

**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

2004

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. Zheyoyou 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 μ M 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.

ACKNOWLEDGMENTS

I would like to express my sincere thanks to my supervisors Dr. Richard W. Bell and Dr. Bernie Dell, and Prof. Yuai Yang and Dr. Longbin Huang for their persistent support and invaluable advice. I am grateful to the technical staff (Max Dawson, Karen Deane, Frank Farrell, Dan Hewitt, Frank Salleo, Luis Soto) for their support in the use of the glasshouse and laboratory analyses. Thanks should be also given to Dr. Caixian Tang and Dr. Qifu Ma for their care during my life in Australia.

I gratefully acknowledge AusAID for providing a postgraduate research scholarship under the recommendation of the Australian Centre for International Agricultural Research Project 9120 (ACIAR 9120).

I would particularly like to thank my wife, Liwen Zheng, for her sustainable support during the 'Long March' of my study.

PUBLICATIONS

The publications listed below originated from the research contained in this thesis:

Ye ZQ, Huang LB, Bell RW and Dell B 2003 Low root zone temperature favours shoot B partitioning into young leaves of oilseed rape (*Brassica napus*). *Physiol. Plant.* 118: 213-220.

Ye ZQ, Bell RW, Dell B and Huang LB 2000 Response of sunflower to boron supply at low root zone temperature. *Commun. Soil Sci. Plant Analy.* 31: 2379-2392.

Ye ZQ, Bell RW, Huang LB, Yang Y and Dell B 1997 Covering plants at night in the winter increased seed yield of transplanted oilseed rape (*Brassica napus* L. cv. Zheyoyou 2) on a low boron soil. *In* Boron in soils and plants. Eds. R W Bell and B Rerkasem, pp. 29-34. Kluwer Acad. Publishers, Dordrecht.

TABLE OF CONTENTS

	Page
Abstract	i
Acknowledgements	vi
Publications	vii
Table of Contents	viii
List of Tables	xv
List of Figures	xix
List of Plates	xx
List of Plant Species Names	xxi
Origin of the project	1
Chapter 1 Review of plant boron nutrition, low temperature physiology and their interaction	3
1.1 Plant boron nutrition.....	3
1.1.1 Forms and functions of B in higher plants.....	3
1.1.1.1 Forms and compartmentation of B in plant cells.....	4
1.1.1.2 Functions of B in higher plants.....	4
1.1.1.2.1 Boron functions: Cell wall.....	6
1.1.1.2.2 Boron functions: Cellular membrane.....	7
1.1.1.2.3 Boron functions: Metabolic roles.....	8
1.1.2 Mechanisms of B uptake and transport in plants.....	9

1.1.2.1 Boron uptake and transport from root to shoot.....	9
1.1.2.2 Boron mobility and redistribution.....	14
1.1.2.3 Requirements of B for plant growth.....	16
1.2 Temperature effects on boron nutrition in plants.....	19
1.2.1 Plant physiological responses to low temperature.....	20
1.2.1.1 Low temperature tolerance of different species and concept of ‘low temperature’.....	21
1.2.1.2 General: Regulation of growth and nutrient requirements by low temperature.....	22
1.2.2 Low temperature effects on plant response to B.....	24
1.2.2.1 Plant responses to low temperature in the field with emphasis on B deficiency.....	25
1.2.2.2 Plant responses under controlled conditions.....	27
1.2.2.2.1 Experiments at sufficient or excessive B supply.....	27
1.2.2.2.2 Experiments at low B supply	30
1.3 Mechanisms for a role of boron in plant injury by low temperature.....	31
1.3.1 Increased sensitivity.....	32
1.3.2 Poorer plant B status.....	33
1.4 Perspective and hypothesis.....	36

Chapter 2 Covering plants at night in the winter increased seed yield of transplanted oilseed rape (<i>Brassica napus L. cv. Zheyouyou 2</i>) on a low boron soil	38
2.1 Abstract.....	38
2.2 Introduction.....	39
2.3 Materials and methods.....	41
2.4 Results and discussion.....	43
2.4.1 Weather conditions in the field.....	43
2.4.2 Effect of cold weather on plant growth rate with different B levels.....	44
2.4.3 Effect of covering plants.....	46
2.4.3.1 Vegetative growth.....	46
2.4.3.2 Reproductive growth.....	46
2.5 Conclusions.....	48
2.6 Implications for further research in controlled environments.....	49
Chapter 3 Low root zone temperature favours shoot B partitioning into young leaves of oilseed rape (<i>Brassica napus L. cv Hyola 42</i>)	53
3.1 Abstract.....	53
3.2 Introduction.....	54
3.3 Materials and methods.....	55
3.3.1 Plant culture.....	55
3.3.2 Temperature treatment	56

3.3.3 Boron treatment.....	56
3.3.4 Data collection.....	57
3.4 Results.....	59
3.4.1 Boron uptake and plant growth.....	59
3.4.2 Boron deficiency symptoms.....	66
3.5 Discussion.....	66
Chapter 4 Warmer root zone temperature exacerbates boron deficiency of oilseed rape plants (<i>Brassica napus</i> L. cv. Hyola 42)	73
4.1 Abstract.....	73
4.2 Introduction.....	74
4.3 Materials and methods.....	75
4.4 Results.....	77
4.4.1 Development of B deficiency symptoms.....	78
4.4.2 Plant B response to RZT (d0-d11)	78
4.4.3 ‘Residual’ plant B response to RZT (d11-d15)	82
4.5 Discussion.....	82
Chapter 5 Response of sunflower (<i>Helianthus annuus</i> L. cv. Hysun 25) to boron supply at low root zone temperature	87
5.1 Abstract.....	87
5.2 Introduction.....	88
5.3 Materials and methods.....	90
5.3.1 Experiment 1.....	90

5.3.2 Experiment 2.....	91
5.3.3 Other data collection.....	92
5.4 Results.....	93
5.4.1 Experiment 1.....	93
5.4.1.1 Plant growth.....	93
5.4.1.2 Boron uptake.....	95
5.4.1.3 Root and leaf K ⁺ leakage.....	98
5.4.2 Experiment 2.....	100
5.5 Discussion.....	104
5.5.1 Plant growth response to RZT.....	104
5.5.2 Effect of RZT on plant response to B.....	107
5.5.2.1 Effect of RZT on plant dry matter.....	107
5.5.2.2 Boron partitioning within shoots.....	110
5.5.2.3 Boron function in plants.....	111
 Chapter 6 Chilling root zone temperature impairs boron nutrition of oilseed	
rape (<i>Brassica napus</i> L. cv. Hyola 42)	114
6.1 Abstract.....	114
6.2 Introduction.....	115
6.3 Materials and methods.....	116
6.3.1 Plant culture.....	117
6.3.2 Data collection and analysis.....	117
6.4 Results	118

6.4.1 Plant growth response and B uptake.....	118
6.4.2 Plant B concentration and B deficiency symptom.....	122
6.5 Discussion.....	123
6.5.1 Threshold root zone temperature.....	123
6.5.2 Mechanism of chilling RZT inducing impairment of B nutrition.....	124
Chapter 7 The role of boron in oilseed rape (<i>Brassica napus</i> L.) plant response to cold air.....	127
7.1 Abstract.....	127
7.2 Introduction.....	128
7.3 Materials and methods.....	131
7.3.1 <i>Experiment 1</i> Effect of night chilling on solute leakage from detached leaves with varying B concentration.....	131
7.3.2 <i>Experiment 2</i> Effect of shoot cooling at night on plant response to B.....	132
7.4 Results.....	133
7.4.1 <i>Experiment 1</i>	133
7.4.2 <i>Experiment 2</i>	135
7.5 Discussion.....	138
Chapter 8 General discussion: Plant boron nutrition and its response to low temperature.....	143
8.1 Abstract.....	143
8.2 Introduction.....	144
8.3 Influences of B nutritional status on low temperature tolerance.....	146

8.3.1 Direct injury to low B plant by low temperature.....	149
8.3.1.1 Root zone temperature.....	149
8.3.1.2 Air temperature.....	149
8.4 Direct effects of low temperature on B acquisition and utilization in crops.....	151
8.4.1 Root B acquisition	151
8.4.2 Internal requirement.....	153
8.5 Indirect effects of low temperature on B acquisition and utilization in crops.....	154
8.5.1 Biomass partitioning.....	156
8.5.2 Root-to-Shoot B transport and partitioning.....	157
References.....	160

LIST OF TABLES

	Page
2.1 Effect of plastic covers over plants on minimum air temperatures in- and out- side covers ($^{\circ}\text{C}$) and number of days when minimum air temperature was below 0°C outside the cover.....	44
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 (%).....	44
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).....	46
2.4 Effects of boron application (B) and canopy treatment (T) on oilseed rape development and seed yield.....	47
3.1 Glasshouse conditions during the two experiments.....	58
3.2 Effects of root zone temperature (RZT) and boron (B) on plant biomass and shoot to root ratio.....	61
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 (<i>Experiment 1</i>) and winter (<i>Experiment 2</i>) experiments.....	63
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) ($\mu\text{mol B g}^{-1}$ root DW d^{-1}).....	64

3.5 Effects of root zone temperature (RZT) and boron (B) on B partitioning between shoot and root and within the shoot.....	65
3.6 Water use efficiency (water consumption per unit dry matter production, mL g ⁻¹) during treatment period (12 d and 14 d in <i>Experiments</i> 1 and 2, respectively)	72
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.....	79
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).....	80
4.3 Effects of root zone temperature (RZT) and solution boron (B) on B concentrations in the youngest open leaves (YOL) (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.....	81
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).....	86
5.1 Effect of solution boron (B) concentration (µM) and root zone temperature (RZT) on sunflower shoot and root growth.....	94
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.....	96

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).....	97
5.4 Responses of plant growth and boron uptake to root zone temperature (RZT) and boron concentration (μM).....	98
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.....	102
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).....	109
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).....	119
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 growing parts (leaf 6 and shoot younger than leaf 6, ≥ L6) relative to whole shoots (%) affected by root zone temperature (RZT) and boron (B).....	120
6.3 Effect of root zone temperature (RZT) and boron (B) supply on boron	

concentration (mg kg^{-1}) in plant parts.....	121
7.1 One night chilling effect on solute leakage after 2 hr (expressed by electrical- conductivity, $\text{EC } \mu\text{S cm}^{-1}$) of oilseed rape leaves differing in boron (B) status (mg B kg^{-1}) under light or dark.....	134
7.2 One night chilling effect on solute leakage after 4 hr ($\text{EC } \mu\text{S cm}^{-1}$) of leaves of field grown oilseed rape plants.....	135
7.3 Effect of cooling at night on air temperature ($^{\circ}\text{C}$).....	136
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 (<i>Experiment 2</i>) ^a	137
7.5 Parameters for Mitscherlich models ^a fitted to the relationship between solution boron (B) concentration and shoot dry weight (g plant^{-1}).....	140

LIST OF FIGURES

	Page
2.1 Weather conditions at field site.....	40
5.1 Relationship between external boron (B) concentration and shoot dry weight (g plant ⁻¹) or root length (m plant ⁻¹) at different root temperatures (RZT).....	99
5.2 Effect of root zone temperature (RZT) and solution boron (B) concentration (μ M) on root K ⁺ leakage (mg K g ⁻¹ fw 2h ⁻¹) of sunflower at 6 days after treatments commenced.....	100
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) (<i>Experiment 1</i>).....	101
5.4 Effect of solution boron (B) concentration and root zone temperature (RZT) on B partitioning to youngest mature leaf blades (YML).....	111
7.1 Examples of changes of air temperature at night in glasshouse and in open field (from field experiment, Chapter 2).....	130
7.2 Effect of shoot cooling at night on relative humidity (R. H. %) and air temperature (°C).....	134
7.3 Relationship between external boron (B) concentration and shoot dry weight (g plant ⁻¹) at different root temperatures (RZT) under shoot cooling (T0) or ambient (T1) condition.....	140
8.1 Mechanisms of low temperature effects on plant boron (B) nutrition.....	147

LIST OF PLATES

	Page
2.1 Symptoms of boron deficiency in oilseed rape at seedling (upper) and flowering (lower) stages.....	45
5.1 Sunflower plant growth response to root zone temperature (RZT) and boron (B) (<i>Experiment 1</i>).....	93
5.2 Effect of root zone temperature (RZT) on plant growth by day 4 (<i>Experiment 2</i>)	103
5.3 A close-up view of boron (B) deficiency symptoms (<i>Experiment 2</i>).....	104
7.1 Spring frost injury to the low boron (B) leaves of oilseed rape plants.....	141

List of Plant Species Names

<i>Species</i>	Plant
<i>Antirrhinum majus</i>	Snapdragon
<i>Apium graveolens</i>	Celery
<i>Arabidopsis thaliana</i>	
<i>Arachis hypogaea</i>	Peanut
<i>Asparagus officinalis</i>	Asparagus
<i>Avena sativa</i>	Oats
<i>Beta vulgaris</i>	Sugar beet
<i>Brassica napus</i>	Oilseed rape
<i>Brassica oleracea</i>	Broccoli
<i>Citrullus vulgaris</i>	Watermelon
<i>Cucumis melo</i>	Muskmelon
<i>Cucumis sativus</i>	Cucumber
<i>Cucurbita pepo</i>	Squash
<i>Helianthus annuus</i>	Sunflower
<i>Hordeum vulgare</i>	Barley
<i>Lilium longiflorum</i>	Lily
<i>Lycopersicon esculentum</i>	Tomato
<i>Malus domestica</i>	Apple
<i>Manihot esculenta</i>	Cassava
<i>Nicotiana tobacum</i>	Tobacco
<i>Oryza sativa</i>	Rice
<i>Triticum aestivum</i>	Wheat
<i>Vigna mungo</i>	Black gram
<i>Vigna radiata</i>	Green gram
<i>Xenopus laevis</i>	
<i>Zea mays</i>	Corn, maize