

A MINIMUM PLOT SIZE FOR ASPARAGUS CLONAL TRIALS

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ABSTRACT

Individual plants in a trial of asparagus clones, with seven clones and two seed-propagated controls, were harvested for two seasons and yields recorded. The aim was to find the minimum plot size that would give acceptable results for future asparagus clonal trials.

Different size plots were formed by amalgamating yields from varying numbers of adjacent plants. A split-plot analysis was carried out on plot sizes of between two and five plants. Standard errors of the mean were then calculated for each plot size. Another analysis was carried out using the first 2,3...10 plants in each row. The precision of variety means was calculated for each plot size.

A plot size of 7-10 plants was found to be adequate for asparagus clonal trials, with three replications. In initial trials comparing seed-propagated cultivars with clones, a larger plot size of 15 plants is recommended.

INTRODUCTION

There has been interest in establishing asparagus production beds using clones of high yielding plants for a considerable time (Murashige *et al.*, Yang 1977). In a recent paper Nikoloff (1988) indicated that yields from clones of between two and three times those of seed-propagated cultivars were possible.

Micropropagation of asparagus has now developed to a stage that allows the establishment of production beds using clones. (Slimmon *et al.*, 1985, Jamieson *et al.*, 1985).

Little information exists on the optimum plot size for evaluating clones and a number of trials are planned. This paper reports results of a trial that enabled a determination of a minimum plot size for trials of clones, thereby ensuring efficient use of resources for future trials.

MATERIALS AND METHODS

In December 1982, at the start of the experiment, two spears from each of 10 high yielding plants were sent to Forest Research Institute (F.R.I), Rotorua, as explant material for multiplication by micropropagation. Another clone (SW) was included at F.R.I. Six of the original 10 clones and SW were multiplied at F.R.I. (Nikoloff, 1988) and were received at Lincoln in December 1983 as rooted plants. Plants of the control varieties (MW500W and WSUT66) were spare seedlings from a batch grown to establish a breeders trial in December 1983. Plants and clones were a similar size. All plants were repotted into larger containers (Planta Bag, no 5, no supplementary lighting) during the winter of 1984.

There were two reasons for doing this; the clone plants had arrived too late to be planted in the field in 1983 and were in different size containers to those of the seedlings. To maintain them easily, as well as increase their size, plants were repotted and grown in the glasshouse. The trial was established in December 1984. Rows were 1.5 m apart and plants 0.45 m apart in the row. Each row was one plot and contained 10 plants. The trial was designed as a

randomised complete block. Both controls and four of the clones were replicated three times, three clones (65, 66, SW) had only two replications.

Plants were planted into the bottom of a 150 mm deep trench and were irrigated when necessary during the following summer. Two applications of nitrogen (50 kg N/ha each application) were banded down the rows during that summer. One application of herbicide (Methabenzthiauron 0.5 kg ai/ha and Metribuzin 0.2 kg ai/ha) was used to control weeds. In the autumn, the trenches were filled. In subsequent seasons herbicides (Methabenzthiauron 1.6 kg a.i. and Diuron 1.4 kg ai/ha 1985, Terbutylazine/Terbutmeton 2.5 kg ai/ha 1986, Terbacil 1.5 kg a.i. 1987) were applied pre-season to control weeds.

A base dressing of lime (2.5 t/ha, blood and bone (1.3 t/ha, serpentine superphosphate (550 kg/ha) and potassium chloride (250 kg/ha) was applied before planting. Subsequently annually broadcast fertilizer applications of lime (2.5 t/ha), serpentine superphosphate (350 kg/ha), potassium chloride (350 kg/ha) and urea (350 kg/ha), were made to maintain fertility. Potassium chloride and urea were applied in a split treatment; half pre- and half post-harvest. During the summers of 1985 and 1986 berries were removed by hand from plants, of female clones.

Individual plants were harvested, every two days, for 21 days in 1986 and 42 days in 1987. Spears were graded into saleable (6mm - 20+ mm basal diameter and a minimum of 180mm long) and reject (bent, thin, damaged or open bracts), and the number and weight of spears recorded.

Row totals were calculated by amalgamating yields from individual plants. Mean yields for each control variety or clone were then calculated.

Yields from varying numbers of individual clones were added to give various plot sizes. Thus adjacent plots were added to give five, two-plant sub-plots, three, three-plant sub-plots, two, four-plant sub-plots and two, five-plant

sub-plots. A split-plot analysis of variance was then carried out using total weight (saleable + rejects), with sub-plots as the split-plot factor. Since a plot size beyond five plants could not be analysed in this way, a second set of analyses was performed using one sub-plot from each row, comprising the first 2, 3...10 plants in each row and the precision of variety means, using Standard Error, was calculated. Estimated efficiency was then calculated using Standard Error of the Mean (S.E.M.) for the 1987 results. This was calculated using the time taken to perform harvesting and recording tasks on the trial and enables a comparison of the labour efficiency of different size plots.

RESULTS AND DISCUSSION

Set out in Table 1 are the mean saleable and total yields (t/ha) for each of the clones and controls. Saleable yields recorded in the trial were low. The control varieties in a breeders trial, using plants from the same batch of seedlings gave better saleable yields. The total yields recorded give an indication of the yield advantage clones could give over conventional crops.

Calculated standard errors for plot sizes between two and five plants are shown in Table 2. A four plant plot gave the lowest S.E. in 1986 and a five plant plot gave the lowest S.E. in 1987.

TABLE 1: Mean total and saleable yields (t/ha) from seven clones and two controls.

	1986		1987	
	Saleable	Total	Saleable	Total
MW500W	0.56	0.80	0.67	1.32
WSUT66	0.6	1.15	0.95	1.97
Clone65	0.3	0.52	0.22	0.37
Clone66	0.32	0.77	0.42	1.83
Clone67	0.79	2.06	1.53	4.98
Clone70	0.63	1.22	1.25	3.24
Clone71	0.01	0.28	0.01	1.53
Clone73	0.55	1.38	1.11	3.09
CloneSW	0.51	0.98	0.76	2.97
MEAN	0.47	1.02	0.77	2.36
SEM	0.12	0.20	0.14	0.33

TABLE 2: The precision, from an asparagus clonal trial using the standard deviation from the whole trial, assuming that three replications are used.

No. of plants /plot	1986			1987		
	SD. (kg/plot)	S.E.M. (kg/ha)	CV%	SD (kg/ha)	S.E.M. (kg/ha)	CV%
2	90	380	33	210	890	32
3	140	410	37	250	710	25
4	90	200	19	280	590	22
5	140	240	21	320	550	20

TABLE 3: Standard errors of cultivar means from plots containing the first 2,3...10 plants in each row.

No. of plants /plot	plot size (m ²)	1986	1987	1987
		precision from 3 reps SEM (kg/ha)	precision from 3 reps SEM (kg/ha)	precision * from equal harvest time
2	1.35	428	645	528
3	2.02	450	619	520
4	2.70	247	562	489
5	3.37	192	484	432
6	4.05	163	373	341
7	4.75	198	301	281
8	5.40	241	318	305
9	6.07	247	414	405
10	6.75	255	384	380

*the estimated efficiency was calculated using average times in seconds taken to achieve each operation on the trial in 1986 and 1987, using an electronic data collector and weighing and grading at the trial site, with two people used to grade and record results.

Formula = S.E.M. (3 reps) * $1.73\sqrt{4s*n + 60s/300s}$

4s = time to harvest each plant

n = number of plants

60s = time to grade and record each plot

300s = total time to harvest, grade and record three replications of a 10 plant plot.

Since a plot size greater than five plants could not be used in the split-plot analysis, a second set of S.E.'s were calculated using the first 2, 3...10 plants in each row. These are shown in Table 3.

In both analyses, years have been kept separate. It was considered that if data were pooled this would bias the results towards the 1987 yields, since yields increased between 1986 and 1987.

In 1986 the lowest S.E. was obtained with a six plant plot and in 1987 with a seven plant plot. In both years the S.E. increased slightly as the plot size increased to 10 plants, probably because there were missing plants at row ends in some plots. When considering the precision from equal harvest times, a seven or eight plant plot gave the best result, for the time spent. S.E.M.'s for 1987 have been used to calculate this, as the harvest period was longer and the plants more mature, than in 1986.

In other asparagus trials at Lincoln, as plants reached maturity the standard error of the mean reduced (Nikoloff *et al.*, 1986). The present results indicate that a plot size of seven or eight plants could be used successfully in asparagus clonal trials of three replications. But since border effects have a greater impact on a small plot size, it would be preferable to settle for a 10 plant plot size. This would also ensure that there were adequate numbers of plants in a row if some of the plants died.

In trials of clones it may be preferable to have a larger plot size with fewer replication rather than a smaller plot

size with fewer replication, since it is probable an estimate of the uniformity of each clone will need to be made (Nikoloff, 1988). A larger plot size will allow this to be made without bias from the greater number of end plants, than more replications would imply. (For a fuller discussion on plot size verses replication in asparagus trials see Nikoloff *et al.*, (1986). It would be preferable to conduct plot size experiments on a greater number of plants. Because a relatively small number of plants (about 180) were measured in this experiment the results cannot be considered definitive, but they provide a starting point for further trials of asparagus clones. Modifications will be required after further experience has been gained.

One problem which will occur in running the first large-scale clonal trials is that the control varieties will need to be propagated from seed. In previous research (Nikoloff *et al.*, 1986) an adequate plot size for running cultivar evaluation trials was determined to be between 12 and 16 plants, with four replicatons. Nikoloff (1988) found that some clones are more uniform than seed propagated varieties, thus the optimum plot size for comparing clones is too small when conventional seed propagated varieties are included as controls in the trial. In order to ensure that a valid comparison is made, either the plot size of all initial clonal trials should be increased, with a maximum of 16 plants, or more plots of seed grown varieties should be included. If clonal planting material is restricted then smaller plots (10 plants) could be used and the number of control plots doubled.

After the initial trials have been run a stable, uniform clone/s could then be used as the control in further clonal trials.

CONCLUSIONS

A minimum plot size for asparagus clonal trials is 10 plants per plot. This will ensure adequate numbers of plant survive the duration of the trial. Once experience has been

gained with running trials of clones, this may need to be modified.

When planning the first large-scale trials, a plot size of 15 plants for each plot, with four replications, would give an adequate comparison with seed propagated controls.

If clonal planting material is in short supply then four replications, of plots of 10 plants, with twice the number of seed propagated plots, would be a second option.

ACKNOWLEDGEMENTS

Ms J. Aitken-Christie for multiplying the clones at F.R.I.; without her work the trial could not have proceeded.

REFERENCES

- Jamieson, J.L., Slimmon, T.Y., Tiessen, H. 1985. Time required to establish tissue culture clones. *Proceedings of the Sixth International Asparagus Symposium*: 89-66.
- Murashighe, T., Shabde, M.N., Hasegawa, P.W., Takatori, F.H. and Jones, J.B. 1972. Propagation of asparagus through shoot apex culture. 1 Nutrient medium for formation of plantlets. *J. Amer. Soc. Hort. Sci.* 97: 158-161.
- Nikoloff, A.S., Palmer, T.P., Wallace, A.R., and Ensor, P. 1986. An optimum plot size for asparagus variety trials. *N.Z. Journal of Experimental Agriculture* 14: 195-198.
- Nikoloff, A.S. 1988. Clones - a new breeding method for asparagus? *Proceedings of the 9th Australian Plant Breeding Conference*: 231-232.
- Slimmon, T.Y., Jamieson, J.L., Tiessen, H. 1985. Multiplication potential of tissue cultured asparagus. *Proceedings of the sixth International Asparagus Symposium*: 97-104.
- Yang, H. 1977. Tissue culture technique developed for asparagus propagation. *Hortscience*. 12(2): 140