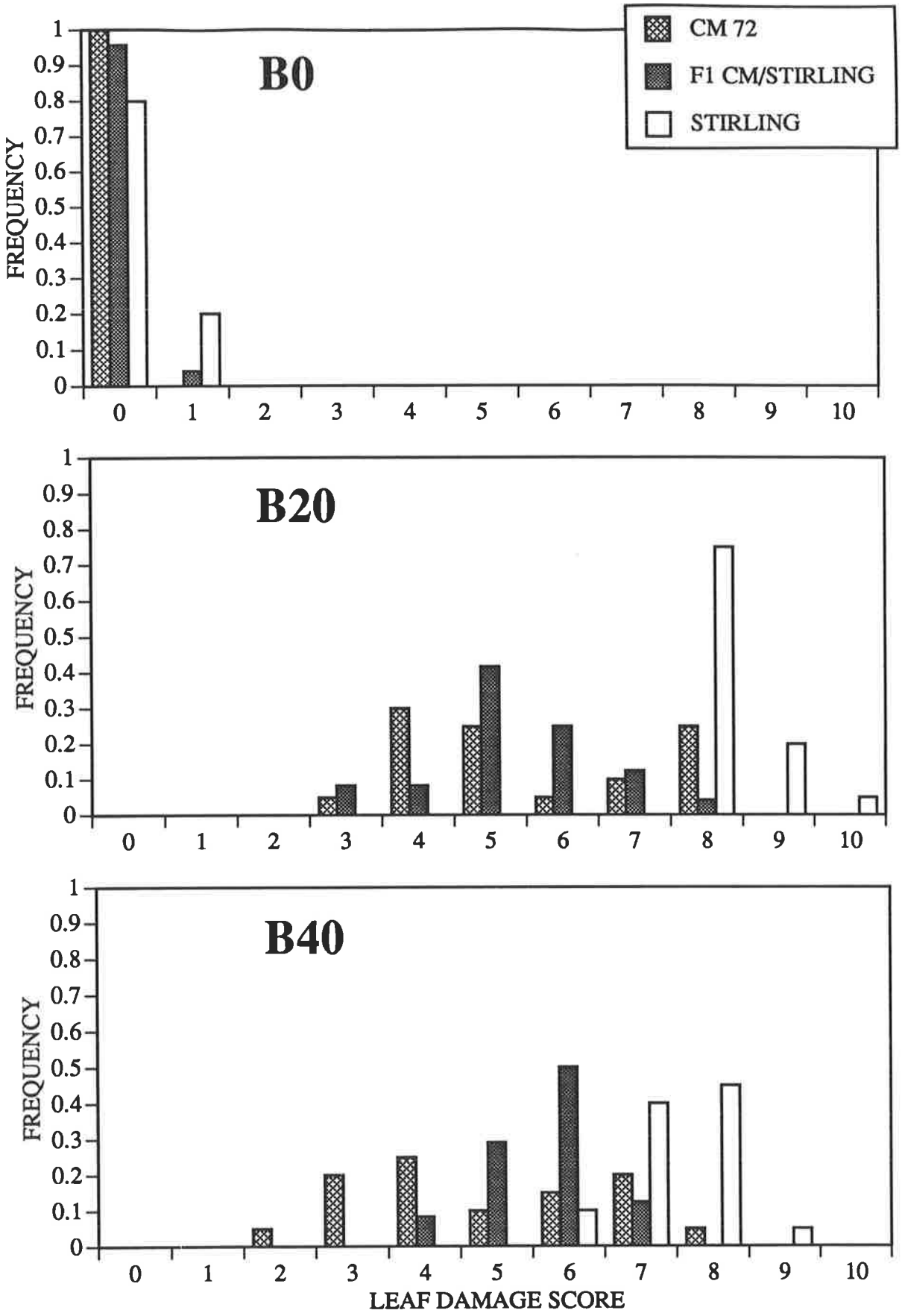


Figure 4.7. Frequency distribution of plants scored for leaf damage due to boron for CM 72, Stirling and their F1 progeny at treatments B0, B20 and B40.

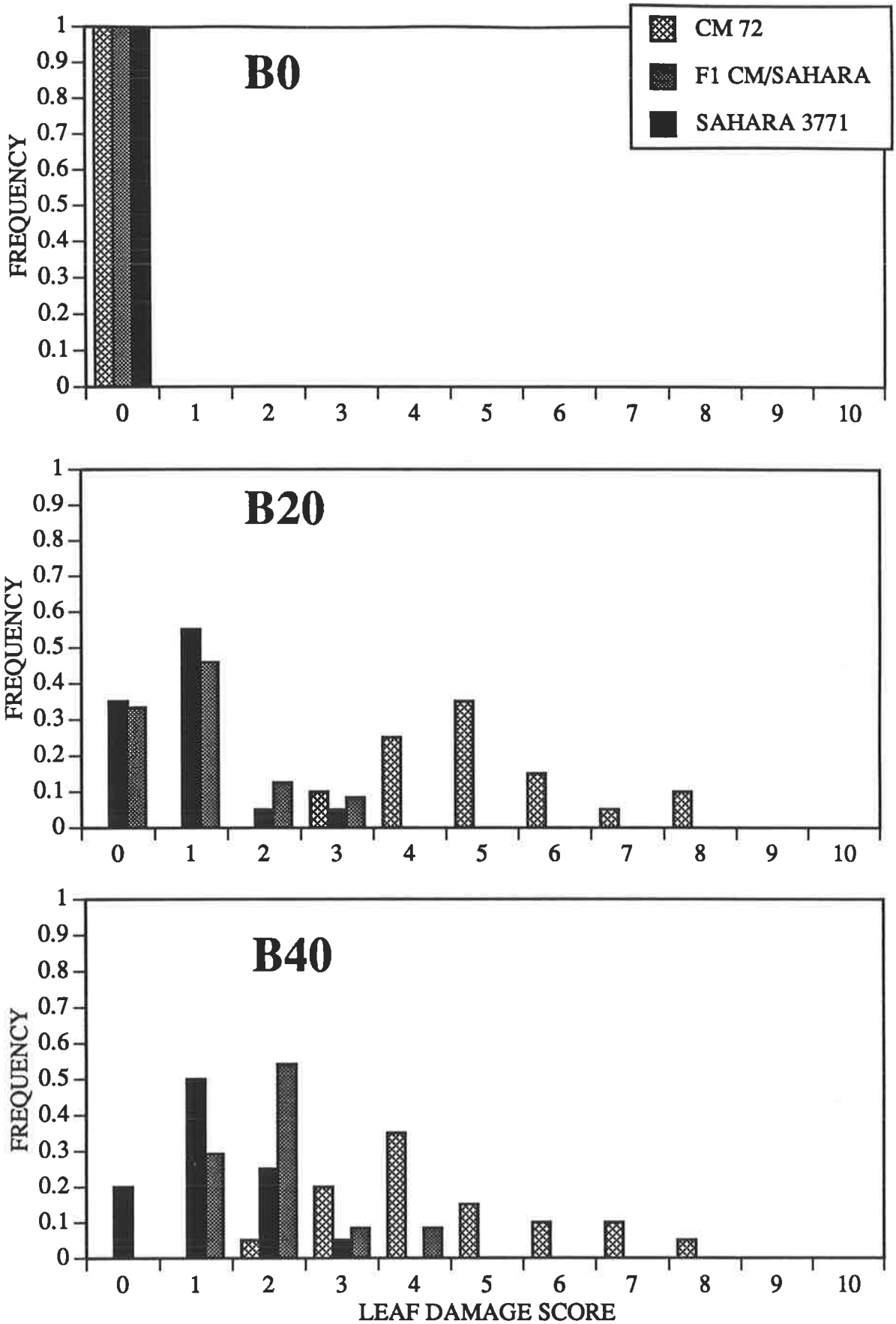


Figure 4.8. Frequency distribution of plants scored for leaf damage due to boron for Sahara 3771, CM 72 and their F1 progeny at treatments B0, B20 and B40.

Parental Lines

The parental lines were ranked the same in their response to B under these conditions as previously described (Boyd *et. al..*, 1988), that is Sahara 3771 showed a high degree of tolerance and CM 72 a moderate level, relative to the intolerant Stirling. In fact, Stirling showed some leaf damage due to B, even at the B0 treatment (Figures 4.6 and 4.7), presumably due to the small amount of B present in the base nutrient solution (Table 4.2).

In both the B20 and B40 treatment the frequency distribution of CM 72 displays a bimodal distribution, rather than a normal distribution (Figure 4.7). CM 72, a presumably genetically uniform line, would be expected to show variation in symptoms due to environmental variations alone. This bimodality arose again within the CM 72 controls for the F_2 distribution for CM 72 x Stirling (Figure 4.9b). It is not clear whether the apparent bimodal distribution within some CM 72 parental classes is significant. It may be indicative of an environmental factor, such at light availablility due to tray position, which is affecting CM 72 because of its intermediate level of tolerance, but not those genotypes with a more extreme response to B. Alternatively, it may be that the original CM 72 population was mixed with respect to the number of B tolerance genes any particular plant is carrying. Any underlying genetic cause of this phenomenon is likely to be complex.

F_1 Frequency Distributions for Leaf Damage Score

Leaf damage scores for the F_1s and the parental controls, at each treatment level, are presented as frequency distributions (Figures 4.6, 4.7 and 4.8). F_1s and reciprocals were pooled, since leaf damage scores were not significantly different. The distribution of the F_1s from the cross Sahara 3771 x Stirling falls closer to the zero leaf damage score of Sahara 3771 at the B20 treatment, but more closely resembles Stirling at the B40 treatment (Figure 4.6). Progeny from the cross between CM 72 and Stirling (Figure 4.7) fell within the range of CM 72 at both treatment levels. The F_1 distribution from the cross CM 72 x Sahara 3771 (Figure 4.8), ~~more~~ is slightly closer to ~~closely resemble~~ the more tolerant parent, Sahara 3771 in its frequency distribution.

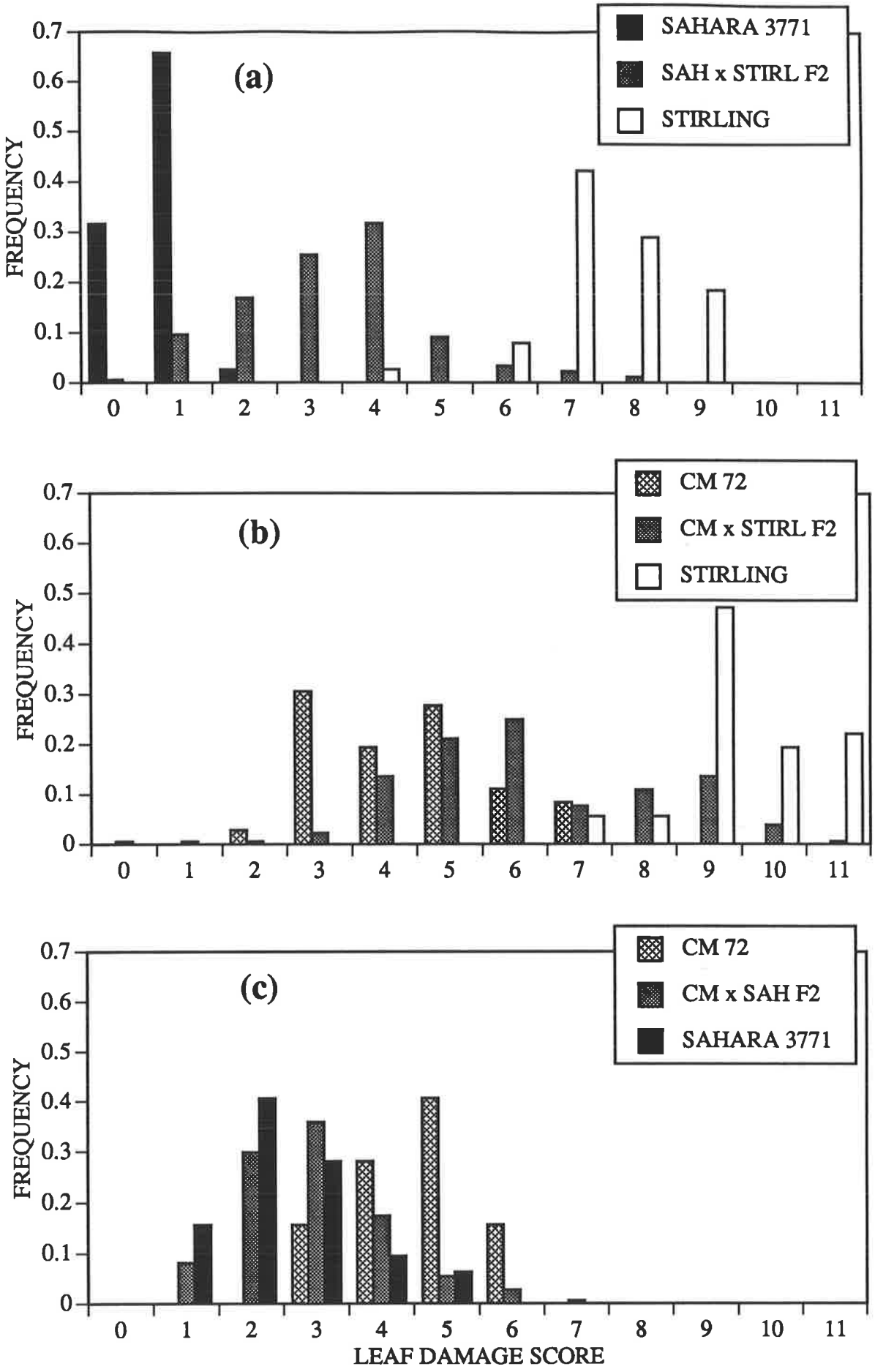


Figure 4.9. Frequency distributuion for parental lines and F2 populations for leaf damage score.

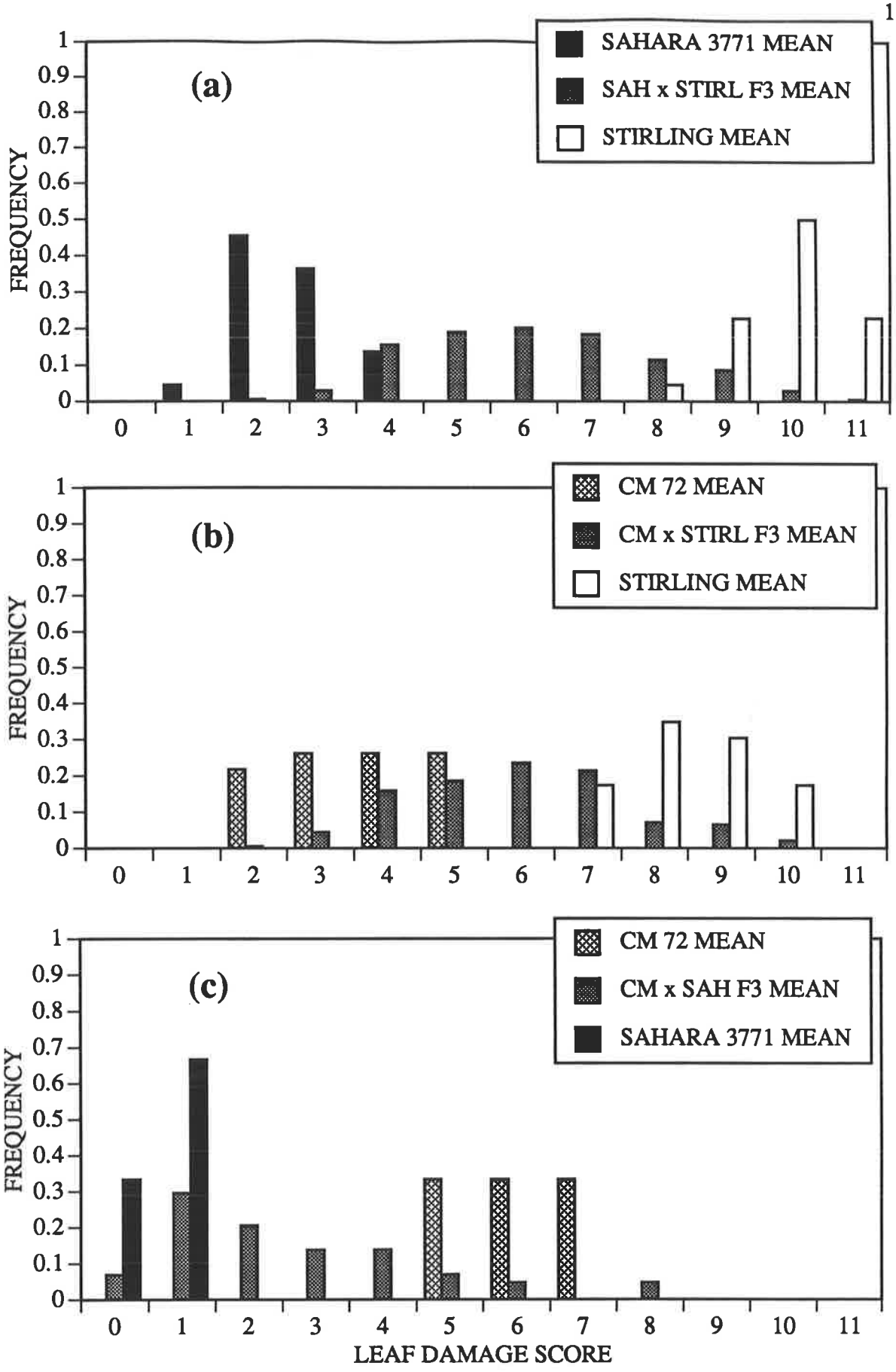
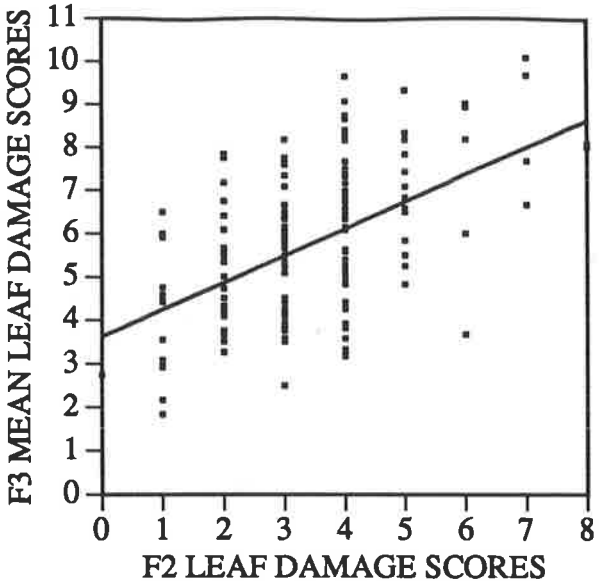


Figure 4.10. Frequency distribution of the mean leaf damage scores for F3 families and their parents, based on means within confectionery trays (see M & M).

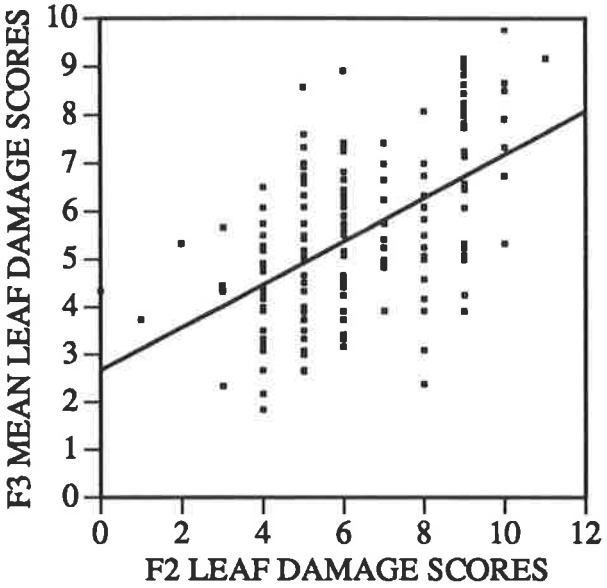
(a)



SAHARA 3771 X STIRLING

f(x) = 0.62x + 3.62
R^2 = 0.27** n = 174

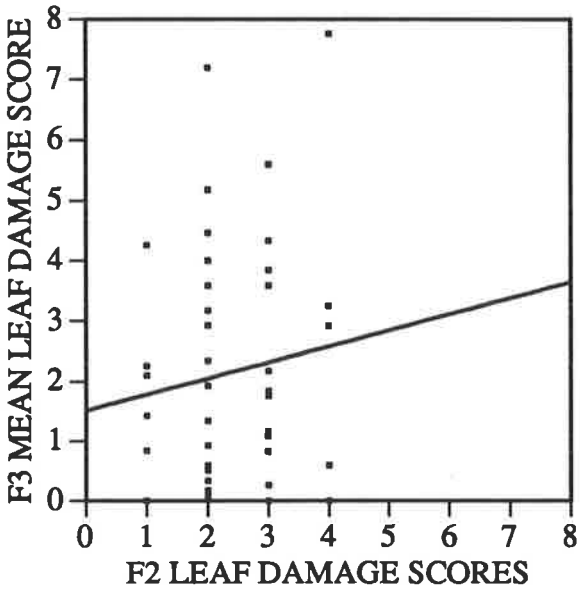
(b)



CM 72 X STIRLING

f(x) = 0.45x + 2.67
R^2 = 0.29** n = 183

(c)



CM 72 X SAHARA 3771

f(x) = 0.27x + 1.51
R^2 = 0.01 n.s., n = 44

Figure 4.11. Regression of F2 leaf damage scores vs F3 mean leaf damage scores for Sahara 3771 x Stirling, CM 72 x Stirling and CM 72 x Sahara 3771.

F_2 Frequency Distributions for Leaf Damage Score

No significant transgressive segregation was observed in the F_2 populations. That is, in no case does a significant proportion of the progeny show less damage than the more tolerant parent, or more severe symptoms than the most susceptible parent. The frequency distribution for leaf damage score for F_2 plants from the crosses, Sahara 3771 x Stirling (Figure 4.9a) and CM 72 x Sahara (Figure 4.9c) showed a continuous distribution. The F_2 population distribution derived from the cross between CM 72 and Stirling (Figure 4.9b) however suggested a degree of bimodality. This F_2 population was derived from one particular F_1 cross, and thus one CM 72 parent only, and so is not the result of mixed parentage.

F_2-derived F_3 Frequency Distributions for Leaf Damage Score

The distribution of the mean leaf damage scores of the Sahara 3771 x Stirling (Figure 4.10a) and CM 72 x Stirling (Figure 4.10b) F_3 families displayed a continuous, normal distribution spanning virtually the entire range defined by each parent. The distribution of the means of the F_3 families from the cross CM 72 x Sahara 3771 (Figure 4.10c) showed a distribution skewed toward the low leaf damage score end of the scale.

Leaf Damage Scores of F_2-derived F_3 Family Means vs F_2 Parents

Mean leaf damage scores calculated for each F_2-derived F_3 family were plotted against the leaf damage scores of the F_2 parents (Figure 4.11). For the cross Sahara 3771 x Stirling (Figure 4.11a) the slope of the fitted line was 0.62 with a y intercept of 3.62. The r^2 was 0.27. For the cross CM 72 x Stirling (Figure 4.11b) the slope of the line was 0.45, the y intercept 2.67 and the r^2 0.29. Both of these regression values are significant. For the cross CM 72 x Sahara 3771 (Figure 4.11c) the r^2 was not significant.

The severity of leaf damage scores produced under the experimental conditions varied with the time of year (Table 4.3 and 4.5). Within each experiment environmental conditions were as uniform as possible, but only limited control could be exercised over temperature and light conditions. Symptoms expressed in the F_1s and their parents tested in August (Sahara 3771, Stirling) and October (Stirling, CM 72 and Sahara 3771, CM 72), the cool months, were less severe than those of F_2s tested in September (Sahara 3771, Stirling), November (CM 72,

Stirling) and December (CM 72, Sahara 3771), in warmer conditions. Conclusions then should be based on comparison of populations with control plants tested simultaneously rather than with other populations.

Table 4.5. Means and variances of parental controls from the three F_2 segregation experiments.

Measure	Parental Genotypes					
	Expt: Sahara 3771 x Stirling		Expt: CM 72 x Stirling		Expt: CM 72 x Sahara 3771	
	Stirling	Sahara 3771	CM 72	Stirling	CM 72	Sahara 3771
Mean	7.5	0.7	4.4	9.5	4.6	2.5
Variance	0.78	0.2	1.8	1.2	0.8	1.1

CONCLUSIONS

Nuclear or Maternal Inheritance

Leaf Damage Score

Reciprocal crosses showed similar distributions and means, with respect to leaf damage scores (Table 4.4). Thus, inheritance is likely to be under the control of nuclear genes rather than cytoplasmic, that is, no maternal inheritance was evident. Since no difference was seen between leaf damage scores for any of the crosses (Table 4.4), the F_1s and their reciprocals were pooled for presentation of frequency distributions, and F_2 populations and F_3 families were derived from a single F_1 crosses.

Tiller Number, Plant Height and Plant Weight

There were some indications of maternal influence on tiller number and shoot dry weight in both the cross between Sahara 3771 and Stirling (Table 4.4) and the cross between Sahara 3771 and CM 72 (Table 4.4). Two explanations come to mind. Firstly, cytoplasmic genes may in part determine the expression of these characters. Alternately, since barley has a triploid

endosperm, a number of seed characters may be influenced by the maternal parent. In this tissue the maternal parent contributes two alleles for each paternal allele. Seed characters influenced by this triploidy may be influencing early vigour, since in each case when these characters differ significantly between the two types of F_1 crosses, it is the line which does not have Sahara 3771 as the maternal parent which appears to show the greater early vigour. Sahara 3771 has a smaller seed size than either Stirling or CM 72. Plant height does not differ significantly between reciprocals for any of the parental pairs.

Shoot Boron Concentration

For the cross between Sahara 3771 and CM 72 (Table 4.4) the t-value for shoot B concentration was significant at the 0.05 level between one F_1 cross and its reciprocal. The genotype with the lower shoot B concentration was on average smaller with a reduced tiller number (Figure 4.5b) and reduced shoot dry weight (Figure 4.5d) compared with its reciprocal. This may have resulted in less transpiration or may reflect a reduced root system resulting in less B uptake . An alternate explanation is that where Sahara 3771 was the maternal parent the B content of the seed would be expected to be lower. Sahara 3771 generally has a lower B concentration in all tissue, regardless of whether the B level in the growing medium is toxic (Nable, 1988). In fact there is some indication that Sahara 3771 may be suffering some growth reduction at the B0 treatment (Figures 4.3d and 4.5d). This small difference in seed concentration may influence concentrations in shoots at this early growth stage, though it is unlikely, since shoot weight exceeds seed weight by at least ten times, and dilution would render seed B relatively insignificant. It is, in fact, the F_1s with Sahara 3771 as the maternal parent which shows the reduced mean shoot B concentration (Figure 4.5e). These seed and seedling characters may be influenced by the triploid nature of barley endosperm.

To distinguish between maternal effects due to cytoplasmic genes or the triploid endosperm, it would be necessary to investigate these traits in F_2 populations derived from the reciprocal crosses. If the trait is controlled by a cytoplasmic gene, it would be expected to persist, that is in every generation the progeny would be expected to resemble the maternal parent for the trait. Alternately, it the trait is determined by the genotype of the endosperm, the effect would not be expected to persist, since the genotype of the endosperm for each generation is determined by

the nuclear genotype of the parental lines. Since the traits found to be influenced by maternal effects in this study are influenced by many genes, it is likely that nuclear genes also play significant roles.

Dominance

Frequency distributions of leaf damage score in F_1 plants derived from the cross between Sahara 3771 and Stirling (Figure 4.46 showed Sahara 3771's tolerance to be partially dominant at the B20 treatment (Figure 4.6b) but recessive at the B40 (Figure 4.6c) treatment with respect to intolerance expressed by Stirling. Drawing conclusions from this observation is complicated by the fact that leaf damage score for Stirling was less at B40 than at B20 (Figure 4.3a). In the cross between Sahara 3771 and CM 72 (Figure 4.8) the frequency distribution for leaf damage score shows a degree of dominance for B tolerance. The uniformly heterozygous F_1s from this cross expressed leaf damage scores closer to the highly tolerant Sahara 3771 than those of CM 72. F_1s with tolerance derived from CM 72, from the cross between CM 72 and Stirling (Figure 4.7), expressed a phenotype more closely resembling the CM 72 parent. It can be concluded then that under most conditions tolerance is partially dominant over intolerance, but that this relationship may change under different conditions.

The dominance relationships are not the same for shoot B concentration as for leaf damage score as reflected in mean scores (Figures 4.3, 4.4 and 4.5) In the cross involving Sahara and Stirling (Figure 4.3), low concentration appears almost completely dominant at both treatment levels. Discrimination is poor at B20 between CM 72, Stirling and their F_1s, but low concentration appears dominant at B40 (Figure 4.4). In the cross between CM 72 and Sahara 3771 (Figure 4.5) the F_1 mean falls almost midway between parents with respect to tissue B concentration at both treatment levels. This suggests that different genetic systems may control shoot B concentration and symptom expression, which has implications for breeding.

The inconsistency of dominance relationships with changing treatment and variable measured is an important factor to be taken into account when trying to establish the number of genes involved in determining tolerance. Based on dry matter yields and shoot B concentrations, Paull (1991a) found that in wheat, tolerance to high concentrations of B was expressed as a

partially dominant character but that there is an interaction between B treatments and the response of an F_1 hybrid relative to its parents. He concluded that it would be expected that the response of heterozygotes in a segregating generation, and therefore the segregation pattern, would also differ with the level of applied B. The level of 20 mgl^{-1} B was adopted as suitable for screening of barley lines and their progeny for tolerance to high B in this series of experiments, since this level generally produced maximum discrimination between parental leaf damage scores. Leaf damage scores were chosen as the most appropriate measure of tolerance to B toxicity. The reasons for this included: symptom expression were found to be more highly correlated with yield in the field (Figure 3.4); a non-destructive method allows plants to be used to produce the next generation; and this method is more efficient in respect of both time and expense, when compared with tissue analysis.

Allelism

No transgressive segregation was observed for leaf damage score in the F_2 populations (Figure 4.9). That is, the segregating F_2 populations fell within the bounds set by their parents. It can be concluded then that the major genes involved in determining tolerance to high B in each of the lines investigated are allelic to each other. That is, the gene or genes coding for tolerance to boron in CM 72 are in common with those of Sahara 3771. It is likely though that many other genes play smaller roles in determining tolerance.

Number of Genes Coding for Tolerance

Chi-square analysis was applied to the leaf damage scores of F_2s and two alternate genetic models were proposed (Figure 4.12). These models took into account the ranking and magnitude of differences in reactions to high B levels between the three test cultivars.

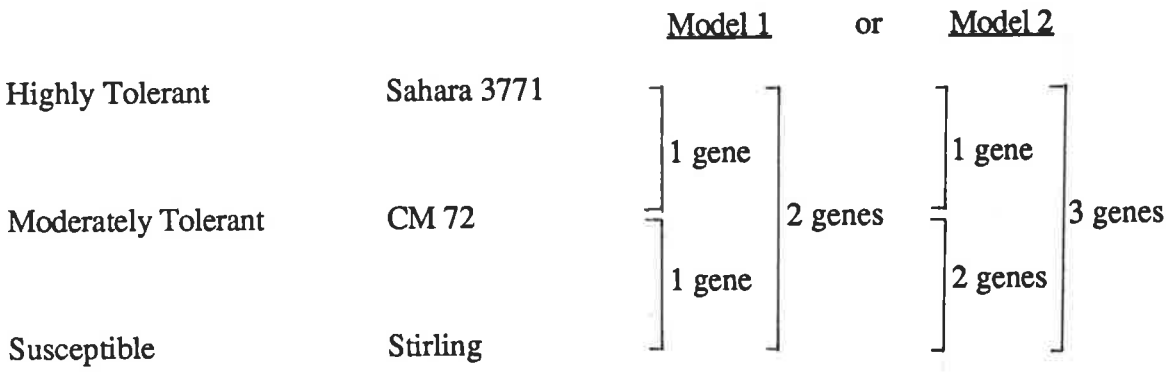


Figure 4.12. Alternative genetic models for B tolerance, as reflected in leaf damage scores.

AaBb * AaBb

gametes

gametes	AB	Ab	aB	ab
AB	AABB	AABb	AaBB	AaBb
Ab	AABb	AAbb	AaBb	Aabb
aB	AaBB	AaBb	aaBB	aaBb
ab	AaBb	Aabb	aaBb	aabb

>>11:5 tolerant:intolerant

AaBbCc * AaBbCc

gametes

gametes	ABC	ABc	AbC	Abc	aBC	aBc	abC	abc
ABC	AABBCC	AABBCc	AABbCC	AABbCc	AaBBCC	AaBBCc	AaBbCC	AaBbCc
ABc	AABBCc	AABBcc	AABbCc	AABbcc	AaBBCc	AaBBcc	AaBbCc	AaBbcc
AbC	AABbCC	AABbCc	AAbbCC	AAbbCc	AaBbCC	AaBbCc	AabbCC	AabbCc
Abc	AABbCc	AABbcc	AAbbCc	AAbbcc	AaBbCc	AaBbcc	AabbCc	Aabbcc
aBC	AaBBCC	AaBBCc	AaBbCC	AaBbCc	aaBBCC	aaBBCc	aaBbCC	aaBbCc
aBc	AaBBCc	AaBBcc	AaBbCc	AaBbcc	aaBBCc	aaBBcc	aaBbCc	aaBbcc
abC	AaBbCC	AaBbCc	AabbCC	AabbCc	aaBbCC	aaBbCc	aabbCC	aabbCc
abc	AaBbCc	AaBbcc	AabbCc	Aabbcc	aaBbCc	aaBbcc	aabbCc	aabbcc

>>59:5 tolerant:intolerant

susceptible phenotype

Figure 4.1[3]. A model for phenotypes for 2 and 3 gene pair differences

Table 4.6. Chi-square values for the Sahara 3771 x Stirling, CM72 x Stirling and CM 72 x Sahara 3771 F_2 populations families divided into classes proposed by the suggested models. The damage scores between which classes are divided are indicated in brackets. For an explantion of the ratios see the text.

Sahara 3771 x Stirling

Classes	Expected Ratio	Expected Values (N= 179)	Observed	D.f.	X^2	Prob.
Model 1 (2genes)						
Tol : Intol	9 : 7	100.7 : 78.3	95 : 84 (3/4)	1	0.61	.30-.50
Model 2 (3 genes)						
Tol : Intol	27 : 37	75.5 : 103.5	95 : 84 (3/4)	1	8.27	.00-.01
Tol : Intol	37 : 27	103.5 : 75.5	95 : 84 (3/4)	1	1.46	.20-.30
Tol : Intol	59 : 5	165.0: 14.0	167 : 12 (5/6)	1	0.17	.50-.70

CM 72 x Stirling

Classes	Expected Ratio	Expected Value (N=184)	Observed	D.f.	X^2	Prob.
Model 1 (1 gene)						
Tol : Intol	3:1	138.0 : 48.0	131 : 53 (7/8)	1	0.12	.70-.90
Model 2 (2 genes)						
Tol : Intol	9:7	103.5 : 80.5	117 : 67 (6/7)	1	3.73	.05-.10
Tol : Intol	11:5	126.5 : 57.5	131 : 53 (7/8)	1	0.41	.50-.70

CM 72 x Sahara 3771

Classes	Expected Ratio	Expected Values (N=184)	Observed	D.f.	X^2	Prob.
M 1&2 (1 gene)						
Tol : Intol	3 : 1	138 : 46	136 : 48 (4/5)	1	0.08	.70-.90
2 gene hypothesis						
Tol : Intol	9:7	103.5 : 80.5	136 : 48 (4/5)	1	22.61	<.001
Tol : Intol	11:5	126.5 : 57.5	136 : 48 (4/5)	1	2.05	.10-.20

Table 4.7. Chi-square values for Sahara x Stirling F_2 derived F_3 families divided into various classes, parental non-segregating (P NS), non-parental non-segregating (NP NS) and segregating (SEG).

Sahara 3771 x Stirling

Classes	Expected Ratio	Expected Values (N=174)	Observed	D.F	X^2	Prob.
Model 1 (2 genes)						
P NS: NP NS and SEG	2 : 14	21.75 : 152.25	4 : 169	1	15.41	<.001
P NS and NP NS: SEG	4 : 12	43.5 : 130.5	11 : 162	1	30.90	<.001
P NS: NP NS: SEG	2 : 2 : 12	21.75 : 21.75 : 130.5	4 : 7 : 162	2	32.09	<.001
Model 2 (3 genes)						
P NS: NP NS and SEG	2 : 62	5.44 : 168.56	4 : 169	1	0.16	.50-.70
P NS and NP NS: SEG	8 : 56	21.75 : 152.25	11: 162	1	5.39	.01-.05
P NS: NP NS: SEG	2 : 6 : 56	5.44 : 16.31 : 152.25	4 : 7 : 162	2	6.32	.01-.05

Table 4.8. Chi-square values for CM 72 x Stirling F_2 derived F_3 families divided into various classes, parental non-segregating (P NS), non-parental non-segregating (NP NS) and segregating (SEG).# Since under this model NP NS are not expected to occur, the 18 families in this class have been divided evenly between the two parental classes.

CM 72 x Stirling

Classes	Expected Ratio	Expected Value (N=183)	Observed	D.F	X^2	Prob.
Model 1 (1 gene)						
P NS: SEG#	2 : 2	91.50 : 91.50	44 : 139	1	48.28	<.001
Model 2 (2 genes)						
P NS: NP NS and SEG	2 : 14	22.87 : 160.12	26 : 157	1	0.34	.50-.70
P NS and NP NS: SEG	4 : 12	45.75 : 137.25	44 : 139	1	0.05	.70-.90
P NS: NP NS: SEG	2 : 2 : 12	22.87 : 22.87 : 137.25	26 : 18 : 139	2	1.49	.30-.50

Table 4.9. Chi-square values for CM 72 x Sahara 3771 F_2 derived F_3 families divided into various classes, non-segregating Sahara 3771 type (NS Sah), segregating (SEG) and CM 72 type (CM). *CM type is defined as families with a mean ≥4. The mean of CM 72 overall was 5.51 with variance 3.19 and the mean of Sahara 3771, 0.18 with variance 0.20.

CM 72 x Sahara 3771

Classes	Expected Ratio	Expected Values (N=44)	Observed	D.F	X^2	Prob.
Model 1&2 (1 gene)						
NS Sah: SEG and CM	1 : 3	11 : 33	15 : 29	1	1.48	.20-.30
NS Sah:SEG: NS CM	1 : 2 : 1	11: 22 :11	15 : 21 : 8*	2	2.31	.30-.50
2 gene hypothesis						
NS Sah: SEG and CM	1 : 15	2.75 : 41.25	15 : 29	1	53.55	<.001

The first model proposes a single gene difference between CM 72 and Stirling, and Sahara 3771 and CM 72 and a two gene difference between Stirling and Sahara. In an F_2 population segregating at a single locus for a trait, a segregation ratio of 3:1 can be expected, where 3 of the 4 expected genotypes posses at least one dominant allele, producing a dominant phenotype. For F_2 plants segregating at two loci for a trait, a segregation ratio of 9:7 can be expected, where 9 of the 16 genotypes possess at least one dominant allele at each loci, and thus express the dominant phenotype, the other 7 phenotypes express the recessive phenotype. These simple Mendelian ratios, and also those predicted in Model 2, assume that the genes act additively (no epistasis), assort independently (the two genes are not linked) and express dominance (in this case that tolerance is dominant). Since boron tolerance in the F_2 families displayed a largely continuous distribution, divisions made between observed tolerant and intolerant plants (shown in Table 4.6 in brackets) reflect parental responses (Table 4.6). The frequency distribution derived from the CM 72 x Stirling F_2 however, expressed a distinctive bimodal distribution.

The second genetic model involves a single gene difference between Sahara 3771 and CM 72, a two gene difference between CM 72 and Stirling, and a three gene difference between Sahara 3771 and Stirling. The 9:7 ratio describes the situation when the dominant phenotype is expressed when at least one dominant allele is present at each of two loci. The 11:5 ratio describes the case where plants expressing tolerance contain at least two dominant alleles, either at the same or at two different loci (Figure 4.13). Two expected ratios, 37:27 and 59:5, were compared with observed results, which predict a three gene difference (Table 4.6). A 27:37 model predicts that of the 64 genotypes expected in a three gene model, the 27 which have at least one dominant allele at each of the three loci, express the dominant phenotype (Figure 4.13). The reversed ratio of 37: 27 was also tested, since leaf damage score for the F_1 population derived from the cross Sahara 3771 x Stirling expressed semi-dominance at B20, but recessiveness at B40 (Figure 4.6). The 59:5 expected ratio is derived if only two of the three classes of genotypes with only one dominant allele fall into the susceptible class (Figure 4.13)

The Sahara 3331 x Stirling F_2 population is consistent with the 9:7 ratio predicted by Model 1, but also fits the 37:27 or 59:5 ratios predicted by the three gene model (Figure 4.13). The CM 72 x Stirling F_2 segregation ratio is consistent with a 3:1, 9:7 or an 11:5 ratio depending on the class division. To distinguish between an 11:5 and a 3:1 ratio a minimum of 860 F_2 plants must be tested for B tolerance (Hanson, 1959), a number beyond the scope of this study. The 3:1 ratio clearly holds for the CM 72 x Sahara 3771 derived F_2 population, which suggests a one gene difference between these genotypes for tolerance to leaf damage scores.

The true situation can be made clearer by classifying F_2-derived F_3 families for B tolerance. The frequency distributions of the average leaf damage scores for F_3 families and their parents are presented in Figure 4.10. The "parental" classification is based on the mean of each parental control within each confectionery tray (see M &M). F_2-derived F_3 families were classified as segregating or non-segregating according to the parental variance. In general, those families with leaf damage scores within a range of three for the cross Sahara 3771 x Stirling and four for the cross CM 72 x Stirling were scored as non-segregating. For the families derived from CM 72 x Sahara 3771 for non-segregating Sahara 3771 type scores fell within a range of three, but the CM type was defined as families with a mean of ≥ 4, due to the large variance shown by the CM 72 parent in this experiment. Parental families were defined as those which straddled the parental mean. The Chi-square analyses are presented in Tables 4.7, 4.8 and 4.9. With most class groupings Model 2 is the most satisfactory.

If the control population of CM 72 was of a genetically mixed composition, as reflected in bimodal frequency distributions (Figure 4.7b,c), this problem appears to have been largely overcome by selecting single F_1 individuals to produce the F_2 populations. With regard to genes determining leaf damage score, it also appears that the CM 72 ancestor of both the CM 72 x Stirling and CM 72 x Sahara 3771 derived populations, were the same, since the segregation data from the F_3 populations do not conflict, and both support Model 2.

Frequency distributions derived for leaf damage scores expressed continuous distributions, thus decisions were made empirically as to where to divide classes for testing against genetic models in the F_2 generation, and in the case of F_3s in defining whether a family is segregating.

This is particularly difficult when an artificial scale, like leaf damage scores, must be used to estimate an underlying phenomenon. To what extent this measure reflects the genetic status of plants is not clear, particularly when the underlying physiological basis for tolerance is still unknown and other parameters like shoot B concentration at times give contradictory results. It was considered since ultimately it is anticipated that the screening method utilised in these experiments be incorporated into breeding programmes on a routine basis, that simplicity of testing, ease of scoring and relatively low cost, favour the visual assessment method.

Minimum family size of progenies to establish the genotype of a phenotype can be calculated (Hanson, 1959). The accuracy of these calculations are in this case however, limited by the extent that underlying genotypes can be predicted from phenotypes, particularly when segregations are not discrete. In the case of CM 72 x Stirling and CM 72 x Sahara 3771 the aim was to distinguish between a one and two gene difference. Were there a one gene difference between these pairs of lines with respect to B tolerance, then of the four genotypes expected in the F_2 generation, two would be segregating. A family size of four is required to detect with a 10% level of probability those families segregating from those non-segregating. In the case of a two gene model, twelve of the sixteen possible genotypes in the F_2 generation are expected to be of a segregating type. A minimum family size of nine is required in this case, to differentiate between a segregating and a non-segregating family at the 10% probability level. In the case of the Sahara 3771 x Stirling cross the aim was to distinguish between models predicting these two lines differing by two or three genes with respect to B tolerance. A family size of nine is required to distinguish segregating from non-segregating families for a two gene model, but for a three gene model Hanson (1959) predicts a requirement of at least eighteen as a family size to predict with a 0.10% probability which families are segregating and which are not. Since it was not practical to screen this number of progeny for all families, it would be expected that some families may have been incorrectly classified as non-segregating when they were in fact of a segregating type. It was the case, however, that for the cross Sahara 3771 x Stirling the non-segregating classes were deficient with respect to the expected values for the three gene difference model. Thus it is likely that there are at least three major genes different between Sahara 3771 and Stirling controlling tolerance to B toxicity.

Genotype x Environment Interaction

The relationship between leaf damage scores of the F_2 plants and the mean leaf damage scores of their F_3 progeny is described in Figure 4.11. Though a clear relationship exists among scores between the two generations for Sahara 3771 x Stirling and CM 72 x Stirling there is a considerable spread of F_3 scores for any given F_2 score. Two major factors are expected to play a role in determining this relationship. Firstly, this range reflects a genotype by environment interaction seen as a result of variation in screening conditions. When selection pressure is strongest, for example in warm weather, a wider range of symptoms would be expected. It can be seen to be the case that the range of scores differs between generations. Since neither line goes through the origin, an overall shift in scores can be seen to have occurred between the two generations. The other factor involved is dominance and other genetic interactions. If the F_2 parent was heterozygous, progeny will segregate, and a change in mean score would be controlled by dominance and other epistatic relationships (departures from an additive relationship) and chance events. The narrower range at F_2 score 7 in the CM 72 x Stirling cross (Figure 4.11) may indicate that this score represents the non-parental non-segregating class, for example in a two gene model, aaBB and AAbb types. Similarly, the F_2 plants scoring either high or low leaf damage scores are more likely to be homozygotes and thus the mean F_3 score would be expected to more closely reflect those of the F_2. This was observed only to a limited extent.

The cross CM 72 x Sahara 3771 does not exhibit a significant correlation. Since both of these parental lines are tolerant to high levels of B the range of responses in segregating offspring is relatively narrow. CM 72 tends to have a broad range of response relative to the other two parental lines in this experiment the overall mean was 5.51 with a variance of 3.19. It is likely that the variation within F_2-derived lines was of a similar order to that between lines. This together with the much smaller number of lines tested may account for the apparent lack of relationship between F2 and F3 mean leaf damage scores.

Summary

It can be concluded then that under high B conditions, leaf damage score, and plant height are determined by nuclear genes, while shoot B concentration, tiller number and plant height are

influenced by either maternal effects or cytoplasmic heredity. This and the fact that dominance relationships also differed between leaf damage score and shoot B concentration lends evidence to the assertion that at least two mechanisms may be involved in conferring boron tolerance in barley, and that these mechanisms may be determined by different genes. Tolerance to leaf damage at high boron was found to behave as semi-dominant in most circumstances, but behaved as a recessive trait in the cross Stirling x Sahara 3771 at B40. The genes determining B tolerance in CM 72 were found to be allelic with those in Sahara 3771. Boron tolerance is a quantitative character, so a number of assumptions must be made when conducting investigations into the number of loci involved in determining B tolerance. It was concluded from the evidence, however, that it is likely that there exists at least two genes determining tolerance to B in CM 72, and three genes in Sahara 3771. Significant genotype x environment interactions and epistatic effects are observed for this trait.

Chapter 5

THE NATURE OF EXPRESSION OF BORON TOLERANCE

INTRODUCTION

A better understanding of the physiological nature of tolerance to boron (B) toxicity would be of major benefit in devising screening techniques and breeding strategies. The mechanism by which barley tolerates high levels of B has been shown to be predominantly by exclusion of B from roots (Nable, 1988). How this is achieved is still not clear.

Genetic differences in tolerance to high levels of B are expressed in wheat (Huang and Graham, 1990) and barley (Huang, pers. comm.) in excised roots and root derived callus. Experiments were conducted to investigate further the nature of the control of B tolerance and to investigate ways to exploit an apparently widespread tissue expression in a breeding programme.

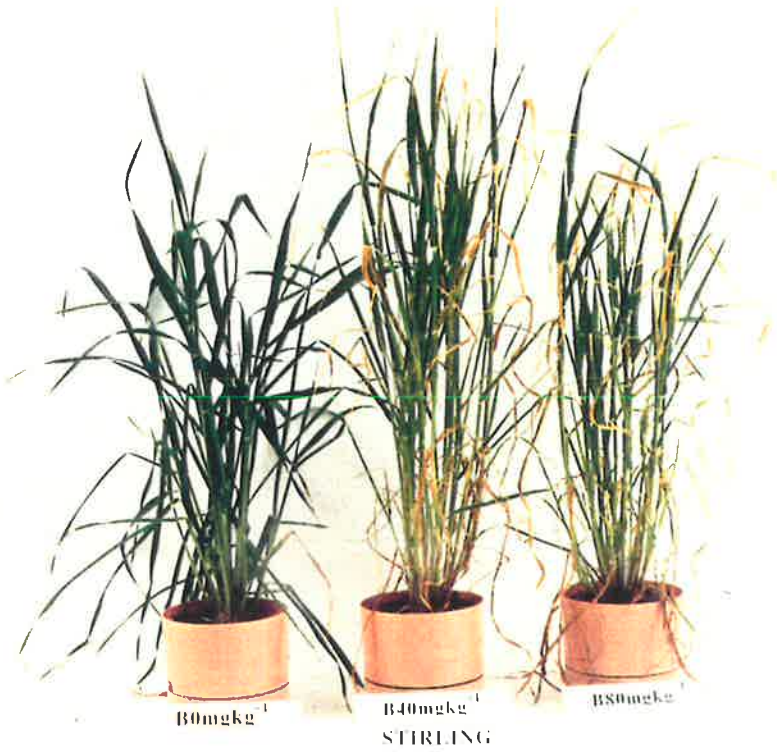


Figure 5.1. Experiment 2: Boron toxicity symptoms expressed by barley cultivar Stirling treated with 0, 40 and 80 mg kg^{-1} B.

Figure 5.2. Experiment 3: *In vitro* pollen germination of barley cultivar CM 72 at B50 (50mg kg^{-1} boric acid).

EXPERIMENT 1: UPTAKE OF BORON BY PROTOPLASTS

INTRODUCTION

It has been suggested that a large amount of tissue B in plants is bound to cell walls, particularly under low B conditions (Smith, 1944). Loomis and Durst (1991) presented evidence for B having a role, or roles, in cell-wall cross-linking. The aim of this study was to find whether protoplasts taken from plant material tolerant to high levels of B at the whole plant and callus level, expressed a difference in B uptake to protoplasts derived from intolerant material. That is, is tolerance to B still expressed in the absence of cell walls.

In order to determine net B uptake, it would be useful to be able to distinguish the B already present in the protoplast from that taken up from the experimental solution. In nature B exists in complexed forms as one of two non-radioactive isotopes; ^{11}B and ^{10}B. ^{11}B is the most abundant at around 80.1%, with ^{10}B 19.9% (Lide, 1991). The more rare isotope ^{10}B, was therefore used as the source of B in the experimental solution. So by measuring ^{10}B concentration in the protoplasts, to a large degree net uptake of B from the solution can be determined. The uptake study procedure in general was based on that of Gronwald and Leonard (1982). By comparing uptake of B by protoplasts with previous studies' investigations of uptake into cells with walls intact, this study will contribute some evidence either for or against a role for cell walls in the expression of tolerance to exposure to high levels of B.

MATERIALS AND METHODS

Protoplast preparation

A method for preparation of protoplasts from barley leaves was modified from that of Thayer and Huffaker (1984) in conjunction with Dr Henning Hu from the University of California, Davis.

Enzyme solution

For 40 ml	
Cellulysin (Calbiochem)	0.4 g
Pectolyase Y-23 (Seishin)	0.04 g
Hemicellulase (Sigma)	0.4 g
Cefataxime (Sigma)	4 mg
in	
MES Buffer (US Biochem) pH 5.6	0.0392 g
Sorbitol (Sigma)	3.648 g
$CaCl_2.2H_2O$ (Fisher)	0.0059 g
then add	
Bovine Serum Albumin (Sigma)	0.2 g
DTT (Sigma)	0.0031 g

The solution was mixed and filtered under vacuum through 0.45 microns (Nalgene™).

Procedure

The barley genotypes used were Sahara 3771, a highly B tolerant barley line, and Stirling, an intolerant line. Plants were grown in potting mix under shade in a glasshouse in the northern summer of 1991. Four to 5 g of five to ten day old leaf material of each genotype was collected. In a laminar flow cabinet, tissue was surface sterilized with a 5% solution of commercial bleach for 1 minute, then rinsed three times with sterile distilled water. The tissue was cut into 0.5 cm diagonal slices and floated on the enzyme solution in four 10 cm petri dishes. The tissue was vacuum infiltrated for 10 minutes, sealed with parafilm, wrapped in foil and put on a 45 rpm rocker at 25°C. The incubation was timed from this point. After about 1.5 hours the condition of the protoplasts was checked under the microscope and the undigested tissue gently teased apart. The plates were returned to 25°C and removed after a maximum of 2 hours total digestion time. The sterile procedure ended here. Care was taken to work quickly to remove protoplasts from the enzyme solution. The protoplast suspensions were handled gently. The opening of disposable pipette tips where increased to 1mm to avoid sheering. The protoplast suspensions were filtered through Miracloth™ (63 micron) or nylon mesh (Fisher 70 micron). The filtered suspension was transferred to 15 ml round bottomed glass centrifuge tubes (5-10 ml per tube) and centrifuged at 170 g for 10 minutes. The enzyme solution was decanted. The protoplasts were resuspended in 10 ml of 0.5M sucrose in HDP (see below) per tube and half was transferred to another tube. The suspensions were carefully overlayed with glucose/sucrose solutions, as described below, using a 5 ml Gillman™ pipette. The tubes were centrifuged at 55 g for 15 minutes, and the purified protoplasts formed a band between the top

two layers. This band was removed using a modified plastic disposable sampling pipette (as described above) and transferred to plastic 15 ml centrifuge tubes, where the volume was made up to 5 ml with 0.5M glucose in HDP if necessary.

Separation solutions

Resuspend in:

5 ml per tube	0.5M Sucrose in HDP
And overlay with:	
2 ml per tube	0.4M Sucrose 0.1M Glucose in HDP
then:	
2 ml per tube	0.3M Sucrose 0.2M Glucose in HDP
then:	
0.6 ml per tube	0.5M Glucose in HDP

HDP: (100 ml)

Hepes to pH 7.5	1.1910 g
DTT	0.0150 g
PVP 40	0.50 g

Uptake study

Wash solutions

Wash solutions were prepared in advance as follows. Five ml of: 0.7M glucose, 2 mM Tris-MES buffer, 1 mM $CaCl_2$ was placed in plastic 15 ml tubes. This solution was underlaid in each tube with 4.8 ml of ficoll/glucose solution (for 48 ml, 0.96 g ficoll, 6.12 g glucose). Tubes were labelled with sample times and genotypes and kept on ice.

Procedure

A trace quantity (<50 mg) of $CaSO_4$ and KCl was placed into each of two plastic 50 ml beakers. The protoplast suspension was added and swirled gently to dissolve chemicals. One ml of solution was taken as the 0 min sample with a Gillman™ pipette with a modified tip as described above, and a drop for a haemocytometer count. To this point then only B present in the system is that in the protoplasts. Then, 0.04 ml of 100 mM ^{10}B solution was added to each beaker, containing 4 ml of suspension, to give a total concentration of 1 mM ^{10}B. The beakers were put on a reciprocation shaker at 45 rpm at 25°C. Each were sampled at 5 minutes (1 ml),

10 minutes (1 ml), 20 minutes (1 ml), 40 minutes (0.5 ml) and 80 minutes (0.5 ml). Each sample was suspended in the top layer of the wash solution and centrifuged at 1000 g for 10 minutes. The 5 minute sampling was kept on ice, and centrifuged with the 10 minute sample. Samples were then aspirated and the pellet resuspended in 1 ml of water. One ml of 2% nitric acid was added and the sample allowed to digest at room temperature for 10 minutes. The sample was frozen, and when ready for analysis, sonicated for 1 hour, then centrifuged at 1000 g for 10 minutes. The B ratios in the supernatant were read using an ICP-MS. This experiment was repeated three times, on separate days.

RESULTS

A graph describing the B content ($\log_{10}$ ng B per 10^5 protoplast) for each isotope, for each genotype over time is presented as Figure 5.3. Each point represents the average measurement from the three replicates, and the error bars represent the standard error. Analysis of variance was performed to determine whether B concentration of in the protoplasts was significantly affected by genotype, B isotope or sample time, using the JMP™ statistical package, applied to the means of replicates (Table 5.1).

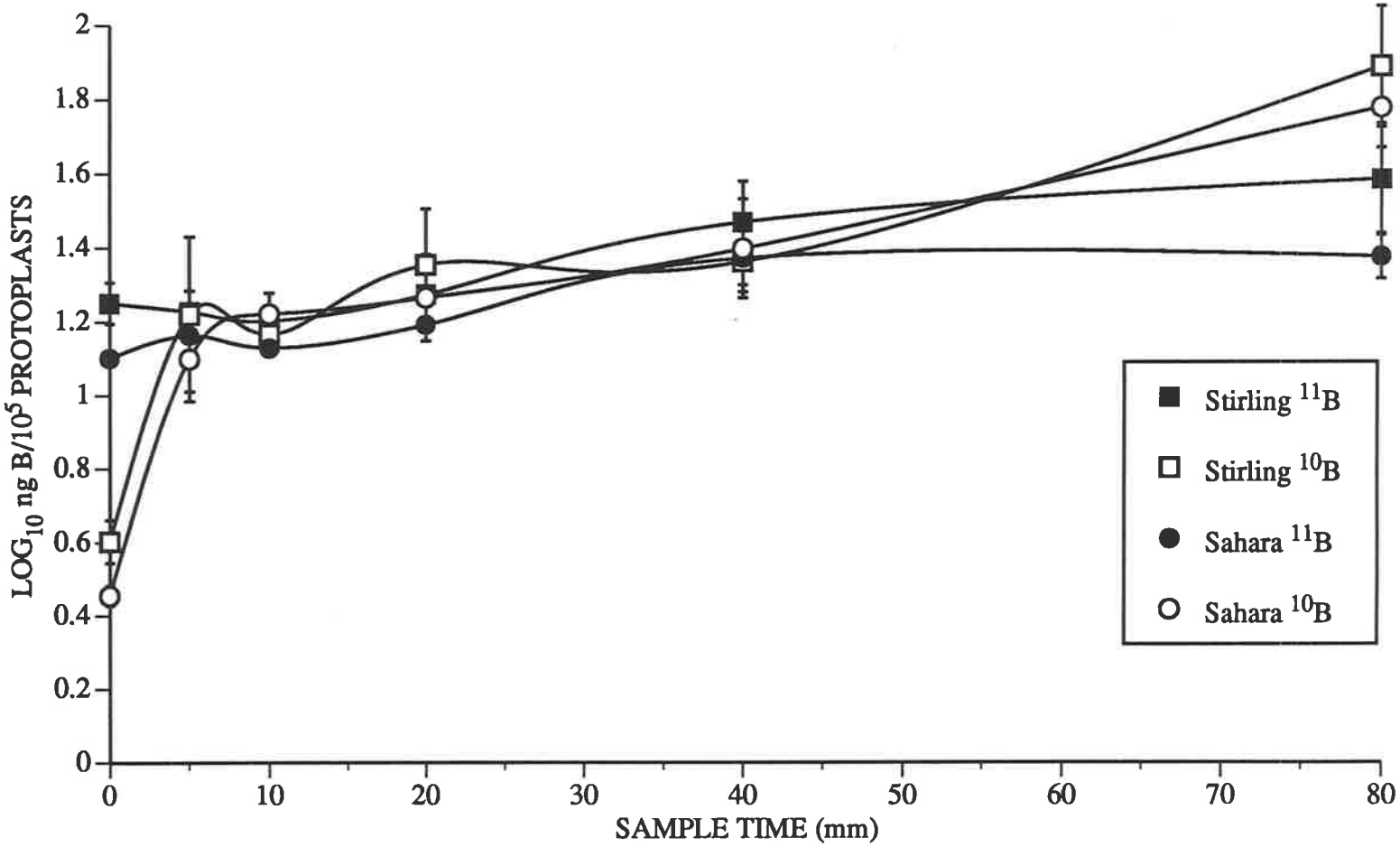


Figure 5.3. Net boron uptake by protoplasts of the two barley genotypes, Stirling and Sahara 3771 is shown. At time zero they were transferred to a solution containing only the B isotope. Samples were taken at 5, 10, 20, 40 and 80 minutes.

Table 5.1. Effect test table describing the probability that each source of variation contributed significantly to determining the B content (log_{10} ng B/10^5 protoplasts) of protoplasts derived from Stirling and Sahara 3771 barley leaves. Analysis of variance was performed to investigate sample time, B isotope and genotype, as sources of variation contributing to B concentration in protoplasts. Analysis was preformed including both isotopes, ^{10}B only and ^{11}B only. * indicates significant difference at the 5% level of significance.

Source of Variation	^{10}B and ^{11}B		^{11}B		^{10}B	
	D.f.	Prob>F	D.f.	Prob>F	D.f.	Prob>F
Including Sample T0						
Genotype	1	0.22	1	0.14	1	0.10
B Isotope	1	0.77	0	NA	0	NA
Sample Time	5	0.00*	5	0.00*	5	0.00*
Excluding Sample T0						
Genotype	1	0.07	1	0.28	1	0.03*
B Isotope	1	0.05*	0	NA	0	NA
Sample Time	4	0.00*	4	0.01*	4	0.01*

CONCLUSIONS

Plot of B Uptake v Time

The difference between Stirling and Sahara 3771 with respect to uptake of ^{10}B is small (Figure 5.3). As expected, at T0 protoplasts produced from Stirling plants were higher in both ^{10}B and ^{11}B, since this cultivar takes up more B from the soil than Sahara 3771. When the protoplasts were exposed to ^{10}B in solution, this difference disappeared within 5 minutes. Not until T80 did a difference become evident, where Stirling showed a somewhat higher concentration of ^{10}B. This difference however was of a similar magnitude to that observed with respect to concentration of ^{11}B. Since no ^{11}B was expected to be present in the system, except that already present inside the protoplasts, this is likely to be an artifact due to settling of protoplasts, causing a higher density of protoplasts in this final sample than indicated by the original concentration estimate. Thus, it is unlikely that this genotypic difference with respect to boron uptake in protoplasts is real over the timespan used in this study. On the other hand, the rank of the two genotypes, with respect to B concentration was not consistent at earlier time intervals, but it may be expected that limitations in precision and reproducibility in the technique would be relatively smaller over 40 minutes, the longest interval, when compared to the total uptake. Thus the genetic difference expressed at T80 may be the beginning of a trend which may have continued over a longer time interval.

The concentration of ^{10}B in the protoplasts increased with time, indicating a net uptake of B through the membranes (Figure 5.3). It must be remembered that the passage of nutrients through membranes is dynamic, in that it is neither one way, nor static. After only five minutes, the protoplasts contain almost equal amounts of each isotope. If suitable methods were available it would be useful to investigate more closely the pattern of uptake within this time. At T0 Stirling protoplasts were higher in both B isotopes. Stirling is expected to have a higher B concentration in shoot tissue than Sahara 3771 under most growing conditions. The average relative concentration of ^{10}B and ^{11}B was calculated for both genotypes at T0. They were: Stirling 18.42% ^{10}B, 81.58% ^{11}B; and Sahara 3771 18.48% ^{10}B, 81.52% ^{11}B. This is close to that cited by Lide (1991). By T80 the protoplasts contain significantly more ^{10}B than ^{11}B (Figure 5.3).

^{10}B and ^{11}B Analysed Together

Firstly, concentrations of both isotopes were analysed together (Table 5.1), that is both isotopes were taken into account. Boron concentration in protoplasts was the Y variable, and sources of variation being considered were sample time, B isotope and genotype. No significant difference in B uptake is evident between leaf protoplasts derived from the barley genotypes Stirling and Sahara 3771, in this analysis. Boron isotope was not significant if sample T0 was included, though it was expected that no ^{11}B was added to the system. It is clear from the plot (Figure 5.3) that this is due to the low initial concentration of ^{10}B compared with ^{11}B in the protoplasts being balanced by the higher concentration at T80. When the initial sample T0 is excluded from the analysis, the two isotopes were significantly different over the uptake period. Concentration of B in the protoplasts changed significantly (Prob>F is $\leq$0.05) with sample time, whether T0 was included or not, indicating that exchange of B is taking place across protoplast membranes.

^{11}B and ^{10}B Analysed Separately

The concentration of ^{10}B and ^{11}B in the protoplasts were analysed separately (Table 5.1). Stirling and Sahara 3771 did not differ overall with respect to ^{10}B concentration, at the 5% significance level, if T0 was included. The two genotypes did however differ significantly if T0 was excluded, thus the relatively steep increase in rate of uptake of ^{10}B over the final time span may correspond with respect to phase of uptake, to the site at which genotypic differences occur.. This result is in contrast to when all data was analysed together, where no significant genotype effect was indicated. The concentration of ^{10}B in protoplasts increased significantly over the time of the experiment (Figure 5.3 and Table 5.1), indicating that exchange of B is occurring across cell membranes.

When ^{11}B was considered in isolation, no difference was observed between genotypes, but unexpectedly the concentration in protoplasts changed significantly with sample time (Table 5.1). This result is however anomalous, since only ^{10}B was present in the solution to which the protoplasts were exposed. The only ^{11}B expected to be present in that system is that initially present in the protoplasts. Two possible explanations for the increase in ^{11}B with later samples is: (1) that the solution to which the protoplasts was exposed was contaminated to an

unacceptable level with extraneous ^{11}B, from the other chemicals used, the water, the air, or (2) that despite efforts to maintain a homogeneous protoplast suspension over time, some settling may have occurred, resulting in progressive samples containing more protoplasts. This sampling error would presumably have occurred in both treatments. The fact that there was no significant difference between the two genotypes tested (Table 5.1) with respect to concentration of ^{11}B is consistent with the increased concentration over time being due to a contamination ~~concentration~~ affect.

Implications

The non-significance of genotype as a factor in determining protoplast B concentration in the joint analysis is not consistent with the differences observed in uptake by whole plants and tissue cultures; two explanations come to mind.

Firstly, cell walls may be involved in the differential uptake of B between tolerant and intolerant lines. A differential growth response was seen in callus between tolerant and intolerant barley lines by Huang (pers. comm.). Callus derived from Sahara 3771 root tissue, suffered much less growth reduction at high B concentrations, than the less tolerant barley cultivars. This difference is presumably due to differential uptake, since on the whole plant basis B tolerance is considered to be, predominantly, an exclusion mechanism (Nable, 1988). Large variation in callus growth, however, was seen between lines at control B levels. No difference in B uptake is seen between the two barley lines tested when cell walls were removed. A better comparison could be made by producing cultured cell suspensions from the roots of barley lines, and performing similar uptake experiments on these suspensions. Thus, cells equivalent in tissue specificity and source could be compared in B uptake with and without cell walls present. Variation in growth and stability of cell cultures often exist between genotypes, however, which may limit such an experiment. A larger scale experiment with split treatments would eliminate the problems caused by subsampling.

Alternately, the genotypes may not be expressing differences in B uptake due to a tissue specificity for this response. Though it is generally assumed that callus is undifferentiated tissue, certain characteristics are likely to be transmitted from the source tissue type. In the

experiments of Huang and Graham (1990) the callus expressing growth response to B was derived from root tissue. The protoplasts used in this experiment were derived from leaf tissue. The B exclusion mechanism may not be expressed in leaf derived protoplasts to the same extent that it is expressed in root tissue. This could be tested by repeating similar experiments using protoplasts derived from root tissue, though it can be difficult to obtain the necessary high protoplast yield from root tissue in some species.

In contrast to when both isotopes were considered, ^{10}B concentration when analysed separately, was significantly different between genotypes at the 5% level when T0 was excluded, and at the 10% level when T0 was included. Thus using this criterion, genotypic differences in B uptake *were* expressed at the protoplast level. If this was accepted as reflecting the method by which whole plants regulate B uptake, it suggests that the cell wall does not act alone in regulating B uptake, and it is likely that cell membranes play at least some role.

It is difficult to draw conclusions from these results. It is believed however, that in combination with other approaches presented here and elsewhere to investigating the mechanism by which the uptake of B is regulated in plants, this information may contribute to the ultimate elucidation of this problem.

EXPERIMENT 2: GAMETOPHYTIC SELECTION

INTRODUCTION

Tolerance to toxic levels of B is expressed in callus (Huang and Graham, 1990). This character may also be expressed in single pollen cells. Mascarenhas *et al.* (1985) estimated 85% of genes expressed in pollen are also expressed in roots and shoots. Mulcahy and Mulcahy (1983) reported that pollen testing may be useful to predict sporophytic sensitivity to *Fusarium* phytotoxins, heavy metals and B. Genetic selection generally takes place in the sporophytic phase of a crop but it can also take place at the gametophytic phase, increasing selection efficiency. Advantages of gametophytic selection include the direct expression of genes in the haploid pollen without the complication of allelic interaction and the opportunity to apply a selection pressure twice in one generation. The aim of this study was to see whether by treating F_1 plants with high levels of B during pollen formation and fertilization, the genetic distribution of response to high levels of B in their progeny could be changed, compared with progeny from control F_1 plants.

MATERIALS AND METHODS

Three *Hordeum vulgare* cultivars were crossed in two B tolerant by intolerant combinations and the F_1 seed derived from these crosses was used in the experiment. The three cultivars were Sahara 3771, CM 72 and Stirling. Sahara 3771 expressed a high level of tolerance to B toxicity and CM 72 a moderate level of tolerance, relative to Stirling a susceptible line (Boyd *et. al.*, 1988). Five pots each of Stirling x CM 72 F_1 and Stirling x Sahara 3771 F_1 and 3 pots each of Sahara 3771, CM 72 and Stirling were planted for each treatment level.

Seed was planted directly into potting soil in Decor™ self watering pots. They represented a closed system with respect to applied nutrients. Each pot was fertilized with 3 g of Nitrophoska™ slow release fertilizer and Topsol™ as required, and watered from below by capillarity, by filling wells with deionised water. At day 56, prior to anthesis, the B treatments were applied, by watering a solution into the well, to apply 0 (B0), 40 (B40) or 80 (B80) mg

kg^{-1} B as boric acid to calculated on the basis of soil weight. This resulted in no, moderate and severe B toxicity symptoms respectively in Stirling (Figure 5.1).

F_2 plants were tested for tolerance to B in a hydroponic system under glasshouse conditions (see Chapter 4). Boron was added to the nutrient solution as boric acid at 20 mg l^{-1}. Seedlings were scored at 3 weeks from zero to twelve based on leaf area damage, as previously described. For the cross Stirling x CM 72, ninety two F_2 plants were tested from each treatment, with sixteen plants each of Stirling and CM 72. For the cross Stirling x Sahara 3771, ninety two plants were tested from the B0 and B80 treatments and ninety one from the B40 treatment. For the B0 and B40 treatments sixteen plants each of Sahara 3771 and Stirling were tested and for the B80 treatment fifteen of each were tested.

RESULTS

F_2 populations from each of the two families, Stirling x Sahara 3771 and Stirling x CM 72 from each treatment, were tested for B tolerance (Figure 5.4 and 5.7). Contingency tables were constructed to compare the frequency distributions of damage scores for each treatment. Some B score classes were grouped to ensure that expected values in each class exceeded five. No significant differences between distributions due to treatment of F_1 plants were observed for the F_2 populations from either cross. For the progeny from the cross Stirling x CM 72, X^2_8 = 11.97 and Stirling x Sahara 3771, X^2_{12} = 10.03.

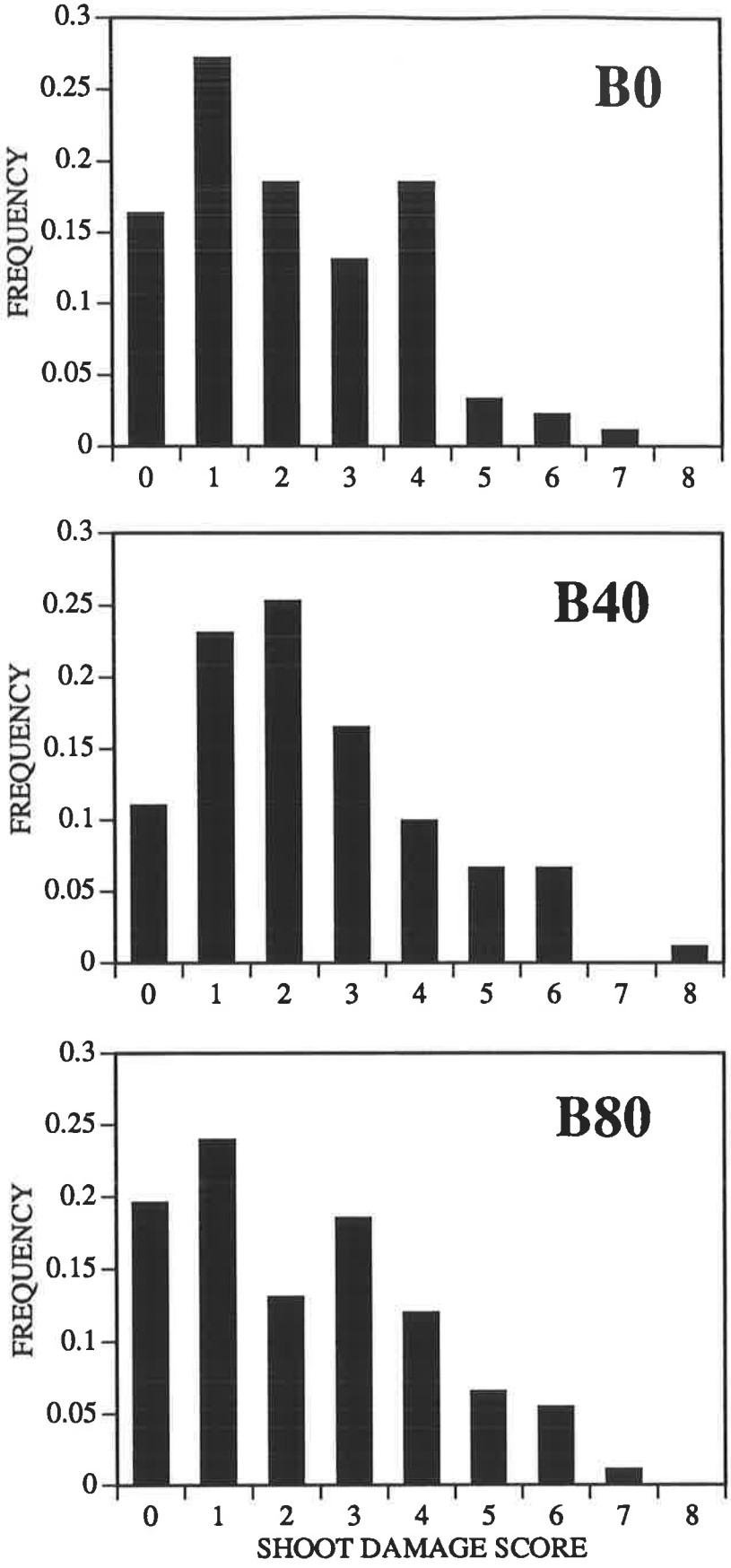


Figure 5.4. Shoot damage scores for F_2 progeny from the cross Stirling x Sahara 3771, when the F_1 parent had been subjected to 0, 40 and 80 $mgkg^{-1}$ boron prior to anthesis. The distributions are not significantly different at the 5% significance level (X^2_{12}= 10.028).

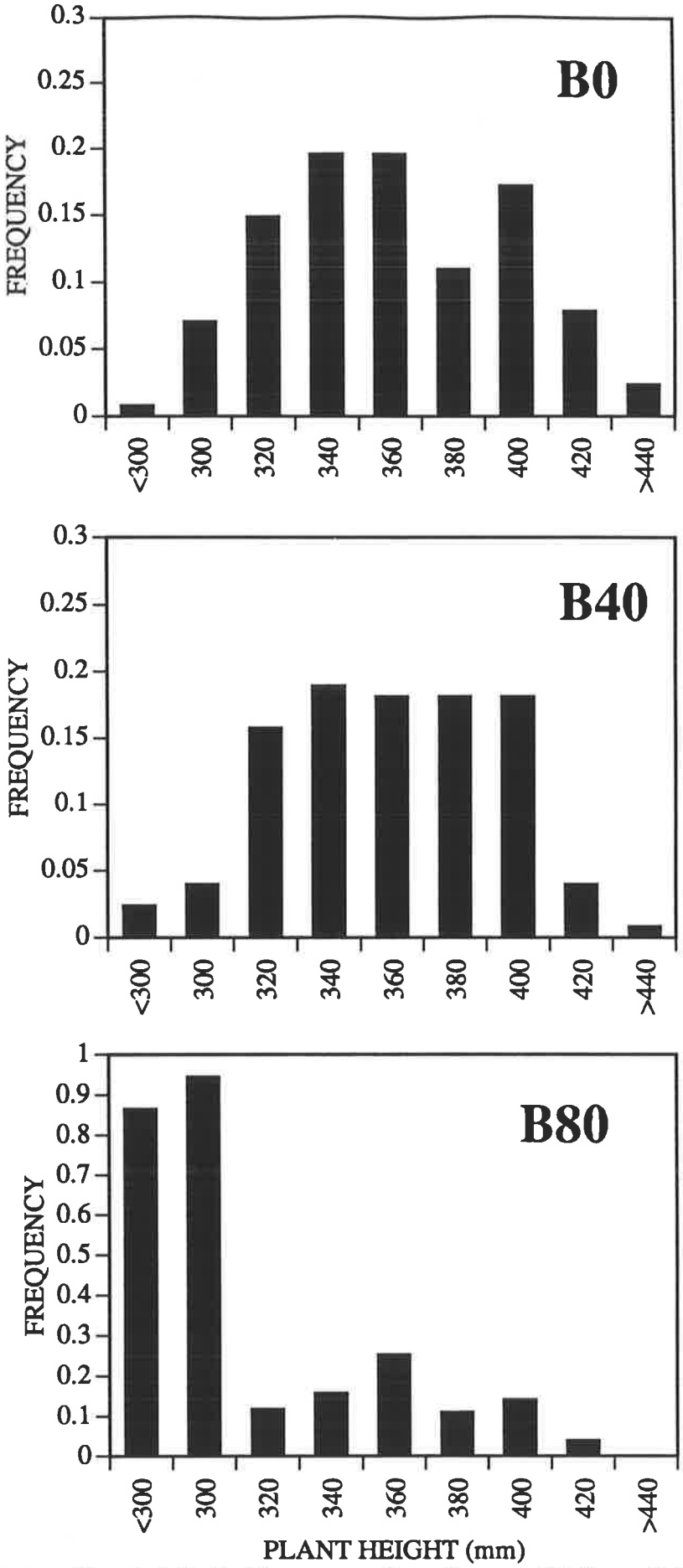


Figure 5.5. Plant height for F_2 progeny from the cross Stirling x Sahara 3771, when the F_1 parent has been subjected to 0, 40 and 80 mgkg^{-1} boron prior to anthesis. The distributions are significantly different at the 5% level ($X^2_{14} = 25.706$).

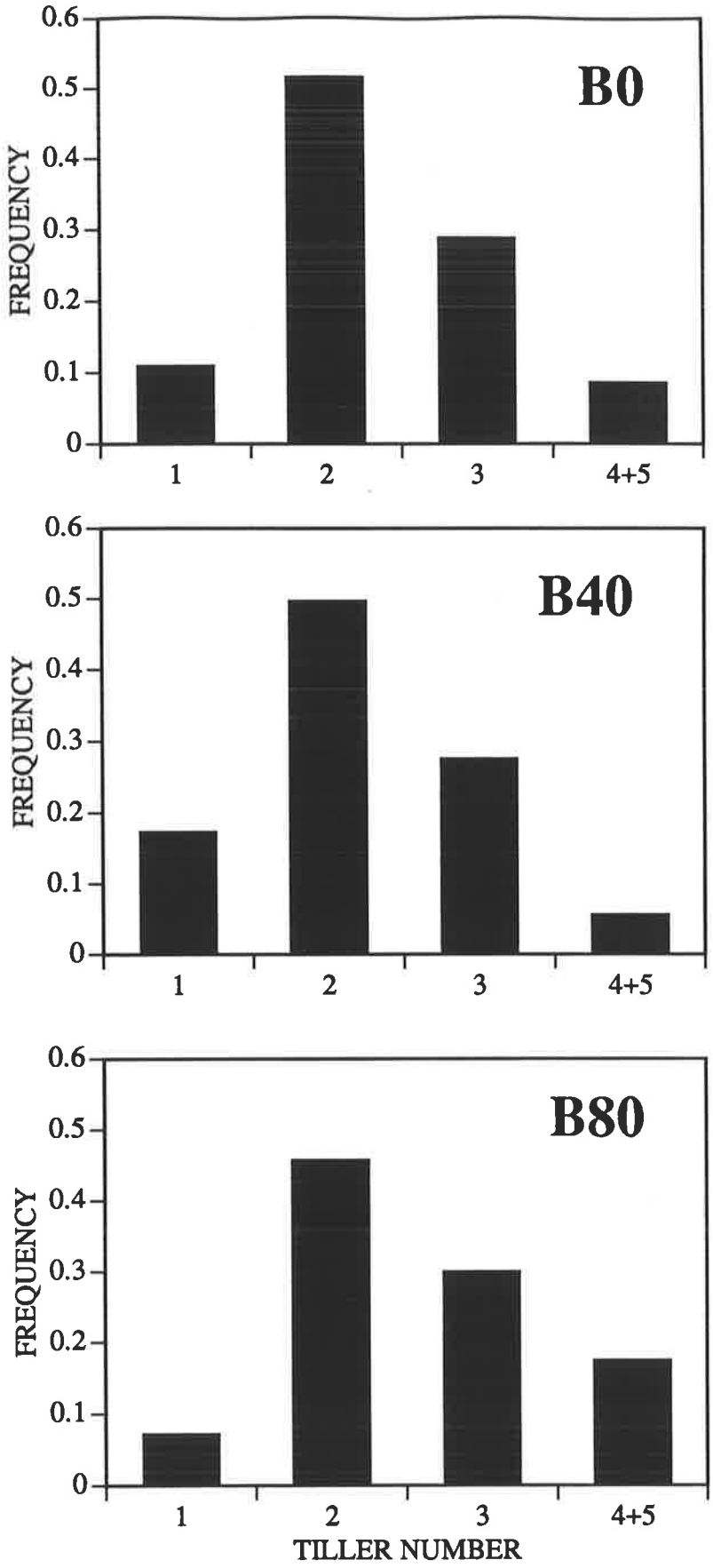


Figure 5.6. Tiller number for F_2 progeny from the cross Stirling x Sahara 3771, when the F_1 prarent has been subjected to 0, 40 and 80 mgkg^{-1} boron prior to anthesis. The distributuions are significantly different at the 5% level ($X^2_6 = 15.464$).

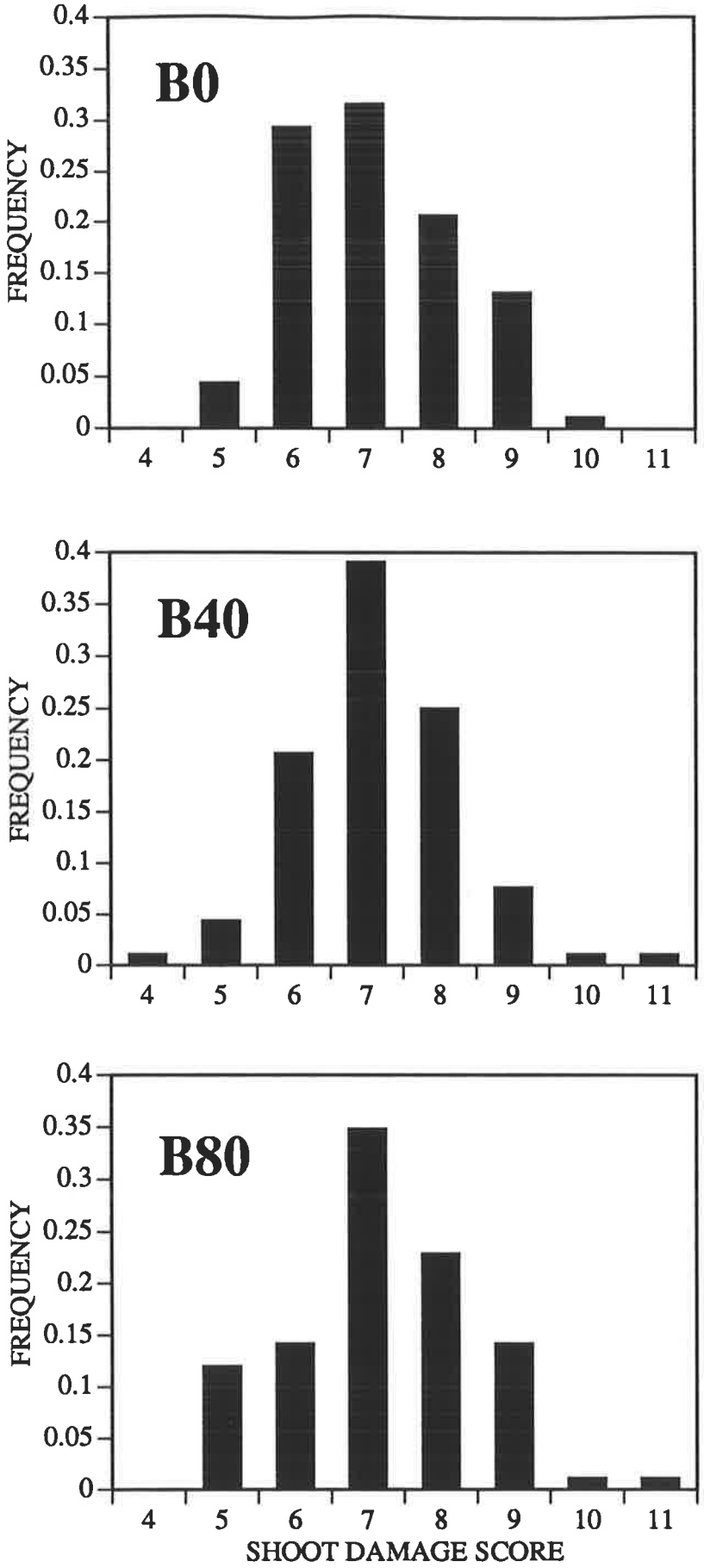


Figure 5.7. Shoot damage scores for F_2 progeny from the cross Stirlng x CM 72, when the F_1 parent has been subjected to 0, 40 and 80 mgkg^{-1} boron prior to anthesis. The distributions are not significantly different at the 5% level ($X^2_8 = 11.973$).

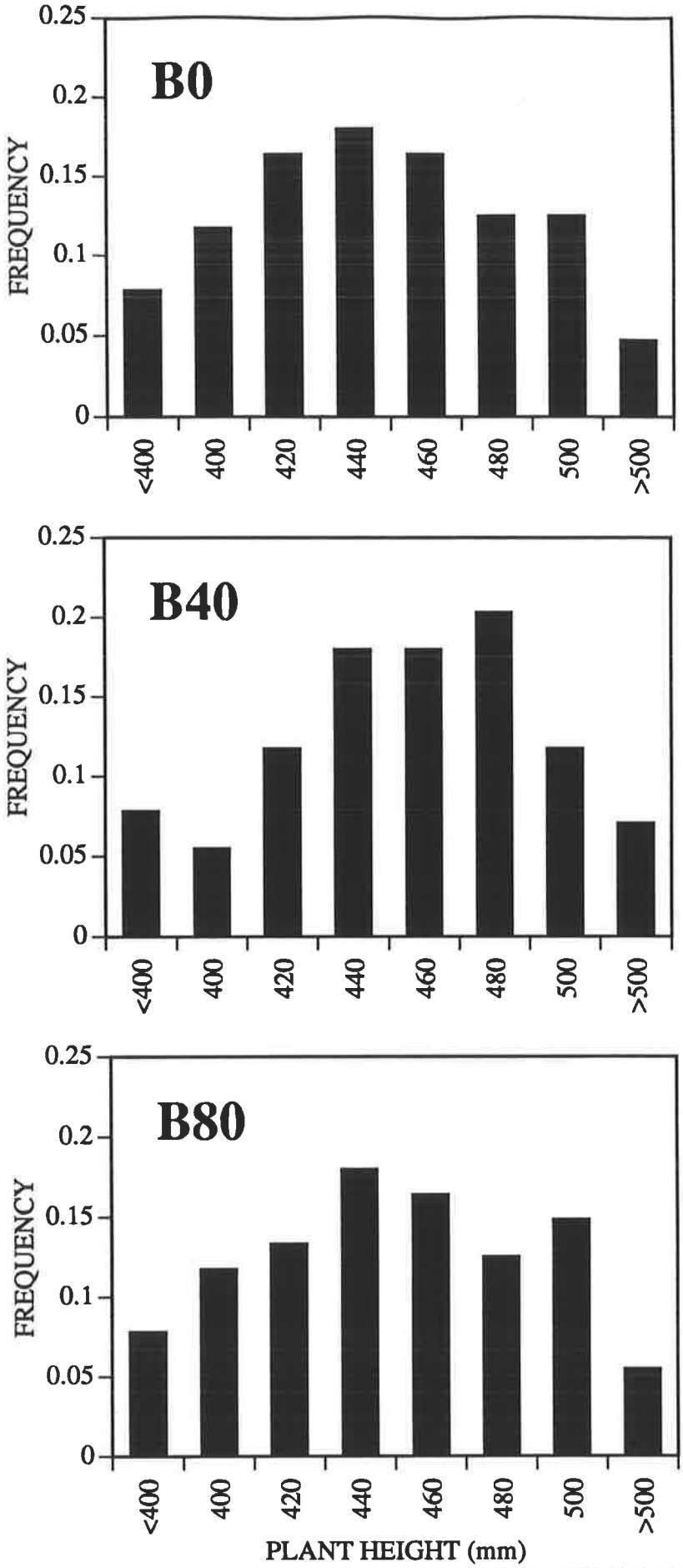


Figure 5.8. Plant height for F_2 progeny from the cross Stirling x CM 72, when the F_1 parent has been subjected to 0, 40 and 80 $mgkg^{-1}$ boron prior th anthesis. The distributions are not significantly different at the 5% significance level ($X^2_{14} = 9.244$).

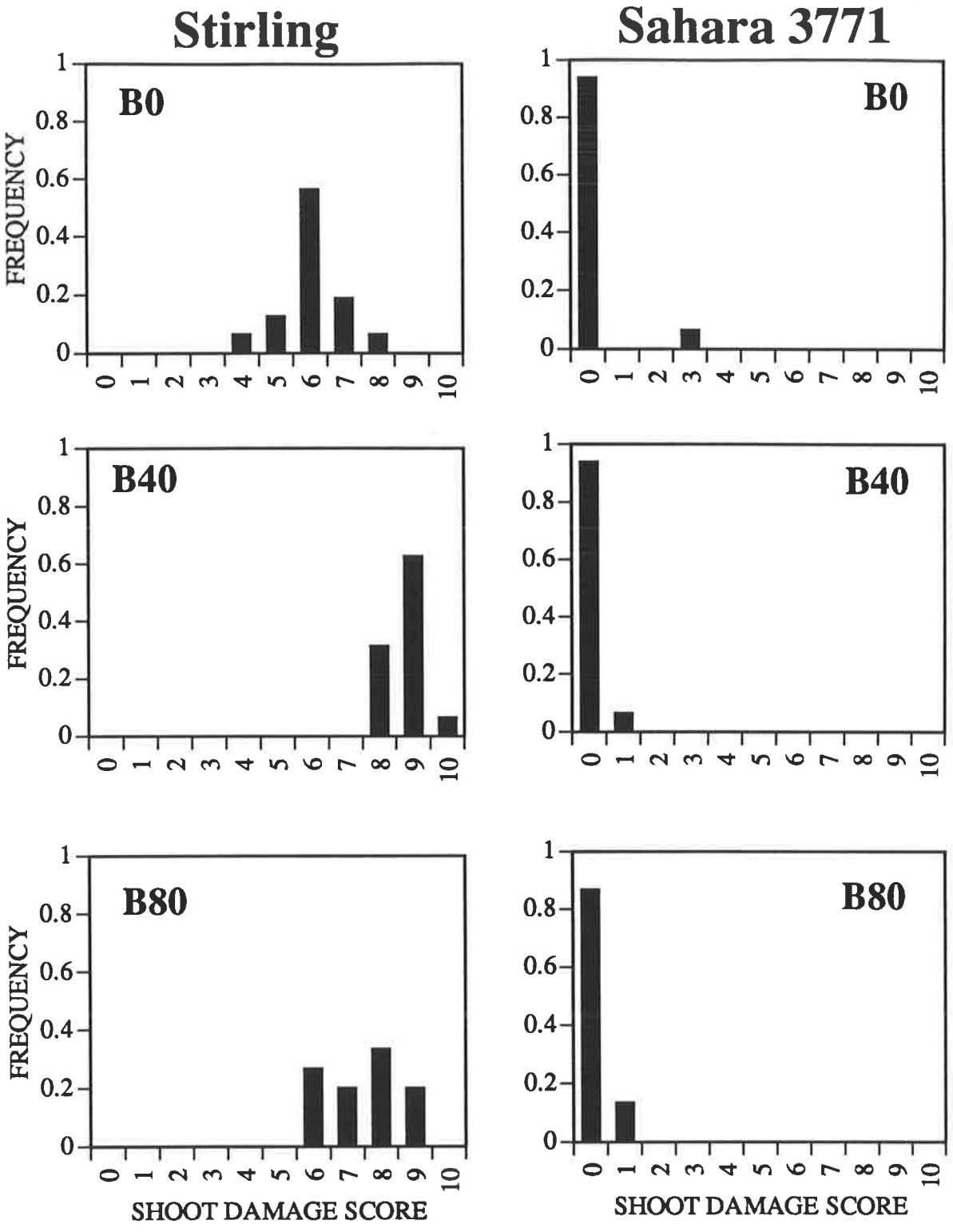


Figure 5.10. Shoot damage scores for parents from the cross Stirling x Sahara 3771, when the previous generation had been subjected to 0, 40 and 80 mgkg^{-1} boron prior to anthesis.

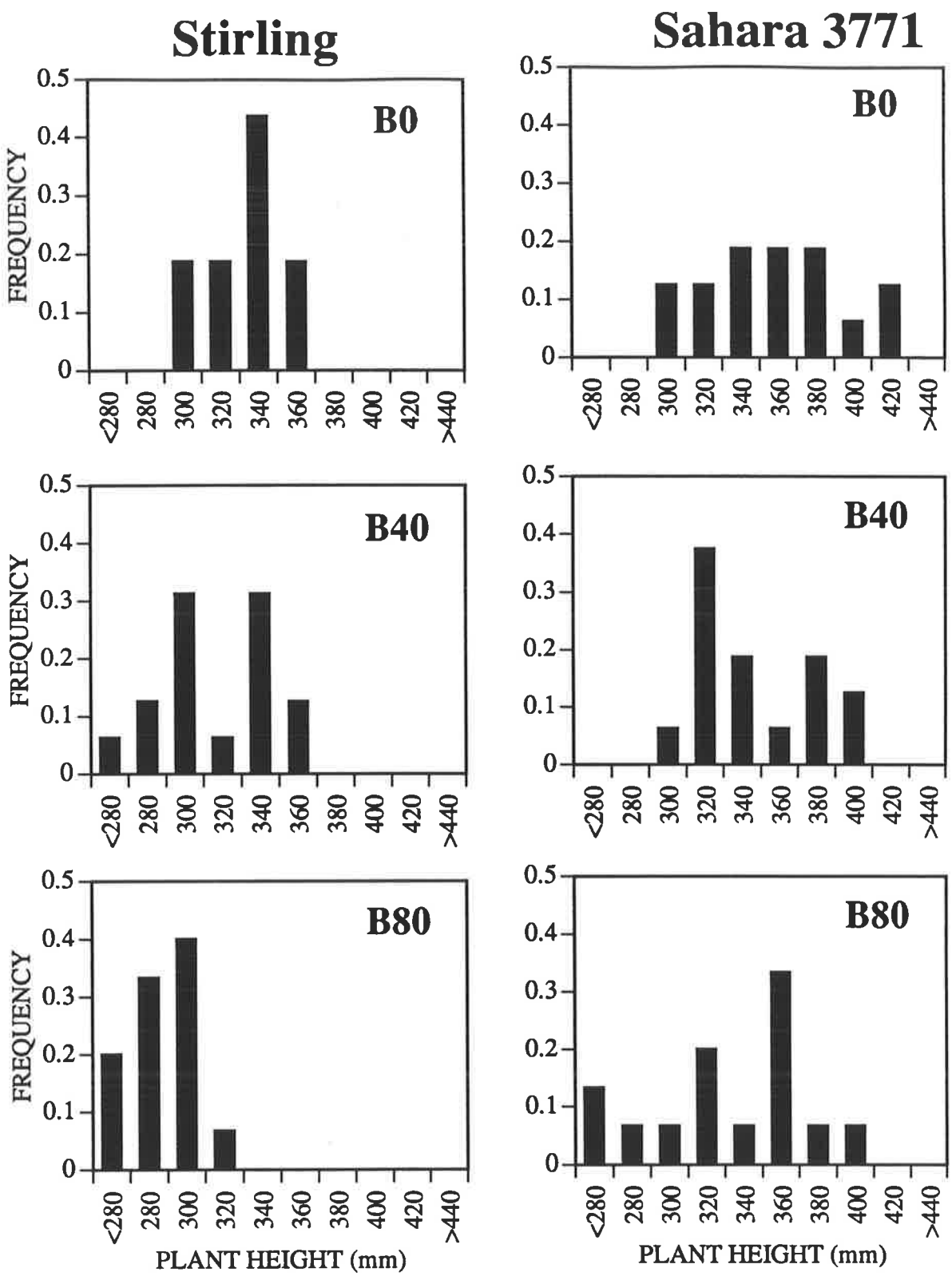


Figure 5.11. Plant height for parents from the cross Stirling x Sahara 3771, when the previous genereation had been subjected to 0, 40 and 80 mgkg^{-1} boron prior to anthesis.

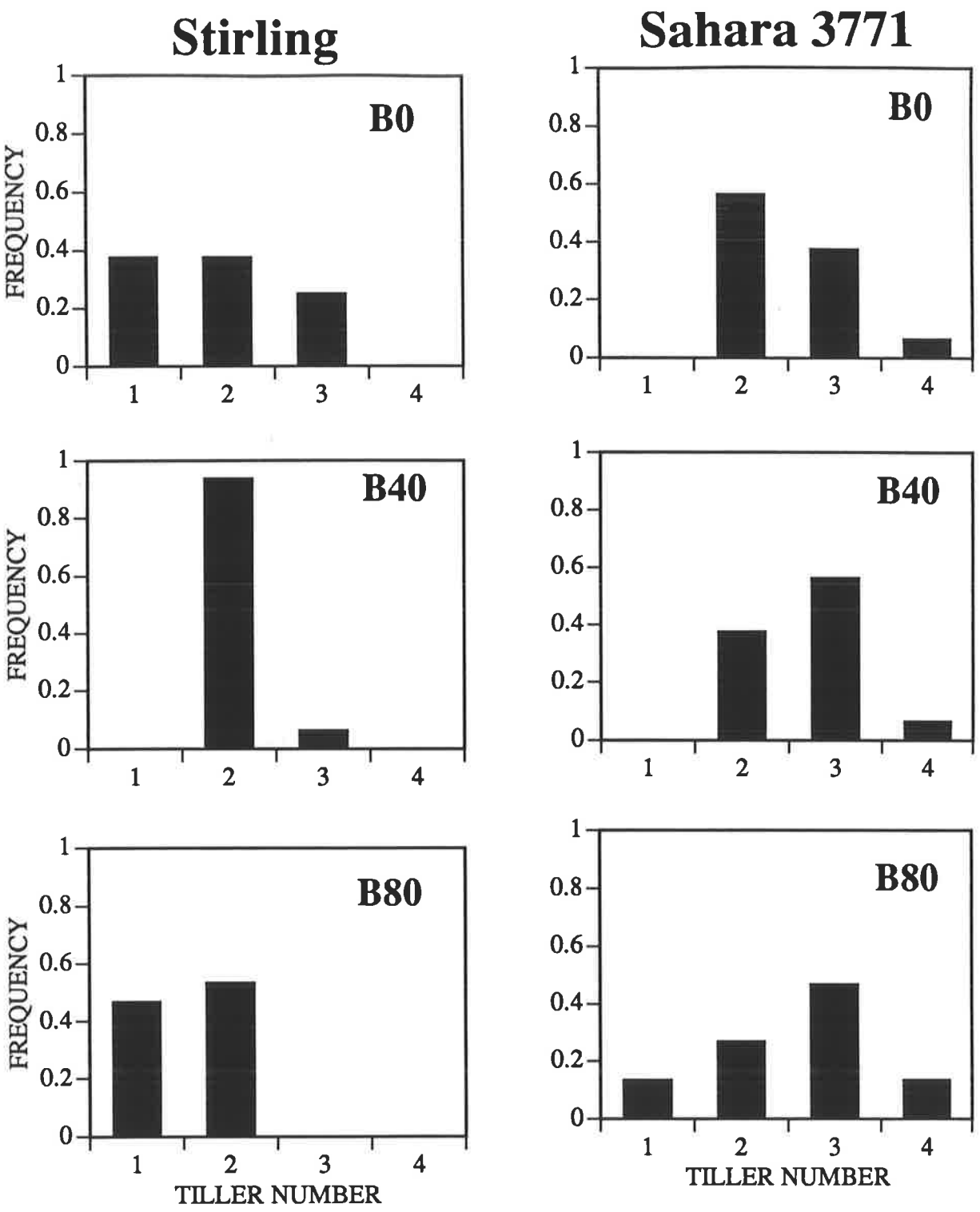


Figure 5.12. Tiller number for parents from the cross Stirling x Sahara 3771, when the previous generation was subjected to 0, 40 and 80 mgkg^{-1} boron prior to anthesis.

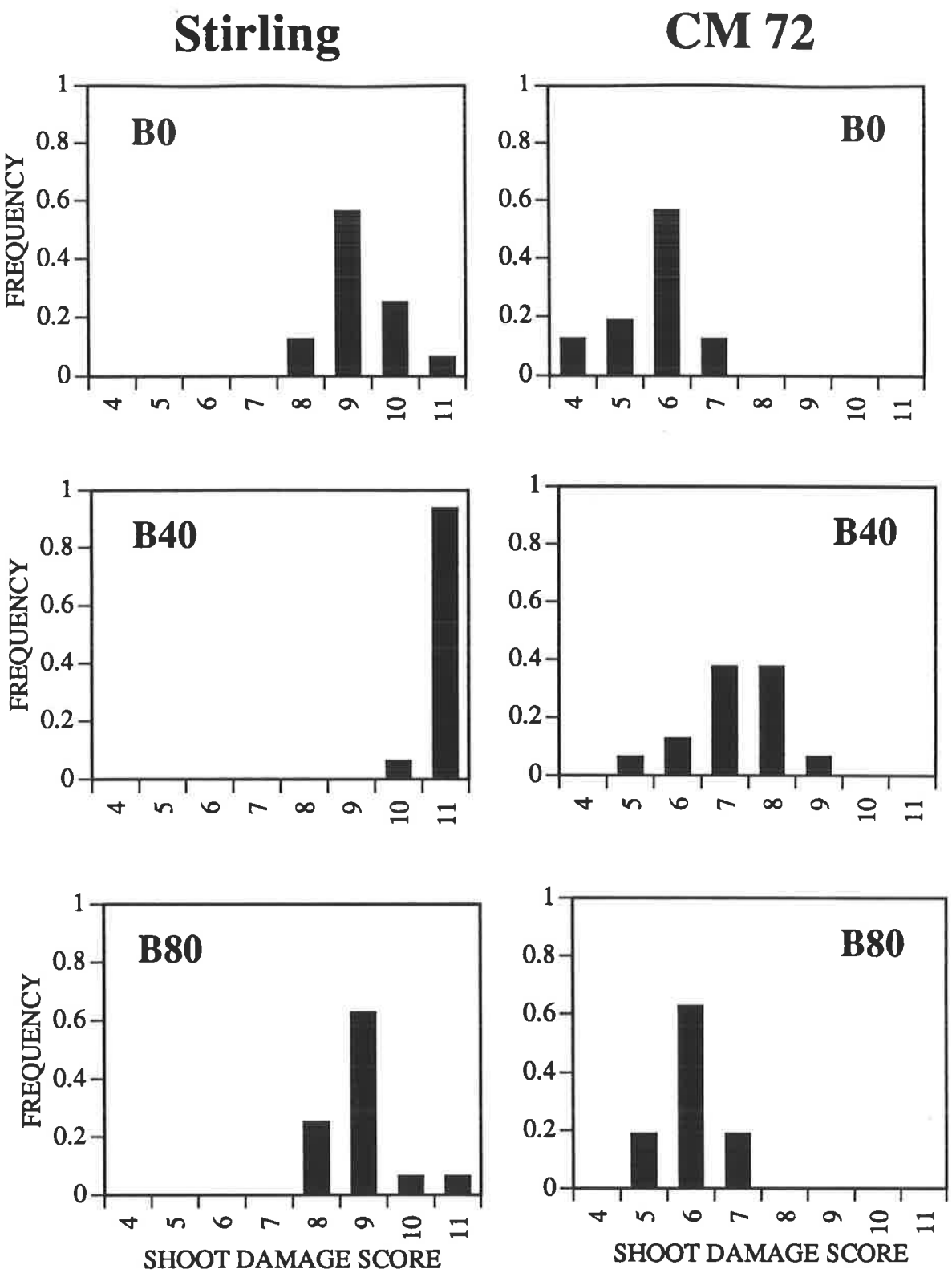


Figure 5.13. Shoot damage scores for parental controls for the cross Stirling x CM 72, when the previous generation was been subjected to 0, 40 and 80 $mgkg^{-1}$ boron prior to anthesis.

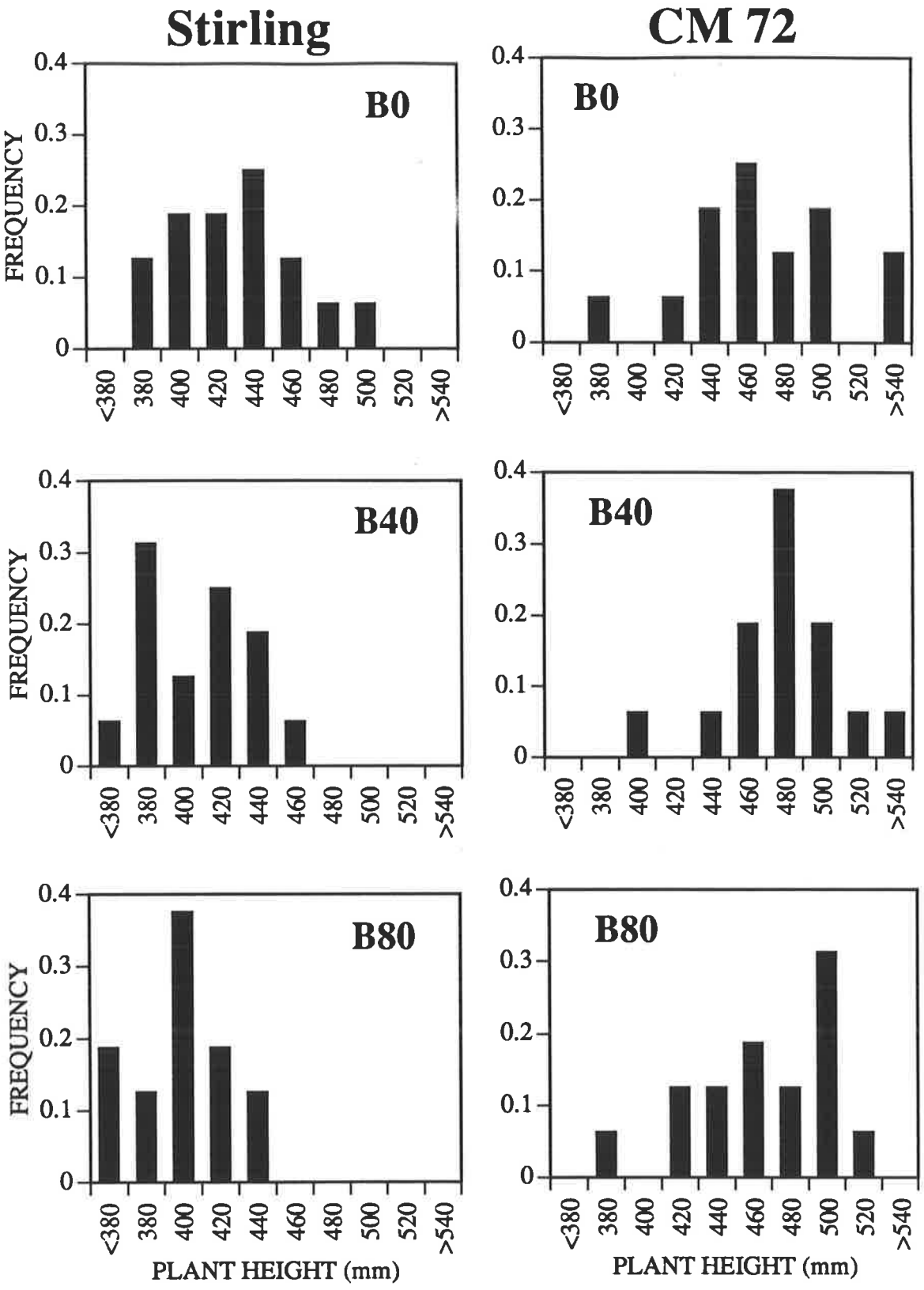


Figure 5.12. Plant height for parents from the cross Stilrling x CM 72, when the previous generation had been subjected to 0, 40 and 80 $mgkg^{-1}$ boron prior to anthesis.

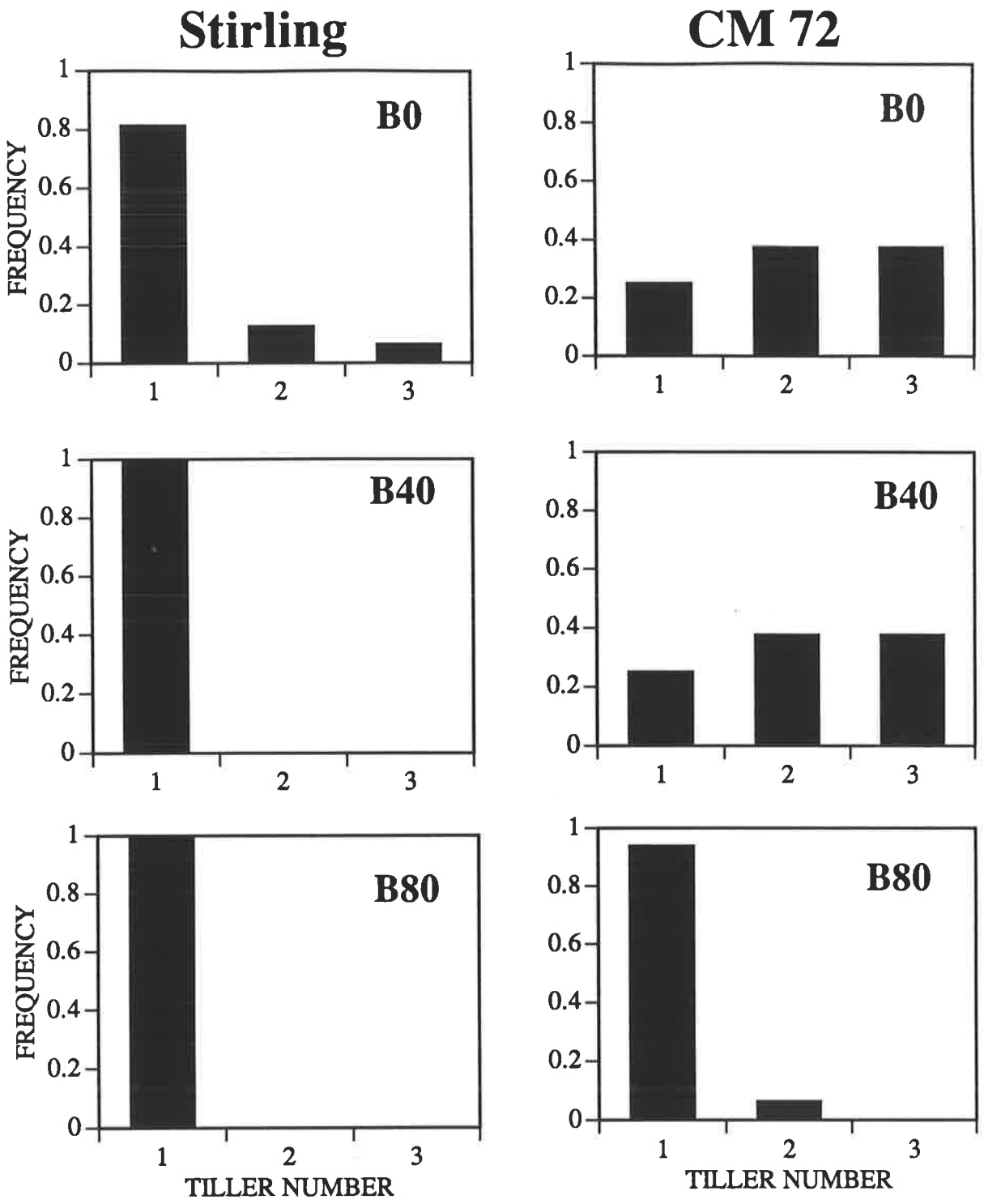


Figure 5.15 Tiller number for parents from the cross Stirling x CM 72, when the previous genereation have been subjected to 0, 40 and 80 mgkg^{-1} boron prior to anthesis.

Contingency tables were also constructed for plant height and tiller number. Significant differences were seen between treatments for the cross Stirling x Sahara 3771, for both plant height (X^2_{14} = 25.70) (Figure 5.5) and tiller number (X^2_6 = 15.46) (Figure 5.6). For the cross Stirling x CM 72 no significant difference was seen between plant height for the three treatments (X^2_{14} = 9.24) (Figure 5.8) but a significant difference was observed between treatments for tiller number (X^2_4 = 57.36) (Figure 5.9).

Frequency distribution for parental lines from the cross Stirling x Sahara 3771 are shown in Figures 5.10, 5.11 and 5.12. Shoot damage scores are highest for Stirling from the B40 treatment, followed by the B80 treatment. Sahara 3771 is not affected. Plant height decreases in Stirling as the B increased in the previous generation (Figure 5.11), but is reduced in Sahara 3771 only by B80 (Figure 5.11). Tiller number is slightly increased in Stirling and Sahara 3771 by B40 and decreased by B80, compared to B0 Figure 5.12. Plots were made of frequency distributions for shoot damage score, plant height and tiller number for the parental lines from the cross Stirling x CM 72 (Figures 5.13, 5.14 and 5.15). For both Stirling and CM 72, shoot damage score was higher at the B40 treatment than at either B0 or B80. Plant height was reduced from B0 to B40 to B80 for Stirling, while little shift was seen for CM 72. For both parents, tiller number was reduced with increasing B treatments applied to the previous generation.

Fifty seeds from each line and treatment were weighed and 1g samples analysed for B concentration (Table 5.1). In general seed size of the three genotypes were ranked, CM 72 > Stirling > Sahara 3771, though at B40 Stirling seed size was less than Sahara 3771. With respect to seed size and treatment, no clear trend emerged. Seed concentration of B was clearly increased from treatment B0 to B40 and B80. The ranking of genotypes however were not consistent either between treatments or with whole plant responses to high B.

Table 5.2. Weight in grams of fifty seeds for each line after treatment. In brackets the concentration of B (mg kg^{-1} B) in seed is given. This measurement was based on a single 1g sample.

Population	Treatment (mg kg^{-1}B)		
	0	40	80
CM 72	2.83 (2.8)	1.71 (4.5)	1.57 (13)
Stirling x CM 72 F_2	2.10 (2.7)	2.48 (5.5)	2.37 (17)
Stirling	2.33 (2.2)	1.06 (7.6)	1.34 (15)
Stirling x Sahara 3771 F_2	1.96 (2.1)	2.43 (3.4)	1.61 (15)
Sahara 3771	1.58 (1.7)	1.08 (7.6)	1.20 (14)

CONCLUSIONS

This experiment was designed to test whether by creating a high B environment for pollen formation and pollen tube growth, intolerant pollen may be selected against or out-competed by tolerant pollen. This has not proven to be the case. For neither cross is there a significant difference between B treatments for shoot damage scores.

Two explanations as to why selection did not occur are proposed.

(1) The genes for tolerance to high levels of B are not expressed in pollen.

(2) Insufficient B is reaching the reproductive parts of the plant to impose a selection pressure.

It would be technically difficult to measure the amount of B reaching the reproductive parts of the plant. Vasil (1987) reported that the stigma, style and ovary, often contain high concentrations of B and that B occurs in pollen at about 0.7 mg per kg dry weight, while the stigma may contain ten times that level. The amount of B in pollen can be increased by the use of B-rich fertilizers, or by irrigation with B-rich water (Vasil, 1987), though these comments may only be relevant to low B situations. Expression of the B tolerance genes in the pollen could be tested by exposing it to different B levels *in vitro*.

Differences were observed between treatments in F_2 families for plant height and tiller number. This may be due to a retarding in early growth due to high seed B content, since it is the high treatment (B80) which shows an overall reduced plant height (Stirling x Sahara 3771) (Figure 5.5) and reduced tillering (Stirling x CM 72) (Figure 5.9), but this relationship does not hold for tiller number in the cross Stirling x Sahara 3771 (Figure 5.6). Nable and Paull (1990), however, found that in wheat, despite up to twenty times normal grain B concentrations, no detectable effect was observed on seedling emergence nor on growth. Seed size may have played a role. No clear trend emerges in relating treatment and seed weight for these lines. Though the B80 treated seeds from the cross Stirling x Sahara 3771 had reduced seed weight (Table 5.2), they produced unexpectedly high tillering plants Figure 5.6). Considering the landrace origins of Sahara 3771 it is possible that high tillering is a response to stress in this line, and its progeny. In fact this trend was also observed in Sahara controls.

The increase in shoot damage score at the B40 treatment for both Stirling and CM 72 (Figure 5.13) can be attributed to a growth/transpiration relationship. For plants derived from the B0 parent, growth and transpiration are normal. For those derived from B40 parents, while growth may be inhibited by increasing seed B, transpiration rate may still be normal, thus more B is taken up per leaf area. This situation would produce a larger proportion of leaf area expressing symptoms. At B80 the effect of the parental B treatment may be sufficiently severe on the seed, to inhibit both growth and transpiration rate in the resultant plants, thus less total B is taken up by plants.

EXPERIMENT 3: *IN VITRO* POLLEN GERMINATION - A PRELIMINARY INVESTIGATION

INTRODUCTION

Boron is required for pollen germination in many species. Cheng and McComb (1992) found that percentage germination of pollen of wheat cultivars differed, depending on the concentration of B in an *in vitro* germination medium. To assess whether tolerance to B toxicity is expressed in pollen, genotypes known to vary in tolerance, were tested for the response of their pollen to high levels of B *in vitro*.

MATERIALS AND METHODS

Media was prepared according to Cheng and McComb (1991) being 0.7% agar containing 300 mg l^{-1} $CaCl_2.2H_2O$ and 0.75 M raffinose. Boron was added at 40 (B40), 50 (B50), 60 (B60) and 70 (B70) mg l^{-1} H_3BO_3. Preliminary experiments (data not shown) indicated this to be the optimal range for *in vitro* pollen germination of barley. Plates were stored at room temperature at high humidity for 4 days.

Pollen from three barley genotypes was studied: Sahara 3771, CM 72 and Stirling. These genotypes are highly tolerant, moderately tolerant and intolerant to B toxicity in the sporophytic form. Plants were grown in standard potting soil with temperature regulated to between 14°C and 17°C, in a glasshouse. Heads bearing near mature pollen were cut from plants, stored in a humid environment at 4°C for 2 days, then ripened to pollen maturity under a 40W desk lamp.

Agar from each B level was cut to approximately 1 cm squares and placed on a microscope slide. This slide sat on a 2 cm square of sponge saturated with water, inside a 9 cm petri dish (Richter and Powells, 1993). Pollen was encouraged to fall directly from heads onto the agar. Dishes were sealed and left at 25°C for 1 - 2 hours.

Pollen grains were classified as either burst, intact or germinated (pollen tube greater than one pollen grain diameter) from five fields from each agar square (Figure 5.2). Percentage germination was calculated as: sum of pollen grains germinated/ total number of pollen grains counted x 100. The experiment consisted of two replicates performed consecutively in a single afternoon.

RESULTS

Table 5.3. Percentage germinated pollen from three barley genotypes on media containing 40, 50, 60 or 70 mg l^{-1} H_3BO_3.

Treatment	Genotypes					
	Stirling		CM 72		Sahara 3771	
	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
B40	**11.1**	6.9	8.0	9.8	28.5	0.0
B50	3.4	4.2	**55.9**	**26.1**	14.3	0.0
B60	7.3	**7.7**	22.8	22.1	**29.1**	**0.8**
B70	2.8	2.9	6.1	20.0	13.8	0.0

The results from the two replicates differed substantially, so they are presented separately. The greatest germination percentage for each genotype has been highlighted.

CONCLUSIONS

The three genotypes responded differently to the four B levels with respect to pollen germination. With the exception of Stirling at B60 in Rep 2, the data suggests a trend whereby the optimum B level for pollen germination of the three types may be rankin in the order: Sahara 3771 > CM 72 > Stirling. This trend is consistent with the pollen from Sahara 3771 requiring or tolerating a higher B concentration in the media than CM 72 and Stirling. Since pollen from Sahara 3771 germinated well at both B40 and B60 in replicate one, the higher rate may not be *required* for optimal germination. An investigation of the range of B concentrations over which pollen will germinate from various genotypes may help to determine whether B toxicity tolerance leads to B deficiency intolerance. This technique could in the future form the basis for a rapid screen for B tolerance, as has been suggested for certain herbicides (Richter and Powles, 1993).

This study should be considered preliminary, since a number of limitations need to be overcome before conclusions can be drawn confidently from this kind of experiment. Firstly, further studies to establish the optimal conditions for *in vitro* pollen germination for barley, are required. Cheng and McComb (1992) reported up to 81.7% pollen germination in wheat, where the maximum germination percentage obtained in this study was 55.9%. Secondly, the overall range in percentage germination varied considerably between the genotypes studied. Factors contributing to this variation include: different genetic backgrounds being expressed in the pollen; differences in flower morphology; and differences in flowering response to environmental factors, particularly different response to day length. This latter factor created difficulties in obtaining comparable pollen from the genotypes. Stirling is an early flowering cultivar, and Sahara 3771 late, so phenologically plants were not equal. Thirdly, pollen density can affect *in vitro* germination. A method needs to be devised to control more closely pollen density and distribution on media. To be able to draw conclusions about the expression of tolerance to B toxicity in pollen, a greater number of genotypes expressing different levels of tolerance to high B should be investigated, in highly replicated experiments, to overcome some of these difficulties.

SUMMARY

Several general conclusions can be drawn from this group of studies:

1. Boron uptake into protoplasts varied little between B tolerant and intolerant genotypes, under the experimental conditions, though a difference in growth response of callus derived from these lines in response to B had been observed (Huang and Graham, 1990). This result is consistent with the hypothesis that cell walls play a role in barley, in regulating B uptake.

2. Treatment of parent plants with toxic levels of B did not effect the level of tolerance of the progeny. This suggests that either pollen formation and germination is unaffected by high levels of B, or that the level of B to which pollen is exposed is restricted by the plant.

3. *In vitro* pollen germination and growth may be affected by the level of B in the media, suggesting that B tolerance *is* expressed in pollen.

Chapter 6

MAPPING OF BORON TOLERANCE GENES

INTRODUCTION

It is vital to locate agronomically important genes within the genome to aid in devising efficient methods for manipulating those genes. A number of factors determine the method(s) most appropriate to achieve this aim and the accuracy with which genes may be located. These include, the number of major genes determining the character, the degree of dominance or recessiveness they express and availability of resources, such as special genetic stocks, known isozymic polymorphisms or molecular probes.

Two approaches were taken to map the major genes determining tolerance to boron (B) toxicity. Firstly, recessive morphological marker stocks were crossed with Sahara 3771, the highly tolerant line, and with CM 72, the moderately tolerant line. An alternative approach involved the use of doubled haploid lines derived from the cross Sahara 3771 x Clipper.

MATERIALS AND METHODS

Linkage with Morphological Markers

The morphological marker stocks were R.I. Wolfe's Multiple Recessive Marker Stock 1 (MS 1) and 3 (MS 3) (Wolfe, 1983). Seed of these stocks was obtained from the Australian Winter Cereals Collection in Tamworth, Australia and are described in Table 6.1. Spike characters can be seen in Figure 6.2.

MS 1 was used as the female parent in crosses with both CM 72 and Sahara 3771. MS 3 was the female parent in a cross with CM 72. F_1 seed was obtained, and self pollinated to produce

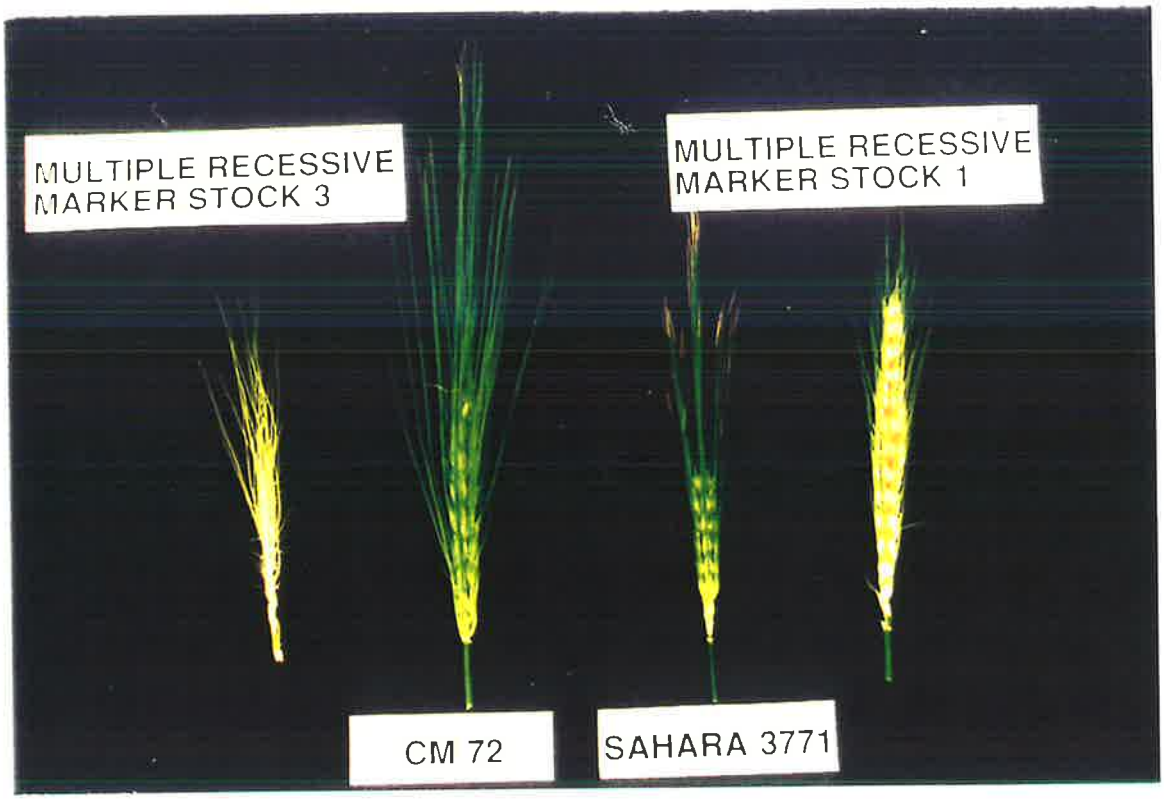


Figure 6.1. Spikes from barley lines MS 3, CM 72, Sahara 3771 and MS 1, expressing differences in awn length and lemma colour.

Figure 6.2. Leaf damage after treatment at 20 mg l^{-1} B of barley lines, Clipper, Sahara 3771 and two doubled haploid lines.

F_2 seed. The segregating F_2 progeny were assessed for tolerance to high B levels using the hydroponic system described in Chapter 4, transplanted to normal potting soil in the glasshouse and grown to maturity. At the appropriate stage the plants were scored for expression of each of the recessive marker characters. Boron tolerance and morphological characters were assessed pair wise for independent segregation using a 2 x 2 chi-square test.

Linkage with RFLP and Other Markers in Doubled Haploid Lines

Sahara 3771 x Clipper doubled haploid lines were kindly provided by Dr. R.K.M. Islam and Dr K.W. Shepherd. They were produced by the *Hordeum bulbosum* method using embryo culture then chromosome doubling using colchicine. Each doubled haploid plant was self pollinated to produce progeny which are homozygous at all loci and genetically identical with each other. Each line reflected the diploid complement of the genotype of F_1 gametes, and was homozygous at each locus for either the Sahara 3771 or Clipper allele. Five plants from each line were tested for tolerance to high levels of B using a randomised block design in the hydroponic system described in Chapter 4, and applying 20 mg l^{-1} B as boric acid. The range of response is shown in Figure 6.2. The parental lines were compared for restriction fragment length polymorphisms (RFLPs), then DNA probes selected and applied to the doubled haploid population. This work was kindly performed by Mr A. Karakousis and Mr N. Kavukis in the laboratory of Dr P. Langridge. Doubled haploid lines were also assessed for head type and tolerance to Cereal Cyst Nematode (CCN). Boron tolerance status and RFLPs were analysed pair wise using Yates' corrected 2 x 2 chi-square analysis for independent segregation.

RESULTS

Linkage with Morphological Markers

The segregation of each morphological character was compared, for each F_2 population, using a Yates' corrected chi-square test, with a 3:1 ratio (Table 6.1). This is the ratio of dominant to recessive phenotype expected for a character determined by a single recessive gene. The probability values express the probability of obtaining the observed deviation between the observed and expected values, by chance alone.

For the F_2 population derived from the cross MS 1 x Sahara 3771 (N = 121 F_2s) 2 x 2 chi-square analysis was performed (using Yates' correction) on data classified for boron tolerance and for expression of morphological markers. For the populations derived from the crosses MS 1 x CM 72 (N = 46 F_2s) and MS 3 x Sahara 3771 (N = 61 F_2s) Fisher's r x c analysis was applied (Hancock, 1975) (Table 6.1). This test does not assume a minimum expected value of 5 for each cell of the contingency table. For each of the three populations, frequency distributions for tolerance to B were compared using Yates' corrected chi-square, with a 3:1, a 9:7 and an 11:5 ratio (see Chapter 4 for an explanation of the ratios). Class divisions were made according to parental performance with respect to leaf damage score. In no case was the resultant value significant. Class divisions of the F_2 population for 2 x 2 analysis then were based on parental means alone. These class divisions were made between the score of 8 and 9 for MS 1 x Sahara 3771; and between 9 and 10 for both MS 1 x CM 72 and MS 3 x CM 72. The value in the "Ind." column describes the probability of obtaining a deviation between the observed and expected values by chance alone, with the expected values being those predicted by a null hypothesis for independent segregation between the morphological character and B tolerance.

The characters for which no probabilities are listed will be scored when the plants reach maturity, but were unavailable for presentation in this publication. Similarly, the F_2 population derived from the cross MS 1 x Sahara 3771 is available for analysis in the future.

Linkage with RFLP and Other Markers in Doubled Haploid Lines

Mean leaf damage scores produced under high B conditions for Clipper x Sahara 3771 doubled haploid lines were compared with ratios expected based on a one, two or three major gene difference between Clipper and Sahara 3771, with respect to B tolerance. For doubled haploid lines these expected ratios are 1:1, 3:1, and 7:1, respectively. In no comparison was the resultant chi-square value significant. The frequency distribution for the 43 lines tested with respect to mean leaf damage score is shown in Figure 6.3.

Table 6.1. Morphological markers combined in Wolfe's Multiple Recessive Marker Stocks 1 and 3, with gene symbols and chromosome locations are listed. This information was tabulated from a number of sources (Franckowiak (1987); Mr M. Mackay (pers. comm.) and von Wettstein-Knoles (1991)). The value in the 3 : 1 column describes the probability of obtaining a deviation between the observed and expected values by chance alone, with respect to a 3 : 1 segregation of dominant to recessive phenotype for each morphological character. The value in the Ind. column describes the probability of obtaining a deviation between the observed and expected values by chance alone, with the expected values being those predicted by a null hypothesis for independent segregation between the morphological character and B tolerance. A significant deviation from the null hypothesis is indicated by *.

Marker Stock	Gene Symbol	Chromosome Location	Character	x CM 72		x Sahara	
				P 3 : 1	P Ind.	P 3 : 1	P Ind.
MS 1	*wx*	1S	waxy endosperm				
	n	1L	naked caryopsis				
	lk2	1L	short lemma awn	>.95	0.29	.7-.9	.5-.7
	*wst ,,k**	2L	white striped	.01-.05*	0.30	.05-.01*	.5-.7
	al	3S	albino lemma & node	>.95	1.00	.7-.9	.5-.7
	o	6L	orange lemma & rachis	>.95	0.71	.05-.01*	>.95
	r	7L	semi-smooth awns	.3-.5	1.00	.2-.3	>.90
	s	7L	short-haired rachilla				
	log =lga	?	short outer glume awn				
MS 3	*lk2*	1L	short lemma awn	.7-.9	0.48		
	n	1L	naked caryopsis				
	yh	4S	yellow head	.2-.3	0.70		
	nec	5L	necrotic leaf spot				
	trd	5L	third outer glume	.7-.9	0.31		
	o	6L	orange lemma & rachis	.7-.9	0.73		
	lgo	?	long outer glume awn				
	s	7L	short-haired rachilla				

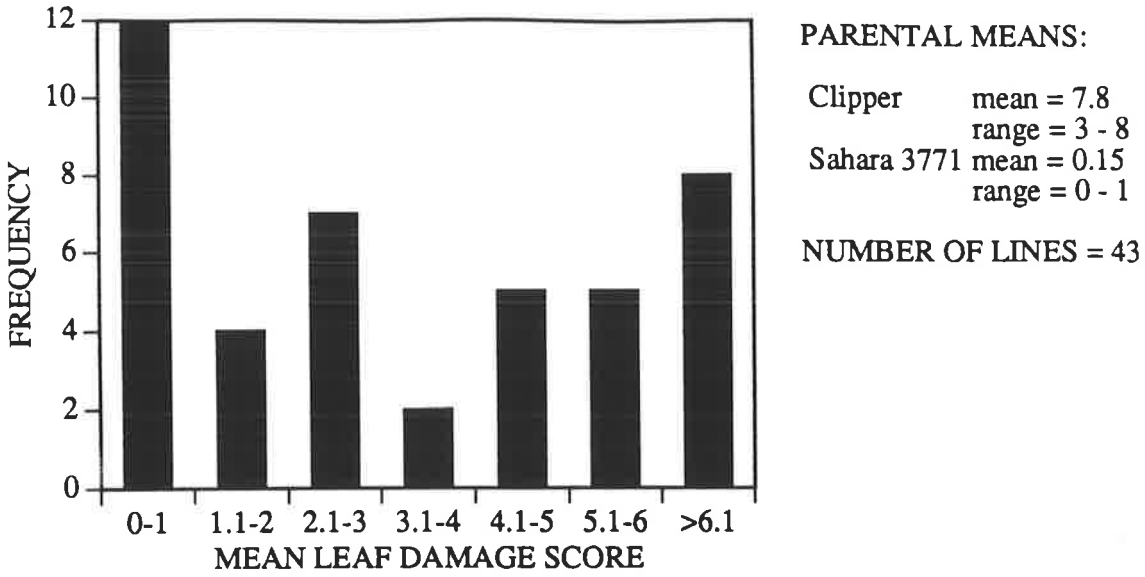


Figure 6.3. Frequency distribution of mean leaf damage score for Clipper x Sahara 3771 double haploid lines.

Jansen (1992) calculated that if a character is determined by two unlinked loci, by producing at least sixteen doubled haploids it can be expected with a probability of 0.95 that all homozygote types are represented by at least one line. If the character is determined by three genes, thirty eight lines are required. It can be expected then that whether the parental lines differ by two or three genes, all genotypes will be represented in our set of lines. Hanson (1959) calculated minimum family sizes required to differentiate between two expected proportions. To compare a 1:1 and a 3:1 ratio 38 lines are required based on 0.05 probability level. However 101 lines are required to distinguish between a 3:1 and a 7:1 at the 0.05 level of significance. More doubled haploid lines therefore need to be tested to distinguish between a two- or three-gene difference model. Alternatively, considering the work involved in producing doubled haploids, analysis of ordinary F_2 plants from a cross between Clipper and Sahara 3771 may be useful in deciding on a two or three major gene difference between these lines.

Since the true nature of the segregation taking place in this population is unknown, class division with respect to B tolerance was based upon the performance of the parental lines Clipper and Sahara 3771. Tolerant lines were defined as those with a score of ≤3, and intolerant as >3, for leaf damage area. Each doubled haploid line was assessed for expression of head type, tolerance to cereal cyst nematode (CCN) and a number of RFLPs. Each of these

traits were tested for genetic independence from B tolerance classification, using a Yates' corrected 2 x 2 chi-square analysis (Table 6.2).

Table 6.2. Chi-square values from 2 x 2 analysis of data from Clipper x Sahara 3771 doubled haploids. The probabilities listed are the probabilities of obtaining the observed results or worse by chance given that the character and B tolerance are genetically independent. Significant deviations from independence are denoted *.

Character	Arm Location	N	X^2_1	Probability
CDO475	1S	42	0.4474	.50-.70
BCD129	1S	27	0.0002	>.95
CDO673	1L	42	0.0057	.90-.95
6row/2row head (*V*)	2L	42	2.47	.10-.20
BCD175	2S	42	1.0082	.30-.50
CDO588 (BAMH1)	2L	31	4.5429	.01-.05*
CDO588 (DRA1)	2L	43	0.3361	.05-.10
WG645	2L	42	0.0100	.90-.95
WG464	4L	42	0.2983	.50-.70
WG114	4L	19	0.0061	.90-.95
KSUG10	4D (of wheat)	37	0.0274	.70-.90
CDO105a	5L	43	0.1010	.70-.90
BCD342	6S	42	0.0649	.70-.90
BCD340	6L	42	0.3141	.50-.70
IPSR154	6L	41	0.5346	.30-.50
CDO745	7S	42	3.9377	.01-.05*
IPSR128	7L	36	1.1496	.20-.30
CCN Tolerance	?	38	0.0854	.90-.95
CDO105b	?	21	0.0358	.70-.90

CONCLUSIONS

Linkage with Morphological Markers

No significant deviations from independence were observed between B tolerance and any of the morphological markers scored so far. Thus it is unlikely that major genes coding for tolerance to B are located near these morphological marker genes on chromosome arms 1L, 2L, 3S, 4S, 5L, 6L or 7L. A more extensive coverage of the genome will be achieved when the remaining characters are scored.

Significant deviations from the expected 3:1 segregation for dominant to recessive phenotype were observed for the white striped character in both MS 1 x CM 72 and MS 1 x Sahara 3771 populations, and for the character orange lemma and rachis for the MS 1 x Sahara 3771 population. A deficiency of plants expressing this character was also observed by Franckowiak (1987), though the character was then thought to be the character ribbon grass (*rb*) (Franckowiak, pers. comm.), and was attributed to poor expressivity. Another explantation could stem from the fact that a small percentage of plants in the MS 1 x Sahara 3771 and MS 3 x CM 72 F_2 populations had not produced heads at all or of sufficient age to to score for morphological characters. If lateness of flowering (or it may be a cold treatment requirement) was linked genetically with orange lemma, it may lead to a deficiency to that phenotype at the time of scoring.

MS 1 has recently been utilized by Shin *et al.* (1990) in a cross with the European two-rowed cultivar Apex, and the progeny assessed for a range of protein and molecular markers. Thus these populations have the potential to be utilized further for the mapping of B tolerance and other agronomically important characters.

Linkage with RFLP and Other Markers in Doubled Haploid Lines

The aim of this study was to gain information concerning the association of major genes coding for tolerance to B with characters of known chromosome location, through linkage analysis. Molecular markers as a rule do not express dominance, they express a high level of polymorphism, they can be scored at early stages of plant growth, and are largely unaffected by

environmental factors. Thus, the use of molecular markers may overcome some of the difficulties experienced in the use of morphological marker stocks for the same purpose. Double haploid lines are homozygous at all loci, and therefore recessive genes are not masked by dominance. Many genetically identical individuals can be produced from a doubled haploid plant, and therefore are able to be assessed for many characters.

Each doubled haploid line was assessed for tolerance to high B and also for head type, response to Cereal Cyst Nematode (CCN), and banding pattern for a range of RFLPs. Association was determined by a simple 2 x 2 chi-square analysis, where doubled haploid plants were classified as either tolerant or intolerant to B, with a leaf damage score of ≤ 3 or >3 respectively; 6-rowed or 2-rowed head type; resistant or non-resistant to CCN; and A type or B type with respect to banding pattern for each RFLP probe tested. Segregation deviated significantly from independence between B tolerance and two of the probes which map to a locus (Table 6.2). These markers are CDO588, located on the long arm of chromosome 2 and CDO745, located on the short arm of chromosome 7. Though the association is not strong, and CDO588 showed non-independence if BAMH1 was the endonuclease, but not with DRA1, it can be concluded that it would be worthwhile to investigate further, association between tolerance to B and other markers located in these chromosome regions. A larger number of doubled haploid families would allow linkage assessments to be made with considerably more confidence.

Chapter 7

DISCUSSION

The following general conclusions can be made about the genetic and physiological determination of boron (B) tolerance in barley.

1. Tolerance to B toxicity is, in the crosses studied, determined largely by genetic factors, and thus is amenable to improvement through selection.
2. In the field, genotype by environment interactions may be large for response to B, and considerable environmental variation is experienced from year to year. Thus, selection based on field data alone could be unreliable, particularly if data are not available for many sites over many years.
3. The degree of genetic variability for tolerance to high soil B in the barley cultivars currently grown in southern Australia is smaller than for wheat (Cartwright *et al.*, 1987); however sources of tolerance are available within the *H. vulgare* and related gene pools. Unlike wheat, however, these sources are often otherwise agronomically unsuited to commercial production in southern Australia.
4. Tolerance to B is expressed as a semi-dominant trait, though like wheat (Paull *et al.*, 1991) under high levels of stress, tolerance can behave as a recessive trait.
5. Genetic effects are likely to be largely additive, although some interaction between loci is proposed, whereby a resistant phenotype is produced in the presence of at least two dominant alleles at any of two or three loci (see Figure 4.11).
6. There appears to be a difference of at least three major genes coding for tolerance to high soil B between barley cultivar Stirling (intolerant) and the landrace Sahara 3771 (highly tolerant). Boron tolerance is quantitative in nature, that is, it displays a continuous distribution of expression. This

suggests that either environmental factors modify gene expression or that an additional number of minor genes are exerting a small effect upon the expression of B tolerance, particularly those influencing root morphology and transpiration rate. Both of these factors are likely to play a role.

7. The location of the major B tolerance genes within the genome is as yet unknown, but genes on chromosome arms 2L and 7S show non-independent segregation with B tolerance.
8. Physiological evidence suggests that tolerance to B is expressed at the cellular level, but may not be fully expressed by leaf protoplasts. This suggests that the cell wall could be involved in expression, or that expression could be tissue specific. In the future, screening methods may be applied on a single cell basis, either to callus culture or to the sporophytic tissue.

These findings can be taken into account when deciding on a suitable breeding method for introducing B tolerance into locally adapted cultivars. Other factors to be considered include; how this new objective can best be integrated into the existing barley breeding strategies for southern Australia; the proportion of resources which could be allocated to this objective; the genetic materials already available for introduction into a breeding programme; and the targeted end use for B tolerant cultivars.

There are two major groups of breeding methods used in barley improvement in southern Australia. These are the progeny and the backcross methods.

Selection, using the progeny method, is based primarily on field performance of families selected for a range of characters, in the F_2 or F_3 generation. This method is most amenable to mechanisation. Though a truer picture of a line's performance may be obtained using field trials, particularly yield, the use of many sites over a number of years is required for reliable evaluation.

The backcross method is most useful for simply inherited characters. In general the parent carrying the desired character is crossed with a locally adapted recurrent parent. At each

generation, expression of both the character and the desirable phenotype of the adapted line, is selected. The selected progeny can then be crossed again to the recurrent parent. Though this method can reduce rapidly the undesirable genetic background from the breeding population, hand crossing and selection at every generation is labour intensive. The backcross method has been successfully used to produce a B tolerant wheat, BT Schomburgk (Paull *et al*, 1992). Since we are selecting for as many as three major genes in barley, linkage drag may be considerable.

In the future, when reliable genetic markers are available for B tolerance genes, the efficiency of the backcross method could be improved. These markers could be isozymes, polymorphisms generated using polymerase chain reaction (PCR), including randomly amplified polymorphic DNAs (RAPDs), or restriction fragment length polymorphisms (RFLPs). RFLPs could additionally be utilized as indicators of which lines most resemble the recurrent parent (Petersen, 1991). Thus, the use of RFLPs would circumvent the need to select genetic material hydroponically. Final testing of selected material could be verified using the hydroponic system. The use of RFLPs may enable a reduction in the number of generations required to achieve a genetic background similar to the recurrent parent. Separate markers for each locus could aid in pyramiding genes, such that the level of tolerance could be more precisely matched with the target growing regions. Molecular markers also enable a precise description of genotype, especially if utilizing RFLPs or PCR generated polymorphisms based on the genes of interest themselves.

It is important to introduce assessment and selection for tolerance to B toxicity into the southern Australian barley breeding programme objectives, in as an efficient way as possible. Two alternative methods are proposed.

A modified progeny method may be appropriate, where single plant selections are delayed until the F_4 or F_5 generations. Some traits such as grain colour, or head type could be selected between non-segregating families as early as the F_3, thus reducing the number of families to be handled. The advantage in selecting at late generations is increased homozygosity and homogeneity within families. Thus, selection could take place more

reliably on a family basis, and selected lines could be prepared for release, with respect to homogeneity more quickly. The main disadvantages with this method is restriction to one or two generations per year, and the need to cope with the unpredictability and heterogeneity of the field environment. It does however, allow selection for many characters, particularly yield, under field conditions, where plots are treated according to local farmer practice.

In reality it is common for backcross and progeny methods to be combined to reap the advantages of both systems. For example, in a case where it is desirable for one or two characters to be introduced to a locally adapted background, one or two rounds of backcrossing and selection, may be followed by three or four generations of selfing after which single plants may be selected.

Alternately, a modified single seed descent method may be appropriate, since an efficient and effective hydroponic screening method has been devised to select for B tolerance, and the character appears to be largely determined genetically. A single seed from each tolerant F_2 plant is grown to produce the next generation. This process is repeated until the F_6, when screening for tolerance to B would again take place. Three generations per year would be possible. By the F_6 each line would be expected to be largely homozygous. These lines would then be intercrossed to produce the desired genotype. Many of the progeny derived from F_2 plants heterozygous for the desired B tolerance genes, however, are likely to loose tolerance over several generations. The advantages in this method is shortening generation time using artificial environments, and a reduced environmental influence under controlled screening. Production of doubled haploid lines is an alternate method of achieving this same end.

In the breeding schemes outlined above, selfing over a number of generations is undertaken primarily to increase the level of homozygosity within the selected families. The process may be shortened considerably by producing doubled haploid plants. This could be achieved using either the *H. bulbosum* method, or by using anther culture. In relation to the single seed descent scheme doubled haploid plants could be produced from the F_1 parent. Thus from the F_1 generation onward, the genotype of the progeny will be homozygous and true breeding. If

doubled haploid plants are produced at an early generation, since each locus will be in a homozygous state, no alleles are masked by dominance effects and thus selection can take place immediately. By selecting as early as the second generation however, some potentially useful recombination is prevented. Population numbers for selection too must be limited by the intensive labour required to produce doubled haploid lines. In practice it is more likely that doubled haploid lines will be screened for both the character of interest, B tolerance in this case, and also assessed for other desirable characters, and the best of these lines backcrossed to a recurrent parent, possibly followed by a second haploidization step (Graner and Foroughi-Wehr, 1991).

The choice of the tolerant parent will strongly influence which of the above methods is most appropriate for introducing B tolerance into a commercial barley cultivar. Sahara 3771 is the most highly tolerant barley yet discovered. It is also a source of resistance to cereal cyst nematode (CCN), a serious problem in many parts of southern Australia (Brown, 1984) and is highly zinc efficient. Sahara 3771 also has poor agronomic attributes being low yielding, six-rowed, and late flowering, with small, poor quality seed, poor straw strength and head retention. A backcross method would be appropriate if B tolerance from Sahara 3771 was to be introduced into locally adapted cultivars.

Boron toxicity occurs in a patchy manner, both temporally, from year to year, (see Chapter 2) and spatially, in the order of square metres (see Chapter 3). Some concern has been expressed (Rathjen *et al.*, 1987) that introducing a high level of tolerance to high soil B may induce susceptibility to B deficiency. A relationship appears to exist between tolerance to B toxicity and susceptibility to B deficiency, where lines tolerant to high levels of soil B are susceptible to B deficiency (Nable *et al.*, 1990a). Thus the number of genes conferring B tolerance introduced to a new barley must be appropriate for the level of soil B to which the cultivar is likely to be subjected.

CM 72 though less tolerant to B toxicity, is considerably more desirable agronomically. It is a six-rowed, feed variety, which reflects the fact that it was bred, selected and grown commercially in the cereal growing regions of California, a Mediterranean environment

having much in common with southern Australia. It is high yielding, is relatively early flowering and is reputedly tolerant of sodic soils (Weltzien and Fischbeck, 1990). A modified progeny breeding method may be more appropriate when using CM 72 as the donor of B tolerance.

The choice between these tolerant parents as the primary donor of B tolerance will depend upon the specifications required of the final B tolerant barley line. Required grain quality characters will determine how much of the donor parent genetic background can be tolerated in a new cultivar, especially if the aim is a malting cultivar. As the number of loci coding for B tolerance to be selected increases, so will linkage drag. The more B tolerance loci being selected, the more undesirable genes will be carried into the next generation. Thus, a larger number of plants per generation or more generations of selection is required to break these linkages. The term loci may refer to one, or a number of closely linked genes.

The breeding methods discussed above, would be the ideal, if a programme for breeding B tolerant barley was now to be initiated, beginning with the original hybridization, and given the knowledge of the genetic and physiological control which has been gained in this research. It is more practical however, to use those populations already available. Three are most promising:

(1) WI 2723 x Sahara 3771 F_2 derived F_7 families

(2) CM 72 x Stirling F_2 derived F_5 families

(3) Clipper x Sahara 3771 F_1 derived doubled haploid lines.

Families derived from the F_2 of the cross, WI 2723 x Sahara 3771 may provide genetic material appropriate to produce a feed cultivar. Individuals could be reseleced within tolerant families, and subjected to further genetic manipulation. Though no selection has taken place, performance of the F_4, F_5 and F_6 generations grown in a high B environment, has been recorded. The line WI 2723 (which is a sister line to the cultivar Chebec) was rejected from the breeding programme due to small seed size and doubt about some quality characters with respect to malt production. Yield however was high, and the line possesses many other desirable traits. Though the next generation will represent the F_7 generation, many families

are still segregating for a number of traits. This reflects the many loci at which the two original parents differed. Information has been recorded on some agronomic characters, such as head type and earliness, as well as yield and tolerance to B. It is suggested that a selection of elite lines with respect of the desirable characters be grown at a number of sites and years, and the best individuals selected from the best families for commercial release. Additionally these individuals should be intercrossed with a malting cultivar, to boost seed size and malting quality.

Families derived from the F_2 of the cross CM 72 x Stirling may be more suitable as a source of material for a programme for the selection of a B tolerant malting barley cultivar. Stirling is grown predominantly as a malting cultivar, and is agronomically well adapted to Western Australian growing conditions. In South Australia however it yields poorly. The degree to which this is the result of intolerance to high levels of soil B is not known. Though six-rowed, CM 72 is also quite well adapted, and possesses desirable characters other than B tolerance, such as resistance to barley yellow dwarf virus. Selected tolerant two-rowed lines could be topcrossed to other malting cultivars for the most promising strategy to achieve a B tolerant malting barley.

The Clipper x Sahara 3771 F_1 derived doubled haploid lines may also be useful source material for production of a B tolerant malting barley cultivar. These lines are homozygous and have been investigated for B tolerance, head type, resistance to CCN and for RFLPs. Thus, with the information currently available alone, it is possible to select lines expressing a high level of B tolerance, a two-rowed head type (that most desired for malting) and an RFLP pattern (and presumably overall genotype) resembling Clipper. Selected lines could be crossed further to another high quality line and the process repeated to achieve the objective.

In summary, the information gained in this research will contribute significantly to devising appropriate breeding strategies for the production of B tolerant barleys. In reality, it is likely that a combination of the breeding methods discussed will be applied to achieving this objective.

In addition to implications for breeding, the presented work also challenges some widely held views concerning the physiological control of B tolerance in barley. It is widely believed that tolerance to B is due to an ability of plants to exclude excessive amounts of this element from the root (Nable, 1988). Though this is largely true, it appears that some genetic families, derived from crosses between B tolerant and intolerant parents, have the ability to achieve high yields, despite either a high level of symptom expression, or a high measured shoot B concentration or both (Chapter 2). It would be interesting to compare over a wide range of environments, the performance of selected lines expressing these characters, to assess whether this ability can be attributed to an internal tolerance to tissue B, or a relatively higher yield potential overall, regardless of soil B levels.

Another useful comparison could be made between field tolerance and that expressed by seedlings under the screening system described in Chapter 4. Though, for those genotypes already tested the correlation is good with respect to rank, some avoidance mechanisms may act in the field which would not be detected in the hydroponic screening system. These mechanisms may involve root distribution in the soil, root morphology in adult plants and particularly, timing of maturity. It has also been suggested that under zinc deficient conditions, zinc efficient cultivars are less affected by B toxicity due to improved membrane integrity. Under zinc adequate screening conditions then, this factor would not be apparent. Thus, although in this study the exclusion aspect of tolerance through the minimization of symptom expression has been the primary focus, potential exists to investigate further internal tolerance and avoidance as additional mechanisms for tolerance to high levels of B in the soil.

This research has established that a yield loss is incurred by growing B intolerant genotypes on B toxic soils (Chapter 2). Since these lines are also segregating for characters other than tolerance to high soil B, it is difficult to estimate yield losses attributable to B toxicity alone. It would be helpful in this regard to develop sets of near isogenic lines, that is, closely related genetic lines differing only in B tolerance, to investigate this problem. Grown in high B environments these lines would enable estimation of yield advantage due to B tolerance. Grown in environments without a toxic level of B these lines would indicate whether the presence of B tolerance genes confers a yield penalty.

Genetic and physiological control of a nutritional character is species- and even cultivar-specific (Chapter 4). Many of the published reports on the critical levels of nutrients for optimum growth are based on one cultivar of a plant species, or even on one species as representative of a family or genus (eg Eaton, 1944). It is clear from the research reported here that the practice of using single cultivars will lead to misconceptions about the nutritional requirements of plants. In barley for example, it is likely that at least four main levels of tolerance exist within *H. vulgare*, and probably other genes for B tolerance are present in related species (refer to Appendix 1). If genes conferring tolerance to high levels of tissue B are acting, then tissue critical levels could vary widely between cultivars. In peas (Mr A. Bagheri pers. comm.) B uptake was found to be controlled by two semi-dominant genes.

It is also clear that the precise conditions under which critical values were established must be reported, since the availability of nutrients to a plant can vary with soil texture, pH, moisture and profile. To what extent a plant will take up and retain a nutrient can also vary with light, rainfall, temperature and the age of the plant. Interactions between B and other nutrients both in the soil and in the plant, particularly elements associated with sodicity, is commonly a confounding factors at high B sites. Interactions between the B nutritional status and various diseases are areas as which are poorrly understood with respect to barley. Thus the concept of a critical range of B concentrations, either in the plant or growth medium, for optimum plant growth at the toxicity end of the scale is of uncertain value.

It is likely in studying the B aquistion by higher plants, that the use of different cultivars and species of plants, over a range of different experimental conditions, is contributing to the confusion about the role, uptake and transport of B. For example, sugar cane, is a plant adapted to low B conditions, acid soils, and a high rainfall environment. Barley, on the other hand, is a plant originating in largely arid regions where high B soils, often alkaline and sodic, are likely to be relatively common. It is certainly not necessarily so that sugarcane should possess the same mechanism for uptake and transport of B as barley. It has been suggested that uptake of many nutrients is multiphasic, with one system of high affinity for B

for uptake from low B environments, and others of progressively lower affinity for high B media (Nissen, 1974). Perhaps plants can use either mechanism as required. Since a requirement for B appears to be widespread for plants if not universal (Raven, 1980), it is more likely that the primary role of B in plants, though as yet not fully defined, involves the same or similar fundamental structures or functions in all plants. The way in which uptake of B differs between tolerant and intolerant barley cultivars is likely to be interesting, since it is widely thought that it is the uncharged form of B that is absorbed by plants, and most investigations have found no evidence for either a carrier or port system of short-distance transport. Evidence suggests that the cell wall may be involved in both the regulation of uptake (Chapter 5) and in a primary role for B (Loomis and Durst, 1991). Though often considered largely in respect of its structural role, it is likely that the cell wall will, in the future, be found to play a more prominant role in metabolic and regulatory functions, often attributed to membranes alone.

Though preliminary results suggest that tolerance to B may be expressed in pollen, *in vivo* selection of pollen is not evident (Chapter 5). Boron is critical for pollen germination in many species, including barley. Thus it is likely that there is some regulating mechanism in place to protect the reproductive mechanism from excess levels of B. It would be interesting to extend this idea to calcium, since it too is essential for *in vitro* pollen germination, and with respect to long-distance transport has much in common with B (Raven, 1980). To what extent variation in B nutrition may affect grain quality is a question yet to be addressed. Particularly if B is found to be involved with cell wall structure in barley grain, B nutrition has the potential to influence many aspects of malting quality.

The current extensive mapping initiatives being conducted around the world has great potential to contribute to knowledge of both the genetic and physiological regulation of many aspects of plant mineral nutrition. Precise characterisation of the genetic complement of plants will allow the function of particular genes to be defined with improved confidence. In particular, genetic differences between lines can be correlated with a phenotypic character and more directly by studying the actual structure of the gene to determine a likely protein product or regulatory function. Once probes have been developed for genes determining

nutritional characters, studies of mRNAs will provide insight into both site and timing of gene expression. The molecular characterisation of the gene itself or closely flanking markers will allow both efficient and precise selection and allow introduction of genes into new backgrounds with little or no linkage drag, since recombination can be monitered precisely. It is clear that before these kinds of studies can be conducted and future research objectives set, a sound knowledge of the basic genetic control of B tolerance in barley is necessary.

It is important that the findings from both fundamental genetic studies and molecular investigations be exchanged and integrated to develop a full understanding of the genetics of nutrient regulation in plants. In the future, through increased knowledge about the genetics and physiology of nutritional characters in plants, and the combination of traditional breeding methods and new technologies, it will be possible to rapidly and efficiently produce new plant cultivars, better adapted to their environment.

Appendix I

SOURCES OF TOLERANCE TO HIGH BORON

INTRODUCTION

It is useful to have available to the plant breeder, a number of sources for tolerance to boron (B). A given level of tolerance, or a particular physiological mechanism for tolerance may be more suitable to one situation than another, depending on the severity of the problem, the degree of patchiness of the problem, and whether the level of B in the grain in considered important. For example, a particularly high soil B area will require a high level of tolerance, but if the problem is spatially patchy, a high level of tolerance may predispose the line to susceptibility to B deficiency, or may impose an unacceptable yield loss under normal B conditions. Furthermore, an internal tolerance rather than an exclusion mechanism may be desirable if too low a level of B is considered detrimental to seed quality. Certain genes may have deleterous pleiotropic effects, and thus prove unsuitable. It may be advantageous for genes from both H. vulgare and interspecific sources tobe combined in one cultivar under some circumstances.

Assessment of response to high levels of B in the media was undertaken for the Waite Agricultural Research Institute barley collection, and for a range of material from other sources, particularly from the Western Australian collection (Boyd *et al.*, 1988). These sources were chosen because either B toxicity symptoms in plants had been observed near the site of origin (eg in Turkey by Dr A.J. Rathjen, pers. comm.); or due to the climate and soil type, the site of origin was considered a likely candidate for as yet unrecognised high B soils to be present eg Cyprus and Israel). The investigation of the origin of the tolerance to boron expressed by the Californian cultivar CM 72 may shed some light on the geographic origins of this source of tolerance. The association between genetic diversity and geographical distribution of tolerance to high levels of soil B in wheat were discussed by Moody *et al.* (1988).

MATERIALS, METHODS AND RESULTS

Waite Collection

In this study almost 350 of the most recent acquisitions of *H vulgare* from the barley collection held at the Waite Agricultural Research Institute were assessed for tolerance to B toxicity. In this initial screening, seven lines proved to be significantly better than Schooner, a moderately intolerant control, with respect to leaf damage. This screening was carried out using the box screening method described below.

Most of the lines acquired earlier by the Waite barley collection, have been screened previously (Lance *et al.*, unpublished). The Sahara series of lines were selected for their high B tolerance. A Japanese *Uzu* dwarf line, Suifu was considered worthy of further investigation with respect to tolerance to high levels of B, because it had been found to be zinc efficient (see Chapter 1, Boron and Zinc). Also identified as potentially B tolerant was Black-Hulless (WI 276), CPI - 18197 and Qi Wu Qi. A number of genotypes were also screened by Boyd *et al.* (1988) for tolerance to B. They selected CM 72 as the most tolerant line tested, with respect to leaf symptoms.

Turkish Lines

Four lines of *H. vulgare* originating from Turkey (three from the Waite Collection and one kindly supplied by Dr A. J. Rathjen) were assessed for tolerance to high boron. The method used in this case was similar to the hydroponic system described in Chapter 4, except that instead of the solution being circulated, punnets were set in a static system. Two of these lines were equal or better than CM 72, showing an absence of leaf damage symptoms.

Israeli *H. spontaneum*

Three or four plants each of 21 lines of *H. spontaneum* originating from Israel (kindly supplied by Dr A.H.D. Brown) were screened as described in Chapter 4, in a flushing hydroponic system at 20 mg l^{-1} added B. Of these, fourteen lines showed leaf damage symptoms less severe than CM 72, six more severe, and one line showed contradictory results in repeat experiments.

Table I.1. List of H. spontaneum lines from Israel, tested for tolerance to B toxicity in relation to CM 72.

H. spontaneum line	Class Compared with CM 72	*H. spontaneum* line	Class Compared with CM 72
Afiq	Better	Rivivim	Better
Akhziv	Better	Rosh Pina	Better
Atlet	Better	Sede Boker	Worse
Bargiyyora A	Worse	Tabigha A	Better
Beit Shean	Contradictory	Tabigha B	Better
Bor Mashash	Better	Talpiyyot A	Better
Damon	Better	Talpiyyot B	Better
Herzliyija	Better	Wadi Qilt	Worse
Maalot	Better	Yerham	Worse
Mechola	Worse	Zefat	Better
Mt Meron	Worse		

Cypriot Material

Seventy lines of *Hordeum vulgare*, *H. agriokcithon* and *H. spontaneum* and some interspecific crosses (kindly supplied by Dr A. Hadjichristodoulou) were tested, under quarantine conditions, in soil boxes for tolerance to high soil B. None of these lines showed as few leaf symptoms under these conditions as CM 72.

Parentage of CM 72

The ancestors of CM 72 are: California Mariout, selected from the original collection from the dry-hill region west of Lake Mariout in Egypt; Club Mariout, selected from the original collection from the irrigated sections of lower Egypt; CI 2376, from Ethiopia, used to introduce tolerance to the barley yellow dwarf virus (Yd2 gene) and tolerance to certain traces of scald *Ryncosporium secalis*; and CI 1179, used to introduce the MIa gene for resistance to powdery mildew (*Erysiphe graminis* DL f. sp. *hordea* Em. Marchal) (Schaller, *et al.* 1977). The two lines used as disease resistance donors were found to be intolerant to high B, and all but one of

those with the Egyptian ancestry to be tolerant. One line known as Mariout (7507) WA 924 from the Western Australian barley collection was found not to be as tolerant as CM 72. Both Club Mariout and California Mariout have Mariout as a synonym.

Screening Methods

Large boxes (2m x 1m x 0.25m) were filled with a bulk sample of surface soil from a red-brown earth with was mixed with 150 mg/kg boron (as H_3BO_3). The B concentration in a boiling $CaCl_2$ extract was estimated to be 58 mg/kg (Moody, pers. comm.). Seeds were imbibed on moist filter paper in petri dishes, for 2 days at 4°C, then pregerminated for 1 to 2 days at 20°C before planting. Six seedlings from each line were sown in rows in the boxes; there were 504 seedlings per box in total. Seedlings were assessed 3 weeks later for leaf damage due to B toxicity relative to Schooner, an intolerant, locally grown barley cultivar.

Assessment of barley lines for tolerance to high levels of boron was also carried out using the hydroponic system described in Chapter 4. Seeds were imbibed at 4° then at 20°C on moist filter paper, then planted into a coarse river sand. A nutrient solution was circulated through this medium for 30 minutes every 6 hours. Boron was added at 20 mgl^{-1} as boric acid, and plants assessed for leaf damage after three weeks.

CONCLUSIONS

The lines listed in Table I.2 may prove to be valuable new sources of tolerance to high levels of B. In the future these lines should be assessed further, and allelism testing applied with respect to Sahara 3771. That is they should be crossed with this line, and the F_2 population inspected for transgressive segregation with respect to B tolerance. Transgressive segregation would indicate that the genes coding for B tolerance were different between the two parental lines. With sources of tolerance available within *H. vulgare*, it is unlikely that the genes from *H. spontaneum* will be required for breeding purposes. It may be useful however to investigate the physiology and genetics of B resistance in *H. spontaneum*, since it may differ from that of *H. vulgare*.

Table I.2. *Hordeum vulgare* genotypes identified as possible source of tolerance to B from the Waite Agricultural Research Institute barley collection.

Line Name	Source	Origin
Black - Hulless	WI 276	USA
CPI 18197	WI 958	Algeria
Jai Ding Hong Jin Zhu Jou	WI 2679	Korea
La Mesita	WI 2492	California, USA (N. Africa)
MC 90	WI 2539	Argentina
Qi Wu Qi	WI 2681	China
Sahara 3763	WI 481	North Africa
Sahara 3769	WI 487	North Africa
Sahara 3771	WI 489	North Africa
Shannon	WI 2599	Tasmania, Australia
Tokak	Dr A.J. Rathjen	Turkey
Tokak 157/37	WI 2514	Turkey
Zafer 160	WI 2523	Turkey

The geographic origins of the lines expressing tolerance is diverse. It is reasonable to expect that tolerance to high B evolved in regions with high soil B, or have been selected by plant breeders for growing in such areas, like California. Though naturally occurring B toxicity has not been officially reported in North Africa, given the similarities in soil types and climate with southern Australia, it could reasonably be expected to be present. The Egyptian origin of the B tolerance expressed in CM 72 supports this idea. The fact that some lines thought to be of Asian origin express some tolerance to high B may seem surprising, but high boron may occur in volcanic areas, as well as sodic, arid zones. Most often this results in high B levels in irrigation waters, rather than in soils *per se*.

Moody *et al.* (1989) found that wheat varieties form the US, Canada, Egypt and NW Europe were mostly sensitive, those from Argentina, Turkey and Iraq had variable sensitivity, while those from Afghanistan, India and Japan were predominantly tolerant. The findings in barley

do not correspond well with that found for wheat (Table I.1). This is likely to be the result of the usually unsystematic way germplasm has been collected until recent years. In addition, a classification of geography according to country is geographically irrelevant, with respect to soil types, climate and agricultural practices, and each of these factors vary enormously within political boundaries.

In summary then, a number of sources of tolerance to high soil boron have been identified, both within *H. vulgare*, and in related species. The genetic and physiological basis of these tolerances are yet to be investigated. The importance of easily accessible germplasm collections has been highlighted in the search for B tolerance in barley. In the future, aquiring nutritional information about genotypes held in collections may be considered a higher priority. More detailed geographic information about the environment in which accessions are collected, would help identify locations likely to suppport new sources of B tolerance.

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