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Accepted Version

Gooding, M. J., Addisu, M., Uppal, R. K., Snape, J. W. and Jones, H. E. (2012) Effect of wheat dwarfing genes on nitrogen-use efficiency. Journal of Agricultural Science, 150 (1). pp. 3-22. ISSN 0021-8596 doi: 10.1017/S0021859611000414 Available at https://centaur.reading.ac.uk/20618/

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To link to this article DOI: http://dx.doi.org/10.1017/S0021859611000414

Publisher: Cambridge University Press

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Effect of wheat dwarfing genes on nitrogen use efficiency

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SUMMARY

Near isogenic lines (NILs) varying for alleles for reduced height (*Rht*) and photoperiod insensitivity (*Ppd-D1a*) in a cv. Mercia background (*rht* (tall), *Rht-B1b*, *Rht-D1b*, *Rht-B1c*, *Rht8c+Ppd-D1a*, *Rht-D1c*, *Rht12*) were compared at one field site, Berkshire, UK, but within different systems ('organic', O, in 2005/6, 2006/7 & 2007/8 growing seasons *v* 'conventional', C, in 2005/6, 2006/7, 2007/8 & 2008/9). In 2007 and 2008, further NILs (*rht* (tall), *Rht-B1b*, *Rht-D1b*, *Rht-B1c*, *Rht-B1b+Rht-D1b*, *Rht-D1b+Rht-B1c*) in both Maris Huntsman and Maris Widgeon backgrounds were added. The contrasting systems allowed NILs to be tested in diverse rotational and agronomic, but commercially-relevant, contexts particularly with regards the assumed temporal distribution of nitrogen availability, and competition from weeds.

With regards to the grain: nitrogen-use efficiency (*NUE*; grain DM yield/available N; where available N = fertilizer N + soil mineral N), recovery of nitrogen in the grain (grain N yield / available N), nitrogen utilization efficiency to produce grain ($NUtE_g$; grain DM yield / above ground crop N yield), nitrogen harvest index (grain N yield / above ground crop N yield), and dry matter harvest index (grain DM yield / above ground crop DM yield) all peaked at final crop heights of 800 to 950 mm. Maximum *NUE* occurred at greater crop heights in the organic system than in the conventional system, such that even adding just a semi-dwarfing allele (*Rht-D1b*) to the shortest background, Mercia, reduced *NUE* in the organic system. Mechanism of dwarfing (gibberellin sensitive or insensitive) made little difference to the relationship between *NUE* and its components with crop height.

With regards to above ground biomass: dwarfing alleles had a greater effect on DM accumulation compared with N accumulation such that all dwarfing alleles could reduce nitrogen utilization efficiency (crop DM yield / crop N yield). This was particularly evident at anthesis in the conventional area when there was no significant penalty for severe dwarfism for nitrogen accumulation, despite a 3 t/ha reduction in biomass compared to the tallest lines. Differences among genotypes for recovery of nitrogen in the grain were thus mostly a function of net nitrogen uptake after anthesis rather than of remobilized N. This effect was compounded as dwarfing, except when coupled with *Ppd-D1a*, was associated with delayed anthesis. In the organic experiments there was greater reliance on nitrogen accumulated before anthesis, and genotype effects on *NUE* were confounded with effects on nitrogen accumulated by weeds, which was negatively associated with crop height. Optimum heights for maximising wheat *NUE* and its components, as manipulated by *Rht* alleles, thus depend on growing system, and crop utilization (i.e. biomass or grain production).

INTRODUCTION

Efficient use of nitrogen by wheat is needed to sustain or increase yield and quality, whilst reducing the negative impacts of crop and fertilizer production on the environment (Hirel *et al.* 2007; Foulkes *et al.* 2009; Sylvester-Bradley & Kindred 2009). Increasing uptake efficiency (*NUpE*) to recover more nitrogen in the above ground crop (*Crop N* yield (*Y)*) reduces pollution with nitrous oxides, volatilized ammonia, and leached nitrate (Raun & Johnson 1999). Recovering more nitrogen in the grain (*Grain NY*) as protein (crudely N × 5.7) is particularly important to maintain bread making potential of higher yielding crops (Loffler *et al*. 1985; Slafer *et al.* 1990; Calderini *et al*. 1995). Increasing the efficiency with which the canopy *uses* nitrogen to produce biomass (Nitrogen utilization efficiency (*NUtE*)) or grain ($NUtE_g$)) is also important to maintain or increase yields whilst constraining nitrogen fertilizer application, and hence improve the energy balance of the crop (White 1981; Murphy & Helal 1996; Rosenburger *et al.* 2001). Moll *et al*. (1982) multiplied *NUpE* and $NUtE_g$ to derive nitrogen use efficiency (*NUE*), i.e. the grain yield of dry matter (*Grain DMY*) divided by the available (fertilizer + soil mineral) nitrogen. There is increasing economic imperative to improve *NUE* as the nitrogen fertilizer : grain price ratio increases in response to rising energy costs to fix nitrogen (Sylvester-Bradley & Kindred 2009).

There is a clear requirement to understand and exploit genetic effects to improve nitrogen-use efficiency (Hirel *et al*. 2007). Reduced height (*Rht*) alleles are widely incorporated in wheat breeding programmes to produce semi-dwarf wheats (Chapman *et al*. 2007). The commonly used *Rht-B1b* and *Rht-D1b* (previously *Rht1* and *Rht2*) semi-dwarfing alleles from ‘Norin 10’ reduce sensitivity to endogenous gibberellins (GA) (Murase *et al*. 2008), and individually often reduce

height by about 15 % (Gooding 2009). In the UK, *Rht-D1b*, has been widely used in elite lines since the 1970's (e.g cvs. Hobbit (1977) and Mardler (1978) were early examples (Austin *et al*. 1980)), although *Rht-B1b* has also been used more recently (e.g. cv. Robigus). More potent alleles, conferring severe dwarfism (>30 % height reduction) are available at both loci: *Rht-Blc* (previously *Rht3*) from 'Tom thumb', and *Rht-D1c* (previously *Rht10*) from 'Ai-bian' (Borner *et al*. 1997). Alternatively, reductions in height can be achieved without reducing GA sensitivity: *Rht8c* confers semi-dwarfism and is present in many Southern European wheats, often linked with *Ppd-D1a* mediated photoperiod sensitivity and more rapid development (Worland *et al*. 1998); *Rht12* is a severe-dwarfing, gamma ray-induced allele from 'Karcagi 522' (Korzun *et al*. 1997).

Dwarfing alleles in wheat must affect *NUE* because they influence *Grain DMY* at constant N availabilities (Flintham *et al*. 1997; Addisu *et al*. 2010). In such circumstances, adding semi-dwarfing alleles to excessively tall backgrounds increases *Grain DMY*, principally through increasing grain populations and the proportion of above ground crop biomass in the grain (Dry matter harvest index; *DMHI*; Flintham *et al.* 1997). Much less is known, however, concerning effects on *NUE* components: *NUpE* and $NUtE_g$. Gale and Youssefian (1985) found *Rht-B1b*, *Rht-D1b* and *Rht-B1c* to either have no effect on *Grain NY* even when *Grain DMY* was increased, or to reduce *Grain NY* more than *Grain DMY* (as was the case for *Rht-B1c*). Gooding *et al*. (1997) found *Rht-B1b* to increase *Grain NY* but they, with Gale & Youssefain (1985), report reductions in grain nitrogen concentrations (or increased *NUtE* of the grain). When Flintham & Gale (1983) found *Rht-B1c* to significantly reduce grain nitrogen concentrations despite also appearing to reduce *Grain DMY* they speculated that this may have been due to reduced availability of crop N for remobilization; Austin *et al*. (1977) had previously suggested that dwarfing may be

associated with reduced *NUpE*, and Wojciechowski *et al*. (2009) found severe dwarfing to be associated with reduced rooting in the field. Cosser (1996), however, found no effect of *Rht-B1b* or *Rht-D1b* on *Crop NY*.

Dwarfing alleles can increase or reduce *DMHI* depending on the height of the background, and the degree of shortening achieved, (Flintham *et al*. 1997; Gooding 2009) with likely implications for $NUtE_g$ (Ortiz-Monasterio *et al*. 1997). Fernandez *et al.* (2009) argue that impaired GA synthesis or signaling in dwarfed cereals would reduce *Crop DMY*, and this can be demonstrated in wheat (Addisu *et al*. 2009), but little is known concerning effects on *NUtE* of the crop biomass.

It is evident that genotype effects on components of *NUE* interact with cropping system. Barraclough *et al*. (2010) found no correlation between a cultivar's ranking for *Crop NY* when no fertilizer N was supplied, compared to when 200 kg N/ha applied around the start of stem extension. Modern, shorter wheats appear better able to recover fertilizer nitrogen when it is applied at and after the start of stem extension (Foulkes *et al*. 1998). However, alleles conferring reduced sensitivity to GA can sometimes reduce earlier nitrogen capture (Addisu *et al*. 2009), and more modern, shorter lines can be poorer at exploiting soil N (i.e. not from contemporary fertilizer applications) (Foulkes *et al*. 1998). These latter effects would be particularly unwelcome in more extensive, and/or organic systems where a greater proportion of N is available in earlier growth stages, particularly if the wheat follows a fertility building phase such as a legume-rich ley or green manure (Dawson *et al*. 2008; Baresel *et al*. 2008; Wolfe *et al*. 2008). It is also likely that dwarfing allele effects on $NUtE_g$ will interact with cropping system as improvements in *DMHI* associated with semi-dwarfing alleles are achieved less reliably in extensive and/or organic systems of production (Addisu *et al*. 2010).

In the work reported here we seek to address three aspects of the effects of dwarfing genes on the nitrogen economy of wheat with the use of near isogenic lines (NILs). Firstly, there is a need to clarify the above responses by assessing the effects of a range of alleles and their combination that confer different height reductions with GA insensitivity (*Rht-B1b*, *Rht-D1b*, *Rht-B1c*, *Rht-D1c*). Secondly, we compare the effects of dwarfing alleles that do not rely on GA insensitivity such as *Rht12* and *Rht8c* + *Ppd-D1a*. Finally, we compare the effect of dwarfing alleles on the nitrogen economy of wheat grown in contrasting production systems ('*conventional*' vs. '*organic*').

MATERIAL AND METHODS

Crop husbandry and experimental design

Seven field experiments compared near-isogenic lines (NILs) of wheat varying for major dwarfing genes, replicated in complete randomized blocks (Table 1). Replication, plot lengths and seed rate varied with experiment (Table 1). All experiments were conducted within the same 10 ha site at the Crops Research Unit, Sonning, University of Reading, UK (51° 29' N, 0° 56' W), on a free-draining sandy loam (9% clay, particle size<0.2 μm; 19% silt, 2-20 μm; 72% sand, 20-2000 μm; pH=6.3-7.1) overlying coarse red-brown sand. The site is split between an area receiving synthetic agrochemicals and fertilizers, managed conventionally, and an area managed organically since 2001 (Addisu *et al*. 2009). The experiments on both the conventional and organic areas followed 2-3 year leys: perennial ryegrass plus clover on the organic area, and unfertilized perennial ryegrass on the conventional area. The aim was to contrast the high

autumn N availability typical after a clover-rich ley in organic rotations, with the more depleted autumn N-availability likely in many conventional combinable crop rotations. Before ploughing, the grass ley in the conventional area was destroyed with 1.8 kg/ha glyphosate; an insecticide, chlorpyriphos (0.45 kg/ha) was also applied in 2006. Soil was tested for pH, phosphorous, potassium and magnesium availability (Anon. 1986), and corrective nutrients applied as appropriate. In the organic area the ley was mown tightly. On both areas, turf was buried by mould-board ploughing to 300 mm. On the organic areas, stale-seed beds were prepared and destroyed 3 times on a 10 day cycle with a power harrow (Lely Roterra), before a final pass with the power harrow on both areas.

Untreated seeds were drilled between 21 September and 4 October at a nominal depth of 50 mm, on 120 mm rows in 2 m wide plots separated by 0.5 m double-width track wheelings. Weather data (Fig. 1) were recorded at an automated meteorological station at the site.

Conventional management of the wheat typically involved: herbicide applications at growth stage (GS, Zadoks *et al*. 1974) 19 and/or 31-32; and fungicide applications at GS 30-31, 39 and 59. No plant growth regulators were applied. Full details of sprays used for 2005/06 to 2007/08 are available in Addisu *et al*. (2010). In each year, 100 kg N/ha + 40 kg S/ha was applied as a mixture of ammonium nitrate and ammonium sulphate at GS 30-31. A further 100 kg N/ha was applied as ammonium nitrate between GS 34-39. In the organic area, wheat was established after two or three years of perennial ryegrass plus clover ley.

All experiments included seven near isogenic lines (NILs) in a cv. Mercia background (*rht* (tall), *Rht-B1b*, *Rht-D1b*, *Rht-B1c*, *Rht8c+Ppd-D1a*, *Rht-D1c*, *Rht12*). Mercia was introduced commercially in 1983, and was the last widely used winter wheat cultivar suited for bread-making in the UK which did not have a major semi-dwarfing gene. Despite the lack of an *Rht*

allele, Mercia was only slightly taller than cv Hereward (*Rht-D1b*; Anon. 1991), its successor as the major bread making wheat cultivar in the UK. Hereward is actually shorter than more recent introductions such as Xi19 (2002; *Rht-D1b*) and Solstice (2002; *Rht-D1b*), and comparable to cv. Gallant (2009) (Anon. 2009). i.e. It can be considered that the height of Mercia without a major *Rht* allele is within the range commonly observed for modern commercial releases in the UK, that have an *Rht* allele. In the last two years, therefore, NILs with taller backgrounds were included in the experiment, i.e. Maris Widgeon (1964) and Maris Huntsman (1969) comprising (*rht* (tall), *Rht-B1b*, *Rht-D1b*, *Rht-B1c*, *Rht-B1b+Rht-D1b*, *Rht-D1b+Rht-B1c*). Source of dwarfing genes and markers used are in Addisu *et al*. (2009).

Nitrogen availability and assessments

Nitrogen availability is calculated as the sum of soil nitrogen supply plus any nitrogen applied as fertilizer. In the conventional area, the soil nitrogen supply is taken as the above ground crop N at maturity of plots receiving robust agrochemical programmes for crop protection, but no fertilizer N (Sylvester-Bradley & Kindred 2009). This value averaged 78 kg N/ha for the four year period 2005/6 to 2008/9, which combined with the fertilizer N, provided 278 kg of available N. In the organic areas soil nitrogen supply was taken as the amount in the above ground crop of cv. Mercia *rht*(tall) lines plus the amount of N in the weeds at crop maturity. This sum averaged 173 kg N/ha for the three year period 2005/6 to 2008/9. Soil total carbon : total nitrogen concentrations (mg/g DM, by oxidative combustion) for the site average 9.0:0.9, 5.8:0.6, and 7.5:0.3, for the 0-300 mm, 300-600 mm, and 600-900 mm depth horizons respectively. Soil mineral N was also determined from bulked samples collected to 900 mm by auger from across

the experimental site at the end of February; and this added to the crop N at the same time gave an assessment of available N before stem extension (Table 1, Anon. 2000).

Crop height was calculated as the mean of three measurements with a rising disc of polystyrene (Peel 1987): at anthesis, the end of grain filling, and at harvest maturity. Ear populations were assessed from between two and four randomly placed $0.1m^2$ circular quadrats per plot during the week before combine harvest. For each of the seven experiments, the central portion of each plot was combine harvested at maturity with a 1.3 m cutter bar, i.e. combined area varied between 13 and 9.75 m^2 for the 10 and 7.5 long plots (Table 1) respectively. The effective distance from the edge of a sampled and harvested area, to the outside row of a neighboring plot was, therefore, maintained at 0.85 m to minimize the shading of short lines growing next to taller lines. Mean grain weights were determined from a divided sample of at least 250 grains per plot. Grain samples (20 g per plot) were dried at 80 ^{o}C for 48 h to determine moisture content, and to adjust yields and mean grain weights to a dry matter basis. Samples (100 g per plot) were milled using a Laboratory Mill 3100 (Perten Instruments AB, Huddinge, Sweden). Resultant wholemeal flour was dried and assessed for nitrogen concentration using oxidative combustion with a LECO FP-528 (LECO Instruments, Stockport, UK). Nitrogen per grain (*NpG*) was calculated from mean grain weight and nitrogen concentration. Results from the combine harvested grain allowed the calculation of *NUE*, and *Grain NY*:

(1)

(2)

Additionally *NUtE* of the grain was calculated as the inverse of grain nitrogen concentration (Foulkes *et al*. 1998):

(3)

In both the conventional and organic areas in 2006 and 2007, and just the conventional area in 2009, nitrogen uptake and partitioning between anthesis and harvest maturity was assessed from destructive samples (1 x 0.5 m quadrat per plot per assessment in 2006 and 2007, and 3 x 1 m row lengths per plot per assessment in 2009). At anthesis (GS 65), above ground crop was collected, dried, weighed and milled. Date of anthesis, and hence date of sampling varied amongst backgrounds and alleles (Fig. 2). At harvest maturity, ears were removed and threshed, with grain and chaff being collected separately. Samples at harvest maturity were taken at the same time for all alleles. Nitrogen concentration was determined for the vegetative and grain tissues as described for the combine harvested grain. The destructive samples allowed the interpretation of effects on *Grain NY* in terms of above ground *Crop NY* and above ground nitrogen harvest index (*NHI*), or more functionally, as the sum of nitrogen lost from the vegetative tissue (assumed to be remobilized N (*RemobN*)), and the net nitrogen taken up in to the above gound crop, between anthesis and maturity (late N uptake (*LNUp*)) (Cox *et al.* 1986; Gooding *et al*. 2005; Hirel 2007):

(4)

(5)

(6)

(7)

Remobilization efficiency (*Remobeff*) of N is expressed as a proportion of N in the crop at anthesis not present in straw (non-grain) components at harvest (Cox *et al*. 1986; Slafer *et al*. 1990):

(8)

The destructive samples also allowed the calculation of *NUpE* of the above ground crop; the *NUtE* to produce biomass and grain ($NUtE_g$); and an assessment of *Grain NY* and *NUtE* of the grain not confounded by any effects of alleles on combine harvesting efficiency.

(9)

(10)

(11)

Statistical analyses

An analysis of Residual Maximum Likelihood (REML) using Genstat 10 was used to investigate main effects and interactions between system and allele in the Mercia background, where system and allele combinations were replicated over year. i.e. the fixed model was System × Allele and the random model comprised Year / System / Block /. This analysis revealed significant interactions between system and allele (Table 2). Analyses using all backgrounds and alleles were therefore assessed within each growing system with the fixed model of Background (Mercia, Maris Huntsman, or Maris Widgeon) × Allele, and the random model of Year / Block /. The relationships between allele effects on height (presumed explanatory variable) and allele effects on response variables were investigated by quadratic regression with background as a grouping factor (Fitted model=pol(ht;2)+background). For presentation purposes the main effects of background on the response variates have been removed by subtracting the background effects on the regression constant so as to rebase the constant to that of Mercia. Finally, to test statistically the effect of system on the height of NILs needed to maximize *NUE*, quadratic regressions were fitted within each year and system and peak height derived empirically before

subjecting estimated peak heights to analysis of variance (Block structure = year ; treatment structure = system).

RESULTS

There were significant (P<0.05) system × allele interactions amongst the Mercia NILs for *NUE* and its components, *NUpE* and $NUtE_g$ (Table 2). Interactions were largely a result of the scale of the differences between alleles, rather than relative differences or ranking position within systems, as demonstrated by high levels of correlation in allele performance between the two systems (Table 2). Nonetheless, *Rht-D1b* in Merica had no effect on the *NUE* in the conventional system (Fig. 3), but significantly reduced yield (compared with Mercia *rht* (tall)) in the organic system (Fig. 4). In contrast, adding semi-dwarfing alleles *Rht-B1b* and *Rht-D1b* to the taller backgrounds Maris Widgeon and Maris Huntsman increased *NUE* in the conventional system (Fig. 3), but not by a statistically significant margin in the organic system (Fig. 4). In both conventional and organic experiments the response of *NUE*, *Grain NY*, *NUtE* of the combine-harvested grain, and dry matter harvest index (*DMHI*) to height deviated significantly from linearity (Fig. 3, 4). Over the range of heights observed the quadratic fits appeared appropriate and in each case the quadratic effect was negative and very highly significant ($P<0.001$). Fitted negative quadratic effects were also significant (P<0.05) for *NUE* and a number of its components in the hand-harvests in the conventional experiments (Fig. 5E,F,G,H,K,L,M; Fig. 6). For the hand harvests in the organic area, only samples from Mercia NILs were available, substantially reducing the range and number of heights available. For the hand harvests, therefore, only linear fits could be supported statistically (Fig. 7) except for $NUtE_g$ and *NHI* (Fig. 8).

Despite very low error degree of freedom (2) for testing the effect of system on peak height for optimizing *NUE* for combine harvested grain the influence was nonetheless significant ($P<0.05$; S.E.D. =19.9 mm); peak height was fitted to be 150 mm lower in the conventional system (Fig. 3) than in the organic system (Fig. 4). Main effects of system, allele and their interaction necessarily derive from effects on yield components. Higher grain yields in the conventional system resulted from greater numbers of ears/m^2, mean grain weights, and for most genotypes, increased grains per ear (Fig. 9). The quadratic response of grain yield and *NUE* to height was largely a reflection of effects on grain numbers per ear (Fig. 9C), as was increased optimal height for *NUE* in the organic system compared with the conventional system. The supra-optimal height of Maris Widgeon and Maris Huntsman *rht* (tall) were particularly associated with low grain numbers per ear, whereas there seemed no penalty for height with regards to ear population (Fig. 9A) nor mean grain weight (Fig. 9B) with generally positive associations observed over the range of heights acheived. In Mercia, *Rht-D1b* was associated with reduced mean grain weight in both systems (Fig. 9B), but this was only compensated by apparently increased grain numbers per ear in the conventional system.

Nitrogen economy in the conventional experiments

Nitrogen recovery in the above ground crop (Nitrogen uptake efficiency; NUpE)

In contrast to effects on *NUE*, nitrogen accumulated in the above-ground crop by anthesis (*Crop* $NY_{anthesis}$) was not closely related to height (Fig. 5A). The shortest lines, i.e. the GA-sensitive *Rht12* and the GA-insensitive *Rht-D1c* (labeled 10 in Fig. 5A), had comparable *Crop* $NY_{anthesis}$ to much taller lines, and to each other. The photoperiod insensitive *Rht8c+Ppd-D1a* combination (8

in Fig. 5A) in Mercia accumulated less *Crop* $NY_{anthesis}$ than the photoperiod sensitive *Rht-D1b* (2 in Fig. 5A) despite being of similar stature. Allele effects on stature were confounded with their effects on rate of development, i.e. dwarfing was associated with delayed anthesis (Fig. 2) whether achieved with GA insensitivity (e.g. *Rht-D1c*), or not (*Rht12*). *Ppd-D1a* with *Rht8c* was associated with earlier anthesis; by more than eight days compared to lines of similar stature but with greater photoperiod sensitivity. Multiple regression (Table 3) suggested that 3 kg N/ha was accumulated for every day that anthesis was delayed. After accounting for this effect, a quadratic response of *Crop* $NY_{anthesis}$ to height could be discerned: the estimated effects of height (Table 3) implying a peak of 160 kg N/ha at 840 mm, declining to 109 kg N/ha at 300 mm, and slightly to 152 kg N/ha at 1050 mm. Whether accounting for anthesis date or not, there was no evidence that adding either of the Norin 10 alleles (*Rht-B1b*, *Rht-D1b*) to the tallest backgrounds (Maris Huntsman and M. Widgeon) reduced *Crop* $NY_{anthesis}$.

Late nitrogen uptake (*LNUp*) between anthesis and maturity was reduced substantially by severe dwarfism (Fig. 5D), only amounting to about 15 kg N/ha for the shortest, and latest flowering line, Mercia *Rht12*, and for Maris Widgeon *Rht-D1b* + *Rht-B1c* (2+3). Semi-dwarfed and *rht* (tall) lines, however, achieved *LNUp* averaging 75 kg N/ha. The GA-sensitive *Rht8c*+*Ppd-D1a*, having accumulated less *Crop* $NY_{anthesis}$ associated with its earlier flowering, compensated with higher *LNUp*, such that *Crop* $NY_{maturity}$ was broadly equivalent to that of Mercia *Rht-D1b*, a line of similar height (Fig. 5F), but achieved with GA-insensitivity. The fitted response in Fig. 4F peaks with a *Crop* $NY_{maturity}$ of 233 kg N/ha, equating to a *NUpE* of 0.84. There was, however, no statistical evidence that adding single semi-dwarfing alleles to tall backgrounds affected *Crop* $NY_{maturity}$. Using the quadratic fits in Figs 5D and F the penalty for reducing crop height from 800 mm to 300 mm on *Crop* $NY_{maturity}$, and thereby also delaying

anthesis by 9 days, was about 80 kg N/ha with 60 kg N/ha of this being accounted for by reduced *LNUp*. At 800 mm, 67 % of *Crop* $NY_{maturity}$ had been accumulated by anthesis.

Nitrogen recovery in the grain (Grain NY)

Nitrogen recovered in the combine-harvested grain peaked at about 800 mm: the maximum fitted value of 160 kg N/ha representing 57.5 % of available N (Fig. 3). A similar response was seen in the hand harvests but with less apparent penalty for excessive height (Fig. 5K). Remobilization efficiency (*Remobeff*) and nitrogen harvest index (*NHI*) peaked at 0.58 (not presented) and 0.72 (Fig. 6) respectively. Despite the quadratic fits, in neither combine-, nor hand-harvested grain was there a statistically significant increase in *Grain NY, Remobeff,* or *NHI* by adding single semi-dwarfing alleles to the two tallest backgrounds. The *Grain NY* of the GA-sensitive dwarfing alleles (*Rht12, Rht8c+Ppd-D1a*) were similar to those of GA-insensitive alleles conferring similar heights (Figs 3 and 5K). The hand harvest (Fig. 5) revealed that effects of alleles on *Grain NY* could be attributed more to *LNUp* than to *RemobN*: using the quadratic fits, there was an 80 kg N/ha increase in *Grain NY* as heights increased from 300 mm to 800 mm; 62 kg of this derived from increased *LNUp*, and only 18 kg from increased *RemobN*. Of the 18 kg attributed to *RemobN*, 11 kg could be ascribed to effects on *Crop* $NY_{anthesis}$ (using a mid point *Remobeff* of 0.55) and 7 kg to improved *Remobeff*. Similarly, the allele effects on *Grain NY* are more a function of *Crop* $NY_{maturity}$ (Fig. 5F) than *NHI* (Fig. 6): about 67 % of the increase in *Grain NY* between 300 mm and 800 mm can be ascribed increased *Crop* $NY_{maturity}$ and the remainder to improved *NHI*. All of the quadratic response of *Grain NY* to height is associated with effects on grain numbers rather than on nitrogen per grain (*NpG*). Indeed, *NpG* for Maris Huntsman NILs increased at heights either sub- or supra-optimal for *Grain NY* (Fig. 3). i.e. For just this

background both the linear (-0.0043 mg/mm; S.E. = 0.00128) and quadratic (0.000030; S.E. = 0.0000094) effects of height were significant ($P < 0.05$) with signs opposite to those for *Grain NY*.

Nitrogen utilization efficiency (NUtE)

Effects of alleles on dry matter accumulation by anthesis (*Crop* $DMY_{anthesis}$; Fig. 5B) contrasted with their effects on *Crop* $NY_{anthesis}$. There was a positive linear association between *Crop* $DMY_{anthesis}$ and height, even as heights exceeded 1 m. When accounting for anthesis date on *Crop* $DMY_{anthesis}$ (about 0.15 t DM/ha/d) deviation from linearity was significant (Table 3) but the predicted response to height still failed to reach a peak over the observed range: the linear and quadratic terms for height (Table 3) implying *Crop* $DMY_{anthesis}$ of 3.5 t/ha at 300 mm, 8.6 t/ha at 800 mm, and 9.5 t/ha at 1050 mm. There is, therefore, an indication that adding any dwarfing alleles, even single semi-dwarfing alleles to tall backgrounds, can reduce *Crop* $DMY_{anthesis}$. This disproportionate effect of dwarfing alleles on *Crop* $DMY_{anthesis}$ compared to on *Crop* $NY_{anthesis}$ produced a close negative relationship between height and crop *NUtE* at anthesis (Fig. 5C), such that there was a significant negative effect of adding single semi-dwarfing alleles to tall backgrounds on *NUtE*. This negative relationship was unaffected by dwarfing mechanism (Fig. 5C).

Net accumulation of biomass between anthesis and maturity increased only as height increased to 800 mm (Fig. 5E). The response of *Crop* $DMY_{maturity}$ to height was similarly curved (Fig. 5G), as was crop *NUtE* at maturity (Fig. 5H). Despite the curvature, crop *NUtE* at harvest was still significantly reduced by single semi-dwarfing alleles (*Rht-B1b*, *Rht-D1b*, *Rht8c+Ppd-D1a*) in Mercia and Maris Huntsman backgrounds, as well as in all the severely dwarfed lines

(Fig. 5H). Again, the negative relationship between height and *NUtE* to produce biomass at maturity appeared unaffected by the mechanism of dwarfing.

Allele and height effects on nitrogen utilization efficiency to produce grain ($NUtE_g$) did vary somewhat from their effects on *NUtE* to produce biomass. $NUtE_g$ (Fig. 6) and *NUtE* of the grain (Figs 3 and 5M) were optimized at around 800 mm, whether achieved by adding single semi-dwarfing alleles to the tallest background, or with the shorter background, Mercia, without a major dwarfing gene. Heights associated with producing the highest *Grain NY* were, therefore, associated with the lowest protein concentrations in the grain (Fig. 3; minimum fitted back transformed crude protein concentration = 136 mg/g). Adding semi-dwarfing genes (*Rht-B1b* or *Rht-D1b*) to both the tallest backgrounds significantly increased $NUtE_g$ (Fig. 6) and/or *NUtE* of the grain (Figs 3 and 5M). Mercia *Rht-D1b* and *Rht8c+Ppd-D1a* produced equally high $NUtE_g$ despite varying in dwarfing mechanism and photoperiod sensitivity. The GA-sensitive *Rht12* did have significantly poorer $NUtE_g$ (Fig. 6) and *NUtE* (Fig. 5M) of the grain than the shortest GA-insensitive line (*Rht-D1c*). Poor $NUtE_g$ (or *NUtE* of the grain) of tall lines and of *Rht12* compared to effects on *NUtE* to produce biomass are necessarily a function of their negative effects on *DMHI* (Fig. 3).

Nitrogen-use efficiency

The fitted maximum combine-harvested grain yield of 6.69 t DM/ha at 810 mm (Fig. 3), equated to a *NUE* of 24.0 kg grain DM/kg available N. As with $NUtE_g$ and *NUtE* of the grain, optimum height for *NUE* was approached by adding single semi-dwarfing alleles to excessively tall backgrounds, or by using a background that had appropriate stature without a major dwarfing allele. A similar response was seen in the hand harvests but with less apparent penalty for

excessive height (Fig. 5L). For neither harvest method was there any evidence that dwarfing mechanism influenced the relationship between height and *NUE*. Differences among alleles and responses to height for *Grain DMY*, and hence *NUE*, can be attributed approximately equally between effects on *Crop* $NY_{maturity}$ (Fig. 5F) and $NUtE_g$ (Fig. 6). Of the 4 t/ha reduction in *Grain DMY* associated with reducing height from 800 mm to 300 mm (Fig. 5L), 1.9 t/ha can be attributed to *Crop* $NY_{maturity}$ (using a midpoint $NUtE_g$ of 23.5 kg DM/kg N) and 2.1 t/ha to $NUtE_g$ (using a midpoint *Crop* $NY_{maturity}$ of 187 kg N/ha).

Nitrogen economy in the organic experiments

Nitrogen recovery in the above ground crop (Nitrogen uptake efficiency; NUpE)

In contrast to the conventional experiments, time to anthesis did not have a significant effect on *Crop* $NY_{anthesis}$. For example, the *Rht8c + Ppd-D1a* line accumulated similar quantities of nitrogen by this stage to that of *Rht-D1b* (Fig. 7A) despite the former flowering nine days earlier (Fig. 2). *Rht12* accumulated the least nitrogen, despite flowering last. In the organic experiments, there was a significant penalty for dwarfism despite dwarfism usually extending the time to anthesis. *LNUp* was much less in the organic- than in the conventional experiments, and again significantly reduced with dwarfism even to the extent that severe dwarfing alleles (*Rht-B1c*; *Rht-D1c*; *Rht12*) were associated with apparent loss of nitrogen from the above ground crop after anthesis. Using the linear fits in Fig. 7, the 78 kg N/ha increase in *Crop* $NY_{maturity}$ that occurred between 300 mm and 800 mm is the sum of 37 kg N/ha from increased *Crop* $NY_{anthesis}$ and 41 kg N/ha from increased *LNUp*. At 800 mm, 84 % of *Crop* $NY_{maturity}$ had been accumulated by

anthesis. The poorer crop nitrogen recoveries in the dwarfed lines were associated with greater quantities being recovered in the above ground weed biomass (Fig. 10).

Nitrogen recovery in the grain (Grain NY)

In the organic experiments, fitted *Grain NY* from the combine harvest peaked at 900 mm with 60.2 kg N/ha or 34.8 % of available N (Fig. 4). Net loss from non-grain tissues between anthesis and maturity (*RemobN*; Fig. 7I) was not closely associated with crop height. i.e. Positive relationships between crop height and *Grain NY* (Fig. 7K) largely reflected effects on *Crop* $NY_{anthesis}$ and *LNUp*. Using the linear fits in Fig. 7, increasing height from 300 mm to 800 mm was associated with an increase in *Grain NY* of 60 kg N/ha; 41.5 kg N/ha deriving from *LNUp* and 18.5 kg N/ha from *RemobN*. Of the *RemobN*, 17.8 kg N/ha can be accounted for by effects on *Crop* $NY_{anthesis}$ with an average *Remobeff* of 0.48. Empirically, 57 % of the increase in *Grain NY* between crop heights of 300 and 800 mm can be ascribed to *Crop* $NY_{maturity}$ (using a mid point *NHI* of 0.45) and the remainder to improved *NHI* (Fig. 8). As with the conventional experiments *Grain NY* effects were unrelated to effects on *NpG* so *Grain NY* was mostly a function of grain population.

Nitrogen utilization efficiency (NUtE)

As in the conventional experiment, dwarfing alleles had greater effects on dry matter accumulation by anthesis (Fig. 7B) than on nitrogen accumulation such that *NUtE* at anthesis was significantly reduced by dwarfism (Fig. 7C). It is notable that dry matter yields at anthesis of the taller Mercia lines were greater in the organic experiments than in the conventional experiments (Fig. 7B *v.* Fig. 5B), despite nitrogen yields being greater in the latter (Fig. 7A *v.*

Fig. 5A). Reduced nitrogen uptake, but greater dry matter production, was therefore associated with much higher *NUtE* at anthesis (around 90 kg DM/kg N for the Mercia *rht* line; Fig. 7C) compared to in the conventional experiments (around 55 kg DM/ kg N for the Mercia *rht* line; Fig. 5C).

As for nitrogen, severe dwarfism in the organic experiments was associated with apparent loss of biomass between anthesis and maturity (Fig. 7C). The negative effect of dwarfing genes on dry matter accumulation both before and after anthesis produced a close negative relationship between height and *Crop* $DMY_{maturity}$ (Fig. 7G) such that even some of the semi-dwarfing alleles (*Rht-D1b*; *Rht8c* + *Ppd-D1a*) in Mercia were associated with statistically significant reductions in biomass production. Again, allele effects on biomass production were greater than on nitrogen accumulation such that there was a negative effect of dwarfism on *NUtE* of the crop at maturity (Fig. 7H). *Rht12* had significantly lower crop *NUtE* at maturity compared with *Rht-D1c*. Effects of dwarfing alleles in Mercia on *GrainDM* (Fig. 7L) and *NUtE* of the grain (Fig. 7M) were similar to their effects on *CropDM* and *NUtE*.

Nitrogen-use efficiency (NUE)

The fitted maximum combine-harvested yield for the organically-grown NILs was 3.38 t/DM/ha at 960 mm representing a nitrogen use efficiency of 19.5 kg grain DM/kg available N. Although the fitted optimum height for *NUE* was apparently taller for the organic crops compared to the conventional crops (Fig. 3 *vs* Fig. 4), and the penalty for excessive height less severe (and not statistically significant, $P>0.05$), fitted peak yields were still achieved by adding dwarfing genes to the tallest backgrounds (Maris Huntsman and Maris Widgeon) or by omitting major dwarfing genes from the apparently suitably-statured background (Mercia). Also in common with the

conventional experiments, GA sensitive alleles produced similar *NUE* to the GA insensitive alleles of similar height. Grain *NUtE* (*Grain DMY*/*Grain NY*) appeared to have a more influential effect on *NUE* in the organic-, compared to the conventional experiments, with much higher values being achieved in the higher yielding lines, and no penalty for the heights of *rht* (tall) M. Widgeon and M. Huntsman being observed (Fig. 4). From the hand harvests, the empirical contributions of *Crop* $NY_{maturity}$ and $NUtE_g$ (Fig. 8) to the *Grain DMY* yield increase of 3.5 t DM/ha between 300 and 800 mm, were approximately equal (46 % attributed to *Crop* $NY_{maturity}$ using the average *NUtEg* of 22 kg DM/kg N).

DISCUSSION

The fitted maximum combine harvested grain yields, equating to 7.9 and 4.0 t/ha at 85 % DM for conventional and organic systems respectively, are consistent with average commercial yields in the UK (FAOSTAT average for UK 2006-2008 = 7.8 t/ha; Lampkin *et al.* (2006) estimate 4.5 t/ha for UK organic). In the conventional area the soil N availability + fertilizer N quantity of 78 + 200 kg N/ha were close to UK estimates of 80 + 187 kg N/ha (Sylvester-Bradley & Kindred 2009). The peak *NUE*, *NHI* and $NUtE_g$ were also close to the calculated averages for UK milling wheat of 22 kg DM/kg N, 0.75 and 33 kg DM/kg N respectively (Sylvester-Bradley & Kindred 2009), and well within the ranges found previously found for wheat grown at this site with similar husbandry (Kindred & Gooding 2004). There is less comparative data for organic wheat. The approach used here to estimate available N (*Crop NY* + *Weed NY*), although comparable to the approach for the conventional wheat (*Crop NY* at zero N fertilizer), is open to criticism. Clearly more soil mineral N is potentially available in the organic system, and a significant amount of this would be at risk of loss. Baresel *et al*. (2008) estimated total mineralized N under

German organic winter wheat following three year fertility building phases to average about 175 kg N/ha, similar to the value we use for available N. However, the German work also calculates 40 kg N/ha to be lost during winter, and it is quite possible that our value underestimates total N availability by a similar quantity. Nonetheless, peak *Grain NY* in the organic wheat presented here is 15-20 kg N/ha less than average values reported by Baresel *et al*. (2008). The peak values of *NUE* and *NUtEg* of 20, and 32 kg DM/kg N for organic wheat presented here, are also lower (24 and 42 in the German work respectively). The performance of wheat in our organic system was compromised by the weeds, with 15 % of available nitrogen being recovered in weeds growing in the plots of Mercia (tall), the most competitive Mercia line. It is possible that *NUE* would have improved if post-establishment weed control measures, such as finger-tine hoeing had been employed, or if crop seed rates had been increased to improve competitive ability, but neither method necessarily increases *Grain NY* or *NUtE* of the grain in organic systems (Samuel & East 1990).

The comparison between organic and conventional results would support previous suggestions that capture of resources made available during early crop growth stages is more important in systems mostly reliant on soil N from previous biological fixation, compared to systems reliant on high synthetic fertilizer inputs timed to periods of peak crop demand (Dawson *et al*. 2008; Baresel *et al*. 2008; Wolfe *et al*. 2008). In the organic experiments this would explain: the lack of an effect of allele anthesis date on *Crop* $NY_{anthesis}$; the reduced contribution of *LNUp* to genotypic effects on $CropN_{maturity}$; and over 80 % of $CropN_{maturity}$ of higher yielding lines being accumulated apparently by anthesis. The 80 % figure is consistent with other field experiments with low levels of synthetic fertilizer (Kindred & Gooding 2004), and broadly with other organic experiments (Baresel *et al*. 2008). In our organic experiments, the poor

performance of the severe dwarfs would have been exaggerated by their reduced competitiveness with weeds (Gooding *et al*. 1997), again emphasizing the need for early resource capture by the crop in such systems (Lemerle *et al*. 1997). We concur with others that organic systems can provide sufficient N for wheat, but that there needs to be better matching of the timing of N availability with that of crop demand (Baresel *et al*. 2008).

We demonstrate that *Rht* effects on *Crop* $NY_{anthesis}$ are confounded with their effects on date of anthesis. We confirm the delay in maturity associated with *Rht12* (Worland *et al*. 1994), *Rht-B1b* (Giunta *et al*. 2007), and *Rht-D1b* (Addisu *et al*. 2009) but further illustrate a more general association between later flowering and degree of dwarfism. Gibberellin sensitivity may affect date of flowering through numerous mechanisms (Mutasa-Göttgens & Hedden 2009), and genetically or chemically induced GA deficiency has delayed anthesis in wheat (Appleford *et al*. 2007). The observation that both GA-sensitive and GA-insensitive alleles can delay anthesis possibly suggests an indirect mechanism mediated through dwarfism *per se* rather than a direct GA signaling effect. The exception to this relationship is clearly the effect of *Ppd-D1a* to reduce plant height and bring forward anthesis by around eight days through photoperiod insensitivity (Worland 1996; Beales *et al*. 2007).

The estimated rate of accumulation of 3 kg N/ha/d around anthesis in the conventional experiments is consistent with previous studies on Mercia in the UK receiving about 200 kg N/ha as fertilizer (Sylvester-Bradley & Stokes 2001). In this comparatively nitrogen rich situation, and even accounting for time to anthesis, we find no evidence that single semi-dwarfing alleles (*Rht-B1b*; *Rht-D1b*) limit pre-anthesis uptake. Even with *Ppd-D1a*, reduced nitrogen uptake pre-anthesis of the *Rht8c* line is compensated for by increased accumulation post-anthesis, and we

find no evidence that mode of dwarfism influences *Crop NY*$_{maturity}$ i.e. effect of alleles are related to their effects on height rather than additional effects of GA-sensitivity.

Our results are broadly consistent with Gale & Yousefian (1985) showing severe dwarfism, such as they report for *Rht-B1c*, can be associated with reduced *Grain NY*. We demonstrate that this negative effect is mostly due to effects on *LNUp* rather than *RemobN* in contrasting production systems independent of the mechanism of dwarfing. The reasons for curtailed *LNUp* require further study, particularly as dwarfing was not a significant impairment to pre-anthesis uptake in the conventional system. Delayed anthesis may have reduced the time for effective uptake if all lines matured into a terminal drought. However, even if it is assumed that all lines completed *LNUp* at the same time, and a maximum uptake of 3 kg N/ha/d was maintained until that time, delayed anthesis would only account for about half of the reduction in *LNUp* observed as crop heights declined below 800 mm. Further, nitrogen can be lost from the crop between anthesis and maturity (Addisu 2009) which leads to an underestimate of the contributions of nitrogen uptake to *Grain NY* and a corresponding over-estimate of the *RemobN* contribution. Rooting may be impaired in the severe dwarf lines (Wojciechowski *et al*. 2009) which could become more important as water and soluble N become depleted late in the season. Finally, the observation that *Grain NY* is a function of grain numbers rather than *NpG* (Le Gouis *et al.* 2000) may infer a degree of sink limitation on *LNUp* (Mi *et al*. 2000). *NpG* in the conventional experiments approached 1 mg N/grain, a level at which uptake efficiency with increased N availability can decline rapidly (Gooding 2005; Gooding *et al*. 2007). Other explanations for the conservatism in *NpG* over a range of *Crop NY* are, however, possible (Gooding *et al*. 2007) and it is notable that *NpG* did not contribute greatly to genotypic effects on *Grain NY* in the organic system despite *NpG* being significantly less than 1 mg N/grain. In a comparison of genotypes of

different vintage, Acreche & Slafer (2009) found more modern varieties to have reduced *NpG* associated with increased grain numbers compared with their predecessors. In the results presented here, however, *NpG* remained comparatively stable over a wide range of grain populations generated by the range of dwarfing alleles.

As with Gooding *et al*. (1997) and Gale & Yousefian (1985), we find no evidence that semi-dwarfing, either with GA-insensitivity or not, reduced *Grain NY*. We concur with Le Gouis *et al*. (2000) that most genotypic variation in *Grain NY* is closely related to variation in *Grain DMY*. *Grain NY* and *Grain DMY* are thus maximized broadly at the same heights.

We reiterate the penalty for biomass yield of dwarfism in cereals, and show clear implications for *NUtE* of the above ground crop biomass. As with Fernandez *et al*. (2009) we would therefore question the value of any *Rht* alleles to meet possible increased demand for lignocellulosic biomass for the production of biofuel. Although single semi-dwarfing alleles often reduced *NUtE* of the crop, that they could increase *NUtE* of the grain or $NUtE_g$ when added to tall backgrounds emphasizes the importance of *DMHI* for these characters (Ortiz-Monasterio *et al.* 1997).

The effects of *Rht* alleles on the *NUtE* of the grain are broadly consistent with the frequently observed negative relationships between genetic effects on *Grain DMY* and protein concentration (Zheng *et al*. 2009). There is, therefore, conflict between wheat systems and genotypes targeted for bread making and hence high grain protein concentrations and those for *NUtE* i.e. the inverse of nitrogen concentration (Barraclough *et al*. 2010). Foulkes *et al*. (1998) label the inverse of grain nitrogen concentration as '*crop nitrogen productivity*' (rather than as '*NUtE of the grain*' as used here) presumably as a more readily available, potential indicator of $NUtE_g$. The results here demonstrate the close association between genotype effects on the inverse of grain nitrogen concentration and $NUtE_g$.

It would appear that dwarfing alleles, GA insensitive or not, would have little to contribute to increased protein concentration at high yields, other than to permit greater fertilizer N applications without excessively increasing the risk of lodging. Comparisons of commercially-released varieties that vary in vintage so as to span the introduction of the Norin 10 dwarfing alleles have found that the more modern, shorter varieties: accumulate the same or more *Crop NY*; and to have greater *Grain NY, NHI, Remobeff, NUtE$_g$, NUtE* of the grain, and *DMHI* (Austin *et al.* 1977, Slafer & Andrada 1989; Slafer *et al*. 1990; Ortiz-Monasterio *et al*. 1997; Barraclough *et al.* 2010). Our results, with those presented previously (Gale & Youssefian 1985; Gooding *et al*. 1997) demonstrate that much of these effects can be attributed to the individual dwarfing alleles incorporated. However, these benefits are only seen in comparatively tall backgrounds (represented by Maris Widgeon and Maris Huntsman here), and we find no benefit for reducing plant height below 800 mm with respect to any component of *NUE*, irrespective of the dwarfing mechanism or cropping system employed. It is notable, that even the semi-dwarfing allele, *Rht-D1b* could sometimes reduce grain yield and *NUE* when added to a comparatively short background such as Mercia, a cultivar that was at least as short as many of its successors carrying semi-dwarfing alleles such as Xi19 and Solstice. Given the success of these latter cultivars, and the results presented here, there would appear little potential benefit in reducing cultivar height below 800 mm. We do, however, demonstrate that optimum height to maximize *NUE* and its components, as manipulated by *Rht* alleles, varies with cropping system and crop utilization (i.e. grain or biomass).

The authors are grateful to the Felix Trust for providing a scholarship for M. Addisu, and to RJ Casebow, RE Kiff and DT Smith for technical support.

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Figure Legends

Fig. 1. Monthly weather data at the experimental site. a) Mean of daily maximum and minimum temperature for 2005/06 (□), 2006/07(○), 2007/08 (△) and 2008/09 (▽). b) Rainfall for successive years, left to right. Heavy lines are the 45 year means for the site.

Fig. 2. Near isogenic lines (NILs) differing in dwarfing alleles in cultivar backgrounds of Mercia (triangles, mean of 2006-2009), Maris Huntsman (squares, 2008 & 2009), and Maris Widgeon (circles, 2008 & 2009), on anthesis date. Numerals 0, 1, 2, 3, 8, 10 and 12 correspond to *rht*(tall), *Rht-B1b*, *Rht-D1b*, *Rht B1c*, *Rht8c+Ppd-D1a*, *Rht-D1c*, and *Rht12* respectively. Effects of background and year have been removed; see text for details. Fitted curve (allele 8 omitted from fit) is quadratic. Error bars are single S.E.D.s (132 D.F.) for comparing alleles within Mercia (left) or within the other backgrounds (right).

Fig. 3. Conventionally-grown near isogenic lines (NILs) differing in dwarfing alleles in cultivar backgrounds of Mercia (triangles, mean of 2006, 2007, 2008 & 2009), Maris Huntsman (squares, 2008 & 2009), and Maris Widgeon (circles, 2008 & 2009) on dry matter and nitrogen in combine-harvested wheat grain, dry matter harvest index, and nitrogen utilization efficiency (*NUtE*) in grain. Numerals 0, 1, 2, 3, 8, 10 and 12 correspond to alleles as described in Fig. 2. Effects of background and year have been removed; see text for details. Fitted curves are quadratic. Error bars are single S.E.D.s (132 D.F.) for comparing alleles within Mercia (left) or within the other backgrounds (right).

Fig. 4. Organically-grown near isogenic lines (NILs) differing in dwarfing alleles in cultivar backgrounds of Mercia (triangles, mean of 2006, 2007 & 2008), Maris Huntsman (squares, 2008), and Maris Widgeon (circles, 2008) on dry matter and nitrogen in combine-harvested wheat grain, dry matter harvest index, and nitrogen utilization efficiency (*NUtE*) in grain. Numerals 0, 1, 2, 3, 8, 10 and 12 correspond to alleles as described in Fig. 2. Main effects of background have been removed; see text for details. Fitted curves are quadratic. Error bars are S.E.D.s (84 D.F.) for comparing alleles within Mercia (left) or within the other backgrounds (right).

Fig. 5. Conventionally-grown near isogenic lines (NILs) differing in dwarfing alleles in cultivar backgrounds of Mercia (triangles, mean of 2006, 2007 & 2009), Maris Huntsman (squares, 2009), and Maris Widgeon (circles, 2009), on nitrogen, dry matter and dry matter: nitrogen ratio (Nitrogen Utilization Efficiency; *NUtE*) in: the above ground crop biomass at anthesis (A-C) and maturity (F-H); and the grain at maturity (K-M). D and E show the net increase in the above-ground crop biomass, and I and J show the net loss from the non-grain tissues, between anthesis and maturity. Numerals 0, 1, 2, 3, 8, 10 and 12 in G correspond to alleles as described in Fig. 2. Alleles in other graphs can be deduced as height is consistent, although 2, 8, 10, 12, are always labeled to compare gibberrelin insensitive (2,10) and sensitive (10, 12) alleles at similar heights. Main effects of background have been removed; see text for details. Fitted curves are quadratic (dashed and solid lines correspond to $P>0.05$ and $P<0.05$ for the regression respectively). Error

bars are single S.E.D.s (78 D.F.) for comparing alleles within Mercia (left) or within the other backgrounds (right).

Fig. 6. Conventionally-grown near isogenic lines (NILs) differing in dwarfing alleles in cultivar backgrounds of Mercia (triangles, mean of 2006, 2007 & 2009), Maris Huntsman (squares, 2009), and Maris Widgeon (circles, 2009), on Nitrogen Utilization Efficiency to produce grain ($NUtE_g$; kg grain DM/ kg N in above ground crop biomass), and Nitrogen Harvest Index (NHI; kg N in grain/ Kg N in above ground crop biomass). Numerals 0, 1, 2, 3, 8, 10 and 12 correspond to alleles as described in Fig. 2. Main effects of background have been removed; see text for details. Fitted curves are quadratic ($P<0.05$). Error bars are S.E.D.s (78 D.F.) for comparing alleles within Mercia (left) or within the other backgrounds (right).

Fig. 7. Organically-grown near isogenic lines (NILs) differing in dwarfing alleles in a cv Mercia background (mean of 2006, 2007), on nitrogen, dry matter and dry matter: nitrogen ratio (Nitrogen Utilization Efficiency; *NUtE*) in: the above ground crop biomass at anthesis (A-C) and maturity (F-H); and the grain at maturity (K-M). D and E show the net increase in the above-ground crop biomass, and I and J show the net loss from the non-grain tissues, between anthesis and maturity. Numerals 0, 1, 2, 3, 8, 10 and 12 in G correspond to alleles as described in Fig. 2. Alleles in other graphs can be deduced as height is consistent, although 2, 8, 10, 12, are always labeled to compare gibberrelin insensitive (2,10) and sensitive (10, 12) alleles at similar heights. Dashed and solid lines correspond to $P>0.05$ and $P<0.05$ for the linear regression respectively. Error bars are single S.E.D.s (42 D.F.)

Fig. 8. Organically-grown near isogenic lines (NILs) differing in dwarfing alleles in a cv Mercia background (mean of 2006, 2007), on Nitrogen Utilization Efficiency to produce grain ($NUtE_g$; kg grain DM/ kg N in above ground crop biomass), and Nitrogen Harvest Index (NHI; kg N in grain/ Kg N in above ground crop biomass). Numerals 0, 1, 2, 3, 8, 10 and 12 correspond to alleles as described in Fig. 2. Fitted lines are quadratic. Error bars are single S.E.D.s (42 D.F.).

Fig. 9. Near isogenic lines (NILs) differing in dwarfing alleles in cultivar backgrounds of Mercia (triangles, mean of 2006, 2007 & 2008), Maris Huntsman (squares, 2008), and Maris Widgeon (circles, 2008), on yield components when grown conventionally (solid symbols) or organically (open symbols). Numerals 0, 1, 2, 3, 8, 10 and 12 in A correspond to alleles as described in Fig. 2. Alleles in other graphs can be deduced as height is consistent. Main effects of background have been removed; see text for details. Fitted curves are quadratic. Error bars are single S.E.D.s (168 D.F.) for comparing alleles within growing systems in Mercia (left) or in the other backgrounds (right).

Fig. 10. Organically-grown near isogenic lines (NILs) differing in dwarfing alleles in a cv Mercia background (mean of 2006, 2007 and samples taken at both anthesis and maturity of the wheat) on nitrogen (kg/ha) in the above ground weed biomass in organically-grown plots. Numerals 0, 1, 2, 3, 8, 10 and 12 correspond to alleles as described in Fig. 2. Error bar is one S.E.D. (42 D.F.)

Table 1. *Experimental details on conventional (C) and organic (O) areas*

	2005/06		2006/07		2007/08		2008/09
	C	O	C	O	C	O	C
No. of blocks	4	4	4	4	3	3	3
Backgrounds included	Mercia		Mercia		Mercia M. Huntsman M. Widgeon		Mercia M. Hunt. M. Widg.
Plot lengths (m)	10	10	10	10	7.5	7.5	7.5
Sowing rates (seeds/m^2)	300	300	300	300	250	250	300
Soil mineral N to 900 mm by end of Feb.	29	67	10	10	25	15	33
N in crop at end of Feb. (excluding weeds)	30.5	37.1	22.9	45.0	11.7	22.3	9.2
Dates of nitrogen (kg/ha) applications:							
GS (Zadoks *et al*. 1974) 30-31	4 April		1 April		1 April		30 March
GS 34-39	11 May		1 May		28 April		6 May
Assessments made on:							
Combine harvested grain	✓	✓	✓	✓	✓	✓	✓
Whole crop samples at anthesis and maturity	✓	✓	✓	✓			✓

Table 2. *F probabilities and correlation coefficients for effects of reduced height allele and system (organic or conventional) in near isogenic lines of Mercia winter wheat for components of nitrogen use efficiency*

	Residual maximum likelihood			Correlation coefficient between allele means in the two systems (7 observations)	
	System	Allele	Allele × System	*r*	*P*
Effects replicated over three years (2005/6-2007/8)*					
Final crop height	0.834	<0.001	<0.001	1.00	<0.001
NUE (Grain DM/available N)	0.153	<0.001	0.014	0.95	<0.001
N recovered (Grain N/available N)	0.049	<0.001	0.539	0.97	<0.001
DMHI (Grain DM/Above ground crop DM)	<0.001	<0.001	0.060	0.91	0.004
NUtE of the grain (Grain DM/Grain N)	0.015	<0.001	<0.001	0.93	0.003
Nitrogen per grain	0.046	<0.001	0.285	0.94	0.002
Effects replicated over two years (2005/6 & 2006/7)*					
NUpE (Above ground crop N/available N)	0.617	<0.001	0.006	0.98	<0.001
$NUtE_g$ (Grain DM/Above ground crop N)	0.590	<0.001	<0.001	0.94	0.002

* Denominator D.F. for effects of Allele and Allele × System for three and two year analyses = 120 and 84 respectively; Denominator D.F. for main effect of System is 2 and 1 respectively.

Table 3. *Effects of crop height and time of anthesis on above ground crop nitrogen and dry matter of winter wheat at anthesis. Data were the 19 allele x background means presented in Figs. 2, 5A and 5B.*

Parameter	Above ground at anthesis			
	Nitrogen (kg/ha)		Dry matter (t/ha)	
	Effect	S.E.	Effect	S.E.
Constant	35.5	31.7	-1.67	1.34
Linear height (mm)	0.298	0.0911	0.0199	0.00385
Quadratic ht. (mm)	-0.00178	0.000625	-0.000088	0.000026
Days to anthesis	3.03	0.994	0.148	0.0421

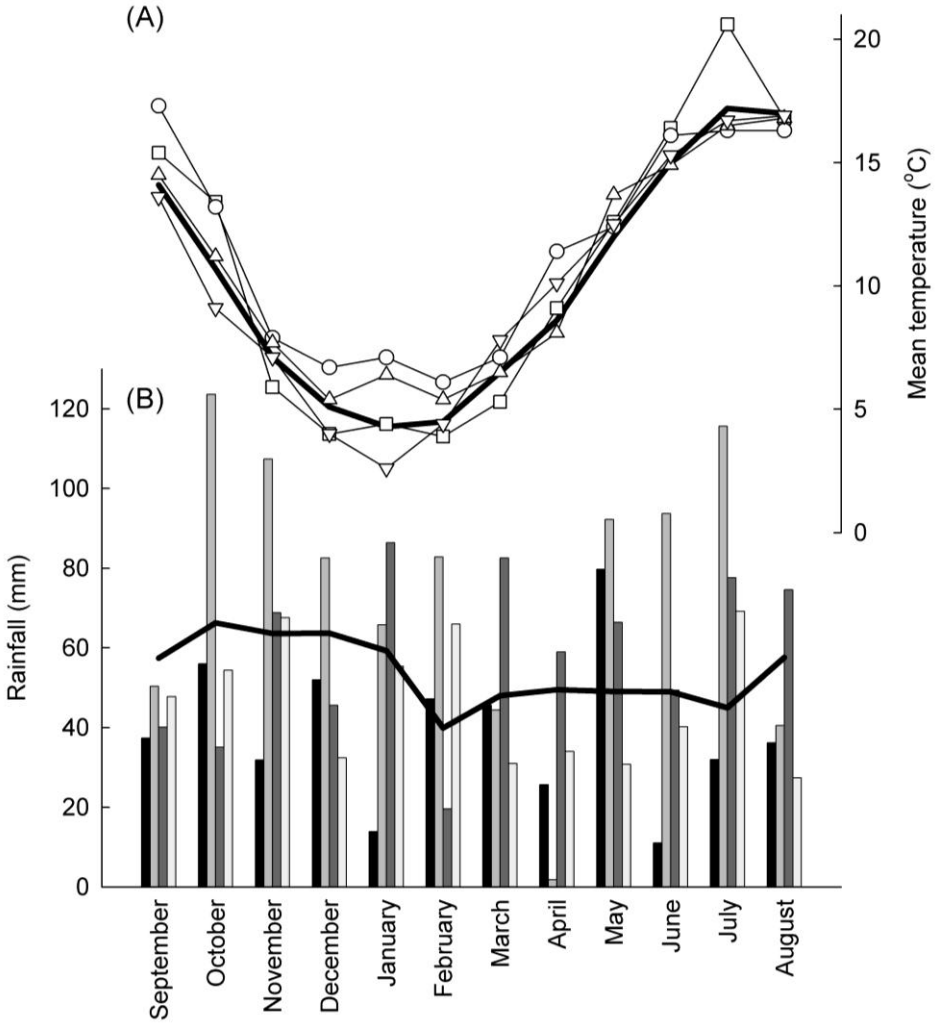
(A)
(B)
Mean temperature (°C)
Rainfall (mm)
0
5
10
15
20
0
20
40
60
80
100
120
September
October
November
December
January
February
March
April
May
June
July
August

Fig. 1.

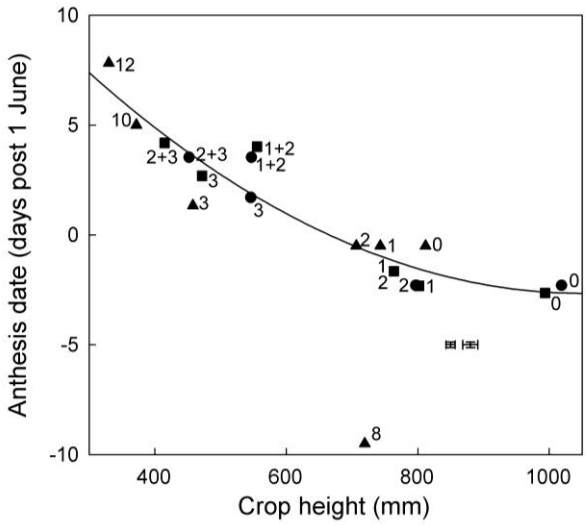
Anthesis date (days post 1 June)
Crop height (mm)
10
5
0
-5
-10
400
600
800
1000
12
10
2+3
2+3
3
3
1+2
1+2
3
2
1
0
1
2
2
1
0
0
8

Fig. 2.

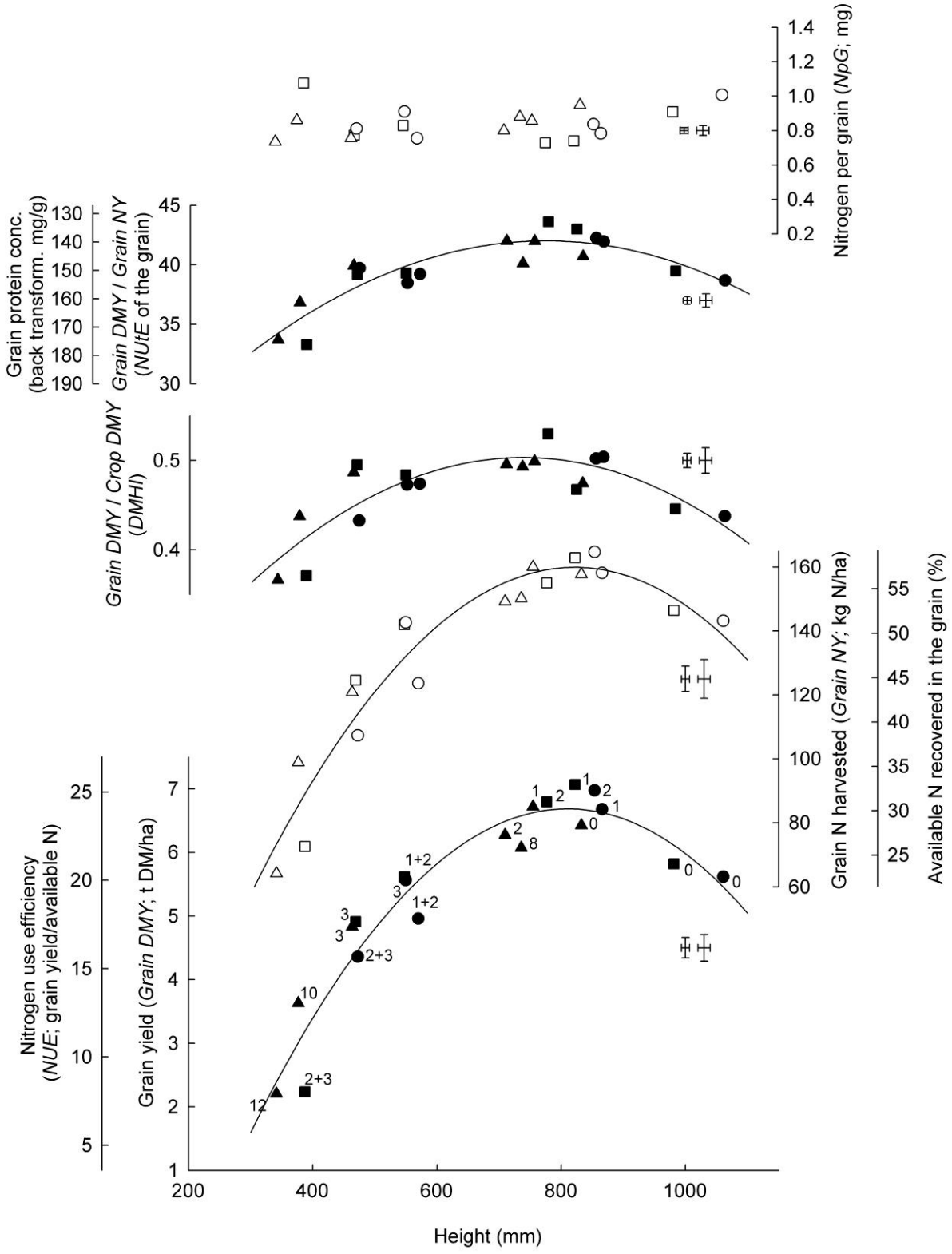
Grain protein conc. (back transform. mg/g)
130
140
150
160
170
180
190
Grain DMY / Grain NY (NUtE of the grain)
45
40
35
30
Nitrogen per grain (NpG; mg)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
Grain DMY / Crop DMY (DMHI)
0.5
0.4
Grain N harvested (Grain NY; kg N/ha)
160
140
120
100
80
60
Available N recovered in the grain (%)
55
50
45
40
35
30
25
Nitrogen use efficiency (NUE; grain yield/available N)
25
20
15
10
5
Grain yield (Grain DMY; t DM/ha)
7
6
5
4
3
2
1
200
400
600
800
1000
Height (mm)

Fig. 3.

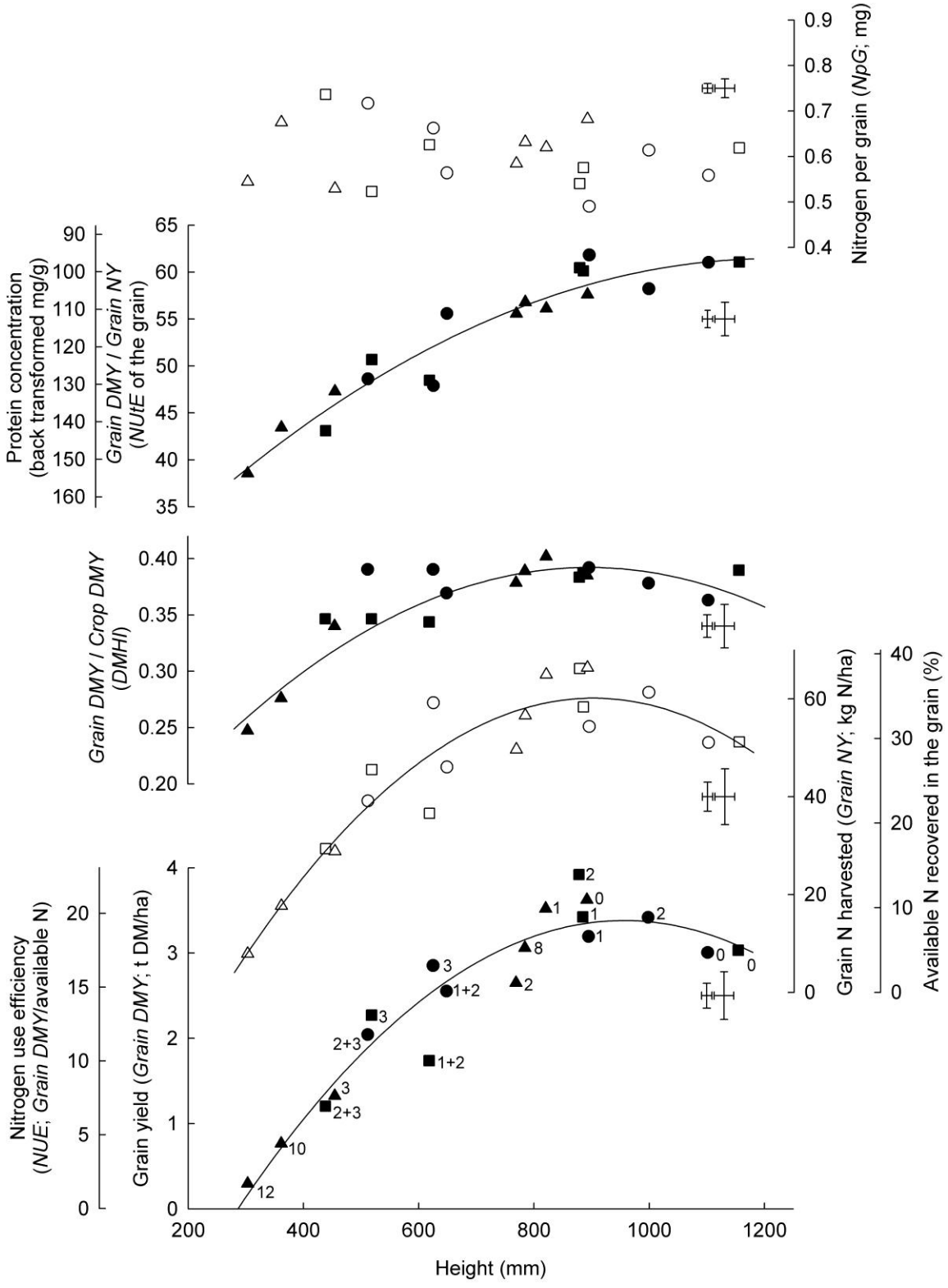
Protein concentration (back transformed mg/g)
Grain DMY / Grain NY (NUtE of the grain)
Nitrogen per grain (NpG; mg)
Grain DMY / Crop DMY (DMHI)
Nitrogen use efficiency (NUE; Grain DMY/available N)
Grain yield (Grain DMY; t DM/ha)
Grain N harvested (Grain NY; kg N/ha)
Available N recovered in the grain (%)
Height (mm)

Fig. 4.

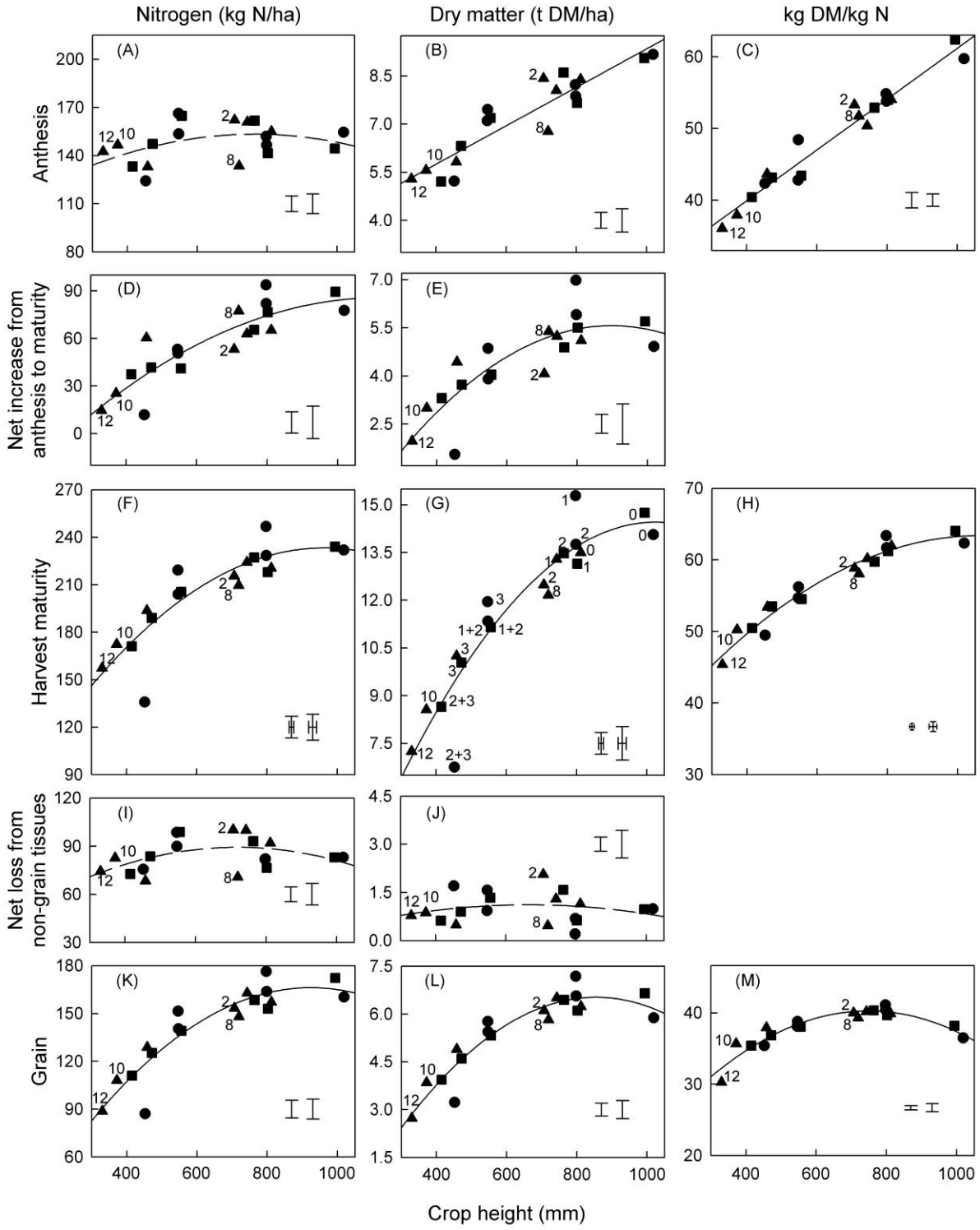
Nitrogen (kg N/ha)
Dry matter (t DM/ha)
kg DM/kg N
(A)
(B)
(C)
(D)
(E)
(F)
(G)
(H)
(I)
(J)
(K)
(L)
(M)
Anthesis
Net increase from anthesis to maturity
Harvest maturity
Net loss from non-grain tissues
Grain
Crop height (mm)

Fig. 5.

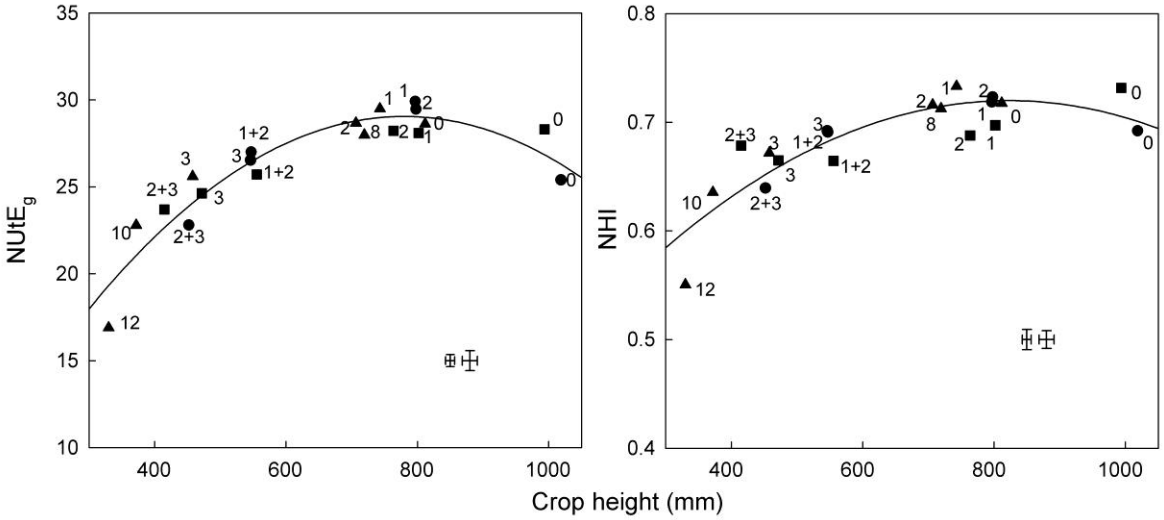
NUtE$_g$
NHI
Crop height (mm)
35
30
25
20
15
10
0.8
0.7
0.6
0.5
0.4
400
600
800
1000

Fig. 6.

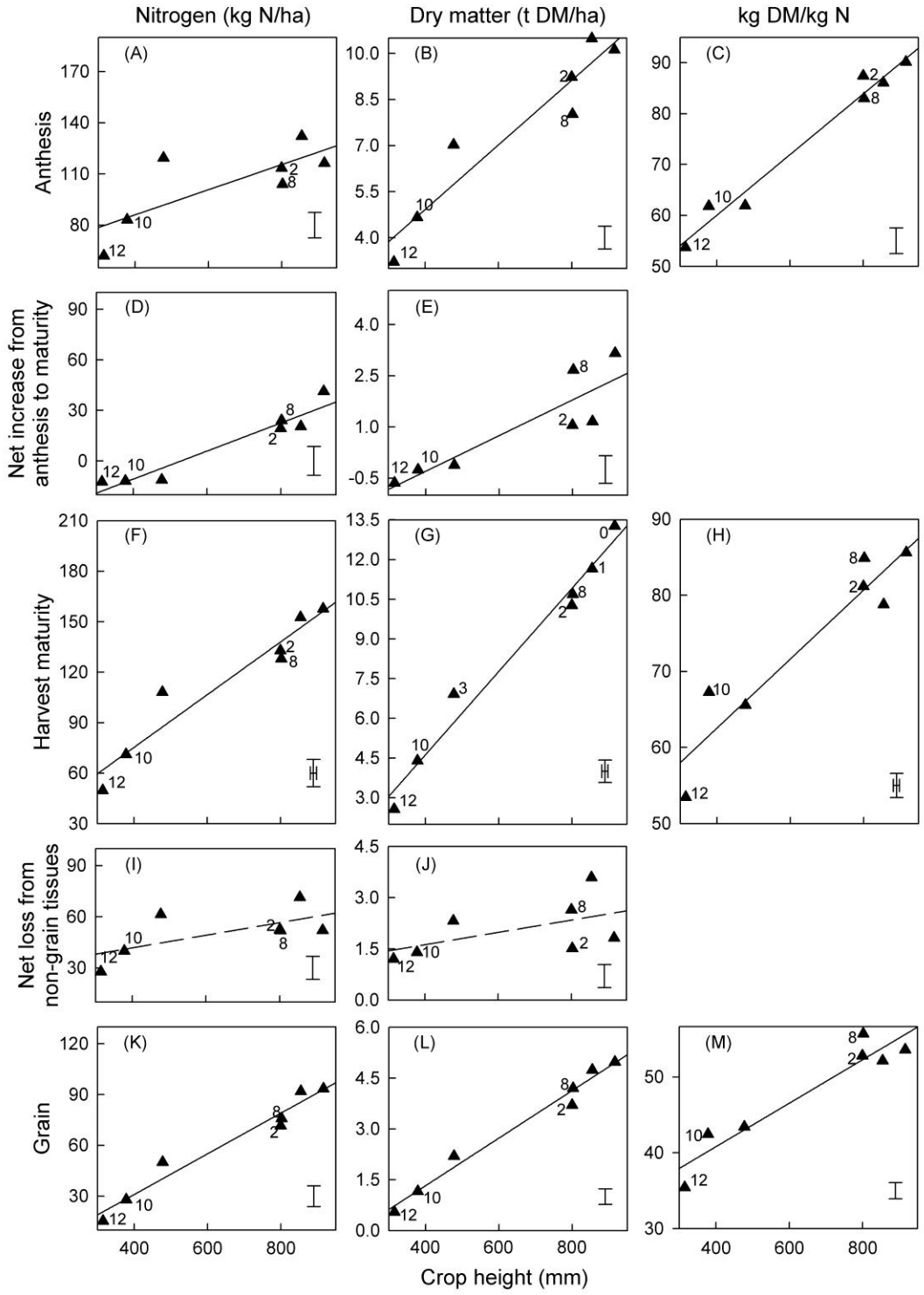
Nitrogen (kg N/ha)
Dry matter (t DM/ha)
kg DM/kg N
Anthesis
Net increase from anthesis to maturity
Harvest maturity
Net loss from non-grain tissues
Grain
Crop height (mm)
(A)
(B)
(C)
(D)
(E)
(F)
(G)
(H)
(I)
(J)
(K)
(L)
(M)

Fig. 7.

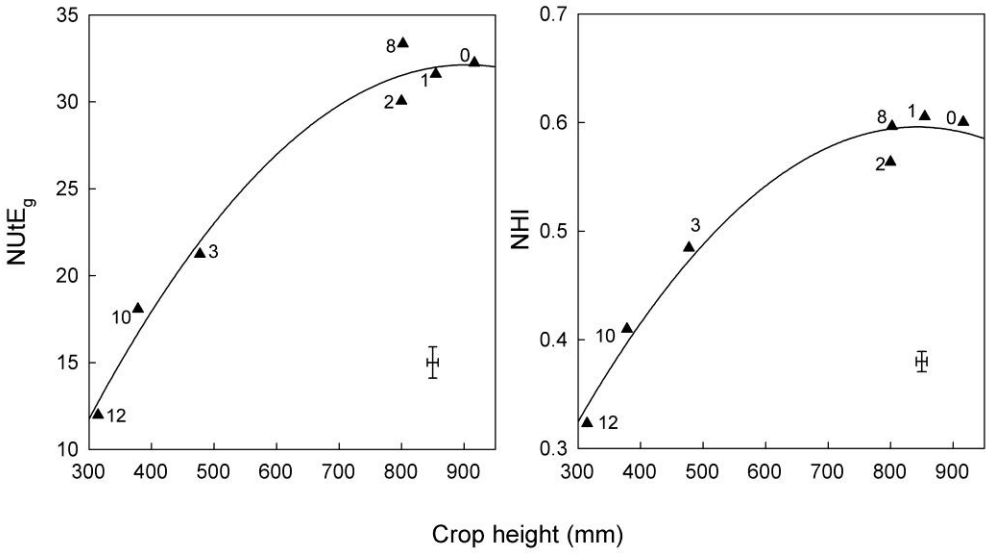
NUtE$_g$
NHI
Crop height (mm)
35
30
25
20
15
10
0.7
0.6
0.5
0.4
0.3
300
400
500
600
700
800
900
0
1
2
3
8
10
12

Fig. 8.

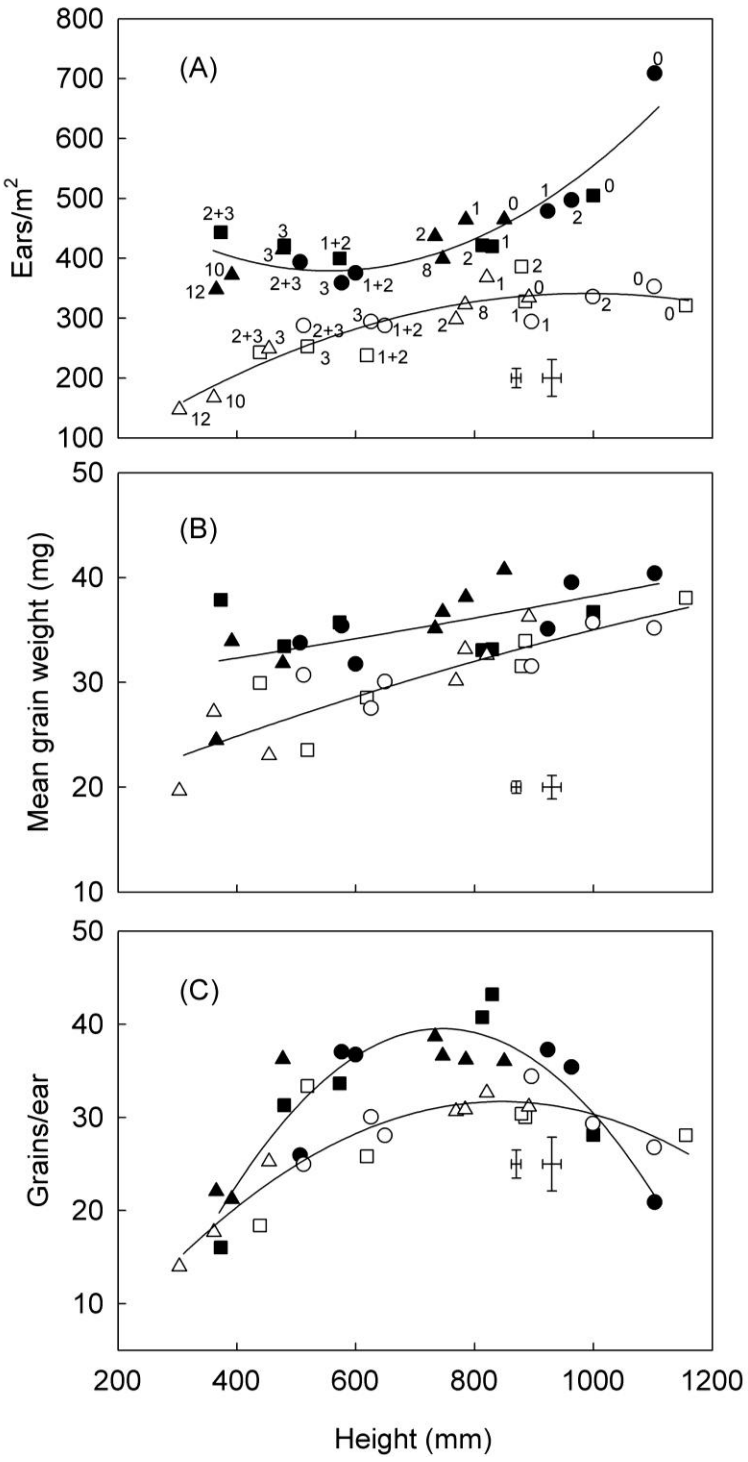
(A)
Ears/m^2
800
700
600
500
400
300
200
100
(B)
Mean grain weight (mg)
50
40
30
20
10
(C)
Grains/ear
50
40
30
20
10
200
400
600
800
1000
1200
Height (mm)

Fig. 9.

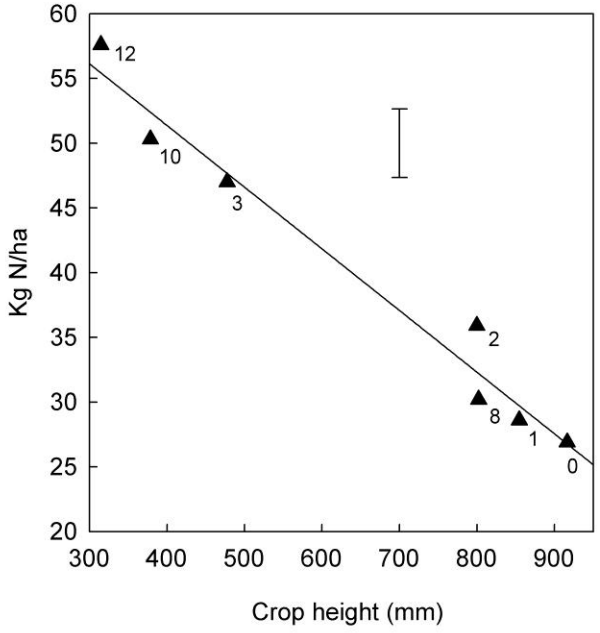
60
55
50
45
40
35
30
25
20
Kg N/ha
12
10
3
2
8
1
0
300
400
500
600
700
800
900
Crop height (mm)

Fig. 10.