Developing protocols to quantify biomass in commercial faba bean crops

Antony Swan[1], Mark Peoples[1], Timothy Crews[2], Peter Matthews[3] and Robert Forrester[4]

[1]CSIRO Plant Industry, GPO Box 1600 Canberra, ACT 2601. www.csiro.au Email tswan@csiro.au
[2]Environmental Studies, Prescott College, Prescott, AZ 86301, USA. Email tcrews@prescott.edu
[3]NSW Agriculture, Temora Research Station, PO Box 304 Temora, NSW 2666. www.nsw.gov.au Email peter.matthews@agric.nsw.gov.au

[4]CSIRO Mathematical and Information Sciences, PO Box 664, Canberra, ACT 2601. www.csiro.au Email bob.forrester@csiro.au

Abstract

To quantify the amount of nitrogen fixed by commercial legume crops, an unbiased estimate of plant biomass with some know precision is required. This study assessed 4 faba bean (Vicia faba L.) crops located in the Temora region of New South Wales (NSW), by examining the variance components of sample size and sample number in relation to plant biomass.

In each faba bean paddock, a 1-2ha representative area was selected for sampling at mid pod fill. All above-ground material was harvested from 10 different locations within each of these selected areas. Each of the 10 replicates consisted of a single row of faba beans. The total length of row sampled was determined by row spacing to represent an area equivalent to $1m^2$. Each $1m^2$ sample was collected as 10 individual $0.1m^2$ sub-samples. Further sampling protocols included 4 plants collected at random (4Pr) near each replication, and 4 plants retained from nominated sub-sample positions (4Pm).

All faba bean crops were highly variable. Ten replications of sampling size $0.7m^2$ provided a reasonable estimate of plant biomass with a CV of 33.6%. Four random plants (4Pr) significantly overestimated faba bean biomass when compared to 10 replications of a $1m^2$ sampling size in 2 of the 4 crops, but there was no significant difference in biomass between 4Pr, 4Pm and $1m^2$ sampling size where the crops were more uniform. The number of replicates and sub-sample size required to obtain a good estimate of plant biomass from commercial crops is discussed.

Introduction

Many farmers across the wheat belt of southern NSW include pulse crops such as faba beans (*Vicia faba* L.) in their rotations to act as a break crop and provide additional nitrogen (N) from symbiotic N_2 fixation. The amount of N_2 fixed by a legume is regulated by the amount of N accumulated during growth and the proportion of that N derived from symbiotic N_2 fixation (1). The amount of N accumulated by the crop is calculated from estimates of crop biomass and tissue N content. It has been suggested that the largest source of error in calculating N_2 fixation, is accurately estimating above-ground biomass (1,2).

The objective of this study was to quantify the size and the number of faba bean samples required to be collected from commercial paddocks in southern NSW to obtain a reasonable estimate of crop biomass.

Methods

Properties

In October 2001, a survey was conducted of faba bean paddocks in the Temora region of NSW. Four separate properties were selected with paddock sizes ranging from 10-60ha. Three of the crops were grown on Vertosols and one crop grown on a Kandosol (3).

Sampling procedure

A 1-2ha representative area within each paddock was selected and all above-ground material was harvested from 10 locations within each crop at mid pod fill (assumed to be around the time of peak biomass). The first of these 10 sample points was randomly selected with subsequent replications being determined in a W pattern at 20m intervals in both directions. Each replicate consisted of a single row of faba beans, with the length of row sampled being determined by row spacing so that the total area harvested was equivalent to $1m^2$. Once the position for the replication had been determined, a tape measure was laid out parallel with a row to the required length starting from a randomly selected plant and the $1m^2$ area was then collected as 10 individual $0.1m^2$ sub-samples. The sub-samples were labeled positions 'A' to 'J' (position 'A' being the first $0.1m^2$ sub-sample). Plant population was determined from the total number of plants counted in each $1m^2$ sampling area.

Measures of crop dry matter accumulation determined from $1m^2$ were also compared to estimates calculated from 4 individual faba bean plants. Two alternative approaches were considered: a) randomly selected by eye (4Pr) within the vicinity at each replication, and b) from 4 nominated sub-sample areas along the sampled row (4Pm). The 4Pm plants were selected from positions 'A', 'D', 'G' and 'J'. All plant samples were dried at 70^oC for 48 hours before dry weights were measured.

Statistical method

All analysis was carried out using Genstat (Genstat 5.0). Position 'A' in each replicate is the only independent sample. The accuracy and precision associated with the mean biomass from position 'A' (first $0.1m^2$ sub-sample) for each property were compared to the biomass and error from the combined positions of 'A' and 'B' ($0.2m^2$), 'A' to 'C'… 'A' to 'J' ($1m^2$ area), with linear mixed models being fitted using REML. The standard error of difference (SED) between property means was determined for each sub-sampling area to examine the precision at each sampling area/size. The coefficient of variation (CV) was calculated to compare the sampling errors of this study relative to other authors.

A fixed sample size of $0.5m^2$ and $1m^2$ were compared using 10 replications via ANOVA. This analysis contained non-independent data, as the 10 replications of the $0.5m^2$ sampling area were part of the 10 replications of the $1m^2$ sampling area.

The accuracy and precision associated with randomly selecting 4 individual plants by eye (4Pr) and from predetermined locations (4Pm) were compared via ANOVA. 4Pr was also compared to the 10 replications of a $1m^2$ sampling area. The 4 properties were split into 2 farm types for analysis of 4Pr compared to 4Pm and 1 m^2. In one grouping (farm properties 1 & 2), the crops were more uniform with fewer weeds, similar low plant populations and plant heights (designated F_{unf}). The second grouping (farm properties 3 & 4) consisted of either weedier paddocks and/or excessively tall plants; both constraints introduced more variability in the choice of individual plant (designated F_{var}).

Results

Biomass yield, precision and Nfixed

There was a decreasing underlying linear trend in faba bean biomass yield with increasing sampling area ($r^2 = 0.977$), with significant curvature at $P<0.001$. The SED between the 4 properties reduced from 3.40t/ha at $0.1m^2$ to 1.01t/ha at $1m^2$ (Fig.1), illustrating the increase in precision through increasing sampling area. The CV ranged from 28.9% ($1m^2$) to 59.4% ($0.1m^2$) between the 4 properties, with the CV from a sampling area of 0.3-0.4m^2 being 37%, and 33.6% at $0.7m^2$. The CV for individual properties from 10 reps X $1m^2$ ranged from 17-35%. There was also a decreasing linear trend in faba bean biomass yield with increasing replication $r^2 = 0.925$, with the SED between the 4 property means decreasing from 1.51t/ha to 1.01t/ha (Fig 2).

When 10 replications of $0.5m^2$ were compared against 10 replications of $1m^2$ sampling size, there was a significant difference in biomass yield at $P=0.025$. Although this analysis was performed on non-

independent data (0.5m^2 being part of the 1m^2 sampling), it suggests that greater than 10 replications of a sampling size of 0.5m^2 is required to obtain a reasonable estimate of faba bean biomass.

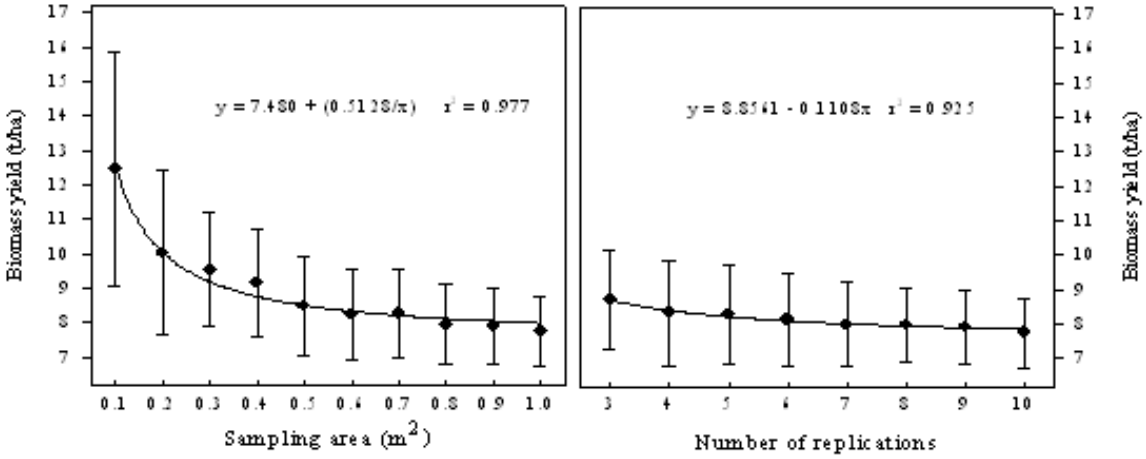

Fig. 1: Faba bean biomass and SED measured between 4 properties, from 10 replications of sub-sampling areas ranging from 0.1m^2 to 1m^2.

Fig. 2: Faba bean biomass and SED measured between 4 properties, for 3 to 10 replications of a 1m^2 sampling area.

Comparison of sampling methods for determining biomass (4Pr vs 1m^2 and 4Pm vs 4Pr)

When 10 reps of 4Pr were compared against 10 reps of 1m^2, there was a significant difference in faba bean biomass at P<0.001, a significant effect of farm groupings at P=0.027, and a significant interaction between farm groups and methods (P<0.001) (Table1). The CV was higher when derived from the 4Pr method compared to the 1m^2, and was similar to that obtained from a 0.3 to 0.4m^2 sampling size (37%). The 4Pr method significantly overestimated mean plant biomass compared to the 1m^2 for group F_{var}. There was no significant difference in faba bean biomass yield between the 4Pr and the 4Pm sampling methods, but there was a significant farm group effect at P = 0.016. The 4Pm sampling method determined mean biomass for group F_{var} to be 9.74t/ha compared to 11.75t/ha from the 4Pr method.

Table 1: Plant population and standard error of mean (se), the mean biomass from individual properties and grouped property data, plus the overall mean biomass determined from 10 replications of 4Pr or 1m^2. (Letters in parentheses indicate significant difference between rows and numbers between columns at P<0.05).

Property	Property type (group)	Plant population		Mean Biomass (t/ha)	
		(plants/m^2)	se	4Pr	1m^2
1	F_{unf}	11.2	1.07	7.02	7.37
2	F_{unf}	10.9	1.08	7.25	7.33
3	F_{var}	11.8	0.84	12.37	7.91

4	F_{var}	16.1	0.81	11.12	8.43
	Group F_{unf}		7.14 (*a*)(*1*)		7.35 (*a*)(*1*)
	Group F_{var}		11.75 (*a*)(*2*)		8.17(*b*)(*1*)
	Mean biomass t/ha, CV (%)		**9.44 (39%)**		**7.76 (29%)**

Discussion

Field-based investigations of N_2 fixation have determined legume shoot dry matter on the basis of sub-samples consisting of replicates of 4-5 individual plants, combined with measures of plant population (4,5), or sampling areas of a crop ranging from 0.25-0.5m^2 (6,7), 0.8-1m^2 (8,9) to 1->2m^2 (10,11). Yet despite being identified as potentially the largest source of error (1), relatively few studies have considered the impact of sampling protocols on estimates of above-ground biomass. Hanway and Weber (12) compared 0.31 and 0.62m^2 sampling strategies with soybean and suggested that the smaller sample size tended to overestimate dry matter yield. Similarly Hunt *et al.*(2) concluded that 0.75-1.5m^2 was required to adequately determine soybean biomass, and that measures based on small sub-samples (0.225m^2) or estimates from 4 randomly selected plants were imprecise. Both these investigations were undertaken on experimental plots, and in one case (2) used irrigation. As such, these crops might be expected to be more uniform than large scale, dryland crops.

Our results indicate that growth within commercial faba bean crops can be highly variable. However, results from the current study indicate that the sampling protocols based on 10 replications of 0.7m^2 or greater provided estimates of dry matter accumulation with reasonable precision, irrespective of crop uniformity. The precision attained (CV) for this sampling area was 33.6%, which reduced to 28.9% for 10 reps by a 1m^2 sampling size. This was considerably greater than that obtained in a study (11) from 3 districts in northern NSW- 11% (Walgett), 13% (Moree) and 16% (Gunnedah). However, this particular investigation sampled 5 properties per district, with 10 replications of a sampling size greater than 1m^2. It is interesting to note that the authors' concluded that it would be wasteful of man-power and resources to attempt to measure biomass with any greater precision (11).

Where the crop is more uniform, it may be possible to use a smaller sampling size as we found no significant difference between faba bean biomass determined from 10 replications of 4 randomly and 4 pre-nominated plants per paddock compared to 10 replications of 1m^2. In the current study, a sampling protocol using 4 plants per replication represented an equivalent area of 0.36m^2 in the weed free paddocks. However, in general, we would not recommend the 4 individual plant method for determining biomass.

Conclusion

Ten replications of a 0.6- 0.7m^2 sampling size provided a reasonable estimate of plant biomass with some precision in variable faba bean crops. In some instances, smaller sampling sizes could be used, but the error associated with these sampling sizes relative to a larger sampling size cannot necessarily always be pre-determined. The current investigation should be considered as a pilot study, and that a different sampling design might need to be implemented to fully test sampling protocols across a wider range of commercial legume crops.

References

(1) Peoples, M.B., Schwenke, G.D. and Herridge, D.F. (1997). Pp141-153 in proceedings of an International Workshop on Managing Legume Nitrogen Fixation in the Cropping Systems of Asia, 1996, ICRISAT, India.

(2) Hunt, P.G. Burnham, K.P. and Matheny, T.A. (1987). Agron. J., 79:425-428.

(3) Isbell, R.F. (1996). The Australian soils classification system. CSIRO Publishing: Melbourne.

(4) Bergersen, F.J., Turner, G.L., Gault, R.R., Chase, D.L. and Brockwell, J. (1985). Aust. J. Agric. Res., 36:411-423.

(5) Unkovich, M.J., Pate, J.S. and Hamblin, J. (1994). Aust. J. Agric. Res., 45:149-164.

(6) Evans, J., Turner, G.L., O'Connor, G.E. and Bergersen, F.J. (1987). Field Crops Res. 16: 309-322. (7) McNeill, A.M., Pilbeam, C.J., Harris, H.C. and Swift, R.S. (1996). Aust. J. Agric. Res., 47:1061-1073.

(7) Evans, J., Fettell, N.A., O'Connor, G.E., Carpenter, D.J. and Chalk, P.M. (1997). Biol Fertil Soils., 24:87-95.

(8) Rochester, I.J., Peoples, M.B., Constable, G.A. and Gault, R.R. (1998). Aust. J. Exp. Agric., 38:253-260.

(9) Schwenke, G.H., Peoples, M.B., Turner, G.L. and Herridge, D.F (1998). Aust. J. Exp. Agric., 38:61-70.

(10) Schwenke, G.D., Murison, R.D. and Harden, S (1997). pps 321-331 in Proceedings of an International Workshop on Managing Legume Nitrogen Fixation in the Cropping Systems of Asia, 1996, ICRISAT , India.

(11) Hanway, J.J. and Weber, C.R. (1964). Agron. J., 63: 227-230.