Effect of Legume/Grass Association on the Growth and Symbiotic Nitrogen Fixation of Leguminous Forages

Jinko Jo*, Shigekata Yoshida** and Ryosei Kayama**

Synopsis


Ladino clover and orchard grass were grown in 1/2000 a pots at the sowing ratios of 10:0, 8:2, 5:5, 2:8 and 0:10 (ladino clover: orchard grass) in order to investigate the variation of growth and symbiotic N fixation capacity of ladino clover in mixed swards. In addition, ladino clovers were grown in 1/10,000 a pots at the different shading levels (100%, 71%, 45% and 23% of full sunlight) to elucidate the effect of mutual shading in mixture swards in a separate experiment.

The effect of NO3-N concentration on the symbiotic N fixation capacity of ladino clover was also investigated in a water culture. Results obtained are as follows.

1. The growth of ladino clover was markedly decreased in both top and root by association with orchard grass, and it was more critical at high N treatment than at the low N treatment whereas the top growth of orchard grass did not reveal the large differences among the different sowing ratios in any N treatment. The root growth of orchard grass, however, showed a gradual increase with the increment of sowing ratios.

2. The legume ratios calculated from the yield of both forages were higher at the low N treatment than at the high N treatment. At both low and high N treatments, the legume ratios were reduced rapidly with the decrease of sowing ratios of ladino clover from 10:0 to 8:2, but no significant differences were present among the sowing ratio treatments of 8:2, 5:5 and 2:8. The similar tendency was observed on root.

3. Symbiotic N fixation capacity/pot was reduced drastically with the decrease of sowing ratios of ladino clover at high N treatment whereas they revealed the almost same values as that of ladino clover pure culture at low N treatment until the sowing ratios of ladino clover decrease to about 50%.

4. A shading imposed on ladino clover (about 70% of full sunlight) resulted in a remarkable decrease of yield and symbiotic N fixation capacity/pot. Nodule formation/plant was not affected by shading to the 45% of full sunlight.

5. In a water culture of ladino clover, symbiotic N fixation capacity was reduced with the increase of NO3-N concentration in the culture solution, and the N fixation capacity was recovered by the removal of NO3-N from the media.

6. The symbiotic N fixation capacity of ladino clover grown in 10 ppm NO3-N culture solution was higher in the without renewal plot of culture solution than that in the renewal plot with weekly intervals and the different values between the two plots were supposed to be resulted from the different concentrations of NO3-N in the media of both plots.

Key words: Legume/Grass association, Symbiotic nitrogen fixation.

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Introduction

A legume/grass association has several advantages to their pure stands. Some part of nitrogen fixed by legumes is utilized directly or indirectly by grass associated, and the difference of root system and nutritional requirement between them acts effective utilization of soil nutrients. Therefore, higher production in quality and quantity, and small seasonal fluctuation in productivity are expected in such a legume/grass association.

It is often difficult, however, to maintain the mixture ratio of legume and grass in favorable range through the years. High level cutting and infrequent one encourage grass, and lower level and frequent cutting favor legume growth in mixed sward. Application of nitrogen in large quantity depressed legume and that of small quantity reduces grass. The variation of legume–grass ratio (ladino clover–orchard grass ratio) is generally recognized to be affected by the different susceptibility to mutual shading and to applied nitrogen.

However, little attention has been paid to the effect of grass on the symbiotic N fixation of legume in legume/grass mixture. Therefore, some experiments were conducted under pot cultured conditions in order to elucidate the factors affecting symbiotic N fixation of ladino clover in mixed culture.

Materials and Methods

1. Conditions for Plant Culture

1-1. Soil Culture (Experiment 1)

Fifteen kg of a soil with pH 6.5 was filled in each 1/2,000 a Wagner pot. Potassium chloride and fused phosphate were applied at a rate of 1 g and 5 g as basal dressing, respectively. For N treatment, 1 g and 3 g of urea/pot were applied as low and high N level, respectively. All of the fertilizers were mixed well with 7.5 kg of upper layer of soil in pot. Seed mixture of ladino clover and orchard grass were sown at different ratios of 10:0 (ladino clover 0.5 g: orchard grass 0 g), 8:2 (0.4:0.1 g), 5:5 (0.25:0.25 g), 2:8 (0.1:0.4 g) and 0:10 (0.0:0.5 g). Plants were grown in the glass house from Oct. 16, 1979 to Jun. 26, 1980 and plant tops were cut four times during that period. Potassium chloride (1 g/pot) and urea (1 g and 3 g/pot for low and high N level, respectively) were top dressed again on May 28, 1980 after the 3rd cutting. An investigation of growth and symbiotic N fixation was conducted at the 4th cutting when the effect of mixed culture was thought to be fully developed. No rhizobia was inoculated in this experiment since the native ones fully existed in the experimental soil.

1-2. Sand Culture of Ladino Clover and Shading Treatments (Experiment 2)

Ladino clover seeds (0.2 g) were sown in 1/10,000 a pots containing each 1 kg of river sand. Sands were inoculated by pouring rhizobial suspension into pots. The nutrient solution was applied every other day. Pots were placed in iron beam frames (2 m×2 m×2 m) covered with several sheets of cheese cloth in a glass house for 6 weeks from Oct. 28, to Dec. 13, 1980. Light intensity was adjusted by cheese cloth at 72% (white cheese cloth 1 ply), 45% (black 1 ply), and 23% (black 2 plies) of control (average 29, 500 lux during the
experimental period at no shading of natural sunlight). Plant tops were cut and weighed, and nodule numbers were counted after measuring symbiotic N fixing capacity at harvesting time (Dec. 13, 1980). All of experiments were triplicated and data shown indicate averages.

1-3. Water Culture of Ladino Clover and Nitrogen Treatment in Culture Media (Experiment 3)

A small plastic basket filled with quarz sand was suspended in a 1/5,000 a Wagner pot containing 2l of nutrient solution. Seeds were sown at the rate of 0.2 g/pot. Pre-grown in the N-free nutrient solution for four weeks after seeding, plants were stored at the culture solutions containing 0, 10 and 80 ppm of NO₃-N, respectively for one week. Then, plants were transferred to N-free solution again for a week. Culture solution was renewed every week. Symbiotic N fixation capacity was measured three times, before and after one week cultivation at various levels of NO₃-N concentration and at the end of plant cultivation.

A separate experiment with the same method as above was made to investigate the relationship between the decrease of N concentration in the media and symbiotic N fixation. Plants were with and without renewal of culture solution containing 10 ppm of NO₃-N. The renewal of culture solution was carried out every week and water loss by evapo-transpiration was complemented with distilled water. The pH of culture Solution was uncorrected during the experimenal period. Sand was inoculated with the same rhizobia as described above. The change of NO₃-N concentration in media was measured at each renewal time of culture solution and N fixing capacity was measured at 25 days after sowing.

Analysis

Symbiotic N fixation capacity of ladino clover was measured on the intact plants by acetylene reduction method, and NO₃-N in the media was by colorimetric method with Grjess-Romijn. reagents⁴).

Results and Discussion

1. Effect of Orchard Grass Association on the Growth and Symbiotic N Fixation of Ladino Clover (Experiment 1)

Figure 1 shows the growth of ladino clover and orchard grass at the 4th cutting in the pure and mixed culture. The top growth of orchard grass was higher in high N plot than in low N plot (P<0.01). The difference of growth was not significant among pure and mixed cultures or among the different levels of mixture ratio except for the pure culture of low N treatment.

On the contrary, the growth of ladino clover showed a significant difference between pure and mixed cultures (P<0.01). In mixed culture, the high N treatment resulted in markedly low growth compared with the low N treatment (P<0.01). The depressed growth of ladino clover in mixed culture was thought to be due partly to the shading effect of orchard grass whose growth was accelerated by nitrogen application in the high N plot.
Fig. 1. Effect of various association ratios on the growth of ladino clover and orchard grass with low and high N treatment in a pot experiment

An experiment to certify the shading effect was conducted separately, and it will be discussed later.

The root growth showed a similar trend with the top growth in ladino clover, whereas that of orchard grass showed a gradual increase with the increase of mixture ratios of orchard grass in both low and high N treatments (low N treatment: \( Y=21.86+15.98X \), and high N treatment: \( Y=121.3+31.66X \), where \( Y \) is root growth and \( X \) is the mixture ratio of orchard grass).

On the other hand, the symbiotic N fixation capacity/pot was almost constant among plot A, B and C at the low N treatment, although the yield of ladino clover and the legume ratio were lower at the sowing ratios of 8:2 (plot B) and 5:5 (plot C) than those of the sowing ratio of 10:0 (plot A).

Generally, in the legume/grass association, the restriction of leguminous photosynthesis by shading of taller grass is known to depress the symbiotic N fixation owing to poor supply of photosynthetic assimilates to the root nodules\(^3\). The results of this experiment, however, were not necessarily agreed with above generalization, that is, symbiotic N fixation

### Table 1. Effect of ladino clover/orchard grass association on the symbiotic N fixation capacity of ladino clover

<table>
<thead>
<tr>
<th>Plot</th>
<th>Sowing ratio</th>
<th>ARC ((\mu)mol/pot/hr)</th>
<th>ARC ((\mu)mol/g root/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ladino clover : Orchard grass</td>
<td>low-N</td>
<td>high-N</td>
</tr>
<tr>
<td>A</td>
<td>10 : 0</td>
<td>13.5</td>
<td>14.6</td>
</tr>
<tr>
<td>B</td>
<td>8 : 2</td>
<td>13.4</td>
<td>2.7</td>
</tr>
<tr>
<td>C</td>
<td>5 : 5</td>
<td>14.9</td>
<td>1.4</td>
</tr>
<tr>
<td>D</td>
<td>2 : 8</td>
<td>6.1</td>
<td>0.5</td>
</tr>
<tr>
<td>E</td>
<td>0 : 10</td>
<td>0.1</td>
<td>0.2</td>
</tr>
</tbody>
</table>

* ARC: Acetylene Reduction Capacity
capacity/g root was significantly different among sowing ratio treatments in both low and high N treatments (Table 1). The inhibition of symbiotic N fixation capacity/g root by shading of orchard grass was less severe in low N treatment because the yield of orchard grass was less in the low N treatment. This may be one of the causes that the symbiotic N fixation capacity of the low N treatment was higher than those of high N treatment (P < 0.01). Some other unknown factors, however, would be concerned in that the symbiotic N fixation capacity/g root did not change significantly despite the decrease of the legume ratio in the low N treatment.

2. Effect of Shading on the Symbiotic N Fixation of Ladino Clover (Experiment 2)

Mixed culture may act ineffectively on photosynthesis of legumes, because the plant height of legume is generally shorter than that of grass. Therefore, it may ultimately result in the depression of growth and N fixation of legume. It is significant to investigate under the modelled condition if the growth and symbiosis of legume in the mixed sward is really depressed.

Table 2. Effect of shading on the growth, nodule numbers, and ARC*** of ladino clover

<table>
<thead>
<tr>
<th>Shading*</th>
<th>Light** intensity (%)</th>
<th>Growth (g)</th>
<th>Nodules/Plant</th>
<th>ARC (μmol/pot/hr)</th>
<th>ARC (μmol/g root/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Top</td>
<td>Root</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>100</td>
<td>13.7±2.0</td>
<td>11.4±2.1</td>
<td>15.4±4.9</td>
<td>13.4±1.4</td>
</tr>
<tr>
<td>2</td>
<td>71</td>
<td>4.6±0.6</td>
<td>5.8±0.8</td>
<td>19.6±7.5</td>
<td>4.9±0.8</td>
</tr>
<tr>
<td>3</td>
<td>45</td>
<td>3.3±1.0</td>
<td>2.9±1.0</td>
<td>12.6±4.2</td>
<td>2.5±0.6</td>
</tr>
<tr>
<td>4</td>
<td>23</td>
<td>2.7±1.6</td>
<td>1.4±0.7</td>
<td>5.7±1.6</td>
<td>1.0±0.3</td>
</tr>
</tbody>
</table>

* 1: No shading, 2: white shading cloth 1 ply, 3: black 1 ply, 4: black 2 plies
** Light intensity of no shading was about 29,000 lux.
*** ARC: Acetylene Reduction Capacity

Table 2 shows the growth and N fixation of ladino clover grown at various light intensities from sowing to the harvest. Both growth and N fixation/pot were strongly depressed with the decrease of light intensity, that is, at the 75% of light intensity to control, remarkable depressions of both growth and N fixation were observed. But the decrease of light intensity until 45% did not affect significantly the nodule formation/plant, and the acetylene reduction capacity (ARC)/g root was not variable among the treatments. There may be two factors reducing plant yield by shading, one of which may be a depression of the growth of individual plant and the other may be that of plant numbers/pot. Plant numbers/pot were observed to be decreased at higher shading treatment in this experiment. Therefore, the shading treatment was thought to reduce the symbiotic N fixation capacity through the decrease of plant number. From this result, the symbiotic N fixation of ladino clover in mixed sward was considered to be depressed by the shading of associated tall grass, of which the plant height was promoted by the increase of N application.

In the low N treatment, however, it was not obviously observed that the association of grass was necessarily inhibitory for the symbiotic N fixation even though the growth was depressed as shown in Table 1. Consequently, another factor other than the shading effect might be related to the N fixation capacity of leguminous forages in the mixed sward.
Figure 2 indicates the effect of sowing ratio on legume ratio. The legume ratio was calculated as the percentages of ladino clover yield to total yield (ladino clover + orchard grass on the fresh weight basis). At both low and high N treatments, the legume ratio in top decreased drastically with the decrease of sowing ratio of ladino clover from 10:0 to 8:2, but among sowing ratio treatments of 8:2, 5:5 and 2:8, the legume ratio did not show the significant difference. Between low and high N treatments, the legume ratio showed a significant difference (P<0.01). The legume ratio of root gave a similar trend with that of top at both sowing ratio and N treatments.

Table 1 shows the symbiotic N fixation capacity of ladino clover. In the high N treatment, the symbiotic N fixation capacity/pot decreased with the decrease of sowing ratio of ladino clover (Y=0.36X-0.27, where Y is symbiotic N fixation capacity, and X is the sowing ratio of ladino clover). The regression coefficient between sowing ratio treatment and symbiotic N fixation capacity was highly significant (P<0.01). In the pure culture of ladino clover of the high N treatment (plot A), however, the symbiotic N fixation capacity/pot was not low compared with that in low N treatment, though the heavy application of N fertilizer is recognized to inhibit the symbiotic N fixation. This may indicate that the depression of symbiotic N fixation capacity/pot in mixed culture in the high N treatment were not caused by the direct effect of N applied but by the growth depression of ladino clover.

3. Effect of Nitrogen Concentration in the Medium on the N Fixation of Ladino Clover (Experiment 3)

A moderate amount of soil nutrients activates the nodule formation and N fixation, but a large amount of N fertilizer is known to depress nodule formation and N fixation chara-
Symbiotic Nitrogen Fixation under Plant Competition

Table 3. Effect of NaNO₃-N levels in the media on the ARC of ladino clover

<table>
<thead>
<tr>
<th>NaNO₃-N in medium (ppm)</th>
<th>ARC (pmol/pot/hr)**</th>
<th>N-free**</th>
<th>N-free**</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.9±0.3</td>
<td>3.9±0.8 (100)</td>
<td>8.8±1.5</td>
</tr>
<tr>
<td>10</td>
<td>4.1±1.7</td>
<td>2.6±1.3 (63)</td>
<td>8.4±1.0</td>
</tr>
<tr>
<td>80</td>
<td>4.0±0.5</td>
<td>1.4±0.1 (43)</td>
<td>6.4±0.8</td>
</tr>
</tbody>
</table>

* 1): ARC of the plants cultivated in a N-free nutrient solution for four weeks from sowing.
2): ARC of the plants treated with various levels of NO₃-N solution for a week after a 4-week cultivation in N-free solution.
3): ARC of the plants restored and cultivated in a N-free solution for a week after one week treatment of various NO₃-N levels.
** ARC: Acetylene Reduction Capacity.

ceteristically²). The absorption of soil nutrients by orchard grass associated with ladino clover may affect the symbiotic N fixation capacity through the decrease of soil nutrients. Especially the absorption of soil nitrogen by orchard grass associated has a possibility to increase the symbiotic N capacity of ladino clover through the decrease of inorganic N in soil. In this point of view, high values of symbiotic N fixation capacity of ladino clover grown with orchard grass in the low N treatment will be understandable without any contradiction.

In order to elucidate that the symbiotic N capacity of ladino clover was accelerated with the decrease of nitrogen in rhizosphere, a hydroponic experiment was conducted, and the result is shown in Table 3. When plants were transferred from N free solution to the various levels of nitrogen solution and incubated for one week, the symbiotic N fixation capacity was depressed rapidly e.g. even in a 10 ppm of NO₃-N solution, the relative value to control revealed only 63%. When plants were restored to N free solution again and incubated for one week, the capacity was rapidly recovered. This result suggests the symbiotic

![Fig. 3. Variation of ARC for ladino clover grown with or without renewal of 10 ppm NaNO₃-N solutions at weekly intervals in water culture method](image)

![Fig. 4. Variation of NaNO₃-N concentration in culture solutions for plots with or without renewal of 10 ppm NaNO₃-N solution at weekly intervals in growing ladino clover in a water culture method](image)
N fixation capacity of ladino clover have rapid and sensitive response to the variation of rhizosphere N status.

Figure 3 shows the symbiotic N fixation capacity of ladino clover grown at the 10 ppm NO₃-N solution for four weeks with or without renewal of the solution at weekly intervals. The capacity of without-renewal plot showed a 3-fold higher value than that of renewal plot.

As written previously, the increase of essential elements at the moderate range is known to promote the nodule formation and the symbiotic N fixation capacity whereas nitrogen has reverse effects on them. In this point of view, the higher capacity at the without-renewal plot was thought to be induced by the decrease of N concentration in the media through absorption by plants. Actually, the nitrogen in the media at the without-renewal plot was almost absorbed by plants and nitrogen was not detected in the media at the end of the experiment as shown in Figure 4. In the renewal plot, the culture solution after a week incubation contained a small amount of nitrogen since this experiment was conducted at a low N concentration (10 ppm NO₃-N). The great variation of symbiotic N fixation capacity even at the low N concentration (10 ppm NO₃-N) suggests that the symbiotic N fixation capacity of ladino clover is very much susceptible to the variation of nitrogen concentration in the media. Accordingly, the result of this experiment supports that the higher symbiotic N fixation capacity at the low N treatment as shown in Table 1 would be due largely to the absorption of soil mineral nitrogen by orchard grass associated with ladino clover.

Acknowledgement

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References

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ラジノクローバの共生窒素固定におよぼす混播の影響と，それに関与する要因の解析

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ラジノクローバとオーチャードグラスを1/2000aポットに単播あるいは混播比率を変え栽培し，ラジノクローバの生育，共生窒素固定におよぼす共存オーチャードグラスの影響について調査した。

モデル試験を行うことによりその影響を光および土壌窒素の競合の面より解析することを試みた。得られた結果は下記のとおりである。

1. オーチャードグラスと単播したラジノクローバの生育は著しく抑制され，その抑制度合いは低窒素施用区に比べて高窒素施用区において著しかった。

両牧草の混生重量を算出されるマメ科率は高窒素区に比べて低窒素区で高く，しかも広い範囲の混播比率内でいずれもほぼ同程度の高い値を維持していた。

ラジノクローバの共生窒素固定量は高窒素区では混播比率の低下に伴って急激に低下したが，低窒素区では混播比率が50%に低下するまでほぼ単播区と同程度の高い値を示していた。

2. 単播したラジノクローバを材料として行った砂耕および水耕試験の結果，遮光処理はその強度に対応して生育量や共生窒素固定能の低下をもたらすこと，および共生窒素固定能は培地中窒素濃度の増減に対応して上下するが，ごく低濃度の範囲（0−10 ppm）内の変動にも敏感に反応することを認めた。

3. これらのモデル試験の結果から，混播したオーチャードグラスは共存するラジノクローバの共生窒素固定に対して光競合を通じて抑制的に働くのみならず，土壌中の可結態窒素の吸収収奪を通じて促進的に働く場合のあることが推察された。

キーワード: ラジノクローバ，窒素固定。

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