CHAPTER 8

INTERFERENCE BETWEEN TWO POPULATIONS OF TRIFOLIUM REPENS IN RELATION TO SOIL NITROGEN
INTRODUCTION

The white leaf markings in *Trifolium repens* are very distinct and can be used to identify the individual clones (Harberd, 1963). Several studies have been done to show the role of predation on the dominance of one clone over the other (Charles, 1968; Walton et al., 1970; Crawford-Sidebotham, 1972; Angseesing, 1974; Cahn and Harper, 1976). Some studies have been done involving edaphic ecotypes of *T. repens* occurring on calcareous and acid soils (Snaydon, 1962), and altitudinal ecotypes differing in photosynthetic CO$_2$ fixation products (Machler, Nosberger and Erismann, 1977). However, no serious attempt seems to have been made to investigate the competitive behaviour of the clones of *T. repens* in relation to soil nitrogen status although grass-legume interactions have been studied at different nitrogen regimes (Stern and Donald, 1962a; Whitehead, 1970; Litav and Zeligman, 1978).

MATERIALS AND METHODS

The stolons of the two populations of *Trifolium repens* differing in leaf morphs were collected from the natural swards of Shillong.

The two populations wherever they occur, form a mosaic in the local swards. The morph with conspicuous 'V' shaped white mark on the leaflets can be easily distinguished from the one with no mark on the leaflets, in the field situation. The stolons
of the two morphs were cut into 2 cm pieces of 0.2 cm diameter, each with a node in the middle to serve as propagules for raising the populations. The dry matter of the stolon pieces of both the morphs was found to be in the range of 14-15 mg (average value based on 10 measurements). The stolons pieces of the two morphs were sown on 27 November 1978 in separate pots filled with garden loam soil. The stolon pieces sprouted after 1-3 weeks after sowing. The sprouts of uniform size (with one leaf) were selected and 8 of such sprouted pieces of each morph raised the pure populations. The sprouts were grown at a distance of 2 cm from each other in a circular manner. In the case of mixed stands, 4 sprouted pieces of each of the two morphs were planted per pot. The pieces of the two morphs were planted alternating with each other in circular fashion maintaining 2 cm distance between any two pieces. The planting and thinning dates were same as on the pure stands.

The pure and mixed populations of the two morphs were grown at two soil nitrogen regimes. The low nitrogen regime represented the garden soil (%N in the garden soil = 0.27%) and in the case of the high soil nitrogen regime two doses each of 600 mg of nitrogen per pot (equivalent to c. 270 kg/ha) in the form of NaNO₃ were added to the original garden soil after 12 and 17 weeks from the planting dates. The low nitrogen regime represented by the garden soil was equivalent to c.30 kg/ha. There were 3 replications for each of the two nitrogen treatments in case of the pure and mixed populations of the two morphs.
The experiment was conducted in plastic pots of 21 cm diameter, kept in an unheated net house roofed with polythene sheet. The maximum temperature (30°C) was recorded in May and minimum temperature (4°C) in January. The first harvest (H₁) was taken after 14 weeks from planting as the growth of the two populations was restricted due to low temperature in winter months. The second (H₂) and third (H₃) harvests were taken after 23 and 32 weeks of growth. The experiment was terminated on August 1, 1979. At each harvest, the stolon length, leaf area per plant, number of fertile shoots and dry weight of different plant parts viz. root, stolon, leaves (leafletlets and petioles) and flowers and fruits were estimated. The dry weight was determined by drying the plant material in an oven at 80°C to constant weight.

Chlorophyll content of the fresh leaves and nitrogen content of the above ground plant parts of T. repens were determined. Chlorophyll extraction was done in acetone and its content was determined by the method outlined by Allen (1974) and nitrogen by micro-kjeldahl method as outlined by Misra (1968).

RESULTS

Leaf area:

The total leaf area per plant of the white morph was greater in mixture than in pure at low N regime while at high N regime the reverse was generally true (Fig. 8.1). The leaf area per plant of the unmarked morph was, however, greater in pure
than in mixture (significant at 5%). The average size per leaf of the white morph was greater in mixture at low nitrogen level while at high nitrogen level there was reduction in leaf size in the mixed stands (Table 8.1). The unmarked population was also characterized by a decrease in leaf size in the mixture at high nitrogen level at $H_3$. At low nitrogen level, however, the reduced leaf size in mixture was apparent only at initial growth stage (significant at 5%, $t = 2.65$).

**Length of stolon:**

The total stolon length per plant in both the clones was significantly greater in the mixed stands than in pure after 32 weeks of growth at low nitrogen (Fig. 8.2). The stolon production by the white morph was stimulated by the addition of nitrogen (significant at 5%, $t = 3.40$) whereas the unmarked morph behaved differently particularly in mixture. After 32 weeks of growth, the stolon production by the unmarked morph was higher as compared to the corresponding pure and mixed stands of the white leaf morph at low nitrogen regime (significant at 5%), while at high nitrogen there was no significant difference.

**Dry matter production:**

The unmarked morph after 32 weeks of growth was found to accumulate more dry matter per plant than the white marked morph at the low nitrogen level irrespective of the nature of stand ($p < 0.05$). The white leaf morph responded better to the addition of nitrogen by accumulating greater amount of dry matter at high
Table 8.1: Average size (sq. cm) per leaf of the two populations of *Trifolium repens* grown in
pure and in mixture under high and low nitrogen regimes (average values ± S.E.).

<table>
<thead>
<tr>
<th>Nature of stand</th>
<th>Nitrogen regimes</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>H1</strong></td>
<td><strong>H2</strong></td>
<td><strong>H3</strong></td>
<td><strong>Low</strong></td>
</tr>
<tr>
<td>White marked in pure</td>
<td>4.97±1.26</td>
<td>5.46±0.73</td>
<td>7.64±1.07</td>
<td>2.16±0.10</td>
</tr>
<tr>
<td>White marked in 50:50 mixture</td>
<td>1.19±0.16</td>
<td>4.91±0.20</td>
<td>5.29±0.17</td>
<td>2.19±0.24</td>
</tr>
<tr>
<td>Unmarked in 50:50 mixture</td>
<td>1.66±0.61</td>
<td>3.80±1.15</td>
<td>4.98±0.07</td>
<td>1.64±0.20</td>
</tr>
<tr>
<td>Unmarked in pure</td>
<td>1.55±0.16</td>
<td>4.72±0.74</td>
<td>5.49±0.15</td>
<td>2.38±0.11</td>
</tr>
</tbody>
</table>

The values at **H1** are significantly different (at 5% level) from those at the later harvests whilst the difference between the data at **H2** and **H3** are insignificant.

The data marked with an asterisk in pure of a given morph are significantly different at 5% from those marked with an asterisk in mixture of the same morph.
Fig. 8.1: Total leaf area per plant of the two populations of *T. repens* after 14, 23 and 32 weeks from planting under two nitrogen regimes. White marked in pure (●—●) and in mixture (○—○) and unmarked in pure (▲▲) and in mixture (Δ--Δ).
Fig. 8.1

High N

Low N

Total leaf area/plant (sq.cm)

Period in weeks

L.S.D, P=0.05

L.S.D, P=0.05
Fig. 8.2: Total length of stolon per plant of the two populations of *T. repens* after 14 (*H*₁), 23 (*H*₂) and 32 weeks (*H*₃) under two nitrogen regimes. White marked grown in pure (■) and in mixture (□); unmarked grown in pure (■) and in mixture (□). Vertical lines (I) indicate L.S.D. at *P* = 0.05.
Fig. 8.2

**High N**

- Total length of stolon/plant (cm)
- Harvests: H1, H2, H3

**Low N**

- Total length of stolon/plant (cm)
- Harvests: H1, H2, H3
nitrogen level as compared to its dry matter at low nitrogen level (significant at 5%, \( t = 2.42 \)). At high nitrogen level, both the morphs showed an increase in dry weight in mixed stands relative to their corresponding pure stand yields whilst the reverse trend was observed at low nitrogen regime although the differences were insignificant (Fig. 8.3). The white leaf morph showed higher mixture yield at high nitrogen level than at low N level, and the difference in yield of the two morphs was narrowed down at high nitrogen regime. The mixture yield of the white morph at high N regime was greater than its pure yield while at low N level the mixture yield was lesser which indicates that the outcome of competition is dependent on the soil nitrogen level.

The relative performance of the two morphs in mixture:

The total yield per pot in mixture at both the N levels was intermediate between the pure stand yield of the two morphs. An increase in soil nitrogen resulted in greater yield per pot of the white morph both in pure and mixture but the unmarked morph was almost unaffected by the addition of nitrogen (Fig. 8.4).

The relative yield of the two clones and the quotient of their relative yield (Table 8.2) were calculated to compare the competitive success of the two morphs. At the high nitrogen regime, the relative yield of the unmarked morph seems to be higher in the early stage of growth, but at later harvests white marked morph wins over the former, which is also indicated by the
Fig. 8.3: Dry weight per plant of the two morphs of T. repens after 14, 23, and 32 weeks from planting under two nitrogen regimes. White marked grown in pure (O---O) and in mixture (0---0); unmarked grown in pure (Δ--Δ) and in mixture (Δ--Δ). Vertical lines (I) indicate L.S.D. at P = 0.05.
Fig. 8.4: Replacement series diagram based on total biomass per pot (g) of two morphs of *T. repens* at final harvest grown at two soil nitrogen levels. The symbols, (---) and (---o---), represent the yield of white marked and unmarked populations respectively. The total yield per pot in mixture (---o---) is also given.
Fig. 8.4

High N

Low N

Total biomass/pot (g)

Ratio grown

0 0.5 1

0 0.5 1
Table 8.2: The relative yield of the two morphs and quotient of their relative yield at the three harvests under high and low nitrogen regimes.

<table>
<thead>
<tr>
<th></th>
<th>High N</th>
<th>Low N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harvests</td>
<td>H1</td>
<td>H2</td>
</tr>
<tr>
<td>White marked</td>
<td>0.78</td>
<td>1.20</td>
</tr>
<tr>
<td>Relative yield</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unmarked</td>
<td>0.89</td>
<td>0.46</td>
</tr>
<tr>
<td>Quotient of relative yield of white marked to unmarked</td>
<td>0.87</td>
<td>2.60</td>
</tr>
</tbody>
</table>
quotient of relative yield. This competitive behaviour of the
two morphs is, however, reversed at the first two harvests under
low nitrogen regime although at H₂, almost the same trend was
observed at both the nitrogen regimes.

The allocation of resources:

The allocation pattern in white marked morph seems to be
unaffected due to competitive interaction between the two morphs
at low N regime (Fig. 8.5b), while the unmarked morph allocates
more of its resources to the sexual reproductive parts (Flowers
and fruits) in the mixture as compared to the pure stand where
the allocation to leaf was found to be higher. It seems that the
white marked morph allocates relatively greater proportion of its
resources to leaves while the unmarked morph allocates more food
reserves to the sink tissue (root and stolon). The allocation to
the leaf decreases with age whilst there was increased allocation
to the roots and stolons with passage of time.

The allocation pattern of white marked morph gets slightly
changed due to added soil nitrogen, particularly at the early
stage when the allocation towards leaves was much less and to
stolons much greater than the corresponding values at low N level
(Fig. 8.5a). The allocation to the leaves and to the flowers and
fruits by the white marked morph at high nitrogen level was
greater in pure stand than in mixture. At both the nitrogen
levels there was a tendency for greater allocation towards stolons
at H₂ while the allocation to leaves decreased considerably at
Fig. 8.5a: Dry matter allocation to various plant parts of the two populations of *T. repens* grown in pure and in mixture under high N regime at three harvests taken after 14 (1), 23 (2) and 32 weeks (3) from planting.
Fig. 8.5a

Percentage dry matter

<table>
<thead>
<tr>
<th>Pure</th>
<th>High N</th>
<th>Mixture</th>
<th>Unmarked</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standing dead</td>
<td>Flowers &amp; Fruits</td>
<td>Leaves</td>
<td>Stolon</td>
</tr>
</tbody>
</table>

Harvests

1 2 3
Fig. 8.5b: Dry matter allocation to various plant parts of the two populations of *T. repens* grown in pure and in mixture under low N regime at three harvests taken after 14 (1), 23 (2) and 32 weeks (3) from planting.
Fig. 8.5b

Pure

Low N

Mixture

Percentage dry matter

Standing dead, Flowers & Fruits, Leaves, Stolon, Root

Unmarked

Harvests

1 2 3

1 2 3
this harvest. However, the magnitude of increase in energy allocation to stolons from H₁ to H₃ was much greater in case of white marked morph at low nitrogen level. It seems that the interaction between the two morphs does not affect the energy allocation pattern.

**Chlorophyll content:**

In the marked population, chlorophyll content was greater in mixture than in pure stand at both the nitrogen regimes. However, no definite trend was observed in the unmarked population. At both the nitrogen regimes, leaves of the unmarked population generally contained more chlorophyll compared to the marked population (Table 8.3). Chlorophyll content was highest at H₂ in both the populations.

**Nitrogen content:**

The percentage nitrogen content of marked population in the above ground parts was greater in mixture than in the pure stand at high nitrogen regime. This trend was, however, reversed at low nitrogen regime. The nitrogen content of the aboveground parts of the marked population at low nitrogen did not show significant difference between pure and mixed stands. The unmarked population, on the other hand contained less nitrogen in mixture than in pure stand at high nitrogen regime, but at low nitrogen regime no definite trend was observed (Table 8.4).

**Flowering and seeding behaviour:**

Both the marked and unmarked populations produced more
Table 8.3: Chlorophyll content (mg/g of fresh leaves) of the two populations of *T. repens* grown in pure and mixed stands under two nitrogen regimes.

<table>
<thead>
<tr>
<th>Nature of stand</th>
<th>High N</th>
<th>Low N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$H_1$</td>
<td>$H_2$</td>
</tr>
<tr>
<td>White marked in pure</td>
<td>2.63</td>
<td>24.73</td>
</tr>
<tr>
<td>White marked in 50:50 mixture</td>
<td>2.45</td>
<td>41.02</td>
</tr>
<tr>
<td>Unmarked in 50:50 mixture</td>
<td>3.41</td>
<td>66.35</td>
</tr>
<tr>
<td>Unmarked in pure</td>
<td>3.73</td>
<td>48.40</td>
</tr>
<tr>
<td>F:variance ratio</td>
<td>1.06NS</td>
<td>38.18**</td>
</tr>
</tbody>
</table>

The values of white marked population at $H_2$ and $H_3$ under high N regime are significantly different (5% level) from those of low N regime, while the difference due to harvests in unmarked population are insignificant.

** indicate significant differences at $P = 0.01$

* indicates significant differences at $P = 0.05$

NS Not significant.
Table 8.4: Nitrogen content (%) of the aboveground parts of the two populations of *T. repens* grown in pure and mixed stands at two nitrogen regimes.

<table>
<thead>
<tr>
<th>Nature of stand</th>
<th>High N</th>
<th></th>
<th></th>
<th></th>
<th>Low N</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$H_1$</td>
<td>$H_2$</td>
<td>$H_3$</td>
<td>$F$ variance ratio</td>
<td>$H_1$</td>
<td>$H_2$</td>
<td>$H_3$</td>
</tr>
<tr>
<td>White marked in pure</td>
<td>1.16</td>
<td>1.68</td>
<td>1.64</td>
<td>2.73 NS</td>
<td>2.38</td>
<td>2.35</td>
<td>1.75</td>
<td>20.70 **</td>
</tr>
<tr>
<td>White marked in 50:50 mixture</td>
<td>1.96</td>
<td>2.21</td>
<td>2.42</td>
<td>11.64 *</td>
<td>0.99</td>
<td>2.35</td>
<td>1.75</td>
<td>63.58 **</td>
</tr>
<tr>
<td>Unmarked in 50:50 mixture</td>
<td>1.11</td>
<td>1.27</td>
<td>1.28</td>
<td>1.76 NS</td>
<td>2.98</td>
<td>2.03</td>
<td>1.83</td>
<td>22.01 **</td>
</tr>
<tr>
<td>Unmarked in pure</td>
<td>1.95</td>
<td>2.04</td>
<td>2.73</td>
<td>34.90 **</td>
<td>0.75</td>
<td>2.07</td>
<td>1.74</td>
<td>52.93 **</td>
</tr>
<tr>
<td>$F$ variance ratio</td>
<td>66.64 **</td>
<td>11.82 **</td>
<td>15.93 **</td>
<td>27.83 **</td>
<td>6.82 *</td>
<td>0.25 NS</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The values of both the populations under high nitrogen regimes are significantly different at 5% level from those of the low nitrogen regime.

** indicate significant differences at $P = 0.01$

* indicates significant differences at $P = 0.05$

NS Not significant.
fertile shoots in mixture at high nitrogen regime while at low nitrogen more fertile shoots were produced in monoculture (Table 8.5). In white marked population the number of fertile shoots increased at high soil nitrogen regime both in pure and mixed stands. The unmarked population also showed similar response in mixture but in pure stands, fertile shoot production was reduced at high nitrogen level.

Per pot seed production in the white marked population in mixture was more than in pure at low nitrogen regime whilst at high nitrogen regime seed production in pure stand yield was more than in mixture as well as that of the monoculture of unmarked morph (Fig. 8.6). In pure, the seed output of the marked population was higher than that of the unmarked population at high nitrogen regime, but the unmarked population did not show any significant increase due to soil nitrogen.

DISCUSSION

The accumulation of dry matter in the mixed stands by the white marked morph was lesser than in the pure stand at low N level in spite of greater leaf area in pure stand. Similarly, in the unmarked morph as well, the wide difference in leaf area values in the pure and mixed stands is not reflected in dry matter yield which is almost equal in the two types of stands (Fig. 8.3). At high N level, the dry matter yield of the marked morph at H3 was higher in mixture than in pure whereas the photosynthetic area was greater in pure stand. The same trend
Fig. 8.6: Replacement diagram based on total seed output per pot of the two populations of T. repens after 32 weeks from planting. The symbols —•—, —x— and —o— stand for marked population, unmarked population and combined seed output respectively.
Table 8.5: 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 24 weeks (*H*$_3$) from planting grown in pure and mixed stands at two nitrogen regimes (average value ± S.E.).

<table>
<thead>
<tr>
<th>Nature of stand</th>
<th>Nitrogen regimes</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High N</td>
<td>Low N</td>
<td></td>
</tr>
<tr>
<td>Pure marked (100%)</td>
<td>10.01±0.60</td>
<td>12.90±0.25</td>
<td></td>
</tr>
<tr>
<td>Mixed marked (50%)</td>
<td>14.72±2.0</td>
<td>6.37±1.56</td>
<td></td>
</tr>
<tr>
<td>Mixed unmarked (50%)</td>
<td>7.22±0.33</td>
<td>7.10±1.0</td>
<td></td>
</tr>
<tr>
<td>Pure unmarked (100%)</td>
<td>6.31±0.50</td>
<td>10.60±1.10</td>
<td></td>
</tr>
</tbody>
</table>
was repeated by the unmarked morph also at high N level. It appears, therefore, that the efficiency of resource utilization increases in the mixed stands. Similarly, lesser chlorophyll content of the marked population in pure than mixed stand suggests that this population suffers from intra-population competition in respect of resource utilization for the synthesis of chlorophyll in the plant body while the unmarked population does not suffer to the same extent in pure stand (Table 8.3). Lower chlorophyll content of the marked population may be attributed to the air spaces present within the palisade tissue. However, increased soil nitrogen appears to have stimulated chlorophyll synthesis in the marked population, both in pure and mixed stands. Chlorophyll content of leaves in the unmarked population was not affected by soil nitrogen.

The stolon length of the two morphs at both the nitrogen regimes was relatively greater in the mixture than in monoculture which indicates that the interference amongst the individuals of the same population causes greater suppression in stolon production. The increase in stolon length in the mixture is much higher at high N regime particularly at H₂ which shows that the intensity of competition in the monocultures is more severe under increased soil nitrogen. At H₃ the unmarked morph showed greater increase in stolon length in mixture at low N level.

Allocation pattern shows that a greater proportion of the assimilates is allocated to leaf tissue at low nitrogen
level in the white marked morph. In spite of this, the dry matter yield of this morph is much lower than that of the unmarked morph (Fig. 8.3), which may be attributed to the presence of lesser amount of 'sink' tissue (Stolon in this case) in the white marked morph (Fig. 8.5b). The difference in the dry matter yield of the two morphs as exhibited at low nitrogen regime is considerably narrowed down at high nitrogen regime (Fig. 8.3) where the allocation to 'sink' tissue in the two morphs is also not much different from each other in both pure and mixed stands (Fig. 8.5a) indicating that the energy allocation to different structures is also modified by the soil nitrogen.

The dry matter data suggest that the competitive success of the two morphs is controlled, at least in part, by the soil nitrogen regime. This is revealed by the poor competitive ability of white marked morph at low nitrogen level and its better performance at high N level (Fig. 8.3). The relative yield values also confirm this fact (Table 8.2). In spite of accumulating more dry matter, the unmarked morph seems to be a weak competitor than the white marked morph indicated by the data on relative yield and quotient of relative yield at the final harvest (Table 8.2). Thus, the aggressiveness of the two morphs may be greatly modified by the soil nitrogen. The increased soil nitrogen caused considerable increase in yield of the white marked morph both in pure and mixed populations whilst yield of the unmarked morph was practically unaffected.
Thus, the present study is partly in contrast to the findings of Cowling (1961) who reported that the nitrogen fertilizer does not result in the increased yield of the white clover. The yield response of the white marked morph of *T. repens* to applied nitrogen is, however, in conformity with the results reported by Dilz and Mulder (1962) in case of red clover growing in acid soils and by Drysdale (1966) in white clover. It is clear that the nitrogen requirement of the white marked morph is greater than the unmarked morph. This is also reflected in the nitrogen content (%) of the two populations. At high soil nitrogen, nitrogen content of the marked population had more nitrogen in mixture than in monoculture showing its competitive superiority over the unmarked population at high soil nitrogen regime. Conversely, the unmarked population suffers more from inter-population competition at high soil nitrogen regime, although at low soil nitrogen regime the nitrogen content of this population was greater in mixed than pure stand. It could be argued that the nitrogen uptake by a given population of *T. repens* depends not only on the soil nitrogen level but also on competition from the other population. Vallis *et al.* (1977) 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 the populations in mixture at high nitrogen regime and a decrease at low nitrogen
level indicate the modifying influence of soil nitrogen on competitive interaction. The magnitude of increase or decrease in mixture over the corresponding monoculture 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 (Turkington and Cavers, 1978). 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 low nitrogen level, the reproductive allocation in the marked population was always greater in mixture than in pure stand, while allocation to the other population in pure and mixed stands was almost same. It could be argued that under nutrient-stressed situation the unmarked population may be more successful in mixed stands in terms of reproductive allocation which may confer an advantage on this population in the maintenance and spread of its population. Incidentally the field observations on relative abundance of marked and unmarked populations also indicate that the former occurs more abundantly in fertile soils (Chapter 2).

The chlorophyll content in the marked population was comparatively less than in the unmarked population but it appears that the marked population has a subtle way of utilizing resources and owing to its greater allocation to stolons it might be able to regenerate rapidly in the established swards.
Cahn and Harper (1976b) 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 morphs may be determined by the soil nitrogen as well.