EFFECT OF LOW TEMPERATURE ON BORON NUTRITION OF
OILSEED RAPE AND SUNFLOWER

This thesis is submitted for the degree of Doctor of Philosophy

Submitted by
Zhengqian Ye (B. Agric. Sci., M. Agric. Sci.)

Division of Science and Engineering
Murdoch University

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DECLARATION

I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary educational institution.

Zhengqian Ye
ABSTRACT

Several reports appear in the literature linking low temperature damage in plants with boron (B) deficiency and alleviation of low temperature injury with B application has been reported in some crops and trees. These results imply that low temperature might increase plant B requirements, beside the reduction of B uptake by plant roots, or that low B tissues might be more sensitive to cold temperature damage than B adequate tissues. In controlled experiments, it has been shown that low root zone temperature (RZT) induces B deficiency in cassava, a tropical root crop. Apart from this, there are few definitive detailed investigations on low temperature effects on B nutrition of plants, including temperate species which are more tolerant of low temperature.

Winter oilseed rape (Brassica napus L.), a crop sensitive to low B supply, is a major crop in the middle and lower Yangtse river basin, China, where low B soils are widespread. Appearance of B deficiency in oilseed rape often coincides with cold weather during its winter and spring growth. However, the incidence and severity of B deficiency of oilseed rape plants and the efficacy of B fertilization varies from year to year and location to location in ways that are not explained simply by differences in cultivar, agronomy or soil B levels. Low temperature is probably one of the important environmental factors influencing growth and yield of oilseed rape in relation to B nutrition.
Therefore, the objective of the studies in this thesis was to investigate mechanisms of low temperature effects on B nutrition of plants with emphasis on oilseed rape. Field and glasshouse experiments were carried out and the physiological basis of plant response to B at different air and root temperatures is discussed.

A field experiment with oilseed rape cv. Zheyouyou 2 was carried out on a red soil (Hapludult, US Soil Taxonomy) with low B availability in Zhejiang province, China. Canopy covers made from transparent plastic sheets, which increased night temperatures by up to 1.5 °C around shoots for 15 days in early February, strongly increased shoot dry weight at all levels of B supply. Furthermore, covering plants increased shoot dry weight of B deficient plants without increasing their leaf B concentration. This suggests that internal B requirements were decreased by canopy covering, possibly due to higher temperatures within the canopy.

Experiments conducted to investigate the effect of RZT (10 and 20°C) on oilseed rape cv. Hyola 42 response to B in solution culture, in summer and winter, showed that regardless of canopy conditions, low RZT (10 °C) promoted the distribution of shoot B towards the actively growing leaves, especially when B supply was low. At low B supply, B deficiency symptoms appeared later at 10 °C than 20 °C RZT and B concentrations in the youngest fully opened leaves (YOL) were higher in plants grown at RZT of 10 °C than that at 20 °C. Growth of plant dry weight (DW) was not affected
by RZT in the summer but was greatly reduced at 10 °C than 20 °C in winter. In B adequate plants, shoot to root ratio (S/R ratio) was not affected by RZT regardless of canopy conditions. By contrast, S/R ratio was smaller in low B plants at 10 °C than 20 °C. In addition, low RZT delayed occurrence of plant B deficiency symptoms regardless of plants’ pre-treatment RZT (either 10 or 20 °C). These results appeared to contradict the response to low RZT found in previous studies with cassava.

In a subsequent experiment, low RZT of 5 °C not only greatly reduced plant DW production of oilseed rape, but also accentuated plant B deficiency. Partitioning of B into the young growing shoots was also depressed and a significant decrease of B concentration in the youngest shoot parts was caused by 5 °C RZT in comparison with that at the control RZT (10 °C). Similar results were also observed in sunflower (Helianthus annuus L. cv. Hysun 25). But B deficiency symptoms in sunflower were induced by RZT as high as 12 °C, when plants were supplied with 0.25 µM B, whilst these plants were free from B deficiency at warmer RZT (17 - 27 °C). Higher external B concentrations were required at such RZT (Chilling temperature) for plant growth free from B deficiency. Therefore, there is a RZT threshold below which an increased response to B is expected in plants of oilseed rape and sunflower. And in the range of chilling RZT, the external B requirement for shoot growth increased with lower RZT. The threshold RZT was considerably higher in the chilling-sensitive plant species, sunflower, than in oilseed rape, a chilling-resistant plant species.
At chilling RZT, leaf functioning was impaired by low B supply as measured by potassium (K) leakage from the youngest mature leaf blade (YML) of sunflower, whereas it was much less directly affected by RZT, and there was no effect of RZT on B-adequate plants. By contrast to leaves, root function was impaired more by chilling RZT than low B.

Despite their different threshold RZT, in both oilseed rape and sunflower, the rates of B uptake (BUR) and B translocation from root to shoot (BTR) were dramatically depressed by chilling RZT especially at low B supply (0.2 µM B): being only 30% of those at the control (5 °C vs 10 °C RZT) in oilseed rape and 33% (10 °C vs 20 °C RZT) in sunflower, respectively. By contrast, there was little or no difference over a range of warmer RZT (10 - 20 °C for oilseed rape, and 20 – 27 °C for sunflower). It is predicted that higher rates of B application will be required for plant growth when soil temperature is below a critical threshold, which is between 5 and 10 °C for oilseed rape, and about 17 °C for sunflower, respectively. Below the threshold RZT plant B deficiency was induced and accentuated due to impairment of B translocation into growing shoot parts besides the decrease of B uptake rate and B transport rate and greater shoot to root ratio.

In comparison with RZT, little is known about causal mechanisms linking cold air temperature and B nutrition. Experiments in this thesis showed not only B transport to the shoot was strongly reduced by low night air temperature during a 6 day period (11.7
– 19.4 vs 15.5 – 23.5 °C), but also that an overnight chilling (at 0 °C) could cause more severe injury to low B than adequate B leaves of oilseed rape plants, expressed by higher solute leakage, in comparison with control (at 10 °C). Moreover, after chilling treatment, solute leakage from low B leaves was increased by exposure to light, which suggests that low temperature injury to leaves in low B plants after a freezing night in the field is at least partly a consequence of light induced damage of leaves.

In summary, at chilling temperature, B uptake, transport and partitioning into growing shoots are strongly impaired, and B use efficiency in the growing tissues might be reduced as well. Low temperature contributes to plant B deficiency also by increasing S/R ratio, so that shoot B demand is not satisfied by available B. Furthermore, low air temperature might increase the internal B requirement for shoot growth. To further understand mechanisms of low temperature, especially the air temperature, effects on plant responses to B, more research is needed, such as the relationship between low temperature and B incorporation into cell walls which may play an important role in leaf tolerance to chilling temperature.
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The publications listed below originated from the research contained in this thesis:


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Effect of low temperature on plant boron nutrition of oilseed rape and sunflower

Origin of the project

The present study originated from a research project of the Australian Centre for International Agricultural Research (ACIAR 9120 project) ‘Management of boron and zinc in oilseed rape in China’. In field experiments in Southeastern China, it was observed that freezing damage in leaves of oilseed rape plants was more apparent in plots without boron (B) fertiliser application. In contrast, leaves of plants of improved B nutrition from B fertiliser were either free of freezing damage symptoms or expressed only a very low frequency of the symptoms. A similar phenomenon has been reported in winter crops, such as in sugar beet under field conditions (Stoker and Tolman, 1941).

China is the largest producer of oilseed rape (Yang et al., 1993) and oilseed rape production is mainly concentrated in the mid-lower Yangtze river valley in Southeastern China where soils are often low in B status (Liu, 1996; Wei et al., 1998). Oilseed rape is reputed to be sensitive to low B supply (Shelp, 1993; Yang et al., 1993; Wei et al., 1998; Xue et al., 1998). As a winter crop, oilseed rape plants are exposed to persistent or episodic low temperature which commonly declines to < 0 °C in these areas (Ye et al., 1997). As a result, these two abiotic stresses may limit the vigour of this winter crop, leading to the reduced seed yield.

Low temperature damage in plants has been speculated to be associated with low B status in plants, in field crops (Stoker and Tolman, 1941), fruit trees (Hanson and
Breen, 1985) and also forest trees (Cooling and Jones, 1970). However, mechanisms responsible for this association or interaction are yet to be revealed (Shorrocks, 1997). In addition, low temperature damage in plant canopies may be influenced by other environmental factors, such as dry weather and sunlight intensity (Braekke, 1983). Therefore, well-defined controlled experiments should be conducted to examine physiological mechanisms involved in the interaction between low temperature and plant B nutrition, in order to formulate effective agronomic measures in the field for profitable oilseed harvest. The present research project focuses on how low root zone temperature (RZT) influences B uptake and utilisation in chilling-sensitive (sunflower) and chilling-resistant plant species (oilseed rape) and on the other hand, how B nutrition status in the leaves influences plant tolerance to low air temperature.
Chapter 1

Review of plant boron nutrition, low temperature physiology and their interaction

1.1 Plant boron nutrition

The essential requirement for boron (B) in plants was first confirmed as early as 1923 by Warington (Loomis and Durst, 1992) and subsequently B deficiency in plants has been reported worldwide (Shorrocks, 1997). Numerous reviews of B in plants and in soils have been reported, and B functions in plant physiological, biochemical and molecular processes have been proposed only some of which has been supported by direct evidence (e.g. Lewis, 1980; Dugger, 1983; Pilbeam and Kirkby, 1983; Shkolnik, 1984; Keren and Bingham, 1985; Lovatt, 1985; Loomis and Durst, 1992; Gupta, 1993; Shelp, 1993; Tanada, 1995; Goldbach, 1997; Blevins and Lukaszewski, 1998; Brown et al., 2002; Dannel et al., 2002). In particular, progress on defining B forms, functions and mobility have been presented at the International Symposia on ‘Boron in Soils and Plants’, held at Chiang Mai, Thailand in 1997 (Bell and Rerkasem, 1997; Dell et al., 1997), and at Bonn, Germany in 2001 (Goldbach et al., 2002a). More recently, a molecular mechanism governing B transport in Arabidopsis has been demonstrated by Takano et al. (2002).

1.1.1 Forms and functions of B in higher plants
1.1.1.1 Forms and compartmentation of B in plant cells

Boron is present in plant cells in all subcellular compartments, in the apoplasm, cell walls, cytosol and vacuoles with the majority of cellular B bound in cell walls (Dannel et al., 2002). Cellular B exists in water soluble and water insoluble forms (Matoh, 1997). A small proportion of cellular B is present as water soluble B in the apoplastic space as boric acid and the size of this B pool decreases with less B supply to plants (Skok and McIlrath, 1958; Shive and Barnett, 1973). In plant species with phloem-mobile B, the water soluble B fraction may be largely present in the form of B-polyol complexes (Hu et al., 1997; Hu and Brown, 1997b). Though the proportion of mobile B relative to total B is small, it is of key importance for plant growth (Brown et al., 1999).

The fraction of water insoluble B is largely complexed with rhamnogalacturonan-II (RG-II), and the complex is ubiquitous in higher plants, but the water insoluble B is mostly located in cell walls. Most of the B in cell walls is associated with pectin via the B-RG-II complex, a borate di-ester (Kobayashi et al., 1996). Recent results have shown that B-RG-II and homogalacturonan (HG) are covalently linked together in pectin (Ishii and Matsunaga, 2001). RG-II is the only confirmed B-containing polysaccharide isolated from biological materials (O’Neill et al., 1996) and is probably the only form of B structurally complexed within cell walls (Matoh, 1997).

1.1.1.2 Functions of B in higher plants

Boron is continuously required for growth of most plants, otherwise, plants grown in low B media exhibit B deficiency symptoms, of various forms (Gupta, 1993). Boron deficiency symptoms can be observed in vegetative and reproductive parts, such as growth inhibition of root and shoot tips, inhibition of flower development, reduced
setting and malformation of fruits and seeds, male sterility, and seed abortion (Dell and Huang, 1997). These morphological symptoms induced by B deficiency are linked with the structural role of B in cell walls and the poor mobility of B to the growing terminals in most species. However, observations at physiological and biochemical levels suggest that B may also play a key role on membrane structure and functions (Cakmak and Römheld, 1997).

Boron functions may include metabolic, structural and non-structural roles (Marschner, 1995). The postulated roles of B in plant physiological and biochemical processes include sugar transport, lignification, carbohydrate metabolism, ribonucleic acid (RNA) metabolism, respiration, indole acetic acid (IAA) metabolism, phenol metabolism, cell wall synthesis, cell wall structure and cell membrane functions, cell division and elongation, and so on (Marschner, 1995). On the basis of recent research findings, however, most of the above postulated roles on metabolic responses are considered to be at least secondary reactions to B deficiency in plants. The physiological role of B is still not yet fully understood although much progress has been made (Dell and Huang, 1997). Boron functions in higher plants have been reviewed several times recently (Goldbach, 1997; Blevins and Lukaszewski, 1998; Dannel et al., 2002; Brown et al., 2002). To date, direct evidence confirming the primary role of B is only demonstrated in cell wall structure (Matoh, 1997; Matoh and Kobayashi, 1998). The function of B in plasma membrane is also well described but no direct evidence has been reported so far (Cakmak and Römheld, 1997; Mühling et al., 1998; Kobayashi et al., 1999).
11.1.1.2.1 Boron functions: Cell wall

Loomis and Durst (1992) proposed a model for B involvement in cell walls in which borate was cross-linked in the middle lamella, probably related to calcium (Ca) cross-linking. Matoh and Kobayashi (1998) confirmed the essentiality of both B and Ca to maintain the integrity of cell walls through binding to the RG-II regions. Borate and Ca$^{2+}$ cross-linking in the RG-II region retain so-called chelator-soluble pectic polysaccharides in cell walls (Kobayashi et al., 1999).

The structural function of B in cell walls is directly supported by the role of B in B-RG-II complexation or cell wall formation (Matoh, 1997). Boron in cell walls is to maintain cell wall structure and integrity (Teasdale and Richards, 1990; Findeklee and Goldbach, 1996; Fleischer et al.; 1998) and it is essential for the normal pore structure of the wall matrix and for mechanical stabilization of the wall at growth termination so as to maintain cell viability. Besides the structural role of B, some reports have shown, however, that B may also involve in cell wall metabolism, such as cell wall synthesis (Bolaños et al., 1994; Behrendt and Zoglauer, 1996; Bolaños et al., 1996). Cassab (1998) also pointed out the importance of B in protein assembly in the cell wall. Extensin is the most well-studied cell wall structural protein of plants (Cassab, 1998). Extensin cross-links into the cell wall but exactly how has not been determined. In B deficient root nodules hypoxyprolene/proline (Hyp/Pro)- rich proteins are not covalently bound to the walls, thus Bonilla et al. (1997) also suggested that B has a role in the assembly of some wall protein components.
1.1.1.2.2 Boron functions: Cellular membrane

Boron is proposed to play an important role both in structural and functional integrity of membranes but the structure or form of B within and/or on plasma membranes has yet to be identified. Brown et al. (2002) suggests that the effect of B on plasma membranes may like that in cell walls (Matoh et al., 1998) involve complexation reactions that are important for the structure of the membrane: this suggest that both B and Ca are essential for stabilizing cell walls and membranes through B complexes. Brown et al. (2002) suggested that B plays a key role in maintaining proper membrane structure by forming cross-links with glyco-proteins and glyco-lipids in the membrane “rafts”, membrane sub-domains where these compounds are most abundant.

Some cellular B is located in membranes although the amount is a small component of the total B pool (Pollard et al., 1977; Torchia and Hirsh, 1982; Parr and Loughman, 1983; Tanada, 1983). The form of B in the membranes is possibly the same as B in the cell walls (Cakmak and Römheld, 1997; Blevins and Lukaszewski, 1998). Responses of membrane functions to B deprivation and those of B- deficient tissues to B addition are rapid, usually within a few minutes (Goldbach et al., 1990; Schon et al., 1990; Barr et al., 1993). Responses include membrane potential, ferricyanide-dependent H⁺ release, adenosine triphosphatase (ATPase) activity, reduced nicotinamide adenine dinucleotide (NADH) oxidase activity, ion transport, and membrane permeability (Cakmak and Römheld, 1997; Blevins and Lukaszewski, 1998). Pfeffer et al. (1998) suggest that B may play direct functional roles in the cellular membrane, possibly by regulating the activity of membrane-related enzymes, or altering membrane structure or integrity. By examining effects of B on the plasma membrane of ungerminated lily pollen grains, Obermeyer et al. (1996) showed that B stimulates the plasma membrane H⁺-ATPase.
They suggested a bimodal mechanism by which B stimulates germination and growth of pollen tubes: firstly, B may stimulate the plasma membrane H\textsuperscript{+}-ATPase, secondly, B stabilizes the tip of the elongating pollen tube. The putative structural role of B in cellular membranes may be similar to that in cell walls through formation of B complexes (Mühling et al., 1998).

### 1.1.1.2.3 Boron functions: Metabolic roles

Besides the roles of B in cell walls and cell membranes, B may have roles in plant physiological and biochemical processes (Shelp, 1993; Marschner, 1995; Cakmak and Römheld, 1997). The important processes include nitrogen (N) metabolism and enzymatic activity (eg., inhibition of nucleic acid synthesis; enhanced ribonuclease activity); carbohydrate metabolism and photosynthesis and respiration (eg., impaired transport of carbohydrate; chloroplast damage); phenol metabolism (eg., accumulation of phenolics; increased activity of polyphenoloxidase) and hormone metabolism (eg., increase of IAA content; reduction of production and export of cytokinins (CKs) from the roots to the shoots) (Shelp, 1993; Marschner, 1995; Cakmak and Römheld, 1997; Goldbach, 1997).

Most of the responses above are considered not to be the primary or the direct effects of B deficiency because these responses (eg., phenol metabolism) follow well after those related to B responses in the cell wall and membranes (Shelp, 1993; Goldbach, 1997). Some of the metabolic effects may be related directly to perturbation of cell wall or plasma membrane functions, such as the impaired ascorbate metabolism by B deficiency which could link with plasma membrane electron transport (Lukaszewki and Blevins, 1996). The difficulty in separating a structural role of B in cell walls from
those in cellular membranes and cellular metabolic processes does not yet allow any conclusive assertions about any direct B roles in metabolic processes.

1.1.2 Mechanisms of B uptake and transport in plants

1.1.2.1 Boron uptake and transport from root to shoot

Mechanisms of B uptake by plants and B translocation within the plant have been contentious for a long time (Bowen and Nissen, 1976, 1977; Hu and Brown, 1997a), but recent work has begun to clarify the processes (Dannel et al., 1997, 2000 and 2002; Pfeffer et al., 1997, 1999; Stangoulis et al., 2001; Brown et al., 2002). Although B uptake is mainly a passive process (Hu and Brown, 1997a), Pfeffer et al. (1999) suggested possible active B uptake by roots at low B supply, but the evidence was complicated by the difficulty separating the initial phase of B complexation in cell walls from the apparent active uptake through cell membrane. For plants receiving low B supply, the transport may be governed by other mechanisms and even the mechanism of xylem loading still remains unclear (Pfeffer et al., 1999), although this is facilitated by B transporters as reported just recently by Takano et al. (2002).

Pfeffer et al. (1999) examined the B uptake mechanisms by using young sunflower plants. When plants were supplied with low B (1 µM), the concentration of free boric acid in the root cell sap was fourfold higher (3.8 µM). Moreover, the concentration of free boric acid in the root cell sap was remarkably lowered by 2,4- dinitrophenol (DNP), an inhibitor of metabolic processes regardless of B treatment levels (1 or 100 µM) for 2 h of B treatment. By contrast, in plants continuously supplied with 100 µM B, the concentration of free boric acid in the root cell sap was not affected by DNP. Therefore,
two mechanisms for B uptake into the roots were suggested: passive diffusion when B supply is high, and active process when B supply is low.

It is now clearer how B traverses plant cell membranes at the molecular level. There are possibly two passive mechanisms of plant B uptake. Boron uptake is facilitated by passive diffusion through lipid bilayers as well as B channels which belong to the aquaporin group (Dordas et al., 2000; Dordas and Brown, 2001; Stangoulis et al., 2001). By contrast, no evidence of active B uptake was observed under the examination of B uptake by squash plants in a range of B supply from 0 to 50 µM (Dordas and Brown, 2001). Earlier, using isolated membrane vesicles from squash roots it was shown that B transport through plant membranes was in part by a passive process via lipid diffusion and in part by B channels (Dordas et al., 2000), and results of further studies on intact squash seedlings were in agreement with this (Dordas and Brown, 2001). Boron uptake is strongly limited by mercuric chloride (HgCl₂), and other channel inhibitors. Boric acid permeation of the plasma membrane vesicles was reduced by 30 – 39 % (Dordas et al., 2000), and B uptake was reduced up to 90 % in squash seedlings (Dordas and Brown, 2001) by HgCl₂. The B uptake inhibition by HgCl₂ was attributed to the blocking of B channels (Dordas et al., 2000; Dordas and Brown, 2001)

New shoot growth needs a continuous B supply because of the restricted phloem B mobility, in most species. For example in tomato (Oertli, 1993) severe B deficiency can be exhibited in the young shoot tops following transfer to low B solution whilst old leaves contained excessive B from a prior episode of high B supply. Therefore, once B enters into roots, the controls on the long distance transport of B from roots to shoots are of key importance for shoot growth.
Boron transport from root to shoot is substantially driven by transpiration water flow, but not entirely (Raven, 1980; Marschner, 1995). Thus, on the one hand, B distribution is closely related to water loss from shoot parts: B concentrations in shoot parts (between organs and even within a particular leaf) generally correspond with their transpiration rates and age (Shelp, 1993; Marschner, 1995). When enough B is supplied, gradients in B concentrations between shoot organs follows the order of leaves > pods >> seeds, and within the same leaf: margins and tips > middle of lamina > petioles and midribs (Shelp, 1993; Oertli, 1994). When excessive levels of B are supplied to the plants, B toxicity symptoms appear on margins and tips of mature leaves (Oertli, 1993, 1994). However, ‘no research has directly compared the distribution of B among shoot organs with their respective transpiration rates or shoot B accumulation with transpiration to estimate the B content of xylem sap from intact plants’ (Shelp, 1993).

Boron translocation from root to shoot may also differ from different plant species and cultivars (Brown and Jones, 1971; Bellaloui and Brown, 1998; Takano et al., 2001). Boron partitioning into shoots has been found to be greatly different between (Bellaloui and Brown, 1998) and within species even when grown under the same environmental conditions such as in celery and tomato (Brown and Jones, 1971; Bellaloui and Brown, 1998) and Arabidopsis thaliana (Takano et al., 2001). The different capacity of B partitioning into and within shoots results in differential external B requirements. In celery and tomato, a large proportion of B is retained in the roots in B- inefficient cultivars; whilst more B is transported into shoots in B- efficient cultivars (Brown and Jones, 1971; Bellaloui and Brown, 1998). The distribution of B to leaves and stems of celery (cv. Emerson Pascal and S48-54-1) was much higher in Emerson Pascal (B-
efficient cultivar) than that in S48-54-1 (B- inefficient cultivar) regardless of external B levels (0.01 – 1 mM $^{10}$B) (Bellaloui and Brown, 1998). Grown at 0.01 mM B for 72 h the B- inefficient tomato (cultivar: T3238) developed B deficiency symptoms whilst the B- efficient cultivar (Rutgers) was free of B deficiency symptoms. Hence, the B- inefficient cultivars require more B than the B- efficient cultivars for normal growth of the shoots. This was also evident in *Arabidopsis thaliana* which had greater B partitioning into the shoots in the wild-type plants than the mutant *bor-1* (Takano et al., 2001). Tests of reciprocal grafts demonstrated that B transport from roots to shoots was controlled by the roots rather than the shoots in tomato (Brown and Jones, 1971).

However, B translocation to shoot tips may also be influenced by mechanisms other than transpiration flow or phloem transport. In *Arabidopsis thaliana*, Noguchi et al. (1997a) have observed that B transport to the inflorescence at the shoot apices is probably due to sink activity since the continued production of cell walls creates a continuously expanding sink for B. In a short-term (48 h) experiment of B uptake and translocation in *Arabidopsis thaliana* by using $^{10}$B, the results showed that no B was retranslocated from old to young organs, but the absorbed B during the 48 h was partitioned into the growing parts (Noguchi et al., 1997a). This was attributed to their higher sink strength. In wheat, when the young ear was still enclosed within the leaf sheaths without any significant transpiration activity, the gain of ear B was mainly transported from the nutrient solution through root uptake (Huang et al., 2001), and attributed to the existence of xylem-to-phloem transfer of B during transport from root to shoot which would deliver B to the non-transpiring new growing tissues through the phloem.
Among cultivars of *Brassica* and wheat, variation in distribution of B into the reproductive plant parts has been reported (Shelp, 1995 for *Brassica*; NaChiangmai et al., 2004 for wheat). In these studies the increased partitioning of B into the reproductive parts was attributed not to increased phloem mobility of B, but to xylem-to-phloem transfer of B absorbed by roots. The mechanism underlying the increased apparent xylem-to-phloem transfer has not been determined, but variation in this trait is apparently so large in wheat as to allow the B efficient cultivar Fang 60 to exhibit no decrease in grain set in low B soils, whereas the B inefficient cultivar SW41 has greatly reduced pollen viability and grain set in the low B soil (NaChiangmai et al., 2004).

Moreover, Noguchi et al. (2000) and Takano et al. (2001) reported that the wild-type plant of *Arabidopsis thaliana* was able to transport B from root to young shoot only under low external B, and it was suggested that another mechanism(s) besides the transpiration flow would be involved (Takano et al., 2001).

Further studies comparing the wild type and *bor-1* mutant of *Arabidopsis thaliana*, have shown that differences in B transport and distribution, are due to differences in expression of B transporters (Noguchi et al., 1997b, 2000, 2003; Takano et al., 2001 and 2002). *Arabidopsis thaliana* mutant *bor1-1* requires higher external B for normal growth (Noguchi et al., 1997b) due to the failure to efficiently transport B from root to the growing parts when B supply was low (3 µM). Considerably less B transport from root to shoot (Takano et al., 2001) was demonstrated with the use of $^{10}$B in the solution culture experiments. In contrast, the root absorbed B was preferentially translocated into the young leaves compared to the old leaves in low B supplied wild-type plants and this was regulated by the BOR1 gene. Efficiency of B distribution towards growing parts is
central to B requirements (especially external requirements) because continuous supply of B is necessary for their growth but not for the mature plant parts (Brown et al., 1999).

Recently active B transport through a B transporter was confirmed by molecular biology (Takano et al., 2002). The transporter is assumed to be located in cell membrane of cortex cells for unloading B into the xylem in roots. Studies on B uptake by *Arabidopsis thaliana* showed that xylem loading is the key step for B transport from root to shoot when B supply is low (≤30 µM) and a B transporter, BOR1, is responsible for the xylem loading (Takano et al., 2002). BOR1 facilitates B transport from root to shoot by increasing B concentrations in xylem sap against a concentration gradient.

Therefore, under low B supply, it still remains uncertain whether active B uptake takes place by plant roots, but it is now clear that active B transport into the xylem is mediated by a B transporter.

**1.1.2.2 Boron mobility and redistribution**

In the past, B was considered as a phloem immobile nutrient in plants (Raven, 1980; Shelp et al., 1995) based on the occurrence of both B deficiency and B toxicity symptoms appearing in terminal plant parts: B deficiency symptoms occurred in young growing tissues (young leaves and shoot tips) and B toxicity symptoms in margins or tips of mature leaves in most cases.

Phloem mobility of B is critical for plants grown in low B media because of limited available B in the environment. The speculation that B moved via the phloem was raised by some researchers (Husa and McIlrath, 1965; Oertli and Richardson, 1970;
Campbell et al., 1975). Because of an increase of root B content after the transfer of tomato seedlings from B supplied media to B free media, Oertli (1993) concluded that a small amount of B was transported from the tops to the roots. However, the possible mechanism was not examined. Earlier, in peanut, Campbell et al. (1975) reported that B concentration of peanut seeds was higher when plant roots or fruits were supplied with B in comparison with those without B supply, and it was suggested that B was translocated via the phloem. But there was a lack of techniques to demonstrate such movement directly until the introduction of B stable isotopes and various types of mass spectrometry (spark-source, laser-probe) and inductively coupled plasma (ICP) mass spectrometry (Martini and Thellier, 1980; Chamel et al., 1981; Chamel and Andreani, 1985; Hanson, 1991).

Boron mobility in the phloem of most plant species is limited, although the small amount of mobile B in the phloem may still be significant for growth (Shelp et al., 1995). Phloem B is the B source for the new shoot growth of some plant species when external B supply is low, such as in broccoli (Shelp, 1988), particularly during the early reproductive growth (Marentes et al., 1997).

It is now clear that B mobility differs greatly among plant species (Brown and Shelp, 1997): in most plant species, B mobility in the phloem is very low, whilst B is freely mobile in plant species that transport photo-assimilates as polyols such as sorbitol, mannitol and dulcitol. The polyols facilitate B transport in the phloem by the formation of B-polyol complexes (Brown and Hu, 1996; Hu et al., 1996, 1997). By using transgenic tobacco enhanced in sorbitol synthesis, Brown et al. (1999) reported that foliar B supply alone to three mature leaves enabled the normal growth and seed yield
of tobacco plants producing sorbitol, but resulted in a poor growth and great loss of seed yield in tobacco plants which did not produce sorbitol. The latter plants could grow normally and produce seed only when supplied adequate B to the roots. This demonstrated convincing evidence of long distance transport of B via the phloem, facilitated by sorbitol.

Because the growing plant parts can receive nutrients from mature parts if they are readily mobile within the phloem when external nutrient supply is in deficit, the internal requirement of nutrient for growth may diminish with the higher phloem mobility of the nutrient. Plants featuring high B mobility can sustain their growth with less supplied B and even could be satisfied with the recycling of plant B that has been already acquired without further supply of external B, as has been discussed above.

The identification of a mechanism for B mobility in the phloem of some plant species provides the possibility that important crops with low phloem mobility of B at present may become genetically engineered to have increased B mobility and reduce their B requirement, since the mature leaves have little need for further B once they reach full size (Brown and Hu, 1997; Brown et al., 1999).

1.1.2.3 Requirements of B for plant growth

Requirements of B for plant growth vary widely among species and within species, and with plant growth stage as well (Gupta, 1993; Marschner, 1995; Rerkasem and Jamjod, 1997; Shorrocks, 1997). Some plants, mainly the Graminaceous species, require only a little amount of B, usually between 2 and 5 mg kg$^{-1}$ in leaves. By contrast, other plants such as some latex-producing species require more than 80 mg B kg$^{-1}$ in leaves. The
remainder require intermediate B levels. This group includes non-grass monocotyledons and dicots. Generally, dicotyledons require more B than monocotyledons (Gupta et al., 1985). Legumes and crops of Cruciferae, Chenopodiaceae and Umbellifereae families have high B requirements (Martens and Westermann, 1991; Gupta, 1993). Plant species with high pectin content usually require high tissue B concentration: plants of the Graminaceous species which contain low cell wall pectin content, also have very low B requirements, e.g., 7 % pectin (expressed as uronic acid as a % of cell wall) and 4 - 10 mg B kg\(^{-1}\) dry weight (DW) for barley, and 20 % pectin and 43 - 55 mg B kg\(^{-1}\) DW for asparagus, respectively (Hu et al., 1996).

Regardless of plant species or varietal differences in B requirements, reproductive growth requires more B than vegetative growth (Gupta, 1993; Rerkasem and Jamjod, 1997; Rerkasem et al., 1997). Since the functions of B in higher plants are not fully clear, it is difficult to conclude at this stage why reproductive growth has higher B requirements, and whether there are extra functions in reproductive tissues for which B is required. But since the internal B requirements are likely to be determined by composition of cell walls such as cell wall pectin content (Hu et al., 1996), control of B partitioning to shoot from root and within shoot, and mobility of B in phloem (as has been discussed in the previous two sections), these factors are likely to have a significant bearing on B requirements for reproductive growth.

The requirement of B for plant growth is affected by a number of environmental factors, such as temperature, light and humidity, and soil water conditions (Moraghan and Mascagni, 1991; Gupta, 1993; Shorrocks, 1997). Although it has long been recognized
that B deficiency interacts strongly with the environmental factors, the mechanisms
governing these interactions are poorly understood.

Appearance of B deficiency symptoms in many crops can be associated with dry
weather (Hopmans and Flinn, 1984; Noppakoonwong et al., 1997; Pant et al. 1998; Xue
et al., 1998). Soil B availability generally decreases with soil drying (Fleming, 1980)
and low soil water, especially as topsoil dries out, depresses B uptake by plants (Hobbs
and Bertramson, 1949; Huang et al., 1997). In oilseed rape, dry condition increased root
growth relative to shoot (R/S ratio) and decreased root B uptake in soil without B
addition but not in soil with B addition (0.45 mg B kg\(^{-1}\)), however, it was unable to
offset the decrease of root B uptake (Huang et al., 1997). Two explanations linking B
deficiency with the dry weather are proposed (Moraghan and Mascagni, 1991): (a) as
topsoil dries out, less B becomes available to plants due to the relative abundance of B
in topsoil (e.g. as demonstrated by Hobbs and Bertramson, 1949) and the limited
phloem mobility of B in many plant species; (b) secondly, it is suggested that there is
decreased release of B from mineralization of soil organic matter (Bell, 2000).
However, so far there is a lack of direct evidence to support the latter explanation.

Rainy weather also impacts on plant B nutrition. Besides its effect on soil B leaching
rate, low vapour pressure deficits in the atmosphere during the rainy season slow down
the transpiration rate, this may inhibit B uptake and transport into growing plant parts
(Oertli, 1994; Rawson, 1996a, b). When transpiration was interrupted at the critical
stage of pollen development in wheat ears, even an adequate external B supply was
unable to prevent the development of sterility, a known symptom of B deficiency
(Rawson, 1996b).
High light intensity accentuates development of B deficiency in sunflower (Cakmak et al., 1995) and increases internal B requirement of black gram (Noppakoonwong et al., 1993). The higher B requirement in plants exposed to high light intensity is suggested to relate to greater plant growth rate, and elevated phenol content and cell wall carbohydrates which immobilize B by formation of stable borate complexes (Cakmak and Römheld, 1997).

Temperature is one of the key environmental factors which affects plant B nutrition and because of its centrality to the present thesis, it will be discussed in detail in the following sections.

1.2 Temperature effects on boron nutrition in plants

Temperature is one of the main environmental factors that not only affects plant growth itself, but also plant responses to low B supply. However, the mode of low temperature and low B supply interaction varies with plant species due to their varied low temperature tolerance (Moraghan and Mascagni, 1991). The mechanisms linking low temperature and B response still remain unclear (Shorrocks, 1997). A better understanding of the mechanism of temperature x B interactions may eventually assist farmers to grow better crops under unfavourable temperature conditions.

Although direct involvement of temperature stress in inducing or exacerbating plant B deficiency has been observed in experiments under controlled conditions in sunflower at high temperature (46 - 47 vs 28-30 °C) (Bozhenko et al., 1972) and in cassava at low
temperatures (18 - 23 vs 28 - 33 °C) (Forno et al., 1979), both species are chilling sensitive. In the literature, reports about studies conducted under controlled conditions concerning the interaction between low temperature and B nutrition in plants is limited. So far, studies on low temperature effects on plant B deficiency have been reported only on cassava (Forno et al., 1979), wheat (Huang et al., 1996a; Subedi et al., 1998b, 2001) and sunflower and oilseed rape in present study.

Boron status may affect plant tolerance of low temperature. Plants with adequate B are reported to be more resistant to low temperature injury (Stoker and Tolman, 1941; Combrink et al., 1995; Ye et al., 1997; Wang et al., 1999). In field experiments, B supplied sugar beet and oilseed rape plants were free of, or had reduced frost injury compared to those receiving no B application (Stoker and Tolman, 1941). In muskmelon, application of B improved plant growth and fruit quality, and in addition the incidence of chilling injury decreased in harvested fruit during cold storage at 5 °C (Combrink et al., 1995).

As a result, B nutritional status of plants may have played a crucial role in their tolerance of low temperature, but how physiological mechanisms are involved remains unsolved. In particular there has been limited research has been conducted on how low temperature affects root functions in B uptake, shoot transpiration for B transport and B partitioning.

1.2.1 Plant physiological responses to low temperature
1.2.1.1 Low temperature tolerance of different species and concept of ‘low temperature’

Plants have a wide temperature range for optimal growth where the plant is in a ‘zero-stress’ state (Mahan et al., 1995). Optimal temperature for plant growth varies among plant species and within one species, and also between plant parts in one plant (Cooper, 1973). For example, the optimum temperature for maximum production of oats is as low as 5 °C, but as high as 26 °C for corn (Singh, 1998), and root zone temperatures (RZTs) of 10 - 25 °C are optimal for oilseed rape shoot DW (Macduff et al., 1987a and b).

The terms ‘low temperature’ or ‘suboptimal temperature’ are frequently used without a clear definition. Usually they mean that the temperature is below the optimal temperature range for plant growth (Mahan et al., 1995; Greaves, 1996). ‘Low temperature stress’ is defined as ‘Any drop in temperature that can evoke reversible or irreversible functional disturbances or lethal injuries’ (Larcher and Bauer, 1981). Thus, low temperatures include positive temperatures and below 0 °C temperature. ‘Chilling temperatures’ are low, non-freezing temperatures, i.e. low positive temperatures (Larcher and Bauer, 1981; Raison and Lyons, 1986). ‘Chilling sensitive plants’ are injured at low temperatures in the absence of freezing (Larcher and Bauer, 1981), i.e. at chilling temperatures. ‘Cold resistance’ is ‘Ability to resist low temperature stress (chilling, frost) without injury (Larcher and Bauer, 1981). Therefore, most tropical plants are damaged at chilling temperatures of 10 - 25 °C (Raison and Lyons, 1986), while these temperatures do no harm to cold resistant plants.
Temperature below the optimal range is then ‘low’ or ‘suboptimal’. ‘Suboptimal temperature stress can be defined as ‘any reduction in growth or induced metabolic, cellular or tissue injury that results in limitations to the genetically determined yield potential, caused as a direct result of exposure to temperature below the thermal thresholds for optimal biochemical and physiological activity or morphological development’ (Greaves, 1996). However, temperatures below 10 - 15 °C are generally regarded as ‘low’ temperatures, since they causes chilling for most tropical and subtropical plants and causes direct injury to the plants, whereas temperatures below 0 °C are ‘freezing’ (Levitt, 1980; Raison and Lyons, 1986; Pollock and Eagles, 1988; Murata and Los, 1997).

In the remainder of the thesis, and in accordance with the general concept of ‘low temperature’ in the literature, ‘low temperature’ is generally regarded as that temperature below 10 - 15 °C regardless of plant tolerance/resistance of low temperature. While ‘cold’ temperature is used instead of ‘low’ temperature to imply the (possible) injury to plants at any temperature including subzero degree, and ‘chilling’ temperature is similar to the ‘cold’ temperature but above 0 °C when the plant is injured or at risk of injury. Temperature below 0 °C is ‘freezing’. ‘Chilling-sensitive plants’ and ‘chilling-resistant plants’ refer to the plants which are injured and without injury at low temperatures of 10 - 15 °C, respectively.

1.2.1.2 General: Regulation of growth and nutrient requirements by low temperature

A range of metabolic (physiological and biochemical) and structural (anatomical and morphological) changes occur in plants in response to low temperature (Cooper, 1973;
Levitt, 1980; Pollock and Eagles, 1988). The physiological and biochemical changes include rates of cell division, photosynthesis, respiration, hormone synthesis and translocation, lipid synthesis, and properties of membrane, proteins and enzymes, as well as nutrient uptake (Berry and Raison, 1981; Graham and Patterson, 1982; Long and Woodward, 1988; Howarth and Ougham, 1993). Structural changes may include root branching and root length, and cell wall properties (Cooper, 1973; Bowen, 1991; McMichael and Burke, 1996, 1998; Rapacz, 1998; Equiza et al., 2001). Consequently, low temperature affects many aspects of plant growth and development, such as growth rate and morphological development (Berry and Raison, 1981; Long and Woodward, 1988). Nutrient requirement can also be altered by low temperature through its influence on shoot to root ratio (S/R ratio) or other mechanisms (Clarkson et al., 1988).

Some processes of plant growth are more sensitive to low temperature than the others, and the severity of low temperature effects on the same process varies with plant species and even within species (Miedema, 1982; Paul et al., 1990). For example, carbon assimilation is more depressed than photosynthesis in the cold, and photosynthesis in rape is more tolerant of low temperature than growth (Paul et al., 1990). Sunflower leaf expansion is more affected by low temperature than cell division (Granier and Tardied, 1998). Reduction of dry matter production in barley plants grown at 5 °C in comparison with those at 15 °C RZT was attributed to reduced water uptake rather than to lower photosynthesis (Sharratt, 1991). Nutrient uptake is affected more by low temperature than plant growth (DW production) (Cooper, 1973).

The biochemical and physiological changes induced by low temperature may have profound implications for nutrient demand for new growth and for nutrient acquisition
capacity in the root. The impact of low temperature on resource supply to the new growth will depend on the balance between the reduction in demand of nutrient and water and in root acquisition capacity. When the supply of water/nutrient is most limiting, plant growth is also affected by low temperature indirectly via its influence on water and nutrient availability (Russell, 1977). Indeed changes of plant water status and water properties, such as decrease of water conductivity in plant, induced by low temperature may be the key biophysiological processes that relate to plant growth and nutrition, especially at low RZT (Clarkson et al., 1988; Bowen, 1991).

Low temperature may alter nutrient concentrations in plant parts and concentrations of nutrients in plants have been reported to be increased or decreased in a growing environment of low temperature (Cooper, 1973; Engels, 1993). Low temperature may reduce plant growth rate and thus nutrient demand for new growth (Engels, 1993). However, plants may have a higher nutritional requirement for growth at low temperatures, because low temperatures may result in deficiency of nutrients, such as phosphorus (P) (Cumbus and Nye, 1985) and B (Forno et al., 1979), and increased supply of nutrient may improve or correct these disorders (Forno et al., 1979). Plant nutrient deficiency occurs when the rate of nutrient supply to growing tissues falls below the rate of demand and usually occurs when the nutrient supply is reduced.

1.2.2 Low temperature effects on plant response to B

Field studies have shown that the frequency of B deficiency in crop plants was increased in cold winters (Stoker and Tolman, 1941). Boron deficiency in cassava induced by low temperature is suggested to be due to a combination of decreased B
uptake rate and enlarged size of shoot relative to root (Forno et al., 1979). Yet, little is known about low temperature effects on plant B deficiency in low temperature tolerant plant species. Here, information linking B deficiency with low temperature is summarized and the possible underlying mechanisms are discussed.

### 1.2.2.1 Plant responses to low temperature in the field with emphasis on B deficiency

Damage to growth of plants under cold weather and low B has been reported in several countries in forest trees, fruit trees, as well as field crops (Stoker and Tolman, 1941; Thompson and Liu, 1973; Chaplin et al., 1977; Callan et al., 1978; Braekke, 1983; Hanson and Breen, 1985; Subedi et al., 1998a; Švagždys, 1995; Blevins et al., 1996).

The observed symptoms of damage by low temperature stress at low B includes greater pollen sterility by chilling (8/2 °C day/night, d/n) in wheat (Subedi et al., 1998a), reduced fruit set by frost (Hanson and Breen, 1985), killing off of the youngest leaves and shoot tip dieback in fruit and forest trees by frost (Cooling and Jones, 1970; Braekke, 1983; Blevins et al., 1996), and freezing injuries to crop leaves as in sugar beet (Stoker and Tolman, 1941) and in oilseed rape (Ye et al., 1997).

Freezing injury is more severe in plants with low B status than those with adequate B status. As early as in 1941, field experiments showed that leaves of sugar beets with B deficiency were severely injured by frost (coldest night temperature was -0.5 °C); whilst almost no frost injury was noted in B supplied plants (Stoker and Tolman, 1941). In forest trees, symptoms of ‘killed apical shoots’ of trees damaged by radiation frost and ‘shoot dieback’ accentuated by dry weather were often observed in forest plantations in Norway (Braekke, 1983). It was found that low leaf B concentrations were closely
related to the frost damage and shoot dieback, and the incidence was reduced by B fertilization (Braekke, 1983). These reports suggest that improving B nutrition may enhance plant tolerance to low temperature stress.

Low soil B may exacerbate the negative effects of low temperature on plant growth (Rerkasem et al., 1990). In a low B soil in northern Thailand, black gram seeds produced more than 75% abnormal seedlings when the seeds were sown in the field in December. By contrast, the same batch of seeds produced about 50% abnormal seedlings when the seeds were sown in January (Rerkasem et al., 1990). Moreover, only 9 - 22% and almost no abnormal seedlings were produced in B treated soil when the seeds were sown in December and January, respectively. It was suggested that higher percentage of abnormal seedlings produced in the December planting is probably due to lower air and soil temperature from 7 days after sowing which would have affected the emerged seedlings. Increasing soil B supply and sowing high B seeds substantially overcame the effects of low temperature.

In considering low temperature injury of low B plants and cold weather effects on plant B deficiency, it is necessary to consider both short-term (i.e. direct) and long-term effects of low temperature on the B status of plants. However, complex interactions can develop in the field, such as dry conditions coinciding with low temperature, confounding responses to low temperature. The temperature itself also varies during day and night, and differs from soil to air temperature, so that it is difficult to draw firm conclusions from the existing field work on the relationship between low temperature and low B in relation to plant growth.
1.2.2.2 Plant responses under controlled conditions

Unlike field experiments, controlled environment experiments may avoid the multiple factors affecting B and temperature interactions with plant growth. There are numerous reports from controlled environment experiments on temperature responses in plants, but few reports dealing with B deficiency.

The nature of the temperature and B nutrition interaction in plant growth and physiology may vary with plant species and temperature regime (day/night; above/below ground, etc.) (Oertli, 1963; Jensén and Perby, 1986; Perby and Jensén, 1986; Clarkson et al., 1988; Moraghan and Mascagni, 1991). The complexity of these factors may have led to different conclusions regarding particular physiological processes that respond to temperature treatments at different levels of B supply to the roots.

1.2.2.2.1 Experiments at sufficient or excessive B supply

In barley seedlings (Oertli, 1963), B concentrations increased with increasing RZT from 9 to around 25 °C, but decreased when RZT was further increased to 39 °C RZT. The intensity of the RZT effect also varied with different plant parts. Similarly, Vlamis and Williams (1970) found that B concentrations in old leaves were higher in barley plants grown in Hoagland solution at 15 °C than at 10 or 20 °C, but root B concentrations were not affected. Nable et al. (1990b) also found that leaf B concentrations of barley increased with RZT from 10 to 20 °C when excessive B was applied in the solutions (1 mM B), however it was also shown that RZT (ranges from 5 to 25 °C) had no effect on B concentrations in barley plants when supplied with adequate B (0.015 mM B) (Nable et al., 1990a). Moreover, Mahalakshmi et al. (1995) reported that lowering soil
temperature resulted in higher B concentrations in barley shoots and caused B toxicity symptoms to appear earlier than that at higher soil temperature (5 vs 10 and 15 °C) due to low growth rate relative to B uptake at low soil temperature.

Shoot B contents in maize seedlings grown in a B sufficient soil changed little when increasing soil temperatures from 12 to 20 °C although the shoot DW increased about sevenfold, but further increases in soil temperature (20 - 32 °C) resulted in increased B uptake (Walker, 1969). Correspondingly, shoot B concentrations decreased from 91 to 16 mg kg\textsuperscript{-1} as soil temperatures increased from 12 to 20 °C and then increased at higher soil temperatures. However in a solution culture when supplied with 0.5 mM B, B concentrations in maize plants were not affected by RZT although total B uptake increased with increasing RZTs (21 °C vs 9 and 15 °C) (Mozafar et al., 1993).

In a B- withdrawal experiment with initial concentration of 0.093 mM B in nutrient solution, uptake of B by tomato shoots did not respond to RZTs (over the range of 10 - 37.8 °C) although it showed a trend towards lower B uptake at 10 °C and 37.8 °C than at 15.6 - 32.3 °C. However, the tomato shoots contained significant lower B concentration at 10 °C (35.8 mg kg\textsuperscript{-1}) than at RZTs of 15.6 - 37.8 °C (39.0-46.6 mg kg\textsuperscript{-1}) (Tindall et al., 1990). The 10 °C and 37.8 °C RZT are sub- and super- optimal temperatures, respectively, which resulted in shoot DW of about 7 g plant\textsuperscript{-1} at either of these two RZTs, (about 50-50 % of those at RZTs of 15.6 - 32.3 °C).

In snapdragon (Hood and Mills, 1994), a cut flower plant, when supplied with sufficient B in solution (0.05 mM B), B concentrations in whole plants decreased with increasing RZTs in range from 8 to 22 °C (from cold to optimal RZTs) although both dry weight of
plant growth and B uptake by snapdragon increased. This was suggested to be a ‘dilution’ effect.

Effects of temperature alone on soil environment could complicate the interpretation of soil trials like that of Walker (1969), as increasing temperature may have increased soil microbial activity and nutrient mineralisation, leading to increases in B bioavailability to the roots. In contrast, the depletion of B concentrations over time in solution culture experiments may decrease B uptake in response to increasing temperature and plant growth rate.

Day/night temperature and light regimes can influence the effect of RZT on B response (Greenfield and Smith, 1973; Mozafar et al., 1993; Behboudian et al., 1994). Boron concentrations in leaves of apple trees grown in half-strength Hoagland solutions (containing a high B concentration of 0.9 mM B) were higher at high day-time RZT (34 °C vs CK: 20 - 25 °C). In contrast, when RZT was maintained throughout the day/night cycle (i.e. 24 h/d), leaf B concentrations decreased at 34 °C (Behboudian et al., 1994). Boron uptake by maize increased with increasing RZTs but this effect was diminished by shortening photoperiod (Mozafar et al., 1993).

In summary, the results above were from experiments that tested plants with adequate or excessive B, and most were on the cereal crops which have a relatively low B requirement (Marschner, 1995; Hu et al., 1996). As a consequence these studies were not likely to provide direct evidence of the interaction between plant B deficiency and low temperature.
1.2.2.2 Experiments at low B supply

Only a few studies have explored, through experiments in the glasshouse and controlled environments, the mechanisms of low temperature effects on plant B nutrition. The experiments were conducted on cassava (Forno et al., 1979), wheat (Subedi et al., 1998b, 2001), cucumber (Wang et al., 1999).

In solution culture experiments with cassava (Forno et al., 1979), at low RZT (18 - 23 °C) plant symptoms of B deficiency were induced or accentuated, whilst plant B deficiency was alleviated or absent when B supply was sufficient. Boron deficiency induced by low RZT was attributed to a combination of reduced B uptake rates and greater size of shoot relative to the root (Forno et al., 1979). However, B concentrations in the whole shoots rather than the growing plant tissues (the growing leaves and shoot tips) were analysed in their study. Hence, the results of Forno et al. (1979) cannot be used to interpret the RZT effects on B transport to the growing shoot parts which continuously require B and on the relationship between B status and plant growth.

In wheat, maintained at a common shoot/root temperature regime, cold temperature (8/2 or 10/2 d/n °C) impaired wheat plants response to B treatment, for example, grain set and sterility were affected by temperature but B treatment had little effect on them at these temperatures (Subedi et al., 1998b, 2001). Low temperature (10/2 d/n °C) decreased flag leaf B concentration of wheat plants (Subedi et al., 2001). However, B concentrations in growing tissues of wheat plants were not affected by low night temperature (>5 °C compared to the control, >10 °C), and consequently, low night temperature delayed B deficiency in plants after they were moved to B free nutrient solution because the growth rate of plants declined at low temperature (Huang et al.,
Therefore, a beneficial effect of B on plant tolerance to low temperature was not observed in these experiments.

In cucumber (Wang et al., 1999), the seedlings were treated for 3 d with either no B (B0) or B supply (B+) prior to temperature treatment by growing them in controlled environment chambers at 7 - 8/5 °C (d/n) (low temperature) or 25/20 °C (control), for 0 - 60 h. It was demonstrated that potassium (K) leakage from plant leaves (expressed as mg K⁺ g⁻¹ fresh weight min⁻¹) increased at low temperature as well as at B0, with maximum leakage at B0 plus low temperature, and minimum from B supplied plant leaves at 25/20 °C. By the end of treatment (60 h), leaf K⁺ leakage in plants of B0 at 25/20 °C, and B+ and B0 at 7-8/5 °C was 1.6, 1.8 and 3.3 times leakage of the control (B+ at 25/20 °C), respectively. These results showed the beneficial effect of B application for cucumber plants to resist low temperature injury. Unfortunately, neither plant B uptake nor leaf B concentrations were reported in this research.

From the research results above, it seems that the nature of interactions between low temperature and B on plant growth may vary with plant species and temperature range as well.

1.3 Mechanisms for a role of boron in plant injury by low temperature

Direct effects of low temperature on plant responses to B may be due to increased sensitivity of plant to low temperature injury and poorer plant B status.
1.3.1 Increased sensitivity

Low B plant parts or plant products are more sensitive to low temperature injury (Combrink et al., 1995; Wang et al., 1999). It has been shown that plants require higher B concentrations to avoid symptoms developed at low temperature (Combrink et al., 1995; Wang et al., 1999). During cold storage at 5 °C, fruits produced from B supplied melon (cv. Galia) had reduced development of chilling injury in comparison with those from plants grown in a coarse river sand with less or no B supplied (Combrink et al., 1995). However, it is as yet unknown if low temperature elevates internal requirement of whole shoots or young growing shoots.

It may be postulated that low B plants/plant parts are less tolerant of low temperature stress because of structural impairment of cell walls and especially to damaged membrane integrity in low B plants. The primary site of low temperature injury to plants is the cell membrane (Lyons, 1973; Levitt, 1980).

Phase change of the membrane lipids is one of the initial forms of damage in the cell membrane at low temperature (Nishida and Murata, 1996). Low B also results in malfunction of cell membrane (Cakmak and Römheld, 1997). For example, P uptake rate was lower in B deficient maize than in the control, and the decrease of P uptake rate was more severely inhibited at temperatures below 20 °C at which temperature the Arrhenius plot showed a discontinuity in P uptake, which is regarded as a reflection of membrane phase change (Parr and Loughman, 1983). Moreover, B deficiency resulted in a higher temperature (1 - 2 °C higher than the control, ie., -B vs +B) at which the Arrhenius plot discontinuity occurs. These results imply that the phase change happened at higher temperature in low B than in adequate B plants. In other words, B deficiency
reduced plant tolerance to low temperature. These findings, indirectly, imply that cold injury to membrane will be more severe in low B plants.

1.3.2 Poorer plant B status

Low temperature reduces B uptake and/or translocation and induces B deficiency in plants (Forno et al., 1979). Consequently, higher external B supply is required for plant growth so that the supply of B to the growing tissues can meet growth demand at low temperature.

Water is the major medium for nutrient uptake and transport in plants. Recent research findings have revealed that water transport is largely dependent on water channels (aquaporins) (Baiges et al., 2002; Tyerman et al., 2002) and B uptake is partly facilitated by aquaporins (Dordas et al., 2000; Dordas and Brown, 2001; Stangoulis et al., 2001). Consequently, any factor such as low temperature that slows down water transport through plasma membrane lipid bilayers may also slow down plant B uptake and transport regardless of other specific mechanisms of B uptake. Because plant B uptake is a passive process at adequate external B concentrations and is probably an active process when external B is at critical or lower concentrations (Hu and Brown, 1997a; Pfeffer et al., 1999), the impact of low temperature is expected to be greater at low than at adequate B supply. For similar reasons, active B transport into the xylem at low B (Takano et al., 2002) implies a greater response to low temperate when B supply is marginal or deficient.
Boron deficiency in cassava was induced by low RZT (Forno et al., 1979) even at B supply that was adequate for plants at higher RZT and higher external B concentration was needed to overcome this problem. In cassava (Forno et al., 1979), B deficiency induced by low RZT is attributed to a combination of reduced rates of B uptake and greater size of shoot relative to the root.

Boron is continuously required by the growing plant tissues. For plants with low B mobility, B concentrations in the whole plant shoots cannot properly represent the status of B in the growing parts. Therefore, B status (concentration) in the growing plant tissues is critical to examine plant B response to low temperature. Unfortunately, amongst the experiments that involve a combination of low temperature and B treatments, little information is often reported on plant B concentrations or distribution. Relationships between status of plant B and plant growth response to low temperature are therefore difficult to interpret.

The above discussion has emphasised RZT, but air temperature affects plant growth and plant B uptake and transport as well. Boron uptake and transport may be more affected by air temperature when the passive process of B uptake predominates since under these conditions, B uptake and transport accompany water flow (transpiration flow) which is mainly governed by vapour pressure deficit in the plant canopy which in turn is dependent on air temperature. In general, except for injurious low air temperature that causes leaf damage, lowering air temperature slows down plant activity of growth and B uptake. However, quantitative effects of air temperature on relationships between B demand for growth and B acquisition have not been described.
When a whole plant is transferred from a warmer to a cooler environment, the S/R ratio is usually decreased but it may be also increased in some plant species or cultivars (Clarkson et al., 1988). A decrease of S/R ratio at lower temperature may reduce shoot B requirement. In addition, the rate of B supply relative to the growth rate of the growing shoot tissues, reflected as B concentration, is of key importance for shoot growth since only the growing plant parts require a continuous supply of B. In B supplied wheat plants, Subedi et al. (2001) reported that the extreme low temperature (10/2 d/n °C) greatly reduced leaf B concentrations with time (from about 30 mg kg\(^{-1}\) at d0 to about 15 mg kg\(^{-1}\) at d3). But equivalent leaf B data for the control plants (21/10 d/n °C) and for S/R ratio were not reported.

When shoot and root are treated with different temperatures, which is common in the open field, plant nutrient and growth responses to temperature are more complex (Clarkson et al., 1988). However, it seems that no information exists for studies on B nutrition at RZTs and air temperatures controlled separately. Besides the inconclusive previous research results on temperature and B interactions, the differences among plant species in B requirement (eg., monocotyledon vs dicotyledon) and more especially in tolerance to low temperature (chilling-sensitive plants vs chilling-resistant plants), suggest that mechanisms of low temperature effects on plant B nutrition need to be examined in various plant species.

Research on B uptake and/or transport that involve a combination of low temperature and low B treatments have only involved wheat (Huang et al., 1996a; Subedi et al., 1998b, 2001) and cassava (Forno et al., 1979): the former has low external and internal B requirements (Asad et al., 2001) and is mildly tolerant of low temperature, and the
latter has a higher B requirement and is a tropical root crop (Howeler et al., 1982). Forno et al. (1979) has concluded that B deficiency induced by low RZT is due to lowered B uptake rate and enlarged S/R ratio. However, effects of RZT on B uptake rate in wheat were not reported in the experiments of Huang et al. (1996a) and Subedi et al. (1998b, 2001). Hence the plant B response to low temperature may depend on plant tolerance to low temperature or low B but this has not been adequately investigated.

1.4 Perspective and hypothesis

Significant gaps exist in understanding the mechanisms of plant B response to temperature. Research focused on plants which are susceptible to low B is most likely to reveal mechanisms. Oilseed rape and sunflower are important oil crops in the world and have relatively high B requirements (Blamey et al., 1978, 1979; Yang et al., 1993; Huang et al., 1996b, 2000a). Whereas oilseed rape is a chilling-resistant plant species with low-temperature tolerance, sunflower is a chilling-sensitive plant species. The different temperature tolerances of these oilseed species make them useful test crops. The objective of the present study was to explore mechanisms of plant B response to low temperature, with emphasis on RZT and its effects on B status in growing plant parts. The hypotheses are that ‘low RZT induces plant B deficiency’ in both oilseed rape, a cold temperature tolerant species and sunflower, a chilling-sensitive crop. Secondly, ‘low RZT increases plant external requirement of B, or alternatively, increasing RZT reduces external B requirement’, and thirdly the elevated B requirement at low RZT is not only due to combination of lowered B uptake rate and enlarged S/R ratio (Forno et al., 1979), but also partly due to the reduced B transport into the growing...
plant parts. In addition, the possibility that the internal requirement of B is altered by RZT was also examined.
Chapter 2

Covering plants at night in the winter increased seed yield of transplanted oilseed rape (*Brassica napus* L. cv. Zheyouyou 2) on a low boron soil

2.1 Abstract

Winter oilseed rape is a major crop in the middle and lower Yangtse river basin, China, where low boron (B) soils are widespread. Appearance of B deficiency in oilseed rape often coincides with cold weather during its winter growth. To understand effects of cold weather on plant response to low soil B, a field experiment with oilseed rape cv. Zheyouyou 2 grown in a red soil with low B availability was conducted in Zhejiang province, China. Canopy covers made from transparent plastic sheets were used only at night to modify the microclimate during the early reproductive growth between pre-exposure of flower buds and green bud stage. Canopy treatment (T) increased the minimum air temperature inside the cover by up to 1.5 °C when the minimum air temperature in the open was below 0 °C in early February. Covering plants for 15 days in early February strongly increased shoot dry weight (DW) at all levels of B supply. That covering plants increased shoot dry weight of B deficient plants without increasing their leaf B concentration suggests that internal B requirements were decreased. However, because later plant responses at maturity gave contradictory responses, it was

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concluded that further study is required to understand the mode of interaction between low temperature x B under controlled growth conditions.

2.2 Introduction

Low temperature in winter and early spring is a major constraint in winter crop production especially during the reproductive phase when it may cause the failure of reproduction (Lardon and Triboi-Blondel, 1994). Low boron (B) supply to seed crops may also result in the failure of seed production (Rerkasem et al., 1993). Moreover, plant response to B may be affected by environmental factors, such as temperature (Moraghan and Mascagni, 1991). Reports on the nature of the interaction between B and temperature have been inconclusive. Studies by Mahalakshmi et al. (1995) and by Walker (1969) which were done at 5 °C and 12 °C, respectively, were of uncertain relevance to the present study because they examined effects on B toxicity in the former and on B adequate plants in the latter case. Whilst increasing root temperature alleviated or even overcame B deficiency in cassava plants’ responses occurred in the range 19 - 33 °C (Forno et al., 1979), which is well above the range of interest for winter crops.

Winter oilseed rape is reputed to be sensitive to B deficiency (Yang et al., 1993). It is a major winter crop in the middle and lower Yangtse river basin in China, where the weather in winter and early spring is cold (see Figure 2.1), and soils with low B level are widespread (Yang et al., 1993; Wei et al., 1998). However little is known about how low temperature modifies B response in winter oilseed rape.
Figure 2.1 Weather conditions at field site. Key to symbols: — max. relative humidity (R.H. %), — — min. R.H. %, • Total rainfall (mm day$^{-1}$), — — max. air temperature ($^\circ$C), --- min. air temperature ($^\circ$C), ○ Total solar radiation (MJ m$^{-2}$ day$^{-1}$).

To reduce night time heat loss and protect plants from the cold, covering is often used in
winter crop production (Mather, 1974). Therefore this present study, which aimed at understanding if cold weather modifies plant response to B deficiency, involved covering treatment plants with a plastic canopy at nights for 15 or 17 consecutive nights in late winter and early spring. The hypothesis in the present study was that changing the plant's micro-climate by imposing a night time canopy cover improves plant growth, and decreases plant B internal requirement; or, conversely, increasing B supply to plants improves their resistance to low temperature.

2.3 Materials and Methods

A field experiment was conducted in Zhejiang province, China in 1994 - 1995, in a red soil (Hapludult, US Soil Taxonomy) with: pH (H_{2}O) 5.5; organic matter, 2.24 %; hot 0.01 M CaCl_{2} extractable B, 0.26 mg kg^{-1} soil (Spouncer et al., 1992). Oilseed rape cv. Zheyouyou 2 seedlings were transplanted from a seedling bed (seed sown on September 26, 1994) into main fields at the 6 to 7 leaf stage on November 5 at a spacing of 27 x 31 cm. Plants received the following basal fertilizers (kg ha^{-1}): NPK compound fertilizer (15:15:15), 450; urea, 100; MgSO_{4}7 H_{2}O, 75; CaSO_{4}, 75; ZnSO_{4} 7 H_{2}O, 15; CuSO_{4} 5 H_{2}O, 10; MnSO_{4} H_{2}O, 10; Na_{2}MoO_{4} 2 H_{2}O, 1.5. Additional fertilizers were applied at seedling stage (kg ha^{-1}): NPK compound (15:15:15), 450; and at stem elongation stage: urea, 150.

Treatments were arranged in a split plot design with main plots (5.4 x 3.7 m) comprising soil application of 0, 1.5 or 15 kg borax ha^{-1} (B0, B1.5, B15), and sub-plots comprising an area of 3.7 x 1.8 m which were either uncovered (T0), or covered
overnight at T1 or T2 (see details following). Each treatment had four replicates. Canopy covers made from clear plastic sheets were used to cover the plants only during the night (between sunset and the following sunrise) to modify microclimate. The canopy was about 40 cm high. This treatment is referred to as canopy treatment (T) in the following text. It was applied over two periods determined according to plant growth stage and weather conditions: T1, Jan 27 - Feb 11, 1995 (GS 3,1 - 3,3) (Flower buds enclosed by leaves – flower buds visible from above (green bud)); T2, Feb 11 - 28, 1995 (GS 3,3 - 3,4) (Green bud – flower buds level with leaves) (GS: Growth Stage, after Sylvester-Bradley, 1985). The non-canopy treatment was used as the control (T0).

An automatic weather station was used to record air (2.5 m above ground) and soil (10 cm below soil surface) temperatures, rainfall, solar radiation, relative humidity and wind speed. The soil temperature sensor did not work properly when the temperature was below 0 °C. The weather station was located about 50 m away from the experimental site. As only one set of sensors was available, the weather station was only used to record the climate data in the open. Air temperatures in- and outside plastic covers were recorded daily with the use of minimum/maximum thermometers.

Plant samples were collected at seedling stage, and before and after canopy treatments from control and treatment plots (The sampling times are shown in Tables 2.2 and 2.3.). Youngest open leaf blade (YOL, after Huang et al., 1996b) samples were collected at each of these sampling times, and dried at 70 °C. They were digested in concentrated nitric acid at 130 °C and concentration of B was determined by inductively coupled plasma atomic emission spectrometry (ICP-AES) after nitric acid digestion (Zarcinas et al., 1987). Percentages of plants at specific growth stages were recorded. Plants were
harvested on 24 May 1995. Seed yields were recorded at maturity.

2.4 Results and discussion

2.4.1 Weather conditions in the field

Weather conditions from late winter to spring, including relative humidity, air temperature, total rainfall, and total solar radiation, are shown on Figure 2.1. Over the whole growing season, coldest temperatures occurred in February and early March. The spring weather fluctuated markedly. Minimum temperatures \(< 5\, ^\circ C\) continued into late March and even in mid April. There was a little rainfall in the winter, but the soil contained sufficient water in the winter due to a perched watertable 70 cm below the ground surface. The rainy season started in mid March.

The coldest temperatures occurred during Jan 29 to Feb 7, within the period of T1. The daily minimum air temperature in this period ranged from \(-6.9 \sim -1.0\, ^\circ C\). Whilst it was much warmer during the period of T2, minimum air temperatures below 0 \(^\circ C\) occurred on Feb 26 and 27. The mean minimum and mean maximum air temperatures during the period, T1 and T2, were -2.0 and 9.4 \(^\circ C\), and 3.8 and 9.7 \(^\circ C\), respectively. Canopy treatment increased the air temperature inside the cover. The minimum air temperature inside the cover increased by up to 1.5 \(^\circ C\) when the minimum air temperature in the open was below 0 \(^\circ C\) in early February (Table 2.1).
2.4.2 Effect of cold weather on plant growth rate with different B levels

Plant growth was severely inhibited and 25% of plants died in low B soil without B application (Table 2.2) (Plate 2.1 shows plant symptoms of B deficiency). In B0 plots, the plants had YOL B concentrations < 10 mg B kg\(^{-1}\) which is the minimum B concentration required for adequate leaf function (Huang et al., 1996b).

Table 2.1 Effect of plastic covers over plants on minimum air temperatures in- and outside covers (°C) and number of days when minimum air temperature was below 0 °C outside the cover.

<table>
<thead>
<tr>
<th>Period</th>
<th>Outside</th>
<th>Inside</th>
<th>Days of air temperature ≤ 0 °C outside the cover</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan 27 - Feb 11</td>
<td>-7 ~ 3</td>
<td>-5.5 ~ 3.5</td>
<td>10</td>
</tr>
<tr>
<td>Feb 11 - Feb 28</td>
<td>0 ~ 8</td>
<td>0.5 ~ 9.5</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 2.2 Effect of boron (B) application on youngest open leaf blade (YOL) B concentration (mg B kg\(^{-1}\) dry weight), shoot dry weight (g DW plant\(^{-1}\)), and survival rate of transplanted seedlings (%). Values are means of four replicates with standard errors in parentheses \(^a\).

<table>
<thead>
<tr>
<th>B (kg borax ha(^{-1}))</th>
<th>Shoot dry weight</th>
<th>YOL B</th>
<th>Seedling survival rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Dec 24, 94) (Jan 27, 95) (Feb 11) (Feb 28)</td>
<td>(Dec 24, 94)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2.8(0.2) 4.3(0.4) 3.9(0.3) 7.0(0.3)</td>
<td>6.7(1.0) 75(5)</td>
<td></td>
</tr>
<tr>
<td>1.5</td>
<td>6.9(0.7) 8.8(1.0) 9.4(1.1) 13.9(0.7)</td>
<td>19.2(1.8) 94(1)</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>7.0(0.4) 8.4(1.2) 9.4(1.1) 13.6(2.4)</td>
<td>29.5(6.1) 94(1)</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Shoot and leaf samples were from non-canopy treatment at the date of sample collection shown.

Plant growth slowed down when ambient temperature declined. The effect of cold
weather on plant growth was more obvious in plants at B0. Without canopy treatment, the plants at B0 decreased in dry weight from Jan 27 to Feb 1 due to the loss of old leaves; by contrast, plants with B supply continued growing even during the coldest period. Plant growth rate significantly increased with increasing ambient temperature in late February. Net biomass increment from Feb 11 to Feb 28 was more than 4 times that from Jan 27 to Feb 11.

*Plate 2.1* Symptoms of boron deficiency in oilseed rape at seedling (upper) and flowering (lower) stages
2.4.3 Effect of covering plants

2.4.3.1 Vegetative growth

Covering plants during T1 improved plant vegetative growth (Table 2.3), while YOL B concentration was not affected by canopy treatment. By contrast, when it became warmer, the canopy treatment did not increase plant growth (T2); neither did it affect leaf B concentrations.

Table 2.3 Effects of boron (B) and canopy treatment (T) on shoot dry weight (g DW plant\(^{-1}\)) and youngest open leaf blade (YOL) B concentration (mg B kg\(^{-1}\) dry weight).

Values are means of four replicates with standard errors in parentheses.

<table>
<thead>
<tr>
<th>B</th>
<th>T (^{a})</th>
<th>Shoot dry weight</th>
<th>YOL B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Feb 11</td>
<td>Feb 28</td>
</tr>
<tr>
<td>B0</td>
<td>T0</td>
<td>3.9(0.3)</td>
<td>7.0(0.3)</td>
</tr>
<tr>
<td>B0</td>
<td>T1/T2</td>
<td>7.0(1.3)</td>
<td>8.2(1.7)</td>
</tr>
<tr>
<td>B1.5</td>
<td>T0</td>
<td>9.4(1.1)</td>
<td>13.9(0.7)</td>
</tr>
<tr>
<td>B1.5</td>
<td>T1/T2</td>
<td>15.3(1.3)</td>
<td>16.6(1.3)</td>
</tr>
<tr>
<td>B15</td>
<td>T0</td>
<td>9.4(1.1)</td>
<td>13.6(2.4)</td>
</tr>
<tr>
<td>B15</td>
<td>T1/T2</td>
<td>11.8(1.2)</td>
<td>15.1(2.6)</td>
</tr>
</tbody>
</table>

F test \(^{b}\)

<table>
<thead>
<tr>
<th>B</th>
<th>T</th>
<th>Shoot dry weight</th>
<th>YOL B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Feb 11</td>
<td>Feb 28</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>T</td>
<td></td>
<td>**</td>
<td>ns</td>
</tr>
<tr>
<td>B x T</td>
<td></td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

\(^{a}\) T: T0, control; T1/T2, plants covered at nights during either 27 Jan ~ Feb 11 (T1) or Feb 11 ~ 28 (T2). Date shown was the plant collection time, i.e., at the end of each canopy treatment.

\(^{b}\) B, T and B x T represent the main effects of B and T, and their interaction, respectively.

ns = non-significant; *, ** significant at 5 % and 1 % probability levels, respectively.

2.4.3.2 Reproductive growth

After removal of the canopy cover, residual effects of canopy treatment continued to
influence growth of the B fertilized plants (Table 2.4). In B1.5 and B15 plots, T1 or T2 treated plants set pods earlier than T0, resulting in more seeds. The effect of canopy treatment in B0 plots was not observed as severe B deficiency strongly depressed setting and filling of pods.

Table 2.4 Effects of boron application (B) and canopy treatment (T) on oilseed rape development and seed yield. Values are means of four replicates with standard errors in parentheses.

<table>
<thead>
<tr>
<th>B</th>
<th>T</th>
<th>Pods visible (^a) (% of plants)</th>
<th>Pods plant(^{-1})</th>
<th>Seeds plant(^{-1})</th>
<th>Seed weight (g plant(^{-1}))</th>
<th>Seed yield (kg ha(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>B0</td>
<td>T0</td>
<td>0(0)</td>
<td>58(33)</td>
<td>519(307)</td>
<td>2.5(1.5)</td>
<td>225(131)</td>
</tr>
<tr>
<td>B0</td>
<td>T1</td>
<td>0(0)</td>
<td>104(48)</td>
<td>951(537)</td>
<td>4.9(2.4)</td>
<td>469(222)</td>
</tr>
<tr>
<td>B0</td>
<td>T2</td>
<td>0(0)</td>
<td>39(23)</td>
<td>353(214)</td>
<td>1.8(1.1)</td>
<td>169(99)</td>
</tr>
<tr>
<td>B1.5</td>
<td>T0</td>
<td>2.3(0.5)</td>
<td>258(55)</td>
<td>2434(109)</td>
<td>13.0(0.8)</td>
<td>1445(91)</td>
</tr>
<tr>
<td>B1.5</td>
<td>T1</td>
<td>5.7(1.7)</td>
<td>298(35)</td>
<td>2889(256)</td>
<td>15.6(1.3)</td>
<td>1620(102)</td>
</tr>
<tr>
<td>B1.5</td>
<td>T2</td>
<td>4.3(0.9)</td>
<td>267(17)</td>
<td>2898(205)</td>
<td>15.0(1.4)</td>
<td>1725(170)</td>
</tr>
<tr>
<td>B15</td>
<td>T0</td>
<td>3.6(0.9)</td>
<td>316(21)</td>
<td>2402(90)</td>
<td>13.3(0.2)</td>
<td>1480(45)</td>
</tr>
<tr>
<td>B15</td>
<td>T1</td>
<td>7.5(2.0)</td>
<td>499(138)</td>
<td>4635(803)</td>
<td>25.0(5.3)</td>
<td>2125(164)</td>
</tr>
<tr>
<td>B15</td>
<td>T2</td>
<td>7.4(2.8)</td>
<td>346(57)</td>
<td>3136(327)</td>
<td>16.8(2.0)</td>
<td>1905(152)</td>
</tr>
</tbody>
</table>

F test \(^b\)

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>T</th>
<th>B x T</th>
</tr>
</thead>
<tbody>
<tr>
<td>**</td>
<td>*</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>**</td>
<td>ns</td>
<td>*</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) % of plants with visible pods (≥ 2 cm long) on main stem was assessed on April 1, 1995. The other data were recorded at final harvest.

\(^b\) B, T and B x T represent the main effects of B and T, and their interaction, respectively. ns = non-significant; *, ** significant at 5 % and 1 % probability levels, respectively.

Responses of seed and pod numbers per plant suggest that canopy treatment and adequate B supply improved plant reproductive growth (Table 2.4), and that both low temperature and low B depressed reproductive growth. Whereas covering plants and B application interacted on seed set and seed yield per plant, canopy treatment had no
effect on numbers of pods set. These results suggest that the short term canopy treatments mainly affected seed development, while B influenced both pod and seed development. These beneficial effects of canopy cover may be attributed to the positive effects on pollen development and viability, leading to higher fertilization rate. Critical reproductive stages are usually very sensitive to environmental stresses such as low B supply and low temperature. Coinciding with ‘Green Bud’ stage, pollen mother cells in anthers are undergoing meiosis which in other species is the most sensitive stage to disturbances from B deficiency (Huang et al., 2000b) and low temperature (Rawson, 1996b). Thus canopy treatment reinforced reproductive growth of B1.5 and B15 plants exposed to cold.

2.5 Conclusions

From the seed yield results, there was no support for the hypothesis that increasing B supply to plants increased their resistance to low temperature. Whilst it might be argued that the increase in air temperature inside the plastic canopy was relatively small, it was nevertheless effective in stimulating vegetative growth by the end of a 15 day period (T1). And the canopy cover had no effect on vegetative growth at the end of T2 when temperature was the same inside as outside the canopy and temperatures were generally warmer. That shoot dry weight increased at the end of T1 despite no increase in leaf B suggests that increasing night temperatures may have decreased internal B requirements of oilseed rape plants. In contrast, to the shoot dry weight results which represent the short term response of oilseed rape to canopy covering over a 15 - 17 day period, the responses of pod set, seed set, and seed yield were rather more complex to interpret.
Unlike the direct responses of shoot dry weight to B and changed canopy microclimate, seed yield responses were affected by a combination of factors acting on pod set and pod filling subsequent to the canopy treatment period as well as the indirect consequences of the canopy treatment period. It is concluded that further investigation of the interaction between low temperature and B supply needs to be conducted in controlled environments for defined periods of plant growth.

2.6 Implications for further research in controlled environments

Although the influences of canopy treatment on plant responses to B supply appeared very complex in the present study, several possible effects of the canopy treatment on micro-climate, including soil and air temperatures, might have direct and/or indirect effects on plant growth and plant B nutrition.

A small temperature increase might greatly improve plant growth at low temperature. For example, besides increased plant growth rate (Macduff et al., 1987a, d), a small increase in root zone temperature (RZT) (3, 5, 7 °C) of oilseed rape for 14 d had significant effect on root morphology (root hair density, root hair length, root surface area, etc., Macduff and Wild, 1986) and nutrient (nitrogen, N) uptake (Macduff et al., 1987b, c, d).

The most significant environmental factors altered by canopy treatment at night are the warmer air and soil temperatures (Wolfe et al., 1989). Warmer night may have significant effect on plant physiological and biochemical activities (Albrizio and
Steduto, 2003) such as increase of respiration rates (Tjoelker, 2001; Atkin and Tjoelker, 2003; Turnbull et al., 2004), photosynthesis (Maleszewski et al., 1993; Allen and Ort, 2001), photosynthetic capacity (Maleszewski et al., 1993), transpiration and water uptake (Tranquillini, 1981), and nutrient uptake (Wien et al., 1993). The night temperature change also affects hormone synthesis and transport (Savé et al., 1995; Farkhutdinov et al., 1997), activities of enzymes such as the pyruvate dehydrogenase complex (PDC), nicotinamide adenine dinucleotide (NAD\(^+\)) - malic enzyme (ME) (Atkin et al., 2000), and sucrose phosphate synthase (Jones et al., 1998). In addition, plant morphological and anatomical characteristics such as decreased specific leaf weight (g cm\(^{-2}\)) are effected by warmer temperature (Papadopoulos and Hao, 2000). Consequently, warmer night may affect plant nutrient status (Papadopoulos and Tiessen, 1987) and result in increase of plant growth rate and yield (Seddigh and Jolliff, 1984a and b; Merritt and Jr Kohl, 1989). Furthermore, even one single night treatment of warm or non-injurious low temperature could result in significant effects on plant activities, such as photosynthetic rate (Ward and Lawlor, 1990), leaf chlorophyll content (Savé et al., 1995), function of photosystem II (PSII) (Warner and Burke, 1992), and transpiration (Peoples and Koch, 1978). Therefore, two aspects of the field canopy covering at night need to be examined: the protection from low temperature injury in the short term – plant response to overnight cold temperature, and on plant growth and B nutrition in the long term.

In the open field, low temperature injury to plants is mainly initiated during the night but becomes more evident under sunlight following a cold night. The appearance of the effects of cold temperature in the dark on subsequent photosynthesis (Welander et al., 1994; Allen and Ort, 2001) is attributed to overproduction of active oxygen species
(AOS) (Dan and Imada, 2002). Damage in cell membranes is thought to be the primary injury by low temperature probably due to phase change of the lipids and protein denaturation in the membrane (Levitt 1980; Nishida and Murata, 1996; Thomashow, 1998). The photosynthesis center, PSII is the pigment protein embedded in the thylakoid membrane (Hankamer et al., 1997) which is more susceptible to low temperature under high light than low temperature alone (Krause et al., 1988; Allen and Ort, 2001). There are several factors which contribute to increase plant resistance to low temperature, such as increased concentrations of soluble carbohydrates (Levitt, 1980) and phenolic compounds (Dan and Imada, 2002). Moreover, cell walls also have roles to protect the plasma membrane from freezing injury (Krause et al., 1988; Yamada et al., 2002).

Boron has key roles in maintaining cell wall structure (Matoh, 1997; Matoh and Kobayashi, 1998) and plasma membrane integrity (Cakmak and Römheld, 1997), as well as probably other physiological functions (Marschner, 1995) (see Chapter 1). These functions of B especially in maintenance of integrity of membranes and cell walls might have led low B- plants to be more sensitive to low temperature injury, and plant injury by low temperature becomes more serious in high light after night freezing due to increased internal B requirements of plants (Noppakoonwong et al., 1993).

Apart from the overnight injury to plants by freezing, non-injurious low temperature may have adverse effect on plant growth (as discussed above) as well as B uptake and transport because water uptake and transpiration continue through the dark period (Mankin et al., 1998), even though the night time transpiration rate is very small compared to the daytime (Seginer, 1984). These overnight adverse effects could have a significant impact on plant growth and B uptake in the following day (Ward and Lawlor, 2002).
In the longer term, even short exposure to low temperature may have residual effects on growth due to reduced leaf area, or damage to apical meristems especially when injury coincides with critical periods of plant growth such as microsporogenesis or anthesis.

Understanding the relative contributions of cold air and soil temperatures to plant B response, and the underlying mechanisms remain for further research.
Chapter 3

Low root zone temperature favours shoot B partitioning into young leaves of oilseed rape (*Brassica napus* L. cv. Hyola 42)\(^1\)

3.1 Abstract

In previous studies with chilling-sensitive plant species, low root zone temperature (RZT) induced boron (B) deficiency, but it is not known if the same response to RZT will be expressed in chilling-resistant plant species like oilseed rape. The present experiments investigated the effect of RZT (10 and 20 °C) on oilseed rape (cv. Hyola 42) response to B in solution culture, in summer and winter. Regardless of canopy growth conditions, low RZT (10 °C) promoted the partitioning of shoot B towards the actively growing leaves, especially when B supply was low. However, low RZT did not significantly alter plant biomass and net B uptake rate. Low RZT decreased the shoot to root ratio, countering the effects of low B which increased it, leading to a decreased demand for B in the shoot. At low B supply, B deficiency symptoms appeared later at 10 °C than 20 °C corresponding with higher B concentrations in the youngest fully opened leaves (YOLs) in plants grown at RZT of 10 °C. The 10 °C RZT increased the tolerance to low B supply. As a result, it is concluded that the effect of decreasing RZT on the responses of the temperate species, oilseed rape, to low B supply depends on whether the low RZT is above or below the critical root temperature range for growth.

\(^1\) An earlier version of this paper first appeared as Ye, Z. Q., and Huang, L. B., Bell, R. W., and Dell, B. (2003). Low root zone temperature favours shoot B partitioning into young leaves of oilseed rape.
3.2 Introduction

A number of environmental factors which fluctuate spatially and seasonally are known to affect plant response to B (Moraghan and Mascagni, 1991; Shorrocks, 1997). Temperature is one of the important factors which may affect plant B response in the field (Blevins et al., 1996; Hanson and Breen, 1985; Chapter 2). Temperature influences plant growth, and B uptake and B requirements (Forno et al., 1979). Furthermore, chilling temperatures may cause physical damage to vegetative and reproductive tissues which are actively growing (Lardon and Triboi-Blondel, 1994), and therefore increase the severity of B deficiency as well (Forno et al., 1979). Field-grown winter oilseed rape experience a wide range of root and air temperatures both diurnally (in autumn and spring) and across the growing seasons (such as in winter and early spring). As a result, temperature may be one of the key environmental factors that affects the variable expression of B deficiency in winter and spring crops.

Low RZT affects both root and shoot growth, as well as nutrient uptake and its translocation from root to shoot (Clarkson et al., 1988; Macduff et al., 1994; Bigot and Boucaud, 1996). Forno et al. (1979) reported that low RZT (19 °C) resulted in B deficiency in cassava, a tropical species, even though no B deficiency was evident when plants were grown at higher RZT (26 °C) at the same B level. By contrast, little is known about how low RZT affects plant response to B deficiency in temperate species that are better adapted to low temperature conditions.

In Chapter 2, it was found that covering oilseed rape plants at night in the winter improved their growth on a low B soil in south-east China. The beneficial effects of covering the canopy at night in winter or mulching (Wang and Li, 1987) on plant growth may be the consequence of improved B nutrition in the plants due to increased RZT or increased air temperature (Forno et al., 1979). However, in a field experimental system, it is difficult to differentiate the mechanisms of the effect of low RZT temperature from other effects, due to the multiple roles of covering or mulching treatments, including effects on soil nutrient availability and moisture. A well-defined experimental system is required to define the effects of low RZT on plant responses to B supply. Therefore, in the present study, experiments were conducted in summer and in winter in the glasshouse, to understand effects of RZT on oilseed rape seedlings’ responses to B under different canopy temperature regimes. To maintain relatively stable B concentrations in culture solutions, programmed nutrient addition (NUTRADD) (Asher and Blamey, 1987) was used in the first experiment, and B resin buffered solution culture was adopted in the second experiment (Asad et al., 1997a and b; Huang et al., 1999).

3.3 Materials and Methods

3.3.1 Plant culture

Oilseed rape (cv. Hyola 42) seeds were germinated on paper towel moistened with 1.0 mM Ca(NO$_3$)$_2$ and 0.01 mM H$_3$BO$_3$ solution at 20 °C for 72 h in the dark. Then, they were transferred to nutrient solution. The full-strength basal nutrient solution used in the experiments was the same as used previously (Huang et al., 1996b), except that the iron
(Fe) concentration was 80 µM in the winter experiment. The germinated seedlings were first transferred to 1/3- full strength nutrient solution in a 8-l plastic tray and were grown in a glasshouse until they had 2 green cotyledons. Uniform seedlings were then randomly selected and transplanted into 2/3- full strength solution in each 5-l pot lined with a plastic bag, with 12 plants per pot for 6 days before being transferred into full strength nutrient solution. Solutions were aerated continuously with filtered air. Nutrients (apart from B in the winter experiment) were replenished according to plant demand as calculated by the NUTRADD program (Asher and Blamey, 1987). All chemicals were AR grade. Macronutrient stock solutions were prepared from AR grade chemicals and were purified further by passing through B specific resin (IRA-743, Sigma Chemical Co.).

3.3.2 Temperature treatment

Seedlings were grown in the glasshouse with RZT controlled at 20 °C by placing pots in a water bath until the 3rd (summer experiment) or the 4th (winter experiment) leaf was fully opened. Roots were then rinsed 3 times in 1mM Ca(NO$_3$)$_2$ prior to imposing treatments. Plants were treated with 2 RZT regimes, ie. low (10 - 12 °C) (referred to as 10 °C RZT) and adequate (20 - 22 °C) (referred to as 20 °C RZT) by placing in temperature controlled water baths. The general management procedures were the same as described previously (Huang et al., 1996b).

3.3.3 Boron treatment

*Summer experiment (Experiment 1)* Plants were treated with 3 B levels with initial B concentrations: 0.1, 0.3 and 5 µM H$_3$BO$_3$ (referred as B0.1, B0.3 and B5, respectively) with 5 replicate pots. Culture solutions were renewed on the 6th day. Solution pH was
maintained at 6.0 ± 0.2 with adjustments as required using 0.1 M H$_2$SO$_4$ and 0.1 M NaOH. Plants were harvested on days 0, 3, 6, 9 and 12 after imposing B and RZT treatments, and oven dried at 70 °C.

Winter experiment (Experiment 2) Boron concentrations in the culture solutions were controlled by the B-specific resin (Huang et al., 1999) at 0.13 ± 0.02, 0.19 ± 0.02 or 8.3 ± 0.2 µM B (means with standard errors), with four replicate pots. A preliminary experiment showed RZT did not affect B concentration established in the culture solution by B-specific resin (data not shown). Plants were harvested on 7 and 14 days after treatment.

3.3.4 Data collection
Growth conditions including temperature, light and humidity inside the glasshouse were recorded continuously during the experiment with an automatic weather station. The recorded data showed that both minimum and maximum temperatures in glasshouse were about 9 °C lower in winter than in summer and light intensity was lower also in the winter than the summer (Table 3.1). By contrast, humidity was much higher.

Plant parts were separated into the youngest open leaf blade (YOL) (after Huang et al., 1996b), 3rd (in Experiment 1) or 4th (in Experiment 2) true leaf blade (L3, L4) and remainder of shoot and root. Roots were rinsed 3 times, for 3 minutes each rinse, with B-free water at harvest. Plant fresh and dry weights (DW) were recorded. Boron in oven dried plant materials was analyzed by ICP-AES after acid digestion in concentrated nitric acid (Zarcinas et al., 1987). Plant water use was estimated by daily weighing and calculating the water loss of each pot.
Table 3.1 Glasshouse conditions during the two experiments.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Air temperature (°C)</th>
<th>Relative humidity (%)</th>
<th>Daytime radiation (W m(^{-2}))</th>
<th>Light intensity (PAR) (MJ m(^{-2}) d(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Summer</td>
<td>mean 28.4</td>
<td>53.4</td>
<td>117</td>
<td>233</td>
</tr>
<tr>
<td></td>
<td>max. 36.7</td>
<td>83.9</td>
<td>700</td>
<td>1154</td>
</tr>
<tr>
<td></td>
<td>min. 20.5</td>
<td>37.6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Winter</td>
<td>mean 20.7</td>
<td>82.6</td>
<td>117</td>
<td>274</td>
</tr>
<tr>
<td></td>
<td>max. 27.6</td>
<td>101.8</td>
<td>406</td>
<td>1179</td>
</tr>
<tr>
<td></td>
<td>min. 12.0</td>
<td>56.3</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Relative growth rate of plants (RGR), and B uptake rate (BUR), and B translocation rate from root to shoot (BTR) were calculated (Engels and Marschner, 1996; Forno et al., 1979; Willits et al., 1992). The data were analyzed for the significance of treatment effects and their possible interactions (Super Anova, USA).

The functions used for calculating the above parameters are listed below:

\[ \text{RGR} = \frac{\ln(W_2/W_1)}{(t_2-t_1)} \]

\[ \text{BTR} = \frac{((\ln W_{r2}-\ln W_{r1})(M_{s2}-M_{s1}))}{((t_2-t_1)(W_{r2}-W_{r1}))} \]

\[ \text{BUR} = \frac{((\ln W_{r2}-\ln W_{r1})(M_{2}-M_{1}))}{((t_2-t_1)(W_{r2}-W_{r1}))} \]

Where: \( W_2, W_1 \), whole plant dry weights (g plant\(^{-1}\)) at harvest 2 and 1; \( s \), Shoot; \( r \), Root; \( W_{r2}, W_{r1} \), Root dry weights (g plant\(^{-1}\)) at harvest 2 and 1, respectively; \( M_{s2}, M_{s1} \), Shoot B content (µmol plant\(^{-1}\)) at harvest 2 and 1; \( t_2, t_1 \), day after treatment at harvest 2 and 1; \( M_{2}, M_{1} \), whole plant B contents (µmol plant\(^{-1}\)) at harvest 2 and 1, respectively.

Analysis of variance was carried out to examine the effects of B, temperature treatments, and time (days after treatment) and their interaction on the parameters described above (Super-ANVOVA, Abacus Concepts, USA). When significant interactions between time and B or temperature treatments were detected, two-way analysis of variance was then performed on the data for each individual harvest, to reveal the time-dependent effects.
3.4 Results

3.4.1 Boron uptake and plant growth

The contrasting experimental conditions (winter vs summer) altered the nature of low RZT treatment effects on plant responses to low and adequate B supply. In summer (Experiment 1), the levels of B supply predominantly determined B uptake in the plants, B concentrations in the actively growing leaves (YOL), and general plant growth parameters (Tables 3.2 – 3.5). Low RZT alone had no significant effect on net B uptake and B partitioning between the root and shoot, as indicated by the total B content per plant, net B uptake rate per unit root mass, B translocation rate from root to shoot, and the ratios of shoot B content to root B content (Tables 3.3 – 3.5). However, lowering RZT resulted in a significantly higher proportion of shoot B partitioned into the actively growing leaf, YOL (Table 3.5). This led to the relatively higher B concentrations in the YOL at 10°C RZT regardless of B supply levels in the solution, especially at day 3 and day 6 (Table 3.3). Although lowering RZT did not suppress plant growth (shoot and root dry weights and root RGR), it significantly decreased the shoot to root ratio from day 6 onwards (Table 3.2). There was no significant interaction between B supply and low RZT on B uptake and plant growth.

In contrast, under winter conditions (Experiment 2), RZT modified some aspects of plant responses in B distribution and growth to low B supply in the nutrient solutions. Specifically, lowering RZT from 20 to 10°C significantly \((P \leq 0.001)\) decreased the net B uptake in the whole plant and relative B uptake rate per unit root mass in the B- adequate
treatment, but had little effect when the B supply in the solution was low (0.13 – 0.19 µM B) (Tables 3.3 and 3.4). In general, there was no effect of low RZT on the B translocation rate from shoot to root, though lowering RZT significantly decreased shoot B content (P ≤ 0.001) to a greater extent than root B content, as indicated by the decreased ratio of shoot B to root B content (Table 3.5). This negative effect of low RZT on the shoot to root B content ratio first occurred at day 7 in the B- adequate treatment (8.3 µM B), but not in lower B supply treatments until day 14.

It was also found that under winter conditions (Experiment 2), lowering RZT alone significantly increased the proportion of shoot B partitioned into the YOL, regardless of B supply levels (Table 3.5), which was similar to the observations described earlier for the summer experiment. There was a significant (P ≤ 0.05) interaction between B supply and low RZT treatment, where low RZT either increased (at day 7) or had no significant effects (at day 14) on B concentrations in the YOL when B supply levels were low (0.13 and 0.19 µM), but decreased them on both occasions when B supply was adequate B (8.3 µM) (Table 3.3).

Under the winter condition, lowering RZT in general, significantly (P ≤ 0.01) decreased shoot biomass at day 7 and 14, but had less negative effects on root RGR (only significant at day 7) and root biomass, resulting in a consistent decrease in shoot to root ratio (Table 3.2).

As expected in both the winter and summer experiments, increasing B supply in the nutrient solution significantly increased B uptake rate over the experimental period.
Table 3.2a Effects of root zone temperature (RZT) and boron (B) on plant biomass and shoot to root ratio. Values are means of five (Experiment 1) or four (Experiment 2) replicates in each treatment, with a standard error in the parentheses. ANOVA = Analysis of variance.

**Experiment 1 (Summer)**

<table>
<thead>
<tr>
<th>Initial B supply (µM)</th>
<th>0.1</th>
<th>0.3</th>
<th>5.0</th>
<th>Two-way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>RZT (°C)</td>
<td></td>
<td></td>
<td></td>
<td>B</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td>ns</td>
</tr>
<tr>
<td>20</td>
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<td></td>
<td>ns</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td>ns</td>
</tr>
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<td>20</td>
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<td></td>
<td></td>
<td>ns</td>
</tr>
<tr>
<td>10</td>
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<td>ns</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td>ns</td>
</tr>
</tbody>
</table>

**DAT (day)**

<table>
<thead>
<tr>
<th>Shoot DW (g plant⁻¹)</th>
<th>0-3</th>
<th>3-6</th>
<th>6-9</th>
<th>9-12</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.48 (0.04)</td>
<td>0.53 (0.03)</td>
<td>0.51 (0.04)</td>
<td>0.54 (0.02)</td>
<td>0.55 (0.04)</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.82 (0.03)</td>
<td>0.93 (0.04)</td>
<td>0.85 (0.05)</td>
<td>0.86 (0.04)</td>
<td>0.91 (0.07)</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.34 (0.10)</td>
<td>1.33 (0.12)</td>
<td>1.42 (0.10)</td>
<td>1.55 (0.10)</td>
<td>1.52 (0.10)</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.92 (0.07)</td>
<td>1.67 (0.09)</td>
<td>2.49 (0.05)</td>
<td>2.70 (0.16)</td>
<td>2.88 (0.07)</td>
</tr>
</tbody>
</table>

**Three-way ANOVA**

<table>
<thead>
<tr>
<th>Shoot to root (S/R) ratio</th>
<th>0-3</th>
<th>3-6</th>
<th>6-9</th>
<th>9-12</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11.0 (0.7)</td>
<td>12.0 (0.3)</td>
<td>11.7 (0.9)</td>
<td>10.8 (0.5)</td>
<td>12.1 (0.9)</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.9 (0.4)</td>
<td>13.2 (0.2)</td>
<td>10.5 (0.7)</td>
<td>11.2 (0.3)</td>
<td>10.2 (0.5)</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13.6 (0.6)</td>
<td>14.9 (0.4)</td>
<td>11.9 (0.6)</td>
<td>13.9 (0.6)</td>
<td>10.7 (0.4)</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13.0 (0.4)</td>
<td>14.4 (1.0)</td>
<td>11.1 (0.5)</td>
<td>13.0 (0.5)</td>
<td>9.4 (0.4)</td>
</tr>
</tbody>
</table>

**Relative growth rate (RGR g g⁻¹ d⁻¹)**

<table>
<thead>
<tr>
<th></th>
<th>0-3</th>
<th>3-6</th>
<th>6-9</th>
<th>9-12</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.29 (0.03)</td>
<td>0.32 (0.02)</td>
<td>0.31 (0.03)</td>
<td>0.33 (0.01)</td>
<td>0.33 (0.02)</td>
</tr>
<tr>
<td>3-6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.18 (0.04)</td>
<td>0.19 (0.02)</td>
<td>0.18 (0.03)</td>
<td>0.15 (0.02)</td>
<td>0.17 (0.04)</td>
</tr>
<tr>
<td>6-9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.15 (0.03)</td>
<td>0.11 (0.03)</td>
<td>0.17 (0.02)</td>
<td>0.19 (0.02)</td>
<td>0.17 (0.04)</td>
</tr>
<tr>
<td>9-12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.12 (0.02)</td>
<td>0.08 (0.04)</td>
<td>0.19 (0.03)</td>
<td>0.19 (0.04)</td>
<td>0.22 (0.02)</td>
</tr>
</tbody>
</table>

(Continued)
Table 3.2b Effects of root zone temperature (RZT) and boron (B) on plant biomass and shoot to root ratio. Values are means of five (Experiment 1) or four (Experiment 2) replicates in each treatment, with a standard error in the parentheses.

<table>
<thead>
<tr>
<th>Initial B supply (µM)</th>
<th>0.13</th>
<th>0.19</th>
<th>8.3</th>
<th>Two-way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>RZT (°C)</td>
<td>10</td>
<td>20</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Shoot DW (g plant⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DAT (day)</td>
<td>7</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.87 (0.02)</td>
<td>1.16 (0.30)</td>
<td>0.85 (0.05)</td>
<td>1.15 (0.10)</td>
<td>0.98 (0.10)</td>
</tr>
<tr>
<td>2.08 (0.07)</td>
<td>2.50 (0.16)</td>
<td>2.28 (0.08)</td>
<td>2.85 (0.23)</td>
<td>3.12 (0.09)</td>
</tr>
<tr>
<td>Three-way ANOVA</td>
<td>DAT ***</td>
<td>B ***</td>
<td>RZT ***</td>
<td>DATxB ***</td>
</tr>
<tr>
<td>Root DW (g plant⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DAT (day)</td>
<td>7</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.07 (0.00)</td>
<td>0.08 (0.01)</td>
<td>0.07 (0.00)</td>
<td>0.08 (0.01)</td>
<td>0.08 (0.01)</td>
</tr>
<tr>
<td>0.14 (0.00)</td>
<td>0.14 (0.01)</td>
<td>0.19 (0.01)</td>
<td>0.14 (0.01)</td>
<td>0.26 (0.01)</td>
</tr>
<tr>
<td>Three-way ANOVA</td>
<td>DAT ***</td>
<td>B ***</td>
<td>RZT ns</td>
<td>DATxB ***</td>
</tr>
<tr>
<td>Shoot to root (S/R)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DAT (day)</td>
<td>7</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13.6 (0.48)</td>
<td>14.7 (0.87)</td>
<td>11.7 (0.61)</td>
<td>13.8 (0.36)</td>
<td>12.1 (1.08)</td>
</tr>
<tr>
<td>14.8 (0.46)</td>
<td>18.4 (1.32)</td>
<td>12.1 (1.10)</td>
<td>20.1 (0.85)</td>
<td>12.2 (0.48)</td>
</tr>
<tr>
<td>Three-way ANOVA</td>
<td>DAT ***</td>
<td>B ***</td>
<td>RZT ***</td>
<td>DATxB **</td>
</tr>
<tr>
<td>Relative growth rate (RGR g g⁻¹ d⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DAT (day)</td>
<td>0-7</td>
<td>7-14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.16 (0.00)</td>
<td>0.20 (0.00)</td>
<td>0.16 (0.01)</td>
<td>0.20 (0.01)</td>
<td>0.18 (0.01)</td>
</tr>
<tr>
<td>0.12 (0.01)</td>
<td>0.11 (0.01)</td>
<td>0.14 (0.01)</td>
<td>0.13 (0.02)</td>
<td>0.17 (0.01)</td>
</tr>
<tr>
<td>Three-way ANOVA</td>
<td>DAT ***</td>
<td>B ***</td>
<td>RZT ***</td>
<td>DATxB **</td>
</tr>
</tbody>
</table>

Note: In the table, ns = non-significant; *, **, *** significant at 5 %, 1 % and 0.1% probability levels, respectively; DAT = Days After Treatment.
Table 3.3 Effects of root zone temperature (RZT) and boron (B) on B uptake by oilseed rape and B concentrations in plant parts in summer (Experiment 1) and winter (Experiment 2) experiments. Values are means of five (Experiment 1) or four (Experiment 2) replicates in each treatment, with a standard error in the parentheses.

**Experiment 1 (Summer)**

<table>
<thead>
<tr>
<th>Initial B supply (µM)</th>
<th>0.1</th>
<th>0.3</th>
<th>5.0</th>
<th>Two-way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>RZT (°C)</td>
<td>10</td>
<td>20</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>DAT (Day)</td>
<td>B</td>
<td>RZT</td>
<td>B x RZT</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>7.2 (0.5)</td>
<td>7.7 (0.3)</td>
<td>7.8 (0.4)</td>
<td>17.3 (1.1)</td>
</tr>
<tr>
<td>6</td>
<td>7.6 (0.2)</td>
<td>8.9 (0.6)</td>
<td>8.9 (0.5)</td>
<td>28.2 (2.4)</td>
</tr>
<tr>
<td>9</td>
<td>8.7 (0.9)</td>
<td>12.7 (1.0)</td>
<td>12.1 (0.9)</td>
<td>51.9 (3.4)</td>
</tr>
<tr>
<td>12</td>
<td>10.0 (0.5)</td>
<td>18.9 (0.3)</td>
<td>18.6 (0.9)</td>
<td>85.8 (2.4)</td>
</tr>
</tbody>
</table>

Three-way ANOVA: DAT ***  B ***  RZT ns  DATxB ***  DATxRZT ns  BxRZT ns  DATxBxRZT ns

Youngest open leaf blade (YOL) B concentration (mg kg⁻¹)

<table>
<thead>
<tr>
<th>Initial B supply (µM)</th>
<th>0.1</th>
<th>0.3</th>
<th>8.3</th>
<th>Two-way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>RZT (°C)</td>
<td>10</td>
<td>20</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>DAT (Day)</td>
<td>B</td>
<td>RZT</td>
<td>B x RZT</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>8.8 (0.4)</td>
<td>10.0 (0.5)</td>
<td>10.1 (0.4)</td>
<td>36.3 (1.2)</td>
</tr>
<tr>
<td>6</td>
<td>4.0 (0.5)</td>
<td>5.6 (0.2)</td>
<td>4.4 (0.2)</td>
<td>34.4 (3.0)</td>
</tr>
<tr>
<td>9</td>
<td>1.7 (0.2)</td>
<td>5.5 (0.3)</td>
<td>4.2 (0.2)</td>
<td>40.4 (1.2)</td>
</tr>
<tr>
<td>12</td>
<td>2.3 (0.1)</td>
<td>6.4 (0.3)</td>
<td>5.3 (0.3)</td>
<td>37.7 (1.4)</td>
</tr>
</tbody>
</table>

Three-way ANOVA: DAT ***  B ***  RZT ***  DATxB ***  DATxRZT ns  BxRZT ***  DATxBxRZT ns

**Experiment 2 (Winter)**

<table>
<thead>
<tr>
<th>Initial B supply (µM)</th>
<th>0.13</th>
<th>0.19</th>
<th>8.3</th>
<th>Two-way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>RZT (°C)</td>
<td>10</td>
<td>20</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>DAT (Day)</td>
<td>B</td>
<td>RZT</td>
<td>B x RZT</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>7.2 (0.2)</td>
<td>8.8 (0.3)</td>
<td>8.9 (0.4)</td>
<td>24.8 (2.7)</td>
</tr>
<tr>
<td>14</td>
<td>9.3 (0.2)</td>
<td>11.7 (0.7)</td>
<td>11.7 (0.9)</td>
<td>81.7 (2.8)</td>
</tr>
</tbody>
</table>

Three-way ANOVA: DAT ***  B ***  RZT ***  DATxB ***  DATxRZT ns  BxRZT ***  DATxBxRZT ns

Youngest open leaf blade (YOL) B concentration (mg kg⁻¹)

<table>
<thead>
<tr>
<th>Initial B supply (µM)</th>
<th>0.13</th>
<th>0.19</th>
<th>8.3</th>
<th>Two-way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>RZT (°C)</td>
<td>10</td>
<td>20</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>DAT (Day)</td>
<td>B</td>
<td>RZT</td>
<td>B x RZT</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>3.5 (0.2)</td>
<td>5.0 (0.2)</td>
<td>4.1 (0.3)</td>
<td>25.0 (0.7)</td>
</tr>
<tr>
<td>14</td>
<td>1.6 (0.1)</td>
<td>2.2 (0.2)</td>
<td>1.2 (0.1)</td>
<td>31.4 (0.3)</td>
</tr>
</tbody>
</table>

Three-way ANOVA: DAT ns  B ***  RZT ns  DATxB ***  DATxRZT ns  BxRZT ***  DATxBxRZT ns

Note: In the table, ns = non-significant; *, **, *** significant at 5 %, 1 % and 0.1% probability levels, respectively; DAT = Days After Treatment.
Table 3.4 Effects of root zone temperature (RZT) and boron (B) on B translocation rate from root to shoot (BTR) and B uptake rate (BUR) (µmol B g⁻¹ root DW d⁻¹). Values are means of five (Experiment 1) or four (Experiment 2) replicates in each treatment, with a standard error in the parentheses.

**Experiment 1 (Summer)**

<table>
<thead>
<tr>
<th>Initial B supply (µM)</th>
<th>0.1</th>
<th>0.3</th>
<th>5.0</th>
<th>Two-way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>RZT (°C)</td>
<td>10</td>
<td>20</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DAT (Day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-3</td>
<td>1.03 (0.56)</td>
<td>1.07 (0.19)</td>
<td>1.67 (0.24)</td>
<td>1.58 (0.32)</td>
</tr>
<tr>
<td>3-6</td>
<td>0.25 (0.31)</td>
<td>0.01 (0.14)</td>
<td>0.54 (0.24)</td>
<td>0.53 (0.20)</td>
</tr>
<tr>
<td>6-9</td>
<td>0.32 (0.23)</td>
<td>0.40 (0.18)</td>
<td>1.20 (0.28)</td>
<td>1.09 (0.22)</td>
</tr>
<tr>
<td>9-12</td>
<td>0.40 (0.21)</td>
<td>0.33 (0.31)</td>
<td>1.15 (0.20)</td>
<td>1.26 (0.29)</td>
</tr>
<tr>
<td>Three-way ANOVA</td>
<td>DAT ***</td>
<td>B ***</td>
<td>RZT ns</td>
<td>DATxB ***</td>
</tr>
</tbody>
</table>

|                       |     |     |     |              |   |     |        |
|                       |     |     |     |              |   |     |        |
| 0-3                  | 0.86 (0.55) | 0.88 (0.18) | 1.49 (0.21) | 1.30 (0.28) | 11.28 (1.15) | 10.64 (0.77) | *** | ns | ns |
| 3-6                  | 0.13 (0.29) | -0.09 (0.13) | 0.37 (0.21) | 0.43 (0.18) | 4.94 (1.08) | 5.41 (0.84) | *** | ns | ns |
| 6-9                  | 0.24 (0.20) | 0.31 (0.14) | 1.05 (0.27) | 0.94 (0.20) | 6.17 (1.28) | 5.86 (0.99) | *** | ns | ns |
| 9-12                 | 0.29 (0.19) | 0.25 (0.27) | 0.93 (0.19) | 1.03 (0.28) | 4.66 (0.97) | 5.46 (0.48) | *** | ns | ns |
| Three-way ANOVA     | DAT *** | B *** | RZT ns | DATxB *** | DATxRZT ns | BxRZT ns | DATxBxRZT ns |

**Experiment 2 (Winter)**

<table>
<thead>
<tr>
<th>Initial B supply (µM)</th>
<th>0.13</th>
<th>0.19</th>
<th>8.3</th>
<th>Two-way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>RZT (°C)</td>
<td>10</td>
<td>20</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DAT (Day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-7</td>
<td>-0.05 (0.06)</td>
<td>0.22 (0.10)</td>
<td>0.46 (0.10)</td>
<td>0.42 (0.10)</td>
</tr>
<tr>
<td>7-14</td>
<td>0.26 (0.05)</td>
<td>0.31 (0.09)</td>
<td>0.24 (0.08)</td>
<td>0.31 (0.08)</td>
</tr>
<tr>
<td>Three-way ANOVA</td>
<td>DAT **</td>
<td>B ***</td>
<td>RZT ***</td>
<td>DATxB ***</td>
</tr>
</tbody>
</table>

|                       |     |     |     |              |   |     |        |
|                       |     |     |     |              |   |     |        |
| 0-7                  | 0.06 (0.06) | 0.34 (0.10) | 0.55 (0.10) | 0.53 (0.10) | 4.94 (0.44) | 7.85 (0.42) | *** | *** | *** |
| 7-14                 | 0.28 (0.04) | 0.33 (0.09) | 0.31 (0.09) | 0.34 (0.14) | 4.98 (0.43) | 5.65 (0.23) | *** | ns | ns |
| Three-way ANOVA     | DAT ** | B *** | RZT *** | DATxB ** | DATxRZT ** | BxRZT *** | DATxBxRZT ** |

Note: In the table, ns = non-significant; *, **, *** significant at 5 %, 1 % and 0.1% probability levels, respectively; DAT = Days After Treatment.
Table 3.5 Effects of root zone temperature (RZT) and boron (B) on B partitioning between shoot and root and within the shoot. Values are means of five (Experiment 1) or four (Experiment 2) replicates in each treatment, with a standard error in the parentheses.

**Experiment 1** (Summer)

<table>
<thead>
<tr>
<th>Initial B supply (µM)</th>
<th>0.1</th>
<th>0.3</th>
<th>5.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>RZT (°C)</td>
<td>10</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>DAT (Day)</td>
<td>B content in youngest open leaf blade (YOL) relative to shoot B (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>7.44 (0.46)</td>
<td>5.62 (0.32)</td>
<td>8.29 (0.76)</td>
</tr>
<tr>
<td>6</td>
<td>4.71 (0.51)</td>
<td>3.09 (0.19)</td>
<td>5.25 (0.32)</td>
</tr>
<tr>
<td>9</td>
<td>2.59 (0.27)</td>
<td>2.66 (0.39)</td>
<td>5.46 (0.42)</td>
</tr>
<tr>
<td>12</td>
<td>3.03 (0.28)</td>
<td>2.40 (0.32)</td>
<td>6.41 (0.25)</td>
</tr>
<tr>
<td>Three-way ANOVA</td>
<td>DAT ***</td>
<td>B ***</td>
<td>RZT ***</td>
</tr>
<tr>
<td>7</td>
<td>12.53 (0.70)</td>
<td>12.27 (0.37)</td>
<td>13.49 (0.87)</td>
</tr>
<tr>
<td>14</td>
<td>11.1 (0.8)</td>
<td>11.5 (0.8)</td>
<td>16.4 (2.9)</td>
</tr>
<tr>
<td>Three-way ANOVA</td>
<td>DAT *</td>
<td>B ***</td>
<td>RZT **</td>
</tr>
<tr>
<td>7</td>
<td>11.1 (0.8)</td>
<td>13.0 (0.7)</td>
<td>8.56 (0.3)</td>
</tr>
<tr>
<td>14</td>
<td>11.1 (0.8)</td>
<td>13.0 (0.7)</td>
<td>8.56 (0.3)</td>
</tr>
<tr>
<td>Three-way ANOVA</td>
<td>DAT *</td>
<td>B ***</td>
<td>RZT **</td>
</tr>
</tbody>
</table>

**Experiment 2** (Winter)

<table>
<thead>
<tr>
<th>Initial B supply (µM)</th>
<th>0.13</th>
<th>0.19</th>
<th>8.3</th>
</tr>
</thead>
<tbody>
<tr>
<td>RZT (°C)</td>
<td>10</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>DAT (Day)</td>
<td>B content in youngest open leaf blade (YOL) relative to shoot B (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>4.32 (0.46)</td>
<td>3.73 (0.32)</td>
<td>5.20 (0.29)</td>
</tr>
<tr>
<td>14</td>
<td>2.91 (0.37)</td>
<td>1.95 (0.05)</td>
<td>3.98 (0.26)</td>
</tr>
<tr>
<td>Three-way ANOVA</td>
<td>DAT ***</td>
<td>B ***</td>
<td>RZT ***</td>
</tr>
<tr>
<td>7</td>
<td>11.1 (0.8)</td>
<td>11.5 (0.8)</td>
<td>16.4 (2.9)</td>
</tr>
<tr>
<td>14</td>
<td>11.1 (0.8)</td>
<td>13.0 (0.7)</td>
<td>8.56 (0.3)</td>
</tr>
<tr>
<td>Three-way ANOVA</td>
<td>DAT *</td>
<td>B ***</td>
<td>RZT **</td>
</tr>
</tbody>
</table>

Note: In the table, ns = non-significant; *, **, *** significant at 5 %, 1 % and 0.1% probability levels, respectively; DAT = Days After Treatment.
3.4.2 Boron deficiency symptoms

In summer, the earliest B deficiency symptom was observed in roots on day 6 in 0.1 µM B at 20 °C RZT, followed by leaf deficiency symptoms on day 7. With the onset of B deficiency, root tips became slightly brown first, then dark brown, followed by physical degeneration. By comparison with the B- adequate plants, young B- deficient leaves were dark green. With the development of B deficiency, the midrib of the young leaf remained abaxially recurved rather than flat. Leaf B deficiency symptoms in plants with 0.1 µM B supply at 10 °C RZT appeared later on day 9.

In the winter experiment, B deficiency symptoms in roots also appeared first in plants supplied with 0.13 µM B at 20 °C RZT on day 6, and leaf B deficiency symptoms appeared on day 8, whilst no leaf B deficiency symptoms were observed in plants at 10 °C RZT with the same level of B supply until day 11.

3.5 Discussion

Root zone temperature influenced B partitioning in the shoot through altering the amount of shoot B distributed into the actively growing leaves – the youngest open leaf. In both the summer and winter conditions, lowering RZT to 10 °C resulted in an increased distribution of B in the shoot into the YOL, which led to the higher B concentrations in the YOL,
especially when B supply was low (0.13 and 0.19 µM). Lowering RZT generally decreased shoot to root ratios, regardless of B supply, which is contrasted with the increased shoot to root ratio under low or deficient B supply. Moreover, the nature of the B and RZT interaction and the effects of low RZT alone on plant growth and B uptake parameters were affected by the canopy conditions, namely winter and summer canopy conditions.

The results suggest that lower RZT actually benefited the plants grown in low B supply under both the winter and summer conditions, by partitioning more B in the shoot to the young growing leaves. As a result, in both experiments, the occurrence of B deficiency symptoms in root tips and young leaves was delayed in plants at 10 °C RZT, compared to 20 °C RZT at low B supply. This is in contrast to the previous findings with tropical species such as cassava, in which lowering RZT exacerbated B deficiency (Forno et al., 1979). Forno et al. (1979) found that cassava plants exhibited B deficiency at 19 °C RZT, but not at 26 °C RZT, even with the same level of B supply. By contrast, a temperate species like oilseed rape grown at high RZT (20 °C) may be more likely to develop into B deficiency, due to the B distribution in the shoot favouring older leaves rather than the YOL. This is particularly important when plants experience a low or deficient B supply. Hence the present results demonstrate that the increase in sensitivity to B deficiency under low RZT is not a universal response of plants, and in oilseed rape even a RZT of 10 °C had no such effect.
The increased B partitioning into the actively growing leaf – YOL is probably related to the effects of low RZT on transpiration of mature leaves and different B transport and possibly B unloading mechanisms in immature shoot tip and YOL from those in mature leaves. Ali et al. (1998) found that decreasing root temperature to 12 - 16/8 - 12 °C (day/night) reduced shoot transpiration. Boron transport into and accumulation in mature leaves is very much a transpiration-driven process, in contrast, B may be transported into the immature leaves and shoot tip of Brassica species through the phloem (after xylem-to-phloem exchange) and then symplasm pathways (Brown and Shelp, 1997). If this was the case in the present experiments, more B would be available at low RZT in the xylem stream in the upper stem close to shoot tip and YOL because less B is withdrawn by transpiring mature leaves. Similarly in wheat, Huang et al. (2001) found that one of the actively transpiring organs, the flag leaf, was the main competing sink against the young ear when the latter was enclosed within the sheath and had weak or minimal transpiration activity. In wheat, decreasing transpiration from leaves increased the relative partitioning of B to the ear. From 10B labelling studies it was concluded that B delivered to the ear required xylem-to-phloem transfer in the stem following root uptake. Hence there appears to be a dynamic connection between B distribution in shoots and the rates of water fluxes within the canopy. Increasing water distribution to mature leaves may have adverse consequences for B delivery to the growing tissues depending on external B supply.

Whether low RZT enhances plant sensitivity to low B supply may be dependent on the
low temperature tolerance of the test species. Tropical species like cassava are expected to be sensitive to low temperature and to have a higher RZT threshold below which root growth and functions are impaired, than those for temperate species such as oilseed rape. The RZT of 10 °C is unfavourable for the root growth and development of tropical species (Raison and Lyons, 1986). Chilling stress was also observed in watermelon plants at 10 °C, which had lower shoot growth than plants at 35 °C (Rivero et al., 2002).

In contrast, Kacperska and Szaniawski (1993) found that root growth of oilseed rape was not negatively affected by RZT as low as 3 °C, regardless of canopy air temperature, but this low RZT decreased shoot growth, leading to a reduction in shoot to root ratio. The root zone temperature of 10 °C has been considered to be optimal for oilseed rape growth (Macduff and Wild, 1986; Macduff et al., 1987 b and d). Therefore, the low temperature tolerance of the root of oilseed rape may increase the avoidance of the occurrence of B deficiency at lower RZT when B supply to the roots is temporarily low or deficient.

Previous studies on B deficiency have shown that decreasing B supply to plants (e.g. oilseed rape, Huang et al., 1996b) increased the shoot to root ratio. High shoot to root ratios may increase B demand in the shoot exacerbating the need for increased B uptake by the root. In contrast, low RZT significantly reduced shoot to root ratios in the present experiments and in that of Kacperska and Szaniawski (1993), and decreased B demand in the shoot. Thus low RZT could offset the effects of low B supply. As suggested by the results of the present experiments, B uptake rates were not decreased by low RZT at
low B supply levels, unlike the case described for sunflower in which lowering RZT decreased active B uptake (Pfeffer et al., 1999). The insensitivity of relative B uptake rates to RZT was unexpected given the evidence from several recent studies that B uptake is predominantly active at low external B concentrations (Pfeffer et al., 1999; Asad et al., 2001; Brown et al., 2002). At adequate to high solution B concentrations, active B uptake processes are repressed, and passive processes dominate. It is not known whether RZT could affect passive B uptake processes by altering membrane properties but there was no evidence that it did so in oilseed rape at 10 °C, which is close to the optimum root temperature for this species. On the other hand low RZT depressed the shoot to root ratio, and possibly transpiration, both of which would affect the rate of passive B uptake and B distribution in the shoot. At low external B concentrations, there may be sufficient capacity for up-regulation of the active B uptake processes to offset the effects of low RZT. As a result, the enhanced tolerance to low B in the plants at low RZT in the present experiments may have been due not only to increased partitioning of B to the YOL, but also to the reduced sink demand for B and at the same time, the lack of effect of this RZT treatment on B uptake rate.

The decreased shoot to root ratios in the oilseed rape at low root temperature may be related to root temperature-induced changes in phytohormone transport in the xylem stream into the shoot. Cytokinin and gibberellin contents in the xylem exudate decreased in the plants exposed to low root temperature in oilseed rape and soluble carbohydrates accumulated in roots and shoots (Ali et al., 1998). This implied that the
reduced shoot growth was not the direct consequence of assimilate limitation, but of hormone controlled processes.

Regardless of root temperature and B supply levels, the canopy conditions will no doubt change shoot growth potential, growth rate, and shoot transpiration (in particular the transpiration of older leaves relative to the young, immature leaves). Plants grown under summer conditions used twice as much water as those in winter per unit of dry matter (Table 3.6). The summer conditions not only increased the total water transpired, but also may have altered the transpiration intensity in the mature leaves, relative to the young and immature leaves. When B supply is in the adequate range, B uptake by plants is a passive process, mainly driven by transpiration (Daniel et al., 1997; Pfeffer et al., 1997).

In conclusion, the optimal root temperature for a test species appears to determine plant responses to low RZT at different B supply levels. For a temperate species like oilseed rape, the lower optimum RZT of 10 °C (compared to 20 °C) actually facilitated the avoidance of B deficiency under low B supply, by increasing the distribution of B transported into the shoot towards the actively growing leaves, the YOL, and therefore increasing B use efficiency in the shoot. The mechanism by which low RZT increases B partitioning into the YOL remains to be explored in future research. However, the present Chapter suggests that a dynamic linkage exists in plants between the pattern of
water flux within the canopy and B distribution between old leaves and the growing plant parts.

Table 3.6 Water use efficiency (water consumption per unit dry matter production, mL g\(^{-1}\)) during treatment period (12 d and 14 d in Experiments 1 and 2, respectively).

<table>
<thead>
<tr>
<th>Experiment 1 (Summer)</th>
<th>Initial B supply (µM)</th>
<th>0.1</th>
<th>0.3</th>
<th>5.0</th>
<th>Two-way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>RZT (°C)</td>
<td>10 20 10 20 10 20</td>
<td></td>
<td></td>
<td></td>
<td>B  RZT  B x RZT</td>
</tr>
<tr>
<td>Water use efficiency</td>
<td>280 271 268 273 302 290</td>
<td>(54)</td>
<td>(31)</td>
<td>(8)</td>
<td>(13)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Experiment 2 (Winter)</th>
<th>Initial B supply (µM)</th>
<th>0.13</th>
<th>0.19</th>
<th>8.3</th>
<th>Two-way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>RZT (°C)</td>
<td>10 20 10 20 10 20</td>
<td></td>
<td></td>
<td></td>
<td>B  RZT  B x RZT</td>
</tr>
<tr>
<td>Water use efficiency</td>
<td>140 150 131 146 133 160</td>
<td>(8)</td>
<td>(12)</td>
<td>(5)</td>
<td>(10)</td>
</tr>
</tbody>
</table>

Note: In the table, ns = non-significant, *, **, *** significant at 5 %, 1 % and 0.1% probability levels, respectively.
Chapter 4

Warmer root zone temperature exacerbates boron deficiency of oilseed rape plants (*Brassica napus* L. cv. Hyola 42)

4.1 Abstract

In a low boron (B) soil, a previous field experiment (Chapter 2) showed that growth of oilseed rape was improved by covering plants at night for 15 consecutive days. That covering plants increased shoot dry weight of B deficient plants despite no increase in leaf B suggests that increasing night temperatures may have decreased internal B requirements of oilseed rape plants. However, results of Chapter 3 suggest that increasing root zone temperature (RZT) decrease proportion of shoot B partitioned into the actively growing leaf, regardless of B supply levels. In the present experiment, the effect of soil temperature on plant response to B was further examined in solution culture. To mimic treatment of the field experiment, oilseed rape (cv. Hyola 42) seedlings were first grown at 10 °C (simulating winter) RZT with B supply from deficient to adequate B levels. At the time when growth of low B plants just began to slow down half of the pots were transferred to 20 °C RZT for 11 days before they were moved back to 10 °C RZT for the final 4 days. Both plant dry mass and B uptake increased after plants were exposed to 20 °C RZT. Boron concentrations in the youngest open leaf blades (YOLs) were higher in B- adequate plants at 20 °C than 10 °C RZT. By contrast, YOL B concentrations in low B plants were decreased by warmer RZT (20 °C) and plant B deficiency was accentuated. These temperature effects persisted after plants were moved back from 20 to 10 °C RZT for 4 days. Consequently, plant B deficiency
became more severe in low B plants pre-treated at warm RZT (T20/10). In combination with previous glasshouse experiments (Chapter 3), it is concluded that effect of RZT on oilseed rape plant response to B is not altered by the pre-growth conditions of the plants. Whether low RZT induces B deficiency or not is dependent on RZT and its relationship to the critical threshold for that species.

4.2 Introduction

Oilseed rape is particularly sensitive to low B supply and B fertilizer responses in the field are often reported in China (Yang et al., 1993; Ye et al., 1997; Xue et al., 1998; Yang et al., 2000). Winter oilseed rape plants experience a great fluctuation of root and air temperatures both diurnally in autumn and spring and, in seasonal growth between winter and early spring. Covering plants in the winter at night (Chapter 2) or mulching (Wang and Li, 1987) improve plant growth possibly by improving plant B nutrition by increased RZT or/and air temperature (Forno, et al. 1979). In a field experiment in Thailand (Rerkasem et al., 1990), early seedling growth of black and green gram in a low B soil had lower incidence of abnormal seedlings sown in January compared with December. This was attributed to higher air and soil temperatures prevailing during the January sowing.

To understand the effect of RZT on B sensitive species of temperate origin such as oilseed rape rather than the tropical species like cassava (Forno, et al. 1979) or plants that require minimal B like wheat (Huang et al, 1996a), the effect of low RZT (10 °C vs 20 °C) on oilseed rape plants in response to B has been examined in the previous
experiments (Chapter 3). It has been shown that low RZT (10 °C) minimized and warm RZT (20 °C) accentuated B deficiency of oilseed rape plants although the effect of warm RZT was more intense in summer growing conditions. These results are contrasting to those reported for the tropical species, cassava (Forno, et al. 1979).

In the field, oilseed rape plants often experience low B soon after transplanting in autumn, and this stress is followed by low temperature in winter. Plant growth response may be influenced by their pre-growth conditions, such as temperature regime and plant B status (Greenfield and Smith, 1973). This might be specifically important for plant response to B when low temperature is imposed because plants require several days or longer to acclimate to the low temperature, the acclimation includes changes of shoot to root (S/R) ratio and nutrient uptake and transport (Clarkson et al., 1988). In the previous two experiments (Chapter 3) oilseed plants were grown at 20 °C RZT prior to RZT and B treatments. To investigate if warmer RZT during the pre-treatment phase has contributed to response to B and its residual effect as observed in the field experiment (Chapter 2), a solution culture experiment was conducted in winter in the glasshouse, and oilseed rape plants were grown first at 10 °C RZT and supplied with external B levels from deficiency to adequacy prior to commencing the RZT treatment (10 °C vs 20 °C).

4.3 Materials and Methods

Oilseed rape (cv Hyola 42) seeds were germinated on paper towels soaked with 1.0 mM Ca(NO₃)₂ and 0.01 mM H₃BO₃ solution at 20 °C for 3 days in the dark. Ten
uniformly germinated seedlings were transferred to full strength nutrient solution (except B) in each 5-l pot lined with a plastic bag with RZT controlled at 10 - 12 °C (referred as T10 thereafter) by a water bath. Plants were thinned to 6 per pot after 10 days to achieve uniform seedlings.

Boron concentrations in the culture solutions were buffered by B specific resin (Huang et al., 1999) at three B levels (B µM) (means with standard errors): 0.18 ± 0.01 (severe low), 0.27 ± 0.01 (mild low) and 8.1 ± 0.3 (adequate), and nutrient solutions in the pots were bubbled continuously with filtered air. Nutrient supply and other general procedures were the same as the previous experiments (Chapter 3).

After growth at T10 for 16 days, when the third true leaf became the youngest open leaf (d0) and leaves in low B plants became noticeably darker green, half of the pots were transferred to another water bath with RZT controlled at 20 - 22 °C (T20), kept there for 11 days (d11), then they were moved back to T10 for the final 4 days for plant growth (T20/10); the other half of the pots remained at 10 °C throughout the experiment as the control RZT treatment (T10/10).

At each temperature shift, all culture solutions including control pots had solutions renewed.

**Data collection**

Plant samples were collected at each temperature shift (d0 and d11) and at the end (d15) of the experiment. Plant parts were separated into the youngest open leaf blade (YOL) (as defined by Huang et al. 1996b), the remainder of the shoot, and root (roots were
rinsed for 3 times for 3 minutes each, with B-free water). The plant samples were oven
dried at 70 °C and their dry weights were recorded. Boron in oven dried plant materials
was analyzed by ICP-AES after digestion with concentrated nitric acid (Zarcinas et al.,
1987).

Relative growth rate of plants (RGR), shoot demand, B uptake rate (BUR) and B
translocation rate from root to shoot (BTR) were calculated (Forno et al. 1979; Willits
et al., 1992; Engels and Marschner, 1996). The data were analyzed for the significance
of treatment effects and their possible interactions with two-way analysis of variance
(Super Anova, USA).

The functions used for calculating the above parameters are listed below:

RGR = (lnW2-lnW1)/(t2-t1)

Shoot demand = ((lnWr2-lnWr1) (Ws2-Ws1))/ ((t2-t1) (Wr2-Wr1))

BTR = ((lnWr2-lnWr1)(Ms2-Ms1))/((t2-t1)(Wr2-Wr1))

BUR = ((lnWr2-lnWr1)(M2-M1))/((t2-t1)(Wr2-Wr1))

Where: W2, W1, whole plant dry weights (g plant⁻¹) at harvest 2 and 1; s, Shoot; r, Root;
Wr2, Wr1 and Ws2, Ws1, Root and shoot dry weights (g plant⁻¹) at harvest 2 and 1,
respectively; Ms2, Ms1, Shoot B content (µmol plant⁻¹) at harvest 2 and 1; t2, t1, days
after treatment at harvest 2 and 1; M2, M1, whole plant B contents (µmol plant⁻¹) at
harvest 2 and 1, respectively.

4.4 Results
4.4.1 Development of B deficiency symptoms

Prior to RZT treatment (d0), dry weights (DW) of plants amongst the three B levels were similar (shoot and root, 0.11 and 0.01 g plant\(^{-1}\), respectively), though with different B concentrations (for shoot, 4.0, 6.6, 21.6; and for root, 7.0, 9.1, 11.0 mg B kg\(^{-1}\) DW, in 0.18, 0.27 and 8.1 µM B, respectively). No obvious B deficiency symptom was observed, except the young leaf colour in 0.18 µM B plants was slightly darkened.

After plants were exposed to RZT treatment, symptoms of B deficiency appeared in 0.18 µM B plants at both T10 and T20 by d11. On d15, 0.18 µM B T20/10 plants developed more severe symptoms of B deficiency than T10/10 plants, and young leaves of 0.27 µM B T20/10 plants also started to show symptoms of B deficiency but not in 0.27 µM B T10/10 plants.

4.4.2 Plant B response to RZT (d0-d11)

Boron and RZT strongly affected plant growth (Tables 4.1 and 4.2). Plant growth was promoted by warmer RZT but inhibited by low B. The greatest plant DW was created by plants grown under adequate B (8.1 µM B) at warmer (20 °C) RZT. The least plant DW resulted from low B supply (0.18 µM B) at 10 °C RZT which nearly halved plant DW in comparison to the plants at higher RZT.

Boron uptake by plants was increased dramatically with increasing external B supply and warmer RZT (Table 4.3), the effect of RZT on plant B uptake was more accentuated in adequate B plants than in lower B supplied plants. Plant B content was more than doubled by warmer RZT in adequate B supplied (8.1 µM B) plants, whilst the increase of plant B content was less than 50 % in low B plants.
Table 4.1 Effects of root zone temperature (RZT) and boron (B) on plant biomass (DW g plant$^{-1}$) and shoot to root (S/R) ratio. Values are means of four replicates in each treatment, with the standard error in parentheses.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Harvest 1</th>
<th>Harvest 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoot</td>
<td>Root</td>
</tr>
<tr>
<td>B (µM)</td>
<td>RZT (°C)</td>
<td></td>
</tr>
<tr>
<td>0.18</td>
<td>10</td>
<td>0.64(0.02)</td>
</tr>
<tr>
<td>0.18</td>
<td>20</td>
<td>1.15(0.06)</td>
</tr>
<tr>
<td>0.27</td>
<td>10</td>
<td>0.85(0.03)</td>
</tr>
<tr>
<td>0.27</td>
<td>20</td>
<td>1.13(0.05)</td>
</tr>
<tr>
<td>8.1</td>
<td>10</td>
<td>0.94(0.05)</td>
</tr>
<tr>
<td>8.1</td>
<td>20</td>
<td>1.18(0.06)</td>
</tr>
</tbody>
</table>

F test  | B | * | *** | *** | *** | *** | *** |
| RZT | *** | *** | ns | *** | * | *** |
| B x RZT | * | ns | ** | ns | ns | ns |

Note: In the table, ns = non-significant; *, **, *** significant at 5 %, 1 % and 0.1 % probability levels, respectively.
Table 4.2 Effects of root zone temperature (RZT) and boron (B) on plant relative growth rate (RGR d⁻¹) and the ratio of RGR of shoot to RGR of root (RGRs/r), and shoot demand (d⁻¹). Values are means of four replicates in each treatment, with standard errors in parentheses.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Harvest 0-1</th>
<th>Harvest 1-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>B (µM)</td>
<td>RZT (°C)</td>
<td>RGR</td>
</tr>
<tr>
<td>0.18</td>
<td>10</td>
<td>0.16(0.00)</td>
</tr>
<tr>
<td>0.18</td>
<td>20</td>
<td>0.21(0.01)</td>
</tr>
<tr>
<td>0.27</td>
<td>10</td>
<td>0.18(0.00)</td>
</tr>
<tr>
<td>0.27</td>
<td>20</td>
<td>0.21(0.00)</td>
</tr>
<tr>
<td>8.1</td>
<td>10</td>
<td>0.19(0.01)</td>
</tr>
<tr>
<td>8.1</td>
<td>20</td>
<td>0.21(0.01)</td>
</tr>
</tbody>
</table>

F test

<table>
<thead>
<tr>
<th>B</th>
<th>RZT</th>
<th>B x RZT</th>
</tr>
</thead>
<tbody>
<tr>
<td>**</td>
<td>***</td>
<td>ns</td>
</tr>
<tr>
<td>ns</td>
<td>***</td>
<td>**</td>
</tr>
</tbody>
</table>

Note: In the table, ns = non-significant; *, **, *** significant at 5 %, 1 % and 0.1 % probability levels, respectively.
Table 4.3 Effects of root zone temperature (RZT) and solution boron (B) on B concentrations in the youngest open leaves (YOL) (B mg kg\(^{-1}\)), plant B content (B µg plant\(^{-1}\)) and B uptake rate (BUR) (µmol B g\(^{-1}\) root DW d\(^{-1}\)) by oilseed rape. Values are means of four replicates in each treatment, with standard errors in parentheses.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>YOL B (mg kg(^{-1}))</th>
<th>Plant B content (B µg plant(^{-1}))</th>
<th>BUR (µmol B g(^{-1}) root DW d(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B (µM)</td>
<td>RZT (°C)</td>
<td>Harvest 1</td>
</tr>
<tr>
<td>0.18</td>
<td>0.18</td>
<td>10</td>
<td>4.3(0.24)</td>
</tr>
<tr>
<td>0.18</td>
<td>20</td>
<td>3.5(0.26)</td>
<td>1.8(0.1)</td>
</tr>
<tr>
<td>0.27</td>
<td>10</td>
<td>7.6(0.33)</td>
<td>5.6(0.5)</td>
</tr>
<tr>
<td>0.27</td>
<td>20</td>
<td>7.1(0.42)</td>
<td>2.7(0.1)</td>
</tr>
<tr>
<td>8.1</td>
<td>10</td>
<td>23.1(0.83)</td>
<td>21.4(0.8)</td>
</tr>
<tr>
<td>8.1</td>
<td>20</td>
<td>30.3(0.80)</td>
<td>23.4(0.5)</td>
</tr>
</tbody>
</table>

F test | B | *** | *** | *** | *** | *** | *** |
| RZT | **** | ns | *** | *** | *** | ns |
| B x RZT | **** | *** | *** | *** | *** | ns |

Note: In the table, ns = non-significant; *, **, *** significant at 5 %, 1 % and 0.1 % probability levels, respectively.
Although YOL B concentrations were increased with greater B supply (Table 4.3), warmer RZT also increased them in B adequate plant but decreased them in low B plants. Consequently, plant B deficiency was accentuated or exacerbated by higher RZT.

4.4.3 ‘Residual’ plant B response to RZT (d11-d15)

Increasing RZT had not only direct effects on plant growth, its effect also persisted after the return of RZT to 10 °C. The residual effect of increased RZT persisted on the shoot growth but not on the root growth. Shoots continued growing at a high rate after plants returned from 20 to 10 °C RZT (Table 4.2), but root growth was greatly reduced. Thus S/R ratio was higher in T20/10 plants than T10/10 plants on d15 especially in low B plants.

In low B supplied plants, YOL B concentrations decreased with growth time and were more severely lowered by warm RZT treatment (T20/10 vs T10/10) (Tables 4.3).

4.5 Discussion

Similar to the previous winter experiment (Chapter 3), the results in present experiment showed that although both RZT and B supply affected plant growth, RZT had a more
acute influence on plant growth than B treatment did (Table 4.1). During d0-11, increasing RZT to 20 °C produced 77, 32, 31 % more shoot DW than those at 10 °C RZT in plants supplied with 0.18, 0.27 and 8.1 µM B, respectively.

Root zone temperature affects growth of both root and shoot (Cooper, 1973; Pollock and Eagles, 1988; Bowen, 1991; McMichael and Burke, 1996), and the assimilate partitioning responds very quickly to RZT (Minchin et al., 1994 a and b). When the temperature does not induce direct injury to the plant tissue, the lower growth rate at low temperature results from the reduced thermal input (Hurry et al., 1995) and/or the loss of substrate carbon conversion efficiency (Criddle, 1997). Hence S/R ratio is subject to change in response to RZT. In the previous two experiments (Chapter 3) where plants were treated by lowered RZT, a decrease of S/R ratio was induced by low RZT especially in winter weather regardless of B supply. However, in present experiment a smaller S/R ratio at low than at warm RZT was only shown in severe low B (0.18µM)- plants (Table 4.1). Since low B induces higher S/R ratio (Marschner et al., 1996), a smaller S/R ratio in the plants at low RZT is more attributed to more severe inhibition of the root growth relative to the shoot at 20 °C RZT (Table 4.1).

The persistent faster growth of shoots of T20 plants than T10 may be caused by the internal hormonal changes (Ali et al., 1997, 1998). However, root growth was greatly restricted by returning from 20 to 10 °C, and this resulted in higher S/R by d15 in
T20/10 plants than the control. Root zone temperature may influence shoot growth directly by affecting the air temperature around the growing point of oilseed rape plant and also indirectly by affecting uptake and transport of nutrients and water (Russell, 1977; Klepper, 1991; Lainé et al., 1993), and other physiological and biochemical properties of plant (Voorhees et al., 1981; Laine et al., 1994; Ali et al., 1997, 1998; Logan et al., 1997; Ryyppö et al., 1998).

The differential responses to RZT between shoots and roots vary with plant species (Cooper, 1973; Wilson, 1988), the duration of treatment (Clarkson et al., 1988) and the aerial conditions (Chapter 3). Thus S/R ratio may be increased or decreased by warm RZT, as shown by Cumbus and Nye (1982, 1985), Macduff et al. (1987a and b) and Kacperska and Szaniawski (1993) in oilseed rape. However, the change of S/R ratio in response to RZT is important for plant nutrient uptake to meet shoot demand (Clarkson et al., 1988). Greater S/R ratio, i.e., smaller size of root relative to shoot, increases the difficulty for plants to acquire sufficient nutrients for shoot growth under adverse conditions.

Although warmer RZT increased B uptake, it also resulted in higher growth potential and more B was required to meet growth demands. However, due to higher S/R ratio induced by low B, when B supply was especially low (0.18 µM), shoot B demand at 20 °C exceeded that at 10 °C RZT (Table 4.2). Moreover, increased rates of B uptake (BUR)
and B translocation from root to shoot (BTR) at warmer RZT were only evident in plants supplied with adequate B but not at low B (Tables 4.3 and 4.4). Even worse for low B plants was that B partitioning into the growing tissues (YOLs) was always greatly reduced by warm RZT. The decrease of B transport to the YOLs is in agreement with previous results (Chapter 3). Consequently, the development of B deficiency occurred more rapidly in T20/10 plants than the control (T10/10). As were shown for the low B plants in the final four days of growth (d11-15), both the B partitioning and YOL B concentrations decreased by half or more in T20/10 plants. By contrast, the decreases were much weaker in T10/10 plants.

Nevertheless, except for a different response of plant S/R ratio to RZT, results of this experiment were in accordance with the previous two experiments (Chapter 3), and showed again that 20 °C RZT accentuates plant B deficiency in comparison to 10 °C RZT, regardless of plants’ pre-growth conditions or weather conditions. Whether lower RZT induces B deficiency or not is dependent on the range of RZTs tested and their relationship to the critical threshold for that species.
Table 4.4 Effects of root zone temperature (RZT) and boron (B) on B partitioning between shoot and root and within the shoot, and on B translocation from root to shoot (BTR) (µmol B g\(^{-1}\) root DW d\(^{-1}\)). Values are means of four replicates in each treatment, with standard errors in parentheses.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>S/R(^a)</th>
<th>YOL(^b)</th>
<th>BTR (µmol B g(^{-1}) root DW d(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B (µM)</td>
<td>RZT (°C)</td>
<td>Harvest 1</td>
</tr>
<tr>
<td>0.18</td>
<td>10</td>
<td>7.42(0.35)</td>
<td>5.22(0.12)</td>
</tr>
<tr>
<td>0.18</td>
<td>20</td>
<td>6.02(0.43)</td>
<td>5.79(0.46)</td>
</tr>
<tr>
<td>0.27</td>
<td>10</td>
<td>7.66(0.34)</td>
<td>6.10(0.60)</td>
</tr>
<tr>
<td>0.27</td>
<td>20</td>
<td>5.79(0.26)</td>
<td>5.01(0.27)</td>
</tr>
<tr>
<td>8.1</td>
<td>10</td>
<td>18.58(1.16)</td>
<td>11.04(2.02)</td>
</tr>
<tr>
<td>8.1</td>
<td>20</td>
<td>19.03(1.00)</td>
<td>17.34(1.00)</td>
</tr>
<tr>
<td>F test(^c)</td>
<td>B</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>RZT</td>
<td>ns</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>B x RZT</td>
<td>ns</td>
<td>**</td>
</tr>
</tbody>
</table>

\(^a\) Ratio of content of B between shoots and roots;

\(^b\) Boron content in the youngest open leaf blade (YOL) relative to shoot B (%).

\(^c\) ns = non-significant; *, **, *** significant at 5 %, 1 % and 0.1 % probability levels, respectively.
Chapter 5

Response of sunflower (*Helianthus annuus* L. cv. Hysun 25) to boron supply at low root zone temperature

5.1 Abstract

In the previous chapters, results of the glasshouse experiments have shown that low root zone temperature (RZT) at 10 °C always delayed or alleviated boron (B) deficiency symptom in oilseed rape plants, regardless of pre-growth conditions of the plants, which contrasts with the hypothesis (Chapter 1) of ‘low RZT induces plant B deficiency’, and also with results of Forno et al. (1979). In Chapter 3, it was postulated that ‘whether low RZT enhances plant sensitivity to low B supply may be dependent on the low temperature tolerance of the test species. Tropical species like cassava are expected to be sensitive to low temperature and to have a higher RZT threshold below which root growth and functions are impaired, than those for temperate species such as oilseed rape’.

In this Chapter, the hypothesis of a RZT threshold below which an increased B response is expected was examined, in sunflower which is also sensitive to low B but much less tolerant to low temperature than oilseed rape. Using B-specific resin to buffer B concentrations in solution, B deficiency symptoms in sunflower were induced by low RZT of 12 °C and lower when plants were supplied with 0.25 μM B whilst plants at the
same solution B concentration were free from B deficiency at warmer RZT. Decreasing RZT from 27 to 12 °C for 6 days depressed sunflower dry matter, root length, root to shoot ratio and B uptake. Boron uptake into the shoots was depressed at ≤ 12 °C RZT to a greater relative extent than dry matter. Furthermore, B partitioning to young leaves decreased with low RZT, particularly at ≤ 12 °C RZT. The external B requirement for shoot growth increased with low RZT from 0.2 to 0.6 µM. Leaf functioning was impaired by low B supply as measured by increased potassium (K) leakage from the youngest mature leaf blade, whereas it was much less affected by RZT, and there was no effect of RZT on K⁺ leakage from leaves of B - adequate plants. By contrast, root function as measured by K⁺ leakage was impaired more by low RZT than low B. The overall pattern of response by sunflower to B and RZT was not changed by a 11-day pre-treatments period at 10 or 20 °C RZT. Therefore, it is predicted that higher rates of B application will be required for plant growth when soil temperature is below a critical threshold, which for sunflower was about 17 °C. Conversely, practices which increase soil temperature above the threshold may also improve plant B nutrition.

5.2 Introduction

Suboptimal RZT of 19 °C induced B deficiency of cassava in solution culture, a response which was attributed by Forno et al. (1979) to lowered nutrient (B) uptake rate (BUR) and reduced root size relative to the shoot. By contrast, in previous experiments with oilseed rape (Chapters 3 and 4), B deficiency in low B plants was always

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accentuated by warm RZT (20 °C) in comparison to 10 °C regardless of pre-growth conditions of the plants such as RZT and B supply.

By comparing the results of Walker (1969) on maize, Tindall et al. (1990) on tomato and Forno et al. (1979) on cassava, and oilseed rape (Chapters 3 & 4), it is inferred that the effect of RZT on plant response to external B may be determined by the species susceptibility to low RZT. In cassava, low RZT induced B deficiency even though the same B supply was adequate at optimal RZT (Forno et al., 1979). In contrast, when external B supply was excessive, lower RZT resulted in higher B concentrations in barley shoots and caused B toxicity symptoms to appear earlier than at higher root temperature (5 vs 10 and 15 °C) (Mahalakshmi et al., 1995). These apparently contradictory reports suggest the possibility that the interaction of B x RZT may relate to the sensitivity to cold temperature of different plant species. For example, increase in dry matter is inhibited in cassava and maize at RZT of 19 °C (Forno et al., 1979) and 10 °C (Marton et al., 1997), respectively, but the growth of barley persists at 5 °C RZT (Mahalakshmi et al., 1995). Therefore, it is hypothesized that there is a RZT threshold below which an increased response to B is expected.

Similar to oilseed rape, sunflower is reputed to be sensitive to low B (Schuster and Stephenson, 1940; Blamey et al., 1978, 1979), but unlike oilseed rape, sunflower is subject to chilling injury at about 15 °C (Seiler, 1998). However, temperature near or below 10 °C often appears in the field in the sunflower growing season (d'Andria et al., 1995; Thompson and Heenan, 1994) making it an ideal species to test the hypothesis. Boron supply, from severely deficient to adequate levels controlled by B specific resin (Asad et al., 1997a; Huang et al., 1999), and RZT, from chilling to optimum (12 to 27
°C), were applied in the first experiment. A 6-day treatment was imposed in the glasshouse to determine the short term responses of sunflower plant growth to B supply and RZT. In a second experiment with sunflower the effect of pre-treatment at either 10° C or 20 °C RZT was imposed on sunflower plants before they were subjected to B and RZT treatments.

5.3 Materials and Methods

5.3.1 Experiment 1

Sunflower cv. Hysun 25 seeds were germinated on paper towels soaked with 1.0 mM Ca(NO₃)₂ and 0.01 mM H₃BO₃ solution at 20 °C for 4 days in the dark. Four day-old seedlings were transferred to full strength nutrient solution in a 10-l tray with RZT maintained at 20 - 22 °C by a temperature-controlled water bath. Four days later, when the seedlings’ first pair of true leaves appeared, five uniform seedlings were transplanted to 5-l full strength nutrient solution (excluding B) in each pot lined with a plastic bag, and exposed to RZT and B treatment with 3 replicates for each treatment (d₀). By placing pots in temperature-controlled water baths, the RZT were maintained at 11 - 13, 16 - 18, 21 - 23 and 26 - 28 °C (termed 12, 17, 22 and 27 °C, respectively, hereafter). Plants received five B concentrations in culture solutions controlled by B-specific resin. Boron concentrations in the culture solutions remained constant between d₀ and d₆: means with standard errors were 0.08 ± 0.00, 0.16 ± 0.01, 0.25 ± 0.01, 0.62 ± 0.03 and 6.5 ± 0.2 µM B, respectively. Plants were harvested on d₆ when their growth showed a response to both B and RZT. By that time, the first pair of true leaves had become the youngest fully mature leaves in B-adequate plants.
Membrane permeability of the youngest mature leaf blades (YML) and roots was measured at 20 °C in a dark room by leakage of solutes into distilled water after 2 hr. Potassium (K) in the solutions were analyzed following Cakmak et al. (1995) and Brown and Hu (1997). Youngest mature leaves and the remainder of the shoots were oven dried at 70 °C for 48 hr and their dry weights (DW) were recorded. Boron in oven dried plant materials was analyzed by ICP-AES after digestion with concentrated nitric acid (Zarcinas et al., 1987). Root fresh weight was determined after blotting with absorbent paper to remove free water and length of the fresh roots was measured using a Comair root length scanner.

5.3.2 Experiment 2

The general procedures were the same as in Experiment 1. The germinated sunflower (cv. Hysun 25) seedlings were pre-grown at either at 20 - 22 °C (RZT20) or 10 - 12 °C (RZT10) (Pre-RZT). Eleven days later, RZT20 plants' 2nd pair of true leaves (L2) were close to the youngest mature leaves, and L4 was just emerging; in RZT10 plants L1 became youngest mature leaves and L3 just emerged. Three uniform seedlings were transplanted to full strength nutrient solution (excluding B) in each 5-l pot lined with plastic bag. The plants were supplied with low or sufficient B (0.19 ± 0.01; 12 ± 1 µM) (means with standard errors) and grown at 10 or 20 °C RZT with 3 replicates (d0).

On d4, when the young leaves in 0.19 µM B plants at 10 °C RZT started to exhibit symptoms of B deficiency regardless of the pre-growth temperature, all plants were harvested. Plant samples were separated into the young shoots (YS) (above the youngest mature leaves), remainder of the shoots, and roots. They were oven dried at 70
"C and their dry weights were recorded. Boron in the oven dried plant materials was
analyzed by ICP-AES after digestion with concentrated nitric acid as described above.

5.3.3 Other data collection

Relative growth rate of plants (RGR), relative elongation rate of the roots (RER), B
uptake rate (BUR) and B translocation rate from root to shoot (BTR), and relative
accumulation rate of B in the shoots (RAR) were calculated (Forno et al. 1979; Willits
et al., 1992; Engels and Marschner, 1996). The functions used for calculating the above
parameters are listed below:

\[
\text{RGR} = \frac{\ln(W2 - W1)}{(t_2 - t_1)}
\]

\[
\text{RGRs} = \frac{\ln(W_{s2}/W_{s1})}{(t_2 - t_1)}
\]

\[
\text{RERr} = \frac{\ln(L2/L1)}{(t_2 - t_1)}
\]

\[
\text{RAR} = \frac{\ln(M_{s2}/M_{s1})}{(t_2 - t_1)}
\]

\[
\text{BTR} = \frac{(\ln(L2/L1)) (M_{s2}-M_{s1})/ ((t_2-t_1) (L_2-L_1))}{(M_{r2}-M_{r1})}
\]

\[
\text{BUR} = \frac{(ln(Wr2-lnWr1)(Ms_{s2}-Ms_{s1}))/((t_2-t_1)(Wr_2-Wr_1))}{((t_2-t_1)(Wr_2-Wr_1))}
\]

Where: W2, W1, whole plant dry weights (g plant\(^{-1}\)) at harvest 2 (t2) and 1 (t1); s, Shoot; r, Root; Ws2, Ws1 and Wr2, Wr1, Shoot and root dry weights (g plant\(^{-1}\)) at
harvest 2 and 1, respectively; L2, L1, Root length (m) at harvest 2 and 1; Ms2, Ms1, Shoot B content (nmol plant\(^{-1}\) in Experiment 1 and µmol plant\(^{-1}\) in Experiment 2) at
harvest 2 and 1; t2, t1, day after treatment at harvest 2 and 1; M2, M1, whole plant B
contents at harvest 2 and 1, respectively.
The data were analyzed for the significance of treatment effects and their possible interactions with two- (Experiment 1) or three- (Experiment 2) way analysis of variance (Super Anova, USA).

5.4 Results

5.4.1 Experiment 1

5.4.1.1 Plant growth

Symptoms Plants at low RZT developed pale green leaves, especially at 12 °C. The shoot apices and the first pair of the true leaves which were emerging on d0 were severely stunted by d6 due to severe B deficiency in plants at 0.08 µM B regardless of RZT (Table 5.2, Plate 5.1). At 0.16 µM B, increasing RZT decreased the severity of symptoms of B deficiency. And leaf B deficiency symptoms in plants at 0.25 µM B were present at 12 °C RZT but absent at 17 - 27 °C RZT.

Plate 5.1 Sunflower plant growth response to root zone temperature (RZT) and boron (B) (Experiment 1).
Dry weight Plant growth increased with increasing RZT and with increasing B supply (Table 5.1, Figure 5.1). In the range of RZT from 12 to 27 °C, only 12 °C RZT restricted root growth markedly in B- adequate plants. Roots of plants at 12 °C RZT had dramatically smaller size and had reduced length per g fresh weight (Table 5.1). Shoots of B- adequate plants required RZT of 22 to 27 °C for maximum shoot dry matter.

Table 5.1 Effect of solution boron (B) concentration (µM) and root zone temperature (RZT) on sunflower shoot and root growth. Values are means of three replications with standard errors in parentheses (Experiment 1).

<table>
<thead>
<tr>
<th>RZT (°C)</th>
<th>B0.08</th>
<th>B0.16</th>
<th>B0.25</th>
<th>B0.62</th>
<th>B6.5</th>
<th>F test&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoot dry weight (mg plant&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>180(10)</td>
<td>185(10)</td>
<td>226(10)</td>
<td>256(10)</td>
<td>264(20)</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>179(10)</td>
<td>220(20)</td>
<td>313(20)</td>
<td>298(10)</td>
<td>323(20)</td>
<td>B ***</td>
</tr>
<tr>
<td>22</td>
<td>181(10)</td>
<td>268(10)</td>
<td>359(10)</td>
<td>303(10)</td>
<td>361(40)</td>
<td>RZT ***</td>
</tr>
<tr>
<td>27</td>
<td>179(10)</td>
<td>295(10)</td>
<td>318(10)</td>
<td>361(20)</td>
<td>343(10)</td>
<td>B x RZT **</td>
</tr>
<tr>
<td></td>
<td>LSD 0.05</td>
<td>30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Root length (m plant&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>1.03(0.13)</td>
<td>2.25(0.18)</td>
<td>3.33(0.16)</td>
<td>3.77(0.57)</td>
<td>4.08(0.67)</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>1.28(0.08)</td>
<td>4.32(0.43)</td>
<td>7.82(0.46)</td>
<td>7.72(0.42)</td>
<td>8.97(0.62)</td>
<td>B ***</td>
</tr>
<tr>
<td>22</td>
<td>1.33(0.32)</td>
<td>4.77(0.20)</td>
<td>8.42(1.12)</td>
<td>8.37(0.47)</td>
<td>9.80(1.65)</td>
<td>RZT ***</td>
</tr>
<tr>
<td>27</td>
<td>1.52(0.12)</td>
<td>4.95(0.40)</td>
<td>9.17(0.86)</td>
<td>10.53(0.71)</td>
<td>9.25(0.73)</td>
<td>B x RZT **</td>
</tr>
<tr>
<td></td>
<td>LSD0.05</td>
<td>1.29</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ratio of shoot dry mass to root length (mg m&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>180(19)</td>
<td>83(8)</td>
<td>68(2)</td>
<td>70(8)</td>
<td>67(7)</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>139(2)</td>
<td>54(2)</td>
<td>40(1)</td>
<td>39(3)</td>
<td>36(1)</td>
<td>B ***</td>
</tr>
<tr>
<td>22</td>
<td>145(20)</td>
<td>56(2)</td>
<td>44(6)</td>
<td>36(1)</td>
<td>38(5)</td>
<td>RZT ***</td>
</tr>
<tr>
<td>27</td>
<td>119(5)</td>
<td>60(4)</td>
<td>35(2)</td>
<td>35(2)</td>
<td>37(3)</td>
<td>B x RZT ns</td>
</tr>
<tr>
<td></td>
<td>LSD0.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ratio of length to fresh weight in roots (m g&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>1.89(0.30)</td>
<td>2.25(0.32)</td>
<td>2.64(0.10)</td>
<td>2.61(0.22)</td>
<td>2.90(0.18)</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>2.46(0.09)</td>
<td>3.47(0.18)</td>
<td>3.68(0.12)</td>
<td>3.62(0.43)</td>
<td>3.78(0.38)</td>
<td>B ***</td>
</tr>
<tr>
<td>22</td>
<td>2.34(0.08)</td>
<td>3.85(0.31)</td>
<td>3.65(0.42)</td>
<td>4.17(0.15)</td>
<td>3.83(0.26)</td>
<td>RZT ***</td>
</tr>
<tr>
<td>27</td>
<td>2.74(0.23)</td>
<td>3.59(0.37)</td>
<td>4.82(0.73)</td>
<td>4.33(0.29)</td>
<td>3.60(0.45)</td>
<td>B x RZT ns</td>
</tr>
</tbody>
</table>

<sup>a</sup> ns = non-significant; *, **, *** significant at 5 %, 1 % and 0.1 % probability levels, respectively. LSD0.05 represents the Fisher’s Least significant difference at 5 % probability level for the B x RZT interaction if the F test was significant.
Plant growth also increased with increasing B supply when RZT was optimal at 22 - 27 °C. Shoot dry matter in B-adequate plants was twice that in low B by d6 (Table 5.1, Figure 5.1). More dramatically, root length was 4 - 7 times greater in B adequate plants than in low B. Root fresh weight followed the same pattern as root length but with a dampened response to B (data not shown).

Root zone temperature substantially influenced plant response to B supply. At RZT of 12 °C, both roots and shoots had least response to B supply. Similarly, at extremely low B supply (0.08 µM B), both roots and shoots did not respond to RZT (Table 5.1). In contrast, in the range of 0.16 to 6.5 µM B, increasing RZT by 5 °C, from 12 to 17 °C, doubled root length, and also markedly increased shoot growth. The most apparent evidence of a B x RZT interaction on plant growth was observed at 0.25 µM B when an increase in RZT from 12 to 17 °C, overcame B deficiency symptoms (Plate 5.1), increased YML B concentrations (Table 5.2) and stimulated dry matter and root length (Table 5.1).

Maximum root growth was achieved at RZT of 22 and 27 °C when B supply was adequate (0.62 and 6.5 µM B; Table 5.1; Figure 5.1). Shoot dry matter showed a similar pattern of response as root, but was less responsive. Shoot dry matter plateaued at RZT ≥ 22 °C with ≥ 0.25 µM B (Figure 5.1; Table 5.3).

5.4.1.2 Boron uptake

Boron uptake in the shoots increased with higher RZT and also with increasing external B except at 0.08 µM B (Table 5.2). Boron concentrations in YML and shoots also
Table 5.2 Effect of solution boron (B) concentration (µM) and root zone temperature (RZT) on B deficiency symptoms, B concentration in the youngest mature leaf (YML) and shoots, and B uptake in sunflower. Values are means of three replications with standard errors in parentheses (Experiment 1).

<table>
<thead>
<tr>
<th>RZT (°C)</th>
<th>B0.08</th>
<th>B0.16</th>
<th>B0.25</th>
<th>B0.62</th>
<th>B6.5</th>
<th>F test *a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score b of B deficiency symptoms in YML</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>5</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>5</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>5</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

YML B concentrations (mg B kg⁻¹) c

|       |       |       |       |       |      |           |
| 12    | 6.0(0.7) | 6.1(0.3) | 7.0(0.5) | 11.7(0.2) | 24.9(1.4) |           |
| 17    | 5.4(0.3) | 5.8(0.5) | 10.9(1.4) | 26.3(0.6) | 29.1(3.5) | B ***     |
| 22    | 5.4(0.0) | 5.3(0.0) | 12.3(0.9) | 29.6(2.2) | 33.4(1.9) | RZT ***   |
| 27    | 5.2(0.3) | 5.9(0.2) | 9.4(0.6)  | 32.6(1.9) | 32.3(2.1) | B x RZT *** |
|       |         |         |         |         | LSD0.05  | 2.71      |

Boron concentrations (mg B kg⁻¹) in the remainder of shoots

|       |       |       |       |       |      |           |
| 12    | 11.6(0.3) | 14.4(0.7) | 17.9(0.1) | 20.8(1.0) | 24.4(1.0) |           |
| 17    | 11.4(0.4) | 14.8(0.6) | 17.2(0.8) | 25.4(0.7) | 30.8(0.8) | B ***     |
| 22    | 11.8(0.4) | 14.6(0.6) | 17.9(0.1) | 26.6(0.8) | 36.8(1.5) | RZT ***   |
| 27    | 10.4(0.9) | 13.8(0.8) | 18.0(0.6) | 28.5(1.7) | 35.0(1.1) | B x RZT *** |
|       |         |         |         |         | LSD0.05  | 1.70      |

Net boron uptake (µg plant⁻¹) in the shoot over 6 d

|       |       |       |       |       |      |           |
| 12    | 0.26(0.09) | 0.60(0.09) | 1.51(1.00) | 2.84(0.20) | 4.89(0.77) |           |
| 17    | 0.17(0.06) | 0.92(0.17) | 2.96(0.55) | 6.03(0.52) | 8.04(0.57) | B ***     |
| 22    | 0.23(0.14) | 1.32(0.12) | 3.98(0.32) | 6.79(0.61) | 11.21(1.82) | RZT ***   |
| 27    | 0.05(0.17) | 1.62(0.17) | 2.81(0.15) | 9.30(0.36) | 9.92(0.44) | B x RZT *** |
|       |         |         |         |         | LSD0.05  | 1.09      |

a ns = non-significant; *, **, *** significant at 5 %, 1 % and 0.1 % probability levels, respectively.

b Score: 5- YML unable to expand and the leaf base became dark brown (bronzing), and shoot apex was severely stunted; 4- symptom of dark brown (bronzing) strip appeared close to YML base; 3- similar to ‘4’ but midrib remained greenness; 2- leaf bronzing evidently; 1- slight B deficient symptoms (mild leaf bronzing); 0- without symptom of B deficiency. The photo plate (Plate 5.1) shows the corresponding symptoms.

c Shaded data represent leaves with symptoms of B deficiency.
increased with B supply, but the influence of RZT varied with external B concentrations. Boron concentrations tended to slightly decrease with increasing RZT when B supply was low (0.08 and 0.16 µM B). At 0.25 µM B, increasing RZT from 12 to 22 °C strongly increased YML B concentrations but further increases in RZT decreased YML B concentration: in shoots, increasing RZT had no effect on B concentrations. By contrast, B concentrations increased markedly with increasing RZT when plants were supplied with 0.62 and 6.5 µM B. The strongest effect of RZT on increasing B concentration was at 0.62 µM B with RZT between 12 and 17 °C: YML B concentration at 17 °C was more than double that at 12 °C (26 vs 12 mg B kg⁻¹). Net B uptake in the shoots after 6 d growth was also more than doubled at 17 °C compared 12 °C. However, in the optimum range of RZT (22 - 27 °C), the effect of RZT in increasing B uptake and concentration diminished. Similarly, rates of B translocation from root to shoot (BTR) and B accumulation in the shoot (RAR) also increased with higher RZT and this was most significant at 0.62 µM B with RZT between 12 and 17 °C (Table 5.4).

Table 5.3 Parameters for Mitscherlich models a fitted to the relationship between solution boron (B) concentration and either shoot dry weight (g plant⁻¹) or root length (m plant⁻¹). The fitted relationships are shown in Figure 5.1 (Experiment 1).

<table>
<thead>
<tr>
<th>RZT (°C)</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>R²</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoot</td>
<td></td>
<td></td>
<td></td>
<td>Root</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>0.26</td>
<td>0.10</td>
<td>2.38</td>
<td>0.70</td>
<td>3.91</td>
<td>4.18</td>
<td>4.81</td>
<td>0.68</td>
</tr>
<tr>
<td>17</td>
<td>0.32</td>
<td>0.29</td>
<td>8.90</td>
<td>0.78</td>
<td>8.52</td>
<td>15.56</td>
<td>9.30</td>
<td>0.91</td>
</tr>
<tr>
<td>22</td>
<td>0.34</td>
<td>0.51</td>
<td>14.10</td>
<td>0.72</td>
<td>9.26</td>
<td>17.06</td>
<td>9.34</td>
<td>0.81</td>
</tr>
<tr>
<td>27</td>
<td>0.35</td>
<td>0.46</td>
<td>12.40</td>
<td>0.93</td>
<td>10.05</td>
<td>18.39</td>
<td>9.25</td>
<td>0.89</td>
</tr>
</tbody>
</table>

a Mitscherlich model: f(x) = a - b*exp(-c*x), where: x, solution B concentration (µM); f(x), shoot dry weight (g per plant) or root length (m per plant).
Table 5.4 Responses of plant growth and boron uptake to root zone temperature (RZT) and boron concentration (µM) (Values are means of three replications with standard errors in parentheses) (Experiment 1).

<table>
<thead>
<tr>
<th>RZT (°C)</th>
<th>B0.08</th>
<th>B0.16</th>
<th>B0.25</th>
<th>B0.62</th>
<th>B6.5</th>
<th>F test *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RGR, g g⁻¹ d⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>0.20(0.00)</td>
<td>0.21(0.01)</td>
<td>0.24(0.00)</td>
<td>0.26(0.01)</td>
<td>0.26(0.01)</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>0.20(0.01)</td>
<td>0.23(0.01)</td>
<td>0.29(0.01)</td>
<td>0.28(0.01)</td>
<td>0.30(0.01)</td>
<td>B ***</td>
</tr>
<tr>
<td>22</td>
<td>0.20(0.02)</td>
<td>0.27(0.00)</td>
<td>0.32(0.01)</td>
<td>0.29(0.01)</td>
<td>0.31(0.02)</td>
<td>RZT ***</td>
</tr>
<tr>
<td>27</td>
<td>0.20(0.01)</td>
<td>0.28(0.00)</td>
<td>0.30(0.00)</td>
<td>0.32(0.01)</td>
<td>0.31(0.00)</td>
<td>B x RZT ***</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Relative elongation rate of the roots (RER, m m⁻¹ d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
</tr>
<tr>
<td>17</td>
</tr>
<tr>
<td>22</td>
</tr>
<tr>
<td>27</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Relative accumulation rate of boron in the shoots (RAR, d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
</tr>
<tr>
<td>17</td>
</tr>
<tr>
<td>22</td>
</tr>
<tr>
<td>27</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Rate of boron (B) translocation from root to shoot (BTR) (nmol B m⁻² d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
</tr>
<tr>
<td>17</td>
</tr>
<tr>
<td>22</td>
</tr>
<tr>
<td>27</td>
</tr>
</tbody>
</table>

* ns = non-significant; *, **, *** significant at 5 %, 1 % and 0.1 % probability levels, respectively.

5.4.1.3 Root and leaf K⁺ leakage

After the 6-d growth period, K⁺ leakage from roots was mainly determined by RZT, the effect of B supply was much weaker (Figure 5.2). At 12 °C RZT, K⁺ leakage from roots was greatest, while 22 and 27 °C RZT minimized K⁺ leakage.

In contrast to roots, K⁺ leakage from leaves strongly responded to both B supply and RZT (Figure 5.3A). Leaf leakage of K⁺ increased in plants supplied with low B (0.08 and 0.16 µM B). Leakage in YML of plants at 0.25 to 6.5 µM B was not affected by...
increasing B supply or RZT. Overall, YML B concentrations < 7 mg B kg\(^{-1}\) were associated with a marked increase in K\(^+\) leakage, which was in accordance with B deficiency (Table 5.2, Plate 5.1).

Figure 5.1 Relationship between external boron (B) concentration and shoot dry weight (g plant\(^{-1}\)) or root length (m plant\(^{-1}\)) at different root zone temperatures (RZT). The parameters of the fitted Mitscherlich relationships are shown in Table 5.3 (Experiment 1).
Figure 5.2 Effect of root zone temperature (RZT) and solution boron (B) concentration (µM) on root K⁺ leakage (mg K g⁻¹ fw 2h⁻¹) of sunflower at 6 days after treatments commenced. The vertical bar represents the LSD₀.₀₁ for the B x RZT interaction (Experiment 1).

5.4.2 Experiment 2

Sunflower plants at 10 °C RZT were small compared to 20 °C RZT (Table 5.5). During the 11 days of pre-RZT treatment, the plants grown at pre-RZT of 10 °C RZT had fewer root branches and root hairs than those at pre-RZT of 20 °C RZT, and the root tips became brown and black, and the effect of low RZT (10 °C) on root morphology was also very evident even in the further 4 days of treatment (Plate 5.2).
Figure 5.3 Effect of root zone temperature (RZT) and boron (B) on K⁺ leakage from the youngest mature leaf blade (YML) of sunflower (mg K g⁻¹ fw 2h⁻¹) (Experiment 1).

A: Effect of RZT and solution B concentration on K⁺ leakage from the YML (the vertical bar represents the LSD_{0.01} for the B x RZT interaction). Values refer to the YML B concentrations or the range of B concentrations (B mg kg⁻¹) for each treatment. F test for the treatment significance: ns = non-significant; *, **, *** significant at 5 %, 1 % and 0.1 % probability levels, respectively.

B: Relationship between B concentration and K⁺ leakage from the YML.
Table 5.5 Effect of pre-treatment root zone temperature (Pre-RZT) and boron (B) and root zone temperature (RZT) on plant growth, B uptake and B distribution in sunflower. Values are means of three replications with standard errors in parentheses (Experiment 2)\textsuperscript{a}.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Growth\textsuperscript{b}</th>
<th>B content</th>
<th>B distribution\textsuperscript{c}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Growth</td>
<td>B content</td>
<td>B distribution</td>
</tr>
<tr>
<td></td>
<td>B content</td>
<td>S/R ratio</td>
<td>S/R</td>
</tr>
<tr>
<td></td>
<td>B distribution</td>
<td>YS/S (%)</td>
<td></td>
</tr>
<tr>
<td>Pre-RZT (°C)</td>
<td>B (µM)</td>
<td>RZT (°C)</td>
<td>DW (g plant\textsuperscript{-1})</td>
</tr>
<tr>
<td>10</td>
<td>0.2</td>
<td>10</td>
<td>0.54(0.02)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>0.75(0.04)</td>
</tr>
<tr>
<td>12</td>
<td>10</td>
<td>0.56(0.04)</td>
<td>0.26(0.02)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>0.76(0.18)</td>
</tr>
<tr>
<td>20</td>
<td>0.2</td>
<td>10</td>
<td>1.21(0.07)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>1.37(0.08)</td>
</tr>
<tr>
<td>12</td>
<td>10</td>
<td>1.32(0.09)</td>
<td>0.37(0.02)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>1.75(0.11)</td>
</tr>
</tbody>
</table>

F test\textsuperscript{d} | Pre-RZT | *** | *** | ns | *** | ns | *** |
| B | ns | ns | ns | *** | ** | *** |
| RZT | ** | ** | ns | *** | ns | *** |
| Pre-RZT x B | ns | * | ** | ** | ns | ** |
| Pre-RZT x RZT | ns | ns | ns | ns | ns | * |
| B x RZT | ns | ns | ns | ns | ns | ns |
| Pre-RZT x B x RZT | ns | ns | ns | ns | ns | ns |

\textsuperscript{a} Pre-treatment conditions: Sunflower plants were treated with different root zone temperature (RZT) for 11 days (Pre- RZT) before exposure to a combination of B and RZT treatment for a further 4 days.

\textsuperscript{b} Plant growth in dry weight (DW), relative growth rate (RGR) and shoot to root (S/R) ratio.

\textsuperscript{c} B distribution between shoot and root: Boron (B) content in shoot to root (S/R); Percent of B content in the youngest shoot part relative to the whole shoot (YS/S, %), the youngest shoot part was defined as the shoot part above the youngest mature leaf at the end of B and RZT treatment (d4), i.e. L2 and younger, and L3 and younger for pre- RZT 10 and pre- RZT 20 plants, respectively.

\textsuperscript{d} ns = non-significant; *, **, *** significant at 5 %, 1 % and 0.1 % probability levels, respectively.
Plant growth responses to B and RZT treatment were greatly influenced by pre-RZT growth conditions that showed warm pre-RZT plants had faster growth rate (Table 5.5). This indicated that it took time for plant recovery from chilling RZT after transfer to 20 °C RZT. Regardless of pre-RZT conditions, plant DW increase was determined by RZT during the short period (4 d) of growth in treatments, and B had little effect (Table 5.5, Plate 5.2). Boron deficiency symptoms appeared in the young leaves in low B supplied plants at 10 °C RZT by d4 (Plate 5.3). In contrast, plants supplied with sufficient B were free of B deficiency.

Plate 5.2 Effect of root zone temperature (RZT) on plant growth by day 4 (Experiment 2). Sunflower plants were first grown at RZT of either 20 or 10 °C for 11 days, then they were transferred to RZT treatment at either 10 or 20 °C (20/10, 10/20), or maintained at 20 or 10 °C (20/20, 10/10) for 4 days.
Boron uptake and B concentrations in the shoots and roots were affected by pre-RZT, B and RZT, with least B uptake and B concentrations in pre-RZT grown plants supplied with low B at 10 °C RZT (Tables 5.5 and 5.6). The symptoms of B deficiency in the young leaves were consistent with their B concentrations.

5.5 Discussion

5.5.1 Plant growth response to RZT

The research on plant response to cold air temperatures has been extensive (e.g. Graham and Patterson, 1982; Murata and Los, 1997; and the references therein), however, the mechanism of plant response is still not yet fully understood. There is much less work reported on plant response to low RZT (Bowen, 1991).
Low temperature reduces plant growth. However, non-chilling temperatures only delay plant growth without injury to the plant tissues, and eventually, plants are able to reach their potential yield after exposure to the required amount of thermal time. In fact, at low, non-chilling RZT, it has been reported that the same amount of nutrient uptake by plants produced higher dry matter than at higher RZT (Cumbus and Nye, 1985).

The direct injury of plant roots by low RZT is considered to be located, primarily, in the plasma membranes (Lyons, 1973). At the cellular level, meristematic cells are more susceptible to chilling than old tissues, and the rapid inhibition of root elongation is observed due probably to the injury or death of these cells at chilling temperatures (Rab and Saltveit, 1996). Consequently, low RZT influences root morphology and root growth. In sunflower, low RZT decreased root length, but increased root diameter as expressed by the ratio of root length per unit of root fresh weight (m g$^{-1}$) (Table 5.1). These responses are consistent with previous authors who reported that roots respond to RZT through anatomical (Kiel and Stamp, 1992) and morphological changes (Seiler, 1998).

Plant tissue leakage of K$^+$ is widely used to measure the membrane injury by stresses (Whitlow et al., 1992), such as low temperature (Reyes and Jennings, 1994). Low RZT increases membrane permeability and this is an indication of membrane injury (Reyes and Jennings, 1994). At 12 °C RZT, K$^+$ leakage from sunflower roots substantially increased indicating that membrane integrity was impaired (Figure 5.2). And 17 °C RZT caused only slight membrane injury to the roots. Thus, according to the definition suggested by Raison and Lyons (1986), in sunflower, 12 °C RZT was in the range of chilling temperatures, 17 °C RZT was near to the critical or threshold temperature, and
22 and 27 °C RZT were optimal temperatures for B-adequate plants. In the present experiments, sunflower root length and root relative growth rate were also strongly depressed at 12 °C RZT or lower, and close to optimum at 17 °C RZT (Tables 5.1 and 5.3, Figure 5.1). Root temperature was reported previously to be optimum for root growth of sunflower at 20 °C (Cooper, 1973). Similarly, Aguirrezdal and Tardieu (1996) reported that the critical temperature for sunflower root growth from germination to the 2-leaf stage was 18 - 20 °C.

Seiler (1998) investigated the effect of temperature on primary and lateral roots of young sunflower seedlings. In that experiment, by 10 days after planting, root growth was strongly inhibited at 10 and 15 °C, compared to growth at 25 - 30 °C. It was suggested that low temperature directly inhibits root growth and therefore indirectly influences water and nutrient uptake.

Sunflower shoot dry matter of B-adequate plants increased with higher RZT, and reached its maximum value at 22 °C RZT (Table 5.1, Figure 5.1). That is, the most marked response of shoot growth to RZT was between 12 and 17 °C RZT, and there was little response at RZT of 17 - 27 °C. This was consistent with the results by Szaniawski (1983), who showed that growth rates of leaves and the whole shoot of sunflower strongly increased with RZT from 10 to 20 °C, and plateaued from 20 to 30 °C RZT. The small dry matter gain in plants at 12 °C RZT in the present study could be ascribed to depressed metabolic activities both for maintenance and growth respiration (Criddle, 1997) and possibly to impaired leaf photosynthesis (Paul et al., 1991; Szaniawski, 1983) since leaves in plants at 12 °C RZT were extremely pale.
There are suggestions that RZT can directly affect the shoot temperature (Tew et al., 1963). However, at 10 °C soil temperature, Tew et al. (1963) found that temperatures of sunflower leaves and ambient air temperatures were identical, and leaf temperature was always closer to air temperature than root temperature. Thus, low RZT generally does not injure the leaves directly except at extremely low temperatures and perhaps when the meristem is close to the soil surface. Also, leaf K$^+$ leakage was not affect by RZT in B- adequate plants (Figure 5.3), which suggests that the effect of RZT on leaf growth was mainly indirect rather than direct. Finally, the fact that the ratio of shoot dry matter to root length (S/R ratio) was higher at 12 °C than RZT of 17 - 27 °C also suggests that inhibition of shoot growth at 12 °C RZT was indirect rather than direct.

The above discussion strongly suggests there is a threshold RZT of about 17 °C for sunflower response. Root zone temperature below the threshold causes chilling injury to the roots, and significantly alters root functions, such as membrane integrity: indirectly it also impairs shoot growth.

5.5.2 Effect of RZT on plant response to B

Besides the inhibition of plant growth by low RZT, low RZT also depresses B uptake by plants (Forno et al., 1979). However, the effect of low RZT from chilling to optimal on B response has not been previously investigated in detail.

5.5.2.1 Effect of RZT on plant dry matter

Chilling RZT reduced sunflower shoot B concentrations and induced B deficiency (Tables 5.2 and 5.6). The response of shoot dry matter and root length to B supply was inhibited by decreasing RZT. To achieve maximal shoot dry matter at a given RZT,
higher external B concentration was required at 12 °C RZT than at 22 - 27 °C RZT (Figure 5.1; Table 5.3). Besides lower B uptake rates, the response of shoot dry matter to low RZT can be attributed to depressed rates of both B accumulation in the shoots (RAR) and B translocation from roots to shoots (BTR) (Tables 5.4 and 5.6).

As discussed earlier, chilling RZT impaired root growth and caused direct injury to the roots. These direct effects of chilling RZT on roots could directly impair B uptake by roots if B uptake is an active process (Dannel et al., 1997; Pfeffer et al., 1999). However, the direct effect of chilling RZT on roots could also impair water uptake by roots and its transport to shoots (Szaniawski, 1983). Thus, B uptake would still be depressed even if B uptake is a passive process (Hu and Brown, 1997a).

By contrast with its effects on roots, chilling RZT did not directly injure the shoots, resulting in a higher S/R ratio at chilling RZT. The small size of roots relative to the shoots and depressed B uptake rate thus combine to induce B deficiency in the shoots. This supports the conclusion of Forno et al. (1979) that suboptimal RZT induced B deficiency in cassava due to reduced BUR and high S/R ratio.

Boron concentrations in sunflower shoots did not always increase with increasing RZT. It has been shown previously that B concentrations in plant shoots could have little response to RZT, or even decrease with higher RZT in a number of plant species, such as maize (Walker, 1969), barley (Mahalakshmi et al., 1995), cassava (Forno et al., 1979), snapdragon (Hood and Mills, 1994), tomato (Tindall et al., 1990) and oilseed rape (Chapters 3, 4). As B concentrations in plant shoots integrate responses in shoot
Table 5.6 Effect of pre-treatment root zone temperature (Pre-RZT) and boron (B) and RZT on B concentrations in plant parts, rates of B uptake (BUR µmol B g⁻¹ root DW d⁻¹) and translocation from root to shoot (BTR µmol B g⁻¹ root DW d⁻¹) and relative B accumulation in the shoot (RAR, d⁻¹). Values are means of three replications with standard errors in parentheses (Experiment 2) a.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pre-RZT (°C)</th>
<th>B (µM)</th>
<th>RZT (°C)</th>
<th>Shoot B mg kg⁻¹</th>
<th>Root YS</th>
<th>BUR µmol B g⁻¹ root DW d⁻¹</th>
<th>BTR µmol B g⁻¹ root DW d⁻¹</th>
<th>RAR d⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>4.7(0.2)</td>
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F test c

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<th>RZT</th>
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<th>Pre-RZT x RZT</th>
<th>B x RZT</th>
<th>Pre-RZT x B x RZT</th>
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</thead>
<tbody>
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<td>***</td>
<td>ns</td>
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</tr>
</tbody>
</table>

a Pre-growth conditions: Sunflower plants were treated with different root zone temperature (RZT) for 11 days (Pre- RZT) before exposure to a combination of B and RZT treatment for a further 4 days.

b YS: the youngest shoot was defined as the shoot part above the youngest mature leaf at the end of B and RZT treatment (d4), i.e., L2 and above, and L3 and above for pre-RZT 10 and pre- RZT 20 plants, respectively.

c ns = non-significant; *, **, *** significant at 5 %, 1 % and 0.1 % probability levels, respectively.
dry matter gain and B uptake, the variations of B concentrations in the shoots in response to B supply and RZT suggest that the relative sensitivity of B uptake and plant growth responses to RZT were different. If the response of RAR in shoots to RZT is smaller than RGR in shoots, B concentrations in the shoots will decrease, and the risk of B deficiency will be enhanced if external B supply is below adequacy. However, B status in the shoots is not merely determined by shoot activity. Boron acquired in the shoots is translocated from roots, thus, it also depends on the ability of roots to absorb B and transport it to shoots.

5.5.2.2 Boron partitioning within shoots

Low RZT resulted in decreased B partitioning into young growing tissues (YML, YS) and especially at low external B was associated with an apparent B deficiency in YML (Figure 5.4, Table 5.5). As there is continuous demand for B in meristematic tissues (Lovatt, 1985), continuous B supply is required to meet B requirements for the development of the young plant tissues. Higher RZT promotes growth rate and potentially results in higher rates of transpiration and respiration (Szaniawski and Kielkiewicz, 1982). Consequently, more B would be transported into new leaves with increasing RZT due to the increased transpiration (Raven, 1980). Moreover, higher growth rate creates a stronger sink for B: thus, more B would also be translocated into new tissues. Therefore, the present results add to those of Forno et al (1979) by showing that besides the direct inhibition of root activities (BUR and S/R ratio) by chilling RZT, the depressed B partitioning into young plant parts may also contribute to the impaired B nutrition in plants at chilling RZT.
Figure 5.4 Effect of solution boron (B) concentration and root zone temperature (RZT) on B partitioning to youngest mature leaf blades (YML) (vertical bars represent the LSD_{0.01} for the B x RZT interaction) (Experiment 1). F test for the treatment significance: ns = non-significant; *, **, *** significant at 5 %, 1 % and 0.1 % probability levels, respectively.

A: Boron content in YML, the first pair of true leaf blades (µg B per plant).

B: Boron content in YML as a percentage of that in the whole shoot.

5.5.2.3 Boron function in plants
Boron is closely involved in the maintenance of membrane integrity (Cakmak and Römheld, 1997), and leaf $K^+$ leakage is used to indicate membrane injury by low B (Cakmak et al., 1995; Brown and Hu, 1997). In agreement with Cakmak et al. (1995) and Brown and Hu (1997), in the present experiment, regardless of RZT, low external B concentration dramatically increased leaf cell membrane permeability (Figure 5.3A). However the effect of RZT on leaf $K^+$ leakage was complex. Root zone temperature did not affect leaf $K^+$ leakage from the YML of B adequate plants. By plotting YML B concentrations against $K^+$ leakage (Figure 5.3B), it was revealed that the effect of RZT on leaf membrane permeability was closely related to its influence on leaf B concentration, which was the integration of leaf growth and B partitioning.

However, by increasing RZT from 17 to 27 °C, YML B deficiency symptoms in plants supplied with 0.16 µM B diminished in severity while $K^+$ leakage increased (17 to 22 °C) and then decreased (22 - 27 °C): by contrast neither S/R ratios nor YML B concentrations were changed (Tables 5.1 and 5.2). Similarly, with no added B, Forno et al. (1979) observed that symptoms of B deficiency in cassava tops appeared 10 d earlier at 19 °C than at higher RZT (23 - 40 °C). These results suggest that low RZT increased the functional B requirements in some way which was not explained by YML B concentrations or $K^+$ leakage. Therefore, the evidence that chilling RZT induced plant B deficiency at low B could not be solely attributed to the direct injury of roots by chilling RZT or to the increased S/R ratios.

In conclusion, plant pre-treatment at 10 or 20 °C RZT did not alter the nature of low root temperature effect on plant B deficiency during the main treatment period. In
sunflower, increasing RZT from 12 to 27 °C increases root : shoot ratio, and B uptake and transport into shoots, and also increased B partitioning into young plant parts in the shoots. Clearly several different mechanisms can account for the B x RZT interaction. A threshold RZT for sunflower response to B was approximately 17 °C.
Chapter 6

Chilling root zone temperature impairs boron nutrition of oilseed rape (*Brassica napus* L. cv. Hyola 42)

6.1 Abstract

Low root zone temperature (RZT) has varied effects on plant boron (B) nutrition, depending on whether the RZT is above or below a threshold temperature for that species. In previous experiments, 10 °C RZT, which inhibited B uptake and partitioning into growing points of sunflower, had no detrimental effects on oilseed rape (Chapters 3 - 5). In this study, it is postulated that the critical RZT for oilseed rape is between 5 and 10 °C. Oilseed rape plants were grown in solution culture at 10 °C RZT with adequate B supply till they had seven true leaves. Then they were exposed to RZT (5 – 15 °C) and B treatments and harvested by day 5 when the plant responses to chilling RZT became apparent in plant size and leaf symptoms. Chilling RZT (5 °C) slowed plant growth rate remarkably. The young leaves of the low B (0.2 µM) plants became dark green with symptoms like B deficiency when grown at RZT of 5 °C whilst not at RZT of 10 °C. Leaf B concentrations were reduced by 5 °C RZT. Supplied with the same low B level (0.2 µM), plants at warm RZT (15 °C) also showed a significant decrease of B concentration in the youngest shoot parts in comparison with the 10 °C RZT. Rates of B uptake by plants (BUR) and translocation from roots to shoots (BTR) were greatly reduced by 5 °C RZT, and B partitioning into growing plant tissues within shoot was also inhibited strongly. However, there was little difference between RZTs of 10 and 15 °C in B partitioning. Consequently, the RZT threshold for oilseed rape is between 5 and
Moreover, chilling RZT (< threshold RZT) induced B deficiency in oilseed rape due to the impaired B translocation into growing shoot parts besides the decrease of B uptake rate and B transport rate and greater shoot to root (S/R) ratio. When RZT was above the threshold, increased RZT also accentuated B deficiency in low B plants which was attributed to a higher demand for B to meet growth requirements.

6.2 Introduction

In the previous experiments, sunflower and oilseed rape exhibited contrasting low root zone temperature (RZT) responses to boron (B) nutrition. Boron deficiency symptoms were induced by low RZT (12 vs 17 °C) in sunflower (Chapter 5). By contrast, B deficiency in oilseed rape plants was accentuated by warm RZT (20 vs 10 °C) (Chapters 3 and 4). Sunflower and oilseed rape are both sensitive to B deficiency, but they differ in tolerance of low temperature. Sunflower is a chilling-sensitive crop which exhibits plant growth injury at RZT of 12 °C (Chapter 5) and visual symptoms of low temperature injury are observed at 10 °C RZT (Chapter 5) such as less root branches and root hairs and the browning root tips compared to those at 20 °C RZT. In contrast, oilseed rape plants are more tolerant to cold temperature and RZT of 10 °C is still optimal for growth (Macduff et al. 1987a and d; Chapter 3). However, the results from a field experiment with oilseed rape exposed to low air and soil temperature clearly demonstrated that shoot growth of low B plants were increased by warmer night temperatures through covering plants during night in the cold winter (Chapter 2).

In winter and early spring, which is the growth season of winter oilseed rape in the
field, daily minimum air temperature is well below 10 °C and temperature below 0 °C at night is common: even the daily maximum air temperature is, mostly, well below 15 °C in winter (Chapter 2). It has been suggested that there is a RZT threshold to impair plant B nutrition (Chapters 3 and 5). Chilling RZT (≤ threshold RZT) reduced B concentration in young growing plant parts in sunflower (12 vs 17 °C) (Chapter 5). By contrast, when RZT was above the threshold temperature, decreasing it from 20 to 10 °C resulted in higher B concentration in the youngest open leaves (YOL) in low B plants of oilseed rape (Chapter 3). Some experimental results (Moorby and Nye, 1984; Cumbus and Nye, 1985; Macduff et al., 1987c and d) imply that RZT of about 3 ~ 7 °C is injurious to oilseed rape as expressed by decreased nutrient uptake. Therefore, based on previous results from field and glasshouse experiments (Chapters 2 - 5), to test whether chilling RZT impairs plant B nutrition, oilseed rape plants were treated with RZT in the range of 2 - 17 °C in the present experiment. Effects of constant low RZT versus lower night temperature on plant growth and B nutrition were also examined. It is postulated that there exists a RZT threshold below which low RZT impairs plant B nutrition in oilseed rape, and that chilling RZT induces B deficiency by inhibiting B partitioning into growing plant parts in addition to a slow down of B uptake and translocation to shoot and to a greater shoot to root (S/R) ratio (Forno, et al., 1979; Chapter 5).

6.3 Materials and Methods

6.3.1 Plant culture

The general procedures and nutrient composition were the same as the previous
experiments (Huang et al., 1996b; Chapters 3 - 5). Oilseed rape (cv. Hyola 42) seeds were germinated on paper towels soaked with 1.0 mM Ca(NO$_3$)$_2$ and 0.01 mM H$_3$BO$_3$ solution at 20 °C for 5 days in the dark. The seedlings were then transferred to full strength nutrient solution in 15-l trays with RZT controlled at 10 - 12 °C by a temperature-controlled water bath. When the sixth true leaf (L6) of the plant became the YOL, two uniform plants were transferred to each 5-l pot lined with a plastic bag and treated with low (0.2 µM) or adequate (9.1 µM) B and exposed to RZT treatments. Each treatment was replicated three times (d0). Root zone temperatures were maintained at 2 - 5 (cold), 10 - 12 (control) and 15 - 17 °C (warm) (referred to 5, 10 and 15 °C, respectively, hereafter). In addition, night RZT were imposed by transferring roots to a lower RZT than during the day. A total of 6 day/night RZT treatments were included: 5/5, 10/10, 15/15 and 15/10, 15/5,10/5. Boron concentrations in the culture solutions were maintained at 0.2 ± 0.0 (low B) or 9.1 ± 0.2 µM (adequate B) (means with standard errors) controlled by B specific resin (Huang et al., 1999). On d5, when the effect of RZT treatment on plant growth was obvious at 5/5 RZT, all plants were harvested. By d5, L8 became YOL in most plants or slightly older than YOL but L9 was much younger than YOL in all plants.

6.3.2 Data collection and analysis

Fresh weights (FW) of roots and shoots were determined at harvest. Plant shoots were separated into leaf blade six (L6, numbered from the base), L7, L8 (L8 and young shoot above L8), and the remainder of the shoots (leaves 1 - 5 plus stem and petioles). The root and shoot samples were oven dried at 70 °C and their dry weights (DW) were recorded. Boron in the samples was analyzed by ICP-AES after digestion with concentrated nitric acid (Zarcinas et al., 1987).
Plant water use was estimated by daily weighing and calculating the water loss of each pot.

Relative growth rate of plants (RGR), and rates of B uptake (BUR) and translocation from root to shoot (BTR) were calculated (Forno et al. 1979; Chapter 5).

Since the effects of night RZT on most of the parameters observed were either not significant or compensated by warmer day RZT, the day and night RZT treatments were then combined and averaged as five RZT (°C) treatments of 5 (5/5), 7.5 (10/5), 10 (10/10 and 15/5), 12.5 (15/10) and 15 (15/15), respectively. The data were analyzed for the significance of treatment effects and the possible interactions between B x RZT (Super Anova, USA). Further analysis of RZT effects as a one factor experiment was carried out when B had no significant effect.

6.4 Results

6.4.1 Plant growth response and B uptake

Although only treated for a short period of 5 days, growth of plants (DW and RGR) was markedly slowed down by low RZT especially at 5 °C RZT but not by low B (Table 6.1). Root growth was more severely depressed by low RZT than shoot growth and this
Table 6.1: Effect of root zone temperature (RZT) and boron (B) on plant growth (dry weight, DW g plant\(^{-1}\); relative growth rate, RGR g g\(^{-1}\) d\(^{-1}\)) and shoot to root (S/R) ratio and proportion of DW in leaf 6 and younger shoot parts (≥ L6) (as a % of shoot DW). Data are pooled across B treatments as the B effects were not significant. Means followed by dissimilar letters were significantly different at 5 % probability level.

<table>
<thead>
<tr>
<th>RZT (°C)(^{a})</th>
<th>Shoot DW g plant(^{-1})</th>
<th>Root DW g plant(^{-1})</th>
<th>RGRs</th>
<th>RGRr</th>
<th>S/R ratio</th>
<th>% DW in young shoot</th>
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</thead>
<tbody>
<tr>
<td>5</td>
<td>2.36(0.08)a</td>
<td>0.23(0.01)a</td>
<td>0.15(0.01)a</td>
<td>0.04(0.01)a</td>
<td>10.40(0.42)a</td>
<td>36.5(1.5)a</td>
</tr>
<tr>
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<td>2.78(0.12)b</td>
<td>0.27(0.01)ab</td>
<td>0.18(0.01)b</td>
<td>0.08(0.01)a</td>
<td>10.24(0.26)a</td>
<td>37.2(1.5)a</td>
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<td>41.7(0.6)b</td>
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<td>0.44(0.02)c</td>
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<td>7.60(0.25)c</td>
<td>42.2(1.0)bc</td>
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<td>0.52(0.03)d</td>
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<td>7.12(0.13)c</td>
<td>43.4(1.0)c</td>
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</table>

\(^{a}\) Mean of day and night temperatures.
Table 6.2 Boron (B) uptake (content, µg B plant⁻¹; and rates of B uptake and translocation from roots to shoots, BUR, BTR, µmol B g⁻¹ root DW d⁻¹) and B partitioning in shoot relative to whole plant B content (%) or that in the young shoots (leaf 6 and younger, ≥ L6) relative to whole shoots (%) affected by root zone temperature (RZT) and boron (B). Means in a column followed by dissimilar letters were significantly different at 5 % probability level.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Shoot B (µM)</th>
<th>Root B (µg plant⁻¹)</th>
<th>BUR (µmol B g⁻¹ root DW d⁻¹)</th>
<th>BTR (µmol B g⁻¹ root DW d⁻¹)</th>
<th>Shoot B (µg B plant⁻¹)</th>
<th>B in young shoot (µg B plant⁻¹)</th>
<th>B (µM)</th>
<th>RZT (°C) ( ^a )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>31.9(2.8)a</td>
<td>3.1(0.3)a</td>
<td>0.22(0.03)a</td>
<td>0.17(0.02)a</td>
<td>91.0(0.2)bc</td>
<td>27.7(3.7)a</td>
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</tr>
<tr>
<td>0.2</td>
<td>35.7(3.2)a</td>
<td>3.3(0.2)a</td>
<td>0.56(0.14)ab</td>
<td>0.51(0.13)ab</td>
<td>91.6(0.2)cd</td>
<td>32.3(0.3)ab</td>
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</tr>
<tr>
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<td>36.3(1.4)a</td>
<td>3.9(0.3)ab</td>
<td>0.66(0.22)ab</td>
<td>0.56(0.22)ab</td>
<td>90.2(0.7)bc</td>
<td>35.6(1.9)b</td>
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<tr>
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<td>43.9(1.8)a</td>
<td>5.0(0.3)cd</td>
<td>1.13(0.16)ab</td>
<td>0.96(0.14)ab</td>
<td>89.7(0.4)b</td>
<td>38.4(3.5)bc</td>
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<td>1.53(0.37)b</td>
<td>1.47(0.35)b</td>
<td>93.2(0.1)d</td>
<td>36.8(1.3)b</td>
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<tr>
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<td>60.7(3.3)b</td>
<td>4.1(0.2)abc</td>
<td>2.87(0.33)c</td>
<td>2.74(0.30)c</td>
<td>93.7(0.1)d</td>
<td>41.5(2.5)c</td>
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<td>3.27(0.40)c</td>
<td>3.12(0.39)c</td>
<td>93.7(0.4)d</td>
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<tr>
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<td>6.1(0.4)d</td>
<td>3.78(0.17)cd</td>
<td>3.55(0.19)cd</td>
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<tr>
<td>9.1</td>
<td>99.3(6.2)d</td>
<td>7.1(0.7)e</td>
<td>4.50(0.39)cd</td>
<td>4.23(0.36)d</td>
<td>93.3(0.3)d</td>
<td>45.1(0.4)c</td>
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F test \( ^b \)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Shoot B (µM)</th>
<th>Root B (µg plant⁻¹)</th>
<th>BUR (µmol B g⁻¹ root DW d⁻¹)</th>
<th>BTR (µmol B g⁻¹ root DW d⁻¹)</th>
<th>Shoot B (µg B plant⁻¹)</th>
<th>B in young shoot (µg B plant⁻¹)</th>
<th>B (µM)</th>
<th>RZT (°C) ( ^a )</th>
</tr>
</thead>
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<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
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<td>*</td>
<td>*</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
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</tbody>
</table>

\( ^a \) Mean of day and night temperatures.

\( ^b \) ns = non-significant; *, **, *** significant at 5 %, 1 % and 0.1 % probability levels, respectively.
Table 6.3 Effect of root zone temperature (RZT) and boron (B) supply on B concentration (mg kg\(^{-1}\)) in plant parts. Means in a column followed by dissimilar letters were significantly different at 5 % probability level.

<table>
<thead>
<tr>
<th>B (µM)</th>
<th>RZT (°C)</th>
<th>Shoot</th>
<th>Root</th>
<th>L8 and younger shoots</th>
<th>L7</th>
<th>L6</th>
<th>Remainder</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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<td>Root</td>
<td>Shoot</td>
<td>Root</td>
<td>Root</td>
<td>Shoot</td>
</tr>
<tr>
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<td>5</td>
<td>13.2(0.5)a</td>
<td>13.6(0.8)abcd</td>
<td>11.6(0.4)a</td>
<td>9.7(0.2)ab</td>
<td>8.8(1.2)a</td>
<td>15.0(0.8)a</td>
</tr>
<tr>
<td>0.2</td>
<td>7.5</td>
<td>12.6(0.3)a</td>
<td>12.2(0.3)ab</td>
<td>15.3(0.2)b</td>
<td>11.0(0.6)bc</td>
<td>7.7(0.8)a</td>
<td>13.4(0.5)a</td>
</tr>
<tr>
<td>0.2</td>
<td>10</td>
<td>12.9(0.7)a</td>
<td>12.2(0.2)ab</td>
<td>15.0(0.6)b</td>
<td>9.2(0.4)ab</td>
<td>8.2(0.5)a</td>
<td>14.3(0.9)a</td>
</tr>
<tr>
<td>0.2</td>
<td>12.5</td>
<td>13.1(0.3)a</td>
<td>11.6(0.0)</td>
<td>16.2(0.9)b</td>
<td>10.2(0.7)b</td>
<td>8.3(0.1)a</td>
<td>13.9(0.7)a</td>
</tr>
<tr>
<td>15</td>
<td>0.2</td>
<td>11.7(0.6)a</td>
<td>11.6(0.6)a</td>
<td>12.7(0.4)a</td>
<td>8.1(0.6)a</td>
<td>7.6(0.3)a</td>
<td>13.1(1.2)a</td>
</tr>
<tr>
<td>5</td>
<td>9.1</td>
<td>19.2(0.7)b</td>
<td>14.6(0.6)cd</td>
<td>24.5(0.2)c</td>
<td>18.1(0.4)d</td>
<td>15.7(0.9)b</td>
<td>19.2(0.8)b</td>
</tr>
<tr>
<td>7.5</td>
<td>9.1</td>
<td>22.3(0.9)c</td>
<td>15.0(0.3)d</td>
<td>27.0(0.3)de</td>
<td>24.8(1.3)ef</td>
<td>20.5(0.8)c</td>
<td>21.2(1.5)bc</td>
</tr>
<tr>
<td>10</td>
<td>9.1</td>
<td>23.7(0.5)cd</td>
<td>14.3(0.6)dc</td>
<td>26.5(0.5)de</td>
<td>23.9(0.5)e</td>
<td>21.5(0.4)c</td>
<td>23.3(0.7)cd</td>
</tr>
<tr>
<td>12.5</td>
<td>9.1</td>
<td>24.6(1.2)de</td>
<td>13.5(0.5)bcd</td>
<td>25.4(1.1)cd</td>
<td>23.0(0.8)e</td>
<td>20.5(0.3)c</td>
<td>25.5(1.7)def</td>
</tr>
<tr>
<td>15</td>
<td>9.1</td>
<td>26.4(0.5)de</td>
<td>13.2(0.4)abc</td>
<td>28.1(1.1)e</td>
<td>25.7(0.3)f</td>
<td>24.8(1.1)d</td>
<td>26.4(0.8)f</td>
</tr>
</tbody>
</table>

F test  \(^b\)

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>RZT</th>
<th>B x RZT</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>RZT</td>
<td>??</td>
<td>*</td>
<td>***</td>
</tr>
<tr>
<td>B x RZT</td>
<td>***</td>
<td>ns</td>
<td>***</td>
</tr>
</tbody>
</table>

\(^a\) Mean of day and night temperatures.

\(^b\) ns = non-significant; *, **, *** significant at 5 %, 1 % and 0.1 % probability levels, respectively.
led to an increase of S/R ratio at low RZT. A reduction of DW partitioning into young plant parts (≥ L6) within shoots by cold RZT was also significant, but not between the warmer RZTs (10 - 15 °C).

Water use was only greatly reduced by cold RZT (5 °C) but not B treatment. During the 5 d treatment, plant water use (kg plant⁻¹) was 0.30, 0.53, 0.54, 0.64 and 0.64 at RZT (°C) of 5, 7.5, 10, 12.5 and 15, respectively.

Boron uptake and transport were slowed down by low RZT and became more evident at adequate B supply (Table 6.2). Cold RZT and low B supply resulted in most severe effect on B uptake and transport. At low B supply, both BUR and BTR at RZT of 5 °C were only one third of that at 10 °C. Boron partitioning from root to shoot slightly decreased with decreasing RZT in low B supply plants, but was not affected by RZT in B adequate plants. Shoot B contained 88 - 93 % of total plant B regardless of RZT and B treatment. Boron partitioning into young growing parts (≥ L6) within shoot was only reduced at the lowest RZT regardless of B supply (Table 6.2).

6.4.2 Plant B concentration and B deficiency symptom

In general, B concentrations in the shoots were more greatly affected by RZT than those in the roots. Effect of RZT on shoot B concentrations varied with external B level and plant parts (Table 6.3), but particularly low B concentrations in the youngest shoot parts (≥ L8) were induced by cold RZT regardless of B supply. In adequate B plants, shoot B concentrations decreased with lowering RZT in all shoot parts. In low B plants, B concentrations in the youngest shoot parts (≥ L8) were decreased either by cold or warm RZT, being 11.6, 15.0 and 12.7 mg B kg⁻¹ at RZT of 5, 10 and 15 °C,
respectively. The concentrations of B at either 5 or 15 °C RZT were close to the critical value (Huang et al., 1996b), and these were in accordance with the plant symptoms observed. There were no symptoms of B deficiency in L6 and L7 although their B concentrations were low (< 10 mg kg$^{-1}$). The B concentrations and corresponding leaf symptoms were in agreement with predicted critical B concentrations which were 11, 9 and 7 mg kg$^{-1}$ in YOL, YOL+1 and YOL+2, respectively (Huang et al., 1996b).

### 6.5 Discussion

#### 6.5.1 Threshold root zone temperature

Temperature of 10 - 15 °C causes chilling injury to chilling-sensitive plants (Lyons, 1973; Levitt, 1980; Pollock and Eagles, 1988). However, in oilseed rape, a chilling-resistant plant, RZT of 10 - 15 °C slowed growth but was above the chilling temperature threshold for this species (Moorby and Nye, 1984; Cumbus and Nye, 1985; Macduff et al., 1987a and d; Chapter 3).

Results from the present and the previous experiments (Chapter 3), as indicated by plant water uptake, RGRr, BUR and BTR, for oilseed rape cultivar cv. Hyola 42, suggested the threshold RZT is between 5 and 10 °C. The values of these parameters at 5 °C RZT were dramatically lower than those at 10 - 15 °C. Moreover, plant responses to RZT as reflected by these parameters vanished or became much weaker as RZT increased from 10 to 15 °C. Similarly, it was shown that phosphate uptake by oilseed rape was not affected by RZT at 10 and 23 °C, but was halved by 5 °C (Moorby and Nye, 1984).
Thus for sunflower (Chapter 5), and oilseed rape, there was a remarkable coincidence in plant B nutrition response to RZT below the threshold. At a given level of external B, the impaired plant growth associated with B deficiency was observed at below threshold RZT, between 5 to 10 °C and about 17 °C for oilseed rape and sunflower, respectively. Boron uptake and transport from root to shoot as well as B transport into the young growing shoot parts are slowed at below threshold RZT (Table 6.2; Chapter 5). These seem to coincide with chilling injury to the root as indicated by root K⁺ leakage (Chapter 5).

6.5.2 Mechanism of chilling RZT inducing impairment of B nutrition

Low RZT decreases relative root growth and increases shoot/root ratio (McMichael and Burke, 1996; Ali et al., 1998; Domisch et al. 2002). Increase of S/R ratio caused by cold RZT as shown in present experiment and by Forno et al. (1979) for cassava and for sunflower (Chapter 5) is unfavourable for meeting B demand in the shoot. However, large S/R ratio is not the sole cause of plant B deficiency because B deficiency symptoms in cassava cv. Seda were evident at low RZT (19 °C) when S/R ratio was little affected (19 vs 26 °C) in plants at a B level that was sufficient at warmer RZT (26 °C) (Forno et al., 1979). Therefore B uptake and translocation may be more important processes by which low RZT induces shoot B impairment.

Boron uptake especially with adequate B supply is related to aquaporins in the plasma membrane (Dordas et al., 2000; Dordas and Brown, 2001). Consequently, effects of low RZT on membrane structure and function may be more important than large S/R ratio (Bowen, 1991; Equiza et al., 2001). Changes to membrane properties under low RZT
may affect activity of aquaporins and root hydraulic conductance (Nishida and Murata, 1996; Aroca et al., 2001; Baiges et al., 2002; Tyerman et al., 2002). These effects would directly alter the capacity of roots to absorb B. Recent studies suggest that it is possible to increase B uptake by *Xenopus laevis* oocytes by increasing the expression of membrane intrinsic proteins that form aquaporins (Dordas et al. 2000). Hence specific effects of low RZT on aquaporin density in membranes or on B transport through aquaporins may account for the expression of B deficiency at low RZT.

Moreover, uptake of B is predominantly an active process when external B is low and transporters that facilitate B uptake are located in membranes (Pfeffer et al., 1999, 2001; Stangoulis et al., 2001; Takano et al., 2002). Presumably B transporters, being proteins have optimal temperature ranges for their activity, and their functions would be subject to be inhibition by low RZT below a critical temperature. The fact that chilling RZT had a more profoundly adverse effect on B uptake when external B is low suggests low RZT acts mostly on B uptake through active processes. In the present experiment, BUR at 5 °C RZT was less than half of that at 10 °C RZT, but differed less over the range 10 - 15 °C RZT (Table 6.2).

Furthermore, it was evident that B concentration in the youngest shoot parts (≥ L8) was most severely decreased by the chilling RZT (Table 6.3). This could be due to its high growth rate and decreased B supply as shown by the decreased BUR and BTR. Since the increase of DW and DW partitioning to the growing shoot parts were not affected by low B in the 5- day growth, decrease of DW partitioning at chilling RZT (5 °C) in comparison with higher RZT could be attributed to the effect of RZT. Partitioning of DW and B into the growing shoot parts was equal in B- adequate plants but decreased
at chilling RZT (Tables 6.1 and 6.2). However, percentages of B partitioned into ≥ L6 (28-38%) were much lower than DW (36-43%) in low B plants regardless of RZT with least B partitioning at chilling RZT. As discussed previously, proportional partitioning of DW and B in B- adequate plants could be attributed to coupling of B uptake and transport with water flow (Raven, 1980). In contrast, B uptake may be facilitated by B transporters in low B plants (Takano et al., 2002), so that the proportion of B partitioning to the growing shoot parts was less than that of DW, and this became more serious at chilling RZT suggesting that activity of the B transporters might have been dramatically inhibited.

In summary, the results from present and previous experiments (Chapters 2 - 5) have demonstrated that low RZT has varied effects on plant B nutrition, depending on whether the RZT is above or below a critical temperature for that species. The critical RZT for the test cultivar of oilseed rape is near 5 °C. Chilling RZT (5 °C) not only slowed plant growth rate remarkably but also accentuated plant B deficiency by impairing B translocation into growing shoot parts besides the decrease of B uptake rate and B transport rate and greater shoot to root ratio. When RZT was above the threshold, increased RZT also accentuated B deficiency in low B plants which was attributed to a higher demand for B to meet growth requirements.
Chapter 7

The role of boron in oilseed rape (Brassica napus L.) plant response to cold air

7.1 Abstract

Previous reports suggest that plants grown on low boron (B) soil are more severely injured by low temperature than those with adequate B supply. Chilling root temperature (< 10 °C) accentuates B deficiency in oilseed rape by increasing shoot : root ratio, as well as impairing B uptake and B partitioning to the young expanding leaves. Yet, little is known about causal mechanisms linking cold air temperature and B nutrition. Experiments were conducted to examine the role of cold air temperature in B responses of oilseed rape. In the first experiment, detached leaves of oilseed rape plants with different B concentrations (10 to 25 mg B kg\(^{-1}\) from the glasshouse and 9 to 32 mg kg\(^{-1}\) in the field) collected from field or glasshouse grown plants were treated with an overnight chilling to test the effect of leaf B status on solute leakage. Chilled (at 0 °C) youngest fully open leaves (YOL) with low B concentration had higher solute leakage measured as electrical-conductivity (EC) of extracts than control YOL (at 10 °C). Moreover, after cold treatment, EC in extracts from low B leaves was increased by exposure to light. In the second experiment, in the glasshouse, plants were grown by maintaining roots at either 10 °C (low) or 20 °C (warm) and shoots were exposed to cold air at night for 6 successive days. Night air temperatures in cooled and ambient air were 11.7 – 19.4 and 15.5 – 23.5 °C, respectively. With adequate B, cool air temperature for 6 consecutive nights reduced shoot growth. By contrast, the shoot
response to low air temperature diminished with lower B supply, and no response was shown when no B (the measured B in solution was 0.08 µM) was supplied. Boron transport to shoots was markedly reduced by cool air. Boron concentrations in YOL in low B supplied plants were not affected by air temperature treatment. In summary, growth of oilseed rape plants with adequate B nutrition was more tolerant of cold air temperature.

7.2 Introduction

It has been reported for several decades that B deficiency in the field is associated with low temperature injury to plant because plants grown on low boron (B) soils are more severely injured by low temperature than those with adequate B supply (Shorrocks, 1997). Low root zone temperature (RZT), so long as it is below the threshold for that species, increases plant response to B in several plant species with contrasting tolerance of low temperature (Forno et al., 1979; previous chapters). Yet, little is known about causal mechanisms linking cold air temperature and B nutrition. That covering oilseed rape plants at night improved growth of low B plants without affecting leaf B concentrations might be partly attributed to the warming of the air temperature (Chapter 2). Interactions between low temperature and B nutrition have also been studied under controlled conditions in growth chambers (Oertli, 1963; Subedi et al., 1998b, 2001). However, in the field conditions and in the controlled temperature growth chambers, the effects of low temperature on plant B nutrition may include both low air and low soil temperatures so effects of temperature on roots and shoots are confounded with each other. Therefore, to understand the effect of low air temperature, studies separating air
temperature from the combined effect of air and soil temperatures on plant response are necessary.

Uptake and transport of B may be more influenced by air temperature when B supply is adequate because B uptake would be mainly passive (Hu and Brown, 1997a; Dannel et al., 2002). In addition, distribution of B in the shoot of B-adequate plants may be passive with water flow (Raven, 1980), which is directly affected by air temperature through transpiration. However, at lower B supply, B uptake might be more affected by RZT when B uptake is largely an active process (Pfeffer et al., 1999, 2001).

Adequate B nutrition is reported to increase tolerance to cold air temperature. At low air temperature (7 - 8 °C day/5 °C night), low B leaves of cucumber, a tropical species, were more subject to cold injury than B-adequate leaves as expressed by leaf potassium (K) leakage (Wang et al., 1999). However, there is little available information on effects of low leaf B on low air temperature injury in temperate plant species which are more tolerant of low temperature. Nevertheless in low B plants, oilseed rape and sugar beets have shown leaf injury symptoms after frost while B-adequate plants in the same condition were free of freezing injury symptoms in leaves in the field (Stoker and Tolman, 1941; Chapter 2). Low B leaves of both species after overnight frost expressed loss of turgour and had a flaccid bleached appearance.

Air temperature decreases at night (e.g. see Chapter 2, Figure 7.1). And in the open field, low temperature injury (cold injury) to plants becomes more evident under sunlight following a cold night (Welander et al., 1994; Allen and Ort, 2001). In the present Chapter, two experiments were conducted to examine the role of cold air
temperature in B responses by oilseed rape. In the first experiment, detached leaves of either field or glasshouse grown oilseed rape plants with different B status were treated with 0 °C at night to test the effect on leaf cell membrane integrity as determined by solute leakage. In the second experiment, plants were grown in the glasshouse by maintaining roots at either 10 °C (low) or 20 °C (warm) and shoots either experienced ambient temperature or were exposed to low temperature at night. Shoot growth and B uptake responses to cold air were examined.

*Figure 7.1* Examples of changes of air temperature at night in glasshouse and in open field (from field experiment, Chapter 2).

- - - - - - Air temperature in the glasshouse at ambient conditions and under ice covering during the same time.

- - - - - - Coldest night and early spring frost in the field experiment.
7.3 Materials and Methods

7.3.1 Experiment 1 Effect of night chilling on solute leakage from detached leaves with varying B concentration

*Leaves from glasshouse grown plants* Oilseed rape plants (cv. Hyola 42) were grown at RZT of 10 °C and treated with 0.08 ± 0.00, 0.16 ± 0.01, and 6.7 ± 0.1 µM B (means with standard errors) controlled by B specific resin (Huang et al., 1999) in the glasshouse by solution culture. Detailed procedures were the same as described for Chapter 4. Youngest open leaves from oilseed rape plants were collected. They were treated at 0 °C (chilling) or 10 °C (CK) overnight. Leaf discs were punched in YOL (10 mm diameter) and rinsed with de-ionized water (DI water), then 9 discs were extracted in 30 ml DI water for 2 hours in the dark room at 20 °C or under sunlight inside the glasshouse at 20 °C (by a temperature-controlled water bath), and the leaf leakage was measured by an EC meter.

*Leaves from field grown plants* Youngest open leaves from oilseed rape plants grown in the field (Chapter 2) were collected. The oilseed rape plants received 0, 1.5 or 15 kg borax ha⁻¹ as a basal B application. In late March, the YOL were collected and stored in plastic bags (note: air temperature at the time of collection was 10 - 12 °C) and carried into the laboratory (about 2 h in delivery) and treated at 0 °C (chilling) or 10 °C (CK) overnight. Then leaf leakage (EC) was measured by shaking 10 leaf discs (6 mm diameter) in 5 ml DI water for 4 hours.

Method of EC measurement was based on Lieberman et al. (1958), Patterson et al. (1976), Whitlow et al. (1992) and Prášil and Zámečník (1998).
7.3.2 Experiment 2 Effect of shoot cooling at night on plant response to B

The general procedures and nutrient composition were the same as previous experiments (Chapter 6). Oilseed rape (cv. Hyola 42) seeds were germinated on paper towels soaked with 1.0 mM Ca(NO$_3$)$_2$ and 0.01 mM H$_3$BO$_3$ solution at 20 °C for 5 days in the dark. The seedlings were then transferred to full strength nutrient solution in 15-l trays with RZT controlled at 10 - 12 °C by a temperature-controlled water bath. When the 3rd true leaf (L3) of the plant became the YOL, three uniform plants were transferred to each 5-l pot lined with a plastic bag and treated with B and air temperature treatments. Plants were grown at RZT of either 10 °C (low) or 20 °C (warm). Each treatment comprised three replicates (d0). Boron concentrations in the culture solutions (means with standard errors) were maintained at 0.08 ± 0.00, 0.16 ± 0.01, 0.26 ± 0.02, 0.66 ± 0.02, 6.7 ± 0.1 µM B, respectively, controlled by B specific resin (Huang et al., 1999). Plant shoots were exposed to cool (shoot cooling) or ambient (control) air temperature at night for 6 successive days. The cool air was achieved by surrounding shoots with trays filled with ice throughout the night time. The ice trays extended to 17 cm above the tops of pots. Microclimate at canopy level was recorded with datalogged temperature and humidity either in the ambient or in the cool air treatment taken between the ice tray and the tops of pots. Variation of night temperature and corresponding air humidity under cool or under ambient air conditions are shown in Figures 7.1 and 7.2. Night air temperatures recorded in cooled and untreated air were 11.7 – 19.4 and 15.5 – 23.5 °C, respectively, during the treatment for 6 nights.
Plants were harvested on d6. Blades of the YOLs and the remainder of the shoots were sampled and oven dried at 70 °C. Boron in the samples was analyzed by ICP-AES after digestion with concentrated nitric acid (Zarcinas et al., 1987).

The data were analyzed for the significance of treatment effects and their possible interactions (Super Anova, USA). Split plot design for analysis of variance was performed on the data when two factors (air chilling x B) were adopted in the experiment. Otherwise three-way analysis of variance was performed when three factors (B x air temperature x light in Experiment 1 and B x air temperature x RZT in Experiment 2) were designed in the experiments.

7.4 Results

7.4.1 Experiment 1
Low B YOL had higher EC in extracts than control leaves (Tables 7.1 and 7.2). Cold and light also accentuated leaf leakage by low B leaves. After cold treatment, EC in low B leaves was enhanced by light. The increased solute leakage from low B leaves with exposure to light was consistent with the field symptoms observed in B deficient leaves that appeared pale or bleached of colour under sunlight after a freezing night.
Figure 7.2 Effect of shoot cooling at night on relative humidity (R. H. %) and air temperature (T °C). Symbols: open- Control; filled- shoot cooling.

Table 7.1 One night chilling effect on solute leakage after 2 hr (expressed by electrical-conductivity, EC µS cm⁻¹) of oilseed rape leaves differing in boron (B) status (mg B kg⁻¹) under light or dark. Values are means of four replicates in each treatment, with a standard error in the parentheses (Experiment I).

<table>
<thead>
<tr>
<th>Boron (B, µM)</th>
<th>Air temperature (T, °C)</th>
<th>Leaf leakage (EC, µS cm⁻¹)</th>
<th>Light ¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dark</td>
</tr>
<tr>
<td>0.08</td>
<td>0</td>
<td>1.30(0.04)</td>
<td>1.46(0.08)</td>
</tr>
<tr>
<td>0.08</td>
<td>10</td>
<td>1.10(0.04)</td>
<td>1.32(0.05)</td>
</tr>
<tr>
<td>0.16</td>
<td>0</td>
<td>1.18(0.05)</td>
<td>1.25(0.03)</td>
</tr>
<tr>
<td>0.16</td>
<td>10</td>
<td>1.05(0.03)</td>
<td>1.22(0.05)</td>
</tr>
<tr>
<td>6.7</td>
<td>0</td>
<td>1.15(0.06)</td>
<td>1.18(0.02)</td>
</tr>
<tr>
<td>6.7</td>
<td>10</td>
<td>1.18(0.06)</td>
<td>1.08(0.02)</td>
</tr>
</tbody>
</table>

F test ²: L**, B***, T**, L x B**, L x T ns, B x T ns, L x B x T ns

² ns = non-significant; *, **, *** significant at 5 %, 1 % and 0.1% probability levels,
respectively. Corresponding leaf B concentrations in plants supplied with 0.08, 0.16 and 6.7 µM B were 6.4, 8.2 and 26.6 mg B kg⁻¹, respectively. L, B and T: light, boron and air temperature treatment, respectively.

b The recorded weather data showed that the solar radiation during leakage extraction was 200 - 265 (mean of 231) W m⁻² and 513 - 684 (mean of 589) µmol m⁻² s⁻¹.

7.4.2 Experiment 2

The effects of cooling on air temperature and humidity in the canopy of oilseed rape are shown in Figures 7.1 and 7.2. The changes of air temperature and humidity influenced by ice enclosure varied with the ambient air conditions. During the 6 nights of treatment, the maximum decrease of night temperature by ice enclosure of the canopy at night was 6.9, 4.1, 5.5, 4.9, 5.8 and 6.3 °C, from 1st to 6th night, respectively, when the corresponding ambient air temperature was 21, 16-19, 20, 19, 21 and 24 °C, respectively; while the relative humidity (R. H. %) was > 90 % (Figure 7.2; Table 7.3). The mean night temperature (from 8:00 pm – 7:00 am) was 14.4 and 18.9 °C during the 6 nights in shoot cooling treatment and ambient, respectively.

Table 7.2 One night chilling effect on solute leakage after 4 hr (EC µS cm⁻¹) of leaves of field grown oilseed rape plants. Values are means of four replicates in each treatment, with a standard error in the parentheses (Experiment 1).

<table>
<thead>
<tr>
<th>Borax (kg ha⁻¹)</th>
<th>Leaf leakage (EC, µS cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 °C</td>
</tr>
<tr>
<td>0</td>
<td>84.0 (7.4)</td>
</tr>
<tr>
<td>1.5</td>
<td>44.5 (2.7)</td>
</tr>
<tr>
<td>15</td>
<td>52.0 (3.7)</td>
</tr>
</tbody>
</table>

F test a B***, T ns, B x T***

a ns = non-significant; *, **, *** significant at 5 %, 1 % and 0.1% probability levels, respectively. Corresponding leaf boron (B) concentration in plants supplied with 0, 1.5
and 15 kg Borax ha\(^{-1}\) were 4.0, 7.2 and 18.9 mg B kg\(^{-1}\), respectively. B and T: B and temperature treatments.

**Table 7.3** Effect of cooling at night on air temperature (\(^{\circ}\)C).

<table>
<thead>
<tr>
<th>Number of the night</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambient*</td>
<td>18.8</td>
<td>17.1</td>
<td>17.6</td>
<td>18.7</td>
<td>19.9</td>
<td>21.5</td>
<td>18.9</td>
</tr>
<tr>
<td>Shoot cooling*</td>
<td>13.2</td>
<td>13.3</td>
<td>12.9</td>
<td>14.8</td>
<td>15.1</td>
<td>17.3</td>
<td>14.4</td>
</tr>
<tr>
<td>Difference*</td>
<td>5.6</td>
<td>3.8</td>
<td>4.7</td>
<td>3.9</td>
<td>4.8</td>
<td>4.2</td>
<td>4.5</td>
</tr>
<tr>
<td>Maximum decrease**</td>
<td>6.9</td>
<td>4.1</td>
<td>5.5</td>
<td>4.9</td>
<td>5.8</td>
<td>6.3</td>
<td></td>
</tr>
</tbody>
</table>

* The mean night temperature (from 8:00 pm – 7:00 am) by ice enclosure of the canopy at night (shoot cooling) and the corresponding ambient temperature (Ambient), and their difference (Difference);

**Summary decrease: the maximum decrease of night temperature by ice enclosure of the canopy at night vs ambient temperature.

In plants supplied with adequate B, shoot cooling for 6 consecutive nights reduced shoot growth (Table 7.4). The relative decline in shoot dry matter diminished with lowering B supply, and no response was shown when least B (0.08 µM) was supplied. In addition, when grown at RZT of 20 \(^{\circ}\)C leaves wilted during day time when directly exposed to the sunlight if B supply was low (\(\leq\) 0.26 µM), and DW of these plants was similar or even less than those grown at RZT of 10 \(^{\circ}\)C. By contrast, at higher B supply (\(\geq\) 0.66 µM), plant grew much faster at RZT of 20 \(^{\circ}\)C than 10 \(^{\circ}\)C. The maximum plant DW was obtained at 6.7 µM B and 20 \(^{\circ}\)C RZT without night cooling treatment.

Plant B uptake in the shoots was markedly reduced by shoot cooling but the effect of shoot cooling diminished with lowering B supply (Table 7.4). Boron concentrations in plant shoots were not affected by shoot cooling in low B supplied plants but were
Table 7.4 Effect of shoot cooling at night on plant growth and boron (B) uptake at root zone temperature (RZT) of either 10 °C or 20 °C (Experiment 2) a.

<table>
<thead>
<tr>
<th>Boron B µM</th>
<th>Shoot air T b</th>
<th>Shoot dw (g plant⁻¹)</th>
<th>Shoot B content (µg plant⁻¹)</th>
<th>YOL B (mg kg⁻¹)</th>
<th>(Shoot - YOL) B (mg kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 °C</td>
<td>20 °C</td>
<td>10 °C</td>
<td>20 °C</td>
<td>10 °C</td>
</tr>
<tr>
<td>0.08</td>
<td>0</td>
<td>0.32(0.03)</td>
<td>0.26(0.02)</td>
<td>3.7(0.4)</td>
<td>4.4(0.5)</td>
</tr>
<tr>
<td>0.08</td>
<td>1</td>
<td>0.28(0.01)</td>
<td>0.28(0.02)</td>
<td>3.9(0.0)</td>
<td>4.3(0.3)</td>
</tr>
<tr>
<td>0.16</td>
<td>0</td>
<td>0.29(0.03)</td>
<td>0.25(0.02)</td>
<td>4.0(0.2)</td>
<td>4.2(0.2)</td>
</tr>
<tr>
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<td>1</td>
<td>0.39(0.03)</td>
<td>0.27(0.03)</td>
<td>5.1(0.4)</td>
<td>4.1(0.3)</td>
</tr>
<tr>
<td>0.26</td>
<td>0</td>
<td>0.47(0.02)</td>
<td>0.44(0.09)</td>
<td>5.1(0.3)</td>
<td>4.7(0.8)</td>
</tr>
<tr>
<td>0.26</td>
<td>1</td>
<td>0.53(0.03)</td>
<td>0.41(0.13)</td>
<td>5.8(0.4)</td>
<td>5.2(1.2)</td>
</tr>
<tr>
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<td>0.58(0.02)</td>
<td>6.6(0.9)</td>
<td>8.8(0.5)</td>
</tr>
<tr>
<td>0.66</td>
<td>1</td>
<td>0.53(0.06)</td>
<td>0.74(0.10)</td>
<td>7.8(1.6)</td>
<td>11.1(1.4)</td>
</tr>
<tr>
<td>6.7</td>
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<td>0.66(0.04)</td>
<td>9.1(0.5)</td>
<td>14.6(1.0)</td>
</tr>
<tr>
<td>6.7</td>
<td>1</td>
<td>0.63(0.03)</td>
<td>0.79(0.06)</td>
<td>14.3(0.9)</td>
<td>19.6(1.9)</td>
</tr>
</tbody>
</table>

| F test c | RZT | ns | *** | *** | *** | *** |*** | *** | *** |
|          | B   | *** | *** | *** | *** | *** | ns | ns | ns |
|          | Air T | ** | *** | ** | ns | ns | ns | ns | ns |
|          | B x RZT | *** | *** | ns | ** | ns | ns | ns | ns |
|          | RZT x Air T | ns | ns | ns | ns | ns | ns | ns | ns |
|          | B x Air T | ns | ns | ns | ns | ns | ns | ns | ns |
|          | B x RZT x Air T | ns | ns | ns | ns | ns | ns | ns | ns |

a Shaded data are from which plants wilted under sunlight; 
b Air T = Shoot cooling treatment: 0- cooling, 1- control (ambient); 
c ns = non-significant; *, **, *** significant at 5 %, 1 % and 0.1 % probability levels, respectively.
reduced in adequate B (6.7 µM) plants. Partitioning of DW and B content between YOL and the remainder of the shoot was not affected by shoot cooling (data not shown).

7.5 Discussion

Both low B and low temperature affect the properties of plant cell membranes and cell walls (for B, Cakmak and Römheld, 1997; Matoh, 1997; and for cold, Lyons, 1973; Pollock and Eagles, 1988). The mechanisms of B and temperature effects on plant growth, however, are obviously different. Boron has an important role in maintaining plant cell membrane integrity (Cakmak and Römheld, 1997; Cara et al., 2002). Low temperature may exacerbate membrane injury to low B plants because membranes are also the primary site of low temperature stress (Lyons, 1973; Levitt, 1980).

In the present experiments, both field and glasshouse grown oilseed rape plants exhibited increased solute leakage following chilling temperature overnight in low B compared to adequate B plants, suggesting that low B leaves were more susceptible to injury from low air temperature. Moreover, the cold injury was exacerbated by exposure of the leaf to sunlight after chilling. By contrast, B- adequate plants are more tolerant of cold air temperature. There are previous reports that low B reduced tolerance of leaf to injury from freezing (Stoker and Tolman, 1941; Braekke, 1983) and high light (Cakmak et al., 1995). A role of B in enhancing tolerance of plants to cold weather has been suggested for a long time, however, the mechanism is not yet clear (Shorrocks, 1997).
Besides the direct evidence that low air temperature exacerbates injury to low B plants, low air temperature may also accentuate injury to low B plants through its effect on B uptake and plant growth rate over the long term especially at low RZT (Table 7.5; Figure 7.3). However, in the present experiment, night shoot cooling had little effect on YOL B concentrations of low B supplied plants (Table 7.4). Yet, in the field, both soil and air temperatures are low in winter, and the temperature is much lower (Figure 7.1) than that in present glasshouse experiment. The much colder soil and air temperatures in the open field might have involved more severe stress on oilseed rape plants especially in low B plants as symptoms were observed in the field (Chapter 2). When plants de-acclimatized they become less tolerant to frost (Rapacz, 2002), and this was observed in the field experiment in which young leaves of low B plants become bleached after a spring frost (see photo, Plate 7.1). Similarly, in Experiment 1 the leaf solute leakage by chilling at 0 °C had highest EC in low B leaves (Tables 7.1 and 7.2). Low B leaves of cucumber, a species of tropical origin, also exhibited an increase of leaf leakage at cold temperature (Wang et al., 1999) although the temperature which resulted in greater leakage in B- deficient plants of cucumber (7 - 8 °C /5 °C day/night) was much warmer than in oilseed rape. Table 7.2 shows that in B- adequate oilseed rape leaf leakage showed little response to temperature treatments.
Figure 7.3 Relationship between external boron (B) concentration and shoot dry weight (g plant\(^{-1}\)) at different root zone temperatures (RZT) under shoot cooling (T0) or ambient (T1) condition. RZT10 and RZT20: RZT at 10 and 20 °C, respectively. The parameters of the fitted Mitscherlich relationships are shown in Table 7.5.

Table 7.5 Parameters for Mitscherlich models \(^a\) fitted to the relationship between solution boron (B) concentration and shoot dry weight (g plant\(^{-1}\)). The fitted relationships are shown in Figure 7.3.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>RZT</th>
<th>air T</th>
<th>(a)</th>
<th>(b)</th>
<th>(c)</th>
<th>(R^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>⬜️</td>
<td>10</td>
<td>1</td>
<td>0.585</td>
<td>0.539</td>
<td>7.096</td>
<td>0.78</td>
</tr>
<tr>
<td>⬜️</td>
<td>10</td>
<td>0</td>
<td>0.474</td>
<td>0.283</td>
<td>5.496</td>
<td>0.54</td>
</tr>
<tr>
<td>⬜️</td>
<td>20</td>
<td>1</td>
<td>0.813</td>
<td>0.736</td>
<td>2.637</td>
<td>0.76</td>
</tr>
<tr>
<td>⬜️</td>
<td>20</td>
<td>0</td>
<td>0.658</td>
<td>0.537</td>
<td>2.813</td>
<td>0.81</td>
</tr>
</tbody>
</table>

\(^a\) Mitscherlich model: \(f(x) = a - b \cdot \exp(-c \cdot x)\), where: \(x\), solution B concentration (µM); \(f(x)\), shoot dry weight (g per plant).
Plate 7.1 Spring frost injury to the low boron (B) leaves of oilseed rape plants. Left two leaves, middle and the right leaves of plants received 0, 1.5 and 15 kg borax ha$^{-1}$ respectively (Field experiment, Chapter 2).

As postulated above, uptake and transport of B, as well as plant growth (DW), were more influenced by air temperature when B supply was adequate (Table 7.4), even though the plant shoots were treated only at night and the decrease of air temperature was $< 7 \, ^\circ\text{C}$. This might have been due to the direct effect of air temperature on leaf transpiration, because at B adequacy plant B uptake and transport is a passive process, accompanying transpiration driven water flow (Raven, 1980; Hu and Brown, 1997a; Dannel et al, 2002). The effect of either air temperature or RZT on B uptake in plant shoots diminished with lower B supply. The effects of RZT (10 vs 20 $^\circ\text{C}$) on B uptake were consistent with a previous experiment (Chapter 3). It should be noted that both air temperature and RZT (above 10 $^\circ\text{C}$) were above the threshold temperature for RZT in oilseed rape in the present shoot cooling experiment. However, low B ($\leq 0.26 \, \mu\text{M}$) plants grown at 20 $^\circ\text{C}$ RZT became wilted under exposure to daylight which implies
that high light increases plant B requirement especially when plant growth rate is high at warm conditions. This is worthwhile for further research.

The previous experiment (Chapter 6) has shown that temperature below the threshold RZT (between 5 and 10 °C for tested oilseed rape) impairs plant B nutrition and induces B deficiency in oilseed rape. The effect of lower air temperature (< 10 °C) on B uptake and distribution in oilseed rape plant warrants further study because temperatures at night well below 10 °C and even below 0 °C are common in the growth season of winter oilseed rape in the field (see Chapter 2).

From the results of YOL chilling (Experiment 1), leaves of oilseed rape were very tolerant to low temperature: B adequate leaves were not injured by chilling at 0 °C as indicated by EC (Tables 7.1 and 7.2). In combination with previous results from Chapter 6, B uptake and transport systems may be more affected by chilling temperature (< 10 °C) in the root zone than in the shoot especially when B transporters are involved in B uptake at low B supply. However, the contribution of chilling air temperature to regulation of B uptake and distribution to the young growing shoot parts, specifically at low B supply, remains unknown so far.
Chapter 8

General discussion: Plant boron nutrition and its response to low temperature

8.1 Abstract

Despite a series of reports linking plant boron (B) deficiency with low temperature stress, the mechanisms underlying a connection remain unclear. The involvement of low temperature with B status in the plant can be divided into indirect and direct effects: indirect effects relate to change(s) of root and/or shoot activities by low temperature that alter requirements for assimilation of B in cell walls and other cell components, while direct effects of low temperature act on B uptake and transport as well as internal B requirements. The impairment of these processes by low temperature whether direct or indirect leads to plant B deficiency when temperature drops below a minimum threshold value. Increasing temperature when below the critical threshold might decrease B requirements for plant growth whereas warmer temperatures above the threshold temperature can have the opposite effect. This chapter, integrates the results from previous chapters (Chapters 2-7) with current understanding of functions of B in higher plants and mechanisms of plant response to low temperature stress. Finally, it summarizes progress in understanding how plant B nutrition is regulated at the physiological level by low temperature, and a conceptual model is proposed for describing the observed interactions between B and low temperature.
8.2 Introduction

Plant response to temperature change is very rapid. However, short-term (a few days or less) effects are usually of much less ecological and agronomic significance than the long-term effects of temperature in crop production. Pre-treatment at low temperature may affect plant responses (Macduff et al., 1994) in the short-term and it takes several days for plant acclimation to occur, eg. it requires about 4 - 5 d for nitrogen (N) uptake to adjust to the temperature-regulated growth (Macduff et al., 1994). In the long-term, plants acclimate to the temperature by adjusting nutrient uptake to satisfy the demand for plant growth if conditions are not too severe (Clarkson et al., 1988; Engels et al., 1992; Marschner, 1995). For instance, low root zone temperature (RZT) (10 °C) for oilseed rape plants increased their shoot B demand (d11 - 15) for growth after the plants were treated with warm RZT (20 °C) for 11 days (Chapter 4). Accordingly, B-adequate plants adjusted B uptake rate to maintain plant growth through enhancing rates of B uptake (BUR) and transport (BTR). By contrast, low B plants failed to do so, i.e., BUR and BTR did not change with RZT, and B deficiency was accentuated by warm RZT (20 °C) compared to the control (10 °C).

Low temperature reduces nutrient uptake by plants and can result in either decrease or increase of nutrient concentration in plants (Cooper, 1973; Clarkson et al., 1988). The
decrease of plant nutrient concentration may increase risk of deficiency. The nutrient requirement by plants for growth can be altered by low temperature through its influence on many aspects of plant growth, such as growth rate, shoot to root ratio (S/R ratio) or other mechanisms. Plant nutrient deficiency may be induced by low temperature due to perturbation of the balance between plant growth and nutrient acquisition (Clarkson et al., 1988). Recent evidence of this comes from reports of deficiency of N, phosphorus (P), or potassium (K) in rice (Setter and Greenway, 1988) and B in cassava (Forno et al., 1979). Indirect effects relate to change(s) of root and/or shoot activities by low temperature that change requirements for assimilation of B in cell division and growth, while direct effects of low temperature act on B uptake and transport as well as internal B requirements.

So, on one hand, low temperature may strongly influence plant B nutrition. Some of the processes affected by temperature directly result in change of plant B status: they may be involved in requirements and assimilation of B such as in cell division, or they may mediate B uptake (especially at low B when active process becomes dominant and consumes ATP) and transport (such as water property and water flow within the plant). The impairment of these processes by low temperature might be the direct cause for depression of B uptake, transport and incorporation into plant tissues at a molecular level, and lead to plant B deficiency. On the other hand, plant tolerance to low temperature can vary with nutritional status of plant, eg., regardless of mechanisms
involved, increased supply of K (Marschner, 1995) and B (Shorrocks, 1997) both can increase plant resistance to frost damage.

The possible interactions between B and low temperature in relation to plant reactions at the physiological level are outlined in Figure 8.1.

8.3 Influences of B nutritional status on low temperature tolerance

Field reports of B involvement in frost resistance have been in the literature for nearly half a century, and the damage of growth of plants at cold (including above and below 0 °C) temperature and low B has been reported in several countries in forest trees, fruit trees, as well as field crops (Shorrocks, 1997). Field experiments have shown that incidence of B deficiency symptoms in plants is more prevalent in cold than in warm weather and B has a beneficial influence on plants which suffer from low temperature stress (Stoker and Tolman, 1941; Braekke, 1983; Rerkasem et al., 1990; Chapter 2). Possible mechanisms underlying the susceptibility of low B tissues to low temperature damage are explored below based on the schema presented in Fig. 8.1a,b.
Low B supply

Inhibition of root functions:
B and water uptake and transport; B assimilation

Decrease of sensitivity to low B supply and B deficiency

Decrease of B partitioning into new growing shoot;
Increase of internal B requirement;
Decrease of B use efficiency (e.g., assimilation);

Increase of sensitivity to low B supply and B deficiency

Exacerbation of membrane impairment

Figure 8.1a Mechanisms of low temperature effects on plant boron (B) nutrition: The general.
Below threshold low root zone temperature

Effect on roots: B acquisition

Increase of root hydraulic conductance;
Decrease of water channel abundance and activity;
Impact on B assimilation;
Denaturization, injury to root cell plasma membrane;

Effect on shoots: B transport and translocation

Decrease of leaf stomatal conductance, transpiration rate;
Decrease of transpiration intensity in new growing vs mature tissues.

Effect on relationships between shoot vs root, and new growing vs mature tissues:
Shoot B demand and B availability to the new growing shoot

Inhibition of root B uptake, assimilation, and transport

Decrease of B partitioning into new growing shoot;
Increase of internal B requirement;
Decrease of B use efficiency;
Increase of shoot B demand per unit root for growth.

Impairment of B nutrition to the new growing shoot

*Figure 8.1b* Mechanisms of low temperature effects on plant boron (B) nutrition: Impairment of plant B nutrition by chilling root zone temperature (below threshold low temperature).
8.3.1 Direct injury to low B plant by low temperature

8.3.1.1 Root zone temperature

In previous chapters, a critical temperature has been proposed below which B deficiency is likely to be induced when B supply is marginal. At a given level of external B, the impaired growth of plant that is apparently due to B deficiency was observed only at certain low RZT, between 5 to 10 °C and below 17 °C for tested oilseed rape and sunflower, respectively (Chapters 5 and 6).

The critical RZT for induction of B deficiency seems to coincide with root response as indicated by root leakage (Chapter 5). Below this threshold RZT, B uptake and transport are strongly depressed relative to those at optimal RZT (Chapters 5 and 6). These results suggest that B uptake and transport systems are impaired below threshold RZT, consequently, efficiency of B distribution into plant shoots is reduced and shoot B deficiency is induced or exacerbated if external B supply is marginal or low (Fig. 8.1).

8.3.1.2 Air temperature

In the field, the appearance of leaf injury immediately after a chilling or freezing night should be a direct effect of low temperature damage in low B plants rather than an indirect one. The occurrence of injury in the young leaves is more serious for plant growth than in the old leaves. On one hand, growth of shoot apices and growing leaves requires more B than the older shoot parts and continuous supply of B is necessary for
mericistems of species with little phloem mobility of B, such as oilseed rape. Low B causes disorganization of cell walls and tissue structure (Matoh, 1997; Dell and Huang, 1997). On the other hand, low temperature changes plant cell physical and chemical properties such as tissue plasticity, and wall extensibility (Pollock and Eagles, 1988; Pritchard et al., 1990; Kubacka-Zębalska and Kacperska, 1999). Moreover, meristematic cells are more subject to damage by chilling temperature than the more developed cells. That is, apical tissues are rich in meristematic cells which are the most sensitive to chilling injury (Rab and Saltveit 1996). Furthermore, the primary injury to the plants by low temperature is located in the membrane (Lyons, 1973; Levitt, 1980). However, both plasma membrane and the cell wall contribute plant cell tolerance of low temperature (Yamada et al., 2002). Besides the impairment of cell membrane functioning by low B (Cakmak and Römheld, 1997), the disorganization of cell wall induced by B deficiency might predispose these low B cells to damage at chilling temperature.

Low B plants are less tolerant of low air temperature, such as in oilseed rape where leaf bleaching occurred during a sunny day after a freezing night and this was more evident in low B- than adequate B- plants (Chapter 2). The evidence of short-term (overnight) cold (chilling and freezing) injury was observed via leaf solute leakage which was much greater in low B- than in adequate B- leaves (Chapter 7). However, anatomical and histochemical observation of leaf cells through light- and electron-microscopy would more directly reveal evidence of low temperature injury in the low B leaves. To
avoid weaknesses in previous studies of this sort, the plants should be grown at defined external B concentrations so that the plant B status is controlled during the imposition of low temperature treatments. The B- buffered solution culture system enables this level of control of solution and plant B status (Huang et al., 1999) and would be suitable for the purpose. Secondly, close attention should be paid to the very early effects of B and low temperature so that responses obtained represent primary effects and not secondary and tertiary effects of treatments (Goldbach et al., 2002b).

8.4 Direct effects of low temperature on B acquisition and utilization in crops

8.4.1 Root B acquisition

Temperature may affect B absorption and translocation through affecting transpiration, water viscosity and membrane permeability (direct effects) and root hormones production (indirect effects) (Cooper, 1973; Clarkson et al., 1988; Ali et al., 1998). To meet shoot growth demand, the root must first gain adequate B (except in the case of foliar B spray). Acquisition by the root of adequate B depends on activities of the shoots (e.g. transpiration) and roots (e.g. aquaporins).

Increase of plant root hydraulic resistance, and decreases of leaf stomatal conductance and transpiration rate induced by low RZT are more responsive in the chilling RZT range, while these effects are diminished or even vanish in the optimal RZT range.
(Tew et al., 1963; Cooper, 1973; Pavel and Fereres, 1998). Besides the effects of chilling RZT inhibition on plant transpiration and root water uptake which impair B movement, chilling RZT may damage membrane structure and permeability (Nishida and Murata, 1996), and B uptake and transport systems (aquaporins, B channels). Hence, root B absorption and upward transport may be impaired directly by dysfunction of the root B uptake and transport systems by chilling RZT. Hence at low RZT, plants require higher external B supply for their growth (Chapters 5 and 6). In sunflower, increasing solution B from 0.25 to 0.62 µM, increased B in the youngest mature leaves from 7.0 to 11.7 mg kg\(^{-1}\) when plants grew at 12 °C RZT, which implied that all of plants were B deficient (Asad et al., 2001, reported that the critical B for the same cultivar is 19.7 mg B kg\(^{-1}\)). By contrast, when grown at any RZT between 17 °C and 27 °C, increasing solution B from 0.25 to 0.62 µM successfully prevented B deficiency (Chapter 5).

It is now clear that aquaporins are central components in plant water relations and are enriched in tissues with rapid cell division and expansion (Tyerman et al., 2002), the same tissues that also require more B. Furthermore, B transport is mediated by B transporters belonging to the aquaporin group and located in membranes (Dordas et al., 2000; Dordas and Brown, 2001; Pfeffer et al., 2001; Stangoulis et al., 2001; Takano et al., 2002). Because aquaporins are proteins with an optimal temperature range for their activity, their functions are subject to damage at chilling temperature. However, further
research on properties of the B channels, and direct evidence of chilling RZT affecting B flux across root membranes and upward transport at molecular level is required.

8.4.2 Internal requirement

In oilseed rape, during and after low temperature acclimation, leaf cell wall content increases and the cell walls become much thicker (Kubacka-Zębalska and Kacperska, 1999). The effect of low temperature is attributed to the increase of cell pectins (Kubacka-Zębalska and Kacperska, 1999; Stefanowska et al. 1999). This may be specifically important for B requirement of shoot apices and growing leaves because B accumulates in the pectin layers of the cell wall (Matoh, 1997) and B is mostly incorporated into cell walls at low B supply (Pfeffer et al., 2001). Moreover, B needs to be continually supplied to maintain the growth of the meristems (Dell and Huang, 1997).

Shoot nutrient deficiency at low temperature can also be directly attributed to depressed nutrient assimilation. As in the case where P assimilation was impaired at low temperature this problem might be more difficult to solve by elevating external nutrient supply (Nye and Sofield, 1982; Cumbus and Nye, 1985).

When wheat is grown in growth chamber at low temperature of 8/2 °C (day/night) sterility did not respond to B treatment (Subedi et al., 1998b). Further research suggests that sterility of low B plants is because they are more sensitive to cold (10/2 °C,
day/night) or a failure of B utilisation (Subedi et al., 2001). Failure of B ‘assimilation’ into cell wall components might occur at chilling temperature. More recently, Iwai et al. (2003) has reported that borate cross-linked RG-II dimers, produced in roots, are transported to above-ground organs via xylem sap. This implies that chilling temperature may not only injure root B uptake and transport but also impair synthesis of B compound(s). Consequently, B use efficiency is decreased by chilling temperature.

Therefore, at cold temperature, requirements for B in these plant tissues may increase. The field experiment (Chapter 2) showed that covering plants at night in winter improved the growth of low B plants without affecting B concentrations in the youngest open leaves (YOL). This result implies that warmer night might have decreased internal B requirement. However, direct information on chilling effects on B assimilation needs further exploration.

8.5 Indirect effects of low temperature on B acquisition and utilization in crops

Besides direct effects, temperature may also indirectly affect B absorption and translocation. In the long term, both air and root zone temperatures affect growth of shoot and root via feedback mechanisms, involving metabolism of regulators, nutrient and water uptake and transport (Clarkson et al., 1988; Ali et al., 1998). After acclimation of plant growth to the temperature change, the relationship between plant
nutrient uptake and temperature is suggested to be determined by the effect of temperature on plant growth rate, which affects the plant's demand for nutrients, rather than the direct effect of temperature on the uptake mechanism (Clarkson et al., 1988). Hence, shoot nutrient status is a ‘consequence’ of activity of the shoot relative to the root, a balance between sinks (shoot, nutrient demand for growth) and sources (root, nutrient acquisition). Any change(s) of shoot and/or root activities by low temperature beyond a particular threshold could be the cause(s) of low temperature-induced shoot nutrient deficiency.

Although air temperature, in the long term, may affect plant root uptake of B via feedback mechanisms (Clarkson et al., 1988), B entry to the root at chilling RZT seems to be more directly controlled by the root rather than the shoot temperature according to the results of experiments conducted in different seasons (Chapters 3, 5 and 6).

Chilling RZT (5 °C for oilseed rape and 12 °C for sunflower for tested cultivars) did not result in an increase of critical B concentrations in the young growing shoots (Chapters 5 and 6). This is probably due to weak or little effect of RZT on shoot temperature in comparison with effect of the shoot ambient temperature. At 10 °C soil temperature, Tew et al. (1963) reported that temperatures of sunflower leaves and ambient air temperatures were identical, and while at warmer soil temperature (25 °C) leaf temperature was always closer to air temperature than root temperature. Therefore, in the chilling temperature range, a decrease of internal B requirement by warmer
temperature is possibly attributed to improvement of B assimilation and the warming
of the shoots so that B use efficiency is increased. However, more information is
needed to make this clearly understood.

8.5.1 Biomass partitioning

In the longer term, air and root zone temperatures both affect shoot and root growth
and hence alter growth of shoot relative to root (change of S/R ratio) (Clarkson et al.,
1988; Kacperska and Szaniawski, 1993). The demand of B for shoot growth in relation
to root B acquisition may be therefore changed (Forno et al., 1979). Depressed nutrient
absorption by the root at low temperature if it fails to meet the shoot growth demand
for mineral nutrients leads to shoot nutrient deficiency. The depression in absorption
can be due to soil processes such as a slow down of mineralization, or decreased solute
movement (Nye and Tinker, 1977; Moraghan and Mascagni, 1991). In these situations,
effects on shoot growth may be mediated by raising external nutrient supply or slowing
shoot growth rate. Indeed, if soil nutrient supply is limiting, lowering RZT within the
optimal temperature range for root function will to some extent alleviate or delay the
occurrence of plant nutrient deficiency because of a slow down of plant growth rate
(Cumbus and Nye, 1982 and 1985; see also Chapters 3 and 6).

Reduced growth of root relative to the shoot by low RZT could lead to increase of S/R
ratio and decrease the root absorption area for nutrient uptake which is unfavourable
for uptake of nutrients such as P and B (Forno et al.; 1979; Clarkson et al., 1988;
At chilling RZT, 5 °C and below 12 °C for tested oilseed rape and sunflower respectively, root growth is markedly constrained relative to shoot growth regardless of B supply; and plant B deficiency was accelerated or induced in low B supplied plants compared to those grown at optimal RZT (Chapters 5 and 6).

However, in the optimal RZT range shoot growth requires more B for its proper growth at higher RZT due to the enhanced potential of the shoot growth rate. In this case, because shoot B demand may not be satisfied by available B in low B supplied plants, leaf B deficiency symptoms appears earlier in warmer (10 vs 20 °C RZT) grown oilseed rape plants regardless of S/R ratio, and this response was more extensive in summer than in winter grown plants (Chapters 3 and 4).

8.5.2 Root-to-Shoot B transport and partitioning

Although decreases of both air temperature and RZT reduce B transport to the shoot (Chapter 7), the relative significance of each is not clear.

In the field, air temperature increases more rapidly with the warmer weather than soil temperature. Low soil temperature, therefore, might have more significant impact on plant B uptake and transport than the air temperature.

In the optimal RZT range, plant transpiration response to RZT is small compared with that in the chilling RZT range (Tew et al., 1963; Cooper, 1973). When RZT is in the
range below optimum for a particular species, transpiration dramatically increases with elevating RZT (Cooper, 1973). For example, in sunflower, at 10 °C RZT, leaf environmental conditions had little influence on transpiration (Tew et al., 1963). Accordingly, in B-adequate sunflower (about 0.4 µM B for plants grown at optimal RZT) the increases of shoot B concentration and shoot B accumulation responded greatly to RZTs between 12 °C and 17 °C, but the response diminished or even vanished at RZT range of 17 – 27 °C (Chapter 5).

Boron is primarily required by the growing plant parts and less so in the mature tissues, and B can be recycled from older leaves when it is phloem mobile (Brown and Hu, 1997). Hence, B utilization within shoot, i.e. B partitioning into the young growing shoot tissues and B metabolism within them are of key importance.

In previous experiments (Chapters 5 and 6) regardless of external B supply levels, B partitioning into the young growing plant parts is strongly depressed by chilling RZT. Conversely, more B is partitioned into the young growing plant parts at lower RZT when RZT is in the optimal temperature range (and hence increasing B use efficiency). It is not clear if this is a consequence of competition between the sinks (young vs old plant parts). The inhibition of B partitioning into the actively young growing tissues within the shoot and consequently increasing of shoot B demand at cold RZT may be due to decreased activity of B transport systems. Boron transport is facilitated via water transport systems when at adequate B supply and, at low B, is more dependent on
transport by B channels (aquaporins) (Dordas et al., 2000; Dordas and Brown, 2001; Takano et al., 2002). A closer investigation of carbon (C) metabolism (photosynthesis and respiration, and C allocation) in the young leaves and their relationship to B partitioning may help to clarify the mechanism underlying decreased partitioning of B into young leaves at chilling RZT.

In conclusion, at chilling temperature, B uptake, transport and partitioning into growing shoots are strongly impaired, and B use efficiency in the growing tissues might be reduced by chilling temperature. Low temperature also increases S/R ratio, so that shoot B demand is not satisfied when available B supply is marginal. Furthermore, low air temperature might increase internal B requirements for shoot growth.


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