Diss. ETH No 9809

REGROWTH, PRODUCTIVITY AND SYMBIOTIC NITROGEN FIXATION OF WHITE CLOVER (*TRIFOLIUM REPENS* L.) MONOCULTURES AND WHITE CLOVER RYE-GRASS (*LOLIUM PERENNE* L.) MIXED SWARDS AT TWO CUTTING HEIGHTS.

A dissertation submitted to the
SWISS FEDERAL INSTITUTE OF TECHNOLOGY
ZURICH
for the degree of
Doctor of Technical Sciences

presented by

RUPANJANIE THAKSHALA SERESINHE
dipl. Ing. Agr. ETH Zurich (Switzerland)
born May 9, 1952
Citizen of Sri-Lanka

accepted on the recommendation of
Prof. Dr.J. Nösberger, examiner
Prof. Dr.P. Stamp, co-examiner
Dr.W. Kessler, co-examiner

Zurich,1992

CONTENTS

1. GENERAL INTRODUCTION

In natural and intensively managed grasslands in temperate zones, white clover (*Trifolium repens* L.) is an important forage legume. Lucerne (*Medicago sativa* L.) on the other hand is grown in regions with long dry summers. Besides its higher nutritive quality, white clover is the most important forage legume which can thrive in mixed swards with several grasses in frequently cut meadows and in intensively grazed pastures. For these advantages to be realized white clover grown in mixtures must yield a proportion of 25% to 40% in the harvested herbage. The capability to fix atmospheric nitrogen (together with *Rhizobium trifolii*) and thus to reduce the inherent energy cost of using nitrogen fertilizers is one of the main advantages of white clover, especially when grown as a component of a mixed sward.

White clover shows large fluctuations in yield, not only from year to year but also during a growing season. The reasons for such fluctuations are not completely understood. It is presumed that they are due mainly to white clovers growth requirements and to interactions when grown in mixtures where it should compete with tall growing grasses and other dicotelydons. Apart from its growth habit, its ability to develop and thrive in such a complex ecosystem depends largely on environmental factors and management practices.

There are numerous reports to indicate the effects of single environmental factors on the growth and symbiotic nitrogen fixation of white clover (Kessler *et al.* 1990; Davidson *et al.* 1990; Chapman *et al.* 1990, 1991; Hartwig *et al.* 1987, 1990). Effects of environmental factors on the growth and productivity of white clover under field conditions or in natural grasslands have been studied previously (Schwank *et al.* 1986; Lüscher 1988; Boller and Nösberger 1987). In the field, factors such as temperature, radiation, nitrogen supply and water status in the soil can bring about different responses as compared with controlled environmental conditions. Menzi (1991) reported that the growth rate of clover-grass swards was influenced to a greater extent by climatic factors than by sward composition or management.

The objective of this study was to examine the effects of two cutting heights on the regrowth, productivity and symbiotic nitrogen fixation of white clover grown in monocultures and in clover-grass mixtures over a period of two years. Cutting swards at two heights was to impose the plants to different carbon economies. This stress also affects the energy supply by influencing the residual leaf area. A furthur consequence of cutting height is its effect on nitrogen fixation, which appears to depend largely on current photosynthesis. Hartwig *et al.* (1987) demonstrated that lack of oxygen at the site of nitrogen fixation is responsible for the decrease in nitrogenase activity after defoliation.

In the first part of this study, regrowth and productivity of white clover swards under two cutting heights are described. Cutting height is important for regrowth and for the sward morphology of white clover grown in monocultures and in clover-grass mixtures.

The second part looks at the role of cutting height in the symbiotic nitrogen fixation of white clover. Nitrogen fixing potential of white clover, grown in monocultures and in clover-grass mixtures, is discussed.

This study aids in the understanding of important factors involved in regrowth, sward morphology and symbiotic nitrogen fixation after repeated defoliation of white clover swards under different defoliation intensities.

2. SUMMARY

The aim of this study was to investigate whether regrowth, productivity and symbiotic nitrogen fixation are limited by repeated defoliations. For this purpose, the effects of two cutting heights (4 and 10 cm above ground level, presumably resulting in different residual leaf area) on regrowth, productivity and symbiotic nitrogen fixation of white clover swards under field conditions was investigated. A comparison of clover monocultures and clover-ryegrass (*Lolium perenne* L.) mixtures was made in order to understand the behaviour of clover when grown with a companion grass. There were six harvests per year and the swards were fertilized with 3 g $N \cdot m^{-2} \cdot cut^{-1}$ (equivalent to 30 kg $N \cdot ha^{-1} \cdot cut^{-1}$). Of that amount, 1.6% to 2% ^{15}N was incorporated. After each harvest, the residual clover leaf area index, yield parameters and symbiotic nitrogen fixation (isotope dilution technique) were determined. At periodic harvests, regrowth of leaf area, stolon morphological parameters and starch contents of clover stolons were determined.

After sowing in previous summer, the cutting height in the first production year (1990) had no influence on the regrowth of clover. The clover monocultures and clover-grass mixtures, cut at a height of 4 cm produced higher annual clover dry matter yields during both years; During the second year (1991) the effect was significant in the clover monocultures. The increase in the annual clover dry matter yields during the second production year is a consequence of 4 cm cutting height which increased the number of leaf bearing nodes and the number of growing points. The increased growth of grass in the clover-grass mixtures, cut at 10 cm height during the second production year, had a negative effect on the proportion of clover. In the field the most predictable response of clover to a lower cutting height (4 cm) would be an increase in the number of growing points. The relative response of clover and grass in mixture is also important in this respect.

During both production years, white clover grown in mixture with grass had

significantly higher percentages of nitrogen from symbiotic fixation as compared to clover grown in monocultures. The annual clover nitrogen yields derived from symbiosis were closely related to the proportion of clover in the harvested herbage. During the second production year, the nitrogen apparently transferred from clover to grass accounted for about one fourth of the total nitrogen harvested from grass. The governing element of symbiotic nitrogen fixation in the field is white clover's demand (sink) for symbiotically fixed nitrogen. This parameter is greatly affected by the availability of mineral nitrogen or the competition through associate non-legume plants. Therefore, it can be concluded that symbiotic nitrogen fixation did not limiting clover growth.

ZUSAMMENFASSUNG

Das Ziel dieses Feldversuches war es zu untersuchen, ob Wiederaustrieb, Ertragsfähigkeit und biologische Stickstoff-Fixierung limitierende Faktoren nach wiederholter Entblätterung darstellen. Zu diesem Zweck wurde der Einfluss von zwei Schnitthöhen (4 und 10 cm über dem Grund, die vermutlich zu zwei verschiedenen Restblattflächen führten) auf Wiederaustrieb, Ertragsfähigkeit und biologische Stickstoff-Fixierung untersucht. Der Vergleich von Weissklee-Reinbeständen und Weissklee-Gras-Mischungen wurde angestellt, um das Verhalten von Weissklee mit einem Begleitgras zu verstehen. Es erfolgten sechs Ernten pro Jahr mit je einer anschliessenden Düngung von mit 3 g $N \cdot m^2$ und Schnitt (30 kg $N \cdot ha$ und Schnitt). Diese Gaben bestand zu 1.6% bis 2% aus ^{15}N. Nach jeder Ernte wurden Restblattflächenindex, Ertragsparameter und biologische Stickstoff-Fixierung (Isotopen Verdünnungstechnik) bestimmt. In periodischen Ernten erfolgte die Untersuchung von Blattneubildung, morphologische Parameter und Stärkegehalte der Weisskleestolonen.

Im ersten Hauptversuchsjahr beeinflusste die Schnitthöhe den Wiederaustrieb von Weissklee nicht. Die Weissklee-Reinbestände und die Weissklee-Raigras-Mischungen ergaben bei 4 cm Schnitthöhe beide Jahren höhere jährliche Klee-Trockensubstanzerträge, in zweiten Jahr war dieser Effekt nur in den Weissklee-Reinbeständen signifikant. Das Ansteigen der jährlichen Trockensubstanzerträge von Klee im zweiten Hauptversuchsjahr war eine Folge der 4 cm Schnitthöhe, welche die Anzahl blattbildender Nodien und Vegetationspunkte erhöhte. Das erhöhte Graswachstum in der Klee-Gras-Mischung bei Schnitthöhe 10 cm beeinflusste im zweiten Hauptversuchsjahr den Kleeanteil negativ. Die Schnitthöhe hatte keinen Einfluss auf den Wiederaustrieb von Weissklee. Im weiteren wurde beobachtet, dass unter Feldbedingungen die deutlichste Reaktion von Weissklee auf eine tiefere Schnitthöhe (4 cm) eine Erhöhung der Anzahl Vegetationspunkte war. In der Weissklee-Raigras-Mischung beeinflusste das relative Verhalten von Klee und Gras diese Grösse ebenfalls.

In beiden Hauptversuchsjahren wies Weissklee in der Mischung mit Raigras im Vergleich zum Weissklee-Reinbestand einen signifikant höheren Anteil an Stickstoff aus der biologischen Fixierung auf. Die jährlichen Stickstofferträge aus der Fixierung waren eng korreliert mit dem Weissklee-Anteil im geernteten Pflanzenmaterial. Im zweiten Hauptversuchsjahr betrug der Anteil des Stickstoffs, der vom Klee zum Gras transferiert wurde, ungefähr ein Viertel des im Graserntegut enthaltenen Stickstoffs. Das bestimmende Element für biologische Stickstofffixierung im Feld ist der Bedarf der Weissklees nach Stickstoff. Dieser Parameter ist stark beeinflusst von der Verfügbarkeit an mineralischem Stickstoff oder der Konkurrenz durch begleitende Nichtleguminosen. Daraus folgerten wir, dass die biologische Stickstoff-Fixierung von Weissklee den Nachwuchs nicht beeinflusste.

3. REGROWTH AND PRODUCTIVITY OF WHITE CLOVER *(TRIFOLIUM REPENS* L.) MONOCULTURES AND WHITE CLOVER-PERENNIAL RYE-GRASS (*LOLIUM PERENNE* L.) MIXED SWARDS AT TWO CUTTING HEIGHTS.

---

1 ABSTRACT

In this study, the effect of cutting height (4 and 10 cm above-ground) on the regrowth and productivity of white clover was investigated under field conditions. A two year experiment (1990/1991) was conducted in Eschikon, Switzerland with white clover (*Lolium perenne* L. cv "Ladino") monocultures, white clover-perennial ryegrass (*Lolium perenne* L. cv "Bastion") mixtures and perennial ryegrass monocultures. The comparison of white clover monocultures and clover grass mixtures was made in order to understand the response of clover when grown in mixture with grass. Six harvests were made at four-week intervals. At each harvest, swards were fertilized with 3 g $N \cdot m^{-2}$ (30 kg $N \cdot ha^{-1} \cdot cut^{-1}$ and 180 kg - $N \cdot ha^{-1} \cdot a^{-1}$). After each harvest, the residual clover leaf area index was determined. At periodic harvests, regrowth of leaf area and stolon morphological parameters, such as number of leaf bearing nodes, stolon length and the number of growing points of clover plants, were determined.

During the first production year, the cutting height showed no effect on the regrowth of clover. After cutting, clover grew faster when cut at 4 cm as compared to 10 cm. Clover monocultures, cut at a height of 4 cm produced a higher annual clover dry matter yield as compared to a cutting height 10 cm during both production years. This effect was apparent during the first year but significant only during second production year. Clover grown in clover-grass mixtures also had a higher dry matter yield when cut to 4 cm as compared with 10 cm in both years, but the differences were not significant. This may be due to the change in growth habit of clover when grown in association with grass. The increase in dry matter yields at a 4 cm cutting height during the second year
in clover monocultures and in clover grass mixtures was due not only to the

positive effects of cutting height but was possibly a consequence of increase in both clover leaf bearing nodes and number of growing points. The annual total dry matter yields of clover-grass mixtures were higher than that of the annual clover dry matter yields of clover monocultures. During the second year, grass growth increased in the clover-grass mixtures cut at a height of 10 cm. This had a negative effect on the proportion of clover in the mixture.

The two cutting heights had no effect on the regrowth of clover after defoliation. Regrowth of clover was mainly governed by the increased leaf area index, thereby increasing the current photosynthesis. However, the effect of the cutting height on the number of clover growing points in both clover monocultures and in clover-grass mixtures as well as the competition by grass in the mixtures were more important than the direct effects of the two cutting treatments on the growth of clover.

2.INTRODUCTION

Mechanical defoliation or defoliation by animals impose a periodic stress on white clover throughout the growing season. Height of defoliation affects the amount of photosynthesising tissues removed from a pasture. The residual leaf area acts as an important carbohydrate source by maintaining the photosynthetic activity of plants during the early stages of regrowth. The regrowth of white clover after defoliation depends to a much greater extent on the photosynthesis of remaining leaves than on carbohydrate reserves (Baur-Höch 1988). The defoliation of clover seedlings reduced the size of the leaf and the length of the petiole of subsequent leaves but had little effect on the rate of leaf production (Boatmann and Hagger 1984). King *et al.* (1978) found little effect of defoliation on the rate of clover leaf production though Carlson (1966b) reported that clover leaf removal 'retarded the leaf emergence', whereas the rate of leaf development (separation and unfolding of leaflets) increased.

Height of defoliation is one of the main factors which determines the proportion of white clover in clover-grass mixed swards. Reducing the height of defoliation has often increased the proportion of clover in the clover-grass swards. (Kishi 1973; Clerk *et al.* 1974). Frame (1976) and Brereton and Carton (1985) found that close cutting (about 2.5 to 5 cm above ground level) increased the total herbage yield up to 44% as compared with a lax cutting regime (6 to 10 cm), with adequate recovery periods between defoliations. White clover-grass swards cut at a height of 2.7 cm, produced more dry matter yield in the spring as compared with a cutting height of 4 cm (Fulkerson and Mitchell 1987). In contrast, the results of Briseno and Wilmen (1961) show that white clover yields were low at a cutting height of 4 cm as compared with a cutting height of 8 cm. However, clover apppeared to recover quickly and completely from the effects of reducing the height of defoliation when the 4 cm cutting treatment was discontinued.

The quantity of light reaching the base of the sward is another important factor related to cutting height. Menzi (1988) observed that, the absorption of irradiance by white clover leaves influenced the growth of clover by increasing the clover leaf area and the rate of branching. Schwank *et al.* (1986) also demonstrated that, for white clover, competition for light is the main factor which limits its proportion in mixed swards.

Reducing the height of defoliation increases the number of clover growing points per unit area as observed by Acuna and Wilmen (1988). There was a positive correlation between the number of growing points and the proportion of clover in the harvested herbage. Work done by Patterson and Laidlow (1990) shows that, during the first production year, there were no differences in the number of stolons or growing points per unit area in white clover swards when cut at different heights. However, during the second production year, a lower number of growing points per unit area was observed under the highest cutting height. These differences between the two years were attributed to different management practices in autumn.

Height of defoliation of white clover swards under field conditions has been less well investigated. An understanding of the response and seasonal variation in the proportion of white clover is important for the long-term management of white clover pastures.

The objective of the present study was to monitor the effects of two cutting heights on the regrowth and dry matter accumulation of white clover. The reason for cutting swards to two heights was to expose the plants to different carbon economies. Removal of herbage by cutting at different heights affects the energy supply by changing the residual leaf area. The leaf area index, yield and morphological parameters of white clover grown in monocultures and in clover-grass mixtures were determined. The comparison of white clover monocultures with white clover grass mixtures over a period of two years, increases our understanding of the response of white clover when grass is present as a companion crop. The results indicate that the two cutting treatments did not limit the regrowth of clover after defoliation.

3 MATERIALS AND METHODS

3.1 Site, plant material and design of the experiment

A two year field experiment was conducted in 1990 and 1991 in Eschikon (550 m above sea level) near Zurich, Switzerland. The soil type was eutric cambisol containing a clay content of 19% and a pH of 7.1, with satisfactory levels of P_2O_5 and K_2O. The experimental site had been cropped with maize and oil raddish in 1988 and phacelia in 1989. The experiment was set up in autumn 1989 as follows: After ploughing and levelling the field, 10 g P_2O_5, 30 g K_2O and 3.5 g $Mg{\cdot}m^{-2}$ (100 kg P_2O_5, 300 kg K_2O and 35 kg $Mg{\cdot}ha^{-1}$) were applied as foscal (15% P_2O_5, 30% K_2O) and potassium and magnesium salts (26 to 30% K_2O, 6% Mg). The following species were sown, using a cyclone seeder.

1. White clover (*Trifolium repens* L.) cv. "Ladino"- monoculture ($1.4\ g{\cdot}m^{-2}$).
2. White clover (*Trifolium repens* L.) cv. "Ladino" ($0.4\ g{\cdot}m^{-2}$) and perennial ryegrass (*Lolium perenne* L.) cv. "Bastion" ($1.0\ g{\cdot}m^{-2}$) mixture.
3. Perennial ryegrass (*Lolium perenne* L.) cv. "Bastion"- monoculture ($1.4\ g{\cdot}m{-}^{2}$).

The seeds were pressed slightly with a roller. After germination, 3 g $N{\cdot}m^{-2}$ (30 kg $N{\cdot}ha^{-1}$) was applied as NH_4NO_3 (27.5% N). Before the onset of winter two standardization cuts were done at a height of 4 cm and weeds removed manually.

The experiment was designed as a randomized block with four replicates and 6 treatments. Perennial ryegrass monocultures were used as a reference crop (see chapter 4). The size of the plot was 4 X 1.40 m.

During the experiment, cuttings were done at 4 and 10 cm heights above the ground level respectively.

3.2 Harvest plan

First production year (1990)

At the beginning of the growing season and after each harvest all the plots were fertilized with 3 g $N \cdot m^{-2}$ (30 kg $N \cdot ha^{-1}$) applied as $(NH_4)_2SO_4$ (21% N). The total nitrogen application was 18 g $N \cdot m^{-2}$ (180 kg $N \cdot ha^{1} \cdot a^{-1}$). Phosphorus and potassium application was also done in March as described on page 11. A plot area of 1 m^2 was used to determine the yield. During the last week of April, a standardising harvest was carried out at 4-5 cm stubble height. Thereafter at each harvest, the plots were cut to heights of 4 or 10 cm above ground at four week intervals until mid October. A total of 6 harvests were made. After the last harvest, the clover and clover-grass sub plots were destructively harvested to determine the morphological parameters of clover plants. This was done by digging out three soil cores from 10 X 10 cm areas from the central zone (chapter 3.3) of each plot.

The remaining plot area was cut with a mover and the fresh and dry herbage weights were determined. During the regrowth, after the third harvest residual leaf area was estimated on previously marked stolons from these areas at 0, 3, 5, 10 and 20 days after cutting. The plots were irrigated with a spinkler during the summer.

Second production year (1991)

During the second production year, seven harvests were done at four-week intervals from end of April to mid October. NPK fertilization of the plots were done as described for the first production year. A plot area of 1 m^2 was used to determine the yield. This plot was destructively harvested in April after the regular harvest. The rest of the plot was cut with the mower and fresh and dry herbage weights were determined. For the first harvest the results are shown only for the destructive harvest. In April, a 2 m^2 main plot area was fertilized after each harvest until July. A sub plot of 1 m^2 of the main plot was used to determine the yield parameters and was destructively harvested in July. The remaining area (1 m^2) was cut and the fresh and dry herbage weights were determined. Thereafter, the remaining 1 m^2 was harvested until October. In October this area was destructively harvested too. During the regrowth after the third harvest, clover growing points (stolon tip with at least two unfolded leaves) were determined. In each plot, four areas of 10 X 10 cm were marked using a metal frame, and clover growing points were counted within these areas.

3.3 Herbage sampling

The herbage of the 50 x 50 cm central zone of each 1 m^2 sub-plot was harvested and separated into grass, clover, and unsown species. The separated herbage material was dried at 65°C for 48 h. The herbage yield of the remaining plot area was also harvested and dried at 105°C for 24 h.
Destructively harvested clover plants (refer page 12) were collected, washed and separated into leaves and petioles and stolons. Stolon morphological parameters were determined and the leaf area was measured and estimated for individual leaves.

3.4 Estimation and measurements of residual leaf area

After each harvest, the residual leaf area of clover leaves was estimated in the central zone of each plot in four 10 X 10 cm areas with the help of a metal frame. For leaf area estimations only fully expanded leaves were used (stage 0.8 *and above as described by Carlson 1966a). The estimation of residual leaf area* was made as described by William *et al.* (1964) using a set of photographic clover leaf standards.

To evaluate the accuracy of the method, the residual leaf area estimation as well as leaf area measurements were made at the destructive harvests. The correlation coeffecient (r^2) of estimated and measured residual leaf area was very high during both production years. In 1990, the correlation coefficient was 0.94 and increased 0.99 in the second production year.

3.5 Climatic data

The following climatic data were obtained from a weather station 500 m from the experimental site (Figure 1):

(i) Mean monthly global radiation (sum of days; MJ/m^2; Pyranometer Kipp and Zonen)

(ii) Mean monthly air temperature (day + night ; 2 cm above soil (°C; Yellow spring Thermistors)

3.6 Statistical analysis

Statistical analyses of the experimental data were made using the programme package SAS (Statistical analysis system , SAS Institute, Cary, North Carolina, USA). Correlations were calculated using the means of respective variables.

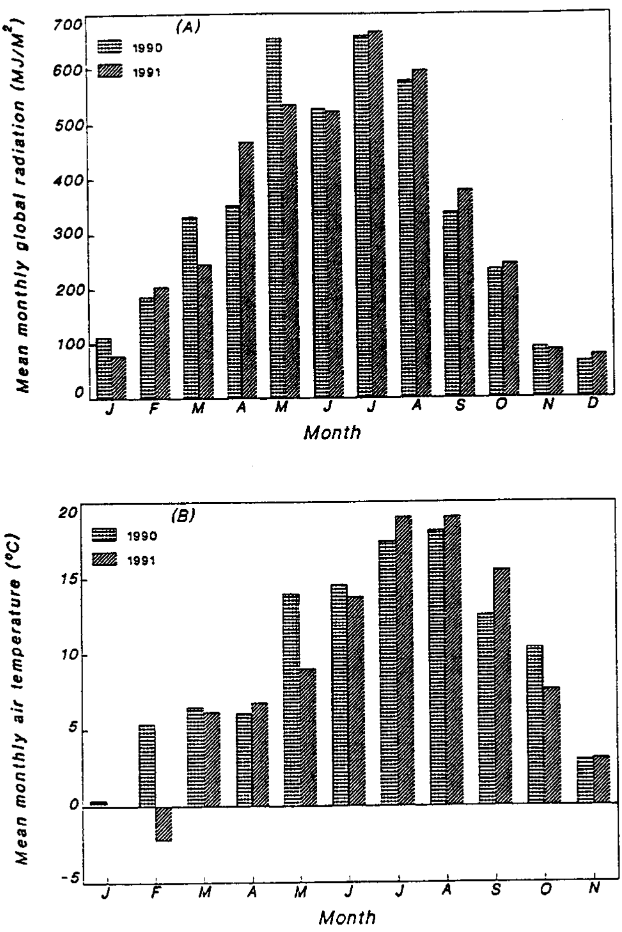

Fig. 1: Climatic data obtained for the first and second production years (1990 and 1991) respectively.

(A) Mean monthly global radiation (MJ/m^2 ; days sum).

(B) Mean monthly air temperature (°C ; day+night)

4. RESULTS

4.1 Dry matter yields

4.11 Clover dry matter yields

In the first production year, the dry matter yields of clover grown in monocultures and cut at a height of 4 cm were significantly higher at fourth and sixth harvests as compared with the 10 cm cutting height (Figure 2A). In the subsequent year, clover grown in monocultures and cut at 4 cm height had higher dry matter yields at all harvests as compared with the 10 cm cutting treatment. However, significant differences were observed only at the first, third and fourth harvests (Figure 2B).

In 1990, the differences in clover dry matter yields of clover-grass mixtures, were not significant for the two cutting treatments (Figure 2A). In 1991, the clover dry matter yields were significantly higher at a cutting height of 4 cm at the fourth and sixth harvests as compared with the 10 cm cutting height (Figure 2B).

4.12 Grass dry matter yields

In the first production year, the grass dry matter yields of grass monocultures were slightly affected by the cutting height (Figure 3A). In 1991, grass monocultures cut at 10 cm had higher though insignificant dry matter yields at all harvest as compared with the 4 cm cutting height (Figure 3B).

In 1990, the grass dry matter yields in clover-grass mixtures followed the same trend as the grass monocultures at individual harvests (Figure 3A). In the subsequent year, the 10 cm cutting treatment had slightly higher dry matter yields at the first harvest and last three harvests as compared with the 4 cm cutting treatment (Figure 3B).

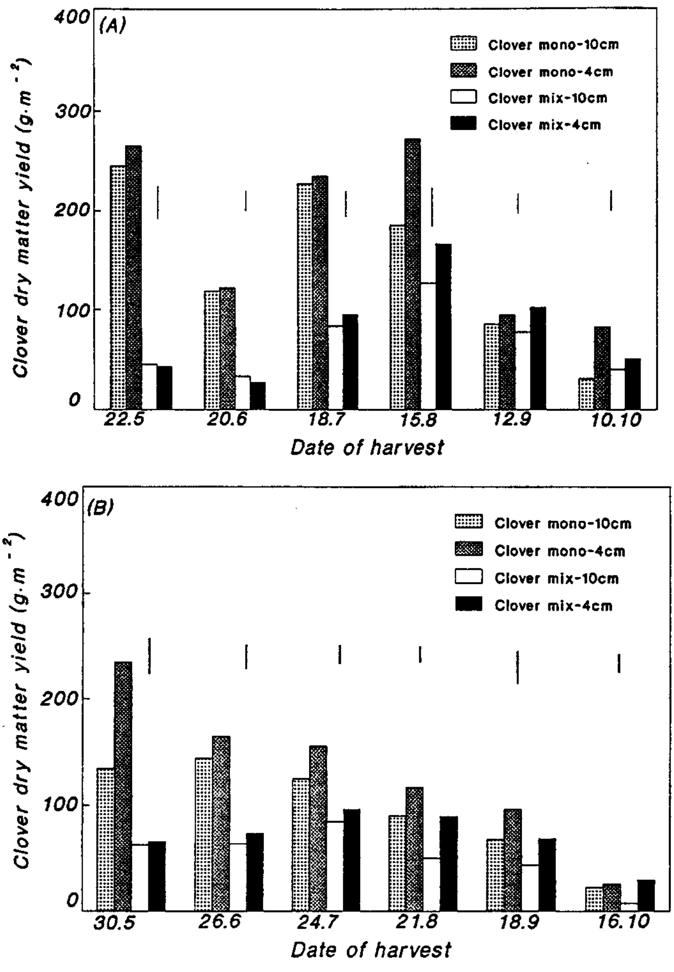

Fig. 2: The course of clover dry matter production at individual harvests for clover grown in monocultures and for clover grass-mixtures respectively. Harvests were done at two cutting heights (10 and 4 cm, respectively).
(A) First production year (1990): (B) Second production year (1991).
Bars indicate least significant differences ($LSD_{0.05}$) for means of 4 replicates.

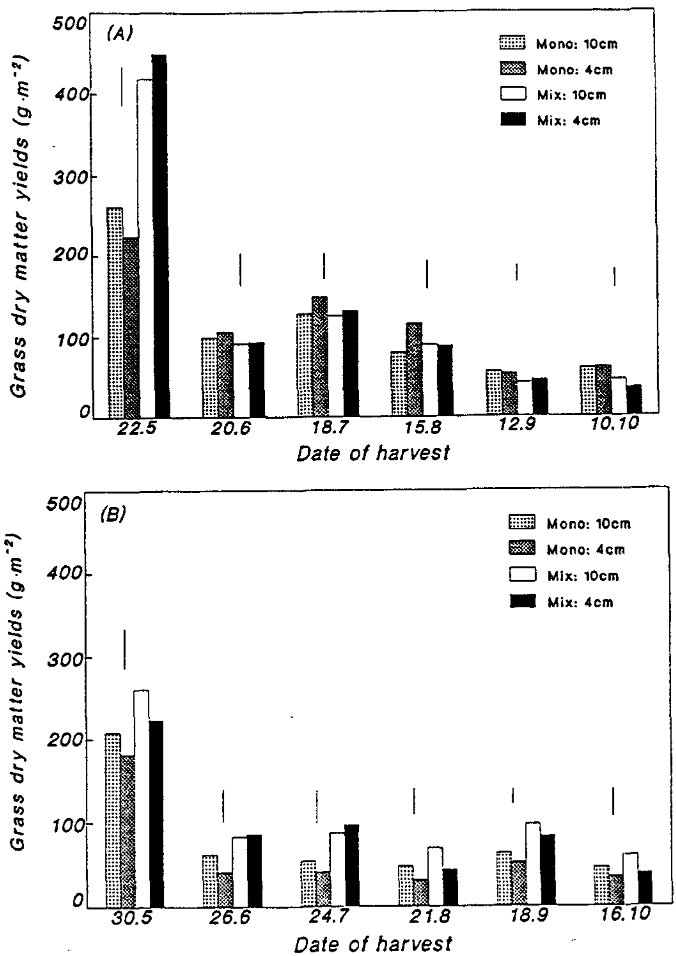

Fig. 3: The course of grass dry matter production at individual harvests for grass grown in monocultures and for clover-grass mixtures respectively. Harvests were done at two cutting heights (10 and 4, cm respectively).

(A) First production year (1990): (B) Second production year (1991).

Bars indicate least significant differences ($LSD_{0.05}$) for means of 4 replicates.

4.13 Annual dry matter yields.

In the first production year, the proportion of clover in the clover-grass mixtures was similar in both cutting treatments (Table 1A). In the subsequent year, the proportion of clover slightly increased in clover-grass mixtures cut at a height of 4 cm (Table 1B). The proportion of clover in the 10 cm cutting treatment decreased during the second production year.

The annual clover dry matter yields in clover monocultures and clover-grass mixtures were higher under the 4 cm cutting treatment as compared with the 10 cm cutting treatment in both years (Table 1). However, clover grown in monocultures had significantly higher dry matter yields at a 4 cm cutting height as compared with the 10 cm cutting height in the second production year only (Table 1B).

The annual total dry matter yields of clover-grass mixtures were also higher at a 4 cm cutting height as compared with the 10 cm cutting height in both years (Table 1). However, a significant increase was observed at a cutting height of 4 cm as compared with 10 cm in the first production year only (Table 1A).

Though not significant, the annual total dry matter yields of clover-grass mixtures were higher than the annual clover dry matter yields of clover monocultures at both cutting heights in both production years (Table 1).

In the first year, the annual grass dry matter yields of grass monocultures and clover-grass mixtures were higher at a cutting height of 4 cm cutting as compared with 10 cm cutting (Table 1A). In 1991, higher dry matter yields were observed when plants were cut to 10 cm as compared with 4 cm height (Table 1B). However, the differences, in both years were not significant. The annual dry matter yields of clover and grass monocultures and clover-grass mixtures are given in Table 1: (1A) First production year (1B) second production year.

Table 1: Annual dry matter (DM) yields of clover and grass grown in monocultures or in clover-grass mixtures, in the first and second production year (Means of 4 replicates).

(1A) First production year				
Type of sward and cutting height (cm)	Clover proportion	Dry matter yield of mixture	Dry matter yield of clover	Dry matter yield of grass
	%	$g \cdot m^{-2} \cdot a^{-1}$		
White clover/ryegrass mixture-10cm	38	1228.2	399.3	815.5
White clover/ryegrass mixture-4cm	37	1400.6	491.3	870.8
White clover monoculture 10cm	96	-	892.9	-
White clover monoculture 4cm	95	-	1060.2	-
Perennial ryegrass mono: 10 cm	-	-	-	735.4
Perennial ryegrass mono: 4cm	-	-	-	781.0
LSD 0.05	9.4	110.2	186.1	112.3

(1B) Second production year				
Type of sward and cutting height (cm)	Clover proportion	Dry matter yield of mixture	Dry matter yield of clover	Dry matter yield of grass
	%	$(g \cdot m^{-2} \cdot a^{-1})$		
White clover/ryegrass mixture-10cm	30	901.9	279.2	632.7
White clover/ryegrass mixture-4cm	41	971.7	387.4	584.3
White clover monoculture 10cm	91	-	529.8	-
White clover monoculture 4cm	96	-	724.9	-
Perennial ryegrass mono: 10 cm	-	-	-	482.4
Perennial ryegrass mono: 4cm	-	-	-	382.5
LSD 0.05	12.6	249.7	171.2	133.9

4.2 Leaf area index

4.21 The removed clover leaf area index at each harvest

In the first production year, leaf area index in clover monocultures cut at 4 cm height was significantly higher at the fourth and sixth harvests as compared with 10 cm cutting height (Figure 4A).
The clover leaf area index of harvested leaves in clover-grass mixtures was significantly higher only at the fifth harvest (Figure 4A).

In the second production year, the leaf area index of harvested leaves in clover monocultures was significantly higher at the first, fourth and sixth harvests at a cutting height of 4 cm as compared with 10 cm (Figure 4B). In the clover-grass mixtures, significant differences were observed only at the fourth and sixth harvests (Figure 4B).

4.22 Estimated residual leaf area index

Clover grown in monocultures and cut to a height of 10 cm had a significantly higher residual leaf area index (after cutting) as compared with a 4 cm height at all harvests during both production years (Figure 5).

The clover in clover-grass mixtures followed the same trend as clover in monocultures during the first production year (Figure 5A). However, in 1991, the clover in clover-grass mixtures cut at 10 cm had a significantly higher residual leaf area index as compared with a 4 cm cutting height only at the second, third and fourth harvests (Figure 5B). The differences between the two cutting treatments (10 and 4 cm) diminished towards the end of the growing season.

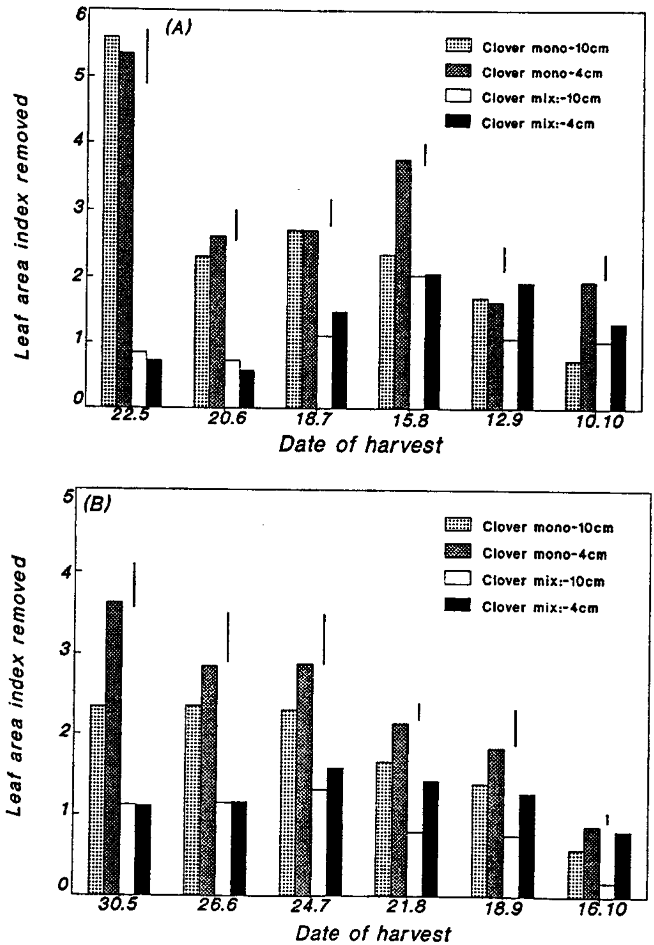

Fig. 4: The course of removed clover leaf area index at individual harvests for clover grown in monocultures and for clover-grass mixtures respectively. Harvests were done at two cutting heights (10 and 4 cm, respectively).
(A) First production year (1990): (B) Second production year (1991).
Bars indicate least significant differences ($LSD_{0.05}$) for means of 4 replicates.

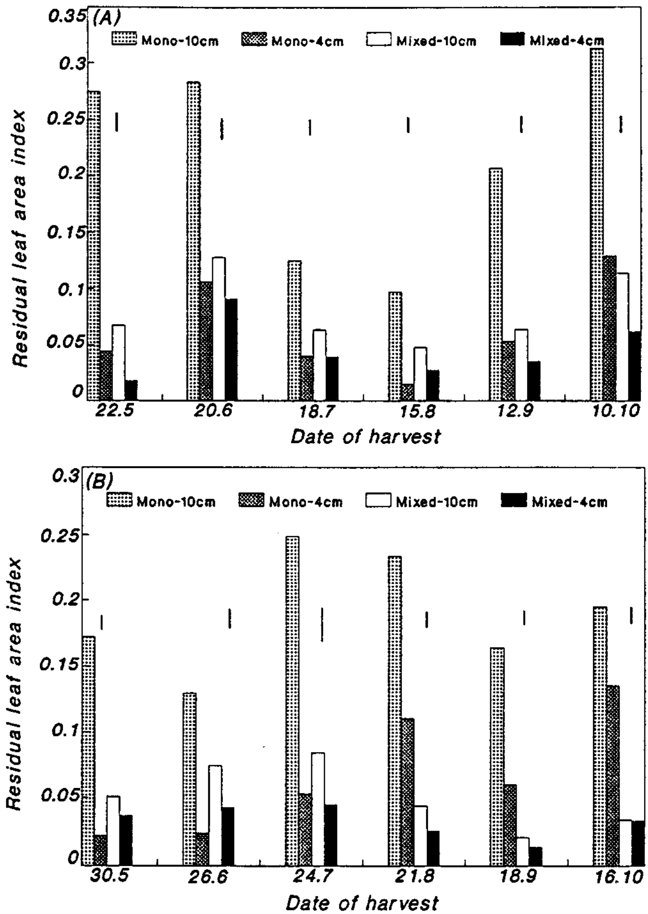

Fig. 5: The course of estimated residual leaf area index of clover (after cutting) at individual harvests for clover grown in monocultures and for clover-grass mixtures respectively. Harvests were done at two cutting heights (10 and 4 cm, respectively).

(A) First production year (1990); (B) Second production year (1991).

Bars indicate least significant differences ($LSD_{0.05}$) for means of 4 replicates.

4.23 Development of clover leaf area index

The development of the clover leaf area index was determined to determine the effect of cutting height on the regrowth of clover. The development of the clover leaf area index during regrowth is shown in Table 2.

Table 2: Development of the clover leaf area index (estimated per 0.1 m^{-2}) in clover monocultures and in clover-grass mixtures during regrowth (0, 3, 7 and 20 days) after the third harvest in the first production year. Harvests were done at two heights (10 and 4 cm). (Means of 4 replicates).

Treatment	Cutting height	Leaf area index 0 day **	Leaf area index 3 days	Leaf area index 7 days	Leaf area index 10 days	Leaf area index 20 days
Clover mono-culture	10 cm	0.062	0.124	1.39	6.58	8.49
	4 cm	0.020	0.039	1.69	6.22	8.84
Clover grass mixture	10 cm	0.032	0.063	1.26	5.09	8.77
	4 cm	0.010	0.034	1.51	5.50	8.56
	$LSD_{0.05}$	0.021	0.021	0.45	0.74	0.99

** immediately after cutting

Immediately after cutting and 3 days thereafter, the residual leaf area index was significantly higher in the 10 cm cutting treatments as compared with the 4 cm cutting treatments both in clover monocultures and in clover-grass mixtures. Thereafter, only slight differences were observed in the leaf area index between the two the cutting treatments both in clover monocultures and in clover-grass mixtures.

4.3 Clover morphology

4.31 Clover stolon parameters

The morphological parameters of clover stolons were determined to study the effects of cutting height on the regrowth of clover in swards. The morphological parameters of clover stolons, determined at destructive harvests, are shown in Table 3.

Table 3: Morphological parameters of clover grown in monocultures and in clover grass mixtures as determined at destructive harvests in the first and second years of production. Harvests were made at two cutting heights respectively (10 and 4 cm). (Means of 4 replicates).

Variable	Harvest Date	Clover mono-culture 10 cm	Clover mono-culture 4 cm	Clover-grass mixture 10 cm	Clover-grass mixture 4 cm	$LSD_{0.05}$
No.of leaf bearing nodes / 0.1 m^2	Oct. 1990	32	33	25	25	5.78
	Apr. 1991	54	83	27	47	19.53
	Jul. 1991	49	71	24	45	7.11
	Oct. 1991	52	75	28	43	13.10
Specific stolon weight mg/cm	Oct. 1990	31	28	20	20	7.1
	Apr. 1991	25	22	13	24	9.1
	Jul. 1991	44	38	23	29	5.3
	Oct. 1991	28	23	19	15	4.7

In October 1990, the number of leaf bearing nodes of clover stolons per unit area was similar for both cutting treatments in clover monocultures and in clover grass mixtures. In April 1991, the number of leaf bearing nodes in clover stolons per unit area was significantly higher in the 4 cm cutting treatment as compared to the 10 cm cutting treatment both in clover monocultures and in clover-grass mixtures. The same trend was observed in July and October 1991.

In October 1990, the specific stolon weight (SSW) of clover was significantly higher in clover monocultures as compared with the clover grass mixtures (Table 3). However, the two cutting treatments had no effect on SSW in clover monocultures and in clover grass mixtures. In April 1991, the specific stolon weight of clover varied only slightly in clover monocultures for the two cutting treatments. In July and October 1991, the SSW in clover monocultures cut at a height of 4 cm was significantly lower than at a 10 cm cutting height. In the clover grass mixtures, the SSW was significantly higher in April and July 1991 at a cutting height of the 4 cm as compared to 10 cm.

4.32 Development of growing points

The growing points of clover were counted to determine the effects of cutting height on the growth of clover in swards. The development of the clover growing points during regrowth is shown in Table 4.

Table 4: Development of growing points per 0.1 m^2 of clover grown in monocultures and in clover-grass mixtures, as determined during regrowth (0, 14 and 28 days after cutting) after the fifth harvest in the second production year. Harvests were done at two heights (10 and 4 cm). (Means of 4 replicates).

Treatment	Cutting height	0 day **	14 days	28 days
Clover monoculture	10 cm	4.3	7	13.2
Clover monoculture	4 cm	6.9	10	19.5
Clover-grass mixture	10 cm	2.7	5.1	7.1
Clover-grass mixture	4 cm	3.9	8.3	10.5
$LSD_{0.05}$		0.99	0.79	3.33

** Immediately after cutting

The number of clover growing points per unit area was significantly higher in the 4 cm cutting treatment as compared with the 10 cm cutting treatment both in clover monocultures and in clover-grass mixtures .

5. Discussion

5.1 The effects of two cutting treatments on regrowth and dry matter accumulation of white clover in the first production year

The two cutting treatments were an effective means of obtaining different residual leaf area indexes (LAI) in clover monocultures and in clover-grass mixtures during the first year of the experiment (Figure 5A). Seventy to seventy five percent of the total clover leaf area was removed from clover monocultures and from clover-grass mixtures when plants were cut to a height of 10 cm. Upto 90% of total leaf area was removed from clover monocultures and in clover-grass mixtures when plants were cut to a height of 4 cm. Since cutting at 4 cm removes more vegetative material, a higher percentage of leaf removal at a 4 cm is to be expected. Therefore, the low LAI observed for about 3 days during the initial regrowth in both clover monocultures and in clover grass mixtures was due to the excessive removal of leaf area. Thereafter, both sward types grew quickly during the first seven days and had slightly more LAI than swards cut to 10 cm height (Table 2). These observations show that the two cutting heights had no effect on regrowth of clover after defoliation. Clover regrew faster when cut to a 4 cm height. In determining the LAI after harvest, leaf stages above 0.8 were considered as described by Carlson (1966a). It was clearly visible that many leaves lower than stage 0.8 were not considered. The advantage of cutting swards to a low level permits more light to reach the base of the swards which results in a more rapid regrowth. In growth cabinet studies with white clover plants Baur-Höch (1988) observed that after the removal of 81.3% leaf mass the plants took as long as 12 days to produce comparable dry matter yields as undefoliated plants. In spite of 90% leaf area removal in this study, a much earlier recovery of clover was probably due to the favourable environmental

conditions which prevailed under field conditions (Figure 1).
In addition, at the end of the first production year, the two cutting treatments had no effect on the number of leaf bearing nodes (Table 3) and starch concentrations in clover stolons (Table 2 page 54). This suggests that the regrowth of clover after cutting depends almost exclusively on current photosynthesis and the starch reserves of stolons are of minor importance. Similar results were also observed by Baur-Höch (1988), where the regrowth of white clover after defoliation depended to a great extent on the photosynthesis of the remaining leaves rather than on the carbohydrate reserves.

Since there was no effect of the two cutting heights on the regrowth of clover in monocultures and in mixtures the annual clover dry matter yields were less affected during the first production year (Table 1). However there is a tendency for dry matter yields to increase following cutting to 4 cm. This increase in clover monocultures was mainly due to the two significant dry matter yields (Figure 2A) obtained at the fourth and sixth harvests and is related to the removal of clover leaf area index (Figure 4A).

5.2 The effects of two cutting treatments on the overwintering of white clover

The effect of the two cutting heights was seen after overwintering. In spring 1991, the number of leaf bearing nodes (Table 3) on clover stolons in clover-grass mixtures cut to a height of 10 cm was significantly lower than in swards cut to a height of 4 cm. Clover-grass mixtures went into overwintering in 1990 with comparatively large amounts of above ground grass stubble and rotted clover leaves and petioles that accumulated at each harvest. Shading by grass and the accumulated stubble could have caused low light penetration to the clover stolons at the beginning of spring. This effect caused a reduction in the number of leaf-bearing nodes (Table 3). Likewise Lüscher (1988) showed that shading (both artificial and by grasses) decreased the density of clover growing points in spring.

Clover monocultures cut to 10 cm also had rotten clover leaves and petioles but to a lesser extent. Since more uncovered clover leaves, petioles and stolons were left as compared with clover-grass mixtures, and would have caused much tissue damage during the over-wintering period. Harris *et al.* (1983) observed that cover provided by the associated grass in clover-grass mixtures reduced the extent of low temperature damage to plants as compared to white clover monocultures. Thus in early spring, clover monocultures cut to a height of 10 cm had to repair the damaged tissues. In spite of presumably low photosynthesis during early spring, the plants had to repair the damaged tissues at the cost of leaf appearence on nodes (Table 3).

5.3 The effects of two cutting treatments on regrowth and dry matter accumulation of white clover during the second production year.

During the second year, significantly higher clover dry matter yields obtained at the first, third and fourth harvests (Figure 2B) in monocultures and at the fourth and sixth harvests (Figure 2B) in the clover-grass mixtures were obtained at 4 cm cutting height. This suggests a cutting height of 4 cm was more effective in increasing clover dry matter yields during the second production year. The significant increase in annual clover dry matter yield in clover monocultures cut at a 4 cm height (Table 1B) as compared with the 10 cm height is a furthur indication of the beneficial effects of cutting the swards to a 4 cm level. The increase in dry matter is a consequence of increasing the number of leaf bearing nodes and number of growing points (Tables 3 and 4). The low specific stolon weight of clover monocultures was due to the significantly higher stolon length per unit area (results not shown) which suggests a higher rate of branching (see to Table 3 page 26). Since a high proportion of a swards production is in the lower layers, herbage production will increase when plants are cut to heights of 4 cm as compared to 10 cm. These results are consistent with those of Patterson and Laidlow (1990), who showed that cutting mixed white clover swards to heights of 3,6 and 9 cm had no effect on the rate of leaf appearence or on the number of stolons during the first production year. However, in the second year these authors too observed that the clover dry matter yields were low when plants were cut to 9 cm cutting height as compared to 3 or 6 cm. They attributed these effects to a lower number of growing points in swards when defoliated at a height of 9 cm. The results of Wolledge and Dennis (1982) showed that better light conditions at the sward base encouraged the increase in clover growing points and promoted photosynthetically efficient leaves.

Irrespective of the cutting height, the higher annual total dry matter yields of clover-grass mixtures in both years (Table 1) indicate that the mixtures are more suitable than monocultures for higher herbage yields. The significantly higher total dry matter yields in 1990, at a cutting height of 4 cm as compared to 10 cm (Table 1A), was due to the slightly higher clover and grass yields and to the contribution of unsown species in the mixture.

Though no effects of cutting height on the proportion of clover in clover-grass mixtures in the first year was seen, in 1991 the clover proportion decreased apparently (from 38%-30%) and increased (from 37%-41%) at the 10 and 4 cm cutting heights respectively (Table 1). This decrease in clover proportion was due to the decrease in the number of leaf bearing nodes and to lower number of growing points (Tables 3 and 4). This decrease was further aggravated by the vigourous growth of the grass (Figure 3B). Furthermore, as a consequence of strong grass growth, the specific stolon weight also decreased (Table 3). In spite of these differences, the annual dry matter yields were not significantly different for the two cutting heights (Table 1B). The development of white clover in the mixture was influenced to a large extent by the grass partner since both compete for light. At a 10 cm cutting height, the competition from grass was stronger. Under such conditions, clover intercepts light by placing the leaves on top of the sward by extending its petioles (Thompson and Harper 1988; Solangaarachchi and Harper 1987; Schwank *et al.* 1986). At cutting, therefore, the amount of leaf area removed for individual harvests as a proportion of leaf area present in the swards was almost the same for both cutting heights except for the fourth and sixth harvests (Figure 4B).

In spite of the lower number of leaf bearing nodes clover grown in monoculture cut at a height of 10 cm had more than twice as much residual leaf area index after each harvest in 1991 as compared with the 4 cm cutting height (Figure 5B). Clover grown in monoculture and cut at a height of 4 cm showed a horizontal growth pattern (sward height 33 cm in summer) and densely growing stolons closer to the ground. On the other hand swards cut at 10 cm showed a more vertical growth habit (38 cm) and produced large clover leaves with long thick petioles. Therefore the remaining leaf area after harvesting was more at a cutting height of 10 cm than at 4 cm.

Clover grown in clover grass mixtures also showed a vertical growth pattern, irrespective of the two cutting treatments. The sward heights, measured, in summer were about 47 and 45 cm for 10 and 4 cm respectively. As indicated previously the residual LAI between the two cutting treatments were small. The findings of Solangaarachchi (1985) show that, when a sward is allowed to grow tall, the growth of clover changes from richly branching to a largely linear growth form, and also the stolons grow into each other or grow into the grass swards.

5.4 The factors governing clover growth in field

It is suggested that the changes in the growth of white clover and yield, as seen in this study, were due to the following factors: The better light interception by white clover monocultures cut at a height of 4 cm increased clover growth by increasing the number of leaf bearing nodes and the number of growing points as observed in 1991. White clover, grown in monocultures and cut at 10 cm invested more resources into increasing the leaf area and extending the petioles which occurred at the cost of branching. This was also the case for clover grown in clover-grass mixtures. It is assumed that the competition for light by white clover was the most important factor for regrowth after defoliation. Menzi (1988) reported that the absorption of irradiance by white clover leaves influenced clover growth by increasing leaf area and the rate of branching. Schwank *et al.* (1986) also demonstrated that, in white clover, competition for light is the main factor which limits its proportion in mixed swards. In addition to the above factors, Solangaarachchi and Harper (1985) and Thompson and Harper (1988) observed that low light intensity, caused by shading in lower layers of the sward causes changes in the quality of light, mainly the reduction of the red/far red ratio (670/730 ηm). This causes a lower rate of leaf appearance and branching of white clover.

5.5 The effects of two cutting treatments on regrowth and yield of grass

Grass cut at a height of 10 cm ensured a better overwintering as compared with at 4 cm. When grass was cut to 10 cm, it had more residual leaf blades and sheaths as compared with the grass cut at 4 cm which had exclusively more leaf sheaths. Possibly the percentage of readily available carbohydrates in the stubble and the proportion of associated root material (the root/shoot ratio) may have been high in the swards cut in 10 cm height too. Therefore, in early spring and after each harvest, regrowth was faster in these swards as compared with the 4 cm cutting height. Therefore, the annual grass dry matter yields (Table 1B) were high though not significant in grass monocultures and in clover grass mixtures in the 10 cm cutting treatment in 1992. Wilman and Shrestha (1985) reported that perennial ryegrass can adapt itself when cut into a higher canopy by extending the length of its leaf blades.

5.6 Seasonal patterns of clover and grass growth

Except for the harvest in June 1990 (Figure 2A) due to increasing temperatures and light intensities (Figure 1), clover growth increased in clover monocultures until August and then declined. In the preceding year there was a continuous decrease in clover yield from May to October (Figure 2B).

During the first year of production it appears that there were two distinct phases in sward growth in the mixtures, one of which favoured the growth of grass over that of clover and vice versa. Firstly, grass grew faster than clover from May until

June and by July both clover and grass growth had attained almost the same levels. In the second phase, the clover grew faster than that of grass between August and October (Figure 2A). The observed differences in growth may be due to changes in light intensity and temperature (Figure 1). These observations indicate that white clover competes strongly with grass. The reduction in grass growth in summer as compared to white clover is probably an indication that clover has the ability to profit from prevailing favourable environmental conditions as compared with grass. Consistent with these findings, Menzi (1991) observed that temperature and radiation had a greater effect on sward growth than did species composition or management in clover-timothy and clover-Lolium multiflorum swards. In the second production year, the growth of clover and grass in mixture was different. Grass showed the highest growth in May. Thereafter, the growth of clover and grass did not differ to any great extent up to October (Figure 2B). Contrary to the previous year, white clover did not seem to benefit more from the favourable environmental conditions. Grass growth was strongest in May in both years. Menzi (1988) also observed strongest grass growth in May and suggested that this was due to the generative growth of grass which was more affeced by temperature than by radiation.

The growth of clover and grass (dry matter yields at individual harvests and annual yields) was low during the second production year as compared with the previous year. These reductions can be explained as follows:

There is ample evidence to support that total herbage production increases with the increasing rest intervals between defoliations (Frame, 1976; Willman and Asiegbu, 1982). Since harvests took place every four weeks in 1990, but without the recommended intervals of 5 to 6 weeks in autumn, the effects were seen in 1992 where the herbage yield was low. This became more pronounced as a result of harvests at every four weeks.

Clover was damaged by the environmental conditions in February 1991 (Figure 1).

Finally the adverse effects of cool spring especially in May 1991 was also an important factor.

5.7 Consequences for management

The 4 cm cutting height used in this experiment can be compared to a continuously stocked sheep grazing system and the 10 cm cutting height can be compared with a rotational grazing system with cattle (reviewed by Frame 1990). Since sheep browse closer to the ground, this type of management results in on intensive removal of herbage as was the case in this study with a cutting height of 4 cm. Under such conditions, the productivity of the swards is also high provided that adequate recovery periods follow harvests.

On the other hand, cattle graze rather selectively, leaving more stubble. Damage to the swards is remarkably high due to treading. Under such conditions, grass growth is promoted to a greater extent than that of clover as observed in this study. The swards become more, vulnerable especially during winter. With such ,management practises the productivity of swards is also low with much left over.

5.8 Conclusions

The two cutting heights had no influence on the regrowth of clover after defoliation. Clover regrew faster when cut to a height of 4 cm as compared to a height of 10 cm.

During the first year of production, the variations observed in clover dry matter yields were less affected by the cutting height. However, during the second year, an increase in clover dry matter yields from clover monocultures and clover-grass mixtures when cut to 4 cm as opposed to 10 cm is related to an increased number of leaf bearing nodes and clover growing points.

Increased growth of grass in clover-grass mixtures cut at 10 cm in the second year also had a negative effect on the clover proportion in mixtures.

These results suggest that, the most predictable response of clover to a lower cutting height (4 cm) in the field is an increase in the number of growing points. In the clover-grass mixtures, the relative responses of clover and grass are also important in this respect. Therefore, white clover yield and persistence can be increased by selecting an appropriate cutting height.

4. SYMBIOTIC NITROGEN FIXATION OF WHITE CLOVER (*TRIFOLIUM REPENS* L.) GROWN IN MONOCULTURES AND IN WHITE CLOVER-RYEGRASS (*LOLIUM PERENNE* L.) SWARDS USING ^{15}N ISOTOPE DILUTION TECHNIQUE AT TWO CUTTING HEIGHTS.

1. ABSTRACT

The aim of this study was to investigate whether symbiotic nitrogen fixation in the field is a factor in limiting plant growth because of repeated defoliation. At the same time, other possible governing factors on symbiotic nitrogen fixation were elucidated. For this purpose the effect of two cutting heights (4 and 10 cm above ground level) on the symbiotic nitrogen fixation of white clover swards under field conditions was investigated. A two-year experiment was conducted in Eschikon, Switzerland, using white clover (*Trifolium repens* L. cv. "Ladino") monocultures and white clover-perennial ryegrass (*Lolium perenne* L. cv. "Bastion") mixtures. After each harvest, the plots were fertilized with 3 g $^{15}N \cdot m^{-2}$ (equivalent to 30 kg $^{15}N \cdot ha^{-1} \cdot cut^{-1}$ or 180 kg $^{15}N \cdot ha^{-1} \cdot a^{-1}$ respectively).

In both production years, white clover grown in mixture with grass had a significantly higher percentage of nitrogen from symbiotic fixation as compared to clover grown in monocultures. This phenomenon is attributed to the strong competitiveness of ryegrass for nitrogen uptake. Consequently, white clover grown in the clover-ryegrass mixtures was more dependent on symbiotic nitrogen fixation as compared to that grown in monoculture. The annual clover nitrogen yields derived from symbiosis were closely related to the clover proportion of the harvested herbage. In the second production year, the nitrogen that was apparently transferred from clover to grass accounted for about one fourth of the total nitrogen being harvested from grass. The cutting height did not influence symbiotic nitrogen fixation selectively as opposed to the uptake of mineral nitrogen from the soil. Therefore it can be concluded that in the present experiment symbiotic nitrogen fixation did not limit clover growth.

It is proposed that symbiotic nitrogen fixation is governed by the demand for total nitrogen and limited availability of mineral nitrogen rather than the availability of carbohydrate reserves. Symbiotic nitrogen fixation can be viewed as an intergrated plant growth element and not as an isolated phenomenon.

2. INTRODUCTION

The ability to fix atmospheric nitrogen and thus reduce the cost of using nitrogenous fertilizers is one of the main advantages of white clover when grown as a component in a sward. The nitrogen advantage for associated grass grown in a sward with white clover (N-transfer - Ledgrad 1991; Boller and Nösberger 1987; Henzell and Vallis 1977) is also an important factor for the maintenance of vigorous perennial pastures. However, the pasture legume, white clover, is inevitably subjected to the rigours of repeated defoliation, which are determined to a large extent by management systems. The physiological processes of both above and below ground organs must adapt quickly to defoliation in order to suppot the growth of new leaf tissues.

One particular consequence of defoliation for legumes is its effect on biological nitrogen fixation. In white clover a complete defoliation leads to a decrease in nitrogenase activity of 80-90% within a few hours, as compared to undefoliated plants (Ryle *et al.* 1985a). Similar response has been observed in other species, including lucerne (Denison *et al.* 1992; Vance *et al.* 1979) and birds-foot trefoil (Cralle and Heichel 1981). Moreover, Ryle *et al.* (1985a) and Heim Hartwig and Nösberger (unpublished data) did demonstrate that the degree of reduction in nitrogenase activity depends on the degree of defoliation: The more leaf area removed, the more nitrogenase activity is depressed. As a result one could expect that under a severe defoliation treatment, symbiotic nitrogen fixation would be less able to keep satisfying the plant's N-demand as compared to a mild defoliation. Consequently, clover growth and competitive ability in the field would depend on the cutting height.

The objective of the present study was to investigate whether defoliation can potentially limit symbiotic nitrogen fixation so that the plant's nitrogen demand can not be satisfied. Moreover we focused on the governing elements of symbiotic nitrogen fixation (^{15}N isotope dilution method) in the field. For this purpose, a clover monoculture and a clover-perennial ryegrass mixture were compared at two cutting heights (10 and 4 cm above ground level, presumably resulting in different residual leaf areas). The results indicate that clover growth is not limit by symbiotic nitrogen fixation; it is tuned to the clover's requirement for symbiotically fixed nitrogen.

3 MATERIALS AND METHODS

3.1 Site, plant material and design of the experiment

A two-year field experiment was conducted in 1990 and 1991 in Eschikon (550 m above sea level) near Zurich, Switzerland. The soil type was eutric cambisol containing a clay content of 19% and a pH of 7.1, with satisfactory levels of P_2O_5 and K_2O. The experimental site had been cropped with maize and oil raddish in 1988 and phacelia in 1989. The experiment was set up in autumn, 1989 as follows: After ploughing and levelling the field, 10 g P_2O_5, 30 g K_2O and 3.5 g $Mg{\cdot}m^{-2}$ (100 kg P_2O_5, 300 kg K_2O and 35 kg $Mg{\cdot}ha^{-1}$) were applied as foscal (15% P_2O_5, 30% K_2O) and potassium and magnesium salts (26-30% K_2O, 6% Mg). The following species were sown, using a cyclone seeder.

1. White clover (*Trifolium repens* L.) cv. "Ladino"- monoculture (1.4 $g{\cdot}m^{-2}$).
2. White clover (*Trifolium repens* L.) cv. "Ladino" (0.4 $g{\cdot}m^{-2}$) and perennial ryegrass (*Lolium perenne* L.) cv. "Bastion" (1.0 $g{\cdot}m^{-2}$) mixture.
3. Perennial ryegrass (*Lolium perenne* L.) cv. "Bastion"- monocultu re (1.4 $g{\cdot}m\text{-}^{2}$).

The seeds were pressed slightly with a roller. After germination, 3 g $N{\cdot}m^{-2}$ (equivalent 30 kg $N{\cdot}ha^{-1}$) was applied as NH_4NO_3 (27.5% N). Before the onset of winter two standardization cuts were done at a height of 4 cm and weeds removed manually.

The experiment was designed as a randomized block with four replicates and 6 treatments. Perennial ryegrass monocultures were used as a reference crop to estimate the symbiotic nitrogen fixation by the ^{15}N-isotope-dilution method. The plot size was 4 X 1.40 m.

During the experiment, cuttings were done at 4 and 10 cm heights from the ground level, respectively.

3.2 Harvest plan

First production year (1990)

In mid-March and after each harvest, all the plots were fertilized with 3 g $N \cdot m^{-2}$ (30 kg $N \cdot ha^{-1}$) applied as $(^{15}NH_4)_2SO_4$ (21 % N, including 600 g of 5 atom % ^{15}N, Isotec Inc, Ohio, USA). Total nitrogen application was 18 g $N \cdot m^{-2} \cdot a^{-1}$ (180 kg $N \cdot ha^{-1} \cdot a^{-1}$ for 6 cuts). The fertilizer was dissolved in 1 L water and applied superficially to the plots.

In the last week of April a standardizing harvest was done at 4-5 cm stubble height. Thereafter, at each harvest, the plots were cut at either 4 or 10 cm heights at four-week intervals until mid-October. A total of 6 harvests were done in this year. After the last harvest, clover and clover-grass sub plots were destructively harvested. This was done by digging out three 10 X 10 cm areas from the central zone of each plot.

Second production year (1991)

During the second production year, seven harvests were done at four-week intervals from the end of April to mid-October. NPK fertilization of the plots were done first in March in the same way as described for the first production year.

For the first harvest the results are shown only for the destructive harvest. However, 226 g of 10 atom % excess of $^{15}N \cdot cut^{-1}$ was applied. Partial destructive harvests were done in April, July and at the end of the experiment in October.

3.3 Herbage sampling

The herbage of the 50 x 50 cm central zone of each 1 m^2 sub-plot was harvested and seperated into grass, clover, and unsown species. The seperated herbage material was dried at 65°C for 48 h. The herbage yield of the rest of the plot was also harvested and dried at 105°C for 24 h. Destructively harvested stolons were freeze-dried.

3.4 Determination of Kjeldhal nitrogen and ^{15}N analysis

Grass and clover material were ground. Kjeldhal N concentration was determined in 500 mg sub-samples digested with concentrated H_2SO_4 (98%) and H_2O_2 (20%) in a pyrex tube for 3 hours at 300°C. Distillation and titration was done by using a Kjeldhal Auto 1030 Analyzer (Tecator, Höganas, Sweden). The ammonium of a sub-sample of the concentrated distillates was oxidized to N_2 with lithium hypobromite in a degassed Rittenberg tube (Sprinson and Rittenberg 1949). In 1990, the atom % excess ^{15}N was determined by mass spectrometry (Consolidated Nier, Modified by Paul Scherrer Institute, Würenlingen, Switzerland). In 1991, the atom % excess ^{15}N was analyzed by the the same method used as in the "Laboratory of Isotope Service"s in Los Alamos, NM, USA.

3.5 Calculation of nitrogen from symbiosis and apparent clover to grass transfer

Percentage of nitrogen as derived from symbiosis ($\%N_{sym}$): This was calculated for each growth cycle according to the following equation described by McAuliffe *et al.* (1958).

$$\%N_{sym} = \left(1 - \frac{\text{atom \% } ^{15}\text{N- excess in white clover [fixing crop]}}{\text{atom \% } ^{15}\text{N- excess in ryegrass [reference crop]}}\right) \times 100$$

In this study grass monocultures were used as a reference crop to calculate the symbiotic nitrogen fixation of clover monocultures as well as that of the clover-grass mixtures.

Apparent clover to grass transfer (% N_{trans}): In the clover grass mixtures, the proportion of N as derived from the transfer of fixed N from white clover to associated ryegrass was estimated from the following equation (Vallis *et al.*1977, and Broadberd *et al.* 1982).

$$\% \text{ N transferred} = \left(1 - \frac{\text{atom } \%^{15}\text{N excess in ryegrass in mixture}}{\text{atom } \%^{15}\text{N excess in ryegrass in pure stand}}\right) \times 100$$

3.6 Analysis of starch reserves in stolons

To remove the mono and the di-sacharides, approximately 50-80 mg sub-samples of ground, freeze-dried stolon material were incubated in plastic tubes with 5 ml 80% ethanol at 60°C for 30 minutes. The samples were then centrifuged for 10 min. The sediment was incubated again with 3 ml ethanol under the aforesaid conditions and centrifuged again.

The pellet left in the tube was heated in a water bath at 100°C after adding 2 ml of distilled water and 1 ml Thermamil solution (Novo ferment AG, Dittigen, Switzerland). After cooling, a buffer (pH 4.6) was added, and the pH of the solution was adjusted to 4.6 using 0.1 M HCl solution. Thereafter, 3 ml of an amyloglucosidase enzyme (Boehringer Mannheim, Germany) was added, mixed well and the volume was adjusted to 25 ml by adding distilled water. The samples were then filtered through a folded filter paper (Schleicher and Schüll, Switzerland, ø 125 mm) into plastic test tubes.

To a sample volume of 0.05 ml (in plastic spectrophotometer curvettes), the following reagents (Boehringer Mannheim, Germany) were added:

0.5 ml buffer solution (pH 7.6)
0.05 ml Adenosine triphosphate (ATP)
0.05 ml Nicotinamide adenine dinucleotide phosphate (NADP)
0.95 ml distilled water
0.01 ml Hexokinase and glucose 6 phosphate dehydrogenase enzyme (HK G 6P DH)

After 15 minutes the absorption of NADP was measured at a wavelength of 340nm, in a spectrophotometer (Hitachi U2000, Japan)). Starch concentration was calculated from NADPH formation according to the equations derived by Amado (1980).

3.7 Statistical analysis

Statistical analyses of the experimental data were made using the programme package SAS (Statistical analysis system , SAS Institute, Cary, North Carolina, USA).

4 RESULTS

4.1 Percentage nitrogen derived from symbiosis (% N_{sym})

The proportion of nitrogen derived from symbiosis (% N_{sym}) was significantly higher in clover grown in clover-grass mixtures as compared to clover grown in monocultures under both cutting heights during both production years (Figure 1). This was the case in all harvests in 1990 (Figure 1A) and, with the exception of the last harvest, in 1991 (Figure 1B).

Clover grown in clover-grass mixtures cut at a 10 cm height assimilated a higher proportion of nitrogen from symbiosis (% N_{sym}) as compared to the plots cut at 4 cm height. This was the case at all harvests, except at the first one, in both production years. Indeed, the values were significant only at the fifth harvest in 1990 (Figure 1).

The annual proportions of nitrogen derived from symbiosis were significantly higher in clover grown in clover grass mixtures as compared with clover grown in monocultures in both production years under both cutting treatments (Table 1).

In addition, the annual proportions of nitrogen derived from symbiosis were higher in clover grown in clover-grass mixtures in the 10 cm cutting treatment as compared with the 4 cm cutting treatment in both production years (Table 1).

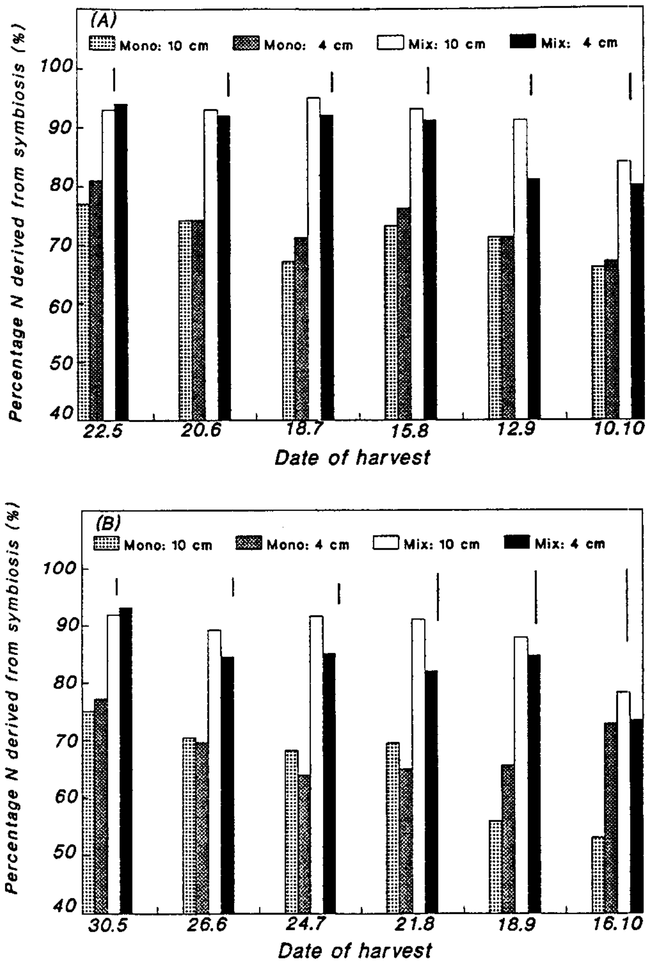

Fig. 1: Percentages of N derived from symbiosis (% N_{sym}) as determined by ^{15}N dilution at individual harvests for clover grown in monocultures and for clover grown in clover-grass mixtures, respectively. Harvests were done at two cutting heights (10 and 4 cm, respectively).

(A) First production year (1990): (B) Second production year (1991).

Bars indicate least significant differences ($LSD_{0.05}$) for means of 4 replicates.

4.2 Clover nitrogen yields from symbiosis.

The time course of the clover nitrogen yields derived from symbiosis at individual harvests followed the same pattern as the clover dry matter yields (Figure 2 page 17). This was the case for clover grown in monocultures and for clover grown in clover-grass mixtures during both production years under both cutting heights (Figure 2).

Clover grown in monocultures reached significantly higher nitrogen yields from symbiosis during the first four harvests in 1990 as compared with clover grown in clover-grass mixtures, in both cutting treatments (Figure 2A). In 1991, the above differences were observed only at the first two harvests (Figure 2B).

Clover grown in monocultures cut at a height of 4 cm reached higher nitrogen yields from symbiosis than clover grown in monocultures cut at a 10 cm height during both years at all harvests, except at the fifth one in 1990. However, these differences were significant only at the fourth and sixth harvests in 1990 (Figure 2A), whereas in the proceeding year significant differences were observed at the first and sixth harvests (Figure 2B).

The effects of the two cutting treatments on nitrogen yields from symbiosis derived by clover grown in clover-grass mixtures during both years were very minor (Figure 2).

In 1990, though not significant, the total annual yields of clover nitrogen derived from symbiosis were higher in 4 cm cutting treatments as compared with 10 cm cutting treatments both in monocultures and in clover- grass mixtures (Table 1).

In 1991, the same trend was observed both in clover grown in monocultures and in clover-grass mixtures. However, the differences between 10 cm and 4 cm cutting treatments were significant for clover grown in monocultures (Table 1).

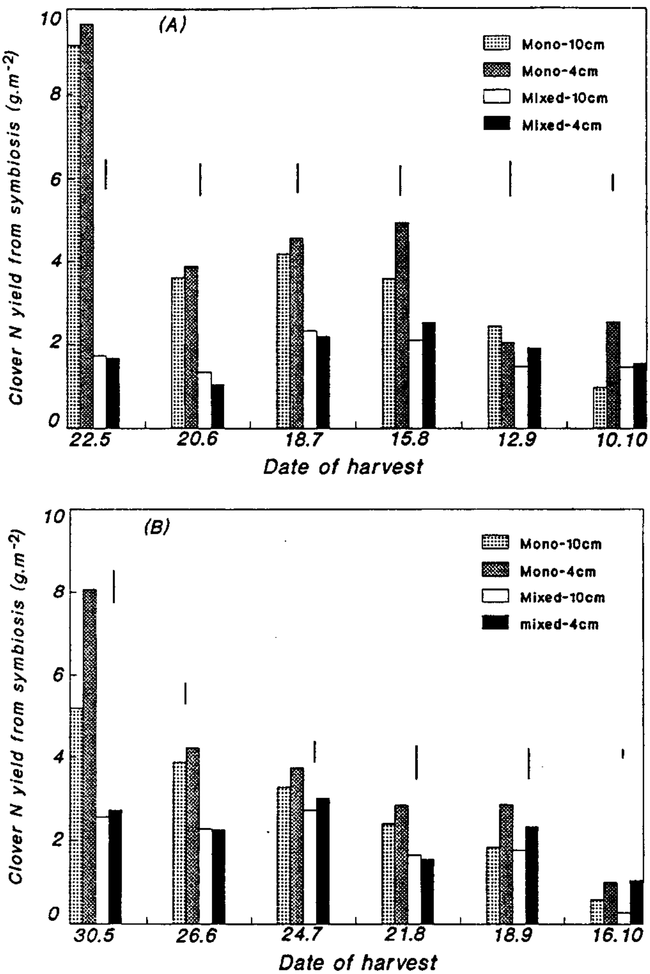

Fig. 2. Clover N yields ($g \cdot cm^{-2}$) derived from symbiosis as determined by ^{15}N dilution at individual harvests for clover grown in monocultures and for clover grown in clover-rye grass mixtures, respectively. Harvests were done at two cutting heights (10 and 4 cm, respectively)

(A) First production year (1990): (B) Second production year (1991).

Bars indicate the least significant differences ($LSD_{0.05}$) for means of 4 replicates.

Total annual nitrogen yields of clover and grass grown in monocultures and in clover-grass mixtures are given in Table 1.

Table 1: Total annual nitrogen yields of clover and grass grown in monocultures or in clover-grass mixtures in the first and second production years, respectively (Means of 4 replicates).

Variable	Cutting height (cm)	mono-culture 1990	mixture 1990	mono-culture 1991	mixture 1991
Total Grass N yield ($g \cdot m^{-2} \cdot a^{-1}$)	10	11.718	19.886	11.034	16.080
	4	11.856	21.564	8.928	14.886
	$LSD_{(0.05)}$	2.136	4.183	3.156	5.208
Grass N yield from transfer ($g \cdot m^{-2} \cdot a^{-1}$)	10	-	1.712	-	4.121
	4	-	2.482	-	3.843
	$LSD_{(0.05)}$		1.269		0.728
Total clover N yield ($g \cdot m^{-2} \cdot a^{-1}$)	10	32.706	11.142	25.254	12.528
	4	36.954	12.570	32.964	16.686
	$LSD_{(0.05)}$	6.419	6.419	4.990	4.990
Clover N yield from symbiosis ($g \cdot m^{-2} \cdot a^{-1}$)	10	23.886	10.224	17.220	11.340
	4	28.194	11.046	22.800	14.082
	$LSD_{(0.05)}$	5.376	5.376	4.140	4.140
The annual % of N derived from symbiosis	10	73	92	68	90
	4	76	88	69	84
	$LSD_{(0.05)}$	5.31	5.31	6.51	6.51

4.3 Nitrogen concentration in grass

Nitrogen concentration increased from spring towards autumn in grass grown in monocultures and in grass grown in clover-grass mixtures at both cutting treatments during both production years (Figure 3). Grass grown in clover-grass mixtures had higher nitrogen concentrations as compared to the grass grown in monocultures during both years. This was always the case except at the fourth harvest in 1990 (Figure 3A) and at the first harvest in 1991 (Figure 3B). However, these differences were less apparent during the second production year. In 1990, the nitrogen concentrations in grass grown in clover-grass mixtures were significantly higher at the last two harvests as compared with the grass grown in monocultures under both cutting treatments (Figure 3A).

Grass grown in clover-grass mixtures produced higher total annual nitrogen yields as compared with grass grown in monocultures under both cutting heights in both production years. In addition, grass grown in monocultures and grass grown in clover-grass mixtures produced slightly higher total annual nitrogen yields under 10 cm cutting height as compared with the 4 cm cutting height in the second production year (Table 1).

4.4 Nitrogen concentration in clover

Clover grown in monocultures had slightly higher nitrogen concentrations as compared with clover grown in clover-grass mixtures during both production years (results are not shown). In spring and in autumn the nitrogen concentrations in clover grown in monocultures and in clover-grass mixtures had almost the same levels (4%-5%) during both production years. However, in summer lower nitrogen concentrations (3%-4%) were observed in all treatments. The two cutting treatments had no effect on the above parameters.

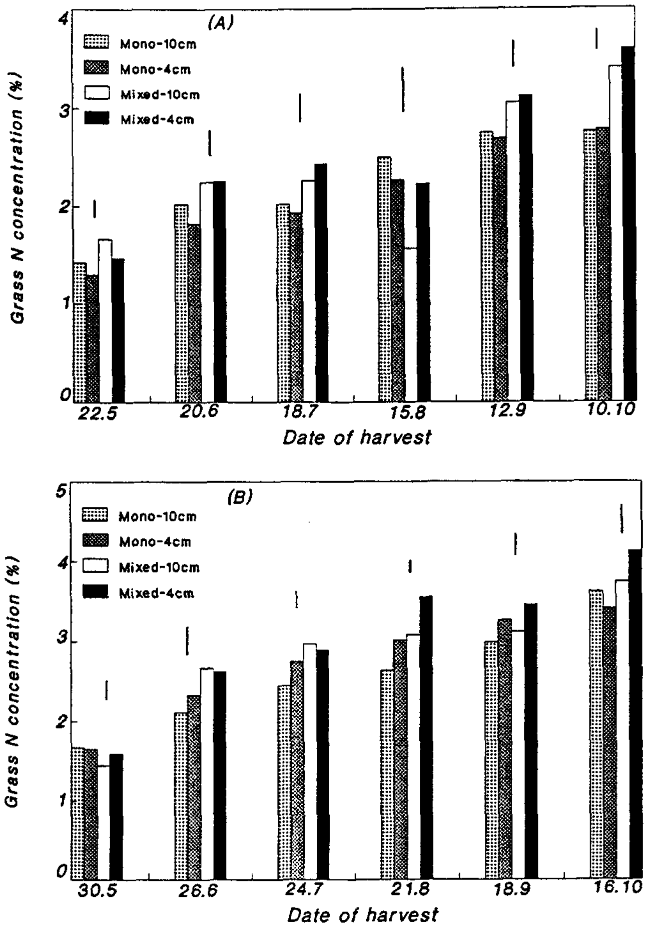

Fig. 3. The time course of N concentration as determined by the Kjeldahl method at individual harvests for grass grown in monocultures and for grass grown in clover-grass mixtures, respectively. Harvests were done at two cutting heights (10 and 4, cm respectively).

(A) First production year (1990): (B) Second production year (1991).

Bars indicate the least significant differences ($LSD_{0.05}$) for means of 4 replicates.

4.5 Apparent nitrogen transfer of fixed nitrogen

Isotopic evidence of fixed nitrogen as being apparently transfered from clover to associated grass was obtained for the last four harvests during the first production year (Figure 4A). The percentage of nitrogen as being apparently transfered from clover to grass showed an increase throughout the second production year (Figure 4B).

In 1990, there was a slight tendency to apparently transfer more nitrogen under the 4 cm cutting treatment as compared with the 10 cm cutting treatment at the last two harvests (Figure 4A).

In 1991, during the first three harvests under the 10 cm cutting treatment the apparent N transfer from clover to associated grass was greater as compared with the values under the 4 cm cutting treatment. However, at the last three harvests the opposite observation was made (Figure 4B). However, all the differences shown between the two cutting treatments in Fig. 4 were not stastically significant.

The contribution of apparent nitrogen transfer from clover to associated grass was about one fourth of the total grass nitrogen yield during the second production year (Table 1).

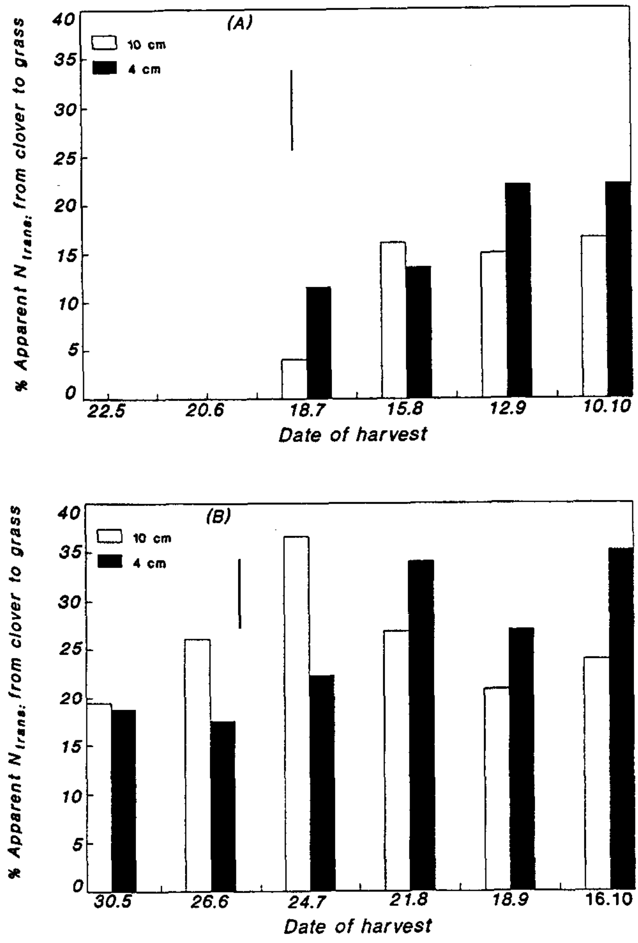

Fig. 4. Percentage of N as being apparently transferred from clover to grass as determined by ^{15}N dilution at individual harvests. Harvests were done at two cutting heights (10 and 4, cm respectively).

(A) First production year (1990): (B) Second production year (1991).

Bars indicate the standard error of means for 4 replicates.

4.6 White clover stolon starch concentrations

White clover starch concentrations were determined so as to observe the effect of the cutting height on the reserve carbohydrates in stolons. White clover starch concentrations determined at destructive harvests are given in Table 2.

Table 2: The effect of two cutting heights (10 and 4 cm) on starch concentration (%) in clover stolons (Means of 4 replicates).

Treatment	October 1990	April 1991	July 1991	October 1991
Mono: 10 cm	21.87	6.18	24.46	22.42
Mono: 4 cm	21.95	8.06	25.74	24.61
Mixture 10 cm	17.13	3.27	21.72	14.85
Mixture 4 cm	17.18	6.33	25.11	18.63
$LSD^{0.05}$	3.33	2.62	2.76	3.95

In October 1990, clover grown in monocultures had significantly higher starch concentrations in their stolons than clover grown in clover-grass mixtures. The two cutting treatments had no effect on the % of starch either in clover monocultures or in clover-grass mixtures (Table 2).

In April, 1991, the % of starch in clover stolons was very low in all the treatments. Clover grown in clover-grass mixtures and cut at a 10 cm height had a significantly lower starch concentration in its stolons as compared with that of clover in the 4 cm cutting treatment (Table 2).

By July, 1991, the starch concentrations were increased dramatically in all the treatments. The clover grown in clover-grass mixtures and cut at a height of 10 cm still had significantly lower starch concentration in stolons compared with that of clover in the 4 cm cutting treatment (Table 2).

As in October 1990, in 1991 clover grown in monocultures had significantly higher starch levels in its stolons than clover grown in clover-grass mixtures. In addition both to clover grown in monocultures and in clover-grass mixtures. The 4 cm cutting treat ments lead to higher starch concentrations in stolons as compared with the 10 cm cutting treatments. These differences were significant in clover grown in clover-grass mixtures (Table 2).

5 DISCUSSION

5.1 The factors governing symbiotic nitrogen fixation in the field.

The results clearly show that both clover grown in monocultures and clover grown in clover-grass mixtures have a high potential to fix atmospheric nitrogen (Figure 1). This was observed in both production years. As shown in the clover grown in monocultures, the application of 3 $g{\cdot}m^{-2}$ (30 $kg{\cdot}ha^{-1}{\cdot}cut^{-1}$) of mineral nitrogen did inhibit symbiotic nitrogen fixation to a level of (average over both years in both cutting treatment) 71.5% N from symbiosis (% N_{sym}). This inhibition can be considered moderate and is consistent with the results of numerous field and pot experiments, including Boller and Nösberger (1987); Nesheim *et al.* (1-990); Davidson *and* Robson (1986). It is generally observed that symbiotic nitrogen fixation in white clover as compared to other legumes (Harper and Gibson 1984), is relatively insensitive to mineral nitrogen fertilization.

Clover grown in clover-grass mixtures fixed a significantly higher percentage of nitrogen from the atmosphere (% N_{sym}) during both years as compared to clover grown in monocultures (Figure 1, Table 1). This phenomenon is attributed to the strong competitiveness of the associated grass for soil and fertilizer nitrogen, in the clover-grass mixtures. Under these conditions much less nitrogen from soil and applied fertilizers was available for clover plants than was for clover grown in the absence of competing grasses. As a result, clover grown in clover-grass mixtures had to depend almost exclusively on symbiotic nitrogen fixation. This finding is consistent with those of Harderson *et al.* (1988), who observed that the percentage of N derived from symbiosis is higher in lucerne grown in a mixture with ryegrass (80% to 95% N from symbiosis) than in pure lucerne swards (55% to 80% N from symbiosis). Ta and Faris (1987) observed similar behaviour in timothy-lucerne mixed swards where the proportion of symbiotically fixed nitrogen by lucerne in the mixture (80% to 84%) N from symbiosis) was higher than for lucerne was grown in monoculture. The fact that clover's symbiotic nitrogen fixation is stimulated by associated grass competition is clearly confirmed when comparing the two cutting treatments: Compared to the 4 cm cutting treatment, the 10 cm cutting height clearly favoured the grass growth and therefore it's competitiveness over white clover (Figure 3 page 18). Under these conditions, clover's dependency on symbiotically fixed nitrogen is even higher since the associate grass competes more for the soil and applied fertilizer nitrogen. In fact, clover grown in mixture with ryegrass cut at a 10 cm height consistently (except the first harvest in both years) had a higher percentage of symbiotically fixed nitrogen as compared to the 4 cm cutting treatment (Figure 1). On an annual basis this effect was clearly apparent but not statistically significant (Table 1). This is consistent with the findings of Nesheim and Boller (1991) who used white clover-ryegrass and white clover-timothy swards and Harderson *et al.* (1988) who used lucerne-ryegrass mixed swards. The above experimenters observed that the proportion of legume nitrogen derived from symbiosis increased when the grass competitiveness is increased through a higher proportion of grass in the mixtures. These results are consistent with the hypothesis that a clover's demand for symbiotically fixed nitrogen is governs biological nitrogen fixation. In the present experiment, such a demand was strongly dependent on the availability of mineral nitrogen in the soil, which obviously was highest in the clover monoculture, lower in the clover-grass mixture under 4 cm cutting height and lowest amid the strongest competition by associate grasses at the 10 cm cutting height in the clover-grass mixture. In fact, more detailed pot experiments are now presenting a thorough set of results confirming this hypothesis: while nitrogenase activity gradually decreases with increasing amounts of mineral nitrogen fertilization (reviewed by Streeter 1988), Jacobson (1984) did show only a very minor nitrate inhibition of nitrogenase activity in nitrate reductase minus pea mutants. Obviosly, under these circumstances, the pea plants could not assimilate nitrate and consequently had to keep fixing nitrogen from the atmo sphere. Likewise, Lindstörm (1984), Kessler *et al.* (1990) and Herdina and Silsbury (1990) demonstrated that the dominating effect of the plant's N-sink activity on nitrogenase activity.

The often suggested idea that carbohydrate concentration in the plant is regulates nitrogen fixation can not be confirmed with the present data. By conversely, the starch concentration in the stolons of white clover (Table 2) were lowest where nitrogen fixation was highest (Figure 1, Table 1). Therefore, it can be concluded that in the present experiment the plant carbohydrate concentration did not affect symbiotic nitrogen fixation. Likewise, Lüscher (1989) has shown that the total non-structural carbohydrates (TNC) in clover stolons had no influence on the % N derived from symbiosis under similar conditions. These results from the field are consistent with the results from growth cabinet experiments by Hartwig *et al.* (1990), who observed no differences in the response of nitrogenase activity to defoliation among plants with different TNC concentrations. The same study further showed no consistency between the time courses of nitrogenase activity and TNC concentrations in roots and stolons during regrowth. Likewise, Vance *et al.* (1979) did not see a correlation between nitrogenase activity and nodule starch reserves after the defoliation of lucerne. Culvenor *et al.* (1989) used CO_2 and supplemented light to recover net photosynthesis after partial defoliation of subterranean clover, but this did not prevent a decline in nitrogenase activity. All these facts represent considerable evidence against the control of nitrogenase activity by carbohydrate availability.

The latter finding does not mean that the energy supply to the nitrogenase does not affect nitrogen fixation; however, recent studies rather to indicate that oxygen supply is the reason why nitrogenase activity decreases after defoliation (Hartwig *et al.* 1987; 1990; Denison *et al*, 1992). Whether this variable oxygen supply to the nodules is influenced by the nodule carbohydrate utilization, as suggested by Vance and Heichel (1991) or is governed by the plant's nitrogen sink, remains open at present.

5.2 Symbiotic nitrogen fixation does not limit the growth of white clover

Clover grown in monocultures produced higher annual nitrogen yields from symbiosis in both production years as compared to clover grown in clover-grass mixtures in both cutting treatments (Table 1). This is not suprising since clover grown in monocultures had nearly 100% of clover content in harvested herbage in both years, as compared to a 30% - 40% clover content in the clover-grass mixture. These results clearly show that the clover content in the harvested herbage is the main factor that determining the yield of fixed nitrogen. Boller and Nösberger (1987) likewise observed that in white and red clover rye grass mixtures, the N yields derived from symbiosis were closely related to the clover content in the mixture. Mallarino *et al.* (1990) observed that in white clover, red clover and birdsfoot trefoil - tall fescue mixed swards the fixed N yields increased linearly as legume proportion increased in red clover-tall fescue and birdsfoot trefoil-tall fescue mixtures.

Table 3: The annual clover N yields from symbiosis per one percent of clover

Treatment	*cutting height*	**1990**	**1991**
Clover monoculture ($g \cdot \%^{-1} \cdot a^{-1}$)	10 cm 4 cm	2620 3060	1840 2400
Clover-grass mixture ($g \cdot \%^{-1} \cdot a^{-1}$)	10 cm 4 cm	2690 2970	3770 3430
$LSD_{0.05}$		189.0	118.0

From numerous pot experiments we know that the complete defoliation of white clover plants leads to a reduction of 80 to 90% of the initial nitrogenase activity within a few hours (Ryle *et al.* 1985a; Hartwig *et al.* 1987). Infact, Heim (personal communication) observed that the nitrogenase activity decreased steadily when 50 percent or more leaves were removed. In the present experiment, the 4 cm cutting height corresponded to a removal of 90% of leaf area, and the 10 cm cutting height to a removal of 75% of leaf area. In the experiments by Heim (personal communication), appropriate defoliation degrees resulted in a reduction in nitrogenase activity of 67% and 25%, respectively as compared to 86% when 100% of the leaf area was removed. As a result, one would expect that under the 4 cm cutting treatment, nitrogen fixation would suffer much more than under the 10 cm cutting treatment. However, in the present experiment the cutting height not neither affect the clover N yield from symbiosis per proportion (%) of clover (Table 3), the percentage of nitrogen from symbiosis (Figure 1, Table 1) the nitrogen concentration of clover (page).

It can be expected that after defoliation the plant's nitrogen demand is temporarily reduced according to the severity of defoliation. If defoliation selectively reduced symbiotic nitrogen fixation and thus favoured nitogen uptake from the soil, one would expect less nitrogen fixation under the 4 cm cutting height as compared to the 10 cm cutting height. However, we cannot confirm this with the present data. It can be suggested that after defoliation, symbiotic nitrogen fixation is tuned by a demand for nitrogen that does not favour symbiotic nitrogen fixation or mineral nitrogen uptake. As a result, it can be concluded that in the present experiment symbiotic nitrogen fixation was not limiting for clover growth.

5.3 Apparent transfer of symbiotically fixed nitrogen from clover to associated grass

In this study, the different isotopic ratios between grass grown in monoculture and in clover-grass mixtures strongly served to indicate that an apparent transfer of nitrogen from clover to grass occurred during both years.

In the first production year, there was initially no apparent N - transfer from clover to associated grass (Figure 5A). However in the later harvests substantial N transfer in both treatments was observed. This relatively low transfer indicates that the dead clover tissues from below or above ground remineralized in the soil with time and contributed to an indirect nitrogen transfer to the associated grass. This is consistent with the findings of Boller and Nösberger (1987) with white and red clover ryegrass mixtures. The amount of nitrogen apparently being transferred from clover to grass increased during the second production year (Figure 5B). In the present study it finally accounted for one fourth of the nitrogen harvested from grass (Table 1). These values are comparable with those obtained by Boller and Nösberger (1987) under the same ecological conditions.

5.4 Conclusions

It is concluded that the governing element for symbiotic nitrogen fixation in the field is, not the availability of carbohydrates, but rather the white clover's demand (sink) for symbiotically fixed nitrogen. This parameter is largely influenced by the availability of mineral nitrogen in the soil or competition from associated non legume plants, respectively.

Though not affected by the cutting height, the nitrogen that was apparently transferred from clover to grass in the second production year, accounted for about one fourth of the total nitrogen harvested from grass. Because of the long time it took for the nitrogen to appear in grass, it is assumed that the decomposition and remineralization of clover material (dead leaves, roots, nodules and plant exudates) was the main pathway for nitrogen transfer.

The cutting height did not selectively influence the symbiotic nitrogen fixation as opposed to the uptake of mineral nitrogen from the soil. As a result, in the present experiment, symbiotic nitrogen fixation went on providing sufficient amounts of nitrogen for the plant growth. Therefore, we can conclude that clover growth was not limited by symbiotic nitrogen fixation; it is tuned to the plant's demand for symbiotically fixed nitrogen. Symbiotic nitrogen fixation can thus be viewed as an intergrated plant growth element and not as an isolated phenomenon.

References

Acuna, H. & Wilman, D. 1988. Some effects of cutting height and phosphorous application on the growth of white clover and perennial ryegrass.- Procce-dings of British Grassland Society. Winter meeting Hurley U.K.

Amado, R. 1980. "Heutiger stand der enzymatischen analyse von lebensmitteln". Mitteilungen Gebiete Lebensmittel Hygiene.- 73: 15.

Baur-Höch, B. 1988. Einfluss von Restblattfläche und Reservekohlenhydrate auf den Wiederaustrieb von Weissklee (Trifolium repens L.).- Diss. ETH No 8580, Ph.D thesis, ETH Zurich, Switzerland.

Boatman, N.D. & Hagger, R.J. 1984. Effects of defoliation intensity on white clover seedling growth.- Grass Forage Sci. 39: 395-399.

Boller, B.C & Nösberger, J. 1987. Symbiotically fixed nitrogen from field grown white and red clover mixed with rye grass at low levels of ^{15}N fertilization.- Plant and Soil 104: 219-226.

Brereton, A.J & Carton, O.T. 1985. The effect of grass tiller density on the performance of white clover.- In Connoly, J. (ed). Nutrition Agronomy and Breeding of White Clover. Proc. EEC Workshop, Wexford, Eire. 72-79

Briseno De La Hoz, V.M. & Wilman, D. 1981. Effects of cattle grazing, sheep grazing, cutting and sward height on a grass white clover sward.- J. Agric. Sci. Camb. 97: 699-706.

Broadbend, F. E., Nakashima, T. & Grace Y. Chang. 1982. Estimation of notro-gen fixation by isotope dilution in field and green house experiments.- Agron. J. 74: 625-628.

Carlson, G.E. 1966a. Growth of clover leaves after complete or partial leaf removal.- Crop Sci. 6: 419-422.

Carlson, G.E. 1966b. Growth of clover leaves - Developmental morphology and parameters at ten stages.- Crop Sci. 6: 293-294.

Chapman, D.F.; Robson, M.J. & Snaydon, R.W. 1990. The carbon economy of developing leaves of white clover (Trifolium repens L.).- Ann. Bot. 66: [illegible] 628.

Chapman, D.F., Robson, M.J. & Snaydon, R.W. 1991. Quantitative carbon distribution in clonal plants of white clover (Trifolium repens L.): Source Sink relationships during undisturbed growth.- J. Agric. Sci. Camb. 116: 229-238

Clark, J., Kat, C. & Santhirasegaram, K. 1974. The effects of changes in height and growth on the digestible organic matter production and botanical composition of perennial pasture.- Grass Forage Sci. 29: 269-273.

Culvenor, R. A., Davidson, I. A. & Simpson, R. J. 1989. Regrowth by swards of subterranean clover after defoliation. 1. Growth and non structural carbohydrate and nitrogen content - Ann. Bot. 64: 545-556.

Cralle, H. T. & Heichel, G. H. 1981. Nitrogen fixation and vegetative regrowth of alfalfa and birdsfoot trefoil before and after successive harvests or floral debudding - Plant Physiol. 67: 898-905.

Davidson, I. A. & Robson, M. J. 1986. Effect of contrasting patterns of nitrate application on the nitrate uptake, nitrogen fixation, nodulation and growth of white clover - Ann. Bot. 57: 331-338.

Davidson, I.A. & Robsen, M.J. 1990. Short term effects of nitrogen on the growth and nitrogen nutrition of small swards of white clover and perennial rye grass in spring.- Grass Forage Sci. 45: 413-421.

Denison, R.F., Hunt, S & Layzell, D.B. (1992). Nitrogenase activity, nodule respiration, and O_2 permeability following detopping of Alfalfa and Birdsfoot trefoil.- Plant Physiol. 98: 894-900.

Fulkerson, W.J. & Michell, P.J. 1987. The effect of height and frequency of moving on the yield and composition of perennial rye grass-white clover swards in the autmn to spring period .- Grass and Forage Sci. 42: 169-174.

Frame, J. & Boyd, G. 1986. Factors affecting production from grass-white clover swards.- West Scotland Agricultural College Annual Report, 173-190.

Frame, J. (1990). Exploiting Grass/White clover swards 1. Agronomy. Training course at Koldkaergård Landboskole, Århus, Denmark, March.

Hardarson, G., Danso, S. K. A. & Zapta, F. 1988. Dinitrogen fixation measurements in alfalfa -ryegrass swards using nitrogen -15 and influence of the reference crop.- Crop Sci. 28: 101-105.

Herdina, & Silsbury, J.H. 1990. The effect of reduction in the number of nodules on nodule activity of Faba Bean (Vicia faba cv. Fiord).- Ann. Bot. 65: 473-481.

Harper, J. E & Gibson, A: H. 1984. Different nodulation tolerance to nitrate among legume species.- Crop Sci. 28: 101-105.

Hartwig, U., Boller, B. & Nösberger, J. 1987. Oxygen supply limits notrogenase activity of clover after defoliation.- Ann. Bot. 59: 285-291.

Hartwig, U., Baur-Höch, B., Boller, B. & Nösberger, J. 1990. The influence of carbohydrate reserves on the response of nodulated white clover to defoliation.- Ann. of Bot. 65: 97-105.

Harris, W. & Rhodes, I. 1983. Observations on environmental and genotypic influences on the over wintering of white clover.- J. App. Ecol. 20: 609-624.

Henzell, E. F. & Vallis, I. 1977. Transfer of nitrogen between legumes and other crops. In Biological Nitrogen Fixation in Farming Systems of the Tropics (eds A. Ayanaba and P.J. Dart), pp. 73-78. J. Wiley & Sons, Chichester.

Jacobson, E. 1984. Modification of symbiotic interaction of Pea (Pisum sativum L.) and Rhizobium leguminosarum by induced mutations.- Plant and Soil 82: 427-438.

Kessler, W., Boller, B.C. & Nösberger, J. 1990. Distinct influence of root and shoot temperature on nitrogen fixation by white clover.- Ann. Bot. 65: 341-346.

King, J., Lamb, W.C. & Mcgregor, M.T. 1978. Effect of partial and complete defoliation on regrowth of white clover plants.- Grass Forage Sci. 33: 49-55.

Kishi, H. 1973. Studies on competition between grass and legumes in a mixed sward 1. The growth of two species in a Cocks foot/Ladino clover mixed sward.- Proc. of the Crop Sci. Soc. Japan. 42: 397-406.

Lindstörm, K. 1984. Analysis of factors affecting in situ nitrogenase (C_2H_2) v activity of Galega orientalis, Trifolium pratence and Medicago sativa in temperate conditions.- Plant and Soil 79: 329-341.

Ledgrad, S. F. 1991. Transfer of fixed nitrogen from white clover to associated grasses in swards grazed by dairy cows estimated using ^{15}N methods.- Plant and Soil 132: 215-223.

Lüscher, A. 1989. Ueberwinterung und Frühlingsaufwuchs von Weissklee (Trifolium repens L.) - Dynamik der Kohlenhydratenreserven und der biologischen N_2-Fixierung.- Diss. ETH No. 8977. Ph. D thesis, ETH Zurich, Switzerland.

Mallarino, A. P., Wedin, W. F., Perdomo, C.H. & West, C. P. 1990. Legume species and proportion effects on symbiotic dinitrogen fixation in legume grass mixtures.- Agron. J. 82: 782-795.

McAuliffe, C., Chamblee, D.S., Uribe-Arango, H & Woodhouse, W.W. jr. 1958. Influence of inorganic nitrogen on nitrogen fixation by legumes as revealed by ^{15}N.- Agron. J. 50: 334-337.

Menzi, H. 1988. Einfluss von Witterung und Bestandstruktur auf das Wachstum verlauf von weissklee (Trifolium repens L.) und die Ertragsbildung von Grass-Weissklee Gemengen.- Diss. ETH No 8702. Ph.D Thesis, ETH Zurich, Switzerland.

Menzi, H., Blum, H & Nösberger, J. (1991). Relationship between climatic factors and the dry matter production of swards of different composition at two altitudes.- Grass Forage Sci. 46: 223-230

Moustafa, Esam, Roger Ball, & Tro Field. 1969. The use of acetylene reduction to study the effect of nitrogen fertilizer and defoliation on nitrogen fixation by field grown white clover.- N.Z. J. Agric. Res. 12: 691-696.

Nesheim, L. & Boller, B.C. 1991. Nitrogen fixation by white clover when competing with grasses at moderately low temperatures.- Plant and Soil 133: 47-56.

Nesheim, L., Boller, B.C., Lehmann, J. & Walther, U. 1990. The effect of nitrogen in cattle slurry and mineral fertilizers on nitrogen fixation by white clover - Grass Forage Sci. 45: 91-97.

Patterson, D & Laidlow, S. 1990. News Letter Issue 25, Working group of detailed sward management IGER, Welsh Plant Breeding Station, Aberyswith, U.K.

Ryle, G. H. A., Powell, C. E. & Gordon, A. J. 1985a. Defoliation in white clover regrowth, photosynthesis and nitrogen fixation.- Ann. Bot. 56: 9-18.

Ryle, G. H. A., Powell, C. E. & Gordon, A. J. 1985b. Short term changes in CO_2 evolution associated with nitrogenase activity response to defoliation and photosynthesis.- J. Exp. Bot. 36: 634-643.

Schwark, O., Blum, H. & Nösberger, J. 1986. The influence of irradiance distribution on the growth of white clover in differently managed canopies of permanent grasslands.- Ann. Bot. 57: 273-281.

Solangaarachchi, M. 1985. The nature and control of branching pattern in white clover (Trifolium repens L.).- Ph.D Thesis University of Wales, Bangor, U.K.

Solangaarachchi, M. & Harper, J.L. 1987. The effect of canopy filtered light on the growth of white clover.- Oecologia 72: 372-376.

Sprinson, D.B. & Rittenberg, D. 1949. The rate of utilization of ammonia for protein synthesis.- J. Biol. Chem. 180: 707-714.

Streeter, J. 1988. Inhibiton of legume nodule formation and nitrogen fixation by nitrate.- C.R.C. critical reviews of plant Science. 7(1): 1-23 by C.R.C. press.

Ta, T.C. & Faris, M. A. 1987. Effects of alfalfa proportions and clipping frequencies on Timothy -alfalfa mixtures 11. Nitrogen fixation and Transfer.- Agron. J. 79: 820-824.

Thompson, L. & Harper, J.L. 1988. The effect of grasses on the quality of transmitted radiation and its influence on the growth of white clover.- Oecologia 75: 343-377.

Vallis, I., Henzell, E.F. & Evans, T. R. 1977. Uptake of soil nitrogen by legumes in mixed swards.- Aust. J. Agric. Res. 28: 413-425.

Vance, C. P., Heichel, G.H., Barnes, D.K ., Brian, J.W & Lois, E.J. 1979. Nitrogen fixation, nodule development and vegetative regrowth of alfalfa (Medicago sativa L.) following harvest.- Plant Physiol. 64: 1-8.

Vance, C. P., & Heichel, G.H., 1991. Carbon in N_2 fixation: Limitation or exquisite adaptation.- Annu. Rev. Plant Physiol. Plant Mol. Biol. 42: 373-392.

Williams, R.F. 1954. Estimation of leaf area for agronomic and plant physiological studies.- Aust. J. Agric. Res. 5: 235-256.

Williams, R.F., Evans, L.T. & Ludwig, L.J. 1964. Estimation of leaf area for clover and lucerne.- Aust. J. Agric. Res. 15: 231-233

Wilman, D. & Asiegbu, J.E. 1982. The effects of clover variety, cutting interval and nitrogen application on herbage yields, proportion and heights in perennial ryegrass-white clover swards.- Grass Forage Sci. 37: 1-13.

Wilman, D. & Shrestha, S.K. 1985. Some effects of canopy height on perennial rye grass and white clover in a field sward.- J. Agric. Sci. Cam. 105: 79-84.

Woledge, J & Dennis, W.D (1982). In Corall A.J (ed). Efficient grassland farming. Proceedings of the ninth General Meeting of the European Grassland Federation published as Occasional Symposium of the British Grassland Society No. 14, pp. 311-312

CURRICULUM VITAE

....

5.9.1952	Born to Charles Warnasuriya and Wimala nee de Silva in Galle, Sri-Lanka
1957-1972	Primary, secondary and Advanced level education in Galle, Sri-Lanka.
1973-1978	Student at the Faculty of Agriculture, University of Peradeniya, Sri-Lanka.
1978	B.Sc. (Agric.) degree with second class honours.
1978-1979	Assistant lecturer in the Dept. of Animal science, Faculty of Agriculture, University of Peradeniya, Sri-Lanka.
1979	Married to Prasantha Seresinhe. Avantha was born in 1981.
1979-1984	Assistant lecturer in the Dept. of Animal Science, Faculty of Agriculture, University of Ruhuna, Matara, Sri-Lanka.
1985-1988	Student at the Faculty of Agriculture, Swiss Federal Institute of Technology (ETH) Zürich.
1988	Diplom Ing. Agronomy (equivalent to M.Sc) in Plant science.
1988-1992	Doctoral student in the Dept. of Plant Science, Faculty of Agriculture, ETH Zürich.

ACKNOWLEDGEMENTS

I wish to express my profound gratitude, heartful appreciation and sincere thanks to Prof. J. Nösberger for selecting an interesting theme, and for the oppurtunity given to work with his group and for his valuable criticisms and guidance at all stages. Above all for understanding my personal situation during my stay where his kindness and helpfulness I appreciate very much.

I am also thankful to Prof. P. Stamp and Dr. Willy Kessler for their criticisms and suggestions as co-examiners.

My sincere gratitude to Dr. Willy Kessler for his great help at the initial stages.
I am also greatful to Dr. U. Hartwig for his invaluable criticisms, discussions, interpretations where his help was essential for the realization of this thesis.
I would also like to extend my personal thanks to Mr. Hans Hänni and Ms. A. Allenbach, Swiss and International students who helped me in various ways during the experimental period and to all my colleages for their helpfulness and cooperation in Eschikon.

My thanks to Dr. H. Blum and Mrs. A. Dürsteler for their kind technical cooperation. To Messers Gägeler and O. Antonsen at the Paul Scherrer Institute, Würenlingen for permitting to work with the Masspectrometer.

Last but not least my thanks to all members of Gruppe Nö. The pleasant atmosphere, and the team spirit of the group during excursions and ski weekends will always remain a happy memory. To Christoph and Beat for the pleasant time and for their moral support especially during the final stages.

A special thank to the Swiss Federal Stipendium Commission for financing my studies all these years and especially the ETH representatives Prof. H.W. Tobler, Dr. L. Badoux and Mrs. E. Schniderlin.

Finally to my husband Prasantha for his encouragement, love and support at all times. To my darling son Avantha for his love, patience and understanding my busy life.