BIOMASS ACCUMULATION AND NITROGEN FIXATION BY A WHITE CLOVER SEED CROP

G.A. Whelan* and J.G.H. White
Department of Plant Science, Lincoln College, Canterbury

ABSTRACT

Dry matter accumulation and nitrogen fixation by a commercial field of volunteer white clover (*Trifolium repens* L.) was assessed from November 1983 to February 1984 at Lincoln, Canterbury. Clover was grown as dense or at a 30 cm row spacing with nil or 75 kgN/ha. Nitrogen fixation was estimated using a partial nitrogen balance method. Soil nitrogen availability after seed harvest was measured by nitrogen uptake of ryegrass grown in undisturbed soil cores in a glasshouse pot trial.

Mean clover dry matter yield was 5,320 kg/ha. Seed yield increased from 44.9 g/m² in dense to 63.2 g/m² in row-spaced clover. Increased seed yield was associated with an inflorescence density of 449/m² in dense and 543/m² in row-spaced clover and a lower proportion of total dry matter as leaf, stem and aerial stolon (233 g/m² for dense and 162 g/m² for row spaced clover respectively).

Clover increased the yield of a succeeding Moata ryegrass crop grown in a pot trial. Mean Moata production after clover was 3.6 g dry matter/pot while ryegrass production after fallow and barley was 2.4 and 2.3 g dry matter/pot respectively.

Clover density and application of nitrogen fertiliser did not significantly affect nitrogen fixation. The mean quantity of N fixed was 223 kgN/ha. Nitrogen in clover consisted of 168 kgN/ha in above ground clover herbage and 27 kgN/ha in seed. Soil nitrogen mineralised during the growth of a succeeding ryegrass crop was 155 kgN/ha after clover and 127 kgN/ha following fallow. Implications of post-harvest management for soil fertility are considered.

Additional Key Words: *soil fertility, clover residues, nitrogen fertiliser, clover density, seed yield*

INTRODUCTION

White clover seed crops have an important role as the fertility restorative phase in many cropping rotations in New Zealand. With the development of intensive cereal growing and elimination of grazed pasture from the cropping sequence, increasing attention has turned to the use of legume seed crops to provide a source of nitrogen (N) through symbiotic nitrogen fixation.

Many estimates have been made of the level of fixation of symbiotic nitrogen by clovers in mixed pastures in New Zealand and the beneficial effects of high producing pasture on succeeding crop yields have been well documented (Sears, 1953; Sears *et al.*, 1965). Fixation values range from 670 kg N/ha in 'cut and carry' systems (Sears *et al.*, 1965) to 180-400 kgN/ha in developed grazed pastures (Hoglund and Brock, 1978; Hoglund *et al.*, 1979). In mixed pastures, companion grasses provide strong competition for available soil nitrogen forcing clovers to rely on symbiotic fixation to satisfy plant N requirements. Consequently estimates of N fixed in mixed pastures are not an accurate measure of fixation in pure legume swards.

No quantitative information is currently available on the beneficial effects of white clover seed crops on soil fertility in New Zealand. The trial reported here was designed to measure the effects of clover density and nitrogen fertiliser on biomass accumulation and nitrogen fixation by a white clover seed crop.

MATERIALS AND METHODS

The trial was conducted in a commercial field of volunteer white clover (*Trifolium repens* L.) on the Lincoln College mixed cropping farm. Two clover densities and two rates of nitrogen fertiliser were compared in a randomised 2 x 2 factorial design with six replicates. Soil type was a Templeton silt loam of 0.16% total N, pH 6.1 and Olsen P 15. The previous cropping history was white clover seed (1980/81), wheat (1981/82), forage brassica (1982) and vining peas (1982/83).

Clover plots (5 m x 3 m) were established on 4 October and dense clover was thinned to provide a 30 cm width of clover spaced 30 cm apart (referred to as row-spaced clover). Plots (3 m x 3 m) of barley harvested for seed and a fallow treatment were established adjacent to the main trial for calculation of a nitrogen balance. Clover top growth was removed from barley and fallow plots by push hoe prior to establishment on 4 October. Weeds were removed from the fallow treatment at regular intervals by push hoe. Calcium ammonium nitrate (26% N) was applied in three split applications (3 x 25 kg N/ha) on 4 and 26 October and 14 November. Clover plots were grazed with sheep for four days prior to closing for seed production on 13 November and all plots were irrigated with 32 mm of water on 29 November. Carbetamide was applied to all plots at 4.5 kg a.i./ha on 12 August to control weeds while Nexion (Bromophos) was applied at 1.5 l a.i./ha on 9 December to

* *Present address — Agricultural Research Division, Ministry of Agriculture and Fisheries, Gisborne.*

control Blue-green lucerne aphid (*Acrythosiphon Kondi Shinji*).

White clover biomass was sampled on 24 December, 20 January and 19 February (41, 68 and 98 days after closing respectively) by taking a 1.0 m^2 quadrat harvested to ground level. Total dry matter yield and the plant components (1) inflorescence and petiole, (2) leaf, stem and aerial stolon and (3) surface stolon were measured. Inflorescence density was measured from two 0.2 m^2 quadrats per plot four weeks prior to the final harvest on 19 February when seed yield was measured. N content of biomass dry matter and seed was determined later.

After harvesting, two undisturbed soil cores (100 mm dia. x 150 mm depth) were taken from clover, barley and fallow plots for measurement of available soil nitrogen in a glasshouse pot trial. 2,4-D was applied at 4.5 kg a.i./ha to all soil cores to kill clover growth. On 14 April, after removal of dead clover and barley stubble, ryegrass (*Lolium multiflorium* cv. 'Grasslands Moata') was sown at 20 seeds per pot. After the ryegrass emerged plants were thinned to 10 plants per pot and grown in a glasshouse at a minimum of 10°C with a 12 hour photoperiod. Soil cores were arranged in a randomised block design with six replications. Dry matter accumulation and N content of the ryegrass was measured in two consecutive harvests over 21 weeks.

Nitrogen fixed by individual clover treatments can be estimated by a nitrogen balance method. The net N yield from white clover can be calculated as:

Net N yield = N removed − N inputs (1)

The nitrogen yield of ryegrass (kg N/ha) was calculated from pot N yields (g N/pot) on an area basis. It was not possible to measure the fate of applied nitrogen in this trial and it is assumed that 70% of applied nitrogen was utilised for clover growth (Steele and Shannon, 1981).

Nitrogen fixed can then be estimated as the difference in the N yield of clover and fallow treatments (Equation 2).

N fixed = clover N yield − fallow N yield (2)

RESULTS

The weather was drier than average in October and November and wetter in all other months. Temperatures were warmer in October and November and cooler from December 1983 to February 1984 (Table 1).

TABLE 1: Mean monthly temperature (°C) and rainfall (mm) from October 1983 to February 1984. Figures in parentheses are deviations from the long term average.

	Month	Temperature	Rainfall	Irrigation
1983	Oct	11.3 (+0.4)	31 (−14)	-
	Nov	13.2 (+0.4)	21 (−30)	31
	Dec	14.0 (−0.8)	87 (+30)	-
1984	Jan	14.5 (−1.5)	86 (+31)	-
	Feb	15.7 (−0.1)	55 (+ 8)	-

Leaf, stem and aerial stolon as a proportion of the total dry matter increased up to 68 days after closing while inflorescence dry matter increased up to the final harvest (Figure 1). At 41 and 68 days after closing inflorescence dry

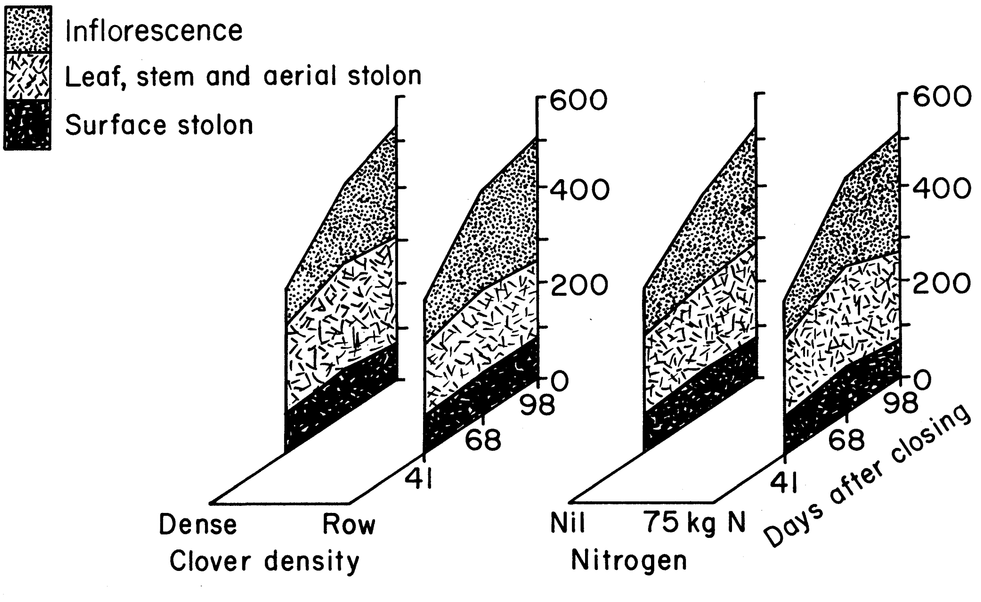

Figure 1: Pattern of dry matter accumulation and the distribution for two clover densities and two rates of nitrogen during the growing season.

matter was higher for row-spaced than dense clover ($P<0.05$), differences being non-significant at the final harvest. Dry matter yield of surface stolon was similar for all clover treatments. Nitrogen fertiliser had no effect on total biomass or on the proportion of dry matter as leaf, stem, inflorescence or stolon.

The seed yield of row spaced clover was 41 percent higher ($P<0.001$) than dense clover (44.9 g/m^2) and was largely due to a higher inflorescence density (Table 2). Nitrogen application had no effect on either seed yield or inflorescence density.

TABLE 2: Inflorescence density and seed yield of white clover as affected by clover density and nitrogen fertiliser.

Treatment	Flowerheads/m^2	Seed yield g/m^2
Clover density		
dense	449	44.9
row spaced	543	63.2
	**	***
Nitrogen		
nil	514	55.3
75 kg N/ha	478	52.7
	n.s	n.s
S$\bar{x}$	18	2.5
C.V. (%)	12.4	15.9

At the final harvest total nitrogen per m^2 in clover herbage was higher in dense than in row-spaced clover. The reverse occurred in the seed where seed N was higher in row-spaced clover (Table 3). There was no effect of nitrogen fertiliser. At final harvest the percentage N was 3.5 and 5.0 in herbage and seed respectively.

TABLE 3: Nitrogen yield (gN/m^2) of white clover seed and herbage as affected by clover density and nitrogen fertiliser.

Treatment	Seed	Herbage
Clover density		
dense	2.24	18.16
row-spaced	3.09	15.52
	***	**
Nitrogen		
nil	2.68	17.16
75 kgN/ha	2.64	16.52
	n.s	n.s
S$\bar{x}$	0.76	0.19
C.V. (%)	15.6	17.3

Total dry matter accumulation of Moata ryegrass was not affected by the various clover treatments (Table 4). However, clover treatments produced 57 percent more dry matter than the mean of barley and fallow (2.31 g dry matter/pot). The fallow treatment yielded only 3 percent more dry matter than the soil which grew barley (2.27 g dry matter/pot).

TABLE 4: Total dry matter accumulation (g/pot) and nitrogen yield (g/pot) of Moata ryegrass as affected by various preceding treatments of clover, barley or fallow.

Treatment		Dry Matter	Nitrogen
Clover			
dense	−N	3.83	0.15
dense	+N	3.60	0.16
row-spaced	−N	3.43	0.12
row-spaced	+N	3.62	0.14
Barley		2.27	0.08
Fallow		2.35	0.10
S$\bar{x}$		0.78	0.015
C.V. (%)		16.3	14.3

Individual clover treatments did not affect the total amount of nitrogen harvested in ryegrass (Table 4). At each harvest, clover produced a higher ryegrass N yield than either barley or fallow treatments ($P<0.05$; data for individual harvests not presented).

The quantity of nitrogen fixed by each clover treatment was estimated by a partial nitrogen balance method (Table 5). The nitrogen removed in seed and herbage was derived from Tables 3 and 4. Nitrogen fixation did not vary significantly over the clover treatments (Table 6). Mean N potentially available to a crop after clover was 196 kg N/ha (Figure 2).

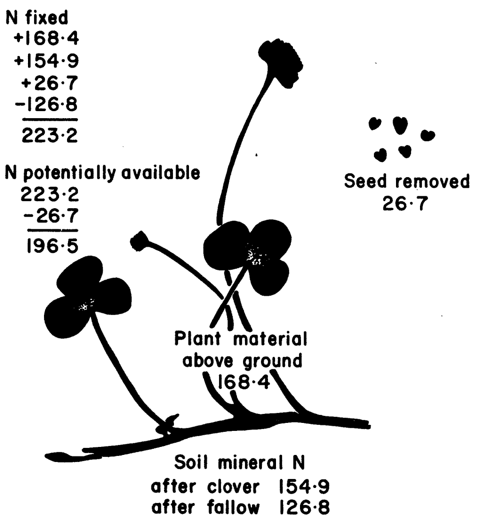

Figure 2: Nitrogen potentially available (kgN/ha) to a crop following white clover in the rotation.

TABLE 5: Nitrogen inputs, removal and nitrogen fixed (kg N/ha) by individual clover treatments.

	Dense −N	Treatment Dense +N	Row −N	Row +N	Fallow	Barley
Nitrogen inputs						
Seed	-	-	-	-	-	2.5
Fertiliser	-	52.5	-	52.5	-	-
Total	-	52.5	-	52.5	-	2.5
Nitrogen removed						
Seed	23.3	21.5	30.6	31.4	-	33.9
Herbage	184.4	178.8	158.1	152.4	-	10.5
Moata ryegrass	189.7	206.3	150.2	178.2	126.8	104.4
Total	397.4	406.6	338.9	362.0	126.8	146.3
Net yield[1]	397.4	351.4	338.9	309.5		
N fixed[2]	270.6	224.6	212.1	182.7		

[1]Net N yield = N removed − N inputs
[2]N fixed = clover N yield − fallow N yield

TABLE 6: Main effects of clover density and nitrogen fertiliser on nitrogen fixation (kg N/ha) by a white clover seed crop.

Treatment	Nitrogen fixed
Clover density	
dense	247.6
row-spaced	197.4
	n.s
Nitrogen	
nil	241.3
75 kg N/ha	203.6
	n.s
S$\bar{x}$	23.2
C.V. (%)	21.8

DISCUSSION

The weather in Canterbury from December 1983 to February 1984 was favourable for growth and the mean dry matter yield of 5,320 kg DM/ha is comparable to the September − January yield (5,500 kgDM/ha) of a pure white clover sward in the Manawatu (Brock, 1973). High rainfall during December and January would have contributed to an increase in the dry matter yield of clover.

Seed yield of dense (44.9 g/m^2) and row-spaced clover (63.2 g/m^2) was above the Canterbury average of 300 kg/ha reported for heavy soil types (Batey, 1980). Growing of white clover in a 30 cm row spacing increased the seed yield 41% which was largely due to a higher number of inflorescences/m^2 (Table 2). High rates of stolon growth into inter-row spaces during flowering would increase the number of sites available for inflorescence development (Clifford, 1979) however, stolon growth up to 41 days after closing was not measured. The lower yield of leaf, stem and aerial stolon dry matter in row-spaced clover (Figure 1) is a direct effect of flower production. Stolon nodes can produce one vegetative auxiliary bud or one flowerhead, but not both. Therefore an increase in the number of inflorescences/m^2 reduces vegetative development as flowerheads develop in auxiliary bud sites (Thomas, 1979). Senescence of leaf and stem tissues at the base of the sward caused the decline in leaf, stem and aerial stolon dry matter at final harvest.

Plant material added to the soil is decomposed by the soil biomass making nitrogen available (Carran, 1983). Some material is more resistant and decomposes only slowly. Wheat ^{15}N derived from medic residues may be only 11-17% of the ^{15}N added after 15 months decomposition (Ladd *et al.*, 1981). Soil organic nitrogen accounted for 72-78% of the ^{15}N added as legume material. Nitrogen added to the soil in clover tops after seed harvest was 168 kgN/ha with the N yield of clover herbage and seed being proportional to the dry matter yield of the two components. The main value of clover residues would appear to be in maintaining levels of soil organic N to be mineralised at slow rates in following years.

Recommendations following recent research suggest that clover seed crops should be grown on soil of low fertility to produce a small leaf size and maximise the potential seed yield (Clifford, 1985). With the use of wide row spacings and management practices to reduce leaf size the amount of vegetative growth present at seed harvest will be reduced. The nitrogen yield of clover residues is proportional to the dry matter yield so the amount of N fixed in clover herbage would also decrease. Post-harvest management could influence the amount and form of nitrogen returned to the soil from clover residues. Grazing of residues with stock may result in an uneven distribution of N in dung and urine and the transfer of fertility to other areas (Gillingham and During, 1973). High contentrations of N in urine could increase the losses of N through leaching (Ball *et al.*, 1979). Total removal of clover residues after seed harvest would severely limit the accumulation of soil N for subsequent crops.

Forage legume and grain legume crops usually improve the soil fertility and productivity of subsequent crops

(Askin, 1983; Janson, 1984; McKenzie and Hill, 1984). This trial has demonstrated that a white clover seed crop was beneficial to a succeeding Moata ryegrass crop compared to fallow and barley (Table 4). Increased ryegrass yield after clover is attributed to an increase in the amount of plant-available N in the soil through symbiotic fixation by nodules on clover roots. Soil ammonium-N and nitrate-N were not measured at the start or completion of the trial or of the soil cores. High nitrogen yield after barley is probably due to the addition of symbiotically fixed N by secondary stolons of clover present in barley plots.

Estimates of nitrogen fixation using the nitrogen balance method assume that N mineralised from soil organic matter and leaching losses were equal for all treatments. Addition of nitrogen fertiliser to the soil has been shown to stimulate the mineralisation of soil organic matter (Broadbent and Nakashima, 1971). Nitrogen mineralised in control or zero-nitrogen plots may not reflect that in nitrogen-treated plots. Losses of N through leaching may also have been greater for fallow than for clover plots. The amount of plant-available soil nitrogen after seed harvest was measured in a glasshouse under controlled conditions. Nitrogen released from soil organic matter and plant material was assessed from a pot trial of only 21 weeks duration. Nitrogen added to the soil by clover may therefore have been greater than that measured. Glasshouse results may not be a reliable measure of nitrogen availability in the field due to variation in soil and climatic conditions. Further trials are required to measure plant-available nitrogen in the field following a white clover seed crop.

The mean quantity of nitrogen fixed by a white clover seed crop (223 kg N/ha) is in the range of values of soil N fixation + clover N yield reported for a ryegrass – white clover pasture (179-222 kg N/ha) from September to December (Brock and Hoglund, 1979). Nitrogen fixation by white clover was measured from November to February only and does not include N fixed prior to the start of the trial or from clover regrowth after the seed harvest. Nitrogen fixation prior to closing is likely to be small. As temperatures rise in spring the amount of mineral N released from soil organic matter increases and N fixation generally decreases (Moustafa *et al.*, 1969). Fixation after seed harvest may be important. If soil moisture is adequate clover regrowth and N fixation is likely to be dependant on the level of available soil nitrogen. The effect of autumn leaching and low rates of mineralisation reduce the amount of available soil N forcing clover growth to be more dependant on symbiotic fixation to satisfy plant N demands (Hoglund *et al.*, 1979).

The quantity of nitrogen available to a crop following a white clover seed crop (196 kgN/ha, Figure 2) is greater than that for field peas (15 kgN/ha), and lupins harvested for seed (55 kgN/ha) or grazed (86 kgN/ha) (Rhodes, 1980; McKenzie and Hill, 1984). High rainfall in December and January would have contributed to an increase in the N yield of clover biomass. Nitrogen fixed in clover residues would be reduced in a drier season. Assuming wheat and barley require 25 kgN to produce 1 tonne of grain (Millner, 1983) a 6 tonne crop has a nitrogen requirement of 150 kgN/ha from soil or alternative sources. Nitrogen derived from soil organic matter and the decomposition of clover residues (Figure 2) should meet the nitrogen demand of a first year cereal crop following clover in the rotation. Residues more resistant to microbial breakdown will contribute N to the organic N pool which will be released slowly in following years.

Elimination of white clover from the rotation could affect the amount of nitrogen accumulated in the soil. The long term effect on soil nitrogen reserves will depend on the balance of other legumes in the rotation and the amount of nitrogen added to the soil from these sources.

CONCLUSIONS

1. White clover seed crops improve soil fertility for succeeding crops compared to fallow and barley.
2. Nitrogen derived from above-ground clover residues forms a substantial proportion of the soil N accumulation for following crops.
3. Post-harvest management could affect the build up of soil fertility from a white clover seed crop.
4. Further research is required on nitrogen fixation prior to closing and after seed harvest and on the effects of post-harvest management on soil fertility.

REFERENCES

Askin, D.C. 1983. Nitrogen fixation in peas (*Pisum sativum*). Ph.D thesis, Lincoln College, University of Canterbury, New Zealand.

Ball, R., Kenny, D.R., Theobald, P.W., Nes, P. 1979. The nitrogen balance of urine affected areas of New Zealand pasture. *Agronomy Journal 71:* 309-314.

Batey, M.R. 1980. White clover seed production. Autumn sown crops on Canterbury soils. AgLink FPP96. MAF Information Series.

Broadbent, F.E., Nakashima, T. 1971. Effect of added salts on nitrogen fixation of pure stands of three pasture legumes with high/low phosphate. *N.Z. Journal of Agricultural Research 16:* 483-491.

Brock, J.L., Hoglund, J.H. 1979. Nitrogen fixation in pasture. VII. Recent alluvium. Palmerston North. *N.Z. Journal of Experimental Agriculture 7:* 27-29.

Carran, R.A. 1983. Changes in soil nitrogen during pasture crop sequences. A review. *Proceedings Agronomy Society of N.Z. 13:* 29-32.

Clifford, P.T.P. 1979. Research in white clover seed production. *In* "Herbage Seed Production". Ed. J.A. Lancashire. Grassland Research and Practice Series No. 1, N.Z. Grassland Association. pp. 64-67.

Clifford, P.T.P. 1985. Effect of leaf area on white clover seed production: recent research. In press.

Gillingham, A.G., During, C. 1973. Pasture production and transfer of fertility within long established hill pasture. *N.Z. Journal of Experimental Agriculture 1:* 227-232.

Hoglund, J.H., Brock, J.L. 1978. Regulation of nitrogen fixation in pasture. *N.Z. Journal of Agricultural Research 21:* 73-82.

Hoglund, J.H., Crush, J.R., Brock, J.L., Ball, R., Carran, R.A. 1979. Nitrogen fixation in pasture. *N.Z. Journal of Experimental Agriculture 7:* 45-51.

Janson, C.G. 1984. Autumn-sown forage legumes for intensive arable cropping systems. *Proceedings Agronomy Society of N.Z. 14:* 61-64.

Ladd, J.N., Oades, J.M., Amato, M. 1981. Distribution and recovery of nitrogen from legume residues decomposing in the field. *Soil Biology and Biochemistry 13:* 251-256.

Millner, J.P. 1983. Agronomy of barley production. *In* "Barley: Production and Marketing". Eds. G.M. Wright, R.B. Wynn-Williams. Agronomy Society of New Zealand Special Publication No. 2. pp. 53-59.

Moustafa, E., Ball, R., Field, T.R.O. 1969. The use of acetylene reduction to study the effect of nitrogen fertiliser and defoliation on nitrogen fixation by field grown white clover. *N.Z. Journal Agricultural Research 17:* 691-696.

McKenzie, B.A., Hill, G.D. 1984. Nitrogen uptake and transfer from spring-sown lupins, barley or fallow to a ryegrass test crop. *Proceedings Agronomy Society of N.Z. 14:* 95-99.

Rhodes, P.J. 1980. Nitrogen fixation by peas and lupins. M.Agr.Sc. thesis, Lincoln College, University of Canterbury, New Zealand.

Sears, P.D. 1953. Pasture growth and soil fertility. IV. The influence on soil fertility of clovers, superphosphate, lime, dung and urine applied to the pastures, as measured by the growth of subsequent forage crops. N.Z. *Journal of Science and Technology 35A. Supplement 1:* 53-67.

Sears, P.D., Goodall, V.C., Jackman, R.H., Robinson, G.S. 1965. Pasture growth and soil fertility. VIII. The influence of grasses, white clover, fertilisers, and the return of herbage clippings on pasture production of an impoverished soil. *N.Z. Journal of Agriculutral Research 8:* 270-288.

Steele, K.W., Shannon, P. 1981. Concepts relating to the nitrogen economy of a Northland intensive beef farm. Department of Scientific and Industrial Research Series.

Thomas, R.G. 1979. Growth of the white clover plant in relation to seed prodution. *In* "Herbage Seed Production." Ed. J.A. Lancashire. Grassland Research and Practice Series No. 1. New Zealand Grasslands Association. pp. 56-63.