Determining sources of variation in yield assessments of fodder beet crops in New Zealand: how many samples are needed?

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Abstract

The rapid increase in the area of fodder beet grazed and sold in the South Island of New Zealand has brought to attention the need for improved yield assessment methods. As a precision planted crop with variable germination and plant survival rates, the number of plants per row metre varies significantly. This makes it more difficult to get a representative sample for yield assessment. Currently there are differing methods for performing yield assessments involving row lengths and multiple rows. These methods are used commercially without any published validation. Therefore, the objective of this study was to determine the effects of increasing row length and increasing sampling sites on measurements of plant counts and dry matter (DM) yields. A novel 'bootstrap' approach was used to assess variation as row lengths and site numbers increased. The sampling area was within a 70 ha crop with seven sites selected at random within a 1 ha block. At each site, two x 7 m adjacent rows were sampled. The number of plants and total fresh weight were recorded for each metre within each row and DM content determined. The results of this study showed strong variation in plant count and DM yield between individual metre lengths sampled, with lower variation in DM content of the individual plants. There was higher variation within sampling rows than between different sites in plant count, DM yield and DM content. A sequential increase in row length up to 5 metres strongly reduced the variation in plant count and DM yield measured, with stable variation beyond that length. A double row of 5 m row lengths x 5 sampling sites provided estimates of DM yield (15.88±1.13 t DM/ha) that were similar to that produced using all samples in the study (15.38±1.17 t DM/ha), suggesting that the methodology provided a suitable balance between accuracy and increased investment for estimation of DM yield in fodder beet crops.

***Additional keywords*:** *Beta vulgaris*, bulb, leaf, dry matter yield, crop sampling, bootstrap analysis

Introduction

Pasture based livestock systems in the South Island of New Zealand routinely use crops as a feed source for winter feeding, as pasture growth during the colder autumn and winter seasons will not sustain a profitable stocking rate (Moot *et al.*, 2007; Gibbs and Hughes, 2008). Such cropping is common across the dairy, beef and sheep industries. Until about a decade ago, winter crops in the south were primarily brassicas such as kale and swedes (de Ruiter *et al.*, 2009). However, comparatively low yields (<15 t dry matter (DM)/ha) and restricted feeding windows with these crops has opened the way for alternatives such as fodder beet.

Fodder beet (FB) (*Beta vulgaris* L.) crops have been grown in New Zealand for more than a hundred years. A limited amount of research into their use as an energy source and as a potential winter crop for stock has been done in New Zealand before the 1990's (Martin, 1983; Bourdôt and Butler, 1985; Magat and Goh, 1988). However, the plant has been used as a stock feed in Europe for five hundred years, with use strongly influenced by the perception that various inherent toxins (e.g. oxalic acid content) produced animal health issues if fed above low rates (>3 kg DM/d for cattle). These ideas governed the use of FB in New Zealand (Williams and Coup, 1959), and clearly limited use of the crop as a winter or lactation feed of any significance.

In the past decade there has been renewed interest in the use of FB in the South Island. This occurred as early industry experience of high DM yields of 20-30 t/ha led to DairyNZ commissioning a specific project on FB use as a primary winter diet (Gibbs, 2011). This project found that grazing management at transition to the crop, not plant toxicity, was the reason for animal health and production issues observed with ruminants consuming the crop. A simple grazing method to overcome transition issues was developed in this work (Gibbs, 2011; Gibbs and Saldias, 2014a). This drove the adoption of FB as a winter, supplementary and finishing feed for both dairy and beef herds (Gibbs and Saldias, 2014b). However, it also increased the need for accurate yield assessments in order to satisfactorily allocate DM during transition to the crop.

The distribution and growth of FB often appears highly variable and 'patchy' across the paddock, with gaps in rows, and plants seemingly at a higher density than the original sowing rate (due to multigerm seeds producing more than one plant). There can also be inconsistencies in how the crop is sampled and reporting of paddock yield estimates (Gibbs, 2011; Matthew *et al.*, 2011). Care is needed when sub-sampling FB in order to extrapolate to estimates of yield per hectare. This is important both for growers, when calculating the amount of feed present and for setting stocking rates. Trial work to compare cultivars, responses to fertilisers, crop losses due to pests and pathogens, and grazing intakes of ruminants on the crop also requires standardised procedures which account for the variation in plant density (Prendergast and Gibbs, 2015).

The industry has no accepted standard protocol for sampling of FB, although there is a strong consensus that multiple replicates of row lengths should be used, and varying sample sizes and number of replicate samples varies widely between studies. In New Zealand field trials for example, Martin (1983) used four replicates of a single 1.5 x 1.5 m quadrat, Bourdôt and

Butler (1985) used ten 1 m lengths of row, Matthew *et al.* (2011) sampled one or two row metres at ten random points in the paddock, Chakwizira *et al.* (2013) used four to six replicates of two rows varying from 2-6 m length, and Gibbs (2011) recommended three replicates of 4 m row lengths. Even recently, DairyNZ were recommending that at least six to eight quadrat samples each a minimum of 1 m^2 should be used for DM yield estimation of FB (Anonymous, 2015).

Determination of optimum sampling protocols for FB that lessens the effects of any extreme values requires two components: the number of different locations ('sites') within the crop, and the minimum limit to the size of each of these samples (e.g. row metres). This leads to a form of multi-level (hierarchical or nested) sampling procedure, where, in FB crops, each site represents the primary or highest sampling level and the metres sampled within each site comprises the secondary level (Snijders and Bosker, 1999). With this sampling structure, each lower level can contribute to the variation observed in the higher level, and thus influence the final estimates of the parameters of interest. However, in FB crops, the lower level samples (row metres of crop) at any sampling location are not added at random to the overall harvested area. This is due to the practical consideration that the samples would increase in size by adding on consecutive metres along the row, thus minimising edge effects, rather than being 'disjointed' within a given sampling zone.

Maas and Hox (2005) stated that there was little guidance for researchers in their multi-level design decisions, however the use of resampling simulation procedures such as Monte-Carlo and bootstrapping has been suggested as having good potential in sample size analysis of multi-level designs (Maas and Hox, 2005; Snijders, 2005).

The aim of this investigation was to examine the effects of increasing sample size (consecutive row metres) and number of replicate samples (sites over the paddock) on estimates of total DM yield of FB. The consequences for accuracy of yield estimation for farmers and statistical power for researchers are discussed.

Materials and Methods

Crop sampling

Fodder beet (cv. Brigadier, Seed Force Ltd, Christchurch) was sown over 70 ha at a dairy wintering unit in Springston, Canterbury (43° 38' S, 172° 20' E) on 1 November 2014 at a sowing rate of 90,000 seeds/ha and row widths of 500 mm. The crop was fertilised at sowing with a tailored fertilisation regime on the basis of soil mineral analyses. The crop received one pre-emergence and two post emergence herbicide and insecticide applications.

On 12 March 2015, a 1 ha area was randomly selected and seven sampling sites chosen by randomly selecting coordinates on a theoretical 100 m x 100 m grid. At each site, two parallel rows (A and B) each 7 m long were used for a plant count and DM assessment. A plant count was performed for each metre of the 7 m row and the number of multigerm plants also recorded so an additional 'corrected' plant count could be included. Plants were harvested and the total fresh weight (FW) of bulbs and leaves in each metre were recorded. Those plants that occurred at the intersection of metre positions were identified and half of the fresh weight was mathematically allocated to each contiguous metre sample. The starting position on row A was always located mid-way between two beets (defining one corner of the quadrat).

A representative plant from each metre was kept for DM (DM%) assessment by the method of Gibbs (2011). Each bulb was cut into longitudinal quarters and two of these quarters then used for duplicate DM estimations. Each quarter was cut into cubes of approximately 2 cm and the fresh weight recorded. Leaves were also cut in half lengthwise for duplicate samples and weighed. Both bulb and leaf samples were placed in a fan forced oven at 65°C and dried to a constant dry weight (DW), approximately 21 days. The DM% of the samples was calculated as:

$$\mathrm{DM\%} = \frac{\mathrm{DW}}{\mathrm{FW}} \times 100$$

DM yield estimations were then made for each sampled metre based on the corresponding harvest FW and DM% estimations for that particular metre.

Statistical analysis

'Best' estimates of the 'true' mean, standard deviation and coefficient of variance (CV%) of each performance variable were initially made by treating all 98 of the one metre samples as independent samples. The proportion of variation due to 'between' and 'within' sampling sites was determined by one way ANOVA, treating site as the main effect and the residual as within-site error.

To examine the effect of increasing the length of the sampled row on consistency of parameter estimation, a non-parametric bootstrap resampling method was utilised (Resampling Stats Add in for Excel software; Resampling Stats, Inc. 2001; Simon, 1997; Bruce, 2000). Mean values were obtained for increasing sampling row lengths based on sampling consecutive metres along the length of each row (and for the average of rows A and B) commencing at the first sampled metre. Thus, the values obtained consisted of a sequence of means, produced by:

$$x1,\ \frac{x1 + x2}{2}, \frac{x1 + x2 + x3}{3}, \ldots, \frac{x1 + x2 + \ldots xn}{n}$$

Values from all seven sites were resampled with replacement 10,000 times and sampling consistency estimated by calculating the between site CV%.

A second set of bootstrap means (10,000) was obtained using these parameters to investigate how many sites in the paddock were required to obtain estimates of means that were within certain limits of the original 'best' estimates. Goodness of estimation was measured by calculating what proportion of the 10,000 bootstrap means were outside of 'acceptable' absolute differences from the best estimate:

$$\frac{\text{Bootstrap mean - true mean}}{\text{true mean}} \times 100$$

The acceptable differences used were: 15% for total plant and corrected plant populations, 5% for leaf and bulb DM%, and 10% for DW per metre measurements.

Results and Discussion

Crop performance

The mean total DW estimate of 769 g per linear metre was equivalent to a crop yield of 15.38 t/ha. The mean growth rate from emergence to final harvest (120 days) was estimated at 128 kg DM/ha/d. A mean DM accumulation rate of at least that figure until a 'mature' crop yield in early June (210 days) is likely on the basis of recent Canterbury fodder beet trials (daily DM increases peak in late summer and early autumn at >300 kg DM/day; de Ruiter, unpublished data), and would suggest final crop yields 25 t DM/ha or greater. This figure would position the crop within the industry-standard commercial target zone for irrigated FB crops with good management and high plant numbers.

Despite this, and the precision sowing at 90,000 plants per hectare, the number of plants harvested per linear metre was highly variable. When plant number was corrected for multigerm seeds the number of plants harvested per metre was only slightly less variable (Table 1). Dry matter assessments tended to be more consistent: while bulb DM% ranged between 6.0 and 13.7% and leaf DM% between 6.3 and 10.8%, the CVs were much lower than that seen in the plant count data (Table 1). This is consistent with industry data from previous South Island wide crop assessments (Gibbs, 2011), and demonstrates the necessity of using known DM% values for individual crop assessment rather than assumed values. In addition, the CV for total DM per metre was 37.6%, suggesting accurate FB crop sub-sampling requires more, rather than fewer, individual plants for DM% and plot DM yield assessment.

For estimates of overall yield, the width of the 95% confidence intervals are positively related to sample variation but negatively related to the square root of sample size. As a consequence, although measuring yield from the cumulative 5 m samples reduced between-sample variation (Table 1), it also effectively reduced the sample size number from 50 samples of 1 m length to five samples of 10 m length. This reduction in sample size number impacts on the confidence intervals. However, from the data collected in this trial, the average overall yield (±95% CI) based on 5 x '5 m double row' samples was 15.88±1.13 t DM/ha, very similar to that obtained from using all of the 98 x 1 m samples of 15.38±1.17 t DM/ha. The practical application suggested by this result is that even in a heterogeneous crop displaying significant variability in plant count and DM weights per metre, the use of 5 m double rows at five sites produces a an accurate estimate of overall yield.

The results of the ANOVA suggest that the majority of variation in FB yield (in terms of plants per m and dry weight) was occurring within the 7 m sampling rows rather than between the distinct sites (Table 1). For most of the response variables, very little variation (R^2 of <6%) was explained due to differences between the seven randomly selected sampling sites (Table 1). This would likely not hold in many commercial crops, where variation in yield across the area sown can be large (Gibbs and Saldias, unpublished data). Only leaf DM% exhibited a statistically significant difference among the seven sites ($P<0.001$; R^2=26.4%), possibly indicating some variability in soil properties (e.g. water content) over the paddock, although the actual magnitude of the difference between highest (9.6%) and lowest (7.8%) mean values was small.

Table 1: Mean, standard deviation (s) and coefficient of variation (%) for fodder beet (cv. Brigadier) yield components grown in Canterbury, NZ. Yield components include: total plants (number/m), corrected plants (multiple germinations from a single-sown seed; number/m), bulb dry weight (DW; g/m), leaf dry weight (DW; g/m), total dry weight (DW; g/m), bulb dry matter (DM; %) and leaf dry matter (DM; %). Values are based on using all of the sampled 98 m as independent samples, the means of the seven sampling sites of seven metres as the sample unit, and the means obtained for five sites obtained from sampling a '5 m double row'. R^2 indicates amount of variation explained by between-site differences estimated from sums of squares produced by one-way ANOVA, scaled 0-100.

	N = 98 sites x 1 m				N = 7 sites x 7 m			N = 5 sites x 5m		
Variable	mean	s	CV (%)	R^2	mean	s	CV (%)	mean	s	CV (%)
Total plants	4.4	1.87	42.4	2.37	4.4	0.31	7.0	4.7	0.61	13.1
Corrected plants	3.2	1.18	37.4	2.50	3.2	0.20	6.4	3.4	0.29	8.7
Bulb DW	504.7	205.2	40. 7	4.38	504.7	46.2	9.2	510.3	42.4	8.3
Leaf DW	264.5	112.5	42.5	2.42	264.5	18.8	7.1	273.8	12.2	4.4
Total DW	769.2	291.7	37.9	3.59	769.2	59.4	7.7	784.1	52.5	6.7
Bulb DM	9.2	1.66	18.1	5.59	9.2	0.42	4.6	9.2	0.64	6.9
Leaf DM	8.6	1.01	11.8	26.41	8.6	0.56	6.5	8.5	0.80	9.4

Sample size

The results of the bootstrap analysis examining the response of between-site CV values to extension of the sampling length are summarised in Figure 1. As expected, when sample size increased the CV values for yield reduced down to the mean values (for each site) and there was a decrease and then a stabilisation of the between-site CVs. However, there were instances where the relationship between CV and sampling length was not maintained (e.g. Figure 1 b, d, h). This was interpreted as an artefact of the sampling process whereby the chance of including an atypical 'metre' in the overall sample increased with increasing sample length. In general terms, the values obtained from the 'double row' sampling produced smaller CVs than each of the single rows, across all of the yield components and also across the increasing cumulative metres that were sampled. While this was not always the case (e.g. Figure 1a and b at two sequential metres of sampling), due to the same reason outlined above, it does suggest that for the yield components assessed in this study there is a measurable benefit in including two rows rather than one in a sampling protocol.

For the yield components assessed in this study, using data from the double row and sampling up to 5 m would appear to produce acceptable results, as the between-site CVs had reduced and typically stabilised by this point in the cumulative addition of row length up to 7 m, with only the partial exception of plant counts (Figure 1a and b). It follows that as a practical measure, the use of extended row length measurements beyond this may not return increased accuracy commensurate with the increased effort and cost involved of sampling.

The between-site CVs for both bulb and leaf DM% (Figure 1c and e) were typically much smaller than the yield variables, showing more consistency between rows and stabilising when fewer metres per row had been sampled. Thus, it is possible that sampling only two or three plants per location might be adequate for estimation of these metrics.

A common source of error in yield estimates is the 'edge effect'. Because of the size of the FB plants many lie on the border of the sampling zone and the individual performing the sampling must decide whether to include the plant in the sample or not. As individual plants can weigh in excess of 20 kg, decisions regarding a plants inclusion in the sample can have significant implications for final yield estimates per unit area. The errors due to edge effects should be reduced by increasing the sampling area, and also by instituting some standardised rules, such as half of the FW of plants falling on the boundary being included in the sample. This was demonstrated in this study by the relative dominance of increased row length measurements as a driver for reduced total variation (Figure 1a and b).

With row length measures as in this experiment, each additional metre is not an independent unit, but necessarily dependent on the previous metre and recognition of this is an important difference in application of these models. The results presented in Table 1 and Figure 1a-g demonstrate the importance of a cumulative increase in 'units' (metres) for accurately assessing FB yield.

Sample sites

The bootstrap analysis, examining how increasing the number of sites improved parameter estimation, used only the values from the 'double row 5 m' sample at each site. For all variables the proportion of bootstrap sample means that differed from the ‘true mean’ by a given percentage decreased, and trends became smoother, as more sites were used to estimate sample means (Figure 2). Although for all variables the percentage of samples deviating from the ‘true mean’ by the stated proportion was still decreasing even after all seven sites were sampled, the trends appeared to stabilise once estimates had been made from between four and six sites. If total DW is taken as the primary measure of yield then for five sites the average difference was only 3.6% that of the best estimate, with a maximum difference of 10.7%, and 95% of differences were below 8.6%. This suggests that the use of a single or very few sites, even with extended row metres, will likely produce crop yield estimates of unacceptable variation.

Consequences for the statistical power of field trials on fodder beet

In statistical significance hypothesis testing, the significance probability (α, usually set at $P<0.05$) is also the probability of committing a Type I error (i.e. rejecting a null hypothesis when it is actually true). The probability (β) of committing a Type II error (i.e. not rejecting a null hypothesis when it is actually false) can be considered equally important, as meaningful differences between treatments, cultivars, etc. may be overlooked. The power of a statistical test to detect a difference of certain magnitude between treatments (i.e. the probability of rejecting the null hypothesis when it is false) is defined as 1-β, and is inversely related to sample size (Sokal and Rohlf, 1995; Zar, 1999).

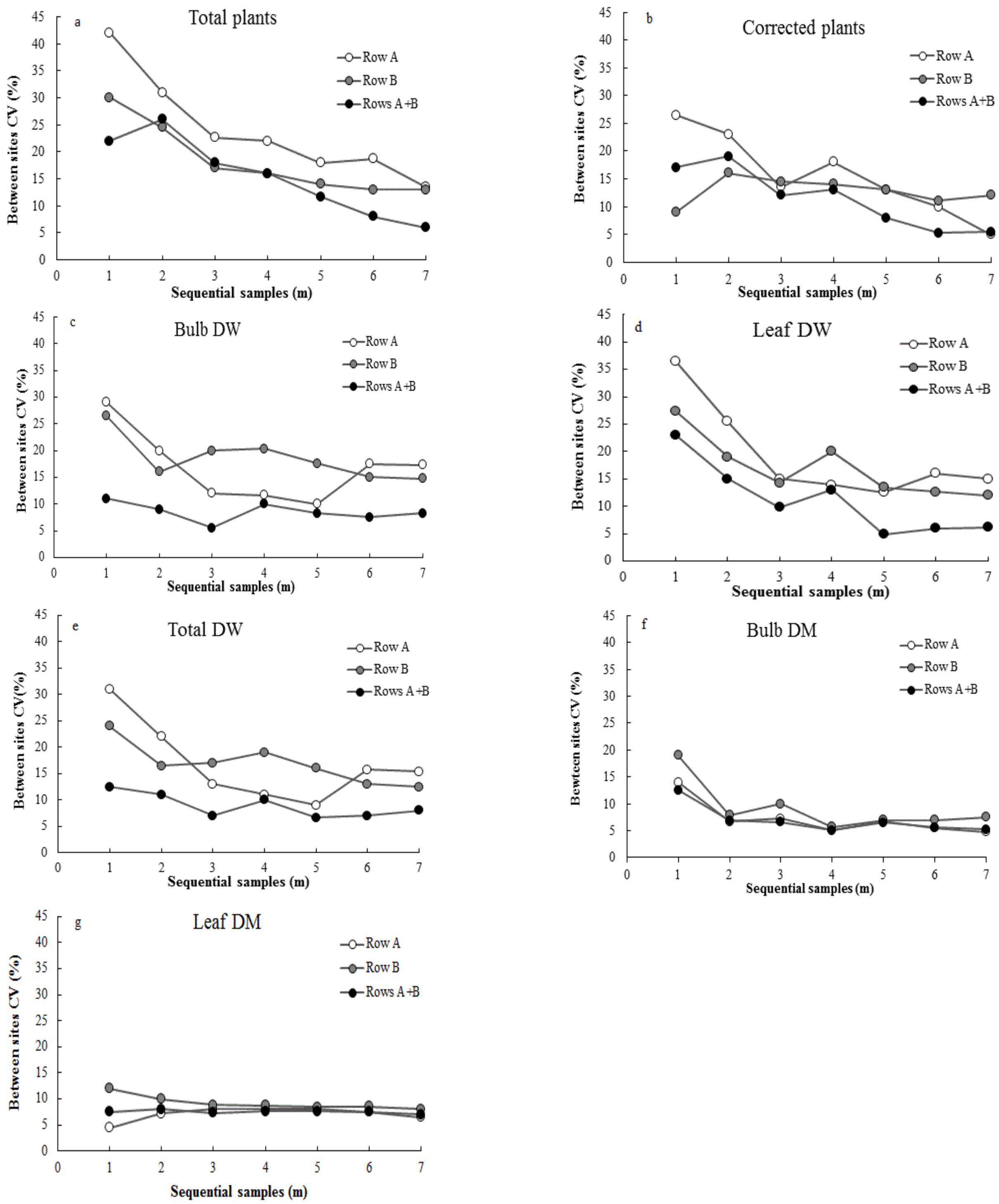

Figure 1: Trends in 'between-site' coefficient of variance (%) and increasing length (m) of sampled row for fodder beet (cv. Brigadier) yield components grown in Canterbury, NZ. Yield components include: total plants (number/m), corrected plants (multiple germinations from a single sown seed; number/m), bulb dry weight (DW; g/m), leaf dry weight (DW; g/m), total dry weight (DW; g/m), bulb dry matter (DM; %), and leaf dry matter (DM; %). Data are shown for individual crop rows (A, B) and the combined average of the 'double row' (A + B).

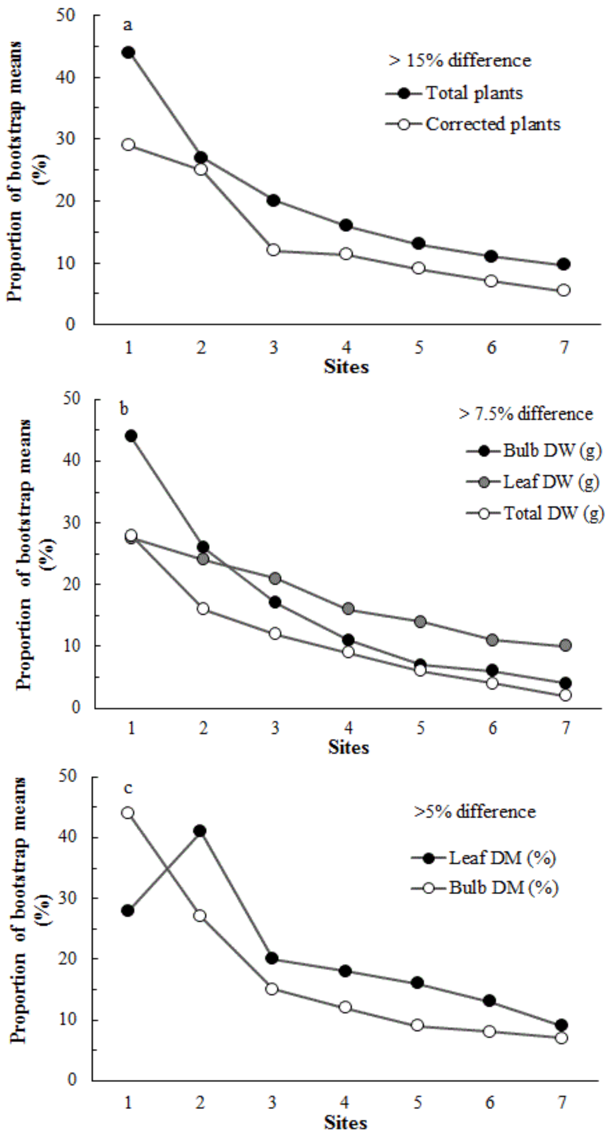

Figure 3: Relationships between the number of sites sampled ('5 m double row') and the proportion of bootstrap sample means (from 10,000) that differ from the 'true mean' by a given percentage (>5, >7.5 or >15%) for fodder beet yield measurements.

Many field trials comparing fodder crops tend to have low replication and thus low statistical power unless large differences between treatments are observed. The difficulty of ensuring adequate power in statistical comparisons of field trial work is not always acknowledged in experimental design of crop assessments (Edmeades and McBride, 2012).

In this trial power analyses were performed on the results from the ‘five sites x 5 m’ sample regime, which provided CVs of 8.3%, 4.4% and 6.7% for bulb DW, leaf DW and total DW respectively (Table 1). Rounding these CV values to 10%, 5% and 7%, setting the significance probability to a typical α=0.05 and the power to 0.8, the analysis indicated minimum detectable differences of 20.2%, 10.1% and 14.2% between treatments based on five samples per group. These differences are all well within those reported in studies comparing cultivars and seasons (Ozkose, 2013), comparing sites (Chakwizira *et al.*, 2013), application of fertilizers (Turk, 2010) and the effects of herbicides (Bourdôt and Butler, 1985) on FB yields.

In this study the use of a multigerm FB crop was deliberate to exacerbate row variability in plant count and DM yield and to use this increased variation to delineate an upper boundary of variability in a well-managed FB crop. By comparison, as genetic monogerm FB crops would be expected to have less variation in plant numbers and individual plant weight.

Conclusions

The use of all samples obtained from the crop estimated a DM yield of >15 t DM/ha at approximately 120 days. This indicates a well-managed crop with good plant counts overall and strong early growth. Despite this, there was considerable variability in plant counts and weights per row metre. The variability observed in both bulb and leaf DM% was lower across the sampling lengths measured. Together this suggests methodologies of yield estimation of FB crops should include more, rather than less, individual plants being counted and weighed, but that fewer plants are required for accurate DM% assessment.

There was more variation within row metres than between sites. This confirms the use of increased metres in each row site, rather than fewer row metres and increased sites, is the preferred method. Also, the estimates of yield developed using all single, independent metre ‘units’ were very similar to those achieved using two rows of 5 m at five sites, giving confidence that the use of that row metre x site number is suitable for FB crop yield estimation. This is additionally supported by the bootstrap analyses, which demonstrate that CVs of DM yield typically stabilise at 5 m within a row, and that gains in reduced CV beyond five sites are minimal when double rows are included.

The deliberate use of a multigerm seed in this trial did increase inter-row variability in plant numbers and weights, but the use of a single hectare in a well-managed crop in this study would be expected to reduce crop variability from that experienced in many commercial scenarios. In addition, variability across soil types, sowing rates, moisture stress periods, fertiliser treatments, and many other factors, will also influence the sampling procedures suitable for FB assessment. While the methodology approach suggested here must therefore only be extrapolated to use across the industry with caution, it appears to provide an advance over current approaches.

This methodology has the statistical power to detect differences in total DM yield of approximately 14% at a detection power of 80%, and this is broadly suitable for most FB crop research purposes. Future work assessing the suitability of this methodology in crops of greater yield heterogeneity is required.

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