

The Effects of Transplanting Time and Plant Density on White Clover (*Trifolium repens* L.) Seed Production

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ABSTRACT

The effects of transplanting time and plant density on seed yield were examined for plug seedlings of white clover (*Trifolium repens* L.). Seedlings transplanted in late autumn (October) did not survive the winter, while seedling survival for early autumn (August) transplants was only 50%. Autumn transplanting resulted in smaller plants which produced fewer flowers and a lower seed yield than plants established from spring transplanting. Time of spring transplanting had little effect on seed production as flower production per plant did not differ for the three transplanting times. Seed yield (16.6 g m⁻²) did not differ at plant densities of 2.7, 6.7 and 13.3 plants m⁻², but was reduced at the lowest density of 1.3 plants m⁻².

Additional index words: transplanting time, plant density, plug seedling, seed yield, white clover.

INTRODUCTION

In Japan white clover (*Trifolium repens* L.) is regarded as one of the most difficult herbage species from which to obtain a good and stable seed yield (Sugita, Fukuoka, Wakamatsu and Miura, 1982). Sowing of white clover at the optimum time is important to obtain a good stand and high yield. Delaying in autumn sowing leads to insufficient growth of seedlings before winter, plant death, and low seed yield, especially when sown at a low plant density (Sugita, Fukuoka, Wakamatsu and Miura, 1984).

Another problem is weed control. White clover seeds are smaller than those of many other herbage species and their growth during the seedling stage can be slow. Therefore, white clover seedlings can be easily damaged by competition from weeds.

White clover flower heads are formed with the extension of stolons and a high plant density inhibits the development of stolons and decreases seed yield (Zaleski, 1961, 1964; Sugita *et al.*, 1982; Clifford, 1985). However, it is not easy to maintain white clover stands established from low sowing rates.

For small areas of high grade (basic) seed, these problems can be resolved by using transplanting as an establishment method. Seed for transplanting can be sown at an optimum time, independent of weather. Seedlings can be reared under intensive care and protected from various kinds of stresses. By transplanting seedlings after they have grown to a size large enough to tolerate stresses, death of plants can be prevented and an even stand at the desired plant density can be established. Competition with weeds can be minimised by transplanting soon after cultivation. Ono, Kanaya, Higuchi and Motomura (1998) showed that white clover seed yield was much improved by transplanting.

To improve the efficiency of raising seedlings, the plug seedling system is widely applied to vegetables and flowers (Tsukada,

1990; Honda, 1995). This system enables a large number of seedlings to be raised in a small area; seedlings can then be mechanically transplanted. In this study, we tried to determine the optimum transplanting time and density of white clover plug seedlings to introduce this system into seed production.

MATERIALS AND METHODS

White clover (*Trifolium repens* L.) cv. Makibashiro, bred at the Tohoku National Agricultural Experiment Station, was used for the following experiments. All experiments were carried out at the Nagano Station of the National Livestock Breeding Center (lat. 36°N and long. 139°E, alt. 700m).

The effect of spring transplanting time on seed yield (Expt 1).

The seeds were sown in 128-hole plug vegetable trays filled with a mix of peat moss and vermiculite. The seedlings were watered regularly and grown in a heated glass house. Three different spring transplanting times were used (Table 1). Seedlings were at the eight-leaf stage for SE and SM and the 12-leaf stage for SL at the time of transplanting. Plot size was 13.5m² (4.5m x 3m) for each treatment. For all treatments, 30 plants per plot (Ono *et al.*, 1998) were grown as spaced plants (0.75m x 0.6m apart in a rectangular pattern which gives 2.2 plants m⁻²). Each treatment was replicated three times.

Superphosphate (18% P), fused magnesium phosphate (20%P, 13%Mg and 0.5%B) and KCl (60%K) were applied at the rate of 148, 148 and 100 kg ha⁻¹ respectively to all treatments after transplanting.

Measurements were carried out at twelve randomly chosen planting spots per plot excluding those in the peripheral area. The time of flowering for each treatment was determined when half of the surviving plants had opened three or more

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florets on at least one head. The flower heads were harvested by hand on 24 August 1995 and dried. The seeds were hand cleaned using sieves and a seed blower. The number of flower heads (flower heads showing white corollas to those which were dark brown) at the time of harvest, and seed weight were recorded for each plot.

The effect of autumn transplanting time on seed yield (Expt 2).

Seedlings were raised as described for Expt. 1, except that a plastic house was used. Three spring transplanting dates (Table 1) were used. The design of plots was the same as for Expt. 1.

Superphosphate, fused magnesium phosphate and KCl were applied at the rate of 150 kg ha⁻¹ of each fertiliser for all treatments on 18 August 1995 and 25 April 1996.

Measurements were carried out at twelve randomly selected planting spots per plot excluding those in the peripheral area. Numbers of surviving plants were counted and the size of individual plants (average of diagonal diameters) measured

for each plot on 2 September 1996. The time of flowering for each treatment was recorded. The flower heads were harvested by hand on 3 September 1996 and the number of flower heads and seed weight were recorded for each plot.

The effect of transplanting density on seed yield (Expt 3). Four plant density treatments were used (Table 2) and each treatment was replicated three times. The seeds were sown in plug trays on 20 August 1996. The seedlings were watered regularly and grown in a plastic house. The seedlings were transplanted to the experimental field on 7 October 1996. A compound fertiliser (15%N, 15%P₂O₅, 15% K₂O), fused magnesium phosphate (20%P and 15%Mg) and calcium carbonate were applied at the rate of 400, 300 and 1200 kg ha⁻¹, respectively at the time of transplanting. Urea (46%N) and fused magnesium phosphate were both applied at the rate of 80 kg ha⁻¹ for all treatments on 16 April 1997.

The number of surviving plants was examined for each plot on 16 April 1997. The time of flowering for each treatment

Table 1. Time of white clover sowing and transplanting for experiments 1 and 2.

Treatment	Abbreviation	Time of sowing	Time of transplanting
Expt.1 Spring early transplanting	SE	30 Jan. 1995	29 Mar. 1995
Spring medium transplanting	SM	8 Feb. 1995	6 Apr. 1995
Spring late transplanting	SL	17 Feb. 1995	17 Apr. 1995
Expt.2 Autumn early transplanting	AE	18 Aug. 1995	28 Aug. 1995
Autumn medium transplanting	AM	28 Aug. 1995	12 Oct. 1995
Autumn late transplanting	AL	8 Sep. 1995	18 Oct. 1995

Table 2. White clover plant density and spacing for experiment 3.

Density (plants m⁻²)	Plant spacing¹	Plot size
1.3	0.75m x 1.0m	4.5m x 5.0m
2.7	0.75m x 0.5m	4.5m x 2.5m
6.7	0.75m x 0.2m	4.5m x 1.4m
13.3	0.75m x 0.1m	4.5m x 0.7m

¹ All plots were transplanted in a rectangular pattern.

Table 3. Weather data for each month over the experimental period and 10 year average.

Month	Mean temperature (°C)		Rainfall (mm)		Sunshine (h)	
	exp. per ¹ .	10 year av.	exp. per.	10 year av.	exp. per.	10 year av.
Mar. '95	2.3	2.5	79	60	165	169
Apr.	8.6	9.0	59	42	168	183
May	14.4	13.6	88	86	133	149
June	16.7	18.2	99	106	68	92
July	22.3	21.8	219	142	110	108
Aug.	24.6	23.1	64	117	191	167
Sep.	17.1	18.8	140	149	128	118
Oct.	12.5	12.4	47	91	163	133
Nov.	4.1	6.0	30	36	201	170
Dec.	-1.4	1.0	3	27	194	177
Jan. '96	-1.6	-1.4	12	27	175	169
Feb.	-2.6	-1.0	17	25	163	157
Mar.	2.0	2.5	46	60	191	169
Apr.	6.4	9.0	24	42	215	183
May	13.1	13.6	87	86	158	149
June	19.1	18.2	83	106	94	92
July	22.1	21.8	124	142	150	108
Aug.	22.5	23.1	60	117	161	167
Sep.	16.8	18.8	195	149	136	118
Oct.	11.4	12.4	80	91	160	133
Nov.	6.6	6.0	56	36	139	170
Dec.	0.9	1.0	29	27	194	177
Jan. '97	-1.9	-1.4	15	27	197	169
Feb.	-1.2	-1.0	10	25	194	157
Mar.	3.5	2.5	67	60	206	169
Apr.	9.6	9.0	68	42	184	183
May	14.9	13.6	97	86	171	149
June	18.7	18.2	110	106	140	92
July	22.7	21.8	111	142	139	108
Aug.	23.2	23.1	48	117	205	167

Table 4. Effects of spring transplanting time on the time of first flowering, number of flower heads per plant and seed yield (Expt 1).

Treatment ¹	Time of first flowering	No. of flower heads per plant	Seed yield		
			g head ⁻¹	g plant ⁻¹	g m ⁻²
SE	16 June	249a ²	0.027a	6.6a	14.8a
SM	30 June	240a	0.020b	4.8b	10.7b
SL	30 June	270a	0.020b	5.4ab	12.0ab

¹ Refer to abbreviations in Table 1.

² Means within a column followed by the same letter are not significantly different at $P < 0.05$.

Table 5. Effects of transplanting density on white clover plant survival, time of flowering, number of flower heads, total above ground yield and seed yield (Expt 3).

Density plants m ⁻²	Survival (%)	Time of flowering ¹	No. of flower heads (m ⁻²)	Total above ground yield (g m ⁻²)	Seed yield (g m ⁻²)	(%)
1.3	96a ²	19 May	339a	522a	8a	1.5a
2.7	97a	19 May	595a	852b	18b	2.1a
6.7	94a	19 May	565a	820b	15ab	1.8a
13.3	90a	19 May	553a	973b	17b	1.7a

¹ The time of flowering for each treatment was determined when half of the surviving plants had opened 3 or more florets on at least one head.

² Means within a column followed by the same letter are not significantly different at $P < 0.05$.

was recorded. A quadrat of 0.75m x 0.75m was set in each plot and the number of flower heads counted. Plants inside the quadrat were cut at ground level on 14 August 1997 and total above ground yield and seed yield were measured.

RESULTS

Weather data during the experiments are shown in Table 3. The rainfall in July 1995 (flowering to seed maturation period for Expt. 1) was higher than average by more than 50%. The temperature from November 1995 to May 1996 was lower than average, which indicates that winter coldness for Expt. 2 was more severe than usual.

The effect of spring transplanting time on seed yield (Expt 1)

All plants survived until harvest. Flowering began first in SE (Table 4).

The number of flower heads per plant at the time of harvest did not differ with time of transplanting. Seed yield per flower head was largest in SE. The largest seed yield was obtained in SE, but the difference between SE and SL was not significant (Table 4).

The effect of autumn transplanting time on seed yield (Expt. 2)

Seedlings were severely damaged by chilling during winter. Survival of plants was only 50% in AE and in the other two treatments, all plants were killed by cold injury. Plant size in AE reached 70cm at the time of harvest, there were 86 flower

heads per plant, and seed yield was 3.4 g m⁻².

The effect of transplanting density on seed yield (Expt 3). Survival of plants was higher than 90% and did not differ significantly among the density treatments. Time of first flowering was also the same for all four densities (Table 5).

Above ground dry matter yield and seed yield were significantly lower in plots of the lowest transplanting density, but did not differ among the other three transplanting density treatments (Table 5). The ratio of seed yield to the total above ground yield did not differ with plant density.

DISCUSSION

Pasumarty, Matsumura, Higuchi and Yamada (1993) and Pasumarty, Higuchi and Murata (1995) showed that white clover seed yields are increased if dry and sunny weather conditions occur during the process of seed maturation. Ono *et al.* (1998) obtained a seed yield of 28 g m⁻² by spring transplanting. The yields in our spring transplanting experiment were much lower than that obtained by them. The high rainfall in July might be one reason for the smaller yields. Higher seed yield per flower head (0.05g) in their experiment suggests better seed set because of more favourable weather conditions.

At Nagano Station, the melting of the snow cover and of soil frozen during the winter means that the soil is very wet in March, and it is therefore not possible to use machinery. However, as shown in Expt. 1, seed yield was not affected by the time of transplanting in spring, so that transplanting in early or mid April when soils have dried out somewhat and machinery for transplanting can be used is a practical option.

In Expt. 2, plants did not survive because of cold injury if the time of transplanting was delayed until after August. The survival rate was only 50% even when seedlings were transplanted in late August. In Expt. 3 and also in the experiment reported by Ono *et al.* (1998) in which the winter was milder than that for Expt. 2, the survival rate of plants transplanted in early to mid August was higher than 90%. From these results, we can conclude that survival of plants during winter is largely affected by the degree of coldness and can easily be decreased by any delay in establishment. To obtain a good stand using the transplanting method, seed must be sown by early August so that seedlings can be transplanted before the end of August.

For experiments 1 and 2 the plant density used (2.2 plants m⁻²) was chosen because Ono *et al.* (1998) demonstrated that at this site, white clover plants at this density reached 100% ground cover before harvest which therefore provided the opportunity for maximal flower production. However, in Expt. 1 an average of only 250 flower heads m⁻² was produced, much fewer than the 500-800 flower heads m⁻² recommended by other authors (Zaleski, 1964; Clifford, 1985) for optimising white clover seed yield. Interestingly however, in the following season (Expt. 3), from a plant density of 2.7 plants m⁻², 595 flower heads m⁻² were produced, once again emphasising the influence of climate (Pasumarty *et al.*, 1995) on white clover seed yield components.

Increasing plant density above 2.7 plants m⁻² to 13.3 plants m⁻² had no effect on seed yield in this trial. Many authors have shown that too high a plant density suppresses white

clover stolon development, reduces the number of flower heads per unit area and decreases seed yield (Zaleski, 1961, 1964; Sugita *et al.*, 1982, 1984; Clifford, 1985). Such a decrease in seed yield at higher plant density was not obvious in our experiment. However, plant densities in direct sowing experiments were generally much higher than those in our transplanting experiment. To determine if such a decrease in seed yield occurs in transplanting treatments, further investigation of seed yields at higher transplanting density must be carried out.

For direct sowing, higher planting density can easily be obtained by increasing the amount of seed sown. But in the case of transplanting, a higher transplanting density is not practical because the labour costs increase with the increase in plant density, even if a transplanting machine is used. The practical transplanting density for seed production should be determined not only by the seed yield but also by production cost efficiency, including that of labour.

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REFERENCES

1. Clifford, P.T.P. 1985. Effect of cultural practice on potential seed yield components of "Grasslands Huia" and "Grassland Pitau" white clover. *New Zealand Journal of Experimental Agriculture* 13: 301-306.
2. Honda, F. 1995. Problems on the use of plug seedlings. 1. Seedling production. *Agriculture and Horticulture* 70: 597-600.
3. Ono, J., Kanaya, T., Higuchi, S. and Motomura, S. 1998. Improvement of white clover (*Trifolium repens* L.) seed production in Japan by transplanting and mulching. *Journal of Applied Seed Production* 16: 101-104.
4. Pasumarty, S.V., Higuchi, S. and Murata, T. 1995. Environmental influences on seed yield components of white clover. *Journal of Applied Seed Production* 13: 25-31.
5. Pasumarty, S.V., Matsumura, T., Higuchi, S. and Yamada, T. 1993. Seed production in white clover (*Trifolium repens* L.). 1. Seed set. *Journal of Japanese Society of Grassland Science* 39: 169-176.
6. Sugita, S., Fukuoka, H., Wakamatsu, T. and Miura, Y. 1982. Studies on the culture for seed production of white clover (*Trifolium repens* L.). 1. Effects of seeding dates, seeding rates, fertiliser levels and cutting treatments. *Bulletin of the Tohoku National Agricultural Experiment Station* 66: 23-35.

7. Sugita, S., Fukuoka, H., Wakamatsu, T. and Miura, Y. 1984. Studies on the culture for seed production of white clover (*Trifolium repens* L.). 2. Seed production in space and row planting and in second seed year. *Ibid* 69: 51-74.
8. Tsukada, M. 1990. Seedling raising system and plug seedlings. *Agriculture and Horticulture* 65: 111-117.
9. Zaleski, A. 1961. White clover investigations. I. Effect of seed rates and cutting treatments on flower formation and yield of seed. *Journal of Agricultural Science* 57: 199-213.
10. Zaleski, A. 1964. Effect of density of plant population, photoperiod, temperature and light intensity on inflorescence formation in white clover. *Journal of the British Grassland Society* 19:237-247.