Plant and Soil 77, 61–72 (1984). Ms. 5468

Competitive interaction between two leaf morph populations of white clover in relation to soil nitrogen

P. PRADHAN and R. S. TRIPATHI
Department of Botany, School of Life Sciences, North-Eastern Hill University, Shillong 793 014, India

Received 21 April 1983. Revised July 1983

Key words Clover Competitive interaction Soil nitrogen *Trifolium repens*

Summary The pure and mixed stands of the two populations of white clover (*Trifolium repens* L.) differing in leaf character were raised in the experimental pots using de Wits' replacement series. Of the two populations, one is characterized by having white 'V'-shaped markings on the leaflets (marked population) and the other does not have any such marking (unmarked population). The competitive interaction between the two populations has been studied at two soil nitrogen regimes. The nitrogen requirement of the marked population seems to be higher than that of the unmarked population. The marked population was found to be more competitive as compared to the other population at high N level as shown by their relative yield. The competitiveness of the former was, however, masked to some extent under low soil nitrogen. This confirmed the differential response of the two populations of *T. repens* to soil fertility as observed in nature.

Introduction

White clover (*Trifolium repens* L.) forms an important pasture component in the grasslands of Shillong and its adjoining areas. It exhibits two common leaf morph populations: one with conspicuous 'V' shaped white markings on the leaflets (marked population) and the other with no such markings (unmarked population). The leaf markings in *T. repens* are very distinct and can be used to identify individual clones[10]. The white 'V' shaped markings in *T. repens* are controlled by the multiple alleles present in one of the two linked groups of genes[4]. Besides, herbivory has also been reported to control the dominance of one leaf morph population over the other in mixed situation[2,3,6].

Some studies have been made on the edaphic ecotypes of *T. repens* occurring on calcareous and acid soils[13] and altitudinal ecotypes differing in photosynthetic CO_2 fixation products[11]. Cowling[5] has reported that the nitrogen fertilizer does not result in the increased yield of the white clover. Since the legumes get additional supply of nitrogen on account of the presence of nitrogen fixing bacteria in their root nodules, it is generally thought that soil nitrogen may not be important in competitive interaction among legume populations. However, the field observations indicate that the two populations of white clover respond differentially to soil nitrogen. An attempt was, therefore, made to investigate the competitive behaviour of the two populations of *T. repens* in relation to soil nitrogen regime.

Materials and methods

The observations pertaining to frequency, density, leaf area and aboveground biomass of the two leaf morph populations of *T. repens* growing on sites with two contrasting soil nitrogen levels were recorded in May 1980, in the swards of Shillong (latitude 25.34°N, longitude 91.56°E and altitude 1500 m). Of the two sites, one was situated quite near to the heap of compost manure and this represented high soil fertility level (% N = 0.81), while the other site situated away from the compost manure, represented low soil fertility level (% N = 0.15) in the same grassland.

Twenty quadrats of 0.25 m^2 (50 cm × 50 cm) area were laid on each of the two sites for estimating density and frequency of the two populations. For leaf area estimation, 10 plants of each of the two populations representing different sizes were taken from each of the 20 quadrats on both sites. Leaf area was computed by multiplying average leaf area per plant by average density per quadrat. For estimating the above-ground biomass, the herbage was removed from 5 quadrats, sorted out into marked and unmarked populations and dried in an oven at 80°C for 2 days and weighed. Seed output was computed by multiplying average number of seeds/fruit by average number of fruits per quadrat. All values are, however, expressed in terms of 1 m^2 area.

For pot culture experiment, stolons of the two morph populations of *T. repens* were collected from the natural swards of Shillong. The stolons of 0.2 cm diameter were cut into 2 cm pieces, each with a node in the middle to serve as propagules for raising the populations. Dry matter of the stolon pieces of both the populations was found to be in the range of 14–15 mg (average value based on 100 measurements).

The propagules were sown on 27 November, 1978, which sprouted after 1–3 weeks from sowing. Sprouts of uniform size (with one trifoliate leaf) were selected and planted on 20 December, 1978 in plastic pots of 21 cm diameter with a basal hole for drainage so as to give a replacement series[7] with an over-all density of 8 plants/pot in the following ratios:

1) 100% marked+0% unmarked *i.e.* pure stand of the marked population
2) 50% marked+50% unmarked *i.e.* mixed stand
3) 0% marked+100% unmarked *i.e.* pure stand of the unmarked population.

The pure and mixed stands of the two populations were grown at two soil nitrogen regimes. Garden soil without additional nitrogen represented low nitrogen regime (% N = 0.27) while in case of high soil nitrogen regime two doses each of 600 mg N per pot in the form of $NaNO_3$ were added to the original garden soil after 12 and 17 weeks from planting. This was equivalent to 80 kg N/ha in the garden soil and 340 kg N/ha in the high nitrogen regime.

The experiment was conducted during late December 1978 to July, 1979, in an unheated nethouse roofed with polythene sheet for protection against rain. The maximum temperature of 30°C was recorded in May and minimum of 4°C in January 1979. The first (H_1), second (H_2) and third harvests (H_3) were taken after 14, 23 and 32 weeks from planting. Since no difference was observed at H_1 due to soil nitrogen, the data for H_1 have not been presented in the paper, and H_2 and H_3 are henceforth considered as H_1 and H_2. The experiment was terminated on August 1, 1979.

At each harvest, stolon length, leaf area per plant, number of fertile shoots, seed output and dry weight of different plant parts *viz* root, stolon, leaves (leaflets and petioles) and flowers and fruits were determined. The dry matter yield was determined as described under field experiment.

The chlorophyll content of the fresh leaves was determined by the method outlined by Allen[1] and the nitrogen content of the aboveground plant parts by the micro-Kjeldahl method described by Misra[12].

Results

Field studies (Table 1)

The marked population showed greater density, leaf area, above-ground yield and seed output at the site with high soil nitrogen level, while the unmarked population behaved just the reverse. At the high soil nitrogen site the difference between the two populations was more obvious than at the low soil nitrogen site. The marked population grew better than the unmarked population at the high soil nitrogen site, whereas nitrogen-poor soil was observed to be more favourable for the latter population.

Pot culture studies

1) *Leaf area* (Fig. 1) The marked population produced a greater leaf area per plant in mixture than in pure stands at the low N regime ($P<0.05$). However, at the high N regime, although at H_1 the difference was insignificant, at H_2 leaf area per plant was more in pure stands ($P<0.01$). The unmarked population, on the other hand, produced greater leaf area in pure than in mixed stands at both low and high N regimes ($P<0.01$).

2) *Length of stolon* (Fig. 2) At the low nitrogen regime, stolon length per plant of both the populations was significantly greater ($P<0.01$) in mixed than in pure stands after 32 weeks from planting. Under the high N regime, the marked population produced greater stolon length in mixture than in pure stands at both harvests (differences significant at 5%). The unmarked population also showed a similar trend but the difference was insignificant. Stolon production by the marked population was stimulated by the addition of nitrogen ($P<0.05$) while the unmarked population behaved differently, particularly in mixture. At H_2, stolon length of the unmarked population was greater than in the corresponding pure and mixed stands of the marked population under the low N regime ($P<0.05$), while under the high N regime the differences were insignificant.

3) *Dry matter yield* (Fig. 3) At the low N regime, dry matter yield per plant of the unmarked population was more as compared to the marked population after 32 weeks of growth ($P<0.05$). The marked population, however, responded better to the addition of nitrogen ($P<0.05$), as a consequence of which the difference in yield of the two populations at the end of the experiment was narrowed down. At the high N level both the populations showed a significant increase in dry weight in mixed stands relative to their corresponding pure stand yields ($P<0.05$), whilst the reverse trend was observed at the low N regime although the differences were insignificant.

4) *Relative performance of the two populations in mixture* Total yield per pot in mixture at both levels of nitrogen was intermediate between the pure stand

Table 1. Frequency, density and growth of the two populations of *Trifolium repens* in relation to soil nitrogen levels in field condition (average value/$m^2 \pm$ S.E.)

	Low soil nitrogen level		High soil nitrogen level	
Parameters	Marked	Unmarked	Marked	Unmarked
Frequency (%)	67	67	100	33
Density	556.61 ± 69.63	765.11 ± 148.84	889.56 ± 92.87	105.00 ± 17.71
Leaf area (sq. cm)	3261.00 ± 75.41	4861.00 ± 92.38	8735.48 ± 501.15	670.95 ± 75.06
Above-ground biomass (g)	12.08 ± 5.17	40.18 ± 2.92	45.61 ± 2.81	6.31 ± 1.33
Seed output	2560 ± 554.27	6832 ± 461.89	12332 ± 727.48	938 ± 161.66

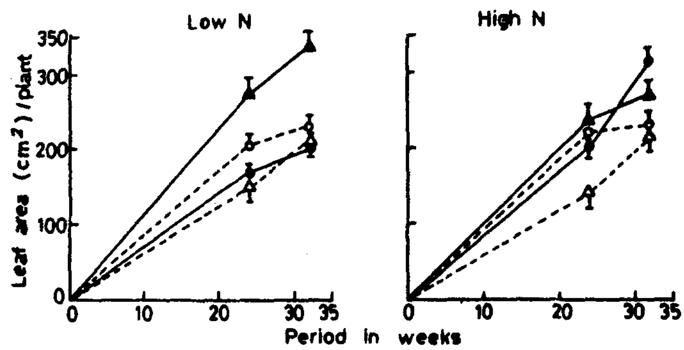

Fig. 1. Total leaf area per plant of the two populations of *Trifolium repens* after 24 and 32 weeks from planting under two nitrogen regimes. Marked population in pure (●——●) and in mixture (O----O) and unmarked population in pure (▲——▲) and in mixture (△----△). Vertical bars indicate S.E.

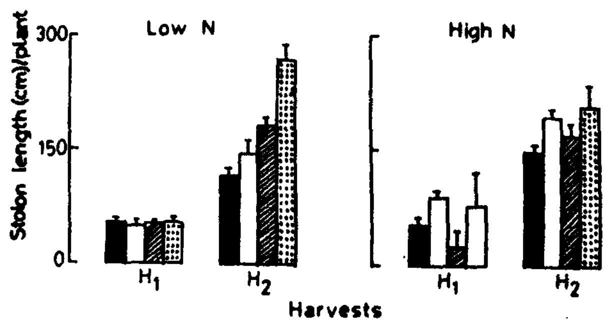

Fig. 2. Total length of stolon/plant of the two populations of *Trifolium repens* after 24 (H_1) and 32 weeks (H_2) from planting under two nitrogen regimes. Marked population grown in pure, (black columns ■), and in mixture (open columns, □); unmarked population grown in pure (hatched columns □) and in mixed stands (dotted columns □). Vertical bars indicate S.E.

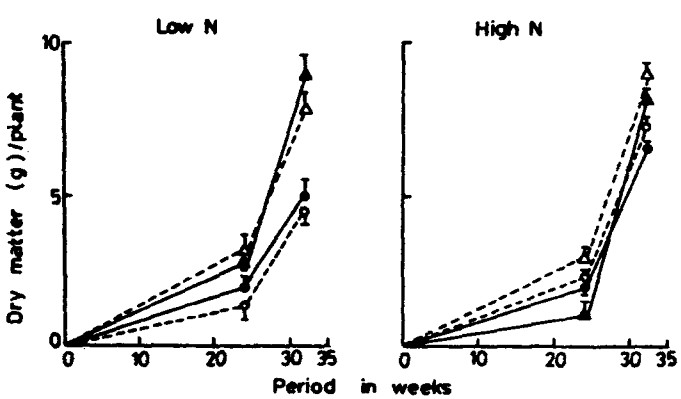

Fig. 3. Dry matter/plant of the two populations of *Trifolium repens* after 24 and 32 weeks from planting under two nitrogen regimes. Marked population grown in pure (●——●) and in mixture (O----O); unmarked population grown in pure (▲——▲) and in mixture (△----△). Vertical bars indicate S.E.

yield of the two populations (Fig. 4). An increase in soil nitrogen resulted in a greater yield per pot of the marked population in both pure and mixed stands ($P<0.05$), but the unmarked population was unaffected by the increased level of soil nitrogen.

The relative yield (Table 2) of the two populations and the quotient of relative yield of the marked to the unmarked population were calculated to compare their competitive success. The unmarked population showed a higher relative yield value at H_1 under a low N regime ($P<0.05$) but the trend was reversed at H_2 although the difference was insignificant. On the other hand, the marked population showed a higher relative yield than the unmarked population at a high N regime ($P<0.01$).

Table 2. Relative yield of the two populations and quotient of their relative yield at two harvests under two nitrogen regimes

	Low N		High N	
	H_1	H_2	H_1	H_2
Relative yield of the marked population	0.78	1.05	1.20	1.14
Relative yield of the unmarked population	0.97	0.98	0.46	0.95
Quotient of relative yield of marked to unmarked population	0.80	1.07	2.60	1.20

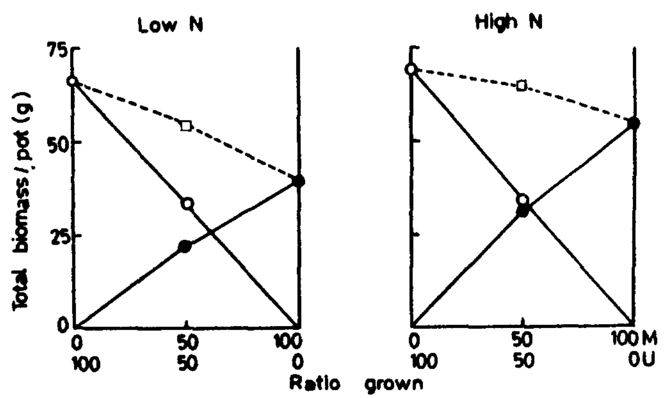

Fig. 4. Replacement series diagram based on total biomass/pot (g) of the two populations of *Trifolium repens* at final harvest grown at two soil nitrogen levels. The symbols (—●—), (—○—) and (---□---) represent the yield of marked population (M), unmarked population (U) and total yield/pot in mixture respectively.

5) *Allocation of resources* (Fig. 5) Allocation pattern of the marked population was significantly changed ($P<0.05$) due to added soil nitrogen. The increase in soil nitrogen resulted in a decreased allocation to leaves and an increased allocation to stolons. However, the resource allocation to other plant parts remained unchanged due to soil nitrogen. Conversely, resource allocation pattern in the unmarked population was not affected by soil nitrogen. However, the unmarked population allocated more resources to flowers and fruits in the mixed stand than in monoculture.

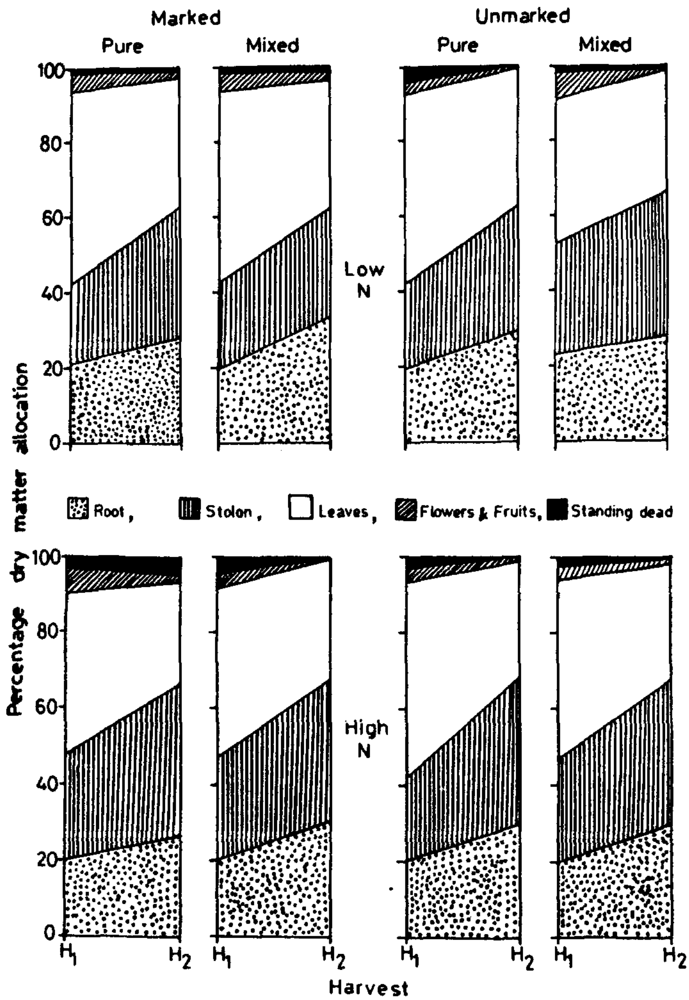

Fig. 5. Dry matter allocation to various plant parts of the two populations of *Trifolium repens* grown in pure and in mixture under low and high N regimes at two harvests taken after 24 (H_1) and 32 weeks (H_2) from planting.

6) *Chlorophyll content of leaves* (Table 3) Both populations showed a higher chlorophyll content in mixed than in pure stands at both N regimes (Table 3). Leaves of the unmarked population contained more chlorophyll compared to the marked population. The chlorophyll content of the latter both in pure and mixed stands increased due to increase in soil nitrogen. The chlorophyll content of the unmarked population also increased with soil nitrogen but only in pure stand.

7) *Nitrogen content of aboveground parts* (Table 4) Nitrogen content (%) of the aboveground parts of the marked population was higher in mixed than in pure stands at a high N regime (Table 4). At a low N regime, however, there was no significant difference in nitrogen content between pure and mixed stands, which was also true in case of the unmarked population. On the contrary, under high soil nitrogen regime, the unmarked population showed a higher nitrogen content in pure stand than in mixture.

8) *Flowering and seed output* The marked population produced more fertile shoots in mixed than in pure stands at high N regime ($P<0.01$) while at a low N regime the trend was reversed ($P<0.01$). A similar trend was exhibited by the unmarked population under a low N regime where the differences in nitrogen content of plants in pure and mixed stands were significant at 5% (Table 5). In mixture, the number of flowering shoots in the marked population increased due to added soil nitrogen while in monoculture fewer shoots flowered. Added soil N had no effect on the number of flowering shoots in the unmarked population in mixture, but in monoculture, it caused reduction in flowering.

Seed output of the marked population increased due to added soil nitrogen ($P<0.05$) while the unmarked population was unaffected (Fig. 6). At a high N regime, the unmarked population suffered more in competition with the marked population in terms of seed output.

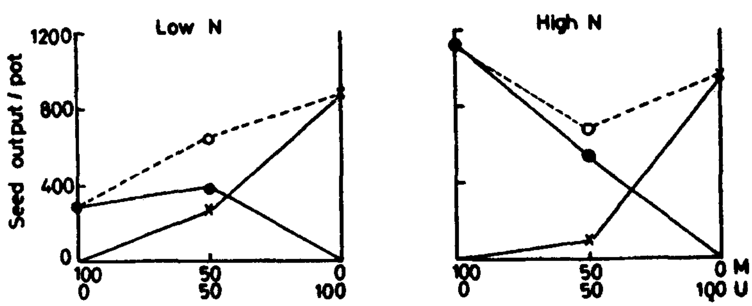

Fig. 6. Replacement series diagram based on total seed output/pot of the two populations of *Trifolium repens* at final harvest grown at two soil nitrogen levels. The symbols (—●—) and (—×—) represent the seed output of marked population (M) and unmarked population (U) respectively. Total seed output/pot in mixture (---O---) is also given.

Table 3. Chlorophyll content (mg/g of fresh leaves) of the two populations of *Trifolium repens* after 24 (H_1) and 32 weeks (H_2) from planting grown in pure and mixed stands at two nitrogen regimes (average value ± S.E.)

	Low N		High N	
Nature of stand	H_1	H_2	H_1	H_2
Marked (pure stand)	**16.23 ± 2.61	*11.84 ± 0.91	***24.73 ± 2.61	*15.91 ± 1.01
Marked grown with unmarked (50:50 mixture)	36.86 ± 3.81	22.17 ± 5.62	41.02 ± 5.83	22.35 ± 4.83
Unmarked grown with marked (50:50 mixture)	77.67 ± 11.61	25.31 ± 1.21	66.35 ± 8.63	15.44 ± 3.18
Unmarked (pure stand)	***26.16 ± 10.2	*20.74 ± 2.06	NS48.40 ± 15.61	NS21.09 ± 3.01

Significant difference at 0.1%, 1% and 5% levels between pure and mixed stands of a population is indicated by ***, ** and * respectively, put on the pure stand of each population. NS indicates insignificant difference.

Table 4. Nitrogen content (% ± S.E.) of the above-ground parts of the two populations of *Trifolium repens* after 24 (H_1) and 32 weeks (H_2) from planting grown in pure and mixed stands at two nitrogen regimes

	Low N		High N	
Nature of stand	H_1	H_2	H_1	H_2
Marked (pure stand)	NS2.35 ± 0.13	NS1.75 ± 0.09	**1.68 ± 0.03	**1.64 ± 0.05
Marked grown with unmarked (50:50 mixture)	2.35 ± 0.05	1.75 ± 0.06	2.21 ± 0.03	2.42 ± 0.12
Unmarked grown with marked (50:50 mixture)	2.03 ± 0.06	1.83 ± 0.07	1.27 ± 0.05	1.28 ± 0.06
Unmarked (pure stand)	NS2.07 ± 0.16	NS1.74 ± 0.12	**2.04 ± 0.09	*2.73 ± 0.26

Significant difference at 1% and 5% levels between pure and mixed stands of a particular population is indicated by **, and * respectively, put on the pure stand of each population. NS indicates insignificant difference.

Table 5. The degree of flowering as indicated by the percentage of fertile shoots to the vegetative shoots (each node with a leaf and root system was considered as an individual shoot) of the two populations of *T. repens* after 32 weeks (H_2) from planting grown in pure and mixed stands at two nitrogen regimes (average value ± S.E.)

Nature of stand	Nitrogen regimes	
	Low N	High N
Marked (pure stand)	**12.90 ± 0.25	**10.01 ± 0.60
Marked grown with unmarked (50:50 mixture)	6.37 ± 1.56	14.72 ± 2.0
Unmarked grown with marked (50:50 mixture)	7.10 ± 1.0	7.22 ± 0.33
Unmarked (pure stand)	*10.60 ± 1.10	^{NS}6.31 ± 0.50

Significant difference at 1% and 5% levels between pure and mixed stands of a population is indicated by ** and * respectively, put on the pure stand of each population. NS indicates insignificant difference.

Discussion

The wide differences in leaf area values in the pure and mixed stands of both populations were not reflected in their corresponding dry matter yield. At a high N level, dry matter yield of both populations was higher in mixed than in pure stands in spite of a smaller leaf area which indicates that the efficiency of resource utilization increases in the mixed stands.

Increased soil nitrogen resulted in an increased chlorophyll content in the marked population both in pure and mixed stands. An increase in chlorophyll content due to added soil nitrogen was not noticed in the unmarked population in mixture. The differential response of the two populations to increase of soil nitrogen in terms of chlorophyll content possibly explains the narrowing down of the differences in their dry matter yield. The increased chlorophyll content of the marked population in mixed stands in response to added soil nitrogen also partly explains its greater abundance and better growth than the unmarked population on nitrogen-rich sites.

The allocation pattern shows that a greater proportion of the assimilates is allocated to leaf tissue at a low N level in the marked population. In spite of this, the dry matter yield of this population is much lower than that of the unmarked population which may be attributed to the lower chlorophyll content and presence of air spaces in leaves of the former.

Increased soil nitrogen also resulted in an increased resource allocation to stolons in the marked population. Both populations, however, showed a greater stolon length in mixture than in monoculture at both nitrogen levels. The marked population, however, showed a greater increase in stolon length with an increase in soil nitrogen. The unmarked population, however, was not affected. If one views the stolons as sink tissue, the narrowing down of the differences between the yield of the two populations at a high N regime could be attributed to greater allocation of resources to the 'sink' (stolons) tissues in the marked population at a high N regime, which might stimulate the growth as argued by Tripathi and Harper[14].

The competitive success of the two populations is controlled, at least in part, by the soil nitrogen regime as revealed by the poor competitive ability of the marked population at a low N level and its better performance at a high N level. This is also confirmed by the relative yield values. In spite of accumulating more dry matter, the unmarked population seems to be a weaker competitor than the marked population in nitrogen-rich soil as indicated by the data on relative yield and its quotient. Thus, the aggressiveness of the two populations may be greatly modified by soil nitrogen. An increased soil nitrogen content caused a considerable increase in yield of the marked population both in pure and mixed stands whereas the yield of the unmarked population was practically unaffected.

The present study is partly in contrast to the findings of Cowling[5] who reported that the nitrogen fertilizer does not result in an increased yield of the white clover. The yield response of the marked population of *T. repens* to added soil nitrogen is, however, in conformity with the results reported by Drysdale[9] in white clover and by Dilz and Mulder[8] in case of red clover growing in acid soils. A higher nitrogen requirement of the marked population than that of unmarked population is also shown by the data on nitrogen content of the two populations. The nitrogen content of the marked population at a high nitrogen regime was higher in mixture than in monoculture showing its competitive superiority over the unmarked population which suffers more from inter-population competition at high soil nitrogen, while at low soil nitrogen the nitrogen content of this population was higher in mixture than in monoculture. It could be argued that nitrogen uptake by a given population of *T. repens* depends both on soil nitrogen level and on competition from the other population. Vallis *et al.*[16] found that the uptake of nitrogen by the legumes (*Lotononis bainesii* and *Trifolium repens*) increased considerably when competition from the grasses (*Digitaria decumbens* and *Chloris gayana*) was reduced.

An increase in flowering of both populations in mixture at a high nitrogen regime and a decrease at a low nitrogen level signify the role of soil nitrogen in competitive interaction between them. The magnitude of increase or decrease in mixtures over the corresponding monocultures was more pronounced in the marked population thus indicating differential response of the two populations to competition at different soil nitrogen levels.

T. repens reproduces both vegetatively and sexually and has both 'r' and 'K' strategies[15]. Reproduction by seeds permits long distance dispersal although at a very high risk for propagules while the vegetative reproduction places daughters close to the parents with low risk. At a low nitrogen level, the reproductive allocation in the unmarked population was always greater in mixture than in pure stands, while the other population showed no such difference in allocation due to nature of the stands.

It could be argued that under a nutrient-stressed situation the unmarked population may be more successful in mixed stands by virtue of increased reproductive allocation, which may confer an advantage on this population in so far as its maintenance and spread in mixed situations are concerned. Incidentally, the field observations on relative abundance of the marked and unmarked populations also indicate that the latter occurs less abundantly in nitrogen-rich soils. Cahn and Harper[2] have shown that animal grazing may be one of the factors for the failure of unmarked morph to dominate over the marked morph. The present study, however, shows that the competitive success of the two populations may be determined by the soil nitrogen as well.

References

1 Allen S E 1974 Chemical Analysis of Ecological Materials. Blackwell Scientific Publications.

2 Cahn M G and Harper J L 1976 The biology of the leaf mark polymorphism in *Trifolium repens* L. 2. Evidence for the selection of leaf marks by rumen fistulated sheep. Heredity 37, 327–333.

3 Charles A H 1968 Some selective effects operating upon white and red clover swards. J. Brit. Grassld. Soc. 23, 20–25.

4 Corkill L 1971 Leaf markings in white clover. J. Hered. 62, 307–310.

5 Cowling D W 1961 The effect of nitrogenous fertilizer on an established white clover sward. J. Brit. Grassld. Soc. 16, 65–68.

6 Crawford-Sidebotham T J 1972 The role of slugs and snails in the maintenance of the cyanogenesis polymorphism of *Lotus corniculatus* L. and *Trifolium repens* L. Heredity 28, 405–411.

7 de Wit C T 1960 On competition. Versl. Landbouwk. Onderz. Ned. 66, 1–82.

8 Dilz K and Mulder E G 1962 The effect of soil pH, stable manure and fertilizer nitrogen on the growth of red clover and red clover associations with perennial ryegrass. Neth. J. Agric. Sci. 10, 1–22.

9 Drysdale A D 1966 A comparison of two sources of nitrogen for grassland with special reference to the grass/clover ratio. Proc. 10th Intern. Grassld. Congr. Helsinki 1966, 255–258.

10 Harberd D J 1963 Observation on natural clones of *Trifolium repens* L. New Phytol. 66, 401–408.

11 Machler F, Nosberger J and Erismann K H 1977 Photosynthetic CO_2 fixation products in altitudinal ecotypes of *Trifolium repens* L. with different temperature requirements. Oecologia Berlin 31, 79–84.

12 Misra R 1968 Ecology Workbook. Oxford and IBH, New Delhi.

13 Snaydon R W 1962 The growth and competitive ability of contrasting natural populations of *Trifolium repens* when grown on acid and calcareous soils. J. Ecol. 30, 439–447.

14 Tripathi R S and Harper J L 1973 The comparative biology of *Agropyron repens* (L.) Beauv. and *A. caninum* (L.) Beauv. I. The growth of mixed populations established from tillers and from seeds. J. Ecol. 61, 353–368.

15 Turkington R A and Cavers P B 1978 Reproductive strategies and growth patterns in four legumes. Can. J. Bot. 56, 413–416.

16 Vallis I, Henzell E F and Evans T R 1977 Uptake of soil nitrogen by legumes in mixed swards. Aust. J. Agric. Res. 28, 413–425.