韓國産 잔디에 있어서 季節과 刈取에 따른 窒酸환원효소와 澱粉분해 효소의 活性度 變化

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Changes in the Activities of Nitrate Reductase and Amylase in Response to the Seasons and Cutting in *Zoysia Japonica* Steud

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摘 要

야외 조건에서 생장한 한국산 들잔디(*Zoysia japonica* Steud)의 잎 · 줄기 · 뿌리 · 지하경의 관부와 절간에 있어서 질산환원효소 활성도(NRA)와 전분분해효소의 활성도(AA)를 측정하였다. 각 기관에서의 효소 활성도는 4월 중순부터 증가하여 6월의 개화기때 최대값에 도달하였다. 그후 효소 활성도는 급격히 감소하여 겨울철에는 최소에 이르렀다. 이 결과는 잔디의 각 기관에서 NR 과 Amylase 는 계절적 활성 변이를 가진다는 것을 제안한다.

NR 과 Amylase 가 낮은 활성도를 가지는 1986년 7월 31일에 6 cm 의 높이에서 잔디의 지상부를 예초(cutting)한 결과, 모든 기관에서의 AA 와 잎 · 줄기에서의 NRA 는 2일간의 지연기 후에 급격히 증가하여 7일째에 최대에 이르렀다. 이 값은 대조구의 효소 활성도 보다 약 3배 높았고, 계절적 변이에서의 최대 활성도의 약 90%에 도달하였다. 예초된 잎에서의 조단백질(CP)의 함량은 예초후 7일째 까지 증가하였으나, 건물생산량(DM)과 총가용성 탄수화물(TSC) 함량은 예초후 4,5일 동안 감소하다가 다시 증가하여 7일째에 예초전의 수준에 도달한 후 일정한 값을 유지하였다. 또한 각 기관에서의 NRA 와 AA 는 예초후 8일째에 정상 수준으로 급감하였다.

이 결과는 완전히 자란 잔디 자체는 NR 과 Amylase 의 활성에 대한 억제제로서 작용하며, cutting 과 같은 외부 성처요인이 개체 수준에서 적용되었을때 손상된 물질을 회복하기 위하여 분자 수준에서 효소가 활성화 된다는 것을 제안한다.

I. INTRODUCTION

The changes of enzyme activities are known to be controlled by genetic and environmental factors (Marcus, 1971). In plants, the dramatic changes in activity of some leaf enzymes, according to vegetative stage of development, have suggested that they might be useful indicators for the developmental stage (Patterson et al., 1980). Although NR and amylase are not directly involved in controlling photosynthesis. they are key enzymes in the metabolic process of green plants. In plants using nitrate, NR has the biochemical characteristic that the reaction it controls is the rate — limiting step of the inorganic phase of nitrogen metabolism (Beevers and Hageman, 1969).

Further metabolism proceeds by way fo other

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enzymatic reactions in which nitrogen is incorporated into a wide range of compounds. Amylase is a key enzyme in the process of breaking down starch accumulated in the plant organs. We therefore examined the seasonal changes of NR and amylase activity for indications that the developmental stage of lawn grass varies according to the season of the field condition.

To understand the growth mechanism and defence reaction against environmental conditions, it is necessary to elucidate the principles of plant response to mechanical injury such as cutting. It has shown that the remarkable changes in wounded tissues were the result of many biochemical events triggered by the plant cell recognition of mechanical wounding (Uritani, 1976). Kahl (1973, 1974) discovered that wound respiration is induced when storage organs are mechanically cut into slices and incubated under conditions of moderate temperature and high humidity. The respiratory rate decreased to the level before wounding after completion of the wound healing layer. Other kinds of organs such as leaves, fruits, rhyzomes, seeds, when activated by cutting, show principally the same metabolic alternations as the storage organs shown in response to cutting (Bireckaet al., 1973). But, in the case of growing plants in the field conditions, few have studied the enzymatic changes after cutting. It had shown in the orchardgrass that phleinase activity increased rapidly during the first 2 days and then kept an almost constant level until it decreased on the 15th day, and invertase activity increased rapidly during the first 3 days and thereafter decreased gradually (Yamamoto et al., 1982).

The object of this paper is to investigate the response mechanism in the enzymatic level during the early stage of regrowth after a completely grown individual was influenced by mechanical cutting.

Ⅱ. MATERIALS AND METHODS

1. Site and Climate

This experiment was carried out on the campus of Seoul National University located on 37° 27′N 126° 50′E. The site chosen for the study is a grassland located at the foot of the northeast slope of Mt. Kwanak which is 230m above sea level. Soil samples, taken at the commencement fo the cutting experiment, had a pH of 5.6, total nitrogen of 0.12percent (Table1). It was slightly lower for best growth condition (Juska, 1959, 1965). Climatic data recorded at the Mt. Kwanak observation station 200m above the experimental site areshown at Fig.1. Annual mean total precipitation was 1,338.3mm. From the middle of April to the beginning of October, it was an appropriate temperature for the growth of *Zoysia japonica.*

2. Sampling Procedures

Samples were taken daily from the same rows between 11.00 and 12.00 a.m. The sampled plants were divided into five parts — leaf, stem, root, crown, and internode of a rhizome — in the laboratory (Fig. 2), and were thoroughly rinsed with sterillized distilled water and excess water removed using filter paper. The second and third leaf from the top of the plants were chosen for the seasonal change experiments. Lawn grass fully grown was cut at the height of 6 cm above the ground with shears on the 31st of July, 1986. Thereafter, sampling was done every day for 15 days.

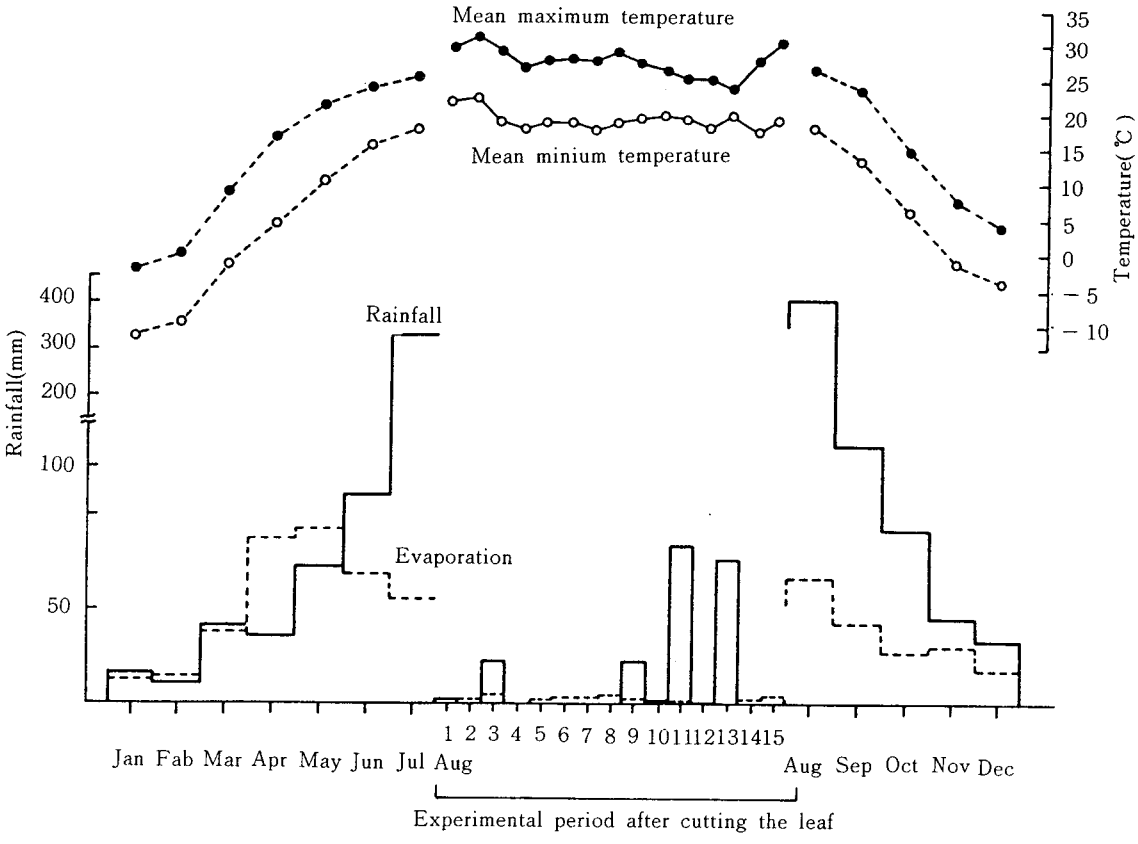

Fig.1. Mean values for meteorological data during experimental period of 1986.

Table 1. Soil ayalysis in the experimental site

Content Sample	Acidity	Organic Matter	Total Nitrogen	Available Phosphate(P_2O_5)	Exchangeable (me / 100g)			Cation Exchange capacity
(depth)	(pH)	(%)	(%)	(P.P.M)	K^+	Ca^{++}	Mg^{++}	(me / 100g)
0~10cm	5.60	2.66	0.12	68.06	0.28	2.78	0.89	5.83
10~20cm	5.75	1.30	0.08	65.69	0.21	2.65	0.89	4.84

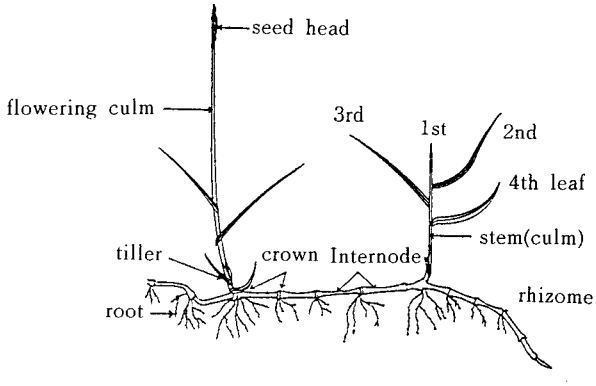

Fig. 2. Morphology and terminology of organ of *Z.Japonica* Steud.

3. Enzyme Assays

1) Extraction of nitrate reductase

Nitrate reductase was extracted from the sample of 1 g. The ice cold extraction medium (total volume of 8 mℓ) was 0.1 M potassium phosphate buffer (pH 7.6), 1mM Na_2 − EDTA, 25mM cystein, 3% (w/v) bovine serum albumine (Schrader et al., 1974), and 0.1% (w/v) PVP − 40. Grinding was carried out at 0 ℃. The homogenate was centrifuged at 20,000 g for 20 minutes at 4 ℃.

2) Assay of nitrate reductase activity

The assay was performed according to that described by Neyra and Hageman (1975). The reaction mixtures contained (in a final volume of 1 mℓ) : 0.2 mℓ of 1M K − phosphate buffer (pH 7.6), 0.1 mℓ of 0.2M KNO_3, 0.1 mℓ of 2mM NADH, 0.2 mℓ of distilled H_2O, and 0.4 mℓ of plant extracts. After 30 minutes incubation at 30°C, the reaction was stopped by the addition of 0.2 mℓ of 0.5M zinc acetate and 0.2 mℓ of phenazine methosulfate (46 mg/1) (Scholl et al., 1974). After standing at room temperature for 10 minutes, it was spun at 1,000 g for 10 minutes, it was spun at 1,000 g for 10 minutes. Nitrite was determined by adding 1 mℓ of sulfanilamide and 1 mℓ of 0.02% N − 1 − naphthyl ethylenediamine dihydrochloride in 1.5N hydrochloric acid and, after 15 minutes, measuring absorbance at 540nm. Nitrate reductase activity was expressed as micromoles nitrate reduced per hour per gram fresh weight.

3) Amylase assay

The amylase assay was based on the method of Varner (1964). The tissue of 1g was ground in a cold 1mM Na − acetate buffer (pH 4.8) of 5 mℓ containing 0.02M $CaCl_2$. The homogenate was centrifuged at 2,000g for 10 minutes. The resulting supernatant fraction was assayed for amylase activity. The amylase assay was carried out at 30 ℃ for 30 minutes with enzyme extracts of 0.15 mℓ. The reaction was started by the addition of 1 mℓ of fresh potato starch solution and stopped by addion 1.0 mℓ of iodine reagent. Five ml of distilled water were added to each tube and after thorough mixing the optical density was read at 620nm.

The decrease in O.D. at 620nm caused by the action of the enzyme is proportional to the quantity of amylase present. One unit of enzyme activity represents the change of 0.1 O.D. unit.

All data reported are the mean values of at least three replications, each with a different sample.

4) Dry matter yield

Dry weight samples were taken from the same rows as the enzyme samples and dried overnight at 80 ℃. Three replicates were sampled for dry weight.

5) Crude protein

The analysis of crude protein was done by the method of the A. O. A. C. (1980).

6) Total soluble carbohydrate

Total soluble carbohydrate was measured by the method of Shaeffersomogyi coperiode metric method (Smith, 1969).

Ⅲ. RESULTS AND DISCUSSIONS

1. Seasonal changes in enzyme activities

Since *Zoysia japonica* is a C_4 − type photosynthetic plant, it has and unusually high minimun growing temperature over 20 ℃ (Youngner, 1961). So leaf growth began in the middle of April and the green period was finished at the beginning of October having a temperature below 20 ℃ (Chang and Kim, 1986). Seasonal change was determined for amylase activity and

nitrate reductase activity in *Z. japonica* grown in field conditions.

A) **Seasonal change of nitrate reductase activity**

Nitrate Reductase (NR) activity in most plants such as corn (Dalling et al., 1975) and CAM plants (Chang et al., 1981) follows a marked diurnal pattern. So all samplings were taken between 11.00 and 12.00 a. m.

The seasonal pattern of NR activity for each organ was shown in Fig. 3. The activity of enzyme was presented on a fresh weight basis. Photosynthetic tissues such as leaf and stem had on an average more high NR activity than underground parts such as root, crown, and internode of a rhizome. The low NR activity in the underground parts may be associated with a defect of stimulating effect of light on induction of NR activity (Beevers and Hageman, 1969).

NR activity in stem and leaf tissues had some high values in the middle of April sending the shoot out. and increased to reach a maximum in the middle of June. Lawn grass has an anthesis and booting – earemergence in June. During this period, high NR activity may contribute to the step of synthesis of nutrient needed for flowering. After booting – ear stage, the NR activity decreased drastically. These results are similar to the report that the seasonal pattern of NR activity for wheat showed a peak of activity at the booting – ear emergence stage of development and then a rapid decline in activity during the grain – filling stage (Dalling et al., 1975). The dramatic changes in the activity of NR enzyme after the anthesis suggest that thev might be useful indicators of flowering and grain – filling in *Z. japonica*.

Although NR activity had decreased to a low level after booting – ear emergence, the green color of the leaf was maintained until late September (Chang and Kim, 1986) and dry matter yield was a high value (Shoji et al., 1973). It suggests that the nutrients required for continuing the growth after the anthesis may be provided by the action of photosynthetic enzymes, and that the ability to form an active nitrate reductase is a prerequisite for fowering and booting – ear emergence.

The rapid fluctuation of NR activity occurring seasonally and the seasonal level of nitrate reductase activity were considered as related with age (Filner et al., 1969) and were under genetic control (Beevers and Hageman, 1969).

B) **Seasonal change of amylase activity**

Differently from the level of NR activity, Amylase Activity (AA) had some high value in all of the organs of *Z. japonica* (Fig. 4). From March, before sending the shoot out, storage organs such as internode and crown had some activity of amylase and increased markedly to the maximum in late June. In the leaf and stem tissue, amylase activity had some high values in the middle of April, thereafter increased gradually to the period of anthesis. Although root tissue had less activity than other organs, all five organs had less activity than other organs, all five organs had a high value of amylase activity during the anthesis period of June. There after, from the beginning of July, the amylase activity had decreased drastically. It means that the increase of amylase activity is a prerequistite for flowering, and it is considered that the use of storage reserves is not necessary after the grain – filling stage and so amylase activity becomes decreased to a very low level.

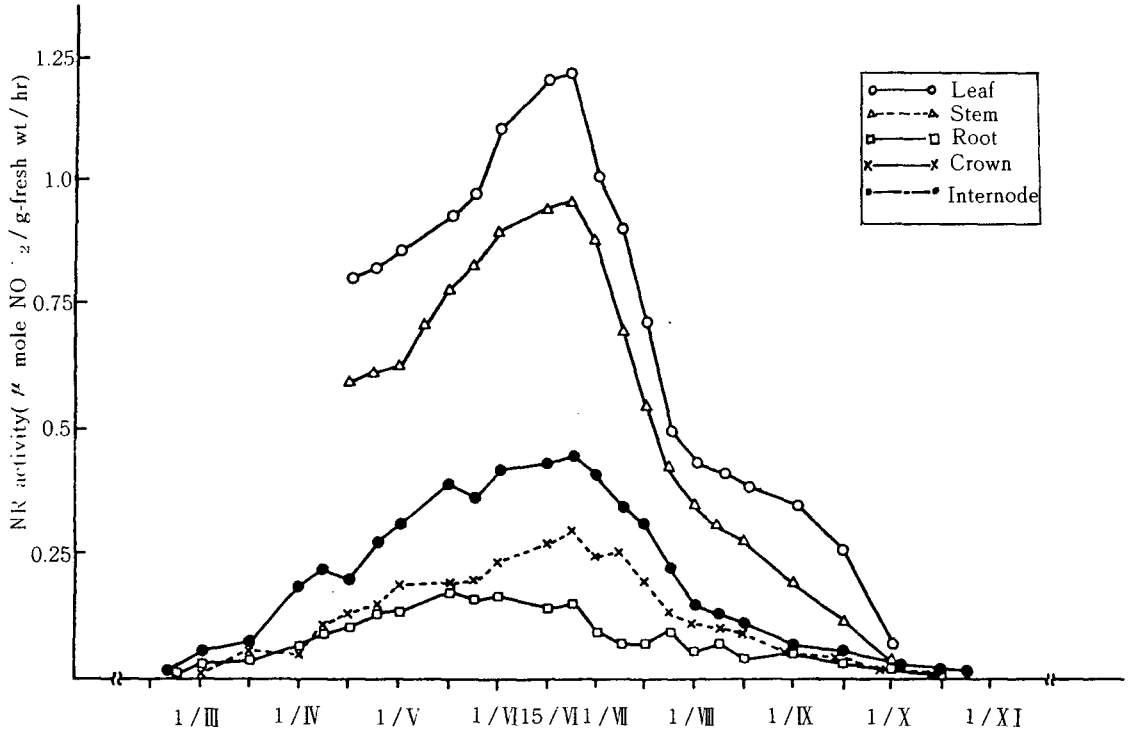

Fig. 3. Seasonal changes in NR activity of each organ in *Zoysia Japonica*.

After the grain — filling stage, new foods which were produced by photosynthesis may be stored at the storage organs such as crown, internode, and root. Since amylase activity has a low activity after the grain — filling stage, starch, which was newly reserved, may not be degraded by amylase.

In conclusion, it is considered that both NR and amylase contribute to the steps of energy supply for the anthesis. When we compared the seasonal changes of AA and NRA with Shoji's study (1973) that dry matter weight has a high value from July to September, photo — synthetic enzymes may be more activated to synthesize the nutrients needed to retain the green period after the booting — ear emergence.

2. Changes in response to cutting the leaves

On the 31st of July 1986, when NR and amylase have a low activity, leaves were cut at the height of 6 cm above ground. A time course study after cutting was done for NRA, AA, Dry Matter (DM) yield, crude Protein (CP), and Total Soluble Carbohydrate (TSC) in each organ during the early stage of regrowth.

A) Changes in NR activity in response to cutting

Changes in NR activity during the early stage of regrowth in response to a specific environmental treatment such as cutting are shown in Figures 5, 6, and 7. In the leaf tissue (Fig. 5), after a lag phase of 2 — days following cutting of leaf tissue, NR activity increased markedly

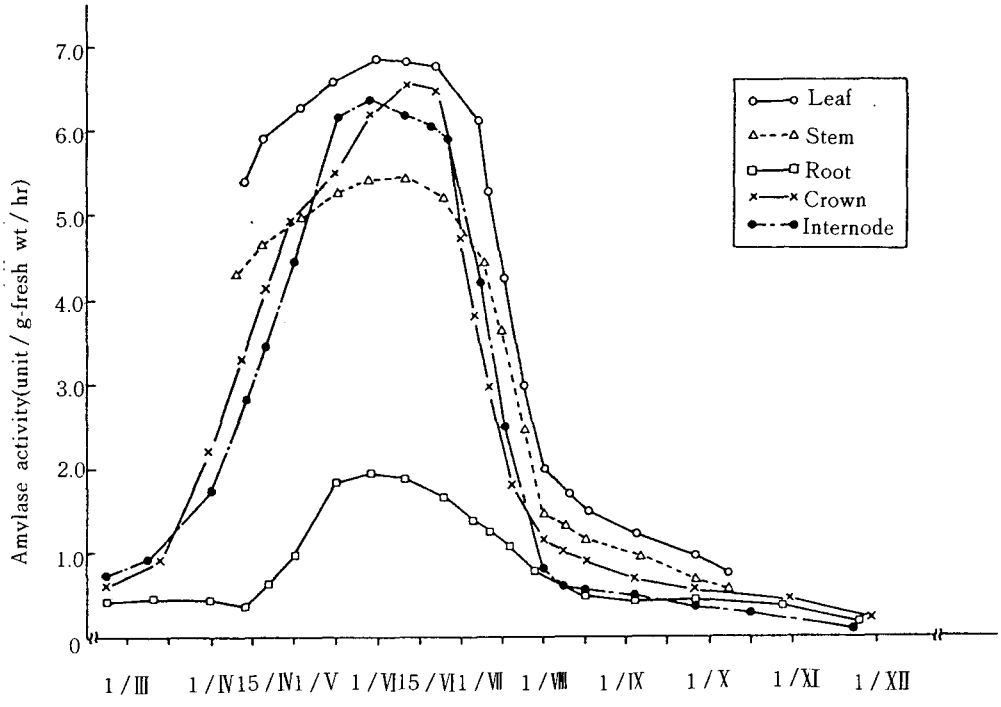

Fig. 4. Seasonal changes in Amylase activity of each organ in *Zoysia Japonica*.

and reached a mzximal value in the 7th day. Its value reached about 80% of the highest value of seasonal pattern in the leaf during a year. Compared it with the value of the control group, which was not cut, its value increased over 2.6 times. After reaching the maximal value, NR activity decreased a relatively constant value thereafter. The level of activity after the 8th day was approximated to the value of the control group.

There was not an increase during 2 — days in a stem after cutting the leaves (Fig. 6). Thereafter NR activity increased gradually and reached a maximum in the 7th day. After the 8th day the NR activity decreased to near the value of the control group. The change pattern in NR activity in the stem tissue was similar to that of leaf tissue. In the under — ground tissues, such as crown (Fig. 7b) and internode of a rhizome (Fig. 7a), there were not significant differences between the values before and after cutting the leaves. Although a root tissue had low NR activity during a year, NR had an increase in the level of activity after cutting as shown in Figure 7c. This result proves indirectly that the increase of NR activity is caused by the increasing of nitrate absorption in the root after cutting of the leaves (Turgeon et al., 1979).

The change of NR activity seems to be accompanied by the change of Crude Protein (CP) after cutting of the leaves. CP levels in cut grass were higher than in uncut *Z. japonica*. In the leaf tissue, CP content increased during the 7 days after cutting, thereafter decreased slowly (Fig. 13). Although not shown here,

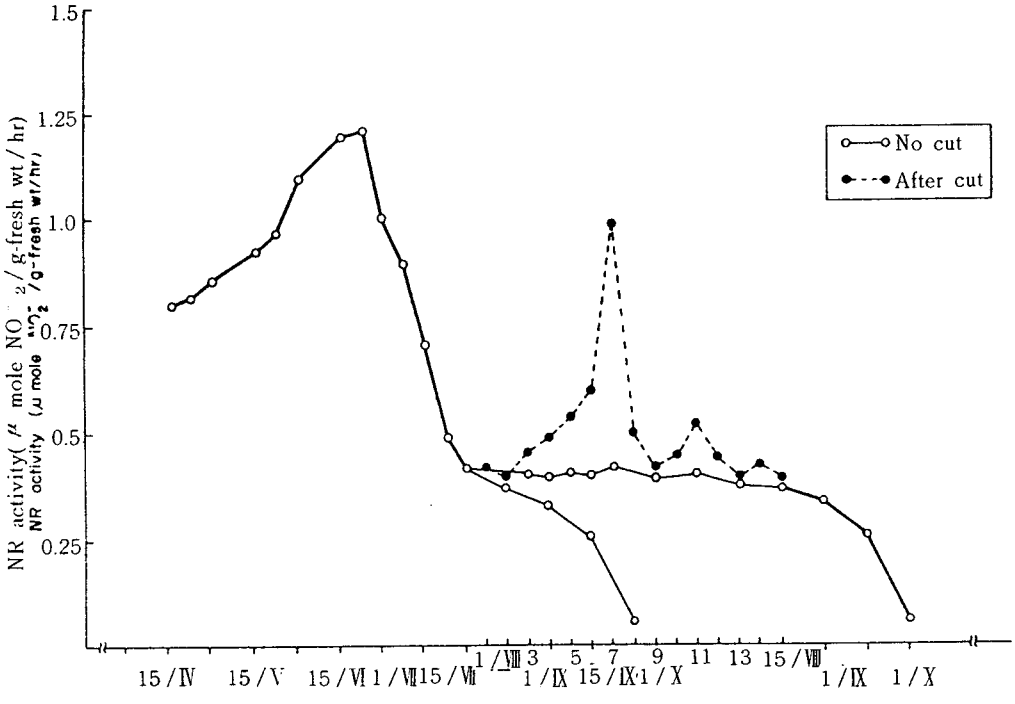

Fig. 5. Seasonal change in NR activity in the leaf and its change after cutting the leaf.

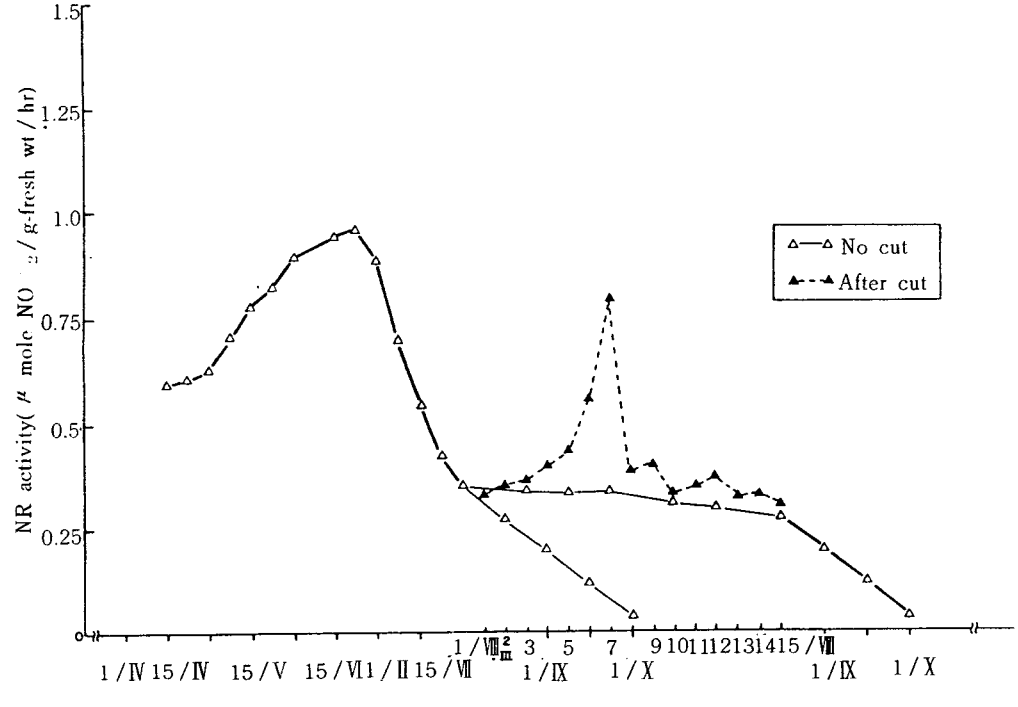

Fig. 6. Seasonal change in NR activity in the stem and its change after cutting the leaf.

when we had a different cutting height such as 1 and 3 cm the CP content was higher at the closer cutting than at the 6 cm cutting. This coincided with the study of Turgeon et al (1979). In the leaf and stem tissues, the content of CP was higher than in other parts. The increased CP content may be contributed to the increase in proportion of cell content during the early stages of regrowth after cutting (Wilman and Wright, 1978a). Since NR is a key enzyme in the first step of nitrate reduction, the change of CP content may be related to the change of NR activity. This experiment indirectly proved that mowing apparently induces physiological changes that account for the higher levels of CP (Turgeon et al., 1979) and nitrate − N in turfgrass (Wilman and Wright, 1978b).

B) Changes in Amylase activity in response to cutting

The remarkable increase of AA was observed during the regrowth after cutting of the leaves. An increase of AA in wounded tissue has shown to have a relationship with stimulation of starch degradation (Kahl, 1974). Amylase activity Jin the leaf tissue increased slowly during the first 5 days. Thereafter increased markedly for 2 days, and then reached 92% of the maximum activity in the seasonal pattern. The maximum value was 3.3 times higher than the control group (Fig. 8). This is considered as a direct response of tissue which was cut. On the 8th day, the activity of amylase decreased drastically and nearly reached the value of the control group. The change pattern in amylase activity in the stem tissue was similar to that of leaf tissue (Fig. 9).

Also, in other storage organs, such as crown (Fig. 10) and internode of the rhizome (Fig. 11), AA increased markedly until the 7th day following the lag phase during the 2 days after cutting of the leaves. It may be related to the breakdown of the abundant starch in the crown and internode (Kahl, 1973).

The root tissue, especially, had a high increase of AA after cutting (Fig. 12). Its maximal value was higher than the maximum in the seasonal change. These results suggest that amylase in the nonphoto − synthetic storage tissues such as crown, internode of the rhizome, and root which were not cut directly was also activated for the suppling of storage nutrients to the cut tissue.

Soluble carbohydrate is of interest as a source of readily available energy within the plants. Kahl (1974) shown that starch is actively degraded to produce soluble sugars at a high rate in response to mechanical cutting. Although photosynthesis complicates the change in the rate of production of soluble sugar in response to cutting (King et al., 1979), stimulation of starch degradation in wounded tissues can be partly ascribed to an increase of amylase activity. The change in the content of the Total Soluble Carbohydrate (TSC) in the leaf after cutting is shown in Fig. 14. Immediately after cutting on day 0, TSC content fell markedly and then continued decreasing for 5 days. The fall of TSC after cutting means that stored carbohydrate is used as a source of energy for the early regrowth (Ehara et al., 1966). From the 6th day after cutting, TSC content in the leaf tissue increased markedly until the 9th day. It changed little thereafter. It can be considered that the increase fo TSC content was caused by the increase of amylase activity and by the photosynthetic products in regrowing leaves.

According to the cutting of the leaves, Dry Matter (DM) yields fell to a very low level

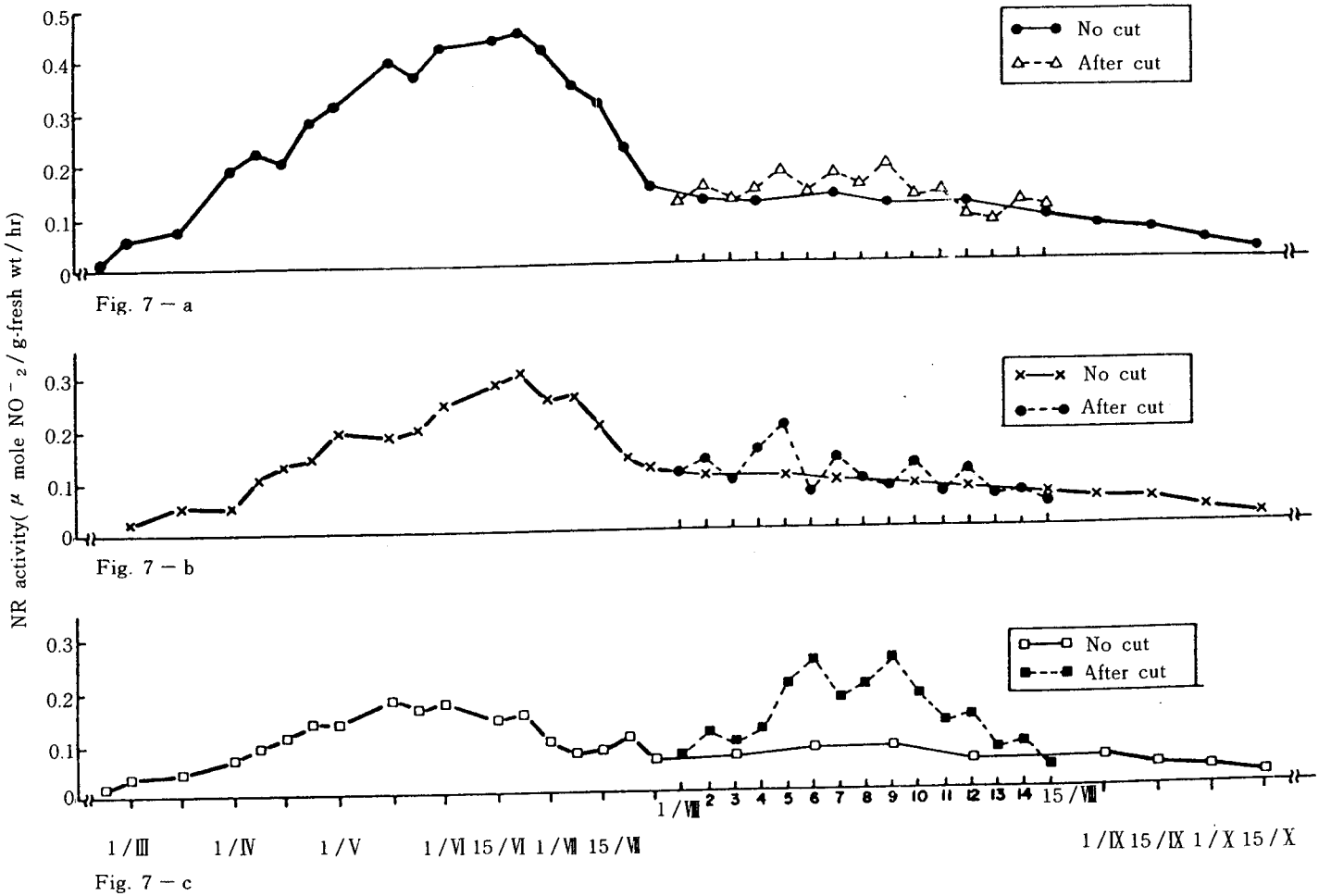

Fig. 7. Seasonal changes in NR activity and their change after cutting the leaf : (a) in the internode of a rhizome. (b) in the crown. (c) in the root.

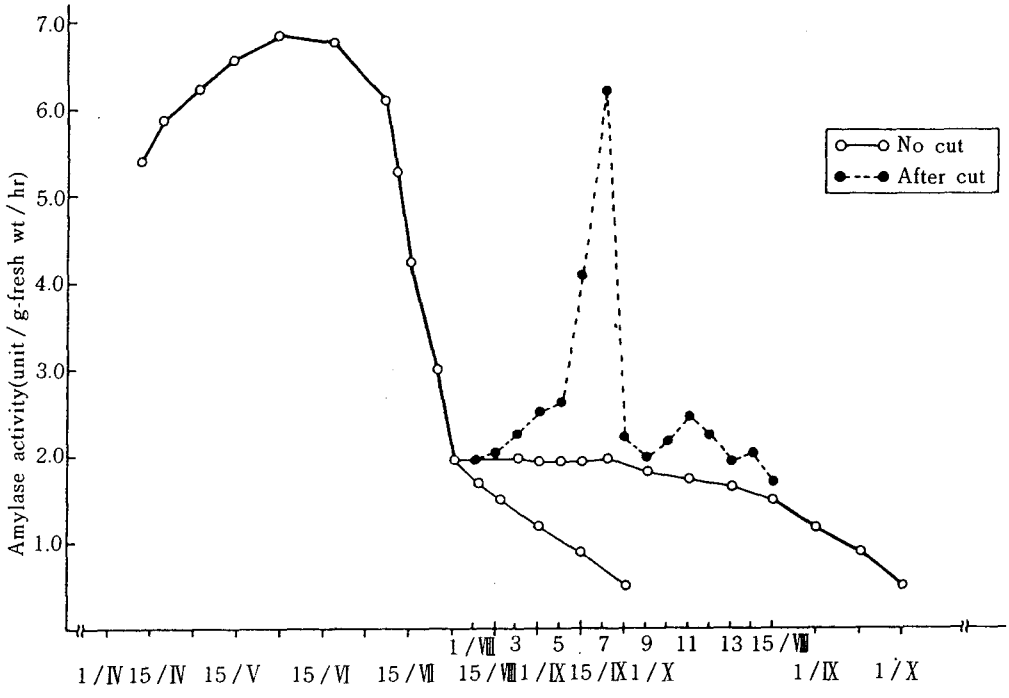

Fig. 8. Seasonal change in Amylase activity in the leaf and its change after cutting the leaf.

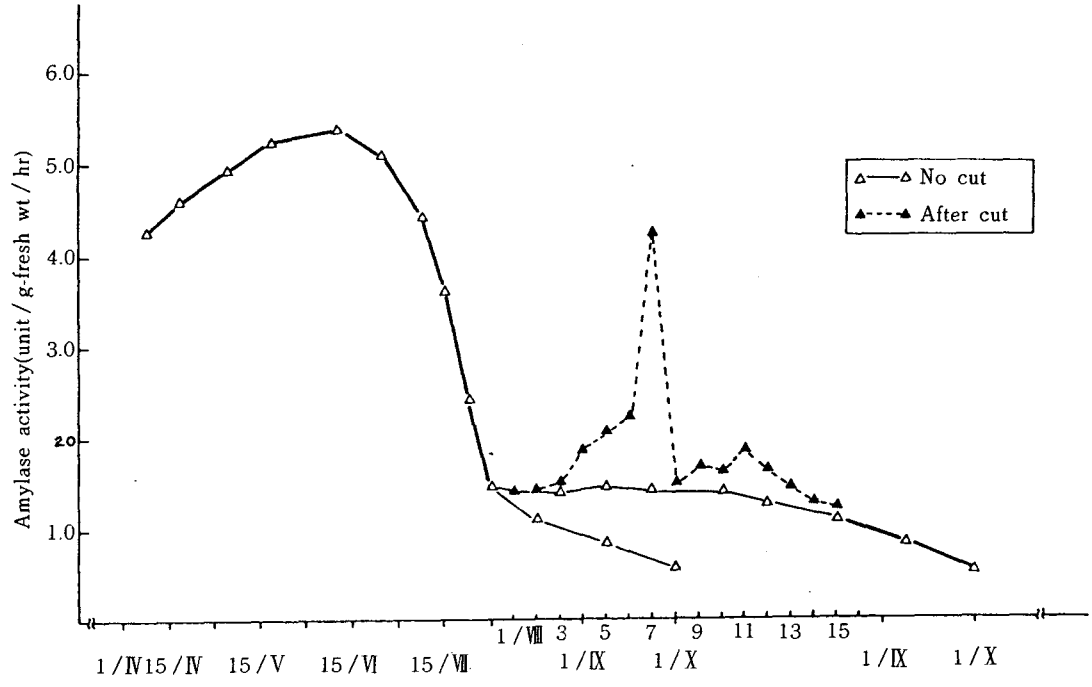

Fig. 9. Seasonal change in Amylase activity in the stem and its change after cutting the leaf.

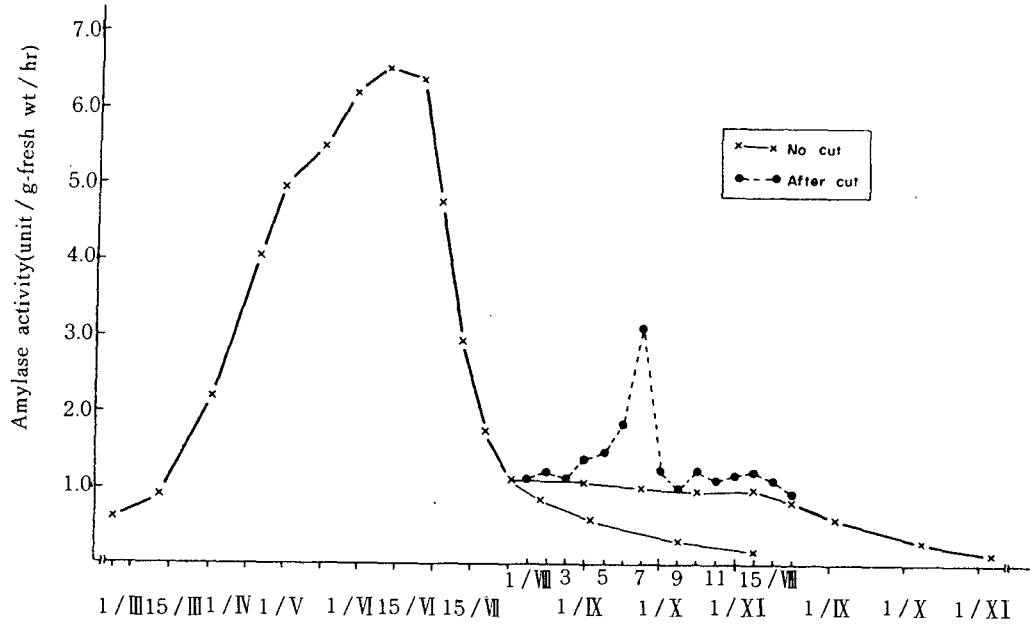

Fig. 10. Seasonal change in Amylase activity in the crown and its change after cutting the leaf.

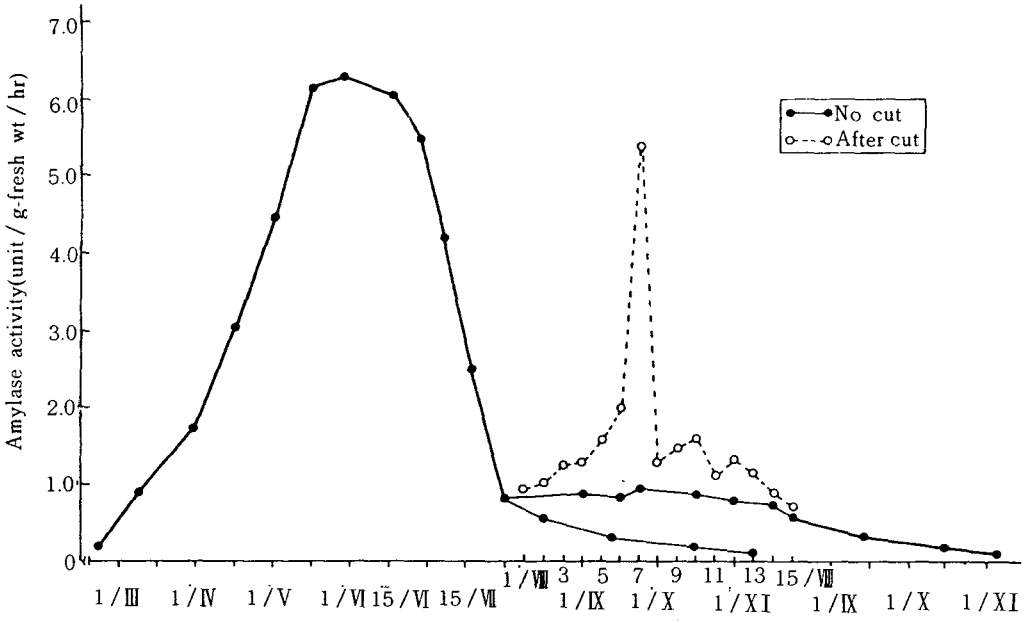

Fig. 11. Seasonal change in Amylase activity in the internode of a rhizome and its change after cutting the leaf.

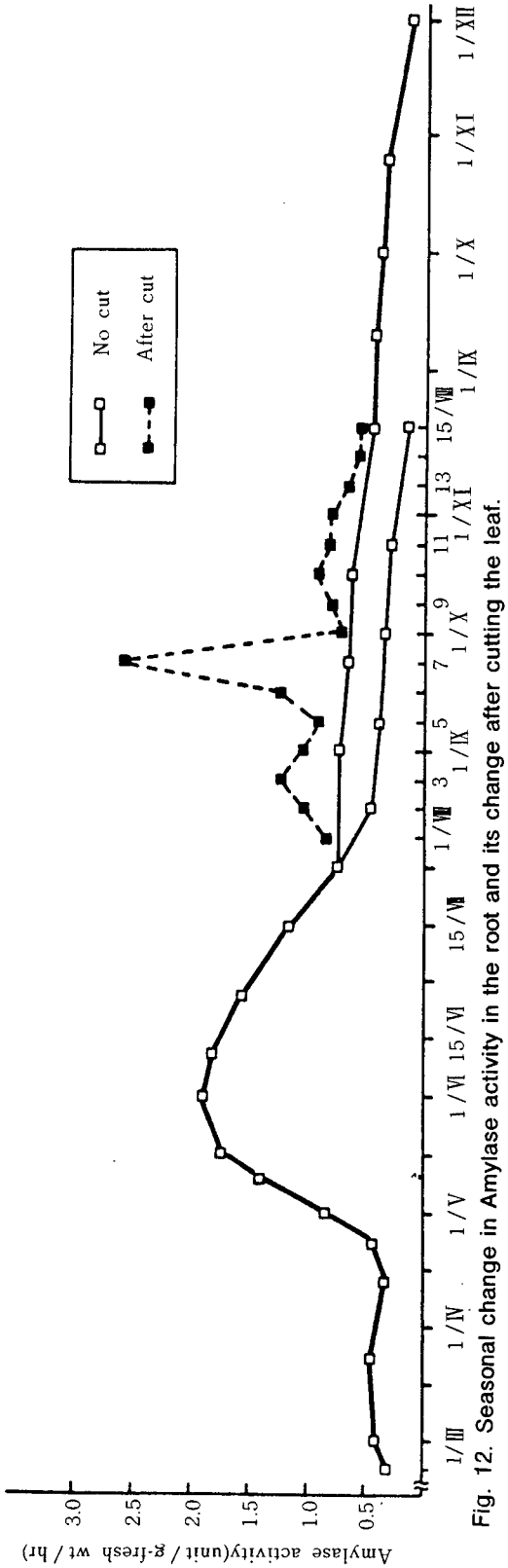

Fig. 12. Seasonal change in Amylase activity in the root and its change after cutting the leaf.

and decreased during the first 4 days (Fig. 15) . This means that much material was used for the regrowth of wounded tissue during the first few days after cutting. DM yield had little change thereafter. The recovery of DM yield may be caused by not only the increase of photosynthetic products according to the re-growth of assimilatory organ (Woledge, 1977) , but also material production by the activation of NR and amylase.

In terms of lawn management, these results suggest that it is not necessary to treat lawn grass with fertilizer for at least 7 days after cutting.

The changes after cutting in this paper may be regarded as a kind of wound effect. It has shown that carbohydrate degradation (Uritani and Kato, 1973) , the activity of the pentose phosphate pathway (Kahl, 1974) , activity of the pentose phosphate pathway (Kahl, 1974) , activation of mitochondrial activity (Greksaket al., 1972) , increase in adenine nucleotides (Takamura and Uritani, 1973) , and synthesis of mRNA and rRNA (Nawa et al., 1970) were stimulated in response to mechanical wounding. When sweet potato root tissue was wounded, acid invertase, which was almost undetectable in fresh tissue, was synthesized after several hours' lag. Its activity reached a maximum at about 18hr (Matsushita and Uritani, 1974) . This is a similar pattern with the experiment for NR and amylase except for the time course change.

The respiratory increase was known to be accompanied by increased carbohydrate catabolism (Crick and Hackett, 1963) . The plant cells may actively decompose carbohydrate to supply chemical energy in the form of ATP. Therefore, amylase contributes to decomposition of starch.

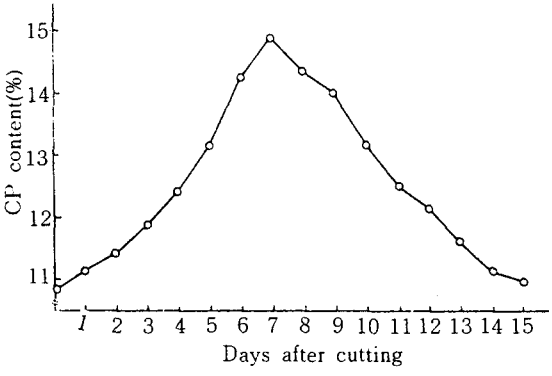

Fig. 13. The change in crude protein content in the leaf with time.

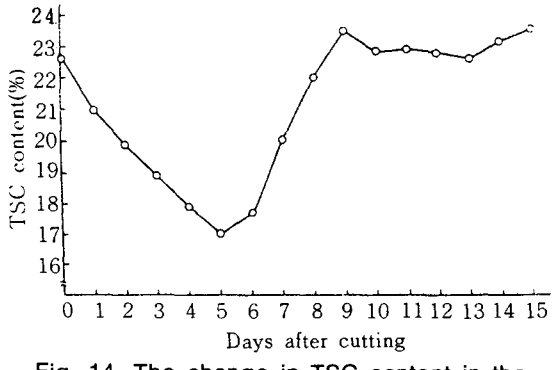

Fig. 14. The change in TSC content in the leaf with time.

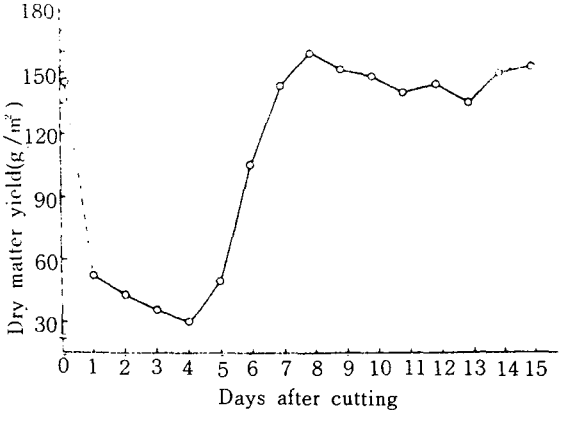

Fig. 15. The change in dry matter yield in the leaf with time.

Uritani and Asahi (1980) suggested that starch degradation may be accelerated by the alteration or loss of the amyloplast membrane surrounding the starch granules, so that the granules become more accessible to the starch — decomposing enzymes.

In respect to the usage of storaged nutrients such as starch, and the synthesis of new nutrients for the regrwoth after cutting, it may be said that NR and amylase decisively contributed to the metabolic process for the regrowth. When growth is considered in terms of material production, the control of activity in NR and amylase after cutting seems to have a relation with DM yield. For the compensation of lost material by partial removal of fully grown leaf tissues, the activity of NR and amylalse increased in most organs of *Z. japonica*. On the 8th day, when DM yield of leaf tissues recovered to the value before cutting, the high activities of NR and amylase were adjusted back to the normal level. Such a change of enzyme activity was also observed in phleinase and invertase activities of orchard grass (Yamamoto and Mino, 1982). Therefore, these results suggest that fully grown leaf tissues containing enough materialact as a repressor at an individual level against the activation of NR and amylase.

Although there was specific regulatory mechanism in higher plants (Filner et al., 1969 ; Marcus, 1971 ; Davis, 1979) the increase of enzyme activity with cutting may be based on one or more of the following mechanisms : (a) the enzyme is newly synthesized *denovo*, (b) the enzyme precursor is activated to the enzyme, or (c) the inactivating system of the enzyme disappears.

Protein inhibitors of invertase were found in potato (Pressley, 1966;Matsushita dnd Uritani,

1977) and in maize (Jaynes and Nelson, 1971)

But, the mechanisms of the increase in the activities of nitrate reductase and amylase in response to cutting, especially in field conditions, should be more investigated.

Ⅳ. SUMMARY

Nitrate Reductase Activity (NRA) and Amylase Activity (AA) in the leaf, stem, root, crown, and internode of a rhizome in Korean lawn grass (*Zoysia japonica* Steud) which was grown in field conditions were measured. The activities in each organ increased from the middle of April, and reached a maximum value at the anthesis period in the middle of June. Thereafter, enzyme activities decreased markedly and had a minimum level during the winter. These results suggest that NRA and AA in each organ in *Z. japonica* have a seasonal change.

On 31st July 1986, when NR and amylase have low activity, the above − ground parts of the *Z. japonica* grass were cut with hand shears at the height of 6 cm. The AA in all of the organs and the NRA in the leaf and stem increased after 2 day lag phase, and rapidly reached maximum on the 7th day after cutting. Their values increased about 3 times higher than the control group and reached about 90% of the maximum level in the seasonal change. Crude protein content in cut leaves increased until the 7th day after cutting, but total soluble carbohydrate content and dry matter yield decreased during the 4 to 5 days after cutting. On the 7th day after cutting, DM yield reached the level before cutting and then maintained a relatively constant value. NRA and AA in each organ decreased drastically to the normal level on the 8th day.

These results suggest that completely grown lawn grass acts as a repressor against the activation of nitrate reductase and amylase, and the enzyme activities at the molecular level are increased to recover the lost material when exogeneous wounding factors such as cutting are applied to the individual level.

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