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Effect of Cutting Height on C and N Reserves and Consequent Regrowth in Frequently Defoliated Turf-Type Perennial Ryegrass(*Lolium perenne* L.)

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잔디형 페레니얼 라이그라스에서 잦은 예취조건하의 예취높이가 저장 탄수화물과 단백질 함량 및 재생에 미치는 영향

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Abstract

Carbohydrate and soluble protein reserves and regrowth characteristics in response to cutting height were investigated over four regrowth cycles of turf-type perennial ryegrass(*Lolium perenne* L. cv. prelude II). When the plants were at the full-vegetative stage (twelve weeks-old), three sequential defoliations at 3, 6 and 9 cm above the root base were imposed at 2-week intervals. Shoot dry weight in all three treatments continuously decreased with progressing regrowth cycle and the decreasing rate was higher as cutting height was lowered. TNC (total non-structural carbohydrate) in stubble at the end of the fourth regrowth cycle in 3, 6 and 9 cm cutting height decreased by 98%, 82% and 27%, respectively, comparing the initial content. TNC in roots also largely decreased with similar pattern in response to cutting height, whereas the absolute amount was much less compared to stubble. Soluble protein in stubble in 3, 6 and 9 cm cutting height decreased by 98%, 82% and 57%, respectively, at the end of fourth regrowth. A significant correlations between TNC (r=0.906) or protein (r=0.879) at the fourth defoliation and dry weight of regrowing shoots at the end of fourth regrowth were observed. These results indicated that cutting height closely influences the levels of organic reserves available for new growth, and that the levels of reserves might provide a useful tool as a determinant for regrowth dynamics.

(**Key words** : Turf-type *Lolium perenne* L., Cutting height, Carbohydrate, Soluble protein, Regrowth)

I. INTRUDUCTION

The importance of organic reserves in the regeneration of new foliage has been generally established. The pool size of organic reserves of turf grasses, which are frequently defoliated during vegetative growth stage, is affected by mowing severity (cutting height, interval). They could serve not only as the determinants of biomass accumulation during regrowth but also as the predictive

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parameters of persistency to cutting stress.

The level of organic reserves in individual plants is determined by the concentration of organic compounds and the biomass of residual plant tissues (Volenec 1986; Morvan-Bertrand et al., 1999). The main reserve organ and principal organic compound are species-specific. Stubble or stem bases in forage grasses (Okajima and Smith 1964; Ourry et al., 1988) and tap roots in lucerne (Kim et al., 1991; Ourry et al., 1994) act as the primary site of storage of organic reserves. It has been reported that soluble proteins and fructans are the main nitrogen (N) and carbon (C) reserves in perennial ryegrass (Ourry et al., 1988; Prudhomme et al., 1992).

A critical role of carbohydrate and N reserves as substrates for regrowth was advocated long ago (Graber et al., 1927; Sullivan and Sprague 1943). Indeed, extensive mobilization of non-structural carbohydrate and nitrogenous compounds (Volenec 1986; Kim et al., 1991; Prudhomme et al., 1992; Ourry et al., 1994) occurs in the residual parts of defoliated plants. The C reserves mobilized support dry matter accumulation of new tissue (De Visser et al., 1997) and respiration and persistence of roots (Hodgkinson 1969). Adaptation to defoliation in many grass species also involves a capacity for mobilization of N compounds stored in roots or stubble, allowing N to be supplied to the growing zones despite the reduced N uptake by roots that usually occurs as a response to defoliation (Ourry et al., 1988). Herdershot and Volenec (1993) reported the extensive depletion of specific amino acids and cetain buffer-soluble proteins from tap roots of *Medicago sativa*. These results suggest that mobilization of specific N pools from vegetative tissues to regrowing foliage is necessary for defoliation tolerance in perennial herbaceous plants (Volenec et al., 1996; Avice et al., 1996).

Therefore, a knowledge of the accumulation and use of organic reserves by grass species is considered to be fundamental to an understanding of management practices. It was anticipated that the levels of reserves might provide a useful tool in developing management strategies for turf grasses, if a define relationship between reserve levels and regrowth could be established.

The current work was initiated to establish 1) the changes in pool size of carbohydrates and soluble proteins in stubble and roots in response to sequential defoliation with different cutting heights; 2) the relationship between reserves pool size and regrowth dynamics.

Ⅱ. MATERIALS AND METHODS

1. Plant growth and experimental procedure

Perennial ryegrass (*Lolium perenne* L.) seeds were germinated in a sand bench. When three-leaf stage was developed, 5 seedlings per 3 L pot were transplanted and grown hydroponically on the nutrient solution described by Kim et al. (1991). The nutrient solution was continuously aerated and renewed every 6 days. Plants were grown in a greenhouse. The natural light was supplemented with 400 W metal halide lamps (250 μM m^{-2} s^{-1} at the canopy height) for 16 h per day. The thermoperiod was 25℃ (day) and 20℃ (night). All plants were grown through two growth cycles before treatment was imposed to allow the plants to develop a satisfactory stubble and root size. When the plants developed at full-vegetative stage (12 weeks-old), the treatment of cutting height (3 cm, 6 cm and 9 cm above the root base) was imposed, and then regrowth allowed for 2 weeks. Thereafter, four sequential defoliations were made by the same cutting height for each treatment at 2-week intervals. Net regrowth height, tiller number and dry matter of regrowing shoot were measured, respectively, throughout 4 regrowth cycles. To estimate the initial and final pool size of organic reserves, plants were harvested with separating roots, stubble and regrowing shoots at the 1st defoliation and at the end of 4th regrowth. Samples were freeze dried, ground to a fine powder and kept under vacuum with $CaCl_2$ for further analyses.

2. Chemical analysis

Soluble sugars were extracted with 92% ethanol. Tubes were shaken for 10 min at room temperature, centrifuged at 14,000 × g for 10 min at 4℃. The supernatant was retained in 10 ml volumetric flask. The ethanol extraction was repeated more than twice, and combined supernatants. The soluble sugars contents from the supernatants were determined with Anthrone reagent (Van Handel, 1968) using glucose as a standard. The residue was dried at 80℃ to remove ethanol. Deionized water was added, and heated to gelatinize the starch. The pH of the solution was adjusted to 5.1 by adding 0.2N Na-acetate buffer. Starch was digested by adding amyloglucosidase (Sigma product A3514) and α -amylase (Sigma product A0273) in the acetate buffer to each sample. Tubes were incubated at 55℃ for 24 h with occasional shaking. Tubes were centrifuged as described and glucose in the supernatant was determined using glucose oxidase (Glucose Trinder, Sigma product 315-100). Starch concentrations were estimated as 0.9 × glucose concentration. Fructans present in the starch extracts was hydrolyzed with 0.1 N H_2SO_4 and fructose released quantified using resorcinol (Davis & Gander 1967). Glucose liberated from the fructan was determined as descrived, and fructan concentration was calculated as the sum of fructan glucose and fructose × 0.9.

Proteins were extracted by suspending 25 mg of freeze dried sample with an equal mass of insoluble polyvinylploypyrolidon (Sigma product P6755) in 100 mM $NaPO_4$ buffer (pH 6.8). Tubes were vortexed for 1 min four times, centrifuged at 14,000 × g for 10 min at 4℃. The supernatant was retained. The pellet was re-extracted and the combined supernatants were centrifuged at 10,000 × g for 10 min at 4℃. Soluble protein in supernatant from the final centrifugation was quantified using dye-binding (Bradford, 1976).

Ⅲ. RESULTS

1. Growth characteristics and dry matter accumualtion

Changes in net regrowth height and tiller number throughout 4 regrowth cycles after defoliation at three cutting heights at 2-week intervals are shown in Fig. 1. Net regrowth height (tiller height at the end of regrowth period cutting height) for the first regrowth cycle was not significantly different between cutting height treatment (Fig. 1A).(Insert Fig. 1 here) From the second regrowth cycle, net regrowth height significantly responded to cutting height treatment. The net regrowth height in 3 cm treatment decreased from 20.7 cm (the first regrowth) to 2.5 cm (the fourth regrowth), and decreased also from 20.1 cm to 13.8 cm in 6 cm cutting height. However, the net regrowth height in 9 cm cutting height tended to increase. For the fourth regrowth cycle, the net regrowth heights in 6 and 9 cm cutting height were 5.5 and 8.6 fold higher compared to that of 3 cm cutting height.

Tiller number was also affected by cutting height throughout 4 regrowth cycles (Fig. 1B). At the end of the first regrowth, tiller number in 6 cm cutting height (36.2/plant) was significantly higher than two other treatments. Tiller number in 3 and 6 cm cutting height largely decreased until the third regrowth, and then remained at same level. The decreasing rate was much higher in 3 cm cutting height. Tiller number in 9 cm cutting height was not significantly different among 4 regrowth cycles. After the fourth regrowth cycle, tiller number in 6 and 9 cm cutting height were 4.5 and 8.3 times higher compared to that of 3 cm cutting height.(Insert Fig.2 here)

Shoot dry weights of 4 regrowth cycles in response to cutting height are presented in Fig. 2. After the first regrowth, the dry weight of regrowing shoot in 3, 6 and 9 cm cutting height was 1.9, 2.5 and 3.5 g/plant, respectively. Shoot dry weight in all

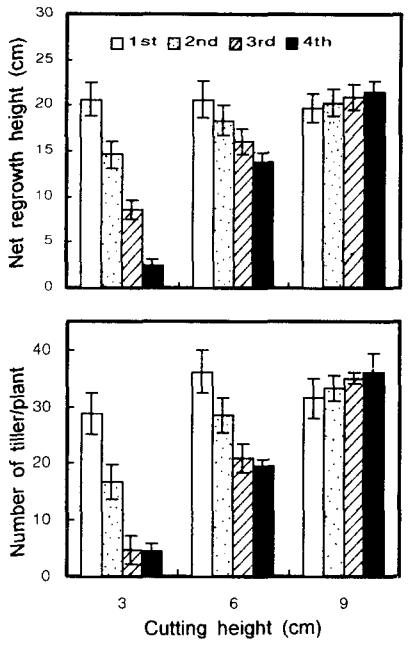

Fig. 1. Changes in net regrowth height and tiller number affected by cutting height during 4 regrowth cycles. Plants were defoliated at 2-week intervals. Each value is the mean± s.e. for n=5.

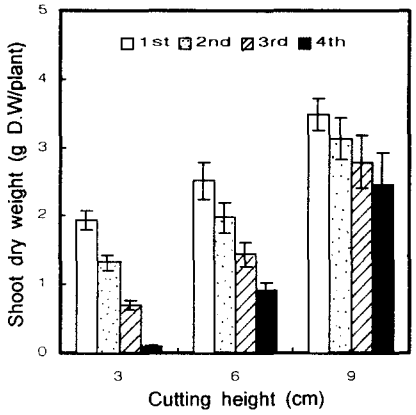

Fig. 2. Changes in shoot dry weight affected by cutting height under 2 weeks of defoliation interval during 4 regrowth cycles. Each value is the mean±s.e. for n=5.

three treatments continuously decreased with progressing regrowth cycle. The decreasing rate in shoot dry weight increased as cutting height was lowered in response to sequential defoliation. Shoot dry weight of the fourth regrowth decreased by 95.3, 61.9 and 29.6% compared to that of the first regrowth. The final shoot dry weight (at the end of fourth regrowth) in 3, 6 and 9 cm cutting height was 0.1, 0.9 and 2.5 g/plant, respectively.

2. Organic reserves in remaining tissues

Initial content of non-structural carbohydrate (TNC, soluble sugars + starch + fructan) at the first cutting in stubble and roots was 65.4 and 14.5 mg/plant, respectively (Fig. 3). TNC in stubble at the end of the fourth regrowth cycle in 3, 6 and 9 cm cutting height decreased by 98%, 82% and 27%, respectively, comparing the initial content (Fig. 3A). All carbohydrate compounds in this organ was nearly depleted at 3 cm cutting height, and they decreased also largely (86.7% soluble sugars, 76.4% starch and 81.4% fructan) at 6 cm cutting height after the fourth regrowth cycle. At 9 cm treatment, the decrease of soluble sugars (48.7% of the initial) was much higher than that of starch (non-significant difference) or fructan (20% decrease). Total content of TNC in stubble in 3, 6 and 9 cm cutting height was 1.2, 11.5 and 47.9 mg/plant, respectively, at the end of fourth regrowth cycle. (Insert Fig. 3 here)

TNC in roots at the end of the fourth regrowth cycle also significantly decreased in 3 all cutting height treatment when compared to the initial content. However the absolute amount decreased during 4 regrowth cycles in roots was much less than stubble (Fig. 3B). At the end of the fourth regrowth cycle, a large portion of soluble sugars (more than 80% of the initial) was depleted at 3 and 6 cm cutting height, while only 20% decreased in 9 cm treatment. Starch was significantly decreased only in 3 cm cutting height. Fructan largely decreased (82.0, 71.6 and 46.1%, respectively, in 3, 6 and 9 cm treatment). Total content of TNC in roots in 3, 6 and 9 cm cutting height was 3.2, 6.0 and 12.9 mg/plant, respectively, at the end of fourth

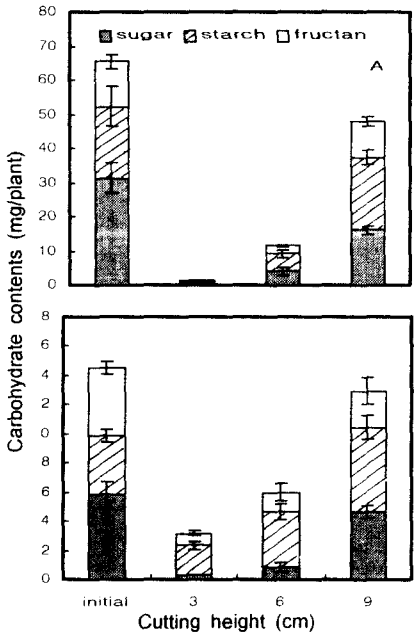

Fig. 3. Changes in non-structural carbohydrate in sugar(■), starch(▨) and fructan(□) in remaining stubble (A) and roots (B) affected by cutting height after the fourth defoliation. Each value is the mean±s.e. for n=5.

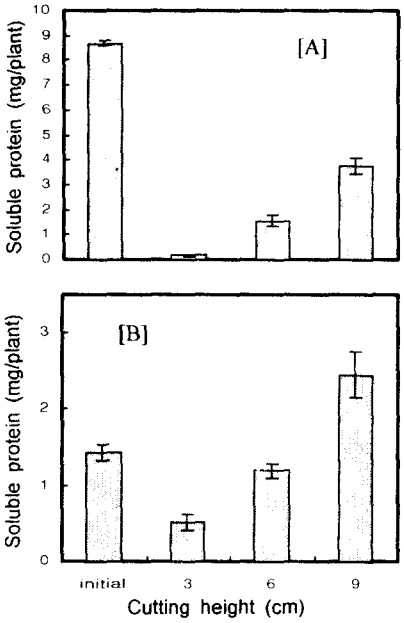

Fig. 4. Changes in soluble protein in remaining stubble (A) and roots (B) affected by cutting height after the fourth defoliation. Each value is the mean±s.e. for n=5.

regrowth cycle.

Initial content of soluble protein in stubble and roots was 8.7 and 1.4 mg/plant, respectively (Fig. 4). Soluble protein in stubble in 3, 6 and 9 cm cutting height decreased by 98%, 82% and 57%, respectively, at the end of fourth regrowth. (Insert Fig. 4 here) The depletion of soluble proteins in roots was much less compared to that in stubble. Soluble proteins decreased by 63 and 16% in 3 and 6 cm cutting height, while significantly increased in 9cm treatment.

IV. DISCUSSION

It is generally accepted that frequent and severe defoliations lead to poor recovery and even death in various grass species, regardless of the most favorable climate and soil environment (Davies, 1965; Richards, 1993). This study showed that regrowth was closely affected by the cutting height when plants was frequently defoliated at 2-week interval. From the second regrowth cycle, net regrowth height and tiller number in 3 cm and 6 cm cutting height continuously decreased with progressing the regrowth cycle, while those in 9 cm treatment was not significantly changed (Fig. 1A and 1B). Shoot dry weights of 4 regrowth cycles much clearly showed the response to cutting height. Shoot dry weight in all three treatments gradually decreased and the decreasing rate was higher as cutting height was lowered in response to sequential defoliation (Fig. 2). This results indicate that regrowth was closely related to the morphological status in remaining stubble (the proportion of active photosynthetic tissues and meristem). Smith and Dale (1974) and Booyen et al. (1975) reported that energy for regrowth of forage grasses was provided by current photosynthate from residual leaf area, and/or

nonstructural carbohydrates stored in roots or stubble. These results are in accordance with the finding of other researchers (Volenec 1986; Busso et al. 1990; Morvan-Bertrand et al. 1999), who showed that when active meristems are present, the presence of high protein and carbohydrate availability in stubble could increase the rate of refoliation.

The results obtained indicate that remaining stubble is a primary storage site of organic reserves, presenting an ample size of C and N reserves in this organ. The content of TNC and soluble protein in this organ at the first defoliation was 4.5-fold and 6.1-fold higher than roots (Fig. 3 and 4). Ourry et al. (1988) and Morvan-Bertrand et al. (1999) observed that a large part of organic reseves accumulated in stubble of perennial ryegrass. In other forage grasses, high concentration of total nonstructural carbohydrate have been reported in leaf sheaths of timothy and switchgrass (Smith and Greenfield 1979) and tall fescue (Volenec 1986). Much higher amounts were depleted in stubble for all biochemical compounds examined during four sequential regrowth cycles, regardless of cutting height treatment (Fig. 3 and 4). The depletion of TNC and soluble proteins in both stubble and roots occurred as the cutting height was lowed (Fig. 3 and 4). All compounds of non-structural carbohydrate and soluble protein (except protein in roots at 9 cm cutting treatment) did not completely replenished after the fourth regrowth cycles. This indicate that the defoliation at 2-week intervals does not give enough time to reconstitute the organic reserves in stubble and roots. Several isotope studies (Ourry et al. 1988; Kim et al. 1991, 1993) showed the re-accumulation of organic reserves mainly occurred after 6 days of regrowth in grasses (10 days in legumes) when photosynthesis, N uptake and the assimilation of exogenous C and N sources begin to actively operate. De Visser et al. (1997) reported that the use of C reserves occurred, with half of total non-structural carbohydrate being remobilized, to support the initiation of regrowth during the first 2 days, and that development of new leaves after 6 days of regrowth favored the reconstitution of C reserves.(Insert Fig. 5)

From the results of regression analysis (Fig. 5), it could be assumed that TNC and protein at the defoliation time are more important factors determining net regrowth. Defoliation caused instantaneous impair of photosynthesis, reduction of

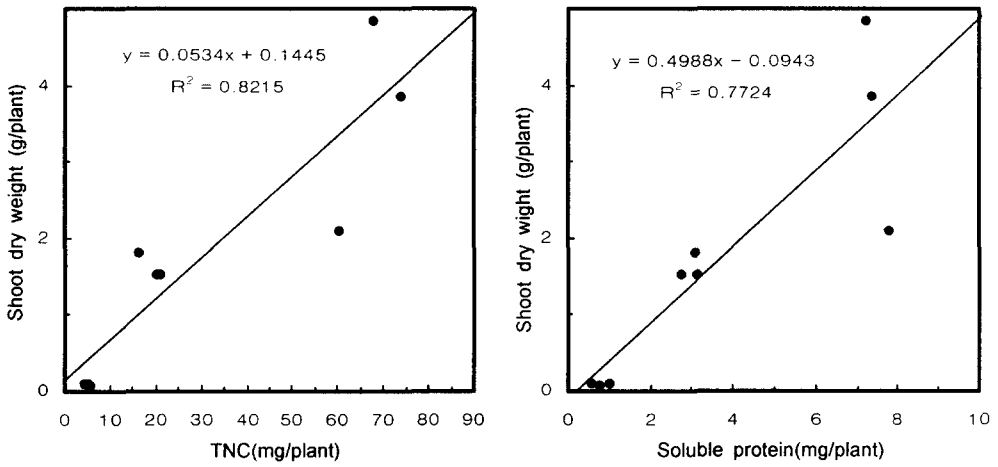

Fig. 5. Correlations between amounts of TNC and regrowth yield(A), Soluble protein and regrowth yield(B) in the regrowth plant after frequent defoliation. Each value is the mean±s.e. for n=3.(P>0.01)

C translocation to the root, and mineral N uptake. Plants depend on endogenous organic reserves to initiate new foliage when exogenous nutrient utilization is ultimately limited (Richards 1993, Volence al. 1996). In perennial ryegrass treated with different C and N supply levels to give different levels of organic reserves, Morvan-Bertrand et al. (1999) reported that initial levels of carbon, water soluble carbohydrates and fructans in elongating leaves significantly related to production of leaf dry matter per tiller during the early 6 days of regrowth, but those of nitrogen and soluble proteins in leaf sheath highly related to leaf dry matter at the 28 day of regrowth. These results suggest that regrowth of perennial ryegrass involves 2 periods with different source organs of organic reserves.

A general conclusion can be drawn that the severity of defoliation modified by the different levels of cutting height influences the regrowth dynamics, which would be affected by the levels of organic reserves available for new growth. The restriction of regrowth in 3 cm cutting height in this study is resulted from an excessive cutting off of active meristems and the repetition of excessive depletion and insufficient reconstitution of organic reserves during sequential regrowth cycle, and consequently from the limitation of organic reserves in these plant tissues.

V. 적 요

잔디형 페리니얼 라이그라스에서 2주 간격의 잦은 예취하의 예취높이가 저장 유기물의 함량에 미치는 영향 및 그에 따른 재생활력을 검토하기 위해 수경재배 조건에서 최고 영양성장기에 도달하였을 때(약 12주령), 뿌리기부에서 3, 6 및 9 cm 높이로 4회 연속 예취 하였다. 3차 재생동안 예취 후 남아있는 기관의 저장 탄수화물과 가용성 단백질 함량과 재생특성이 조사되었다. Shoot의 재생 건물중은 3가지 예취높이 처리구 공히 재생주기동안 감소되었고, 예취높이가 낮을수록 그리고 예취빈도가 높을수록 그 감소율은 높았다. 1차 예취전 (초기수준)의 그루터기에서 비구조적 탄수화물 총량 (Total non-structural carbohydrate, TNC)은 뿌리보다 약 4.5배 높게 축적되어 있었다. 4차 재생후 그루터기의 TNC 함량은 3, 6 및 9 cm 예취구에서 초기 수준에 비해 각각 98%, 82%, 27%의 감소하였고, 뿌리에서의 TNC 또한 각 예취높이구에서 비슷한 비율로 크게 감소하였다. 4차 재생후 그루터기의 가용성 단백질의 함량은 3, 6 및 9 cm 예취구에서 각가 98%, 82%, 57%의 감소하였다. 4차 예취시 뿌리와 그루터기내의 TNC(r=0.906)와 protein(r=0.879) 함량과 4차 재생후 잎의 건물중 간에는 고도의 유의적 상관성이 인정되었다. 이러한 결과는 예취높이가 재생을 위한 유기저장물의 이용수준에 영향을 미치며, 저장물의 수준이 재생활력을 결정하는 주요한 생리적 요인임을 잘 보여주었다.

VI. REFERENCES

1. Avice, J.C., A. Ourry, G. Lemaire and J. Boucaud. 1996. Nirtrogen and carbon flows estimated by 15N and 13C pulse-chase labeling during regrowth of alfafa. Plant Physiol. 112:281-290.
2. Booysen, P.V. and C.J. Nelson. 1975. Leaf area and carbohydrate reserves in regrowth of tall fescue. Crop Sci. 15:262-266.
3. Bradford. M.M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248-254.
4. Busso, C.A., J.H. Richards and N.J. Chatterton. 1990. Non-structural carbohydrates and spring regrowth of two cool-season grasses: interaction of drought and clipping. J. of Range Manage. 43:336-343.
5. Davies, A. 1965. Carbohydrate levels and regrowth of perennial rye-grass. J. Agric. Sci. 65:213-221.
6. Davis, J.S and J.E. Gander. 1967. A reevaluation of the Roe procedure for determination of fructose. Anal. Biochem. 19:72-79.

7. De Visser R, H. Vianden and H. Schnyder. 1997. Kinetics and relative significance of remobilized and current C and N incorporation in leaf and root growth zones of Lolium perenne after defoliation: assessment by 13C and 15N steady-state labeling. Plant, Cell and Environ. 20:37-46.
8. Graber, L.F., N.T. Nelson, W.A. Luekel and W.B. Albert. 1927. Organic food reserves in relation to the growth of alfalfa and other perennial herbaceous plants. Wis. Agric. Exp. Stn. Res. Bull. 80.
9. Hendershot, K.L. and J.J. Volenec. 1993. Taproot nitrogen accumulation and use in overwintering alfafa(*Medicago sativa* L.). J. of Plant Physiol. 141(1):68-74.
10. Hodgkinson, K.C. 1969. The utilization of root organic compounds during the regeneration of lucerne. Aust. J. Biol. Sci. 22:1113-1123.
11. Kim T.H., A. Ourry, J. Boucaud and G. Lemaire. 1991. Changes in source-sink relationship for nitrogen during regrowth of Lucerne (*Medicago sativa* L.) following removal of shoots. Aust. J. of Plant physiol. 18: 593-602.
12. Kim T.H., J. Bigot, A. Ourry and J. Boucaud. 1993. Amino acid content in xylem sap of regrowing alfalfa (*Medicago sativa* L.): Relations with N uptake, N2 fixation and N remobilization. Plant and Soil 149:167-174.
13. Morvan-Bertrand A, J. Boucaud and M.P. Prudhomme. 1999. Influence of initial levels of carbohydrates, fructans, nitrogen, and soluble proteins on regrowth of Lolium perenne L. cv. Bravo following defoliation. J. of Exp. Bot. 50:1817-1826.
14. Okajima, H. and Dale Smith. 1964. Aailable carbohydrate fractions in the stem bases and seed of timothy, smoth bromegrass, and several other northern grasses. Crop Sci. 4:317-320.
15. Ourry A, J. Boucaud and J. Salette. 1988. Nitrogen mobilization from stubble and roots during re-growth of defoliated perennial ryegrass. J. of Exp. Bot. 39:803-809.
16. Ourry A, T.H. Kim and J. Boucaud. 1994. Nitrogen reserve mobilization during regrowth of *Medicago sativa* L. relationships between availability and regrowth yield. Plant Physiol. 105:831-837.
17. Prud'honne, M.P., B. Ganazalez, J.P. Billard and J. Boucaud. 1992. Carbohydrate content, fructan and sucrose enzyme activities in roots, stubble and leaves of ryegrass(*Lolium perenne* L.) as affected by source/sink modifidation after cutting. J. of Plant Physiol. 140:282-291.
18. Richards, J.H. 1993. Physiology of plants recovering from defoliation p. 85-94. In Proc. XVII Int. Grassl. Cong., SIR Publishing, Palmerston North, New Zealand.
19. Smith, Dale. 1974. Growth and development of timothy tillers as influenced by level of carbohydrate reserves and leaf area. Ann. Bot. 38:595-606.13.
20. Smith, D. and S.B. Greenfield. 1979. Distribution of chemical constituents among shoots parts of timothy and switchgrass at anthesis. J. of Plant Nutrit. 1:81-99.
21. Sullivan, J.T. and V.G. Sprague. 1943. Composition of the roots and stubble of perennial ryegrass following partial defoliation. Ibid. 18:656-670.14.
22. Van Handel, E. 1968. Direct microdetermination of sucrose. Anal. Biochem. 22:1341-1346.
23. Volenec J.J. 1986. Nonstructural carbohydrates in stem base components of tall fescue during regrowth. Crop Sci. 26:122-127.
24. Volenec, J.J., A. Ourry and B.C. Joern. 1996. A role for nitrogen reserves in forage regrowth and stress tolerance. Physiol. Plant. 97:185-193.