

Controlled environment research within the project

Effects of Temperature, Light and Humidity during the Phase Encompassing Pollen Meiosis on Floret Fertility in Wheat

H. M. Rawson*, M. Zajac* and R. Noppakoonwong*†

Abstract

This paper examines the effects of temperature, humidity and light, altered during the period surrounding pollen meiosis, on floret fertility of wheat. A companion paper in these Proceedings examines the effects of these environmental variables against a background of marginal and zero soil boron.

There were no significant differences between the two genotypes Fang 60 and SW 41 in their responses to a reduction of light from 950 to 500 $\mu\text{mol}/\text{m}^2/\text{s}$ during the phase from emergence of the second-last leaf ligule to early grain filling. Plant height was marginally increased under less light (2.2%), ear number was reduced (26%), and ears were shorter (6.7%) and had fewer florets (22%), which were less fertile (8.2%). The overall effect of reduced light was a 28% reduction in grain number.

The averaged effects of increasing mean temperature from 12° to 20°C during the phase were large. Plants were 16% shorter and produced 10% fewer ears, which were 8% shorter and had 9% fewer florets; these florets were 9% less fertile, leading to a 16% reduction in grain number per main ear.

By contrast with temperature, the averaged effects of reducing humidity from nominally 80% to 40% were very small and generally insignificant. However, responses to humidity interacted significantly with temperature and radiation. Thus, high humidity always reduced floret fertility (to 83.4% mean) regardless of temperature when light was low; that is, only 21.6 $\text{mol}/\text{m}^2/\text{d}$, or equivalent to field conditions in spring. The reductions were greater at higher temperature (to 73.2% mean) in keeping with earlier findings. The most severe condition for sterility with adequate boron in the growth medium was high humidity combined with high temperature and low radiation. It is calculated that transpiration integrated over the pollen meiosis phase could be reduced by 88% by this combination of treatments compared with that in the contrasting environments.

It is concluded that environmental factors can change floret fertility in the presence of boron in the growth medium. This does not rule out boron limitation in the sexual growth centres as a cause, particularly as boron uptake is largely dependent on transpiration rate, which was changed by the environmental factors in this experiment.

DURING the past two decades, many cereal crops in the subtropics have been observed to be partially sterile (e.g. Singh et al. 1976; Ganguly 1979; Mandal 1994). Some observers have noted that sterility appears to be most severe when temperatures are high, humidities are low and there are hot winds (e.g. Galrao and Sousa 1988). In controlled environments also, high temperatures have been found to reduce grain set (Saini and Aspinall 1982a,b; Saini et al. 1983; Wardlaw 1994), though poorer grain set in those instances has occurred under high rather than low humidity (Bagga and Rawson 1977; Dawson and Wardlaw 1989; Tashiro and Wardlaw 1990). Water stress has been implicated (Saini and Aspinall 1981).

Evidence has accumulated that wheat genotypes can differ markedly in their degree of sterility in equivalent subtropical conditions (Singh et al. 1976; Ganguly 1979; Chatterjee et al. 1980; Rerkasem et al. 1993; Rerkasem and Loneragan 1994). In all these studies, the degree of sterility of the genotypes was linked with their differential requirement for boron when they were grown in severely boron-deficient soils. It is interesting, however, that the genotype Janak, which showed greatest sterility and greatest response to added boron in the field studies conducted between 1971 and 1977 (Ganguly 1979; Chatterjee et al. 1980), was also found to be sterile under high humidity in controlled environment work (Bagga and Rawson 1977). In the controlled environment studies, boron was added daily in a nutrient solution and so should not have been limiting.

There is little doubt that the most common direct cause of devastating sterility in the subtropics is a

* CSIRO Division of Plant Industry, GPO Box 1600, ACT 2601, Australia

† Multiple Cropping Centre, Chiang Mai University, Chiang Mai, Thailand

limitation of boron. In the cereals the sterility seems to show itself as poorly formed anthers or infertile pollen (Cheng and Rerkasem 1993). A clear sign of this is 'gaping glumes': the lemma and palea remain open after anthesis for several days instead of the normal several hours. This same visible response is also characteristic of sterility associated with high humidity. Limiting boron results in poor growth, sterility or both, not only in wheat, but also in a wide range of other species (Berger et al. 1957; Ifteni and Toma 1977; Garg et al. 1979; Mozafar 1987; Galrao and Sousa 1988). The effects can be reduced or avoided by the addition of boron either to the soil or as late foliar sprays (e.g. Chatterjee et al. 1980 in wheat). But even in these other species there is some evidence that factors such as radiation and temperature (Forno et al. 1979) can modify the responses to boron. The overall picture seems to be one in which sterility is often linked with limiting boron but the effects vary depending on environmental conditions.

It has been suggested that boron moves primarily in the transpiration stream (Kohl and Oertli 1961), though small amounts can move in the phloem (Shelp 1987). Consequently, it might be expected that any environmental factor that modifies transpiration rate might also modify the degree of sterility by altering the availability of boron to the sexual growth centres. By corollary, it might also be expected that sterility would vary from year to year in the field where factors affecting transpiration rate also vary, and that the variability would be greatest in areas where boron availability is marginal.

Vapour pressure deficit (VPD) is the environmental variable that has most influence on transpiration rate. In the absence of changes in stomatal aperture, transpiration rate increases linearly with VPD, the slope of the response varying with species (Rawson et al. 1977; Rawson and Clarke 1988). As VPD is determined by temperature and relative humidity, it would be expected that all these factors would modify the uptake of boron into the plant.

The aim of this paper and its companion paper (Rawson and Noppakoonwong, these Proceedings) is to examine the hypothesis that the degree of sterility in wheat is linked with boron availability in the growth medium and the ability of the plant to use that boron as influenced by transpiration rate and rate of growth. Transpiration

rate was ostensibly modified by having two humidity treatments in combination with two temperature regimes (that is, four environment treatments). Rate of growth (that is, rate of demand) was ostensibly modified by changing temperature. Following Bingham (1966) and Saini and Aspinnall (1981, 1982b), it was assumed that the critical phase leading to sterility in wheat is the period surrounding pollen meiosis (Bennet, Finch et al. 1973; Bennet, Rao et al. 1973), which is marked approximately by emergence of the flag leaf ligule (Dawson and Wardlaw 1989). The environment treatments were therefore applied only during the period encompassing that phase. Before the emergence of the second-last leaf ligule, all plants were grown in a single environment.

The first study, reported here, used a growth medium to which a complete nutrient solution was added daily to test whether the four environmental treatments would change fertility in the absence of a 'soil' boron limitation. Two genotypes were tested which contrasted in their responses to boron in earlier studies (Rerkasem and Loneragan 1994). The second study, which is the subject of the companion paper, added boron as a treatment as the two boron concentrations used were considered marginal, unlike in this first study.

Materials and Methods

Plants of two genotypes of wheat, Fang 60 and SW 41, both from Thailand, were grown in a glasshouse in the Canberra phytotron (Morse and Evans 1962). The glasshouse temperature was maintained between extremes of 27° and 13°C following a daily sine curve. Maximum temperature occurred at 1 p.m. The growth medium was a 50:50 mix of vermiculite and perlite in 100 × 150 mm pots (volume 1.18 L) to which was added a full Hoagland solution each morning and tap water each afternoon. Liquid was added until the pots dripped. There were 16 pots of each genotype in each treatment, split into four blocks.

The treatments of the two genotypes were two temperatures by two humidities by two light regimes. One set of plants remained in the initial glasshouse condition throughout. Treatments began when the ligule emerged on the leaf before the flag leaf. This leaf number was determined by dissection of some plants, which were then discarded. At that stage, plants were moved from the glasshouse to artificially lit growth cabinets

maintained at 27°C day / 13°C night (as in the glasshouse) or 17°/7°C sine curve. Both high and low temperature regimes were set for 80% and 40% relative humidity (four cabinets). Continuous monitoring showed that the set temperatures were achieved but the set humidities were not. In the high temperature treatment, actual mean daily relative humidities were $83.4 \pm 0.11\%$ and $49.9 \pm 0.08\%$ (vapour pressure deficits of 3.88 and 11.71 mb respectively), and in the low temperature treatment they were $84.4 \pm 0.07\%$ and $67.7 \pm 1.33\%$ (VPDs of 2.19 and 4.53 mb). The two light regimes were 950 and 500 $\mu\text{mol}/\text{m}^2/\text{s}$ maintained for 12 hours each day. The lower light regime was achieved by the use of frames covered with aluminium-stranded cloth over half the plants in each cabinet.

As each main ear emerged at least 50% above the flag leaf, and well before anthesis occurred, the ear was enclosed in a small paper packet (Table 1). This was to prevent pollination from other ears. Main ears of all 16 replicate plants were enclosed in all treatments. At harvest, which occurred before grains were hard, these ears were mapped for fertility, considering each floret and spikelet separately. A floret was fertile if it contained a grain, and infertile if the floret was gauged large enough to have a grain, yet was empty.

The control study is reported in this paper. The boron study, shown in Table 1 for comparison, appears in the companion paper. Glasshouse light was 31.6 ± 1.5 in the control study and 35.6 ± 1.9 $\text{mol}/\text{m}^2/\text{d}$ in the boron study between sowing and emergence of the second-last leaf ligule (FL - 1), and following that stage averaged 30.0 ± 1.4 and 42.1 ± 3.4 $\text{mol}/\text{m}^2/\text{d}$ respectively. Full light in the cabinets was 42.3 $\text{mol}/\text{m}^2/\text{d}$ in both studies.

Results

Environmental effects on growth and fertility of genotypes in different soil environments

Averaging across treatments in which full nutrient solution was applied daily, Fang 60 was taller than SW 41 (866 v. 833 mm, Table 2). It had around 15% more ears per plant (5.7 v. 4.9), though the ears were shorter (90 v. 106 mm long excluding the awns), with fewer spikelets (14.7 v. 16.6) and fewer florets (48.5 v. 57.3, Table 2). These florets, however, had marginally higher fertility (92% v. 88%). The combined effect on grain number was

that Fang was some 12% less productive per main ear than SW 41 (45.0 v. 50.7).

Table 2 also shows that the effects of reducing boron rates had little effect on height, ears per plant and florets per ear, but ears were progressively shorter, were progressively more sterile and had progressively fewer grains per ear.

Light, temperature and humidity effects

There were no significant differences between genotypes in their responses to a reduction in light from 950 to 500 $\mu\text{mol}/\text{m}^2/\text{s}$ between emergence of the second-last leaf ligule and early grain filling. Plant height was marginally increased under less light (Table 3), ear number was reduced, and ears were shorter. However, the ears had the same spikelet number (this was expected as treatments were started after terminal spikelet initiation) but fewer florets, which were less fertile. The overall effect was an almost 40% reduction in grain number from a 47% reduction in light during this relatively short phase.

The overall effects of increasing mean temperature from 12° to 20°C were also large (Table 3). Plants were shorter, produced fewer ears and shorter main ears, and had fewer florets, which were less fertile. This led to a reduction in grain number of 16% per main ear. These changes were associated with a 43% reduction in the duration of the period between the start of the temperature

Table 1. Days after sowing of events in SW 41 and Fang 60 wheats

Sown	Control 14 Feb. 95	Boron 31 Oct. 94
Days after sowing for:		
Seedling emergence	6	6
Fang 100% FL - 1	31	29
SW-41 100% FL - 1	32	31
Ears bagged*:		
Fang high temperature	43	43
SW-41 high temperature	45	†
Fang low temperature	52	50
SW-41 low temperature	57	†

* Ears were bagged at heading over several days but the day given is the average day when ears were approximately 90% emerged.

† Missing data.

treatment and ear emergence (from 23 to 13 days).

By contrast with temperature, the averaged effects of reducing humidity from 80% to 40% were very small and generally insignificant. However, because of significant interactions, using averages was not meaningful. Thus, although low light always reduced ear number, high temperature reduced ear number only at low humidity, not at high humidity. This is perhaps in keeping with ephemeral effects of aerial or soil water stress. Similarly, though shading always reduced floret numbers, RH had mixed effects. At high temperature, low RH apparently reduced floret number from 51 to 44 (though only under high light). Again, this could stem from brief water stress. By contrast, at low temperature, and under low light, low RH increased floret number from 47 to 51.

Interestingly, there were strong indications that high humidity, regardless of temperature, reduced floret fertility and grain number when light was only 21.6 mol/m²/d, or equivalent to field conditions in early spring (Table 4). In keeping with earlier findings (Dawson and Wardlaw 1989), the reductions in fertility were greater at higher temperature.

Discussion

At the workshop on sterility held in Chiang Mai, Thailand, in 1994, a summary table was compiled of the suspected effects of environmental factors on sterility in the subtropics (Table 5). All participants, apart from the delegate from Pakistan, perceived low boron as a primary cause of sterility (Pakistan has few problems of the type of sterility

discussed at the workshop). High temperature was seen as an important companion cause of sterility at Chiang Mai, and of some importance in Yunnan Province in China. It was not considered significant elsewhere. In Nepal, low temperature was identified as a contributor, whereas in Bangladesh, high humidity and low radiation were considered causes. Data in this paper lend support to the suggestions of high temperature, high humidity and low radiation. Even in the absence of limitations of boron in the growth medium, higher temperature increased sterility. The increases were marginal (3%–4%) under high radiation, but became important under low radiation (10%–15%), particularly at high humidity (Table 4). Low radiation was always of consequence to fertility in this experiment, reaching its greatest effect of 20% if temperature and humidity were both high. It also marginally reduced fertility at low temperature, again if humidity was high.

Though boron was added daily to the growth medium in the current control study, that does not rule out a limitation of boron at the sexual sites as being linked with sterility. In this study, increased mean temperature from 12° to 20°C increased the rate of development, reducing the period from emergence of the second-last leaf ligule to ear emergence from 23.0 to 12.5 days (Table 1). The number of days over which the sensitive stage of pollen meiosis occurred was possibly reduced by a similar proportion. This would halve the time plants could take up boron from the growth medium to fulfil the boron requirements of this phase. Increased humidity reduces transpiration

Table 2. Genotype comparisons. This represents the average response of all environment treatments for the study with boron non-limiting (control). There were no significant genotype by environment interactions. Also shown is the average genotype response for all environment treatments in the study with marginal boron (B1) and extremely low boron concentrations (B0)

Character	Fang 60 control	SW-41 control	Fang 60/SW-41 % change	Mean of control plants	Marginal boron (B1)	'Zero' boron (B0)	B0/control % change
Height (mm)	866	833	+4	850	855	857	0
Ears per plant	5.7	4.9	+16	5.3	5	5.6	+6
Ear length (mm)	90	106	-15	98	82	78	-20
Spikelets per main ear	14.7	16.6	-11	15.7	14	14	-11
Florets per main ear	48.5	57.3	-15	52.9	50	51	-4
Fertility main ear (%)	92	88	+5	90	34	1.6	-98
Grain number per main ear	45.0	50.7	-11	47.9	18.1	0.7	-99

Table 3. Effects of differences in light after emergence of the second-last leaf ligule, and effects of temperature and humidity (average response of Fang 60 and SW 41)

Light ($\mu\text{mol}/\text{m}^2/\text{s}$):	950	500	500/950 (% change)	Temperature 20°/12°C (% change)	Humidity 40%/80% (% change)
Character					
Height (mm)	845	864	+2	-16	-3
Ears per plant	6.2	4.6	-35	-10	-5
Ear length (mm)	103	96	-7	-8	0
Spikelets per main ear	15.7	15.7	0	0	0
Florets per main ear	59.9	46.8	-28	-9	0
Fertility main ear (%)	92.9	85.3	-9	-9	+4 *
Grain number per main ear	55.5	40	-39	-16	+2 *

* Interactions occurred

and the uptake of water (and boron) from the growth medium. A reduction of vapour pressure deficit from 11.7 to 3.9 mb, as in the two humidity treatments at high temperature (see Materials and Methods), would reduce transpiration to a third, from approximately 8.9 to 2.8 ng $\text{H}_2\text{O}/\text{cm}^2/\text{s}$ (Rawson et al. 1977). Similarly, a reduction in radiation from 950 to 500 $\mu\text{mol}/\text{m}^2/\text{s}$ would reduce stomatal conductance (and transpiration) by around 30% (Rawson ('Parameters'), these Proceedings). So the combined effects of low radiation and high humidity at high temperature (compared with high radiation and low humidity) could be to reduce daily boron uptake to 25%, and the duration of the period of development compared with that at low temperature to half. This means that plant transpiration integrated over the period of pollen meiosis would be reduced to 12%

by these treatments. The most severe condition for sterility in the absence of boron limitation in the growth medium was high humidity combined with high temperature and low radiation. Clearly, in a low boron soil, this combination of environmental factors could become highly significant to fertility.

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Table 4. Effects of humidity differences after emergence of the second-last leaf ligule on floret fertility at lower light (22.3 $\text{mol}/\text{m}^2/\text{d}$) (average responses of Fang 60 and SW 41)

Character	27°/13°C			17°/7°C		
	40% RH	80% RH	(% change)	40% RH	80% RH	(% change)
Height (mm)	791	765	+3	953	949	0
Ear length (mm)	92	89	+3	101	99	0
Florets per main ear	44	45	0	51	47	+8
Fertility main ear (%)	84	74	+13	93	89	+5
Grain number per main ear	37	33	+12	48	42	+14

Table 5. Summary of perceived effects of several factors on sterility in wheat. Numbers indicate rankings of importance (from the workshop on sterility in wheat held at Chiang Mai University, 1994)

	Thailand	Nepal	Bangladesh	Pakistan	Yunnan
Soil pH	✓	✓ 2	✓		
Soil boron	✓ 1	✓ 1	✓ 2		✓ 1
Soil N	☆	✓ 4	✓		
Low light			✓ 2	☆	
Water excess	✓	✓ 3	✓ 3	✓	✓ 2
Water deficit	✓ 2		✓ 4	✓	
Low temperature		✓ 1	✓ 4	☆	
High temperature	✓ 1			✓	✓ 3
High humidity			✓ 1	✓	✓ 4
Salinity				✓	

✓ = perceived effect

☆ = suspected effect

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Effects of Boron Limitation in Combination with Changes in Temperature, Light and Humidity on Floret Fertility in Wheat

H. M. Rawson* and R. N. Noppakoonwong*†

Abstract

The aim of this study was to determine whether the effects of low boron in causing floret sterility are accentuated by environmental variables. Plants of two genotypes of wheat (Fang 60 and SW 41) were grown in a glasshouse (27°/13°C) in sand culture using a boron-free nutrient solution until the ligule of the second-last leaf on the main shoot had emerged. They were then transferred to artificially lit growth cabinets and exposed to all combinations of 27°/13°C or 17°/7°C temperature, 80% or 40% relative humidity, marginal or zero boron solution, and 950 or 500 $\mu\text{mol}/\text{m}^2/\text{s}$ light until approximately one week after anthesis. Some plants remained in the glasshouse throughout and others were transferred to the cabinets only until ear emergence (short transfer treatment). The resultant degree of floret sterility was determined during mid grain filling.

Zero boron caused almost complete sterility regardless of genotype or the environmental treatment applied. Marginal boron reduced fertility to around 37% whereas a full boron solution resulted in 90% fertility. Lower light alone reduced fertility by around 8%, but when in combination with a marginal boron supply it amplified the boron effect by some 60%. So these two sterility factors were much more powerful in combination than separately, and the effects were not simply additive. Reduced light had the most deleterious effect at high temperature. There was no evidence that high humidity amplified the effects of marginal boron. Indeed, the reverse applied. This unexpected response is discussed.

Plants remaining in the glasshouse throughout performed the same as plants in the most similar artificially lit treatment, indicating that light quality in the growth chambers did not affect fertility. The short transfer treatment produced equivalent results to the full-duration transfers, demonstrating that the effects of boron and environment on sterility occurred before anthesis.

THE companion paper to this one (Rawson et al., these Proceedings) described the effects of temperature, light and humidity on the performance of wheat plants (genotypes Fang 60 and SW 41) when boron was non-limiting. It was shown that production of grain could be considerably altered by those environmental parameters, even though they were changed only during the period from first appearance of the flag leaf to early grain set. The most deleterious combination of weather parameters was high temperature, low radiation and high humidity. The effects were on number of ears, number of florets per ear (low radiation particularly) and floret fertility, which fell to around 70%. From that controlled environment work it was concluded that weather can alter fertility even when boron in the soil is plentiful.

The present paper examines whether the deleterious weather parameters still induce sterility

when boron is limiting, and whether they act to amplify the boron effect. As discussed in the companion paper, boron is moved primarily with the transpiration stream (Kohl and Oertli 1961), so it might be argued that any environmental factors that reduce transpiration would also reduce the flow of boron into and through the plant. In a low boron soil this could be enough to result in increased ear sterility, as boron is required for fertility of the generative organs. Environmental factors expected to increase the negative effects of low soil boron would include high temperature, which accelerates the rate of plant development, thus leaving less time for boron to be imported for each developmental phase; high humidity, which reduces potential transpiration and possibly boron movement; and low radiation, which reduces stomatal aperture and associated transpiration rate. This paper tests these assumptions by comparing ear sterility of plants grown at low and high temperature, low and high humidity, and low and high radiation, all with or without severely limiting soil boron. To make the work comple-

* CSIRO Division of Plant Industry, GPO Box 1600, Canberra, ACT 2601, Australia

† Multiple Cropping Centre, Chiang Mai University, Thailand 50002

mentary to that in the companion paper where boron was non-limiting, the two boron rates used here were severely limiting and marginal (see Table 2 in the companion paper). Exactly the same environmental treatments were used as in the companion paper. The timing of treatment imposition was the same; that is, when the ligule of the second-last leaf had emerged (leaf 6 or 7). At this time, plants were moved from a common glasshouse condition, in which no boron had been provided in the nutrient solution, into artificially lit growth cabinets, where the different environments were applied and the application of a marginal amount of boron began.

As in the companion paper and in the other experiments done by the ACIAR group, wheat genotypes Fang 60 and SW 41 were used. These are respectively tolerant of and sensitive to low concentrations of soil boron (Rerkasem and Loneragan 1994). The companion paper found the two genotypes to respond very similarly to weather parameters when boron was not limiting.

Materials and Methods

Plants of two genotypes of wheat, Fang 60 and SW 41, both from Thailand, were grown in a glasshouse in the CERES phytotron, Canberra, Australia (Morse and Evans 1962). The glasshouse temperature was maintained between extremes of 27° and 13°C following a daily sine curve. Maximum temperature occurred at 1 p.m. The growth medium was a sand, which had been prewashed for several days, contained in 100 × 150 mm pots (volume 1.18 L) to which was added a nutrient solution (Table 1) each morning and afternoon. All pots were washed in dilute acid followed by boron-free water before planting. There were 16 pots of each genotype in each treatment split into four blocks (four plants per block). While plants remained in the glasshouse and before the environment treatments began, they were watered with a boron-free solution. Plants provided with a full nutrient solution throughout growth were part of the companion study (Rawson et al., these Proceedings).

The treatments of the two genotypes were two boron rates (zero and marginal) by two temperatures by two humidities by two light regimes. One set of plants remained in the initial glasshouse condition throughout. Treatments began when the ligule emerged on the leaf before the flag leaf. This

leaf number was determined by dissection of some plants, which were then discarded. At that stage, plants were moved from the glasshouse to artificially lit growth cabinets maintained at 27°/13°C (as in the glasshouse) or 17°/7°C sine curve. Both high and low temperature regimes were set for 80% and 40% relative humidity (four cabinets). In the high temperature treatment, actual mean daily RHs were 83 ± 0.1% and 49 ± 0.1% (vapour pressure deficits of 3.8 and 11.7 mb respectively), and in the low temperature treatment they were 84 ± 0.1% and 67 ± 1.3% (VPDs of 2.1 and 4.5 mb). The two light regimes were 950 and 500 μmol/m²/s maintained for 12 hours each day. The lower light regime was achieved by the use of frames covered with aluminium-stranded cloth placed over half the plants in each cabinet.

As each main ear emerged at least 50% above the flag leaf, and well before anthesis occurred, the ear was enclosed in a small paper packet. This was to prevent pollination from other ears. Main ears of all 16 replicate plants and many of the subsidiary ears were enclosed in all treatments. At harvest, which occurred before grains were hard, these ears were mapped for fertility, considering each floret and spikelet separately. A floret was fertile if it contained a grain, and infertile if the floret was gauged large enough to have a grain, yet was empty (Bagga and Rawson 1977).

Results

Effects of boron on fertility

If there was no boron in the nutrient solution (B0) during the period of flag leaf expansion to anthesis, as in this study, ears were made almost completely sterile (Table 2), regardless of other environmental parameters. If boron was supplied at a marginal level (B+) during this period, fertility rose to around 37%, even though no boron had been provided during the vegetative phase. But if boron was sufficient throughout (B++), fertility was at least 70% in the most limiting environment and 98% in the best. Consequently, boron had a dominant effect on fertility in these studies. Because boron had such an overwhelming effect in the B0 treatment, that treatment does not help to assess interactions between boron and the environment. However, the B+ treatment is useful.

The lesson from the GH and ST treatments (Table 2) is that plants in the glasshouse performed

Table 1. Composition of the nutrient solution. Stock solutions 1 to 4 and all water used in low boron tanks were purified through a column of borate-specific resin (Amberlite, IRA-743, Sigma Chemical Co.). Nutrient solution pH was 6.88

Stock	Chemical	Mass in the stock solution	Stock solution (L)	Element	Final conc. (ppm)
1	Ca(NO ₃) ₂ ·4H ₂ O	9.6 kg	20	N	114
2	(NH ₄) ₂ HPO ₄	1.371 kg	20	P	16
3	KNO ₃	6.118 kg	40	K	118
4	MgSO ₄ ·7H ₂ O	2.536 kg	20	Mg	13
5	FeSO ₄ ·7H ₂ O	50 g	5	Fe	2
	EDTA	66 g			
	NaOH	13 g			
6	MnCl ₂ ·4H ₂ O	3.99 g	5	Mn	0.056
	ZnSO ₄ ·7H ₂ O	2.20 g		Zn	0.025
	CuSO ₄ ·5H ₂ O	0.51 g		Cu	0.007
	Na ₂ MoO ₄ ·7H ₂ O	0.30 g		Mo	0.006

very similarly to their transferred equivalents (27°/13°C, no shade and 80% RH). These plants with zero boron were fully sterilised and those on a marginal dose approximated 50% fertility. This comparison indicates that light quality in the chambers was acceptable and that the chambers themselves did not have any spurious effects on sterility.

The short transfer from the glasshouse to the 27°/13°C treatment with low humidity and without shade also paralleled the response of plants in the full treatment. From this we can also draw some confidence in the overall data set. We can also conclude that the treatment effects that increased sterility had already occurred by ear emergence. Plants in the main study remained in their regimes until at least seven days after anthesis, or around two weeks more. Thus, the humidity effects were not effects during anthesis.

Effects of reduced light × boron on fertility

Table 3 shows how fertility of the main ears was reduced by shading by an average of only 8.3% in the treatment with adequate boron (100 – 91.7 = 8.3), whereas in the marginal boron treatment (B+), shading reduced fertility by more than 60%. Most surprisingly, the genotype Fang 60, which is little affected by low boron in the field (Rerkasem and Loneragan 1994), was more affected by shading than SW 41 when boron was limiting. The effects of shading were more

deleterious at higher temperature (66%) than at lower temperature (48%). There was limited evidence also that shading reduced fertility more at higher humidity, though only at lower temperature.

Effects of increased temperature × boron on fertility

Table 4 shows that increased temperature had little overall effect on percentage fertility when boron was adequate, but reduced fertility considerably when boron was marginal, particularly under low radiation.

Effects of increased relative humidity × boron on fertility

There was no evidence that high humidity accentuated the effects of limiting boron on sterility, as was expected from theory. In fact, the reverse situation generally applied: there was less sterility under boron limitation. This contrasts with the effect of high humidity reducing fertility at high temperature under low radiation when boron was adequate.

Discussion

The expectation from this work was that the negative effects of low boron on fertility would be amplified by growing plants at high temperature during pollen meiosis (between first appearance of the flag leaf and ear emergence), by maintaining

Table 2. Percentage fertility in main ears of Fang 60 and SW 41 plants provided with no boron (B0), a marginal amount (B+), or an adequate amount (B++) and grown under two temperatures, two humidities and two light levels after emergence of the second-last leaf ligule

Temp.	RH	Light	B0			B+			B++		
			Fang 60	SW 41	Mean	Fang 60	SW 41	Mean	Fang 60	SW 41	Mean
27°/13°C	80	high	0.0	0.1	0.1	48.4	50.9	49.7	90.7	90.7	90.7
		low	1.9	2.7	2.3	5.7	19.3	12.5	74.6	74.3	74.5
	40	high	0.2	0.3	0.3	36.3	38.9	37.6	96.7	92.5	94.6
		low	0.6	0.0	0.3	1.2	15.8	8.5	96.0	91.0	93.5
17°/7°C	80	high	4.7	0.5	2.6	52.3	53.9	53.1	97.9	91.3	94.6
		low	5.0	0.3	2.7	17.5	30.5	24.0	93.7	85.1	89.4
	40	high	0.0	0.0	0.0	56.9	55.7	56.3	93.0	90.1	91.6
		low	5.0	0.0	2.5	29.0	37.7	33.4	87.7	79.8	83.8
27°/13°C	GH	high	0.1	0.1	0.1	49.2	52.4	50.8	98.5	97.0	97.8
		ST		0.0	0.0		40.5	40.5			
Mean					1.3	36.6			90.0		

GH = plants that remained in the glasshouse with natural light throughout growth (i.e. no shading).

ST = plants that were transferred to the artificially lit cabinet at the prearranged stage but were transferred back to the glasshouse at heading time ('short transfer').

lower radiation, and by holding a high relative humidity. The perceived effects of these treatments would be, first, to accelerate development, leaving less time for the plant to take up boron and move it to the generative areas; and second, to reduce the uptake of boron by reducing both transpiration rate (low radiation) and evaporation (high humidity).

The expectations were partially borne out. High temperature accelerated development (see companion paper) and certainly reduced fertility when boron was marginal (Table 4). There was only a minimal effect when boron was plentiful. Low radiation dramatically accentuated the low boron effect as predicted (Table 3), particularly at high temperature.

High humidity effects did not follow expectations, however. In fact, high humidity reduced the effect of low boron on fertility. All we can suggest to explain this response is that high humidity reduced the diurnal water stress that plants might have been suffering, and this in turn reduced stomatal closure, which increased boron uptake. Plants were grown in sand and provided

with water twice each day, but the pots were small. Unfortunately, we cannot confirm this suggestion as we did not measure stomatal conductance. The point remains, though, that the response to humidity will always depend on whether the plants are stressed or not. The same argument will apply to responses to light and temperature. High light, high temperature and water stress are often linked in the field, and it is very difficult to separate their effects on boron uptake unless extensive measurements are taken to characterise both the environment and the plant's degree of stress.

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Table 3. Effect of shading in the B+ and B++ treatments on fertility. Data are presented as (fertility % at 500 $\mu\text{mol}/\text{m}^2/\text{s}$) + (fertility % at 950 $\mu\text{mol}/\text{m}^2/\text{s}$) \times 100

Temp.	RH%	B+			B++		
		Fang 60	SW 41	Mean	Fang 60	SW 41	Mean
27°/13°C	80	11.8	37.9	25.2	82.2	81.9	82.1
	40	3.3	40.6	22.6	99.3	98.4	98.8
17°/7°C	80	33.5	56.6	45.2	95.7	93.2	94.5
	40	51.0	67.7	59.2	94.3	88.6	91.5
Mean		24.9	50.7	38.1	92.9	90.5	91.7

Table 4. Effect of increasing temperature in the B+ and B++ treatments on fertility. Data are presented as (fertility at 27°/13°C) + (fertility at 17°/7°C) \times 100

RH%	Light	B+			B++		
		Fang 60	SW 41	Mean	Fang 60	SW 41	Mean
80	high	92.5	94.4	93.5	92.6	99.3	95.9
	low	32.6	63.3	52.1	79.6	87.3	83.3
40	high	63.8	69.8	66.8	104	102	103
	low	4.1	41.9	25.5	109	114	111
Mean		48.3	67.4	59.5	96.4	101	98.5

Table 5. Effect of increasing relative humidity in the B+ and B++ treatments on fertility. Data are presented as (fertility at 80%) + (fertility at 40%) \times 100

Temp.	Light	B+			B++		
		Fang 60	SW 41	Mean	Fang 60	SW 41	Mean
27°/13°C	high	133	130	132	93.8	98.1	95.9
	low	475	122	147	77.7	81.6	79.6
17°/7°C	high	91.9	96.8	94.3	105	101	103
	low	60.3	80.9	72.0	106	106	106
Mean		190	107	111	95.9	96.9	96.4

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Effects of Boron Deficiency and Low Temperature on Wheat Sterility

L. Huang*, J. Pant†, R. W. Bell*, B. Dell*, and K. Deane*

Abstract

Wheat exhibits sterility in many parts of subtropical and tropical Asia. Boron deficiency is believed to be the cause of sterility in many, but not all, cases. This creates the need for standards to allow B deficiency to be diagnosed and distinguished from other causes of sterility. This study was designed to investigate the responses of functional indicators of B requirements, namely leaf blade elongation rates and potassium leakage rates, to B supply (0 and 10 μM B) and night temperature (ambient: $> 10^\circ\text{C}$ and low: $> 5^\circ\text{C}$). An objective of the study was to develop diagnostic and prognostic standards for B deficiency, based on the functional B requirements for leaf elongation and membrane permeability.

Although the interruption of B supply (-B) significantly decreased B concentrations in leaf blades and ears, it had no consistent effect on rates of leaf blade elongation or leaf K leakage in the two cultivars of wheat tested at either the vegetative or the reproductive stage. Low night temperature also had little effect. However, the grain set index (GSI) was significantly decreased by the -B treatment. Low night temperature had no effect on GSI in +B plants. In -B plants, ambient night temperature decreased GSI apparently by accelerating ear development, so that critical stages of grain set coincided more with the deficiency of B than in -B plants exposed to low night temperatures.

BORON (B) is an essential micronutrient for the growth of plants. Some grasses, such as wheat, have a much lower B requirement for normal growth than dicots (Dugger 1983). Nevertheless, B deficiency causing yield loss has been documented in wheat in southern and eastern Asian countries such as Bangladesh, China, Nepal, India and Thailand (Rerkasem et al. 1993).

Boron deficiency affects the growth and function of male or female reproductive organs of wheat, leading to the failure of fertilisation and grain setting (Rerkasem et al. 1993) and eventual yield loss. B deficiency causes the poor development of anthers and pollen and germination failure of pollen, owing to insufficient B supply in the pollen or in the stigma and style (Cheng and Rerkasem 1993).

Although there is an increasing understanding of the role of B deficiency in wheat sterility, the detection of B deficiency in wheat plants at the reproductive stage often allows no time for correction of the problem. Wheat vegetative growth is insensitive to B deficiency. There are

usually no symptoms in the leaves and no significant growth reduction. Vegetative growth is therefore not a reliable diagnostic indicator of B deficiency. In addition, rate of dry matter accumulation responds to the declining B supply more slowly than leaf B concentrations (Kirk and Loneragan 1988). As a result, it is advisable to explore physiological responses of shoots to B deficiency for its early diagnosis.

Boron plays both structural and functional roles in plant cells (Loomis and Durst 1992; Parr and Loughman 1983). Boron is an essential component of cell wall structure (Loomis and Durst 1992) and B deficiency inhibits cell division and elongation (Hu and Brown 1994). Boron deficiency disturbs membrane integrity and increases membrane permeability (Cakmak et al. 1995; Parr and Loughman 1983).

Based on these functional roles of B in plant cells, it is presumed that leaf elongation and leaf K leakage are sensitive parameters by which to diagnose B deficiency in wheat plants. The response of leaf blade elongation rates to B deficiency has been successfully used to set a critical B concentration for the diagnosis of B deficiency in black gram (Noppakoonwong et al. 1993). In sunflower, leaf K leakage rates were closely correlated with leaf B concentrations

* School of Biological and Environmental Sciences, Murdoch University, WA 6150, Australia

† Institute of Agriculture and Animal Science, Central Campus, Tribhuvan University, Rampur, Nepal

(Cakmak et al. 1995).

Wheat production is increasing in the warmer areas of southern and eastern Asia, such as Bangladesh, Nepal, China and Thailand (Rerkasem et al. 1993). Field observation has shown that wheat plants grown in these areas might be exposed to low night temperature just before or during anthesis, enhancing the failure of grain set (Pant, pers. comm.).

Several reports appear in the literature linking low temperature damage in plants and B deficiency (Shorrocks 1991). These reports are limited to observations of increased damage to shoots by frost or low temperature when plant B status is low, and in some cases, alleviation of the injury with foliar B sprays. Experimental evidence that low temperatures increase sensitivity to B deficiency is limited to one brief report by Parr and Loughman (1983). Parr and Loughman examined the response of P uptake by *Zea mays* to decreasing temperature in the presence or absence of B in solution. In solutions supplied with B, the uptake of P declined with decreasing temperature. However, below 20°C there was a distinct inflection in the curve, which implied a temperature-dependent change in membrane conformation from a fluid to a gel. For plants grown in tropical and subtropical regions, low temperature even above freezing point might cause a change in membrane properties and enhance membrane permeability (Simon 1974). What had not been previously reported was the finding of Parr and Loughman (1983) that the critical temperature at which membrane properties changed, depressing P uptake, was 2°C higher in B-deficient solutions than in B-sufficient solutions. These limited results imply that plants with low or deficient B supply might be more sensitive to cold temperature damage to membranes of growing tissues than plants with sufficient B.

In 1994, at Tonglu in Zhejiang Province, China, we observed oilseed rape plants showing symptoms of frost damage to the leaves. This was in late March, some 10 to 14 days after snowfalls that remained on the ground for two days. It was only in plots without B fertiliser that frost damage occurred; plants treated with B fertiliser at sowing were free of the symptoms. Thus it appears that leaf tissue of oilseed rape was more sensitive to frost damage when low in B. The converse conclusion, that low temperature increases internal

B requirements, has not been demonstrated.

Interesting as these observations are, their relationship to B deficiency and to internal B requirements is not clear. Episodes of low temperature could be the cause of site-to-site and year-to-year variation in internal B requirements of wheat, and therefore in grain set. Further studies to establish a meaningful causal link between low temperature damage and plant B status would be particularly useful. Controlled environment studies would appear to be necessary to establish such a link. Field demonstration of the significance of a low temperature effect is also necessary.

The objectives of the present study were to examine effects of B deficiency and low night temperature on leaf blade elongation and leaf K leakage, and to establish a relationship between these responses and leaf B concentrations. On the basis of this relationship, the study aimed at estimating diagnostic standards for B deficiency in wheat vegetative growth, and prognostic standards for predicting the sterility of florets and grain set failure in wheat plants during the reproductive stage. It was hypothesised that low night temperature might exacerbate B deficiency in wheat plants subjected to low B supply. This paper reports the responses of leaf blade elongation rates, leaf K leakage, B concentrations, spikelet fertility and grain set index to B deficiency and low night temperature for wheat plants at vegetative and reproductive stages.

Materials and Methods

Plant culture

Wheat plants were grown in solution culture in a glasshouse. The full-strength basal nutrient solution used initially contained the following chemicals. Concentrations in parentheses are $\mu\text{mol/L}$: NH_4NO_3 (2000), KNO_3 (2800), $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (1600), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (1000), KH_2PO_4 (100), and K_2HPO_4 (100), FeEDTA (100), NaCl (8), $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (2), $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ (2), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (0.5) and $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ (0.08). Water used for making up the solutions and the macronutrient stock solutions were purified with B-specific resin (IRA-743, Sigma Chemical Co.). Only analytical grade chemicals were used to make up the solutions. The initial pH in the solution was about 6. The pH was buffered at 7.0 to 7.5 by adding 0.1 g CaCO_3 to each pot of solution.

The programmed nutrient addition technique (Asher and Edwards 1983) was used to supplement all nutrients during plant growth, except for B during the B treatment period. The following nutrient concentrations were assumed to be adequate in whole shoots: 4.5% N, 0.4% P, 3.0% K, 0.3% Ca, 0.3% S, 0.2% Mg, 50 mg/kg Fe, 20 mg/kg Mn, 20 mg/kg Zn, 5 mg/kg B, 3 mg/kg Cu and 0.5 mg/kg Mo. The following model was used to predict plant dry matter at a given day:

$$Y = A \times \log_{10}(A) + e^{(RGR \times T)}$$

where Y is the predicted dry weight per plant, A is the existing dry weight (g per plant), RGR is relative growth rate (g/g/day), and T is the number of days. The amounts of nutrients and B needed were calculated by the 'Nutradd' software (Asher and Blamey 1987). The RGR was determined by sequentially harvesting plants in extra pots of the +B treatment.

Experiment 1—Reproductive stage

This experiment was designed to investigate the responses of the permeability of leaf cell membranes (measured as K leakage) and leaf blade elongation in plants at the reproductive stage to the experimental treatments, and to attempt to correlate the responses with pollen viability and grain set index.

Wheat seeds (*Triticum aestivum* cv. SW 41, from Thailand) were soaked in aerated CaSO₄ solution (2 mM) for four days in the dark at room temperature (10°–16°C). The germinated seedlings were then transplanted into a plastic tray containing 8 L of one-third strength basal nutrient solution (with 5 µM B) and placed in the glasshouse. After five days in the trays, the seedlings were transplanted into pots containing 5 L of full strength nutrient solution with 5 µM B. Nine seedlings, with about 1½ leaves each, were planted into each pot. The pots were randomly positioned in a temperature-controlled water bath set at 18 ± 1°C. The pots were repositioned every two days to minimise positional effects on growth.

At 13 days after transfer (DAT), plants were thinned to eight per pot. Dry weights of the thinned plants were recorded at transfer and at 13 DAT, and relative growth rates were calculated using the Nutradd software (Asher and Blamey 1987). Calculated amounts of nutrients were added to the solutions on 15, 22, 44 and 64 DAT. The healthy appearance of the plants in the B-sufficient pots

indicated that the nutrients added were adequate for plant growth.

At 31 DAT (about 8th leaf stage), the B and night temperature treatments were begun using two sets of four replicates in each treatment. The B treatments were 0 µM (–B) and 10 µM (+B) H₃BO₃. The night temperature treatments were ambient glasshouse temperature (> 10°C) and low temperature (> 5°C). The detailed glasshouse environmental conditions are shown in Table 1. A recorded example of the low night temperature profile is shown in Figure 1. During the treatment, the low night temperature averaged 9.2°C (max. 13.7° – min. 5.7°). Low temperatures were imposed by transferring pots every evening to a temperature-controlled room. The room was programmed so that temperatures decreased progressively from ambient at 5:30 p.m. to a minimum of 6°C at 6 a.m. Plants were transferred out of the cold room each day at 7 a.m. to the glasshouse. During the day both sets of plants experienced the same environmental conditions. The plants were exposed to the B and night temperature treatments for 18 days and then B was resupplied to the –B plants until grain set.

The length of the ninth leaf of a main stem was measured daily over seven consecutive days in three plants per replicate from its emergence to its maximum length for the determination of elongation rate. From one set of the plants, the youngest emerging blades (YEB) and the blades immediately older than YEB (YEB + 1) were sampled from the main stems of two plants per replicate for the determination of B concentrations at 1, 3, 7, 12 and 18 days after the start of treatment. At 12 and 18 days after the start of treatment, ears of the main stems were also harvested for the determination of B concentrations. Plant growth stage at each harvest is given in Table 2.

For the determination of leaf K leakage in YEB of the main stem, two plants per replicate were sampled from the other set of plants. The leaves were cut off at the base of the emerged blades. As B deficiency affects growing tissues more than mature ones, the basal part of the YEB was considered to be the most sensitive to B deficiency. The leaf segment up to 5 cm from the base was cut into 1 cm strips. After being washed in three changes of triple-deionised (TDI) water, these leaf strips were placed in a plastic container with 10 mL TDI water and gently shaken for two hours (10 a.m. to 12 noon) at glasshouse temperature in

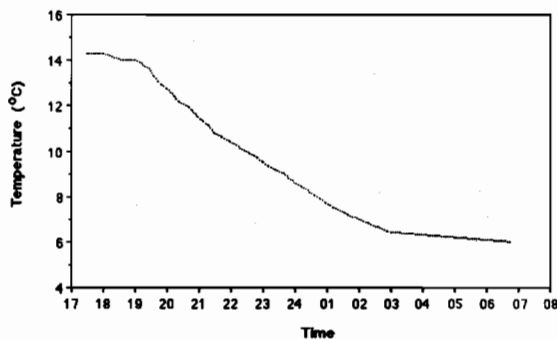


Figure 1. A example of a night temperature ($^{\circ}\text{C}$) profile in the cold room used to expose wheat plants to low temperature. The temperature was set to decrease gradually between 5:30 p.m. and 6 a.m. the next day from ambient temperature down to 6°C .

the light (Navari-Izzo et al. 1989). The solutions were sampled for the determination of K leaked out of the leaf strips. The leaf strips were frozen at -20°C and thawed at room temperature for total membrane disruption in the leaf cells. The thawed leaf samples were incubated in 20 mL TDI water overnight and K concentrations in the incubation solutions were determined by inductively coupled plasma (ICP) spectrometry.

Ears of the main stems were harvested for the

determination of grain set index 52 days after the start of treatment, when full grains were observed in ears of the +B plants.

Experiment 2—Vegetative stage

Wheat cultivar Wilgoyne was used to investigate effects of B supply and low night temperature on responses of leaf blade elongation, leaf K leakage and leaf B concentrations at the vegetative stage for the diagnosis of B deficiency. Wheat seedlings were prepared as described for Experiment 1. Wheat seeds were soaked in the dark at room temperature (about $18^{\circ}\text{--}25^{\circ}\text{C}$) in aerated CaSO_4 solution for three days until germination. Uniformly germinated seedlings were transplanted into a plastic tray with 8 L of one-third strength nutrient solution. After being acclimatised in the glasshouse for six days, ten randomly selected plants were transferred into each plastic pot, which contained 5 L of complete, full-strength nutrient solution.

The treatments of B supply and low night temperature were applied to the plants at 26 DAT. Boron treatments were 0 (–B) and 10 (+B) μM and the temperature treatments were ambient glasshouse temperature ($16^{\circ}\text{--}20^{\circ}\text{C}$) and low temperature ($5^{\circ}\text{--}15^{\circ}\text{C}$). In the –B treatment, B-specific resin (6 g air-dry per pot) was placed in

Table 1. Environmental conditions in the glasshouse during the treatment periods of experiment 1 and 2

		Air T ($^{\circ}\text{C}$)	Water T ($^{\circ}\text{C}$)	RH %	Daily average light intensity ($\mu\text{mol}/\text{m}^2/\text{s}$)	Total radiation ($\text{MJ}/\text{m}^2/\text{day}$)
Experiment 1 (SW 41)						
Day	Mean	21.5	18.3	69	268	6.61
	Maximum	25.2	21.1	83	760	9.41
	Minimum	15.5	16.2	59	64	1.54
Night	Mean	17.3	16.9	80		
	Maximum	21.3	17.9	84		
	Minimum	15.8	16.9	75		
Experiment 2 (Wilgoyne)						
Day	Mean	21.9	19.6	73	291	7.14
	Maximum	25.2	23.0	85	758	8.65
	Minimum	16.0	16.5	64	198	5.28
Night	Mean	17.9	17.5	81		
	Maximum	21.5	18.7	84		
	Minimum	15.8	16.9	75		

the solution to minimise B concentrations in solution. Each treatment was replicated four times. The detailed glasshouse environmental conditions are shown in Table 1. A recorded example of night temperature profile in the cold room is shown in Figure 1. During the treatment, the low night temperature averaged 10°C (max. 14.2° – min. 7.1°) in the cold room.

Potassium (K) leakage was measured in the youngest emerging blades. Two plants per pot were sampled for YEB from the main stems of one set of plants at 0, 3, 6, 9 and 12 days after the start of treatment. The measurements were conducted in the same way as in experiment 1.

The length of the seventh leaf of a main stem was measured daily over seven consecutive days in three plants per replicate from their initial emergence to their maximum length for the determination of their elongation rate.

In the other set of plants, the YEB and YEB + 1 blades were sampled from the main stems of two plants per replicate for the determination of B concentrations at 0, 3, 6, 9 and 12 days after the start of treatment. Plant growth stage at each harvest date is given in Table 2.

Boron determination

The samples were dried at 70°C to constant weight and digested with concentrated nitric acid at 140°C. Boron concentrations in the digest solutions were determined by ICP spectrometry (Zarcinas et al. 1987).

Data analysis

Two-way analysis of variance was applied to the data to detect the effects of B supply and night temperature treatments on leaf blade elongation rates, leaf K leakage and B concentrations.

Results

Leaf blade elongation and K leakage— Experiment 1

At the start of treatments, B concentrations in leaf 8 were relatively low, at 3 to 5 mg/kg (Tables 3a, 3b). Subsequently, in +B solutions, leaf B concentrations in plants at ambient night temperatures increased to 9 mg/kg at 12 days after the start of treatment (DAS) and then declined markedly at 18 DAS to 3 mg/kg. In +B plants at low night temperature, B concentrations in leaf 8

Table 2. Plant growth stages at each harvest in experiments 1 and 2

Harvest	Date	Day	Plant stage
Experiment 1 (SW 41—reproductive stage)			
	9 June	0	8th leaf emerging
Harvest 1	10 June	1	8th leaf emerging, 7th leaf fully emerged
Harvest 2	12 June	3	8th leaf fully emerged, 9th (flag) leaf emerging
Harvest 3	16 June	7	Flag leaf half emerged
Harvest 4	21 June	12	Flag leaf ligule emerged, ear size ≈ 4 cm
Harvest 5	27 June	18	Late booting in Ta plants
(B resupply)			Mid booting in Tl plants
	6 July	27	Anthesis in Ta plants
	12 July	33	Anthesis in Tl plants
Harvest 6	31 July	52	Late milky to soft dough. Main stem grain full
Experiment 2 (Wilgoyne—vegetative stage)			
Harvest 1	23 June	0	6th leaf $\frac{1}{3}$ – $\frac{1}{2}$ emerged
Harvest 2	26 June	3	6th leaf fully emerged, 7th leaf $\frac{1}{4}$ – $\frac{1}{3}$ emerged
Harvest 3	29 June	6	7th leaf $\frac{4}{5}$ emerged
Harvest 4	2 July	9	7th leaf fully emerged, 8th leaf emerging
Harvest 5	5 July	12	8th leaf $\frac{1}{2}$ emerged

Ta = ambient night temperature, Tl = low night temperature

ranged from 4 to 8 mg/kg over the period 3–12 DAS and, as in plants at ambient night temperature, dropped at 18 DAS to 3 mg/kg.

In –B treatments, B concentrations in leaf 8 declined strongly between 1 and 3 DAS and, by 7 DAS, had declined to 2 to 3 mg/kg, where they remained.

In leaf 9, which emerged at 7 DAS, B concentrations in –B plants were 1 to 1.5 mg/kg at emergence and remained there. By contrast, B concentrations in leaf 9 of +B plants were 3 to 7 mg/kg.

At 12 and 18 DAS, low temperature increased B concentrations in the ears of +B plants, and decreased them in –B plants.

No obvious symptoms of B deficiency or injury caused by low temperature were observed in the –B plants or low-temperature-treated plants.

Although the removal of B supply decreased B concentrations to as low as 1 mg/kg dry matter, it did not induce any significant increase in K leakage out of the newly emerged leaf blades (Table 4). The effects of low temperature on leaf K leakage were not consistent over time.

The effects of –B and low temperature on leaf blade elongation rates of the ninth leaf were generally not significant (Table 5).

Leaf blade elongation and K leakage— Experiment 2

Boron concentrations in the YEB were decreased only by the –B treatment, not by low temperature. There was no interaction between –B and low temperature on B concentrations in the leaves (Table 6).

The treatments of –B and low night temperature generally caused no response in K leakage from YEB, except that –B significantly increased K leakage from YEB at ambient temperature compared with low temperature at 6 DAS (Table 7). The elongation rates of YEB were not consistently affected by the B or temperature treatments (Table 8). A significant decrease in leaf blade elongation rates caused by low temperature was observed at 2–3 DAS, but the reverse applied at 4–6 DAS. The young leaves became flaccid when returned to the glasshouse after being exposed to low night temperature.

Spikelet fertility and grain set index

The interruption of B supply significantly decreased spikelet fertility and grain set index

(GSI) in SW 41 plants (Table 9, Fig. 2). There was slower ear development in the low night temperature treatment than at ambient night temperature. The low temperature treatment had no effect on spikelet fertility or GSI of plants with B, but increased them in plants without B. In comparison, the numbers of grain and florets in each spikelet of both –B and +B plants was increased by the low temperature treatment (Fig. 2). These effects of B and night temperature treatments occurred mostly in the central florets (spikelets 3–17).

Discussion

The present study aimed at exploring sensitive physiological responses of wheat to B deficiency and their relationship to B concentrations in shoot parts. However, the relationship between rates of leaf blade elongation and leaf K leakage and concentrations of B in leaf blades and ears could not be established, owing to the lack of response of these two variables to B withdrawal (–B) and low night temperature.

The results indicate that the growth of vegetative parts requires very low internal B supply at both the vegetative and reproductive stages. A previous study with SW 41 (Rerkasem et al. 1993) showed that withdrawing B supply for 12 days also had little effect on the length of YEB. For wheat plants during the vegetative stage, a B concentration of 3 mg/kg dry matter or greater was adequate for growth (Reuter and Robinson 1986). In the present study, –B treatment decreased B concentrations in the YEB to around 1 mg/kg dry matter in the plants at vegetative (Wilgoyne) and reproductive (SW 41) stages, but did not have any effect on rates of leaf blade elongation and leaf K leakage from the YEB. In contrast, dicot crops have much higher B requirement for their growth and much higher critical B concentrations than the adequate B concentrations for wheat (Reuter and Robinson 1986). For example, critical B concentrations in the youngest open leaves (similar to the YEB in wheat plants) of canola plants are as high as 10 to 14 mg/kg dry matter (Huang and Bell 1994). The low internal B requirement of wheat plants gives rise to the practical difficulty of establishing the changes in leaf blade elongation and leaf K leakage rates of YEB in response to the narrow range of leaf B concentrations from deficiency to adequacy.

Table 3a. Boron concentrations (mg/kg dry matter) in the eighth and ninth leaves of SW 41 wheat for selected days after starting the treatment (DAST). Plants were subjected to a factorial combination of temperature and B supply treatments at the reproductive stage. The values are means of four replicates, followed by standard deviation in parentheses

Treatment		1 DAST	3 DAST	7 DAST	12 DAST	18 DAST
Eighth leaf	-B, Tl	3.45 (0.84)	2.82 (0.78)	1.88 (0.50)	2.44 (0.24)	1.92 (0.34)
	-B, Ta	3.76 (1.31)	2.39 (0.26)	2.18 (0.25)	3.13 (0.86)	1.88 (0.56)
	+B, Tl	5.10 (0.42)	4.55 (0.25)	4.05 (0.70)	7.64 (1.04)	3.42 (0.18)
	+B, Ta	3.67 (0.28)	6.38 (0.68)	7.09 (0.47)	9.27 (0.95)	3.30 (0.82)
Ninth leaf	-B, Tl		ND	0.93 (0.65)	1.38 (0.74)	1.08 (0.20)
	-B, Ta			1.37 (0.55)	1.18 (0.84)	1.55 (0.42)
	+B, Tl			3.35 (0.51)	5.67 (0.84)	5.93 (0.69)
	+B, Ta			5.22 (1.37)	5.35 (0.17)	6.94 (1.40)
Ear	-B, Tl		ND		3.13 (1.51)	1.14 (0.53)
	-B, Ta				6.81 (1.12)	2.72 (1.80)
	+B, Tl				7.50 (1.32)	5.48 (0.81)
	+B, Ta				6.28 (0.48)	3.12 (0.24)

-B = 0 μ M boron added, +B = 10 μ M B added, Tl = low night temperature, Ta = ambient night temperature. The treatments of B supply and night temperature started at 31 days after transplanting when the eighth leaf was emerging. The eighth leaf was the youngest emerged blade at the start of treatment. The ninth leaf was the flag leaf. ND = not determined.

Table 3b. Statistical summary of effects of B supply and temperature treatments on B concentrations in the eighth and ninth leaves and the ears in wheat plants (cv. SW 41) for selected days after starting the treatment (DAST). The values are mean squares. Levels of significance are * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$

Source of variation	df	1 DAST	3 DAST	7 DAST	12 DAST	18 DAST	
Eighth leaf	B supply	1	1.9	32.7***	50.0***	128.6***	8.5***
	Temperature	1	1.0	1.9*	11.2***	5.3*	0.0
	Interaction	1	2.4*	5.1**	7.5***	0.9	0.0
	Residual	12	0.5	0.3	0.3	0.7	0.3
Ninth leaf	B supply	1		39.3***	71.6***	104.8***	
	Temperature	1		5.3*	0.3	2.2	
	Interaction	1		2.1	0.0	0.3	
	Residual	12		0.7	0.5	0.7	
Ear	B supply	1			13.7**	22.5***	
	Temperature	1			5.6	0.6	
	Interaction	1			22.2**	14.5**	
	Residual	12			1.4	1.1	

Table 4. Potassium leakage ($\mu\text{g K}^+$ per g fresh weight over 2 hours) out of the youngest emerged leaf blades of wheat (cv. SW 41) for selected days after starting the treatment (DAST). Plants were subjected to a factorial combination of temperature and B supply treatments at the reproductive stage. The values are means of four replicates, followed by standard deviation in the parentheses. Mean squares are shown together with levels of significance: * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$

	Treatment	1 DAST	3 DAST	7 DAST	12 DAST	18 DAST
	-B, Tl	227 (38)	230 (24)	246 (49)	187 (12)	170 (15)
	-B, Ta	185 (18)	295 (53)	194 (64)	189 (23)	206 (22)
	+B, Tl	199 (22)	236 (17)	329 (90)	177 (25)	205 (34)
	+B, Ta	159 (24)	254 (15)	210 (26)	164 (17)	174 (13)
Source of variation	df					
B supply	1	2948	1246	9555	1228	9
Temperature	1	6765 **	6988 *	29223 *	138	17
Interaction	1	2	2266	4523	204	4506 *
Residual	12	720	989	3837	397	518

-B = 0 μM boron added, +B = 10 μM B added, Tl = low night temperature, Ta = ambient night temperature. The treatments of B supply and night temperature started 31 days after transplanting when the eighth leaf was emerging. At 1 and 3 DAST, the youngest emerging blades (YEB) were the eighth leaves; at 7, 12 and 18 DAST, the YEB were the ninth leaves.

Table 5. Elongation rate (cm/day) of the ninth leaf blade of wheat (cv. SW 41) for selected days after starting the treatment (DAST). Plants were subjected to a factorial combination of temperature and B supply treatments at reproductive stage. The values are means of four replicates, followed by standard deviation in the parentheses. Mean squares are shown together with levels of significance: * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$

	Treatment	5-6 DAST	6-7 DAST	7-8 DAST	8-10 DAST
	-B, Tl	4.4 (0.47)	3.9 (0.32)	3.7 (0.40)	6.6 (1.09)
	-B, Ta	5.3 (0.14)	4.5 (0.27)	4.0 (0.92)	5.2 (0.96)
	+B, Tl	4.4 (0.32)	4.1 (0.52)	3.6 (0.24)	6.0 (0.49)
	+B, Ta	5.8 (1.34)	4.2 (0.35)	3.9 (0.30)	5.8 (0.39)
Source of variation	df				
B supply	1	0.23	0.03	0.02	0.0
Temperature	1	5.57 **	0.50	0.36	2.9
Interaction	NS				

Table 6. Boron concentrations (mg/kg dry matter) in the youngest emerged blades of wheat (cv. Wilgoyne) for selected days after starting the treatment (DAST). Plants were subjected to a factorial combination of temperature and B supply treatments at the vegetative stage. The values are means of four replicates, followed by standard deviation in parentheses. Mean squares are shown together with levels of significance: * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$

Treatment	0 DAST	3 DAST	6 DAST	9 DAST	12 DAST	
-B, Tl	2.05 (0.19)	0.93 (0.17)	0.54 (0.14)	0.85 (0.23)	0.89 (0.61)	
-B, Ta	2.05 (0.19)	1.74 (0.18)	0.68 (0.34)	1.01 (0.47)	1.26 (0.44)	
+B, Tl	2.63 (0.21)	3.05 (0.43)	3.55 (0.31)	4.33 (0.39)	4.07 (0.43)	
+B, Ta	2.63 (0.21)	3.60 (0.70)	4.14 (0.65)	5.45 (1.06)	4.36 (0.14)	
Source of variation	df					
B supply	1	1.2***	13.5***	35.8***	57.2***	36.4***
Temperature	1	0.0	1.6*	0.4	1.6	0.4
Interaction NS						

-B = 0 μM boron added, +B = 10 μM B added, Tl = low night temperature, Ta = ambient night temperature. The treatments of B supply and night temperature started 26 days after transplanting, when the seventh leaves were emerging.

Table 7. Potassium leakage ($\mu\text{g K}^+$ per g fresh weight over 2 hours) out of the youngest emerged leaf blades of wheat (cv. Wilgoyne) for selected days after starting the treatment (DAST). Plants were subjected to a factorial combination of temperature and B supply treatments at the reproductive stage. The values are means of four replicates, followed by standard deviation in parentheses. Mean squares are shown together with levels of significance: * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$

Treatment	0 DAST	3 DAST	6 DAST	9 DAST	12 DAST	
-B, Tl	249 (81)	277 (147)	218 (35)	335 (206)	270 (75)	
-B, Ta	249 (81)	334 (273)	412 (70)	268 (57)	273 (58)	
+B, Tl	240 (31)	346 (157)	260 (70)	257 (80)	274 (63)	
+B, Ta	240 (31)	429 (316)	224 (27)	309 (71)	251 (26)	
Source of variation	df					
B supply	1	306	26732	21462 *	1388	342
Temperature	1	0	19600	24806 *	189	420
Interaction	1	0	729	52900 **	14221	676
Residual	12	3794	55491	2962	14273	3401

-B = 0 μM boron added, +B = 10 μM B added, Tl = low night temperature, Ta = ambient night temperature. The treatments of B supply and night temperature started 26 days after transplanting, when the seventh leaf was emerging.

Table 8. Elongation rate (cm/day) of the seventh leaf blade of wheat (cv. Wilgoyne) for selected days after starting treatment (DAST). Plants were subjected to a factorial combination of temperature and B supply treatments at the vegetative stage. The values are means of four replicates, followed by standard deviation in parentheses. Mean squares are shown together with levels of significance: * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$

	Treatment	1-2 DAST	2-3 DAST	3-4 DAST	4-5 DAST	5-6 DAST
	-B, Tl	4.8 (1.45)	4.3 (0.96)	3.9 (0.40)	8.5 (0.28)	3.5 (0.64)
	-B, Ta	5.4 (0.45)	4.5 (0.69)	5.2 (0.80)	7.9 (0.40)	2.7 (0.32)
	+B, Tl	5.6 (1.03)	3.7 (0.46)	4.8 (0.60)	9.2 (0.45)	4.0 (0.33)
	+B, Ta	6.1 (1.99)	5.2 (0.30)	5.0 (0.34)	8.4 (0.62)	2.4 (1.07)
Source of variation	df					
B supply	1	2.4	0.0	0.4	1.4*	0.0
Temperature	1	1.3	2.7*	2.2*	1.9**	6.0**
Interaction NS						

Table 9. Effects of B supply and night temperature treatments for 18 days on the grain set index in wheat plants (cv. SW 41). The values are means of four replicates, followed by standard errors in parentheses

Treatment	CMU %	LAC %
-B, Tl	52.6 (18.2)	53.5 (14.4)
-B, Ta	6.8 (4.6)	19.3 (5.6)
+B, Tl	94.4 (3.0)	81.4 (4.8)
+B, Ta	95.2 (2.5)	80.4 (9.0)

-B = 0 μM boron added, +B = 10 μM B added, Tl = low night temperature, Ta = ambient night temperature. CMU % is defined as the percentage of grain-set florets out of the central 20 florets of an ear (the method devised and used at Chiang Mai University); LAC % is defined as the percentage of grain-set florets out of the total number of florets of an ear (the method used by Lumle Agricultural Research Centre).

In contrast to the responses of leaf blades, spikelet fertility and grain set index were significantly decreased by the -B treatment in the present study. A similar result was also observed in the study by Rerkasem et al. (1993). These effects of B deficiency are attributed to poor pollen viability at low B supply and possibly to low B in the stigma and style of the floret (Cheng and Rerkasem 1993). The depression in grain set index

in plants at low night temperature was associated with leaf B concentrations of 1 to 2 mg/kg and with ear concentrations of 1 mg/kg. In contrast, in plants at ambient night temperature, similar concentrations of leaf B were associated with substantially lower grain set index. Moreover, B concentrations in the ears at 12 and 18 DAT were actually higher in the plants at ambient temperatures than those at low night temperature, whereas grain set index was markedly lower. However, clearly, ear B concentrations of more than 3 mg/kg were adequate for grain set whereas concentrations of less than this at 18 DAT were associated with marked decreases in grain set index.

Low night temperature did not enhance the effects of the -B treatment on leaf blade elongation and K leakage from YEB in plants at vegetative and reproductive stages in the present experiment. It significantly delayed the development of plants at the reproductive stage, however, resulting in about six days' delay in anthesis of the main stems. As a result, the +B plants at low night temperature had fewer florets per spikelet than those at ambient night temperature.

The results suggest that the timing of B supply to the florets is crucial for the development of the reproductive parts and eventual fertilisation. There was a higher spikelet fertility and grain set index in the -B plants at low night temperature than those at ambient temperature. A part of the developmental process of ear and pollen in the -B plants at low night temperature might have coincided

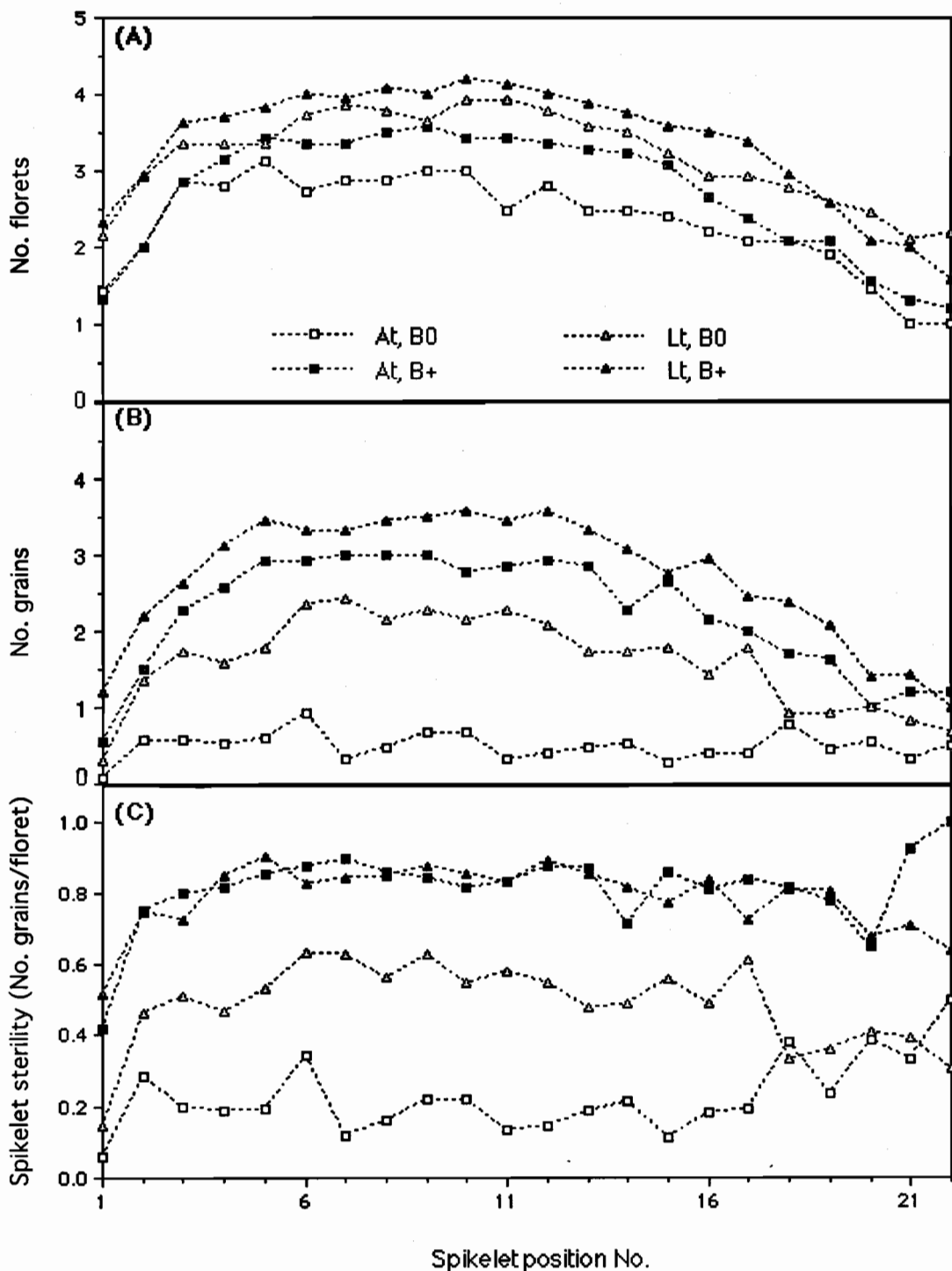


Figure 2. Effects of B supply and night temperature treatments on (A) number of florets per spikelet, (B) number of grain per spikelet and (C) spikelet fertility (number of grains per floret) in wheat plants (cv. SW 41) grown in the glasshouse. Tl = low night temperature; Ta = ambient night temperature, B0 = 0 μ M B added, B+ = 10 μ M B added.

with the resupply of B at the end of the treatment period (18 days after starting treatment). It is important to identify the critical stage of reproductive development when B deficiency causes irreversible damage to the fertility of florets. This knowledge can provide a basis for the correct timing of foliar B fertilisation to minimise the probability of grain set failure in wheat.

In conclusion, the results from the present study further confirmed the low internal B requirement for the vegetative growth of wheat plants. There was a distinct difference in sensitivity to B withdrawal between vegetative and reproductive parts of the same plant. This difference could be caused by the differences in external or internal B requirements, or both.

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