

THE INTERNATIONAL RESEARCH GROUP ON WOOD PRESERVATION

Section 3

WOOD PROTECTING CHEMICALS

Borates and their biological applications

J D Lloyd

Borax Europe Limited, Priestley Road, Guildford, GU2 5RQ, United Kingdom

Paper prepared for 29th Annual Meeting
Maastricht, Netherlands
14-19 June 1998

IRG Secretariat
S-100 44 Stockholm
Sweden

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J D Lloyd

Borax Europe Limited, 170 Priestley Road, Guildford, GU2 5RQ, United Kingdom

Abstract

This paper reviews some of the many biological applications of borates. Boron is a ubiquitous element found widely distributed in the environment and is a normal component of a healthy diet. Elemental boron does not exist in nature, but is always found combined with oxygen in compounds called borates. Boron is an essential micro-nutrient for plants, and there is evidence to suggest that boron is of nutritional importance, if not essential, for humans. Borates possess biostatic activity which enabled their use in medicine and has allowed their continued development as preservatives.

The essentiality of boron in plants has led to extensive biological use in agriculture. The biostatic properties at high doses have enabled their use in biodeterioration control, against insects, fungi, algae and bacteria. Some use is currently being made of borates for insect control in the home.

The application of borates to crops, to alleviate boron deficiency, has resulted in recognized increases in quality and yield. Consideration of the relative safety and effectiveness of borates as biocides, is expected to lead to an increase in the use of these products in the future.

Key Words: Applications; Biochemistry; Biology; Borates; Boron; Chemistry; Micronutrient; Preservative.

Introduction

The objective of this paper is to review some of the large scale commercial applications of boron which interact with biological systems. The relevant chemistry and biochemical interactions, which render borates bioactive molecules, are introduced. Boron chemistry and biochemistry is of particular interest as all of its physiological effects are as a direct result, even though some appear at first to be contradictory.

The two most well known which will be discussed in detail here are the essentiality of borates on the one hand and their toxicity or effectiveness as a preservative on the other. This is perhaps not such a surprise when one considers the ubiquitous nature of borates and the ability of most micro-nutrients to become detrimental at high physiological concentrations.

Boron is a trivalent element widely distributed in the environment, comprising about 0.001% of the earth's crust (The Merck Index, 1989); concentrations average 3-10 µg/g in soil (Adams, 1964; Muetterties, 1967), 4.5 µg/g in ocean waters (Weast, 1983), and about 0.01 µg/g in freshwater (Jenkins, 1980).

Boron is widely distributed in plant and animal tissues and is known to be essential for plant growth (Gouch & Dugger, 1954; Skok, 1958; Skol'nik, 1974; Underwood, 1977; Lovatt & Dugger, 1984). In a review on its toxicity by Murray (1995), it was reported that: the average U.S. daily dietary intake of boron is 1.5 mg B/day. The median boron content of U.S. drinking water is 0.031 µg/g with a maximum of 3.95 µg/g

(EPA, 1994) and the daily boron intake for humans from food and water has been estimated at 0.5 - 3.1 mg for adults (Nielson, 1992).

Chemistry of Boron

Boron is the only non-metal in a family otherwise comprised of active metals, group IIIb of the periodic table. As could be expected, boron exhibits bonding and structural characteristics intermediate to both, as do other elements lying to either side of the metal/non-metal border. Boron (Atomic number 5) also has a tendency to form double bonds and macromolecules, although these bonds are more correctly described as partial double bonds and are due to π electron back bonding into the empty p orbital of boron.

Because of an incomplete electron octet, boron compounds can act as electron pair acceptors and this behavior is demonstrated by the Lewis acid properties of boron. It is this tendency which is fundamental when forming hypotheses attempting to predict the action of boron within biological systems, as will be discussed.

Boron does not occur in nature in its elemental form, but rather as oxygen containing compounds such as boric acid ($\text{B}[\text{OH}]_3$), in some volcanic spring waters and elsewhere, as borates such as borax. These compounds are used as commercial products and for the synthesis of other boron compounds. In this paper and elsewhere, references to boron in the environment and in various applications, have referred to the elemental boron content, which in some cases allows for comparisons between studies and applications.

Oxygen containing compounds of boron are among the most important, comprising nearly all the naturally occurring forms. The structures of these compounds consist mainly of trigonal BO_3 units with sp^2 hybridization, and with tetrahedral BO_4 units with sp^3 hybridization (Cotton *et al.*, 1987). B-O bond energies are 560 - 790 kJ, with the only competition in strength offered by the B-F bond in BF_3 (640 kJ) (Cotton & Wilkinson, 1986). Endless organic derivatives containing boron-oxygen bonds are known; the main examples that include trigonal boron are the orthoborates ($\text{B}[\text{OR}]_3$ e.g. esters; the acyl borates ($\text{B}[\text{OCOR}]_3$); the peroxo borates ($\text{B}[\text{OOR}]_3$); and the boronic acids ($\text{RB}[\text{OH}]_2$). It is appropriate to consider that these are derivatives of boric acid (Cotton *et al.*, 1987).

Boric acid is a colorless, odorless, transparent crystals or as white granular powder (Anon, 1980). It is readily soluble in water, ethanol and glycerol (Merck Index, 1989). Borax is a white crystalline substance and is soluble in water and glycerol, but insoluble in alcohol (Merck Index, 1989). Three other sodium borates are commonly known: sodium metaborate (NaBO_2); sodium perborate ($\text{NaBO}_3 \cdot 4\text{H}_2\text{O}$); and sodium pentaborate ($\text{Na}_2\text{B}_{10}\text{O}_{16} \cdot 10\text{H}_2\text{O}$). Like boric acid and borax, they are soluble in water and glycerol (*ibid.*).

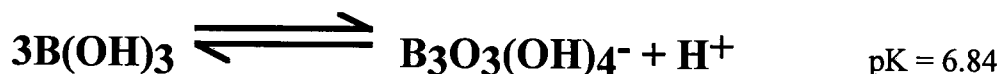
It is these oxygen containing compounds of boron that are traditionally used in biological applications. Boric acid, borax, mixtures of the two or a spray dried mixture equating roughly to disodium octaborate tetrahydrate, being the most commonly used, although organic esters of boric acid such as trihexylene glycol diborate, are also currently in use and boronic acids are used in minor applications. Perborates, boric acid and borates, have been used in the past as general antiseptics or bacteriostats (Anon.,

1980; Merck, 1989) and in fact boric acid was used as an antiseptic by Sir Joseph Lister, the father of modern surgery, in the mid 19th century.

Boric acid is moderately soluble in water, but has a large negative heat of solution so that the solubility increases markedly with temperature. It is a very weak and exclusively monobasic acid that is believed to act, not as a proton donor, but as a Lewis acid, accepting OH^- :



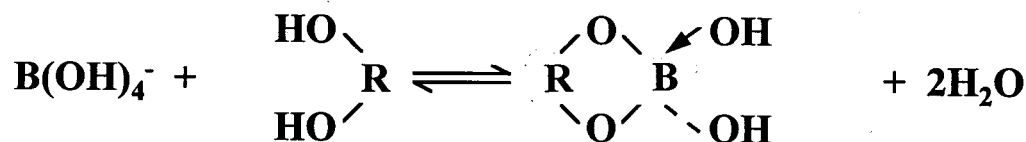
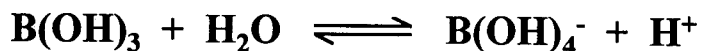
The B(OH)_4^- ion actually occurs in some minerals. At concentrations less than 0.025M, only the mononuclear species B(OH)_3 and B(OH)_4^- exist; but at higher concentrations the pH becomes consistent with the formation of polymeric species such as:



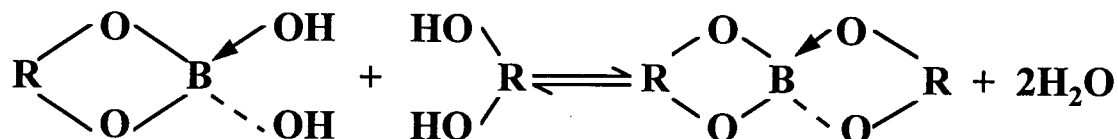
It is also likely that polymers exist in mixed solutions of boric acid and borates, such as:



Boron (in the form of boric acid or more probably ionized as the tetrahydroxy borate anion), is one of the chemical elements whose oxygen compounds will form chelate complexes with certain organic compounds containing *cis* adjacent alcohol groups:



and



These complexes occur in aqueous solution and are well known. Biot as early as 1842, reported that a solution of boric acid became acidic to litmus upon the addition of sugars and Thompson (1893) found that boric acid could be determined by titration in the presence of various polyhydroxy compounds (or polyols).

Many chemical and most biochemical reactions of boron are based on the reactivity of the borate anion with adjacent alcohol groups. These stable complexes formed by rapid esterification with polyols (Nickerson, 1970; Boeseken, 1949), are mainly 1 : 1 and charged. It has also been suggested that similar reactions take place with α -hydroxy carboxylic acids (Kustin & Pizer, 1969) and although this is true of gluconate for example, it has been shown that the complex is only formed as a result of an additional hydroxyl group in the β position as other α -hydroxy carboxylic acids such as lactate do not undergo the same reaction (Lloyd, 1993). The type of complexes formed with polyols depends on pH and on the ratio of the borate ion to the diol (Zittle, 1951) and the acidity of the hydroxy groups. When a low diol to borate ratio exists, it has been proposed that the monoester is prevalent, whilst when the diol to borate ratio is high, then the diester is predominant. The acidity of boric acid is thereby increased as mentioned above. Steric considerations are critical in the formation of these complexes. Thus 1,2- and 1,3-diols in the *cis*-form only, such as *cis*-1,2-cyclopentanediol are active, and only *o*-quinols react. Indeed the ability of a diol to affect the acidity of boric acid is a useful criterion of the configuration where *cis-trans*-isomers are possible.

This specific complex forming ability has led to the use of boric acid in carbohydrate separation and in determining carbohydrate configuration (Boeseken, 1949; Annison *et al.*, 1951; Khym & Zill, 1951; 1952; Popiel, 1961).

Biochemical Effects of Boron

Several compounds of biological importance such as vitamins and co-enzymes can react to form complexes with the borate ion (Zittle, 1951; Aruga, 1985). Reactions with these molecules and others within the cell, have been found to produce dramatic changes in metabolism.

A good example of this is the effect of boron on plant metabolism and lignification. Here the borate seems to play at least one role by partitioning metabolism between the pentose phosphate shunt and glycolytic pathways. Boron deficiency has been shown to result in an accumulation of phenolic compounds in plants (Dugger, 1983; Shkol'nik, 1974). An increase in lignification has also been observed under these conditions (Acerbo *et al.*, 1973). Such metabolic changes appear to be caused by a lack of the inhibition of glucose-6-phosphate and 6-phosphogluconate dehydrogenases, which are normally inhibited in the presence of boron. This inhibition in the case of 6-phosphogluconate dehydrogenase was suggested to result from the formation of a complex with boric acid and the α -hydroxy carboxylic acid, 6-phosphogluconate (Lee & Aronoff, 1967) but has been subsequently shown to be as a result of chelate formation with the co-enzyme NADP^+ which also features in the reaction (Lloyd *et al.*, 1990; Lloyd & Dickinson, 1991; Lloyd, 1993). In the absence of boron, the pentose phosphate pathway is left unregulated and results in an over-production of phenolic acids and other lignin components. The accumulation of such compounds would not only lead to the necrosis of plant tissue, but also to an increased deficiency problem, as some of these phenolics, also having alcohol groups, would complex with boric acid

(Shorrocks, 1990). In the presence of boron, the inhibition of glucose-6-phosphate and 6-phosphogluconate dehydrogenase will restrict both the flux of substrate into the pentose phosphate pathway and the synthesis of phenols. As a result of this, glycolysis and the synthesis, for example of hemicellulose and related cell wall material will increase.

Frost (1942) found that borate forms complexes with the ribityl group of riboflavin and this was then shown to inhibit the activity of the flavoprotein xanthin oxidase (Roush & Norris, 1950). Because of the borate ion complexing with the ribityl group of another flavin containing molecule FAD^+ (flavin adenine dinucleotide) (Shepherd, 1951) the effect of borate on NAD^+ (nicotinamide adenine dinucleotide) requiring enzymes was investigated: borate inhibited yeast alcohol dehydrogenase (Roush & Gowdy, 1961; Weser, 1968; Smith and Johnson, 1976); yeast glyceraldehyde-3-phosphate dehydrogenase (Missawa *et al.*, 1966); and aldehyde dehydrogenase (Deitrich, 1967). Evidence given by some workers suggested that the inhibition was due to complex formation. This hypothesis was suggested as a result of the removal of inhibition by the addition of non-substrate polyols (Roush & Norris, 1950; Roush & Gowdy, 1961). It was later shown that these inhibitory effects were due to the formation of a complex with the ribityl group of NAD^+ (Smith & Johnson, 1976). Johnson & Smith (1976) showed that the same complexation occurred with NMN^+ (nicotinamide mononucleotide), that the reaction took place with the *cis*-adjacent hydroxyls within the ribityl group of NMN^+ and NAD^+ , and that the ribose next to the positively charged nicotinamide moiety in NAD^+ was preferred (NAD^+ has two ribose units). Another important flavoprotein that was found to be effected by borate is cytochrome b_5 reductase (NADH-cytochrome b_5 reductase) (Strittmatter, 1964). It was found that borate again interacted with the nucleotide substrate. It was also shown that the addition of borate buffers to reduced flavoprotein-pyridine nucleotide complexes results in proton transfer from the flavoprotein to the pyridine nucleotide, and release of the nucleotide presumably as the borate complex. It could be conceived that this sort of borate-cytochrome interaction could result in a partial blocking of the electron transport chain.

Other vitamins and co-enzymes found to react with the borate ion include: AD(5)P (muscle adenylate); pyridoxine (vitamin B_6); dehydroascorbic acid (the reversible oxidation product of vitamin C); co-enzyme A; 5-deoxyadenosylcobalamin (vitamin B_{12}) and pantothenate (Zittle, 1951).

In addition, borate appears to be able to act as an inhibitor in a purely ionic manner as shown in the case of alkaline phosphatase (monoesterase) (Cram & Rossiter, 1949; Zittle & Della Monica, 1949). The phosphodiesterase is inhibited by borate too, although this may be due to an interaction with enzyme bound polysaccharide.

Another slightly different form of inhibition is seen in the case of serine-acyl-enzymes. These enzymes, the most noted of which being the serine proteases, contain an unusually reactive serine residue (e.g. serine 195 in chymotrypsin) at the active site of the enzyme. These enzymes have been shown to be inhibited by the formation of a transition state inhibitor, via complex formation between the hydroxyl group of the serine residue and boric, or boronic acids. The close proximity of a cationic histidine residue may also be of importance. The reactive serine residue can be labeled quite

specifically by the formation of an inactive diisopropylphosphoryl/enzyme complex by the addition of the organic fluorophosphate diisopropylphosphofluoridate. Other serine enzymes that are deactivated by diisopropylphosphofluoridate have also been shown to be inhibited by boric and boronic acids (Berezin *et al.*, 1967; Bauer & Pattersson, 1974; Lindquist & Terry, 1974; Mathews *et al.*, 1975), including choline esterase (Garner & Pelly, 1984). Another serine class of enzyme is phosphoglucomutase, which plays a role in glucose metabolism. This is a phospho-enzyme rather than an acyl-enzyme, but still uses the active serine residue at the center of the active site. As could be expected this enzyme has also been shown to be inhibited by borates (Loughman, 1961; Parr & Loughman, 1983).

It is also of interest that enzymes using serine as a substrate can also be inhibited with boric acid by the formation of a transition serine/borate/enzyme complex as with γ -glutamyl transpeptidase (Tate & Meister, 1978). Transpeptidase enzymes are also of the serine class, and this interaction is probably an enzyme-serine/borate/serine complex. Related to this perhaps is the inhibition of β -lactamases (Amicosante *et al.*, 1989). These enzymes attack β -lactams such as penicillin, whose target enzyme happens to be the transpeptidase used for cross-linking the peptidoglycan cell walls of some bacteria. β -lactamases are therefore likely to have a similar active site to transpeptidases, making them accessible to inhibition by boric and boronic acids in a similar way. A number of other enzyme systems not discussed in this review have been shown to be inhibited by borates. Some of these are listed in table I.

TABLE I Some Additional Enzyme Systems Inhibited By Borates

Enzyme System	Reference
Asparagine semi-aldehyde dehydrogenase	Hegemann <i>et al.</i> (1970).
Fructose biphosphatase	Vergnano <i>et al.</i> (1960).
Hexokinase	Wiebelhaus & Lardy (1949)
Lecithinase	Arnaudi & Novati (1957).
Enzyme Q	Gilbert & Swallow (1949).
Sorbitol dehydrogenase	Wolff (1955).
Tyrosine hydroxylase	Quick & Sourks (1974).
UDPG pyrophosphorylase	Griffith <i>et al.</i> (1978).
UDPG starch glucosyltransferase	Augsten & Eichhorn (1976).

Boron compounds may also be able to interact directly with hydroxyl rich compounds in cellular membranes. Any such interaction could result in a change of functional activity. It has been reported that roots and apical meristems grown under boron-deficient conditions contained lower than normal amounts of phospholipids and cell structural organization (Shkol'nik & Kopman, 1970). Membrane bound enzyme activities in plants have also been shown to be altered as a result of changes in membrane permeability induced by boron deficiency (Dave & Kannan, 1980).

Because of the sensitivity of membranes and membrane permeability, to treatments such as low calcium or low temperature, many workers have investigated the role of boron in plants with respect to membrane permeability (Robertson & Loughman, 1974; Pollard *et al.*, 1977; Parr & Loughman, 1983; Dugger, 1983). Artificial membrane

systems (liposomes) have been used by Parr & Loughman (1983), to demonstrate a direct effect of borate on membrane permeability, and they suggest that if boron in plants does contribute to the integrity of the cell membrane, then this could well be its primary role in plants. Direct membrane effects have also been shown in fungal systems using yeast protoplasts, although concentrations required to observe changes were above normal physiological concentrations (Lloyd, 1993).

Boron Essentiality

The biochemistry of boron in the metabolism of vascular plants encompasses a history of about 80 years of research trying to elucidate its primary role. A large proportion of this work that has been carried out since 1910, when it was first suggested that boron was required for plant growth, has been concerned with showing the boron requirement of a variety of plants for both growth and developmental processes (Hewitt, 1963). The majority, however, has been concerned with trying to determine its primary function in metabolism.

Boron in the form of boric acid or borates is an essential mineral element for all vascular plants (Gouch & Dugger, 1954; Skok, 1958; Shkol'nik, 1974; Underwood, 1977; Parr & Loughman, 1983; Dugger, 1983; Lovatt & Dugger, 1984) and diatoms (Lewin, 1966a, b). The essential nature of boron in plants was apparently derived through evolution (Mc Clendon, 1976) and may be due to adaptation to its presence. Neither fungi nor fresh water algae, however, seem to have a measurable boron requirement, although some evidence exists for a role of boron in other organisms, especially those that fix nitrogen (Anderson & Jordan, 1961; Gerloff, 1968). Growth of some other organisms has been stimulated by the presence of boron, although it did not appear to be essential (Davis *et al.*, 1928; McIlrath & Skok, 1958). Schwarz (1974) suggests that because boron is ubiquitous in animal tissues and possesses properties expected of an essential element, it should be classified 'under special consideration' for trace element function. Other evidence has also suggested that boron has importance in human nutrition, and it has recently been considered to be a "probably essential" trace element (WHO, 1996). (Ref: WHO, 1996. "Trace Elements in Human Nutrition and Health, WHO, pp. 161, 175-178, 1996) Further information on the importance of boron in animal and human diets has been reported by Hunt (1994) and Nielsen (1994).

The essential nature of any element for plants can be established according to a set of defined criteria (Arnon & Stout, 1939):

1. the element must be essential to the completion of the life cycle;
2. the element cannot be substituted for or replaced by any other element;
3. the element must have a distinct function.

The first two criteria were fully demonstrated in plants by Sommer & Lipman (1926) and Warrington (1923) respectively, however, the final criteria has yet to be proven, although it is generally accepted that boron is an essential element in plants.

As mentioned previously, boric acid and borates are able to form complexes with compounds containing certain configurations of alcohol groups. Most hypotheses that attempt to explain the role of boron in plants are based upon this reaction and these have been reviewed by Shorrocks (1990). A substantial proportion of the boron content in higher plants seems to be complexed as stable *cis*-borate esters in the cell walls (Thellier *et al.*, 1979). The fact that the boron requirement of dicotyledons is higher than monocotyledons, is presumably related to the higher proportions of compounds with

cis-diol configuration in the cell walls, mainly in the hemicellulose fraction and in lignin precursors (Lewis, 1980). It has been suggested that the function of this apoplastic boron is somewhat similar to that of calcium, in both regulating synthesis and stabilizing certain cell wall constituents, including the plasma membrane (Shorrocks, 1990). According to Lewis (1980) the functions of boron are primarily extracellular and, where intracellular are related to lignification.

Metabolic regulation by boron is hypothesized in plants, again by virtue of its ability to form complexes with polyols. When the borate ion complexes with compounds that are reactants or products of enzymatic reactions in plants, it may stimulate or inhibit the course of specific metabolic pathways. The effect of boron on the pentose phosphate pathway (Lee & Aronoff, 1967), production of phenolics and lignification (Lewis, 1980; Shorrocks, 1990) is a good example of this. These altered pathways may in turn bring about altered metabolite pool sizes, which in their turn can bring about altered plant growth or development. In a review on boron in plant metabolism (Dugger, 1983) a hypothetical sequence of effects, responses, or metabolic events in plants influenced by boron was suggested. This is quite an important model as it demonstrates the difficulty in determining which steps are effected as a primary and direct result of boron interaction and, which are secondary or as a subsequent result of any primary effects.

Other work has been carried out in this area of carbohydrate metabolism, and it has been postulated that boron plays a role in plants in the production and translocation of sucrose (Dugger & Humphrey, 1960; Loughman, 1961), perhaps by the inhibition of starch synthesis (Dugger *et al.*, 1957; Scott, 1960; Augsten & Eichhorn, 1976).

Following the discovery that boron was essential for the growth of vascular plants in the 1920's boron deficiency was soon identified as being the cause of serious crop losses in sugar beet in Germany and apples in both Canada and New Zealand. Since then boron deficiency has been recognized in nearly every country and in many crops and the addition of boron as a fertilizer along with the 6 other micronutrients, 3 secondary and 3 major nutrients is well established and it is believed that boron deficiency is more widespread than deficiency of any other of the micronutrients. This probably reflects the water solubility of borates. In addition to any low original amounts of boron, borates are depleted by rainfall which washes boron from the topsoil and by cropping where it is removed in the harvested material. Natural level in soils can also be rendered less available to plants by high levels of calcium and drought. An estimate of the global coverage of crops susceptible to boron deficiency can be given as over 205 million hectares (compiled from Annon, 1990), although currently the global borate micronutrient application is approximately 60,000 product tonnes per annum, mostly in the form of pentahydrate borax and disodium octaborate tetrahydrate. This figure has grown at a rate of approximately 5 % per year over the last 20 years and is likely to continue at the same rate for at least another 20 (Phillips pers. comms).

Probably as a result of the complex biochemical interactions of boron in plants, the physiological effects are many fold. Boron is needed for many of the normal functions of plant growth, but is particularly involved in meristem development; pollination; tissue viability/stability; frost resistance and disease resistance. These have been briefly reviewed by Shorrocks (1989).

When boron is in short supply normal cell division cannot proceed. Eventually shoot and root apical meristems die or become moribund. Boron is essential for proper seed set and fruit development and problems here often appear as part of the boron deficiency symptoms. It has been established that boron is required for pollen tube growth and the boron is adsorbed as the tube grows through the stigma tissue and in some cases pollen grain germination is boron dependent.

Apart from the degeneration associated with meristems, breakdown of parenchyma tissues are also common in boron deficiency. This is usually manifested by brown discoloration, areas or spots of necrosis and the formation of corky nodules and appears to be associated with the biochemical effects of overproduction of phenolics and lignification.

In a number of situations boron deficiency is also associated with poor frost resistance. This is particularly the case in trees such as Pine and Eucalyptus and other studies have shown a similar situation with apples and grapes. There is no doubt that boron application can significantly increase frost resistance in these situations.

From a microbiological point of view, boron usage in agriculture is particularly interesting with regard to disease resistance and control. There have been many observations of increased disease resistance following boron fertilizer application, including resistance to mildew and aphid attack. It is generally believed that good and healthy plant vigour would render the plants less susceptible to attack by pathogenic organisms, many of which are highly opportunistic. Other reports have associated the enhanced resistance of tomato, capsicum and cabbage to damping off fungi following seed treatment with boron, to the increased activities of polyphenol oxidase and peroxidase, both of which are influenced by boron (Shorrocks, 1989). Some of the observed effects such as the reduction in Ergot caused by the basidiomycete smut *Claviceps purpurea* on barley and rye (Tainio, 1961; Simojoki, 1969) however, could possibly be as a direct fungi static/fungicidal effect and this may also be the case with club root in the Brassicaceae caused by *Plasmodiophora brassicae* (a plasmodial fungus in the Gynomycota).

Boron has been recognized for more than 50 years to be able to influence the impact of club root disease. *P. brassicae* is a soil borne fungal pathogen which exists outside of the host as a resting spore which retains viability for many decades. Disease spread is by zoospore and the devastating secondary stage of the disease only occurs after morphogenesis of the plasmodium into the sporangium containing the zoospores. The plasmodium in the host does not appear to cause the plant a problem. In a review prepared by Dixon (1996) confirmatory evidence obtained in controlled environments and in the field shows that the morphogenic change from plasmodium to sporangium in the root hair or epidermal cell is inhibited by boron. He concludes however, that it is not certain whether this is a direct effect on the fungus or whether it is an effect on the host which subsequently effects the fungus life cycle.

An area where borates provide a definite biostatic control of a pathogenic fungus in agriculture or rather forestry, is in its use for controlling Fomes disease of conifers. This application has been extensively reviewed by Pratt (1996), with recent results in the U.K. being summarized by Pratt & Lloyd (1996). Conifers throughout the northern hemisphere are susceptible to a root and butt rotting disease caused by the

basidiomycete *Heterobasidion annosum*. The fungus spreads over long distances by aerial dispersion of basidiospores, which can be released throughout the year or during summer or winter months, depending on ambient temperatures, from fruit bodies on rotted wood. In managed forests and plantations, freshly cut stump tops are created at regular intervals in thinning and during clear-felling and these are susceptible to infection by *H. annosum* basidiospores. The fungus is a primary colonizer of wood, and the successful infection of a stump top may lead to almost complete colonization of a stump within a few months. This saprophytic phase is of no economic significance *per se*. However, on many soils, the fungus can infect healthy intact roots of standing trees where tree and diseased roots are in close contact. Cortical lesions may be so prolific as to kill the distal portion of a living root, and thus provide *H. annosum* entry into the central xylem of the living tree. From such a position, heartwood in both root and stem can be rapidly decayed following colonization by the fungus. This in turn increases the risk of premature wind-blow and renders the valuable butt sawlogs useless often to a height of many meters. The heartwood decay is cryptic and large numbers of trees can be diseased with no outward symptoms until they are felled. Stumps of decayed trees are themselves potent sources of infection, since the fungus can remain viable within them for many decades (Greig and Pratt 1976). Infection arising from such stumps can spread into replacement crops even before thinning provides more stumps as access for infection. The disease can therefore increase both within and between rotations.

The disease can, however, be controlled by treatment of stump tops to prevent their infection by basidiospores. Because the fungus cannot survive freely in the soil and only has access to the stump for a short period of time, the treatment of stumps with a prophylactic material is an economically viable and effective strategy against the disease. Stump treatment has been used in the U.K. and France to prevent the establishment of the disease, in mainly healthy stands, for the past 30 years.

Whilst borax was adopted for this use in the United States the highly soluble disodium octaborate tetrahydrate has been found to be ideal for the heavily mechanized harvesters used in Europe. Borax is applied as a solid to stump surfaces in the U.S.A. and demonstrates good efficacy to *H. annosum* but is unsuitable for mechanized liquid application because of its solubility characteristics.

The mechanism by which borates act against *H. annosum* is poorly understood but is likely to be similar to their mechanism as borate fungistats and preservatives used in biodeterioration control and timber treatment. Rishbeth (1959) showed that boron penetrated to 5 cm or more below the stump surface of treated freshly cut stumps within a few hours. Thereafter it was depleted from above ground tissues so that after two months it was hardly detectable ($>0.002\%$) above natural background levels. He proposed that the original barrier to fungal colonization was toxic in nature and that the subsequent lowering of concentration allowed the development of fungal species that were both less susceptible to borates and antagonistic to *H. annosum* (e.g. *Peniophora* spp., *Botrytis* spp.).

Boron Toxicity

Although boron in the form of boric acid or borates is an essential element for the growth of vascular plants and has been shown in some cases to improve the growth of other organisms, as the concentration of boric acid or borate is elevated to very high levels, it becomes toxic to all cells (Dreisbach, 1974). The toxicity of boron containing

compounds to most organisms would appear to be due to its ability to form complexes with various organic compounds. This effect in micro-organisms, however, has been shown to be more biostatic than biocidal, with these organisms appearing to 'starve' (Allen, 1929; Cushny, 1940; Goodman & Gillman, 1941; Rosenberg, 1946).

Reports of toxicity in plants are common in areas of naturally high boron content, although often relate to symptoms or a yield reduction rather than actual death of the plant or necrosis of tissues. When borate fertilizers are used at the recommended rates on the specified crops they are completely safe. It is possible to induce toxicity in the laboratory although this is almost completely unknown in the field through soil application other than through gross misuse or overdosing. Some garden crops have displayed slight symptoms at water concentrations as low as 2-4 µg boron/ml (Gupta, 1983). Many plants are however quite tolerant of boron and are able to accumulate high levels.

Bacteria, fungi and insects are also effected by high concentrations of boron. Because of this boron compounds have been used against bacteria in the form of antiseptics and as preservatives in cosmetics and food. Some species of fungi exhibit effects of boron toxicity, resulting in the aborted growth of hyphae, perithecia and ascospores (Bowen & Gauch, 1966) and in the failure of gametes to cleave (Zittle, 1951). Boric acid and disodium octaborate tetrahydrate are used widely as an insecticide against cockroaches and are put down in powder form (Cornwell, 1976; Merck Index, 1989). They are also used against the black carpet beetle and for ant control (*ibid.*) In the past EPA registration has been obtained for use against *Alphitobius* sp. and *Dermestes* sp. in poultry houses along with house fly larvae, latrine fly and in manure. The Farm Chemical Handbook (1984) listed borax as a larvicide against dog hook worm and swine kidney worm.

Boron toxicity to animals and humans has been reviewed in detail . It has been concluded to be of low toxicity with an acceptable daily intake of 18 mg B/kg bw/day. (Ref: (a) IEHR, 1995. "Assessment of Boric Acid and Borax Using the IEHR Evaluative Process for Assessing Human Developmental and Reproductive Toxicity of Agents", NTIS Report No. PB96-156005, March 1995. (b) ECETOC, 1995. "Reproductive and General Toxicology of Some Inorganic Borates and Risk Assessment for Human Beings", Technical Report No. 63, February 1995. (c) Murray, 1995.)

Boric acid and borax have in the past been used as food preservatives, although this use was largely superseded by the widespread introduction of refrigeration during transport and storage (Anon, 1980). The use of these compounds for food preservation is now also prohibited due to their potential toxicity. However, in some countries their use continues, although only in specialized circumstances.

Whilst the potential toxicity of borates has led to their use as preservatives in foods to be discontinued, their broad spectrum activity against both micro-organisms and insects has led to their extended use in biodeterioration control and wood preservation. Boron compounds are used as fungicides, algacides, bactericides and insecticides (Merck Index, 1989) with their use in wood preservation being effective against both fungi and insects. The use of borates as antiseptics has been discredited because of their lack of

effectiveness in this application and behavior as bacteriostats rather than as bacteriocides.

Boron Controls Biodeterioration

The problems of either potential mammalian toxicity as food preservatives or low level of effectiveness in antiseptics, appear to be avoided when borates are used for biodeterioration control in applications such as wood preservation. Borates are much less toxic than most other compounds used and the levels that can be used in treated timber allow borates to be highly effective wood preservatives. As a wood preservative boron compounds are one of the safest in current use, and no fatalities or other harmful effects have occurred due to this or other industrial uses. The earliest recorded use of boron in preservation is probably in the chromium boron preservative developed by Wolman in 1913 for the treatment of timber and in fact a wealth of data and information is available in the literature on this topic. For a comprehensive picture of the use of borates in wood preservation, the reader is directed to the reviews of Carr (1959); Cockroft and Levy (1973); Barnes *et al.* (1989); Dickinson & Murphy (1989); Drysdale (1994); and Lloyd & Manning (1995). Two decades after its introduction by Wolman, boron was proposed as a replacement for dichromate in flame retardant treatment of timber (Falck & Ketkar, 1934), as it possessed fungicidal, insecticidal and flame retardant properties. Promising toxicity data was then produced by Bateman & Baechler in 1937. Three of the four commercial fire retardant formulations reported to the American Wood Preservers Association in 1949 contained boric acid (Harlow *et al.*, 1949) and the most successful flame retardant systems used today still rely on borates and their numerous attributes.

Interest in borates as wood preservatives also arose in Australia, where they were recommended for the preservation of hard woods susceptible to *Lyctus* spp., destined for use in plywood (Cummins, 1938). The Forest Products Research Laboratory of Australia found that boric acid treatment made sapwood immune to attack (Hunt & Garret, 1953; Cockroft & Levy, 1973). Diffusion into veneers and sawn lumber was good, and by 1945 treatments were being carried out on a large scale. Similar work was carried out in New Zealand, but here a treatment for *Pinus radiata* to be used in house construction was needed. The first industrial treatments were carried out in 1949 using a rapidly diffusing boric acid/borax mixture equating to disodium octaborate tetrahydrate. A spray dried formulation of similar chemical composition then became available in the early 1950's and was sold under the trade name of Timbor®. The spray dried formulation was used widely and this process became known as Timborising, although today the formulation has gained widespread acceptance in vacuum pressure treatments. Boron treatment has been adopted almost exclusively for the treatment of rubberwood in South East Asia and this wood is available commercially in the form of furniture and other products on a global basis. Borates find use here, again for their performance against insects but also because of their colorless and odorless features amongst others. Powder post Lyctid beetles are also significant in these products, although the false powder post bostrychid beetles such as *Heterobostrychus* sp. are probably more so. The wood is particularly palatable to these organisms because of its very high natural starch and sugar content, which is typically used as the substrate by wood boring beetles rather than the actual cellulose component. Early research carried out in Europe was concerned primarily with the problems caused by *Hylotrupes* sp. and *Anobium* spp. (Kaltwasser, 1941). The first experiments with boron showed it to be as

effective as fluorine compounds and these results were later confirmed by Schwarz & Rensch (1943).

Efficacy against termites has also been demonstrated. Good results have been obtained by various workers, both in the field and the laboratory (Cockroft & Levy, 1973). The toxicity of boron compounds to termites in Australia has been shown to vary considerably with species, giving high toxicity to *Coptotermes* sp. and low toxicity to *Nasutitermes* sp. (Findlay, 1985); the minimum loading that was found to give protection of Matai and Radiata pine was in excess of 1% (Gay *et al.*, 1958). Although some authors have even suggested that boron compounds are not effective against termites (Richardson, 1978), the general consensus is that they are, but that a retention higher than that needed to control boring beetles is usually needed (Findlay, 1985). The question arises because borates have no repellent qualities, so will allow termites to come and 'taste' the treated wood. Such tasting or 'grazing' can allow some very slight cosmetic damage but could not conceivably result in structural failure of building timbers. Multiple exposure techniques have demonstrated this (Grace & Yamamoto, 1993). More recently borates have been demonstrated to be effective against the Formosan termite *Coptotermes formosanus* (Grace *et al.*, 1992; Grace *et al.*, 1995), and have been adopted in Hawaii where this problem is highly significant.

Specific research on the ability of borates to control wood colonizing fungi has also been extensively reported. There are very many species of fungi that are able to cause problems when they grow on timber but under conditions where borates are used, these can be put into two main categories: the moulds and stainers, which cause only a cosmetic problem; and the decay basidiomycetes. Control against staining organisms has been achieved and indeed, it was for this purpose that simple borates were first used against fungi (Scheffer & Lindgren, 1940). Borates are most effective against these organisms at high pH, so that sodium tetraborate is more effective than boric acid or more soluble mixtures (Richardson, 1978) although the more soluble mixtures are usually used to achieve the normal commercial concentrations required.

The effectiveness of borates against wood decaying basidiomycetes has now been demonstrated in service and in the laboratory by many workers (Carr, 1961), and is accepted. Organisms tolerant to other preservatives, such as copper, arsenate, PCP, creosote and TBTO (tributyl tin oxide) were amongst the first to be tested against borates. The principal organisms used included *Coniophora puteana* and *Coriolus versicolor* with others such as *Poria* spp., *Gloeophyllum trabeum* and *Lentinus lepιδius*. As yet, no wood decaying fungus has been reported to be tolerant to borates at normal preservative retention (Dickinson & Murphy, 1989).

The fungicidal mechanism of action of borates has been investigated (Lloyd *et al.*, 1990; Lloyd *et al.*, 1991; Lloyd & Dickinson, 1991; Lloyd, 1993). It was hypothesized that its primary mode of action was on general metabolism by interaction of the borate anion with polyols of biological significance, and the oxidized co-enzymes NAD^+ , NMN^+ and NADP^+ were suggested as the most likely target of the borate ion.

The hypothesis was tested on *in vitro* enzyme systems and fungal systems and *in vivo* with fungi in decaying wood. It was found that at all three levels of interaction the effects of borate could be completely negated by the addition of non-substrate polyols and that similar effects could be achieved with other chelating metaloids with similar

properties to the borate anion, again at all three levels. The hypothesis was thus upheld and it was concluded that the same mechanism was likely in all organisms, not just decay fungi.

The one restriction that borates have had in timber preservation has been as a result of their natural solubility and the depletion of borates from treated wood which can occur in ground contact applications. For this reason they have been restricted for use in interior situations such as in the treatment of rafter and stud used in the construction of dwellings and other general building use in protected environments, such as painted external joinery. This limitation of boron preservatives is, however, directly linked to one of their greatest assets - their mobility within the treated timber. This allows them to be highly effective in remedial situations in the home, as solid implants into utility poles and in industrial pre-treatment situations for construction timber in protected situations. Borates applied in these applications remain mobile in the treated wood and will continue to penetrate, thus providing one of the most effective wood preservative systems available today.

This restriction has also been overcome by the use of more complex formulations where boron is just one active ingredient in a formulation containing two or more. This is particularly the case with copper chrome boron (CCB) products used in vacuum pressure application for exterior application. These systems were adopted by Germany during the late 1940's instead of the more widely used copper chrome arsenic (CCA) formulations, because of concerns related to the toxicity of arsenic. CCA products are now being replaced across Europe and in other parts of the world, as registration authorities insist upon their removal from the market place and borates are the obvious substitute. In the continued search for friendlier wood preservatives, borates are also used in the next generation of exterior wood protection systems where they are preferred to other less desirable active ingredients.

It is particularly the use of borates in exterior preservative formulations which is likely to prove commercially more significant in the future. Current use of borates in preservation is estimated at approximately 10,000 tonnes per annum, as boric acid, borax and disodium octaborate tetrahydrate, although if arsenic in CCA preservatives were restricted from sale globally, this would likely increase to between 40,000 and 60,000 tonnes per annum, with the increase being made probably in the form of boric acid.

Natural fibre products apart from timber are also protected against biodeterioration by borates. These are receiving increased attention as a result of the ever increasing concern for our environment and the trend towards the use of naturally renewable raw material products. Such materials are now being used in a multitude of different applications including: structural and non-structural construction uses; insulation; fillers and strengtheners of other materials such as plastics and concrete. These can be based on natural cellulosic fibres, including those derived from waste paper, hemp, bagasse, cotton, flax, coconut and straw, and non cellulosic materials such as sheep's wool. The protection of such products is often a well recognised requirement, reflected by national standards. The British standard for straw products used in construction recognises the need to prevent infestation by Psocopterans such as the book louse *Liposcelis* sp. (BS 4046, 1991) and requires a mean boron content not less than 0.1 % B. This factor is probably of relevance

for straw bale construction, which is practised on a small scale in the United States (Bainbridge *et al.*, 1992).

In certain applications national standards and other requirements also require them to give a good performance in the event of fire. This is particularly the case when products are used in the home or in public buildings. Borates have again been found to be ideal for the treatment of these materials and are often used in the multi-functional role to give protection from fire as well as biodeterioration. Other benefits including their long term stability, lack of odour or colour and low toxicity and environmental impact have also supported the use of borates in these applications and the problems of corrosion, chemical attack of the substrate and premature char induction associated with many other flame retardant systems are not observed with borates.

Specific data on the bacteriostatic properties of borates was produced as early as 1929 (Allen) and these properties are still in commercial use today. Good examples of these can be given as their uses in lubrication fluids, as additives to air conditioning systems and their use as in-can preservatives and corrosion inhibitors in paints. Particularly zinc borates and barium metaborate have found specific use in this latter application, and efficacy against the following listed bacteria has been given (Schubert, 1992: Quill, 1992): *Pseudomonas aeruginosa*; *Pseudomonas putida*; *Pseudomonas stutzeri*; *Escherichia coli*; *Enterobacter aerogenes*; *Proteus vulgaris*; *Proteus moranii*; *Aeromonas hydrophila*; *Flavobacterium* sp. *Micrococcus flavus*; and *Alcaligenes* sp. Test paints inoculated with a mixed culture of these organisms were incubated at 30 °C and periodically examined for active bacteria. After 7 days the samples were re-inoculated and monitored for a further 21 days. After the second inoculation it was found that paint containing zinc borate showed no viable bacteria after 3 to 7 days and the barium metaborate samples required between 7 and 14 days to reach no viability. The control continued to maintain a high bacterial population.

The final large scale commercial use of borates in biodeterioration control which will be discussed here, is their use as fuel additives. Microbial growth can occur in liquid fossil fuels contaminated with water as organisms are able to use, particularly long chain hydrocarbons, as substrates for metabolism at fuel/water interfaces. There are a number of problems associated with microbial growth in fuels and these range from simple degradation of the material to more severe problems such as: corrosion of tanks, gaskets or coatings; the blockage of valves and gauges; or even problems in fuel lines and carburation systems which can be devastating depending on the particular application. It is therefore of no real surprise to find that specific action is usually taken as routine in aviation fuels. The deterioration of fuel systems by micro-organisms has been reported by many authors, although the problem and its control has been reviewed by Docks & Bennett (1986). The susceptibility of fuels varies according to a number of factors such as hydrocarbon chain length and the solubility of water in the hydrocarbon. Generally however, it is the amount of undissolved water which is significant and this usually lies in the bottom of storage tanks. Microorganisms whilst using the hydrocarbon as a substrate also require water and nutrients (from the water phase) although once established can be self sustaining for water as a result of its metabolic production. Fuels containing no undissolved water, whilst able to contain a multitude of different organisms, do not allow germination or growth.

Whilst many types of bacteria such as the pseudomonads can also be associated with problems in fuels, particularly corrosion, one of the most interesting organisms taking advantage of this ecological niche is the deuteromycete fungus *Cladosporium resinae*. This is the most common fungal contaminant in aviation jet fuels and, because of its mycelial growth and subsequent effects of fuel supply interruption, is rather significant. This fungus is reported to survive for up to three years in completely water free fuel (Berner & Ahearn, 1977) and can also tolerate other extreme environmental situations. For example, it is also associated with the decay of creosoted utility poles. The actual decay here, is as a result of basidiomycete organisms such as the creosote tolerant organism *Lentinus lepideus* but these organisms cannot usually become established without a certain amount of creosote detoxification, brought about by its utilization as a substrate by *C. resinae*.

The ubiquitous nature of microbial contaminant sources and the difficulty in removing water or trace minerals, means that the at first obvious counter measure against fuel biodeterioration, good house keeping or physical exclusion, is impracticable. Therefore the approach of chemical elimination or control is generally the most appropriate. Whilst many compounds have been assessed for this application, the ability of organisms such as *C. resinae* to detoxify organic biocidal components is an important factor. For this reason the inorganic borates have been most successful in this application. The actual application itself, however, requires an organic boron compound initially, in order to overcome the solubility problems of borates in hydrocarbon fuels such as paraffin/kerosene. Following a study of 97 compounds in 1968, Rogers and Kaplan showed that a mixture of boric acid esters was suitable. Today there are several micro-biocides used in aircraft fuel, including that same mixture of boric acid esters. Optimization of this additive led to the specific mixture of two boresters, which have now been marketed as a fuel additive for the last 30 years under the trade name Biobor[®] JF, and to its incorporation into U.S. Army specifications (MIL-S-53021). The composition of this mixture is a 95 % solution in petroleum naphtha of 2,2'-oxybis(4,4,6-trimethyl-1,3,2-dioxaborinane) and 2,2'-(1-methyltrimethylenedioxy) bis-(4-methyl-1,3,2-dioxaborinane). The boresters are particularly suited to this application as they have a good partition between water and fuel mixtures and boric acid produced as a result of the ester hydrolysis in water is also effective in controlling the causative organisms, when it is delivered to the aqueous phase by the fuel itself. These additives are successfully used to preserve diesel fuel, home heating oils and marine fuels, as well as kerosene products such as aviation fuel for which they were originally developed. Currently, approximately 300 tonnes of boric acid per year is used in their production.

Conclusions

Borates are ubiquitous within the environment, present in small amounts in sea and fresh water, soil and food. They are essential plant micro-nutrients and recent evidence has already suggested that boron is of nutritional importance to humans.

When the chemistry of boron and the reactivity of the borate anion with polyols is considered, the ability of borates to play a regulatory and essential role in plant metabolism is of no surprise. It would also appear reasonable to assume that because of the abundance of important alcohol containing compounds in other biological systems, a similarly important role could be expected in other organisms.

Borates have been commercially applied as micro-nutrients in agriculture since their importance was demonstrated in the 1920's. Their use by plants prior to this probably spans the length of evolutionary history itself. The dramatically increased yield which can be achieved by boron application in agriculture and the improvement in product quality is certain to see a continued increase in the use of borates in this application.

Borates have also been used as active ingredients in timber preservation for many decades, as well as in food and fuel, as antiseptics and as bacteriostats, although the use in food and as antiseptics has been discontinued. Borates are much less toxic than other preservatives used, and the relatively high quantities that can be reached in many treated products allow boron compounds to be effective in bacterial, algal, fungal and insect control. These factors are usually combined with their importance for plant growth, and lead most authors to conclude that in normal handling and use, borate preservatives present no risk to either people or the environment. These positive attributes were recognized in a review of the current expansion of interest in borates as wood preservatives, and Dickinson & Murphy (1989) concluded that the true value of borates in this area will be realized during this decade.

The use of borates to control biodeterioration fits well with the growing emphasis on the use of natural products, the protection of resources and the importance of environmental impact. Their use in biodeterioration control, and subsequent contribution to the quality of our lives, as in agriculture, is sure to continue to expand well into the next millennium.

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