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Effects of Cutting on Nodule Development and Nitrogen Fixation in Alfalfa

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刈取가 알팔파 根瘤의 發達과 窒素固定活性에 미치는 影響

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摘 要

刈取가 알팔파根瘤의 着生 및 發達과 窒素固定活性에 미치는 影響을 檢討하기 위하여 圃場實驗에서는 刈取區와 非刈取區로 구분하여 時期別로 Acetylene還元法에 의하여 根瘤의 窒素固定活性을 測定하였다. 養液栽培實驗에서는 地上部의 50%와 90%刈取, 花芽의 除去, 根瘤의 50%와 100% 除去등을 組合處理後 알팔파 再生과 根瘤의 着生과 發達에 미치는 影響을 檢討하였다.

1. 圃場實驗에서 根瘤의 무게는 1回刈取後 30%, 2回刈取後 25%의 減少가 있었고 2回刈取後 30日부터는 減少가 없었다.

2. 7月初, 8月初, 9月初에 刈取區의 specific nodule activity(SNA)는 非刈取區보다 높았으며 total nodule activity도 刈取區가 非刈取區보다 높았다. 開花期의 刈取는 根瘤의 窒素固定活性 유지에 도움이 되는 것으로 나타났다.

3. 養液栽培實驗에서는 地上部의 50% 刈取는 알팔파의 再生과 根瘤의 發達에 影響이 없었으나 地上部의 90% 刈取는 再生을 느리게 하고 根瘤의 脫落을 심하게 조장하고 着生된 根瘤나 새로이 着生된 根瘤의 發達을 나쁘게 한 것으로 보아 알팔파根瘤의 窒素固定力을 높은 狀態로 維持하기 위해서는 너무 낮은 刈取를 피하는 것이 바람직할 것으로 보여진다.

I. INTRODUCTION

Defoliation by cutting and grazing is a part of the pasture management system. The close involvement of photo synthesis in nodule development and function has been appreciated since early 1930's. A number of investigation have shown that nodules could act as sinks for newly formed photosynthates from the leaves.

In a short-term studies, cutting could cause an immediate decline in N_2 fixation, but the nodule activity was reported to recover in the next 10-20 days after cutting (Chu et al. 1974, Moustafa et al. 1969). In addition, the frequency of cutting affected nodulation, N_2 fixation and the amount of re growth. Differences in response to defoliation were found between tropical and temperate species (Whiteman 1970).

Despite the numerous descriptions of nodule decay and "shedding" following cutting, the fate of the nodule tissue was not known clearly. Wilson (1942) reported that white clover lost about one third of its nodules after harvest. Vance (1978) also stated in his review that the mechanisms controlling nodule degeneration, nodule loss, nodule replenishment after cutting were not understood for forage legumes. Whiteman (1970) suggested that effects of cutting on nodule activity were probably affected by environmental or biological variables, all of which were likely to operate in the field situation.

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The understanding of cutting effect which influence nodule development and activity can provide information about developing the method of cutting management for maximizing nitrogen fixation of nodule. The objectives of this experiment were to evaluate the effects of cutting on nodulation, nodule development and activity in alfalfa.

II. MATERIALS AND METHODS

1. Field experiment

The experiment was conducted at the Experimental Farm of the Agricultural College, Seoul National University, Suweon in 1982.

(1) Field condition; Alfalfa field was established with cultivar, Luna, in the fall in 1980. Basal application of P_2O_5 (10kg/10a) and K_2O (10kg/10a) were broadcasted over the plot area. The soil property before experiment was characterized by acid soil with low cation exchange capacity and low organic matter content.

(2) Treatments; (i) Uncutting control (ii) Cutting: Alfalfa plants were cut twice at 10-15cm height from ground level. First cutting was achieved on June 8, flowering stage and second cutting on July 24, seed maturing stage.

(3) Sampling; Periodical samples were collected at intervals of 15 to 30 days from April 5 to September 26 to measure accumulation of dry matter and acetylene reduction activity of nodules. At each sampling, whole alfalfa plant was dug out and the leaves were washed with water until free of soil. They were oven-dried for 48 hours at 80°C before weighing.

(4) Acetylene reduction assay

N_2-fixing activity of nodule was measured by using the acetylene reduction method by Hardy.[5] Branch test tube included nodules were sealed with rubber stopper and were evacuated and injected with rubber stopper and were evacuated and injected with a syringe containing 0.82% gas mixture (CO_2: 40.3ppm, C_2H_2 1.82%, argon balance) at 0.2 atm. After samples were incubated at 28°C for two hours, one ml mixture gas was injected into a gas chromatograph (Shimadzu Model GC-6A) equipped with a flame inoization detector. C_2H_4 and C_2H_2 were separated from gas mixture in a column filled with porapark®. The column temperature was 45°C and the nitrogen carrier gas was 0.8kg/cm. The retention time of C_2H_4 was four minutes and that of C_2H_2 was three minutes.

Caculations were made as following formulare.

(i) Specific nodule activity (SNA)

$$\frac{\mu\text{moles } C_2H_4 \text{ in test tube}}{\text{fresh nodule weight}} \div \text{incubation time}$$

(ii) Total nodule activity (TNA)

$$\frac{\mu\text{moles } C_2H_4 \text{ in test tube}}{\text{No. of plant}} \div \text{incubation time}$$

2. Solution culture experiment

Alfalfa (*Medicago sativa* L.) cultivar, Luna, seeds were sown in a vat (60x40x10cm) on April 13. At sowing, the seeds were inoculated with a comercial preparation of *Rhizobium meliloti* (Nitragin Co. USA) and macro-and micro-nutrients except nitrogen source were supplied in the vat.

At flowering stage, well-nodulated and uniformly-grown plants were selected and transplanted into the container (75x75x50cm) for solution culture. The container was covered with a plywood with 40 holes and each hole was 7.5cm apart and stopped with sponge sheet for holding plants.

Two air pumps (4.5 Watt) were installed in container for aeration. Nitrogen-free nutrients solution with the following composition was changed every 15 day. Nutrient solution composition: $CaHPO_4$(1g), K_2HPO_4 (1g), $MgSO_4 \cdot 7H_2O$ (0.2g), NaCl (0.2g), $FeCl_3$ (0.18g), H_3BO_3 (5mg), $MnCl_2$ (5mg), $ZnSO_4$ (0.5mg), $CaSO_4$ (0.2mg) Water (1ℓ).

(1) Treatments

Before solution culture there were following treatments; cutting of half and 90% of shoots, removal of flower buds and half, 90% of nodules. As the result of the treatment, the following were the combination of cutting of the shoot and removal of nodules;

Description / Treatment No.	Shoot cutting (%)	Nodule removal (%)
Control (T_1)	0%	0%
T_2	0%	50%
T_3	0%	100%
T_4	0% (Flower buds removal)	0%
T_5	0% (Flower buds removal)	50%
T_6	0% (Flower buds removal)	100%
T_7	50%	0%
T_8	50%	50%
T_9	50%	100%
T_{10}	90%	0%
T_{11}	90%	50%
T_{12}	90%	100%

(2) Investigation

Plant fresh weight and the number and size of nodule were measured at 7 days intervals. The nodule size was measured with vernier caliper. The plants were replanted for continued growth.

III. RESULTS

1. Field trial

(1) Nodule weight

Nodule fresh weight in the field was changed during growing season. At the sampling of 30 days or 45 days after first cutting, nodule weight of cutting plots was reduced by 30% as compared with that of uncutting plots. After the second cutting (15 days after cutting), nodule weight of cutting plots was reduced by 20% as compared with that of uncutting plots. After the 30 days of second cutting, there was no significant difference between cutting plots and uncutting plots (Fig. 1).

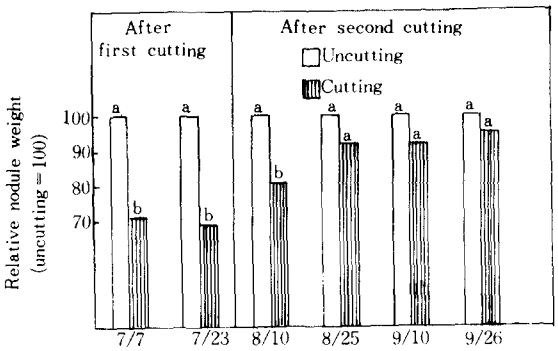

Fig. 1. Changes of relative nodule weight of cutting to that of uncutting as affected by cutting

(2) Nodule activity

Specific nodule activities (SNA) of cutting plots at the beginning of July (30 days after first cutting) and at the beginning of September were 80% and 100% higher than that of uncutting plots respectively. From the end of July to the end of August, SNA decreased to very low levels, showing no significant difference between cutting and uncutting plots (Fig. 2). After first cutting, total nodule activities of cutting and uncutting plots were not significantly different, but total nodule activity of autumn growing season (in the end of August and in the beginning of September) in cutting plots was higher by 40% than that of uncutting plots (Fig. 3).

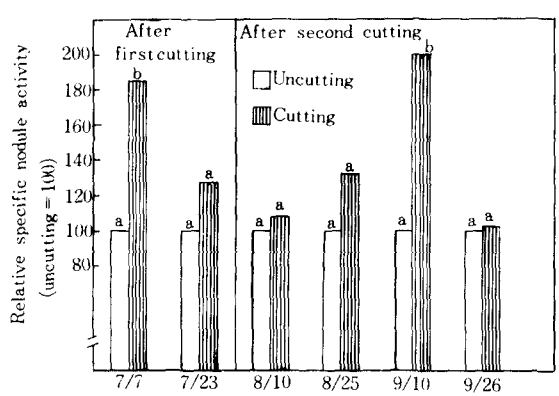

Fig. 2. Changes of relative Specific Nodule Activity (SNA) of cutting to that of uncutting as affected by cutting

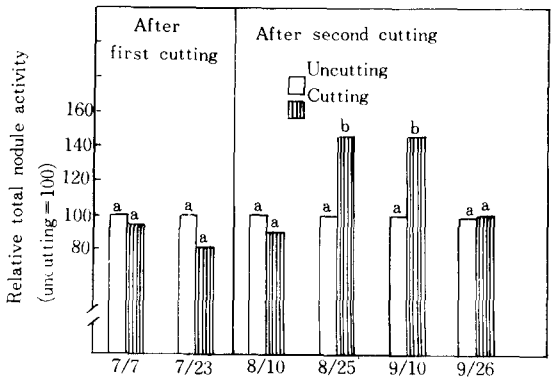

Fig. 3. Changes of relative Total Nodule Activity (TNA) of cutting to uncutting as affected by cutting

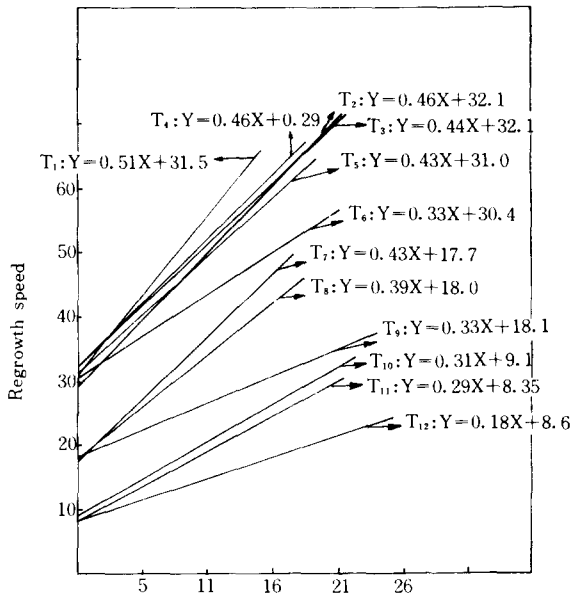

Fig. 4. Effects of cutting and nodule removal on the speed of regrowth

2. Solution culture trial

(1) Regrowth

The plants in solution culture grew slowly for 25 days after cutting. By July 17, the uncutting plants were in full bloom. The plants of uncutting and of removal of flower buds showed similar increase in fresh weight. The speed of plant regrowth in the combination of 50% cutting of shoot and 50% removal of nodules were showed to be similar to that of plants in combination of 50% cutting of shoot and not-removal of nodules, but the speed of regrowth retarded in plants in combination of 50% cutting of shoot and removal of all nodules. The speed of plant regrowth in combination of 90% cutting of shoot and removal of all nodules markedly retarded until 15 days after cutting (Fig. 4.).

(2) Nodule condition and nodule number

At the flowering stage, the nodules were in excellent condition. Each nodules appeared to be functioning perfectly. After the flowering stage, nodules in solution culture were found to be disintegrated. They had lost some of their pinkish or green color. Some nodules were distintegrating and others were being newly formed. Many nodules were present on the surface of crown. But most of these nodules disappeared and new ones appeared on the root down the crown. Five days after cutting, nodule disintegration was initiated and such a phenomenon was severe after eleven days. The nodules of 90% cutting of shoot were undergone nore remarkable decay than those of uncutting plants. Sixteen days after cutting, the number of new nodules began to increase. After all nodules were removed, new nodules were increased in their number at a constant rate. The number of newly formed nodules, when subjected to cutting of shoot and the removal of all nodules, were increased at a fairly slow rate (Fig. 5.).

(3) Nodule size

The nodule size of 50% cutting of shoot which has been described by length x width was similar to that of uncutting of shoot and removal of flower buds, but the nodule size of 90% cutting of shoot was small. After all nodules were removed, the size of newly formed nodules was similar to that of not-removal of nodules (Fig. 6.).

IV. DISCUSSION

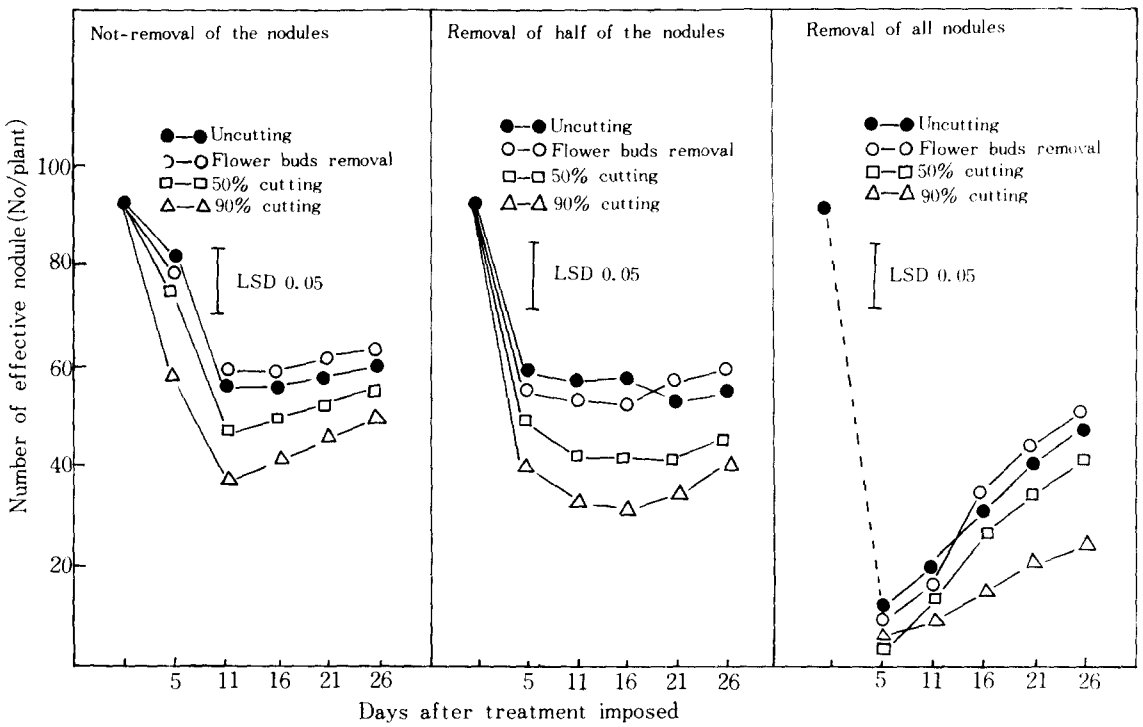

Fig. 5. Effects of cutting and nodule removal on nodule number

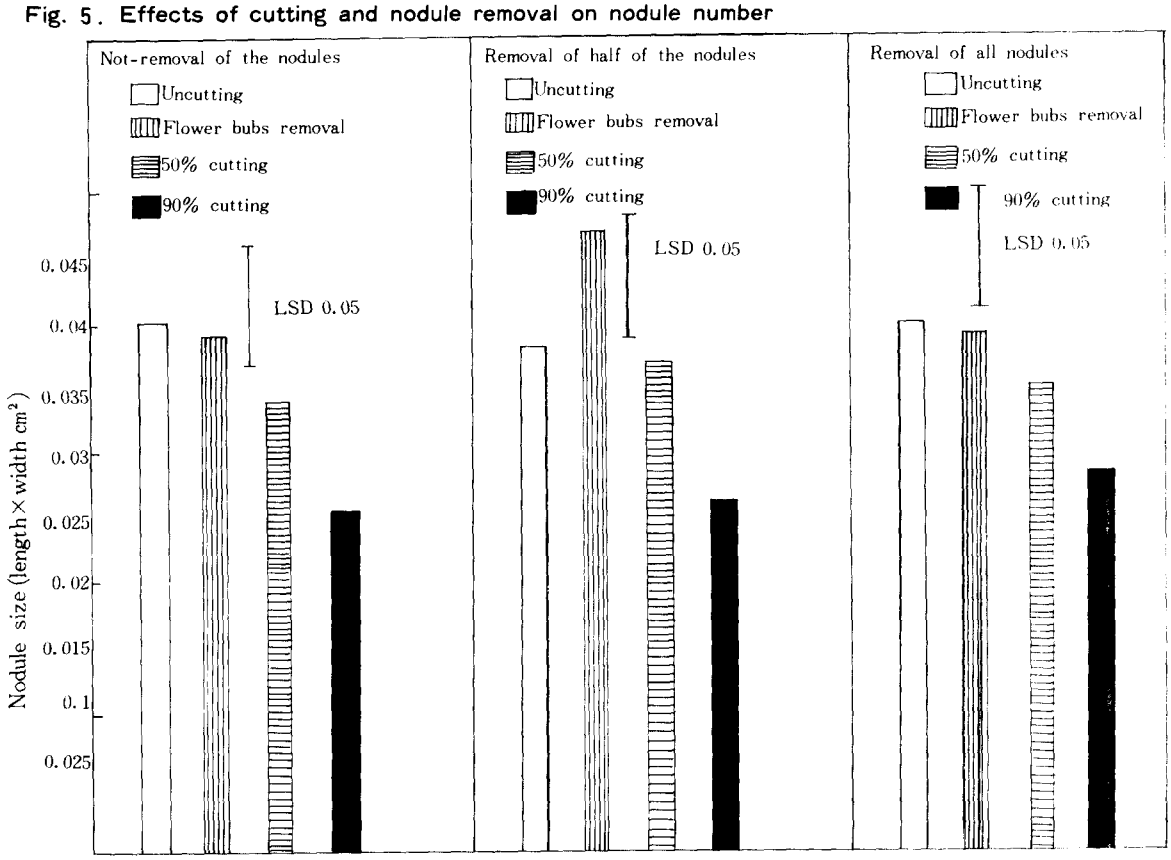

Fig. 6. Effects of cutting and nodule removal on nodule size

The result of this experiment coincided with the suggestion that cutting eventually caused the decrease in nodule weight of temperate species (Wilson 1942, Greenwood 1959). The loss of modules might have been due to the energy cost (Gibson 1966) for maintaining surplus nodules during short periods after defoliation. At field sampling, it was impossible to measure the nodule status. But, cutting in solution culture led to a loss of larger older nodules and initiation of new modules.

Specific nodule activity of cutting plots in the field at the beginning of June and at the beginning of September were 80% and 100% higher than that of uncutting plots respectively. Total nodule activity increased from the end of August and peaked at the beginning of September in cutting plots, but a little increased and did not peak in uncutting plots. The decrease of nodule activity in uncutting plots might have resulted from the eventual senescence of leaves and other tissue including nodule. Cutting of shoot diminished leaf photosynthesis instantly, but it removed competition between seed sink and nodules to secure photosynthates. Shoot cutting mobilized reserved carbohydrate in crown and root, and after a few days the plant regrew, carbohydrate increased in cutting plots, and the nodule activation might have been resulted. Because the decrease of nodule activity in uncutting plots seemed to be related to the onset of flowering and pod filling, it was possible to increase the specific nodule activity by preventing or delaying the decrease through cutting at flowering stage. Defoliation of white clover produced an immediate decrease in nodule activity, but complete recovery occurred within 10 days after cutting in a green-house experiment (Chu and Robertson 1974) and within 15 days in a field experiment (Moustafa et al., 1969).

Nodule disintegration in solution culture was found after cutting. The nodules subjected to 90% cutting of shoot decayed more markedly than those of uncutting of shoot. It was suggested that photosynthates when 90% the shoot cut was insufficient for maintaining nodule number and led to decrease in the number of nodules and insufficient development. Shoot cutting in solution culture appeared to have two processes; first, the severe cutting would cause the loss of activity of original nodule population, and second, prevent new nodule from developing. The major effect of the cutting treatment would appear to be exerted through the reduced carbohydrate supply to the root system.

In this experiment, nodule weight after cutting, decreased, but cutting prevented nodule activity from dropping down in the late period of flowering and led to the higher nodule activity in autumn growing season than that of uncutting plots. But this experiment failed to find the optimum cutting date to maximize nitrogen fixation by means of initiating new nodules.

V. SUMMARY

This experiment was conducted to evaluate the effects of cutting in field and solution culture. Periodical samplings of alfalfa in cutting and uncutting plots were taken to measure nodule development and nodule activity.

Regrowth of plant and nodule development after shoot cutting by different heights and nodule removal at different levels were investigated in solution culture of alfalfa plant.

1. Nodule weight in the field was reduced 30% after the first cutting and 25% after the second cutting, but during the following 30 days after second cutting, there was no significant difference between cutting and uncutting plots.
2. Specific nodule activities of cutting plots at the beginning of June and at the beginning of September were 80% and 100%, higher than those of uncutting plots respectively. Total nodule activities of cutting

plots in late August and early September were 40% higher than those of uncutting plot. The decrease of nodule activity can be prevented by cutting at flowering stage.

3. The decrease of nodules in solution culture when 50% of the shoot was cut, was as much as that when shoot was not cut or flower buds were removered. But when 90% of the shoot was cut, the number of the nodules were decreased more remarkably than the above treatments. New nodules, when 90% of the shoot was cut, were reformed slowly and did not grow fully until 15 days after cutting.

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