

Plot size matters: interference from intergenotypic competition in plant phenotyping studies

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Abstract. Genetic and physiological studies often comprise genotypes diverse in vigour, size and flowering time. This can make the phenotyping of complex traits challenging, particularly those associated with canopy development, biomass and yield, as the environment of one genotype can be influenced by a neighbouring genotype. Limited seed and space may encourage field assessment in single, spaced rows or in small, unbordered plots, whereas the convenience of a controlled environment or greenhouse makes pot studies tempting. However, the relevance of such growing conditions to commercial field-grown crops is unclear and often doubtful. Competition for water, light and nutrients necessary for canopy growth will be variable where immediate neighbours are genetically different, particularly under stress conditions, where competition for resources and influence on productivity is greatest. Small hills and rod-rows maximise the potential for intergenotypic competition that is not relevant to a crop's performance in monocultures. Response to resource availability will typically vary among diverse genotypes to alter genotype ranking and reduce heritability for all growth-related traits, with the possible exception of harvest index. Validation of pot experiments to performance in canopies in the field is essential, whereas the planting of multirow plots and the simple exclusion of plot borders at harvest will increase experimental precision and confidence in genotype performance in target environments.

Additional keywords: border, competition, density, edge, row, validation.

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Introduction

The availability of new high-throughput sequence and genotyping platforms has reduced the cost and increased the speed of delivery of genetic data for many important crops (Bräutigam and Gowik 2010). Subsequent access to an explosion of genetic data is allowing genetic dissection of simple to complex traits such as crop biomass and grain yield (e.g. Mathews *et al.* 2008), and traits contributing to increases in productivity (e.g. transpiration efficiency; Rebetzke *et al.* 2006, 2008a). This activity has extended beyond geneticists and breeders with an interest in genetic control and marker-based selection to pathologists, physiologists and ecologists exploring mechanisms underpinning target traits (e.g. Pinto *et al.* 2010).

Examination of characteristics in plant biology varies from simple visual observations to understanding of the detailed biochemical pathways, and from assessment of yield potential in 'non-limiting' environments to complex responses under

targeted environmental stresses. This range reflects the complexity in the traits and different methodologies developed and used in robust phenotyping. Data are obtained for plants in pots in controlled and semicontrolled environment facilities, or for plants that are spaced in 'hills' or in single rows or small plots in the field (Fig. 1). However, for many traits, there is little evidence that performance in small plots is correlated with that in large field plots. There are some exceptions, where traits with constitutive expression and high penetrance (e.g. tolerance to subsoil constraints including aluminium, boron and salt; e.g. Munns *et al.* 2012) permit nonfield assays that correlate well with field performance. The inference from *ex situ* assessment to field with more complex traits is typically less clear (e.g. Passioura 2006). This is becoming an issue, as there is an increasing trend towards the use of single plants in pots in artificial environments, and small hills or plots containing



Fig. 1. Competition for resources can arise where adjacent plots are small: (a, b) unbordered rows or rods containing lines differing for (a) height and (b) development; (c, d) use of small hills or spaced plants differing in (c) height and (d) maturity.

single or paired rows lacking borders when assessing genotype performance, particularly under stress (Fig. 1). In such cases, genotype response to a studied specific stress (e.g. nutrient deficiency or drought) will be confounded by the artificial environment (e.g. limited light of a growth cabinet) or complicated by competition effects from neighbours that do not reproduce the competition experienced by plants grown in canopies in the field. This viewpoint aims to demonstrate the potential pitfalls with screening diverse germplasm for complex traits in pots, spaced rows and small plots.

Why more is better – increasing population size

The potential for allele segregation at multiple loci requires measurement of large numbers of progeny lines to obtain unbiased estimates of the number, size and location of genomic regions associated with variation for a desired trait (Schön *et al.* 2004; Bonnett *et al.* 2005). For genetically

complex traits, minimum numbers of evaluated lines are commonly of the order of 300–500, with genetic estimates becoming increasingly more robust with the sampling of more lines (Vales *et al.* 2005). However, the shortcomings with an increasingly larger population size are the greater resources required to confidently capture the phenotypes being assessed. This is because as the number of lines increases, so does the size of the experiment, as well as the potential for environmental variation and random error that reduce repeatability and confidence in the phenotype.

An increasing number of papers are being published exploring trait dissection and yield in well watered and water-limited environments, and other stress conditions (e.g. Mathews *et al.* 2008; Rebetzke *et al.* 2008b). These papers have focussed on sampling larger mapping populations with the aim of measuring phenotypes and correlating observed variation with changes in allelic frequency throughout the genome. The genetic complexity of productivity under stress, particularly water limitation

(Mathews *et al.* 2008), requires assessment of large populations when targeting trait dissection. The large area required to phenotype large numbers of genotypes adequately in small plots has encouraged the phenotyping of genotypes in single unbordered rows. Indeed, in a survey of papers reporting grain yield or yield components, less than one-half reported data from bordered plots or where pot-evaluated provided evidence of any validation in the field (G.J. Rebetzke, unpub. data).

Competition and yield potential

Organisms compete for the limited resources available in their immediate environment. Competition is an important driver of fitness, adaptation and the differential reproduction of better suited individuals (Primack and Kang 1989). This is true of both natural and managed agricultural environments, where the resources required for plant, sward or canopy growth are many (e.g. water, nutrients and light), and the limitation of one or more will reduce the capacity to produce seed. In targeting cereal varieties with greater competitiveness against weeds, breeders have gained insight from the ecological literature (e.g. Cousens *et al.* 2003) in selecting breeding lines with increased plant height, greater tillering and more vigorous early growth (faster leaf area development) (e.g. Huel and Hucl 1996; Coleman *et al.* 2001). However, despite this potential benefit with weeds, early generation selection for taller, more vigorous barley (*Hordeum vulgare* L.) plants when grown free of weeds can be associated with reduced yield in some environments (e.g. Hamblin and Donald 1974).

Crops represent monocultures of many individuals, with the same genotype growing together in a 'like' community. 'Communal genotypes' are thought to commit fewer resources to structures promoting competition (e.g. increased plant height, greater leaf and stem size), thereby freeing up assimilate for greater partitioning to reproductive organs (Donald 1968). Indeed, selection for high grain yield potential (e.g. semidwarf cereals) has seemingly promoted a greater communal response. Comparisons among wheat (*Triticum aestivum* L.) varieties has demonstrated that more recent high-yielding wheat varieties lacked competitive ability when grown in pure stands (as in a commercial crop) and showed a limited yield response to removal of interplant competition (Reynolds *et al.* 1994). Older, lower-yielding lines responded to removal of above-

and belowground competition with increases in almost all measured traits including biomass and yield. However, the performance of the older varieties was substantially below that of the newer, less competitive varieties when sown in conventional plots. Similarly, a comparison of historic wheats in southern Australia demonstrated reduced intraplot competitiveness in recent commercial releases, consistent with a more communal plant ideotype (Sadras and Lawson 2011). Evidence can also be found in other crops like maize (*Zea mays* L.) where breeding for high yield in the United States has favoured selection against tillering (Doebley *et al.* 1997) and adoption of agronomic systems aimed at reducing within-crop competitiveness (Duvick 2005).

Extrapolation from pot and container studies to field performance

The ease of controlling stress in growth cabinet or glasshouse conditions has promoted some assessment of populations under controlled environments. Such artificial conditions provide many benefits in the precise screening of responses to specific stresses. The potential to control atmospheric conditions and photoperiod can allow for plant growth at most times of the year. However, pot size and shape are essential criteria for optimising growth that are commonly overlooked. Pots are often too small (Poorter *et al.* 2012) or too shallow (Passioura 2006), leading to root restrictions on shoot growth. Furthermore, pot studies commonly focus on extreme stress, screening for survival, although the environments targeted by breeders are very different, with patterns of stress varying in time and intensity (Chenu *et al.* 2011, 2013; Passioura 2012). Overall, many differences in the growth of plants in pots can make interpretation and extrapolation to the field a challenge (Passioura 2006). This challenge is greater for leaf and canopy characteristics, where small changes in temperature and light quality or irradiance level can reduce the correlation for traits such as transpiration efficiency (e.g. Condon *et al.* 1990), specific leaf area and leaf size (Rebetzke *et al.* 2004), and grain yield (Quail *et al.* 1989) between glasshouse and field studies.

Potential problems of relating the plant growth of pot-grown plants to that of field-grown plants are illustrated for wheat variety Janz grown at low and high nitrogen levels (Table 1). The pot plants were grown outdoors under low and high supplemental

Table 1. Response to supplementary nitrogen of spaced plants (grown in pots) and crops (grown in the field at Pucawan and Wagga Wagga in 1991) of the wheat variety Janz for different traits

Means and percentage change are given (table modified from the data in van Herwaarden *et al.* 1998a and 1998b with permission from the *Australian Journal of Agricultural Research*). WSC, water-soluble carbohydrates; *, the difference between the low and high nitrogen treatments is statistically significant at $P=0.05$; **, the difference between the low and high nitrogen treatments is statistically significant at $P=0.01$; ns, the difference between the low and high nitrogen treatment is not statistically significant at $P=0.05$

| Attribute | Pot plants | | | Field crops | | |
|---|------------|--------|------------|-------------|--------|------------|
| | Low N | High N | Change (%) | Low N | High N | Change (%) |
| Spike density (no. per m ²) | 215 | 244 | +13* | 421 | 530 | +26** |
| Aerial biomass (g m ⁻²) | 548 | 725 | +32** | 874 | 1043 | +19** |
| Grain yield (g m ⁻²) | 219 | 290 | +32** | 341 | 396 | +16** |
| Harvest index | 0.40 | 0.40 | +0.04ns | 0.39 | 0.38 | -0.04ns |
| Stem anthesis WSC (%) | 46 | 45 | -2ns | 30 | 17 | -44** |
| WSC at anthesis per kernel (mg) | 21 | 22 | +5ns | 12 | 5 | -58** |

nitrogen levels (0 g N m^{-2} and 24 g N m^{-2} , respectively) in cylindrical pots 10 cm in diameter and 1.2 m long juxtaposed one from another to simulate a 'field-like' canopy (a density of $\sim 30\text{ plants m}^{-2}$) (van Herwaarden *et al.* 1998a, 1998b). Plants in the field were also cultivated with 0 g N m^{-2} and 24 g N m^{-2} of supplemental N at the standard density of 150 plants m^{-2} . Absolute differences in pot- and field-grown plants were large for most traits, with the exception of partitioning of assimilate to grain (harvest index (HI)) (Table 1). Response to additional nitrogen varied with each trait and was inconsistent between pot- and field-grown canopies. For instance, tillering response to increasing soil nitrogen was large in the field (+26%) but smaller for the pot-grown plants (+13%), despite the relative increase in total biomass. It appears that the tillering capacity of pot plants was high under both low and high N (~ 7 and 8 tillers per plants respectively), probably due to the low plant density resulting from the pot experimental design. By contrast, individual plants in the field only had few tillers (2.8 and 3.5 on average for low and high N, respectively). Additional nitrogen did not affect the high level of water soluble carbohydrates (WSC) at anthesis in pot plants, whereas the already lower WSC (stem WSC and WSC reserves available to each kernel) in field plants was further reduced with high N. Increased tillering under higher N in the field supported an increased investment in structural stem carbon at the expense of stored WSC. In turn, WSC reserves available per kernel at anthesis were greater for spaced than for field-grown plants, probably due to greater light interception per stem.

The reduced assimilate available for remobilisation with increased plant density or tiller number or nitrogen fertilisation observed in the field is consistent with other agronomic and genetic reports for WSC in field crops (Rebetzke *et al.* 2008b; van Herwaarden *et al.* 1998a). Clearly, differential response with supplemental nitrogen and altered canopies compared with pot-grown plants highlights the plasticity of many canopy-related traits. Together, this study underlines the issue of interpretation and extrapolation from pot to field performance for canopy-related traits, and questions the relevance and utility of such studies without proper validation.

Recent technological innovations have seen increasing capacity for high-throughput phenotyping, particularly given the automated systems now available for growing and screening large numbers of lines under controlled conditions (e.g. Montes *et al.* 2007). Despite this greater capacity for environmental control, phenotyping away from the field must be practised with extreme caution, particularly for characteristics that potentially alter the dynamics of yield formation (Table 1). Although improved environmental control and monitoring of individual plant processes will facilitate more accurate data collection, spaced and even simulated canopies rarely translate meaningfully to field response. Any activity undertaken in controlled growth chamber or glasshouse conditions must be properly validated with field measurements to be confident that the data are relevant and of value for field conditions (e.g. Munns *et al.* 2012).

The importance of plot size

Plant breeders have long considered the issue of plot size in their evaluation of elite breeding lines (e.g. Hamblin and Donald 1974). Typically, the more genotypes that can be sampled, the greater the genetic variance captured and the greater the likelihood of bringing favourable alleles together into improved genotypes for potential release as new varieties. Efforts have aimed at reducing plot size to the point where the number of genotypes is maximised to increase genetic variance and selection differential. In turn, optimum plot size can be considered as that which maximises the proportion of controllable (e.g. genotype) to uncontrollable (e.g. environmental) variation.

Plot types as small as unbordered 'hills' or short rod-rows containing only a few seeds (Fig. 1) have been compared with performance in large bordered plots (Fig. 2) and commonly demonstrate a requirement for multirow plots (e.g. yield in rice (*Oryza sativa* L. (Jearakongman *et al.* 2003) and wheat (Fischer 1978)). Variation in plant height and canopy architecture contributes to increased light competitiveness and confounding among adjacent unbordered plots of diverse rice



Fig. 2. Large, bordered plots are critical in minimising competition effects from adjacent plots. Here, tall near-isolines for a major dwarfing gene surround a short near-isogenic sister. In these 10-row plots, the outside border rows will be removed to leave the middle eight rows for harvesting.

and wheat genotypes alike. In wheat, yields are reduced by 0.34% for every 1-cm increase in the height of adjacent plots (Clarke *et al.* 1998), whereas small increases in wheat canopy height have been genetically linked to reductions in canopy temperature (Rebetzke *et al.* 2013). Equally, variation in root size and architecture observed across genotypes (e.g. Fischer 1981; Manschadi *et al.* 2008; Wasson *et al.* 2012) are likely to contribute to belowground competition where water and nutrients are limiting. However, unlike aboveground morphological differences, belowground variation is poorly known and therefore the potential for competition more insidious.

Spaced plants versus large plots

The relationship for grain yield measured in spaced plants (both field and pots) and large plots is summarised for several published sets of wheat genotypes in the field and in one glasshouse study (Table 2). Estimates of heritability and repeatability for grain yield and other traits were high in all studies (data not shown). Under irrigation (Examples 1–3), phenotypic correlations between plant and plot yields were small, never accounting for more than 10% of the variation in plot yield. These small

associations reflect the different response of the genotypes to space, which, under favourable conditions of irrigation and high fertility, represents variation in light interception. The extra light intercepted by the spaced plant was such that the average yield per plant was around 25–45 times the yield per plant under the normal intense intragenotypic competition between plants in a large plot. The traits which favoured spaced plant performance were related to the greater capture of light: increases in grain yield were related more to increases in total dry matter than to HI; in plots, the reverse was true and variation in grain yield better reflected genotypic differences in HI (in Example 1, Table 2). Indeed, the slope in the relationships between grain yield and plant height changed direction for spaced plants to plots in Examples 1 and 2 (Table 2), partly because greater height allows for greater light capture in a spaced planting (e.g. Fig. 1a). Other traits assisting greater light capture in spaced plants such as nonerect leaves and increased tillering or branching also appeared to favour yield in spaced plants compared with plots. The only spaced plant traits showing some significant association with plot yield were plant height (a negative association) and HI (positive association), and then only in some datasets.

Table 2. Phenotypic correlation estimated for spaced plant grain yield (g per plant) and plot yield (g m^{-2}) for experiments containing different wheat genotypes

Other relevant relationships with grain yield are included. DM, dry matter; HI, harvest index; *, correlations are statistically different from zero at $P=0.05$; **, correlations are statistically different from zero at $P=0.01$; ns, correlations are not statistically different from zero at $P=0.05$

| Example | Material | Conditions | Spaced plant arrangement ^A | Spaced plant vs plot yield correlation | Other yield-based correlations | Reference |
|---------|---------------------------------|------------------|---------------------------------------|--|---|--------------------------|
| 1 | 40 varieties | Irrigated | 60 × 60 cm, field | 0.33* | Spaced plant height vs spaced plant yield, $r=0.65^{**}$ Spaced plant DM vs spaced plant yield, $r=0.86^{**}$ Spaced plant HI vs spaced plant yield, $r=0.17\text{ns}$ Plot plant height vs plot yield, $r=-0.22\text{ns}$ Plot DM vs plot yield, $r=0.44^{**}$ Plot HI vs plot yield, $r=0.65^{**}$ Spaced plant HI vs plot yield, $r=0.66^{**}$ | Fischer and Kertesz 1976 |
| 2 | 48 varieties | Irrigated | 40 × 40 cm, field | 0.15ns | Spaced plant height vs spaced plant yield, $r=0.51^{**}$ Spaced plant height vs plot yield, $r=-0.40^{**}$ Spaced plant HI vs plot yield, $r=0.50^{**}$ | Fischer 1978 |
| 3 | 60 F _{2,3} wheat lines | Irrigated | 25 × 50 cm, pots in glasshouse | 0.18ns | Spaced-plant height vs plot yield, $r=-0.36^{**}$ Spaced plant HI vs plot yield, $r=0.18\text{ns}$ | Quail <i>et al.</i> 1989 |
| 4 | 34 wheat varieties | Terminal drought | 30 × 60 cm, field | 0.38* | Spaced plant yield (irrigated) vs plot yield (drought), $r=0.01\text{ns}$ Spaced plant DM (droughted) vs plot yield (drought), $r=0.36^*$ | Fischer 1981 |

^ASpacing represents distances between adjacent plants.

Under drought (Example 4 in Table 2), spaced plant yield was again only weakly associated with plot yield ($r=0.38$, $P<0.05$) as was spaced plant DM ($r=0.36$, $P<0.05$) and no other droughted, spaced plant trait was significantly associated with droughted plot yield. Under drought, water becomes a limiting resource, together with light, and although it is conceivable that some aspects of genetic variation in water acquisition in spaced plants (e.g. rooting depth and distribution) extend to performance in a large plot there was only weak evidence for this.

Spaced single rows versus large plots

The influence of single-row performance on grain yield and components was assessed for a genetically diverse range of commercial and advanced wheat breeding germplasm grown across contrasting environments. A total of 12 lines were assessed, with the results for six lines (a reduced tillering germplasm, 978/951-5, and five commercial varieties) being

summarised in Fig. 3. Plant density was the same for all lines at each site. Lines were evaluated in a replicated study containing an unbordered 36 cm spaced row (one row per genotype) and adjacent large 10.8 m² plots, with 10 rows spaced 18 cm apart. Traits were estimated from the mean of ~11 m length of a spaced row (two repetitions of a 5.5-m row) and the mean of the 10-row plot after removal of outside border rows. The same estimates are given for experiments undertaken at the lower-yielding Condobolin (a mean grain yield of 292 g m⁻²), the intermediate-yielding Moombooldool (393 g m⁻²) and the high-yielding Gundibindyal (604 g m⁻²) New South Wales sites.

Performance in a spaced row is rarely the same and is commonly greater than performance in a plot (i.e. the ratio of performance for the spaced row relative to the plot > 1; Fig. 3). For example, the site mean grain yield ratio ranged from 1.91 for the more favourable Gundibindyal to 2.23 for the drier Condobolin site. Changes in grain yield were mainly due to an

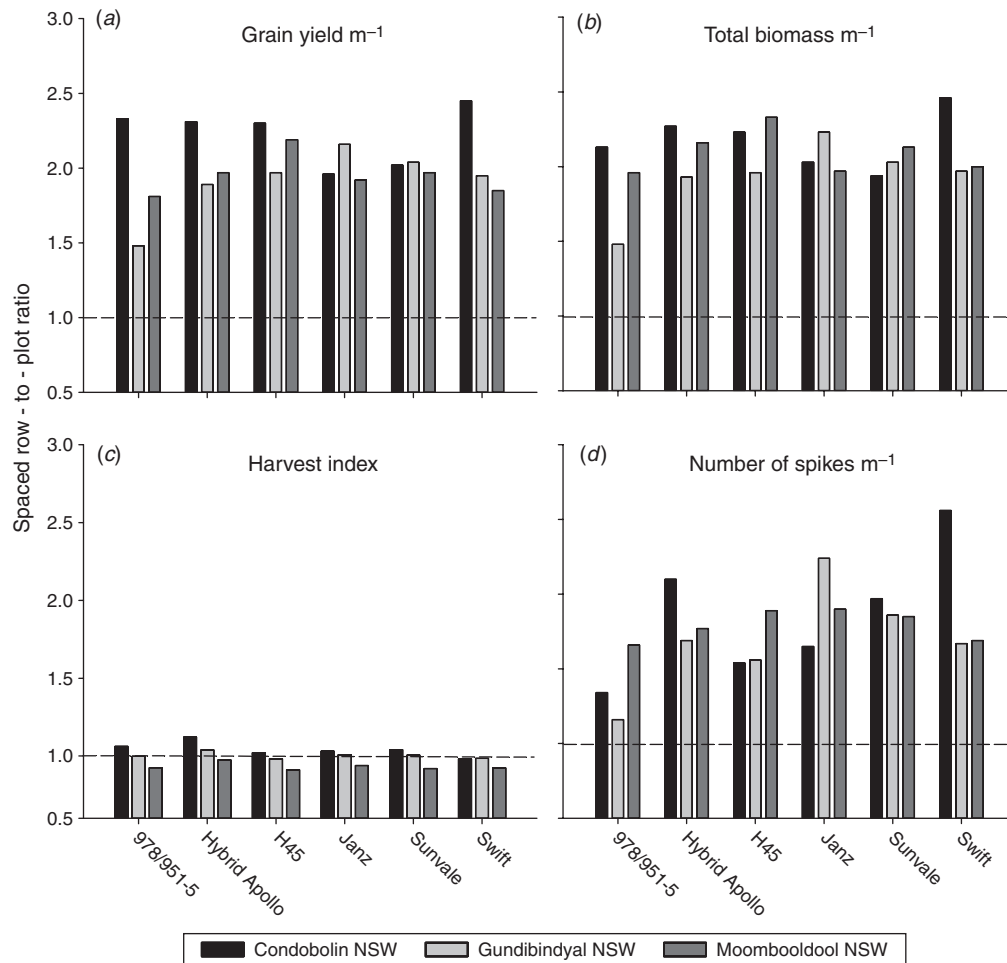


Fig. 3. Relative performance of spaced row and bordered plot yield for four agronomic traits measured on different wheat genotypes assessed at three contrasting sites in 2000: (a) grain yield, (b) total biomass, (c) harvest index and (d) number of spikes. The ratio was calculated as spaced row mean divided by plot mean (8 out of 10 rows) for each trait. The $LSD_{0.05}$ values for genotype \times site were 0.21, 0.28, 0.09 and 0.31 for grain yield, total biomass, harvest index and number of spikes, respectively. Plant height and anthesis were similar among all lines except H45, which flowered 1 week earlier than other lines on average.

increase in spike number and total biomass, but HI remained largely unchanged. Importantly, line ranking changed significantly ($P < 0.05$) across environments for all traits, with the exception of HI (Fig. 3). For all 12 lines considered in the study, phenotypic correlations for spaced row and plot yields were 0.68, 0.55 and 0.32, and genotype rank correlations for yield were 0.76, 0.53 and 0.14 for Condobolin, Gundibindyal and Moombooldool, respectively. By contrast, corresponding rank correlations for HI were similar (0.73, 0.71 and 0.81, respectively) at these sites. Over all sites, the genetic correlations between spaced rows and plots were 0.54, 0.78 and 0.76 for grain yield, total biomass and spike number, whereas the genetic correlations for seed weight and HI were stronger at 0.93 and 0.91, respectively. These associations indicate reduced confidence in the assessment of genotypes in spaced rows for grain yield, total biomass and spike number, but high confidence for HI and seed weight.

Plot borders reduce competition effects

Traits such as plant height, development, tiller and branch number etc. are rarely the same for genotypes across experiments. Morphological and phenological variation can contribute to trait expression (e.g. influence of phenology and height on canopy temperature and transpiration efficiency (Rebetzke *et al.* 2008a, 2013)), and influence plant growth and biomass accumulation to modify resource acquisition (Cousens *et al.* 2003; Reynolds *et al.* 1994). The use of an outside, border or edge row as a buffer between adjacent plots will compensate for much of the interplot competition for finite resources including water, nutrients and light (Fig. 4). Distances between adjacent plots are commonly large enough to give the ‘sunken loaf’ appearance observed in water-limited environments as border rows exploit moisture between plots. Under such conditions, up to half of

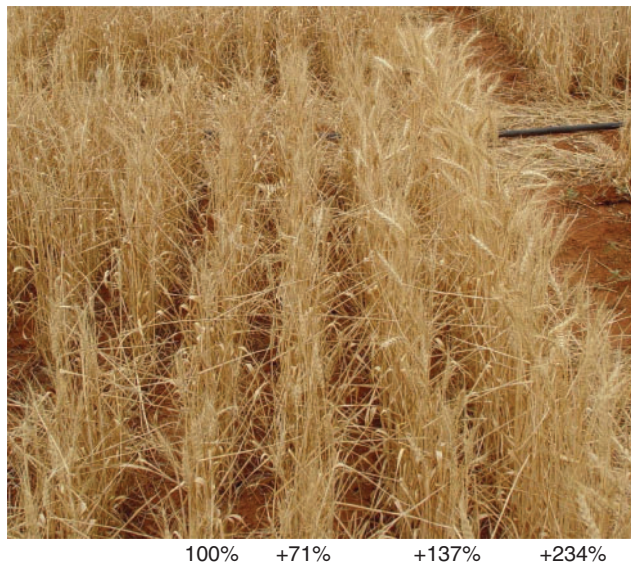


Fig. 4. Close-up of a rainfed plot illustrating the ‘sunken loaf’ appearance and the change in grain yield with row position. Values indicate the row yield relative to the centre row and highlight increasing yield in the outside border rows.

total plot biomass and yield can be due to border-row effects, reflecting increased stem elongation, plant height and greater tillering in response to greater soil water access. Genotypes vary in their capacity to respond to interplot spaces through differential root growth (e.g. Fischer 1981; Manschadi *et al.* 2008; Hammer *et al.* 2009) and to respond to light through differences in tiller number, angle and height (e.g. Evers *et al.* 2006). Competition-based statistical models have also been developed to reduce the correlation between different neighbours particularly in large experiments (e.g. Stringer and Cullis 2002). These should be considered but do not fully compensate for the removal of immediate neighbour effects, particularly where replication is small (which is usually the case).

The association of border and adjacent rows was investigated for two wheat genotypes (variety H45 and a H45 backcross-derived breeding line, B403D). Irrigation treatments were rainfed and a single irrigation treatment applied midway through grain-filling. Each of the 10 rows of the plot were harvested separately and results for the intermediate (third and eighth) and centre (fifth and sixth) rows are summarised relative to the border (first and tenth) rows in Fig. 5. Row spacing was 18 cm and path width between plots was c. 40 cm. Increasing the water availability of the outside border rows was associated with significantly ($P < 0.05$) increased growth and proportionally greater productivity (ratio > 1 ; Fig. 5). In turn, average performance tended to decrease away from the border row, and commonly more so with supplemental irrigation. This reduction was greatest for B403D, particularly for grain yield, HI and grain yield per spike. H45 was largely unchanged by the supplemental irrigation for most traits, and was particularly stable for HI and grain yield per spike (data not shown). The change in yield and biomass between inner plot rows confirms the need for multiple rows and a preference for sampling a larger number of rows for a given plot. Equally, the impact of including border rows in the analysis will be lessened the more rows contained in a plot when assessing under conditions favouring competition for limited resources.

In a separate study conducted under rainfed conditions at Birchip, Victoria, in 1999 (mean grain yield of 269 g m^{-2}), six wheat genotypes were assessed for spike number at two nitrogen levels: basal and a supplemental 40 kg N ha^{-1} predrilled at sowing (Fig. 6). Overall, the outside border rows averaged 56% greater spike number than the centre rows but the change in average spike number with supplementary N was -3 and 18% for the outside and centre rows, respectively. The advantage in spike number for border rows compared with inner rows was greater at low N than for supplemental N, illustrating the argument that border rows have greater access to resources such as nutrients when these may be limited. Individual genotypes responded differently with the largest relative change in spike number associated with the free-tillering Goldmark, Silverstar and CS949 (Fig. 6); the reduced-tillering genotypes CS950 and CS971 were intermediate in their responsiveness; the smallest change with supplemental N was associated with the commercial wheat variety Frame. Importantly, the association for border and centre row spike number over all six genotypes was statistically nonsignificant ($P > 0.05$) at 0.50 and 0.74 for basal and supplemental N treatments, respectively.

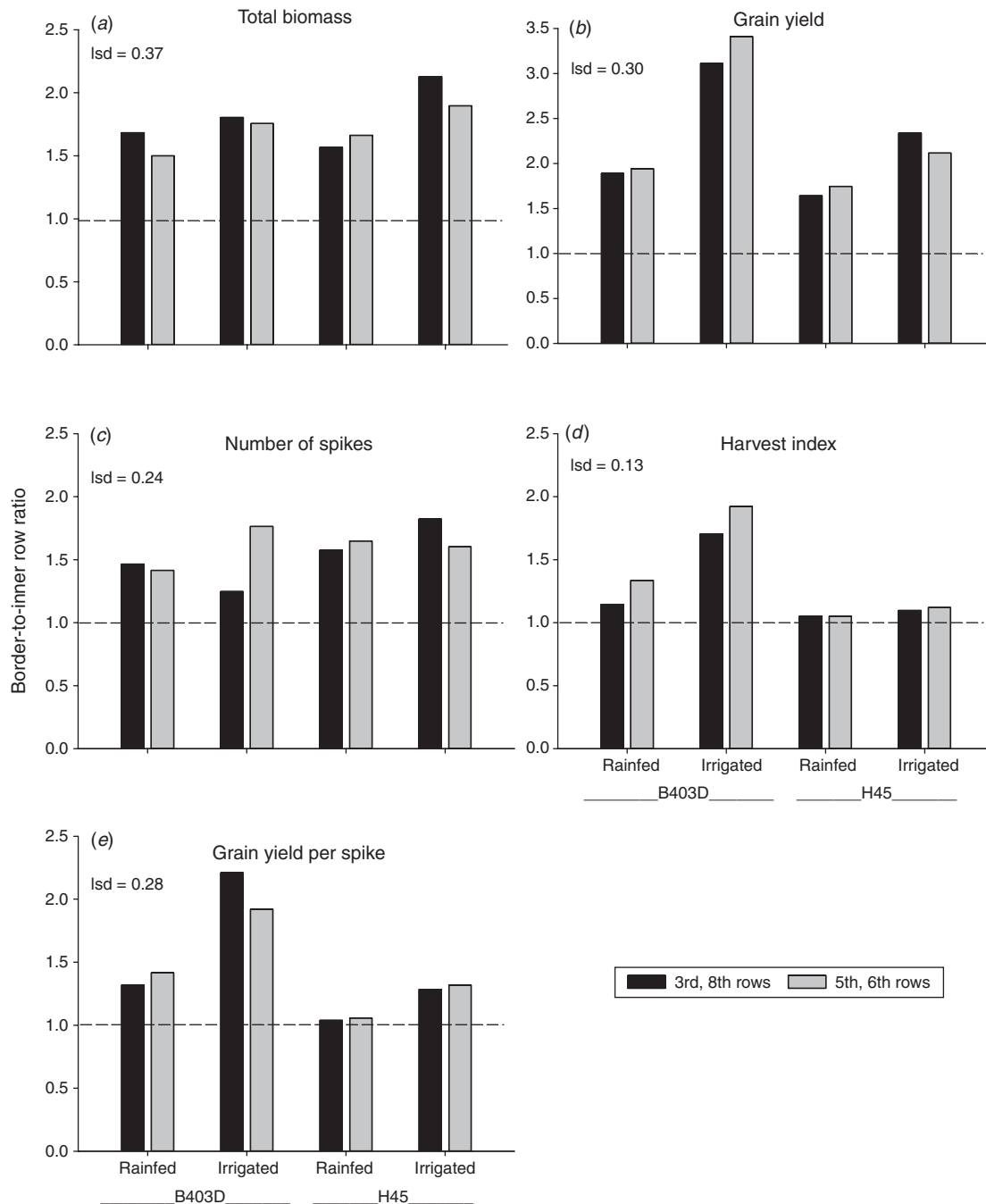


Fig. 5. Border row advantage compared with intermediate (third and eighth) and centre (fifth and sixth) rows for agronomic traits measured on different wheat genotypes (B403D and H45) grown with (irrigated) and without (rainfed) supplemental water: (a) total biomass; (b) grain yield; (c) number of spikes; (d) harvest index; (e) grain yield per spike. The ratio was calculated as border row divided by designated inner row for each trait. Lines were sown at 18-cm row spacing in 10-row plots at Condobolin, New South Wales in 2003. Average grain yields were 48 g m⁻¹ and 60 g m⁻¹, and average total biomass values were 151 g m⁻¹ and 175 g m⁻¹ of row for the rainfed and irrigated treatments, respectively.

Are unbordered mini-plots a useful compromise?

Small plots represent an intermediate planting arrangement between the spaced plant and row, and the large plot. Small plots contain multiple rows (commonly four or six) including border or edge rows that are harvested, and have been used

as a compromise between hills and full plots. However, as demonstrated above, the edge rows are influenced by the genotype's response to the extra resources and competition for these same resources by the edge row of the genotype in the neighbouring plot. In a few cases, there is no interplot path

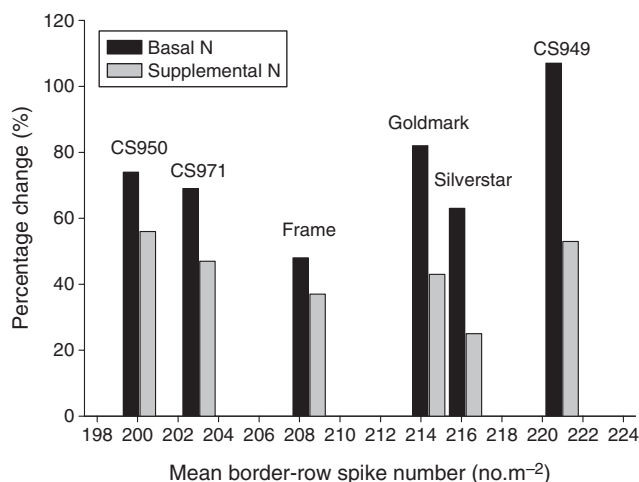


Fig. 6. Border row advantage (as a ratio of the centre rows to the border rows) for maturity spike number measured on six wheat genotypes supplied with basal and basal+ supplemental fertiliser nitrogen (LSD_{0.05} = 7%). Data are for Birchip, Victoria in 1999 (A. van Herwaarden, unpubl. data).

(gap) and hence no extra resources, and then there is simply competition between adjacent edge rows for a ‘normal’ amount of resources.

Under optimal conditions (irrigation and high inputs), Fischer (1978) studied a typical plot design that comprised four × 4.5 m-long rows, spaced 30 cm apart, with one unsown gap row between plots. ‘True yield’ was assumed to be the yield of 3.5 m of the two central rows (equivalent to our ‘large plot’ yield) and this was compared with the yield of the four-row whole plot (no removal of edge rows or plot ends but inclusion of the path in calculating plot area), equivalent to an unbordered small plot. Two tall and two short wheat genotypes were compared, with tall and short plots alternating across the experiment. The whole plots yielded 10% more on average than the true yield, as the plot-end benefit far outweighed any small yield loss due to the 60 cm space between plots. More importantly, the tall genotypes (average final height: 113 cm) yielded 7% more than the short cultivars (height of 79 cm) in the whole plot, whereas their true yield was 3% below that of the short cultivars.

The yield bias (~0.3% cm⁻¹ height difference) arose largely because of competition across the common paths, and was

Table 3. Ranking of robustness for different wheat traits when measured under favourable conditions, and recommended minimum plot sizes and types for robust sampling

A measure of responsiveness and subsequent genotype repeatability is also given for conditions of potential water limitation. LAI, leaf area index

| Trait robustness ranking | Minimum plot size ^A | Water-limited responsiveness ^B |
|---|--------------------------------|---|
| 1. Development score or anthesis date | Short, unbordered row | Stable |
| 2. Plant height | Short, unbordered row | Plastic |
| 3. Anatomical (e.g. xylem number, stomata size) | Short, unbordered row | Stable |
| 4. Morphological (e.g. awn presence, leaf number) | Short, unbordered row | Stable |
| 5. Carbon isotope discrimination | Short, unbordered row | Plastic |
| 6. Harvest index | Short, unbordered row | Plastic |
| 7. Early ground cover | Small, bordered plot | Plastic |
| 8. Tiller number at jointing | Small, bordered plot | Plastic |
| 9. Grain protein concentration | Small, bordered plot | Plastic |
| 10. Light interception or LAI | Small, bordered plot | Plastic |
| 11. Root depth | Small, bordered plot | Plastic |
| 12. Canopy stay-green | Small, bordered plot | Plastic |
| 13. Lodging score | Small, bordered plot | Plastic |
| 14. Grain weight and size | Small, bordered plot | Plastic |
| 15. Ear fertility | Small, bordered plot | Plastic |
| 16. Stomatal conductance | Small, bordered plot | Plastic |
| 17. Osmotic adjustment | Small, bordered plot | Plastic |
| 18. Stem carbohydrate concentration | Small, bordered plot | Plastic |
| 19. Root architecture | Small, bordered plot | Plastic |
| 20. Final spike number | Large, bordered plot | Plastic |
| 21. Biomass at anthesis | Large, bordered plot | Plastic |
| 22. Stem carbohydrate content | Large, bordered plot | Plastic |
| 23. Nitrogen uptake | Large, bordered plot | Plastic |
| 24. Canopy temperature | Large, bordered plot | Plastic |
| 25. Grain number | Large, bordered plot | Plastic |
| 26. Grain yield | Large, bordered plot | Plastic |
| 27. Maturity biomass | Large, bordered plot | Plastic |
| 28. Water use | Large, bordered plot | Plastic |

^AShort unbordered rows can be substituted for hill plots and spaced single plants.

^BThe absolute ranking will depend on the timing and extent of water limitation and will be exaggerated by variation in genotype-specific factors including phenology, seedling vigour and tillering.

^CSmall, bordered plot is minimum size of 3-4 rows, and large, bordered plot is minimum size of 6-7 rows.

close to what would be expected from the extra solar radiation intercepted by the taller whole plot at the expense of the adjacent shorter plot (Fischer 1978). Leaf angle was also implicated in interplot competition, with genotypes producing more horizontal leaves gaining more in competition across a gap path row. Without a separating path, and ignoring plot-end effects, interplot competition in alternating tall–short plots would result in a yield bias estimated at 2.5% cm⁻¹, 1.4% cm⁻¹, 0.9% cm⁻¹ and 0.6% cm⁻¹ height difference for plots with one, two, three or four rows, respectively (cf. 0.3% cm⁻¹ for the four-row plot plus path above). These numbers will depend somewhat on row orientation (east–west here) and the latitude or month of growth, but the bias is always likely to be significant when genotypes differ in height and other traits favouring competitiveness (e.g. greater tillering and horizontal leaf architecture).

Yield biases have also been reported for small plots grown under drought (Fischer 1981). For example, the average benefit of a row bordering open space was little different under a yield-halving terminal drought compared with under full irrigation (about +80% in each case), presumably because of the extra water benefit under drought (through lateral roots accessing the adjacent soil water reserves) about equalled the extra light benefit of edge plants without drought. When plot ends were removed, harvesting 2 × 30 cm plots with implanted path row still led to highly significant biases with respect to true yield, especially under drought. Under a terminal drought reducing overall yield by 46%, the known drought-tolerant wheat cultivar Gabo yielded 27% less than cultivar Cocorit 71 in the small plots, whereas the true yield was 29% greater. In a similar comparison over 2 years, the barley variety CM67 yielded 82% more than the wheat cultivar Yecora 70 in small plots under drought, which reduced overall yield by 50%, but the true yields under this drought were similar between the two genotypes. Thus the small plot bias reversed the drought tolerance of Gabo but exaggerated that of the barley cultivar, presumably because the differing lateral rooting response of the genotypes was unlikely to be of importance in the crop situation.

Which traits are robust?

A greater understanding is required of the relative robustness of different traits when phenotyping in order to assist in the planning and resourcing of future experiments. However, this information is not well documented or readily available. We have summarised, to the best of our knowledge and experience, a ranking of trait robustness for spring wheat evaluated across multiple seasons (Table 3). Traits such as development score and plant height have a strong genetic signal and so are highly repeatable, with genotypes ranking relatively consistently across favourable environments. This robustness leads to greater confidence in phenotyping in small plots or even short rows and hill plots. In contrast, grain yield and maturity biomass are genetically complex and are likely to be more sensitive to both environmental variation and experimental sampling to reduce repeatability (e.g. Quail *et al.* 1989). The greater sampling errors possible in assessing these traits necessitate larger plot sizes to increase confidence in genotype means. Traits including light interception, tiller number and

canopy temperature are less challenging to measure but can suffer from effects of interplot competition. Hence some form of plot border is required in their measurement.

Genotypes commonly change ranking when extended across contrasting environment types. Earlier, we demonstrated the capacity for genotypes to change ranking for yield when density (as row spacing) is altered. Changes in performance arising through differential water use will also affect the ranking of some genotypes for some but not all traits. Importantly, the extent of genotype rank change will depend on the timing and extent of water limitation, and on the variation in genotypic factors, including root growth, tillering, early vigour and development, will magnify the extent of this potential rank change. Table 3 summarises the extent to which traits are stable when assessed in response to changing water availability. Indeed, differential water use among genotypes is likely to influence most traits of economic interest, resulting in a relatively plastic response.

Conclusions

Breeders, geneticists and physiologists, plant pathologists and others are growing larger amounts of germplasm in studies aimed at better exploiting genetic variance and an improved understanding of genetic control. If we ignore the importance of planting arrangement and the sampled unit, the possible confounding effects of interplot competition or border rows are likely to identify germplasm of limited value and genomic regions not relevant to the target trait and target environment. Furthermore, many of the important true genetic effects are very small in size and are unlikely to be detected where confounding may contribute to bias and statistical error. The ecological literature focussing on growth and survival under resource limitation has recognised the importance of above- and belowground competition, and the relevance of this to agricultural fitness is equally valid. This was demonstrated within this paper in the comparisons to plot measures across many independent wheat studies. The experiments collectively demonstrate the need for well planned studies. Investigations into the dynamics of yield formation (particularly canopy-related characteristics) must consider the likely value of plots large enough to permit the discarding of border rows so as to minimise the effects of interplot competition and/or extra space. Finally, research undertaking assessment of canopy-based traits such as biomass, grain yield or grain yield components should include support by a breeder, agronomist or crop physiologist with experience in the phenotyping of these traits.

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