DEFOLIATION MANAGEMENT OF
BIRDSFOOT TREFOIL (*Lotus corniculatus* L.)

A thesis presented in partial fulfilment of the requirements for the
degree of Doctor of Philosophy,
Institute of Natural Resources, Massey University, New Zealand

Walter Ayala
2001

This Thesis is dedicated to my wife Rossy, and our daughters Agustina and Bianca for their love, help and support

ABSTRACT

Ayala, W. (2001). Defoliation management of birdsfoot trefoil (*Lotus corniculatus* L.). Ph.D Thesis, Institute of Natural Resources, Massey University, Palmerston North, New Zealand.

Birdsfoot trefoil (*Lotus corniculatus* L.) is a forage legume widely cultivated around the world, adapted to grow on infertile, drought-prone or acid soils, and with a high feeding value and bloat safe forage. However, its persistence is poor, limited by the management of defoliation and disease incidence. Adjustments in defoliation strategies, reproductive processes and population dynamics are seen as alternatives to increase production and persistence of birdsfoot trefoil swards. The objectives of this research were to determine appropriate defoliation strategies for different birdsfoot trefoil cultivars, in terms of the frequency, intensity and timing of defoliation, and to quantify morphological and physiological adaptations and population changes in response to defoliation. A series of three field and one glasshouse experiments were conducted in Massey University, Palmerston North, New Zealand (latitude 40°23'S) and INIA Treinta y Tres, Uruguay (latitude 33°54' S) from 1997 to 2000. The cultivars evaluated were San Gabriel (Brazil), INIA Draco (Uruguay), Grasslands Goldie (New Zealand) and Steadfast (USA). Management varied in intensity of defoliation from 2 to 10 cm height, in frequency from 20 to 40 days, and in timing the start of defoliation in the first year from vegetative to late mature stages. Also, combinations of rest periods in autumn, winter and summer were studied on pure and mixed birdsfoot trefoil swards.

A preliminary short term study with Grasslands Goldie in New Zealand, showed that hard defoliation (2 cm) in spring reduced birdsfoot trefoil spring production (17%) and plant population (21%) compared with the average of laxer defoliation (6 and 10 cm). Root mass, crown mass, primary and total number of shoots/m^2 and root reserves were all reduced under hard defoliation. Early autumn rest (last cut in April) improved plant root reserves and increased spring herbage production (17%). The effects of intensity of defoliation were confirmed under controlled glasshouse conditions, where lax regimes

(6 and 10 cm) increased herbage production over intensive defoliation (2 cm) when defoliated at 20 day intervals. In spring, intensive and frequent defoliation (2 cm-20 days) reduced production and plant survival, and lax and less frequent defoliation (10 cm-40 days) resulted in herbage losses by excessive accumulation. Limited and short term plant adjustments in relative growth rate, leaf area, specific leaf area and number of leaves per plant were not enough to compensate for the excessive loss of plant tissues under severe defoliation (2 cm). The effects of defoliation intensity increased over time, reducing crown size, root mass, root diameter and root reserves of birdsfoot trefoil plants.

Cultivars evaluated in a two years study in Uruguay differed in plant habit (from semi-prostrate to erect types), winter activity (active and dormant types) and morphology. The presence of rhizomes in cv. Steadfast was observed in 17% of individual plants tested. Cultivars San Gabriel and INIA Draco were 2.6 and 2.5 times more productive than introductions from New Zealand and USA in the first and second year respectively. The group of cultivars tested showed adequate standards of forage quality, varying from 590-720 g/kg DM for digestibility of organic matter, 25-39 g/kg DM for nitrogen, 230-400 g/kg DM for acid detergent fibre and 22-31 g/kg DM for condensed tannins. In contrast with previous results, herbage production was higher for plots defoliated at 4 cm height than at 8 cm during the first year and there were no differences in the second year. Contrasts with previous experiments were attributable to an extended defoliation interval (40 days) and rest periods of approximately 6 months in the year that allowed plants to rebuild enough reserves for successive regrowths.

When growing in competition with white clover, birdsfoot trefoil production was improved by autumn rest and lax grazing (10 cm). Plant density was reduced by intensive grazing (4 cm) and by strategies that grazed swards between 9 and 12 times a year compared with those where grazing was 6 times a year. Summer spelling increased seed production of birdsfoot trefoil, achieving 11110 viable seeds/m^2 if a winter rest was also used. However, seedling emergence from soil seed reserves was only between 5-13% under grazing conditions during autumn and winter, demanding additional management practices to increase recruitment of new individuals.

The results of these studies were used to define practical management strategies to optimise the production and persistence of birdsfoot trefoil swards, and plant characteristics appropriate to Uruguayan and New Zealand conditions.

Keywords: *Lotus corniculatus* L.; birdsfoot trefoil; cultivars; defoliation management; forage production; nutritive value; persistence; plant morphology; carbohydrate root reserves; seed production; soil seed reserves; seedling emergence.

ACKNOWLEDGEMENTS

There are many people that I would like to express my gratitude for their support and contribution to the completion of this project.

I wish to thank sincerely to my chief supervisor, Professor John Hodgson (Institute of Natural Resources, Massey University) for his continuous encouragement, guidance and support over these years, providing a fertile environment of discussions and development of research skills. His accurate supervision from the start to the end, made possible to organize a split Ph.D program between New Zealand and Uruguay, and contributed to extend more the research links between both countries.

Special thanks to my co-supervisor, Dr. Peter Kemp (Institute of Natural Resources, Massey University) for provides always constructive comments, guidance and patience throughout this study. His fine sense of humor made always the difference between peaks of frustration and motivation.

My particular appreciation to my co-supervisors, Professor Ing. Agr. Milton Carámbula (National Institute of Agricultural Research of Uruguay) and Ing. Agr. Diego Risso (Head of Pastures Program of the National Institute of Agricultural Research of Uruguay), for their generous help and suggestions particularly over the trials ran in Uruguay, making this study productive.

I am extremely grateful to the National Institute of Agricultural Research of Uruguay for the support, research facilities and financial assistance, especially to the following members: Mr. J.P. Hounie and Ing. Agr. P. Bonino (past and present presidents of the INIA Board and in their name to the other Board members), Dr. E. Indarte (National Director), Dr. G. Cerizola (Human Resources) and Ing. Agr. H. Duran (Leader of Animal Production group). To Dr. F. Montossi (Head of Sheep Program of the National Institute of Agricultural Research of Uruguay) for his encouragement and help in many stages of this project.

I would like to express my recognition to New Zealand, through the Ministry of Foreign Affairs and Trade (MFAT) for provision of financial support by a NZODA scholarship. My gratitude to Mr. Mac Herrera, consul of New Zealand in Uruguay, who provided early arrangements for this scholarship. Also to the staff of the International Students Office in Massey University for the assistance provided to me and my family.

My thanks to the staff of Natural Resources Institute, and particularly to Pastoral Science Group at Massey University for their friendship and support over these years. The assistance and invaluable criticism provided by Dr. C. Matthew, Mr P. Matthews and Dr I. Valentine in different stages are well recognized. In particular, to Mr M. Alexander, Mr R. Dissanayake, Ms K. Hamilton, Ms H. Kennedy, R. Levy, I. Manley, Mr M. Osborne and others. Also to the staff of the Plant Growth Unit, Mr C. Johnstone, L. Taylor, L. Sylva and H. Logan, and late Charlie Howell of Deer Research Unit of Massey University. Very special thanks to Dr D. Woolley and Mr C. Rawlingson for provide assistance to conduct the root carbohydrate analysis.

My recognition to those people that provided seeds of evaluated cultivars, to Dr. W. Williams (Agresearch, Palmerston North, New Zealand), to Dr P. Beuselinck (USDA-ARS, Columbia, Missouri, USA) and Ing. Agr. J. Bologna (Agronomy Faculty, Uruguay).

My sincere thanks to Ing. Agr L. Helguera and Ing. Agr. G. Zorrilla, past and present Directors of INIA Treinta y Tres, to provide the best technical and human conditions to execute this project. To the staff of the “old” Estación Experimental del Este (INIA Treinta y Tres Research Station, Uruguay) for their assistance, warm support and encouragement to achieve this goal.

I would like to acknowledge the generous field and laboratory assistance given by Mr C. Carmona, Mr G. Ferreira, Mr J. Jackson and Mr N. Serron of Pastures Group of INIA Treinta y Tres, during the trials conducted in Uruguay. Their attention and accuracy during many hours that we shared running these experiments will be never forgotten. Many thanks goes to my colleague of Pastures group Ing. Agr. R. Bermúdez,

that gave me unconditional assistance. Thanks to the students Mr. C. Machado and Mr. I. Nuñez from the Agronomy Faculty of Uruguay, who co-laboured with me collecting and sharing information.

The friendship and encouragement of my fellow graduate students at Massey University provided an excellent environment, especially to Dr. S. Assuero, Dr. S. Bluett, Mr. W. Beskow, Mrs. R. Briant, Mr D. Cabrera, Ms. D. Carvalho, Dr. N. Devkota, Mrs M. Ercolin, Dr. A. Guevara, Mr. C. Hepp, Mr. F. Hughes, Mr. and Mrs. Laborde, Dr. G. Li, Mrs. H. Logan, Dr. I. Lopez, Dr. Z. Nie, Dr. S. Oppong, Dr. C. Poli, Dr. D. Real, Mrs C. Realini, Mr. A. Romera, Mr. J. Rossi, Mrs P. Salles, Mr. T. Pande, Mr. A. Wall and others.

My sincere thanks to my parents and sister for their education, support and continuous encouragement from early steps at the primary school (Escuela Rural No. 84 de Molles de Gutierrez, Lavalleja, Uruguay) to nowadays. The support of our friends, in special to my relations in Uruguay, is greatly appreciated.

Finally I want to express my gratitude to my wife Rossy and daughters Agustina and Bianca. Their technical help, tolerance, sacrifice and motivation throughout these "long and hard" years, accepting the challenge to complete this project, contributed to make this study successful.

Walter Ayala
April 5, 2001

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

1. GENERAL INTRODUCTION

1.1 INTRODUCTION

Lotus corniculatus L. (birdsfoot trefoil, BFT) is one of the most common and widely distributed forage legumes around the world. It can be found in areas across Europe, Asia, Africa, South America, North America and Australasia. BFT shows a high degree of genetic variability that confers the capabilities of a wide range of adaptation and distribution (Kirkbride, 1999; Steiner, 1999).

Approximately 4.5 million hectares of BFT are reported around the world, in mixed pastures or pure stands for grazing or forage conservation, the main areas being concentrated in Uruguay, USA, Argentina, Austria and Italy (Blumenthal and McGraw, 1999). BFT agronomy has been extensively reviewed by MacDonald (1946), Seaney and Henson (1970), Turkington and Franko (1980), Grant and Marten (1985), Jones and Turkington (1986), Blumenthal *et al.* (1994), and Frame *et al.* (1998) among others. It is particularly adapted to grow on low fertility, droughty or acid soils (Seaney and Henson, 1970) as a pioneer or alternative species, gaining the description of "lucerne of poorest soils". Its feeding value is comparable with lucerne (Marten and Jordan, 1979), and it is a bloat safe forage (Blumenthal and McGraw, 1999).

Over decades, the main problem to solve has been the persistence of BFT swards. Major factors that determine BFT stand persistence are grazing management and diseases incidence. BFT is sensitive to intensive and frequent defoliation, and when growing in warm and humid environments it can only maintain dense and productive stands for 2-3 years. The development of new cultivars with improved disease resistance could solve some of the reported problems, but this will not constitute by itself a genuine solution. In practice, there is general acceptance to consider BFT as a short-lived perennial legume under grazing (Pierre and Jackobs, 1953). A strategy to improve sward persistence can be through natural reseeding of existing soil seed banks to replace plant losses (Blumenthal and McGraw, 1999), which is effective in areas where seed production is not limited (Beuselinck and McGraw, 1989).

BFT is used in pastoral systems of Uruguay for grazing in pure or mixed swards with grasses like annual ryegrass (*Lolium multiflorum*), tall fescue (*Festuca arundinacea*) or cocksfoot (*Dactylis glomerata*), and legumes like white clover (*Trifolium repens*). Reduced stand persistence is reported, with declining herbage production and population after the second year (Formoso, 1993; Altier, 1997). Recent attempts to overcome these limitations and increase the productive life of swards are being oriented to develop new cultivars with improved disease resistance, looking for germplasm that shows a stronger root-crown system like the recently released INIA Draco (Altier, 1997). Inadequate management contributes to limited productive potential and successful adoption by farmers. There has been a continuous programme of work to provide management recommendations for new cultivars, often in environments with a certain degree of restriction.

BFT is regarded as an alternative legume species for New Zealand. However, there has been renewed interest in the species during the last decade, based on the influence of condensed tannins on its feeding value (John and Lancashire, 1981). However, the most revolutionary change over traditional cultivars and conceptual models of BFT morphology and physiology is the recent development in the USA of a prostrate cultivar with the presence of rhizomes (Beuselinck and Steiner, 1995). Vegetative reproduction appears as an alternative focus to solve problems of diseases incidence and plant reproduction.

Although there has been extensive study of the agronomy of BFT (Smith, 1962; Smith and Nelson, 1967; Nelson and Smith, 1968; Greub and Wedin, 1971; Alison and Hoveland, 1989; Li, 1989), there is little detailed information on the physiological ecology of the species. Alternative approaches need to be developed to refine BFT management. Understanding physiological and morphological characteristics of BFT plants under defoliation, plant architecture to tolerate grazing, and the manipulation of reproductive processes with a focus on population dynamics, are seen as central issues to increase BFT persistence.

1.2 OBJECTIVES

Recognising the importance of *Lotus corniculatus* in pastoral systems as an alternative legume, and acknowledging the problems in terms of achieving consistency in production, the main aim of this research project is: *to define the best grazing management to achieve adequate production and persistence in reference to New Zealand and Uruguayan conditions.*

Therefore, the **general objectives** of this study are as follows:

i) determine appropriate defoliation strategies in terms of frequency, intensity and timing of defoliation around the year for different *Lotus corniculatus* cultivars.
ii) quantify physiological and morphological adaptations under contrasting management practices.
iii) analyse population dynamics and strategies to improve *Lotus corniculatus* persistence under grazing conditions.

Additionally, a series of **specific objectives** relevant to the use of BFT in the conditions of Uruguay are explored:

i) quantify the nutritive value of BFT herbage for different cultivars pure or mixed and under different management strategies.
ii) determine levels of condensed tannins of cultivars under use.
iii) study seed production of BFT under different grazing strategies, and BFT seedling dynamics to improve plant persistence.

1.3 THESIS OUTLINE

Following a preliminary review of literature, experimental results are presented in five chapters from studies carried out in New Zealand (chapters 3 and 4) and Uruguay (chapters 5 and 6), and a final section (chapter 7) where a general overview of the information and the perspectives are presented. A brief description of each chapter follows:

Chapter 2 reviews the relevant literature on *Lotus corniculatus* management, with a general assessment of its potential as an alternative legume. Focus is on relevant problems that are limiting *Lotus corniculatus* utilisation within pastoral systems in New Zealand and Uruguay.

Chapter 3 focuses on a field experiment oriented to quantify dry matter production, morphological and physiological changes, and plant survival in *Lotus corniculatus*, in response to autumn defoliation and different intensities and frequencies of defoliation in spring. Winter plant survival and spring regrowth capacity are quantified.

Chapter 4 describes an experiment conducted in glasshouse conditions, oriented to evaluate the effects of defoliation intensity on biomass allocation during regrowth of *Lotus corniculatus*. Regrowth rate, biomass partitioning and morphological changes under different residual height treatments are evaluated, as well as biomass accumulation under undisturbed growth conditions. Changes in root carbohydrate reserves at different growth stages and defoliation intensities are reported.

Chapter 5 presents the results of a field experiment conducted to evaluate the productive performance of different cultivars of *Lotus corniculatus*, under contrasting defoliation regimes during the year of establishment and subsequent effects during the second year. At the same time, physiological and morphological plant parameters are described for the cultivars under analysis.

Chapter 6 reports a field experiment using a mixed *Lotus corniculatus – Trifolium repens* sward to evaluate forage production and BFT stand persistence for different management systems under grazing conditions. In addition, it relates to seed production, soil seed bank and seedling emergence of the *Lotus corniculatus – Trifolium repens* mixture, providing information on population dynamics in different grazing systems.

Chapter 7 contains an integrated discussion of the main findings and general conclusions, synthesising the information presented in previous chapters and discussing the essential issues in terms of management recommendations to achieve productive and persistent *Lotus corniculatus* pastures. Perspectives for future research are outlined, emphasising areas that need further detailed study.

1.3 REFERENCES

ALISON, M.W. AND HOVELAND C.S. (1989). Birdsfoot trefoil management. II. Yield, quality and stand evaluation. *Agronomy Journal* **81**(5): 745-749.

ALTIER, N. (1997). Enfermedades del Lotus en Uruguay. (Lotus diseases in Uruguay). INIA La Estanzuela, Uruguay. *Serie Técnica* No. **93**. ISBN: 9974-38-083-9. 16 pg.

BEUSELINCK, P.R. AND McGRAW, R.L. (1989). Environmental considerations for *Lotus* production: seed versus herbage. *Proceedings of the XVI International Grassland Congress.* Vol. 1. Nice, France. pp. 649-650.

BEUSELINCK, P.R. AND STEINER, J.J. (1995). Registration of ARS-2620 birdsfoot trefoil. Electronic address: www.nal.usda.gov /ttic /tektran /data/ 000006/ 33 /00000633-74.html

BLUMENTHAL, M.J., KELMAN, W.M., LOLICATO, S., HARE, M.D. AND BOWMAN, A.M. (1994). Agronomy and Improvement of Lotus. A review. *"Alternative Pasture Legumes 1993".* Edited by D.L. Michalk, A.D. Craig and W.J. Collins. Department of Primary Industries South Australia, Technical Report 219. pp. 74-85.

BLUMENTHAL, M.J. AND McGRAW, R.L. (1999). Lotus adaptation, use and management. *Trefoil: The Science and Technology of Lotus*. CSSA Special Publication Number 28. Ed. by P.R. Beuselinck. American Society of Agronomy, Inc. Crop Science Society of America, Inc. Madison, Wisconsin, USA. pp. 97-119.

FORMOSO, F. (1993). *Lotus corniculatus.* I. Performance forrajera y características agronómicas asociadas. (Productive performance and agronomic characteristics). *Serie Técnica* No. 37. INIA Uruguay. ISBN: 9974-556-69-4 20 pg.

FRAME, J.; CHARLTON, J.F.L. AND LAIDLAW, A.S. (1998). Birdsfoot Trefoil and Greater Lotus. Chapter 6. In *Temperate Forage Legumes*. CAB International ISBN 0-85-199-214-5. pp. 245-271.

GRANT, W.F. AND MARTEN, G.C. (1985). Birdsfoot trefoil. *Forage: The Science of Grassland Agriculture.* Iowa State University. Iowa, USA. Chapter 11, pp. 98-108.

GREUB, L.J. AND WEDIN, W.F. (1971). Leaf area, dry-matter accumulation, and carbohydrate reserve levels of birdsfoot trefoil as influenced by cutting height. *Crop Science* **11**: 734-738.

JOHN, A. AND LANCASHIRE, J.A. (1981). Aspects of the feeding and nutritive value of *Lotus* species. *Proceedings of the New Zealand Grassland Association* **42**: 152-159.

JONES, D.A. AND TURKINGTON, R. (1986). Biological Flora of the British Isles. *Lotus corniculatus* L. *Journal of Ecology* **74**: 1185-1212.

KIRKBRIDE Jr., J.H. (1999). Lotus systematics and distribution. *Trefoil: The Science and Technology of Lotus*. CSSA Special Publication Number 28. Ed. by P.R. Beuselinck. American Society of Agronomy, Inc. Crop Science Society of America, Inc. Madison, Wisconsin, USA. pp. 1-20.

LI, Q. (1989). Seed production in birdsfoot trefoil (*Lotus corniculatus* L.). *Thesis for the Degree of Doctor of Philosophy*. Massey University. Palmerston North, New Zealand.175 p

MARTEN, G.C. AND JORDAN, R.M. (1979). Substitution value of birdsfoot trefoil for alfalfa-grass in pasture systems. *Agronomy Journal* **71**: 55-59.

McDONALD, H.A. (1946) Birdsfoot trefoil (*Lotus corniculatus* L.). Its characteristics and potentialities as a forage legume. Cornell University Agricultural Experimental Station. Mem. 261. Ithaca, New York.

NELSON, C.J.; AND SMITH, D. (1968). Growth of birdsfoot trefoil and alfalfa. II. Morphological development and dry matter distribution. *Crop Science* **8**: 21-25.

PIERRE, J.J. AND JACKOBS, J.A. (1953). The effect of cutting treatments on birdsfoot trefoil. *Agronomy Journal* **45**: 463-468.

SEANEY, R.R. AND HENSON, P.R. (1970). Birdsfoot trefoil. *Advances in Agronomy* **22**: 119-157.

SMITH, D. (1962). Carbohydrate root reserves in alfalfa, red clover, and birdsfoot trefoil under several management schedules. *Crop Science* **2**: 75-78.

SMITH, D. AND NELSON, C.J. (1967). Growth of birdsfoot trefoil and alfalfa. I. Responses to height and frequency of cutting. *Crop Science* **7**: 130-133.

STEINER, J.J. (1999). Birdsfoot trefoil origins and germplasm diversity. *Trefoil: The science and technology of Lotus*. CSSA Special Publication Number 28. Ed. by P.R. Beuselinck. American Society of Agronomy, Inc. Crop Science Society of America, Inc. Madison, Wisconsin, USA. pp. 81-96.

TURKINGTON, R. AND FRANKO, G.D. (1980). The Biology of Canadian Weeds. 41. *Lotus corniculatus* L. *Canadian Journal of Plant Science* **60**: 965-979.

2. REVIEW OF THE LITERATURE

2.1 INTRODUCTION
2.2 AGRONOMIC CHARACTERISTICS
    2.2.1 Plant morphology
    2.2.2 Birdsfoot trefoil habitat
    2.2.3 Herbage production
2.3 NUTRITIVE VALUE
    2.3.1 Digestibility
    2.3.2 Crude protein
    2.3.3 Fibre content
    2.3.4 Mineral content
    2.3.5 Condensed tannins content
2.4 UTILIZATION IN FARMING SYSTEMS
    2.4.1 New Zealand
    2.4.2 Uruguay
2.5 DEFOLIATION MANAGEMENT
    2.5.1 Frequency and intensity of defoliation and growth habit
    2.5.2 Leaf area index and photosynthetic efficiency
    2.5.3 Carbohydrate reserves
2.6 DISEASE INCIDENCE
2.7 PERSISTENCE REVISITED
    2.7.1 Plant strategy
    2.7.2 Improved persistence approach
2.8 CONCLUSIONS
2.9 REFERENCES

2.1 INTRODUCTION

Birdsfoot trefoil (*Lotus corniculatus* L.) (BFT) is a long-lived perennial, tap-rooted and non-bloating legume, widely distributed and adapted around the world (Seaney and Henson, 1970). Many advantages give BFT a recognised popularity for pasture, silage or hay production in temperate environments. However, it has a reduced production and poor persistence under intensive grazing (Smith and Nelson, 1967; Bologna, 1996). Additionally, crown-rot diseases have a high incidence, particularly in warm environments, where poor BFT persistence constitutes an unsolved problem. In fact, avoidance of hard defoliation regimes and the selection of disease resistant cultivars played major roles in early development programmes.

In many regions, BFT has limited acceptance and use when compared with other legumes because of its poor and slow stand establishment. Seed size and seedling vigour are key limitations for successful establishment, sometimes overcome by the use of fertilisers, good inoculation and reducing competition from other species (Twamley, 1967). During the last decade, BFT has gained popularity due to two main comparative advantages. Firstly, the interest in low input systems revalidates the place of BFT as an economic species with low resource requirements. Secondly, BFT feeding value has particular advantages over many other legume species due to the presence of condensed tannins (Waghorn *et al.*, 1998).

In essence, lack of persistence limits BFT use. An integrated approach to improve the production and persistence by focusing on the population dynamics of BFT is under analysis (Olmos, 1996; Bologna, 1996; Emery *et al.*, 1999). As well, the development of potential cultivars of BFT with vegetative reproduction (Li and Beuselinck, 1996; Beuselinck *et al.*, 1996) opened a new window of opportunity for the species.

This chapter will summarise published research on agronomic and nutritional characteristics, management requirements and persistence problems currently highlighted for BFT, with particular reference to factors influencing productive persistence of BFT in New Zealand and Uruguayan pastoral systems.

2.2 AGRONOMIC CHARACTERISTICS

2.2.1 Plant morphology

The main characteristic of the BFT plant is the presence of a deep woody taproot with numerous lateral branches placed in the first 30-60 cm of soil strata, sometimes up to 1 m depth, and accompanied by a dense mass of secondary roots. This structure allows BFT to perennate like other temperate forage legumes such as lucerne or red clover, giving advantages in terms of exploration of resources like water and nutrients (Seaney and Henson, 1970).

From a crown, a series of primary and secondary shoots are developed. Aerial shoots are solid and slender, varying from erect to prostrate and having high variation from glabrous to pubescent (Jones and Turkington, 1986). Branching occurs in the leaf axils of main and secondary shoots. BFT leaf is pentafoliate (Frame *et al.*, 1998), consisting of three leaflets attached at the end of the petiole and two others at the base (Seaney and Henson, 1970).

The BFT inflorescence is a terminal umbel with 4 to 8 florets, attached by short pedicles to a long peduncle (Seaney and Henson, 1970). Pods are long and cylindrical, being from brown to almost black as maturity advances, and placed at right angles to the top of the peduncle as a "bird's foot " (Turkington and Franko, 1980). Seeds are small, with a 1000 seed-weight of 1.2-1.4 g (Frame *et al.*, 1998). High proportions of hard seeds are present in mature seed lots, varying from 90% if the crop is hand harvested to 40% if machine harvested (Brown, 1955, cited by Li, 1989).

Traditionally, two distinct BFT types were recognised: erect European and semi-erect Empire (Seaney and Henson, 1970). European types are suited for hay production, being more erect with faster establishment and regrowth after harvest and earlier maturity than Empire. But they are less tolerant of close grazing, intolerant to poor drainage and less winter-hardy than Empire types (Seaney and Henson, 1970; Turkington and Franco, 1980). Empire is a semi-erect cultivar, with fine stems and 10-14 days later flowering than European types (Grant and Marten, 1985), and it is recommended for grazing because of its prostrate habit and indeterminate growth. However, Empire has slower growth at establishment and subsequently slower regrowth than European types (Van Keuren and Davis, 1968).

New Zealand introductions of BFT have been grouped into five categories: South American, Mediterranean/mild temperate, maritime Europe/cold temperate, continental, and Middle East (Widdup *et al.*, 1987). South American accessions are the most erect types with low shoot density and moderate frost damage, having poor recovery after defoliation. Introductions from Mediterranean and mild temperate areas show an intermediate growth habit, adapted to grazing and forage conservation. They show good tolerance to drought, but decline under cold and infertile conditions. Continental types are more prostrate, with high shoot density, well adapted to grazing and resistant to frost damage. In contrast, the Middle East group is extremely prostrate, but not adapted to grazing, resembling wild BFT ecotypes.

Wild BFT accessions from Morocco showed a difference from traditional types, in that they develop rhizomes (Beuselinck *et al.*, 1996). In crossing Moroccan material with traditional American types the rhizomatous character was transferred, developing a semi-prostrate cultivar with capacity for vegetative reproduction (Beuselinck and Steiner, 1995).

2.2.2 Birdsfoot trefoil habitat

BFT grows in Europe, Canada, United States, South America, Australia, New Zealand, Asia and North Africa (Grant and Marten, 1985; Grant and Small, 1996), having the greatest distribution and variation of all species of the genus *Lotus* (Jones and Turkington, 1986). Its wide distribution is a consequence of adaptability to a broad range of soil conditions: acidic, poorly drained, shallow, drought to infertile conditions. BFT has potential as a replacement for lucerne in less fertile, acid, poorly drained and dryland soils where lucerne has limitations (Scott and Charlton, 1983), and is also potentially suited for low-input systems (Bullard, 1990; Charlton and Belgrave, 1992).

BFT occurs naturally in herb-rich hill and lowland swards, and under adequate conditions of moisture and fertility and at pH near neutrality grows well (Smith, 1975). It is more tolerant to low pH than white clover (Frame, 1992) or lucerne (Grant and Marten, 1985), having a moderate degree of tolerance of drought conditions by virtue of a deep taproot system (Jones and Turkington, 1986). It is more tolerant to waterlogging than white clover, red clover and lucerne among others (Heinrichs, 1970), but waterlogging causes clorosis, senescence, root decomposition, shoot hypertrophy and plant death if the period is extended (Vignolio *et al.*, 1994). BFT is less resistant to waterlogging than *Lotus tenuis* (Vignolio *et al.*, 1994). It is not well adapted to extreme high temperatures (Turkington and Franko, 1980), and performance is poor below 12 °C because of a reduction in symbiotic activity (Kunelius and Clark, 1970).

Described as a long-day plant, BFT requires 16 hours or more daylength for full flowering (Turkington and Franko, 1980). Under short photoperiod, the rate of development and number of floral primordia are restricted. The critical photoperiod lies between 14 and 14 ½ hours, flowering being very sparse and retarded below this level (McKee, 1963). Plant form and height change with photoperiod length, plants being short, compact, dark green and prostrate under 12 hours photoperiod. In contrast, when photoperiod reaches 16 hours, plants are erect, tall and light green (McKee, 1963). Beuselinck and McGraw (1988, 1989) considered areas below 40° degrees of latitude as poor environments for BFT seed production in terms of quantity and quality of production. In these areas, selection of materials with short photoperiod and early flowering when temperatures are cooler could improve BFT success (Beuselinck and McGraw, 1989).

2.2.3 Herbage production

Levels of BFT herbage production can be considered adequate in many regions, because it grows in environments with certain restrictions, where other legumes have a limited performance. In Uruguay, BFT forage production reached a maximum during the second year, with values higher than 9 t DM/ha/year (Carámbula *et al.*, 1996). When seed production and seedling recruitment are not allowed, forage production declines in subsequent years, the best cultivars producing 3.5 t DM/ha in the fourth year (Carámbula *et al.*, 1996). Growth rate, evaluated in different trials over 18 years at La Estanzuela, Uruguay, showed the lowest range of variation compared with white clover, red clover and lucerne, achieving average maximum of 42 kg DM/ha/day during the second spring, the maximum registered being 74 kg DM/ha/day. In winter, growth rate of BFT is lower than 10 kg DM/ha/day (Diaz *et al.*, 1996). Seasonal distribution is influenced by pasture age, spring-summer production increasing and winter contribution reducing as pasture age increases (Formoso, 1993). In Chile, Acuña (1995) reported a range of 6.7-8.9 t DM/ha for the second year, for a group of commercial varieties used in South America (San Gabriel, Ganador, El Boyero and Quimey), achieving highest growth in November-December.

Performance of BFT introductions in acid and infertile East Otago soils, New Zealand, showed good performance from cool temperate and continental types, South American accessions having the lowest performance for all sites (Fraser *et al.*, 1988). Bologna (1996) reported between 7.5-13 t DM/ha/year in Canterbury, New Zealand, depending on grazing interval. Growth rate reached a peak of nearly 80 kg DM/ha/day in November-December, declining to winter, when the minimum was less than 10 kg DM/ha/day from July to early September

2.3 NUTRITIVE VALUE

BFT forage has a nutritive value comparable to species like lucerne, with the ability to maintain high quality to advanced stages of maturity. This provides the flexibility to use BFT in summer when other forage species have low quality, or later as a feed reserve when grass availability is lower (Collins, 1982; Alison and Hoveland, 1989 c).

Nutritive value of BFT is associated with season, length of regrowth, or physiological stage. During vegetative stages, forage contains 60-70% of leaves, declining to maturity where leaves can contribute only 20-30% (Formoso, 1993). Grazing management defines accumulation periods and timing of defoliation, altering the quality of forage grazed.

2.3.1 Digestibility

BFT has lower organic matter digestibility than white clover (71% vs 85%), due to a high lignin content in stems compared with other species (John and Lancashire, 1981), suggesting that to achieve an efficient animal performance grazing management should maximise intake of leaf rather than stem material. Digestibility of leaves remains constant from early growth to full flowering (75%), but stem digestibility declines from 61% during the vegetative stage to 50% at full flowering. After flowering, the decline in digestibility is high, being associated with a reduction in leaf proportion and an increase in lignin content of stems (Lopez *et al.*, 1965). Early harvests to minimise poor quality stems and selection of plants with slow decline in stem digestibility with advancing maturity were proposed by Buxton *et al.* (1985).

2.3.2 Crude protein

BFT crude protein (N x 6.25) content ranged from 22.4% during pre-bloom stage to 14.5% at seed dehiscence stage (Duell and Gausman, 1957). Formoso (1993) identified a decline in crude protein, from 22% in spring to 18% in mid-summer. Crude protein levels of leaves reported by Scheffer-Basso *et al.* (1998) varied from 30.3, 26.1 and 22.9% for vegetative, bloom and full flowering stages, respectively. These authors reported values of 14.8, 11.1 and 8.9% of crude protein for stems during vegetative, bloom and full flowering stages respectively. BFT can retain a high leaf:stem ratio later than lucerne, indicating the potential for a high intake of crude protein at advanced plant maturity by animals (Buxton *et al.*, 1985; McGraw and Marten, 1986).

2.3.3 Fibre content

Levels of acid detergent fibre reported in a group of BFT materials (El Boyero, Ganador, San Gabriel and Quimey) showed a range between 24-26% at early vegetative stage and 31-38% at advanced maturity (Acuña, 1995). Lignin content is lower in leaf than in stem, increasing with plant maturity because of the high proportion of structural tissues in stems (Lopez *et al.*, 1965).

2.3.4 Mineral content

Nutrient accumulation (mg/kg DM) reaches a maximum earlier than the dry matter maximum for N, Ca, Mg, S and Zn, only Cu shows a reduced accumulation during this phase. Accumulation of N, Ca, K, Mg, S, B, Zn and Mn remains stable during seed filling (McGraw *et al.*, 1986). There is a tendency for a reduction in the concentration of all minerals when maturity advances. Seeds are demanding of N, P, S, Zn and Cu with concentration two to four times greater than foliar material, suggesting importance in seed production. The majority of minerals show a greater concentration in leaves than stems, though in K the reverse is true (Pierre and Jackobs, 1953; McGraw *et al.*, 1986).

Concentrations of K are higher than 30 g/kg, the maximum tolerable in cattle, but a sole diet of BFT is unusual (Kallenbach *et al.*, 1996). Mg levels ranged up to 4 g/kg, which is the maximum for beef cattle. P concentrations in leaves increase as soil pH increases, but stems decline, resulting in a deficient diet for animals weighing 450 kg. Concentrations of Al and Ca are associated with soil pH levels, and for Ca means exceed critical levels at high pH. Zn in BFT tissues follows the same tendency as Zn concentration in soil, decreasing with pH increase. Mn concentration in tissues decreases as soil pH increases, but BFT leaves provide adequate levels (30 mg/kg) for beef cattle (NRC, 1984, cited by Kallenbach *et al.*, 1996).

2.3.5 Condensed tannins content

BFT and other *Lotus* species are considered non-bloating legumes because of the presence of condensed tannins (CT) (Kelman and Tanner, 1990; Ehlke and Legare, 1993). CT in forage increases protein digestion and utilisation, protecting forage protein from rumen degradation. Nitrogen retention, protein absorption and essential amino acids absorption in the small intestine are all increased (John *et al.*, 1980; John and Lancashire, 1981; Blumenthal *et al.*, 1994; Wang *et al.*, 1996 c; Min *et al.*, 1998).

Despite the benefits described, high CT concentrations can reduce herbage intake, palatability and digestibility of soluble carbohydrates and hemicellulose. Kelman and Tanner (1990) reported that CT concentrations in leaves of accessions of BFT were at adequate levels to prevent bloating and would not affect dry matter digestibility negatively.

The presence of CT can substantially improve performance in animals eating BFT. For example, an increase of 14% in wool growth rate (Wang *et al.*, 1996 c), 19% in efficiency of wool production and improved wool quality (Min *et al.*, 1998), higher rates of carcass gain and higher carcass dressing-out percentage in lambs (Wang *et al.*, 1996 b), a positive effect in milk yield and milk protein of dairy cows (Harris *et al.*, 1998) and milk production in lactating ewes (Wang *et al.*, 1996 a), have all been observed in comparisons with animals receiving polyethylene glycol to inactivate CT.

The number of protozoa in rumen fluid of lambs grazing BFT decrease, probably as a consequence of the astringent nature of CT, or by an inhibition of rumen bacterial growth and limitations in feed sources for protozoa (Wang *et al.*, 1996 b). In addition, the presence of CT in forage has anti-parasitic effects, suggesting advantages in terms of reduced antihelmintic use (Niezen *et al.*, 1993; Robertson *et al.*, 1995).

Additive genetic effects control CT content (Miller and Ehlke, 1997), which varies widely among *Lotus* species. *Lotus pedunculatus* (GL) shows the highest concentration, (Kelman and Tanner, 1990; Roberts and Beuselinck, 1992), with a range of CT in leaves from 2.5 to 10.7% for a group of 10 accessions (Kelman and Tanner, 1990). BFT leaves contained between 0.1 and 7.3% in 22 materials tested, *Lotus subbiflorus* moderate to low levels (2.37-3.95% in one material) and *Lotus tenuis* the lowest levels (0-0.32% in 2 materials tested). CT production is sensitive to environmental conditions, soil fertility and pH. A reduction of 10 to 40% in tannin production in high temperature regimes was reported in the majority of genotypes of BFT tested by Ehlke and Legare (1993), probably by a disruption in normal plant metabolism. Kelman and Tanner (1990) reported no significant lime effects (pH 5.2), despite a tendency for an increased CT concentration in unlimed conditions (pH 4.3). Other results showed that soil pH effects are associated with CT content of genotypes, low tannin types producing less tannin on acidic soil, while high and medium types responded inconsistently (Ehlke and Legare, 1993).

Recognising the importance of CT in animal production, measurements of CT content should be incorporated in evaluation and breeding programmes of *Lotus* species, particularly in countries where *Lotus* is widely used, as a way to reduce potential problems by high CT concentrations. Additionally, the role of BFT in sward mixtures, improving nutritive value and nutrient absorption, and acting as a bloating control agent is under study and should be confirmed in grazing conditions (Waghorn and Shelton, 1997).

2.4 UTILIZATION IN FARMING SYSTEMS

2.4.1 New Zealand

The earliest description of the use of BFT in New Zealand dates from 1864 (Thomson, 1922), and Levy (1918) first considered its potential agricultural use. However, BFT remains little used by New Zealand farmers (1000 ha sown in the South Island hill country, West *et al.*, 1991 cited by Blumenthal and McGraw, 1999), despite its recognised productive role. This is probably because of an increasing pressure to maximise production (Sheath and Hay, 1989), and a poor understanding of the agronomy and management principles of BFT. Nodulation failures due to ineffective inoculation techniques, inability to survive and slow initiation of symbiotic N fixation are identified problems of BFT establishment in New Zealand environments (Charlton, 1983; Chapman *et al.*, 1990; Patrick and Lowther, 1992). Management recommendations include inoculation at five times recommended doses, using improved *Rhizobium* strains, and drilling within 24 hours of inoculation, at no more 12 mm depth. Also, it is recommended to sow alone or with non-aggressive grasses. Movement of rhizobia in the soil for new seedling infection is poor, <0.25 and 4.0 metres/year laterally and downslope respectively, when periods of favourable moisture occur (Lowther and Patrick, 1993; Douglas *et al.*, 1996).

The role of BFT in New Zealand pastoral systems depends on its adaptability to stressful environments, where it competes with many traditionally used legumes (Scott and Charlton, 1983). The alternative option is for more fertile and productive soils and favourable climate, where it is not a good competitor with traditional mixtures of white clover-ryegrass, that are more productive and persistent under these conditions. In this case, BFT may be regarded as a complementary feed source in periods where growth or quality of commonly used mixtures decline, or as a high quality protein bank for special purposes. The advantages of the presence of condensed tannins reported from many trials can only be exploited in mixtures if BFT of high persistence and competitive capacity is used (Waghorn and Shelton, 1997).

The presence of only one commercially available cultivar of BFT in New Zealand constitutes a weakness, and exploration of other genetic resources would be useful. Successful incorporation of alternative species in pastoral systems requires adequate technical knowledge, as a way to avoid failures. Studies to evaluate the effect of different grazing practices in terms of productivity and long-term persistence will help to clarify the role of BFT in New Zealand.

2.4.2 Uruguay

Native grasses and a reduced proportion of legumes are the main component of grasslands of Uruguay, growing in acid soils with low phosphorus levels and affected by irregular drought and wet periods. In this context, the introduction of "pioneer" legumes like BFT increases productivity, forage quality and nitrogen inputs. As a pioneer, it is widely used (1,100,000 ha, including mixtures and pure stands, Altier, 1995 cited by Blumenthal and McGraw, 1999), from poor to rich environments and from extensive to intensive systems.

Considering its priority use for low input systems, the focus for BFT is to achieve reasonable production and persistence rather than a highly productive performance. Warm and wet conditions altogether result in a high incidence of diseases, reducing BFT stands severely in 2-3 years, particularly in areas with a long BFT cultivation history (Altier, 1997). Research programmes concentrate on improved persistence by management practices, or by selecting new materials with improved diseases resistance (INIA, 1997). The next step should be to clarify the specific conditions that provide the best returns from BFT, since currently it is used in a wide range of situations. Often it is associated with other legumes with different requirements like white clover, resulting in contradictory management requirements. Under these conditions, productive performance never achieves high potential, and opportunities for refined management techniques are limited.

2.5 DEFOLIATION MANAGEMENT

Several studies have illustrated the limited degree of adaptability of BFT to different grazing conditions, particularly its weakness to hard grazing. It can not persist at a productive level under continuous grazing (Van Keuren and Davis, 1968), and its high acceptability to livestock demands some degree of grazing control (Frame *et al.*, 1998). In fact, BFT performance and tolerance to grazing is strongly influenced by frequency and severity of defoliation, plant structure and phase of development at defoliation, all of these factors influencing both short and long-term effects.

2.5.1 Frequency and intensity of grazing and growth habit

Pierre and Jackobs (1953) reported high forage production during the first year of production of BFT under close defoliation (2.5 cm). The height of defoliation is less important under long defoliation intervals, but its effect increases under short intervals (Smith and Nelson, 1967). Yield varied by 30% under contrasting defoliation heights, but by 92% under contrasting defoliation frequency over two years (Smith and Nelson, 1967).

BFT largely regrows from axillary buds of cut shoots, in contrast to lucerne, which is more adapted to growth from new buds from the crown. Lucerne responds better to infrequent cutting, being less tolerant than BFT to hard grazing (Nelson and Smith, 1968 a). Tall stubble preserves a high proportion of plant parts involved in regrowth, influencing the vigour of regrowth and dry matter yield of BFT (Cordeiro de Araujo and Jacques, 1974). Bologna (1996) found that under two-week defoliation intervals, BFT regrowth was dependent on activation of crown buds. Conversely, with longer intervals regrowth occurred from axillary buds of primary branches.

Prostrate types are less affected than erect types by intense defoliation (Pierre and Jackobs, 1953; Alison and Hoveland, 1989 c). Changes in defoliation height from 10 to 2.5 cm caused a reduction in plant population of 16 and 36% for prostrate and erect types respectively (Pierre and Jackobs, 1953). A high residual leaf area on prostrate materials was identified as the main cause of the difference. Also, Smith and Nelson (1967) described how reduction in winter survival is caused by a low biomass and poor protection of crown meristems. Autumn harvests also lowered plant stand survival (Fulkerson, 1982). During spring-summer, stand losses were caused by diseases (Greub and Wedin, 1971 a) or poor plant reserves (Alison and Hoveland, 1989 a).

Despite the drought tolerance described for BFT, careful defoliation during summer is recommended. Under hard defoliation, plants reduce root mass, losing the capacity to capture water and nutrients (Vickery, 1981), and plants prioritise metabolite flux to aerial rather than underground parts. Root mass declines under intensive defoliation

(Greub and Wedin, 1971 a), and under short defoliation intervals (Smith and Nelson, 1967; Alison and Hoveland, 1989 c), the degree of reduction being associated with plant type (Pierre and Jackobs, 1953). In Uruguay, Morales (1992) determined a rapid decline in BFT proportion by the fourth year of pasture if BFT was defoliated in summer.

2.5.2 Leaf area index and photosynthetic efficiency

In BFT, maximum crop growth rate was reached at a leaf area index (LAI) of 1.4 at the end of spring and remained constant to LAI 6, after flowering, when leaf loss started (Nelson and Smith, 1968 b). Growth rate is reduced at the end of summer, leaf losses starting over LAI 4. BFT growth rate increases over the first 20 days of regrowth, and maintains its rate during the following 45 days. After that, regrowth declines to zero at 80 days approximately (Greub and Wedin, 1971 b). In lucerne, maximum growth was at a LAI of 3.5, declining before flowering, and loss of leaves started at LAI 6.5. Maximum LAI values in spring were 6.1 and 6.7 for BFT and lucerne respectively (Nelson and Smith, 1968). In early summer, LAI values between 3-4.5 were reached after 4 weeks of regrowth if plants were defoliated at 3.8 or 11.4 cm height, maximum being 4 after seven weeks of regrowth for all defoliation intensities. In late summer, LAI was approximately 3 for 7.6 and 11.4 cm height defoliation and 2 for 3.8 cm height after seven weeks of regrowth. (Greub and Wedin, 1971 a).

The photosynthetic efficiency of regrowth declines under long defoliation intervals, old basal leaves being less efficient. Beuselinck *et al.* (1984) found that under extended periods of accumulation, leaves are mainly concentrated in the top canopy strata, with high leaf losses starting at 60 days. Under long grazing intervals, intense defoliation could produce a more vigorous regrowth with a greater quantity of new leaves. BFT residual herbage from grazing after an extended accumulation has poor regrowth vigour. It is preferable to use an intensive defoliation to remove old material making a more efficient use of forage (Twamley, 1968). Differences in LAI, crop growth rate and net assimilation rate of BFT and lucerne explain species differences in photosynthetic efficiency (Gregerson *et al.*, 1999).

2.5.3 Carbohydrate reserves

In BFT, carbohydrate reserves (CHO) are low from spring to autumn even in plants that grow without defoliation, and these levels are lower than in lucerne or red clover (Smith, 1962). After cutting, CHO reserves decline, only increasing again following photosynthetic activity, explaining the slow regrowth rates and low tolerance to heavy grazing of BFT (Smith and Soberalske, 1975). In contrast to the situation with lucerne or red clover the indeterminate growth habit of BFT, with a constant production of new shoots, induces a lower CHO accumulation (Smith, 1962). Greub and Wedin (1971 a) reinforced the description of Smith (1962), both studies showing low levels of CHO accumulation and its partial effect on BFT regrowth, that it is more dependent on CO_2-fixation (Gregerson *et al.*, 1999). Greub and Wedin (1971 a) also proposed that the metabolic system of BFT differed from that of lucerne, accumulation of CHO requiring reduction of temperature or photoperiod, conditions achieved in late autumn. Risso *et al.* (1983) mentioned that high temperatures reduced photosynthesis and CHO accumulation in BFT. In red clover, slight growth and high mortality rate of plants growing at high temperatures, was the result of CHO deficiencies (Kendall, 1958). In practice, the reduced patterns of CHO accumulation after BFT defoliation, a difference from other crown-forming species like red clover or lucerne, suggest a high importance of defoliation height (Nelson and Smith, 1968 b), the percentages of CHO in roots increasing with high stubble height following defoliation (Greub and Wedin, 1971 a). High levels of root reserves are believed to be essential to improve plant survival under defoliation or environmental stress. Starch (amylose and amylopectin), sucrose and reducing sugars (fructose and glucose) are the fractions of total nonstructural carbohydrate in BFT, being present in similar relative proportions to those in lucerne (Gregerson *et al.*, 1999). There is limited information about seasonal changes and conversion processes of starch to soluble sugars, as well the incidence of other N-containing root compounds on winter survival and regrowth post-defoliation on BFT. More extensive references are available for lucerne (Fankhauser *et al.*, 1989; Li *et al.*, 1996).

2.6 DISEASE INCIDENCE

A diversity of agents, including fungi, pests, root diseases and viruses, are reported as affecting *Lotus* spp., in many cases appearing simultaneously or sequentially (Turkington and Franco, 1980, Jones and Turkington, 1986). Stress factors like poor soil drainage, low pH, deficiencies in soil nutrients, adverse winter conditions and intense defoliation contribute to increased susceptibility of crown forming species to foliar and root diseases (Bologna, 1996). Diseases are a potential limitation in terms of productivity and persistence of BFT, drastically reducing stands. In warm and humid environments losses in stands between 68 and 88% at the end of the second year were reported as a consequence of disease incidence (Henson, 1962).

In Uruguay, disease incidence is important after the second spring of the BFT pasture, total yield in the third year being 25-33% greater if fungicides and insecticides are applied (Altier, 1997). The effectiveness of this control is mainly in stem and leaf diseases, but it is not effective over crown and root disease complexes which have a higher impact in BFT persistence. Altier (1997) working with spaced plants, reported plant losses of 27%, 93% and 99.9% during the first, second and third year respectively. In Uruguay and Argentina, *Fusarium* species were the main fungi associated with crown and root rot diseases, *Fusarium oxysporum* having the highest relative incidence (54-58%), in diverse geographic areas (Leath, 1989). For stem and leaf diseases the most frequent causal agents are *Stemphylium loti* and *Colletotrichum* spp (Chao *et al.*, 1994; Altier, 1997). Other diseases with minor relevance occur under specific weather conditions, *Sclerotinia trifoliorum* being severe under wet and cool weather in fall and spring (Altier, 1994). Also *Colletotrichum acutatum* produces flower blight when wet weather occurs during flowering, reducing seed yield in BFT more than 36% (Stewart *et al.*, 1994).

In New Zealand, disease research has been concentrated in forage species of agronomic importance, and alternative species like BFT have received little attention (Watson *et al.*, 1989). Despite that, Scott and Charlton (1983) reported cases of crown and root diseases (*Fusarium* species) that severely reduced plant stand. High disease incidence is reported in warmer and humid areas of the southern United States compared with cold areas in the north (Grant and Marten, 1985). Nelson and Smith (1969) found a high incidence of root diseases when plants grew at 32/24 °C. Under intense defoliation, plant losses were associated with the incidence of diseases, especially in treatments defoliated late in summer (Greub and Wedin, 1971 a), and plant survival was associated with high root reserves (Alison and Hoveland, 1989 a,b). Barta (1978) proposed that under high temperature, carbohydrate levels are reduced, making plants more easily affected by pathogen incidence.

Roberts *et al.* (1994) reported a positive correlation between disease resistance and the concentration of chitinase, an antifungal hydrolase associated with disease resistance in other crops. Future disease research will be continuing to develop genotypes with field resistance, focusing selection on clearly defined disease-defence mechanisms and understanding ecological population dynamics to develop innovative management strategies for diseases (English, 1994). In addition, the effect of pasture management on the incidence and severity of BFT diseases could be explored (Altier, 1994).

2.7 PERSISTENCE REVISITED

From previous sections (2.5 and 2.6), a fragile life cycle was identified in BFT caused by constraints in management and diseases incidence, BFT being considered in practice as a short-lived legume under grazing (Pierre and Jackobs, 1953). To improve BFT persistence, the understanding of plant strategy to compete, grow and reproduce is crucial to meet challenges in grazing management oriented to extend productive life of BFT swards.

2.7.1 Plant strategy

The presence of a deep taproot and a thick fibrous root system in the upper subsoil provides BFT, like other crown forming species, the capacity to perennate (Forde *et al.*, 1989). The structure of the BFT plant allows it to develop and compete in closely compact growth units or modules, highly branched and slowly expanding, the extended life cycle and reproductive characteristics being classified as "k-strategies" or "phalanx" species (Pianka, 1983; Hutchings, 1997). From Grime's model, BFT can be described as a competitor species "C", adapted to stable and rich environments (Grime, 1979), characterised by low reproductive effort and high growth rate (Grace, 1991). As a competitor, BFT plants have a well defined growing season, and capacity to store root reserves for shoot and root growth, showing a high morphological plasticity for shoot and root development. (Grime, 1988). Also, the capacity to grow in low resource environments determines that BFT can be defined as a stress tolerator species, with conservative mechanisms of mineral nutrient capture and utilization that constrains carbon nutrition and reproductive activity (Grime, 1988). Stress tolerator plants develop different mechanisms of defence to protect from herbivores and pathogens (Grime, 1988). Tannins and cyanogenic glycosides are produced by BFT, a costly process for the plant that is reflected in reduction of growth and reproduction (Briggs, 1990; Briggs and Schultz, 1990).

BFT invests all its assimilates in the maintenance and growth of the individual plant, traditional BFT types not having the capacity to produce new individuals by vegetative reproduction. The root/shoot ratio is higher in BFT than in white clover, independent of environmental conditions, showing the importance of its tap root (Foulds, 1978). Once established, BFT survives under drought conditions through its strong root system, a high root/shoot ratio, low shoot yield and low seed production.

The presence of a rhizomatous characteristic in a new BFT ecotype would allow it to spread and maintain its population (Beuselinck *et al.*, 1996), as also occurs in red clover (Smith and Bishop, 1998; Hyslop *et al.*, 1998) and tall fescue (Carlson and Hurst, 1989). Rhizomatous BFT is expected to avoid crown and root-rot diseases by vegetative propagation because diseases only affect primary roots. Therefore the plant population could be maintained without heavy dependence on natural reseeding (Beuselinck *et al.*, 1996).

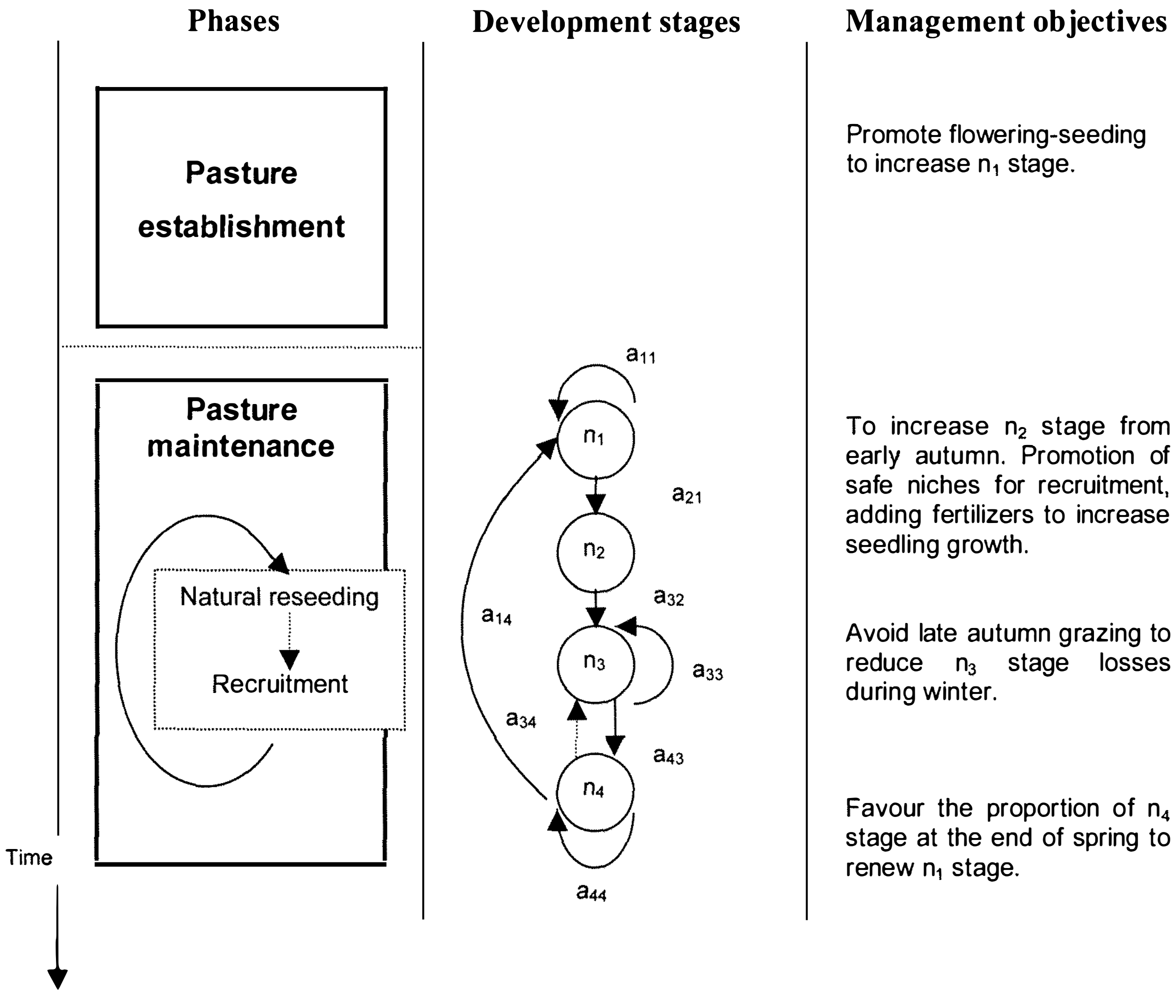
In *Lotus* species of contrasting life cycles and morphological structure, plant investments in belowground/aerial mass vary. A rhizomatous plant like *Lotus pedunculatus* invests a high proportion of nutrients in the root system and a low proportion in the reproductive system, contrasting with an annual species like *Lotus subbiflorus* where resources are assigned mainly to the reproductive process (Olmos, 1996). BFT appears in an intermediate position; reproductive investments are costly, but only at the time of peak reproductive output (Briggs and Schultz, 1990). Lloyd (1980) proposed that in species with indeterminate flowering, the number of flowers is continually adjusted by environmental conditions. Potential reproductive investments are regulated by aborting juvenile fruits, altering the number of flowers, altering number of seeds/fruit, seed mass or intervals between flowering flushes (Stephenson, 1984). The regulation of reproductive investments determines that seed size or mass is little altered, an adaptive aspect of high importance in germination, dissemination and establishment processes (Harper, 1970, cited by Stephenson, 1984).

Reported seed production levels on BFT can approach to maximum of 600 kg/ha, but commonly ranges between 50-175 kg/ha in USA environments (McGraw *et al.*, 1986).

2.7.2 Improved persistence approach

The BFT life cycle has four developmental stages: seeds, seedlings, mature vegetative plants and reproductive plants (Figure 2-1). During the establishment year, seedlings and vegetative plants are the main components, with low reproductive frequency and high mortality. The post-establishment phase is characterised by a continued growth of mature vegetative plants, high frequency of reproduction and low mortality (Emery *et al.*, 1999), population growth being dependent on seed production levels.

Each population is composed of a series of development stages, from seeds to adult plants. Transitional stages represent the portion of a category that change to others in a certain period of time (Caswell, 1989, cited by Emery *et al.*, 1999). Transitional stages in Figure 2-1 comprise the portion of seeds that became seedlings (a_{21}), the portion of seedlings that became adults (a_{32}), the adults that became reproductive (a_{43}), the reproductive success of adult plants improving soil seed reserves (a_{14}), and also the portion of each category that remains stable.



Promote flowering-seeding to increase n_1 stage.

To increase n_2 stage from early autumn. Promotion of safe niches for recruitment, adding fertilizers to increase seedling growth.

Avoid late autumn grazing to reduce n_3 stage losses during winter.

Favour the proportion of n_4 stage at the end of spring to renew n_1 stage.

Figure 2-1. Theoretical model for population dynamics of BFT (partially adapted from Emery *et al.*, 1999). Developmental stages are seed (n_1), seedling (n_2), mature vegetative plant (n_3) and mature reproductive plant (n_4). The references $a_{1 \text{ to } 4}$ represent transitional stages from stages n_1 to n_4.

Promotion of flowering-seeding processes will increase soil seed reserves and encourage the recruitment of new individuals to produce an effective and dynamic replacement of individuals (Figure 2-1). Maintenance of an adequate plant density will depend on the survival rate of established individuals and the recruitment rate of new ones (Jones and Carter, 1989). Young BFT plants often do not reproduce during the first year, but matrix population models indicate that with adequate seed production and recruitment, stand density can be maintained (Emery *et al.*, 1999).

A seed bank like BFT is persistent, where a fraction of the dispersed seed population survives more than one year as a dormant seed (Hutchings, 1997). Population growth is limited because only a portion of the seed population germinates each year, but this ensures that at least some seeds germinate during favourable conditions. In Australia, the size of the seed bank is positively associated with latitude and negatively correlated with mean maximum temperature in January (Blumenthal and Harris, 1993, cited by Blumenthal *et al.*, 1994). The proportion of hard seeds in BFT seed banks is high, 38-41% and 45-54% (Bologna, 1996; Taylor *et al.*, 1973), respectively.

Seedlings of BFT are described as small, poorly competitive and slow growing (Cooper, 1967), usually slower than red clover or lucerne (Grant and Marten, 1985), improving seedling vigour being a breeding objective (McLean and Nowak, 1997). BFT seedlings representing a vulnerable stage in the life cycle are highly intolerant of drought conditions, (Foulds, 1978).

Genetic improvement of seedling vigour may be limited, but major objectives are rapid expansion rate of cotyledons and true leaves, rapid leaf area expansion, improved leaf area ratio, rapid regrowth rate and fast growing root system (Nelson *et al.*, 1994). Seed size appears important as energy storage only during the first 2-3 weeks, other factors achieving relevance after that (Nelson *et al.*, 1994). BFT seedlings initiated growth in late summer or early autumn, under a declining photoperiod length, a disadvantage in competition with aggressive grasses, weeds or companion crops (McKee, 1962).

BFT seedling survival in New Zealand hill and high country is poor. From 128 seedlings/m^2 in autumn, less than 1 seedling/m^2 contributed to the stand after one year (Fraser *et al.*, 1994). Recruitment patterns have a peak in autumn, declining during winter and spring. Despite some advantages in terms of temperature, survival of emerged spring seedlings is low, due to the dry conditions that affect an undeveloped root system (Bologna, 1996). Roberts and Boddrell (1985) identified the spring emergence peak as the most important for *Lotus corniculatus*, *Medicago lupulina*, *Melilotus altissima* and *Trifolium repens*, by breakdown dormancy in winter.

Chapman (1987) reported survival rates lower than 10% in *Trifolium repens*, the rapidly germinable fraction of the soil seed bank having a small effect on short term persistence (Nie, 1997). In Uruguay, seedling emergence of BFT in autumn ranged from 600-1300 seedlings/m^2, but establishment was only 13-90 plants/m^2, an intensive and successive recruitment each year being proposed from predictive models (Olmos, 1996). Phosphorus fertilization increases leaf area index, pod numbers and seed mass of BFT, improving future recruitment (Olmos, 1996).

In fact, long term success of BFT requires careful attention to stand management, to extend the productive life of individuals, minimising stress factors that reduce plant survival to 2-3 years. The manipulation of reproductive processes to develop a seed bank needs to be accompanied by an accurate promotion of seedling recruitment in order to improve plant population. The low efficiency of levels of recruitment supposes successive phases of seeding-recruitment every year, relatively large seed banks, and management practices to increase the opportunity for seedling establishment.

2.8 CONCLUSIONS

Considerable progress has been made in improving BFT in pastoral systems; management practices and the strategic role as a high quality feed source are fields extensively documented in the literature. Despite this, poor persistence remains as an unsolved problem. Diseases and abusive management practices are recognised as the main factors reducing plant populations and consequent productivity. Research is concentrated on alternative procedures to improve persistence by enhanced reproductive processes and disease resistance (Figure 2-2). The interaction of management and disease incidence needs further research, quantifying production losses.

In reproductive processes, efforts are concentrated on aspects of natural reseeding as a way of maintaining population, and grazing management practices to promote natural reseeding and consequent recruitment of individuals. Low efficiency of recruitment processes has been identified in some work, requiring a better definition of the role of the soil seed bank, as well as all processes involved in seedling recruitment and survival.

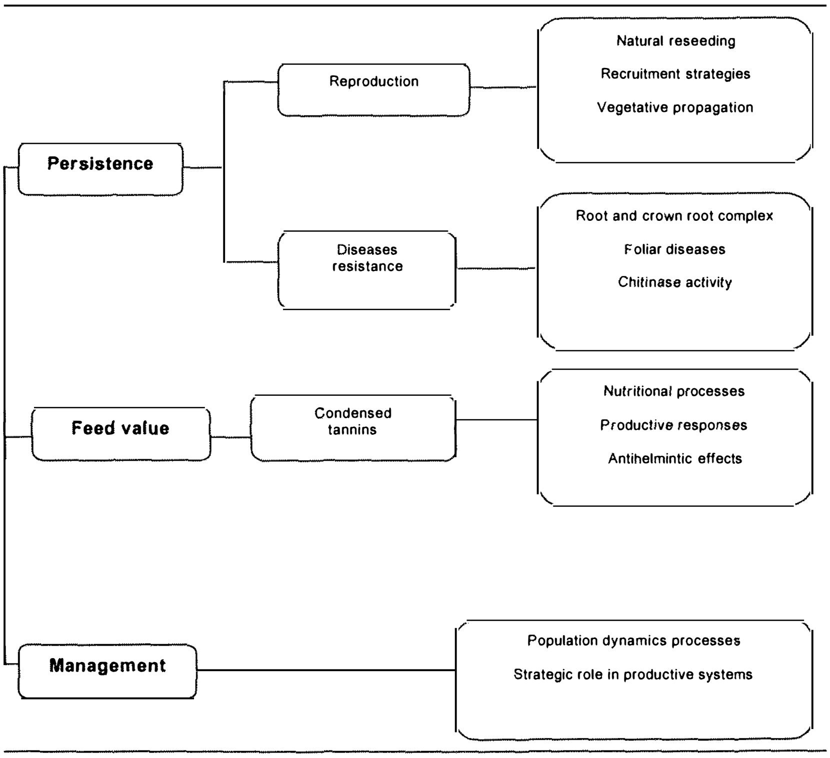


Figure 2-2. Research priorities in birdsfoot trefoil.

Management strategies play a central role, defining seed bank size and seed quality, controlling appearance of vegetation gaps for successful establishment, and directing sward competition in developing stages. The development of a new plant type with presence of rhizomes opens new expectations in terms of increased plant persistence.

The processes highlighted are partially understood, requiring information to enhance accuracy of management practices. Integration of the effects of management decisions on aspects of production and persistence provides the focus for studies reported in this thesis.

2.9 REFERENCES

ACUÑA, H. (1995). Comparación de variedades de tres especies del género Lotus (*Lotus corniculatus* L., *Lotus uliginosus* Cav. y *Lotus tenuis* Wald et Kit.) en suelos de aptitud arrocera. (Comparison of three species of Lotus genus (*Lotus corniculatus* L., *Lotus uliginosus* Cav. y *Lotus tenuis* Wald et Kit.) in soils adapted for rice production). INIA Chile. *Revista Agricultura Técnica* **58**(1): 7-14. ISSN: 0365-2807.

ALISON, M.W. AND HOVELAND, C.S. (1989 a). Birdsfoot trefoil management. I. Root growth and carbohydrate storage. *Agronomy Journal* **81**: 739-745.

ALISON, M.W. AND HOVELAND, C.S. (1989 b). Root and herbage growth response of birdsfoot trefoil entries to subsoil acidity. *Agronomy Journal* **81**: 677-680.

ALISON, M.W. AND HOVELAND C.S. (1989 c). Birdsfoot trefoil management. II. Yield, quality and stand evaluation. *Agronomy Journal* **81**(5): 745-749.

ALTIER, N. (1994). Current status of research on *Lotus* diseases in Uruguay. *Proceedings of The First International Lotus Symposium*. Edited by Paul R. Beuselinck, USDA-Agricultural Research Service and Craig A. Roberts, University of Missouri, Columbia, MO. 22-24 March, 1994. St. Louis, Missouri, USA. pp. 203-205.

ALTIER, N. (1997). Enfermedades del Lotus en Uruguay. (Lotus diseases in Uruguay). INIA La Estanzuela, Uruguay. *Serie Técnica* No. **93**. ISBN: 9974-38-083-9. 16 pg.

BARTA, A.L. (1978). Effect of root temperature on dry matter distribution, carbohydrate accumulation, and acetylene reduction activity in alfalfa and birdsfoot trefoil. *Crop Science* **18**: 637-640.

BEUSELINCK, P.R. AND McGRAW, R.L. (1989). Environmental considerations for *Lotus* production: seed versus herbage. *Proceedings of the XVI International Grassland Congress*. Vol. 1. Nice, France. pp. 649-650.

BEUSELINCK, P.R., PETERS, E.J. AND McGRAW, R.L. (1984). Cultivar and management effects on stand persistence of birsfoot trefoil. *Agronomy Journal* **76**: 490-492.

BEUSELINCK, P.R. AND STEINER, J.J. (1995). Registration of ARS-2620 birdsfoot trefoil. Electronic address: www.nal.usda.gov /ttic /tektran /data/ 000006/ 33 /00000633-74.html

BEUSELINCK, P.R., LI, B. AND STEINER, J.J. (1996). Plant genetic resources. Rhizomatous *Lotus corniculatus* L.: I. Taxonomic and cytological study. *Crop Science* **36**: 179-185.

BLUMENTHAL, M.J., KELMAN, W.M., LOLICATO, S., HARE, M.D. AND BOWMAN, A.M. (1994). Agronomy and improvement of *Lotus*. A review. *"Alternative Pasture Legumes 1993"*. Edited by D.L. Michalk, A.D. Craig and W.J. Collins. Department of Primary Industries South Australia, Technical Report 219. pp. 74-85.

BLUMENTHAL, M.J. AND McGRAW, R.L. (1999). Lotus adaptation, use and management. *Trefoil: The Science and Technology of Lotus.* CSSA Special Publication Number 28. Ed. by P.R. Beuselinck. American Society of Agronomy, Inc. Crop Science Society of America, Inc. Madison, Wisconsin, USA. pp. 97-119.

BOLOGNA, J.J. (1996). Studies on strategies for perennial legume persistence in lowland pastures. *Thesis of Master of Agricultural Science.* Lincoln University. New Zealand. 220 p.

BRIGGS, M.A. (1990). Chemical defense production in *Lotus corniculatus* L. I. The effects of nitrogen source on growth, reproduction and defense. *Oecologia* **83**: 27-31.

BRIGGS, M.A. AND SCHULTZ, J.A. (1990). Chemical defense production in *Lotus corniculatus* L.. II. Trade-offs among growth, reproduction and defense. *Oecologia* **83**: 32-37.

BULLARD, M.J. (1990). The potential of Bird's-foot Trefoil for UK Agriculture. Session 3. Poster 3. Research Meeting 2. In British Grassland Society. Second Research Conference. Scottish Agricultural College, Auchincruive, Ayr, UK. September 1990.

BUXTON, D.R., HORNSTEIN, J.S., WEDIN, W.F. AND MARTEN, G.C. (1985). Forage quality in stratified canopies of alfalfa, birdsfoot trefoil, and red clover. *Crop Science* **25**: 273-279.

CARAMBULA, M.; AYALA, W.; CARRIQUIRY, AND BERMUDEZ, R. (1996). Informe de resultados de la red de evaluación de forrajeras del Cono Sur (REFCOSUR). (Forage evaluation report). INIA Uruguay.

CARLSON, I. T. AND HURST, S. M. (1989). Breeding tall fescue for improved rhizomatous spreading. *Proceedings of the XVI International Grassland Congress,* 4-11 October 1989, Nice, France. Association Francaise pour la Production Fourragere, Centre National de Recherche Agronomique, Versailles, France. pp. 343-344.

CHAO, L.; DE BATTISTA, J. AND SANTIÑAQUE, F. (1994). Incidence of Birdsfoot Trefoil crown and root rot in West Uruguay and Entre Rios. *Proceedings of The First International Lotus Symposium.* Edited by Paul R. Beuselinck, USDA-Agricultural Research Service and Craig A. Roberts, University of Missouri, Columbia, MO. 22-24 March, 1994. St. Louis, Missouri, USA. pp. 206-208.

CHAPMAN, H. M. (1987). Natural re-seeding and *Trifolium repens* demography in grazed hill pastures. II. Seedling appearance and survival. *Journal of Applied Ecology* **24**: 1037-1043.

CHAPMAN, H. M., LOWTHER, W. L. AND TRAINOR, K. D. (1990). Some factors limiting the success of *Lotus corniculatus* in hill and high country. *Proceedings of the New Zealand Grassland Association* **51**: 147-150.

CHARLTON, J.F.L. (1983). Lotus and other legumes. In *Plant Breeding in New Zealand.* Edited by G.S wratt and H.C. Smith. DSIR. ISBN :0409 70137 8. pp. 253-262.

CHARLTON, J.F.L. AND BELGRAVE, B.R. (1992). The range of pasture species in New Zealand and their use in different environments. *Proceedings of the New Zealand Grassland Association* **54**: 99-104.

COLLINS, M. (1982). Yield and quality of birdsfoot trefoil stockpiled for summer utilization. *Agronomy Journal* **74**: 1036-1041.

COOPER, C.S. (1967). Relative growth of alfalfa and birdsfoot trefoil seedlings under low light intensity. *Crop Science* **7**: 175-178.

CORDEIRO DE ARAUJO, J. AND JACQUES, A. (1974). Características morfológicas e producào de matéria seca de cornichào (*Lotus corniculatus* L.) colhido em diferentes estádios de crescimento e a duas alturas de corte. (Morphology and production of dry matter of birdsfoot trefoil (*Lotus corniculatus* L.) at different growth stages and under two defoliation heights). *Revista da Sociedade Brasileira de Zootecnia.* Vol. **3**(2): 138-147.

DIAZ, J.E., GARCIA, J. AND REBUFFO, M. (1996). Crecimiento de Leguminosas en La Estanzuela. (Growth of legumes in La Estanzuela). *Serie Técnica* No. **71**. INIA la Estanzuela, Uruguay. ISBN: 9974-38-054-5.

DOUGLAS, G.B.; WILLS, B.J.; PRYOR, H.N.; FOOTE, A.G. AND TRAINOR K.D. (1996). Establishment of perennial legume species in drought-prone, North and South Island sites. *Proceedings of the New Zealand Grassland Association* **58**: 253-257.

DUELL, R.W. AND GAUSMAN, H.W. (1957). The effect of differential cutting on the yield, persistence, protein and mineral content of birdsfoot trefoil. *Agronomy Journal* **49**: 318-319.

EHLKE, N.J. AND LEGARE, D.G. (1993). The effects of temperature and soil stresses on the production of tannins in birdsfoot trefoil (*Lotus corniculatus* L.). *Lotus Newsletter* Vol. 24.

EMERY, K.M., BEUSELINCK, P., AND ENGLISH, J.T. (1999). Evaluation of the population dynamics of the forage legume *Lotus corniculatus* using matrix population models. *New Phytologist* **144**: 549-560.

ENGLISH, J.T. (1994). Diseases of *Lotus* spp. *Proceedings of The First International Lotus Symposium.* Edited by Paul R. Beuselinck, USDA-Agricultural Research Service and Craig A. Roberts, University of Missouri, Columbia, MO. 22-24 March, 1994. St. Louis, Missouri, USA. pp. 183-186.

FANKHAUSER Jr., J.; VOLENEC, J.J. AND BROWN, G.A. (1989). Composition and structure of starch from taproots of contrasting genotypes of *Medicago sativa* L. *Plant Physiology* **90**: 1189-1194.

FORDE, M.B., HAY, M.J.M. AND BROCK, J.L. (1989). Development and growth characteristics of temperate perennial legumes. In *Persistence of forage legumes.* Edited by G.C.Marten, A.G. Matches, R.F. Barnes, R.W. Brougham, R.J. Clements and G.W. Sheath. Proceedings of the Trilateral Workshop, Honolulu, Hawaii. July 1988. ISBN: 0-89118-098-2. pp. 91-108.

FORMOSO, F. (1993). *Lotus corniculatus.* I. Performance forrajera y características agronómicas asociadas. (Productive performance and agronomic characteristics). *Serie Técnica* No. 37. INIA Uruguay. ISBN: 9974-556-69-4 20 pg.

FOULDS, W. (1978). Response to soil moisture supply in three leguminous species. *New Phytologist* **80**: 535-545.

FRAME, J. (1992). Characteristics of grasses and legumes. Chapter 2. In *Improved Grassland Management.* Published by Farming Press Books. Ipswick. ISBN 0 85236 246 3. pp. 16-17

FRAME, J.; CHARLTON, J.F.L. AND LAIDLAW, A.S. (1998). Birdsfoot Trefoil and Greater Lotus. Chapter 6. In *Temperate Forage Legumes.* CAB International, Wallingford. ISBN 0-85-199-214-5. pp. 245-271.

FRASER, W.J., KEOGHAN, J.M. AND HEENAN, R.P. (1988). Performance of *Lotus corniculatus* cultivars and lines in an altitudinal sequence on the East Otago Plateau. *Proceedings of the Agronomy Society of New Zealand* **18**: 53-57.

FRASER, W.J., OGDEN, S.C., WOODMAN, R.F. AND LOWTHER, W.L. (1994). Role of re-seeding and seedling recruitment for sustainable *Lotus corniculatus* based pastures in dry hill and high country. *Proceedings of the New Zealand Grassland Association* **56**: 139-142.

FULKERSON, R. S (1982). Fall harvest effects on the yield and persistence of birdsfoot trefoil (*Lotus corniculatus* L.). *Lotus Newsletter* **13**: 9-10.

GRACE, J.B. (1991). A clarification of the debate between Grime and Tilman. *Functional Ecology* **5**: 583-587.

GRANT, W.F. AND MARTEN, G.C. (1985). Birdsfoot trefoil. *Forage: The Science of Grassland Agriculture.* Iowa State University. Iowa, USA. Chapter 11, pp. 98-108

GRANT, W.F. AND SMALL, E. (1996). The origin of the *Lotus corniculatus* (Fabaceae) complex: A synthesis of diverse evidence. *Canadian Journal of Botany* **74**: 975-989.

GREGERSON, R.G., ROBINSON, D.L. AND VANCE, C.P. (1999). Carbon and nitrogen metabolism in *Lotus. Trefoil: The Science and Technology of Lotus.* CSSA Special Publication Number 28. Ed. by P.R. Beuselinck. American Society of Agronomy, Inc. Crop Science Society of America, Inc. Madison, Wisconsin, USA. pp. 167-185.

GREUB, L.J. AND WEDIN, W.F. (1971 a). Leaf area, dry-matter accumulation, and carbohydrate reserve levels of birdsfoot trefoil as influenced by cutting height. *Crop Science* **11**: 734-738.

GREUB, L.J. AND WEDIN, W.F. (1971 b). Leaf area, dry-matter accumulation, and carbohydrate reserves of alfalfa and birdsfoot trefoil under a three-cut management. *Crop Science* **11**: 341-344.

GRIME, J.P. (1979). *Plant Strategies and Vegetation Processes.* Wiley, Chichester.

GRIME, J.P. (1988). The C-S-R model of primary plant strategies-origins, implications and tests. Chapter 14. *Plant Evolutionary Biology.* Edited by Leslie D. Gottlieb and Subodh K. Jain.Chapman and Hall.ISBN 0-412-29290 –4. pp. 371-393.

HARRIS, S.L., CLARK, D.A. AND LABOYRIE, P.J. (1998). Birdsfoot trefoil – an alternative legume for New Zealand dairy pastures. *Proceedings of the New Zealand Grassland Association* **60**: 99-103.

HEINRICHS, D.H. (1970). Flooding tolerance of legumes. *Canadian Journal of Plant Science* **50**: 435-438.

HENSON, P.R. (1962). Breeding for resistance to crown and root rots in Birdsfoot Trefoil (*Lotus corniculatus* L.). *Crop Science* **2**: 429-432.

HUTCHINGS, M.J. (1997). The structure of plant populations. In *Plant Ecology.* Edited by Michael J. Crawley. Second edition. ISBN: 0-632-03639-7. Blackwell Science, Oxford. Chapter 11. pp. 325-358.

HYSLOP, M. G., KEMP, P. D. AND HODGSON, J (1998). Preliminary investigations into the persistence of a vegetatively reproductive red clover cv. Astred in mixed swards. *Proceedings of the Agronomy Society of New Zealand.* **28**: 117-122.

INIA (1997). Plan indicativo de mediano plazo. Documento de circulaciön interna. (Research programmes. Internal report)

JOHN, A., ULYATT, M.J., JONES, W.T. AND SHELTON, I.D. (1980). Factors influencing nitrogen flow from the rumen. *Proceedings of the New Zealand Society of Animal Production* **40**: 226.

JOHN, A. AND LANCASHIRE, J.A. (1981). Aspects of the feeding and nutritive value of *Lotus* species. *Proceedings of the New Zealand Grassland Association* **42**: 152-159.

JONES, D.A. AND TURKINGTON, R. (1986). Biological Flora of the British Isles. *Lotus corniculatus* L. *Journal of Ecology* **74**: 1185-1212.

JONES, R.M. AND CARTER, E.D. (1989). Demography of pasture legumes. In *Persistence of Forage Legumes.* Edited by G.C.Marten, A.G. Matches, R.F. Barnes, R.W. Brougham, R.J. Clements and G.W. Sheath. Proceedings of the Trilateral Workshop, Honolulu, Hawaii. July 1988. ISBN: 0-89118-098-2. pp. 139-158.

KALLENBACH, R.L, McGRAW, R.L. AND BEUSELINCK, P.R. (1996). Soil pH effects on growth and mineral concentration of birdsfoot trefoil. *Canadian Journal of Plant Science* **76**(2): 263-267.

KELMAN, W.M. AND TANNER, G.J. (1990). Foliar condensed tannins in *Lotus* species growing on limed and unlimed soils in South-Eastern Australia. *Proceedings of the New Zealand Grassland Association* **52**: 51-54.

KENDALL, W.A. (1958). The persistence of red clover and carbohydrate concentration in the roots at various temperatures. *Agronomy Journal* **50** :657-659.

KUNELIUS, H.T. AND CLARK, K.W. (1970). Influence of root temperature on the early growth and symbiotic nitrogen fixation of nodulated *Lotus corniculatus* plants. *Canadian Journal of Plant Science* **50**: 569-575.

LEATH, K.T. (1989). Diseases and forage stand persistence in the United States. In *Persistence of Forage Legumes.* Edited by G.C Marten; A.G Matches; R.F. Barnes; R.W. Broughman; R.J. Clements; and G.W. Sheath. Second Printing 1989. ISBN 0-89118-098-2. pp. 465-479.

LEVY, E.B. (1918). The birdsfoot trefoil. *New Zealand Journal of Agriculture* **17**: 347-351.

LI, Q. (1989). Seed production in birdsfoot trefoil (*Lotus corniculatus* L.). *Thesis for the Degree of Doctor of Philosophy.* Massey University. Palmerston North, New Zealand.175 p

LI, B. AND BEUSELINCK, P.R. (1996). Plant genetic resources. Rhizomatous *Lotus corniculatus* L.:II. Morphology and anatomy of rhizomes. *Crop Science* **36**: 407-411.

LI, R.; VOLENEC, J.J.; JOERN, B.C. AND CUNNINGHAM, S.M. (1996). Seasonal changes in nonstructural carbohydrates, protein, and macronutrients in roots of alfalfa, red clover, sweetclover and birdsfoot trefoil. *Crop Science* **36**: 617-623.

LLOYD, D.G. (1980). Sexual strategies in plants. I. An hypothesis of serial adjustment of maternal investment during one reproductive session. *New Phytologist* **86**: 69-80.

LOPEZ, J., PRESTES, P.J.Q AND MAGALHAES, E. (1965). A curva de crescimento e a composição em carboidratos solúveis, estruturais, lignina e proteina, e a digestibilidade em cornichão. (Growth and composition in carbohydrates, lignin, protein and digestibility of birdsfoot trefoil). *Proceedings of the IX International Grassland Congress*. Sao Paulo, Brazil. Volume **1**, pp. 851-857.

LOWTHER, W.L. AND PATRICK, H.N. (1993). Spread of *Rhizobium* and *Bradyrhizobium* in soil. *Soil Biology and Biochemistry* **25**: 607-612.

McGRAW, R.L. AND MARTEN, G.C. (1986). Analysis of primary spring growth of four pasture legume species. *Agronomy Journal* **78**: 704-710.

McGRAW, R.L., RUSELLE, M.P. AND GRAVA J. (1986). Accumulation and distribution of dry mass and nutrients in birdsfoot trefoil. *Agronomy Journal* **78**: 124-131.

McKEE, G.W. (1962). Some effects of daylenght on seeding growth and nodulation in birdsfoot trefoil. *Crop Science* **2**: 315-317.

McKEE, G.W. (1963). Influence of daylength on flowering and plant distribution in birdsfoot trefoil. *Crop Science* **3**: 205-208.

McLEAN, N.L. AND NOWAK, J. (1997). In vitro selection for improved seedling vigour in birdsfoot trefoil (*Lotus corniculatus* L.). *Canadian Journal of Plant Science* **77**: 385-391.

MILLER, P.R. AND EHLKE N.J. (1997). Inheritance of condensed tannins in birdsfoot trefoil. *Canadian Journal of Plant Science* **77**: 587-593.

MIN, B.R., BARRY, T.N., MCNABB, W.C. AND KEMP, P. (1998). Effect of condensed tannins on the production of wool and its processing characteristics in sheep grazing *Lotus corniculatus*. *Australian Journal of Agricultural Research* **49**: 597-605.

MORALES, A. (1992). Manejo de la defoliación de *Lotus corniculatus* sembrado en cobertura sobre una pastura natural. (Defoliation management of oversown birdsfoot trefoil). *Tesis Ing. Agr. Facultad de Agronomía*. Montevideo, Uruguay. 71 pg.

NELSON, C.J.; AND SMITH, D. (1968 a). Growth of birdsfoot trefoil and alfalfa. II. Morphological development and dry matter distribution. *Crop Science* **8**: 21-25.

NELSON, C.J. AND SMITH, D. (1968 b). Growth of birdsfoot trefoil and alfalfa. III. Changes in carbohydrate reserves and growth analysis under field conditions. *Crop Science* **8**: 25-28.

NELSON, C.J. AND SMITH, D. (1969). Growth of birdsfoot trefoil and alfalfa. IV. Carbohydrate reserve levels and growth analysis under two temperature regimes. *Crop Science* **9**: 589-591.

NELSON, C.J., HUR, S.N. AND BEUSELINCK, P.R. (1994). Physiology of seedling vigour in birdsfoot trefoil. *Proceedings of The First International Lotus Symposium.* Edited by Paul R. Beuselinck, USDA-Agricultural Research Service and Craig A. Roberts, University of Missouri, Columbia, MO. 22-24 March, 1994. St. Louis, Missouri, USA. pp. 68-71.

NIE, Z. (1997). The influence of pastoral fallow on hill country pastures, emphasising white clover growth behaviour. *Thesis for the Degree of Doctor of Philosophy.* Massey University. Palmerston North, New Zealand.. 185 p.

NIEZEN, J.H., WAGHORN, T.S., WAGHORN, G.C. AND CHARLESTON, W.A.G. (1993). Internal parasites and lamb production – a role for plants containing condensed tannins?. *Proceedings of New Zealand Society of Animal Production* **53**: 235-238.

OLMOS, F. (1996). Estrategias de persistencia y productividad de Lotus. (Strategies for persistence and productivity in Lotus). *Tesis de Maestría en Biología.* Opción Ecología. PEDECIBA. Montevideo. Uruguay. 102 pg.

PATRICK, H.N. AND LOWTHER, W.L. (1992). Response of *Lotus corniculatus* to inoculation and pelleting on a range of Otago tussock grassland environments. *Proceedings of the New Zealand Grassland Association* **54**: 105-109.

PIANKA, E.R. (1983). Evolutionary Ecology, 3rd. Edition. Harper & Row, New York.

PIERRE, J.J. AND JACKOBS, J.A. (1953). The effect of cutting treatments on birdsfoot trefoil. *Agronomy Journal* **45**: 463-468.

RISSO, D.F., COSCIA, P. AND SURRACO, L. (1983). Productividad de un tapiz de Lotus (*Lotus corniculatus* L.) bajo tres manejos del pastoreo. (Productivity of birdsfoot trefoil under three grazing strategies). *Investigaciones Agronómicas* No. **4**. Año 4. No. 1. pp. 50-56.

ROBERTS, C.A. AND BEUSELINCK, P.R. (1992). Condensed tannins in Lotus species. *Lotus Newsletter* . Vol. **23**.

ROBERTS, C.A., KARR, A.L., MOHAMMADI, M., MAREK, S.M. AND BEUSELINCK, P.R. (1994). Chitinases in *Lotus corniculatus. Proceedings of The First International Lotus Symposium.* Edited by Paul R. Beuselinck, USDA-Agricultural Research Service and Craig A. Roberts, University of Missouri, Columbia, MO. 22-24 March, 1994. St. Louis, Missouri, USA. pp. 187-190.

ROBERTS, H.A. AND BODDRELL, J.E. (1985). Seed survival and seasonal pattern of seedling emergence in some Leguminoseae. *Annals of Applied Biology* **106**: 125-132.

ROBERTSON, H.A., NIEZEN, J.H., WAGHORN, G.C., CHARLESTON, W.A.G. AND JINLONG, M. (1995). The effect of six herbages on liveweight gain, wool growth and faecal egg count of parasitised ewe lambs. *Proceedings of the New Zealand Society of Animal Production* **55**: 199-201.

SCHEFFER-BASSO, S.M., JACQUES, A.V.A. AND CASTRO, S. (1998). Qualidade de forragem de especies de *Adesmia Dc.* e *Lotus L.* . I. Proteina Bruta e Digestibilidade in vitro da materia orgánica. (Quality of *Adesmia Dc.* e *Lotus L.* species. I. Protein and digestibility). Anais de XVII Reuniáo do grupo técnico em Forrageiras do Cone Sul. Zona Campos. *Utilizacáo sustentavel é melhoramento de campos naturais do Cone Sul: Desafios para o III Milênio.* Octubro, 1998. pp. 116.

SCOTT, D. AND CHARLTON, J.F.L. (1983). Birdsfoot trefoil (*Lotus corniculatus*) as a potential dryland herbage legume in New Zealand. *Proceedings of the New Zealand Grassland Association* **44**: 98-105.

SEANEY, R.R. AND HENSON, P.R. (1970). Birdsfoot trefoil. *Advances in Agronomy* **22**: 119-157.

SHEATH, G.W. AND HAY, R.J.M. (1989). Overview of legume persistence in New Zealand. In *Persistence of Forage Legumes.* Edited by G.C.Marten, A.G. Matches, R.F. Barnes, R.W. Brougham, R.J. Clements and G.W. Sheath. Proceedings of the Trilateral Workshop, Honolulu, Hawaii. July 1988. ISBN: 0-89118-098-2. pp. 23-36.

SMITH, D. (1962). Carbohydrate root reserves in alfalfa, red clover, and birdsfoot trefoil under several management schedules. *Crop Science* **2**: 75-78.

SMITH, D. (1975). *Forage management in the North* 3rd Edition. Kendall Hunt Publishers Co., Dubuque, Iowa. 237 p

SMITH, D. AND NELSON, C.J. (1967). Growth of birdsfoot trefoil and alfalfa. I. Responses to height and frequency of cutting. *Crop Science* **7**: 130-133.

SMITH, D. AND SOBERALSKE, R.M. (1975). Comparison of the growth response of spring and summer plants of alfalfa, red clover and birdsfoot trefoil. *Crop Science* **15**: 519-522.

SMITH, R. S. AND BISHOP, D. J (1998). Register of Australian herbage plant cultivars: B. Legumes 1. Clover (b) *Trifolium pratense* L. (red clover) cv. Astred. *Australian Journal of Experimental Agriculture* **38**(3): 319-324.

STEPHENSON, A.G. (1984). The regulation of maternal investment in an indeterminate flowering plant (*Lotus corniculatus*). *Ecology* **65**(1): 113-121.

STEWART, S., FORMOSO, F. AND ALTIER N. (1994). A flower blight on birdsfoot trefoil caused by *Colletotrichum acutatum*. *Proceedings of The First International Lotus Symposium.* Edited by Paul R. Beuselinck, USDA-Agricultural Research Service and Craig A. Roberts, University of Missouri, Columbia, MO. 22-24 March, 1994. St. Louis, Missouri, USA. pp. 210-211.

TAYLOR T.H., TEMPLETON JR. W.C. AND WYLES J.W. (1973). Management effects on persistence and productivity of birdsfoot trefoil (*Lotus corniculatus* L.). *Agronomy Journal* **65**: 646-648.

TURKINGTON, R. AND FRANKO, G.D. (1980). The Biology of Canadian Weeds. 41. *Lotus corniculatus* L. *Canadian Journal of Plant Science* **60**: 965-979.

THOMSON, H.G.M. (1922). *The Naturalisition of Animals and Plants in New Zealand.* Cambridge University Press, UK. 399 p.

TWAMLEY, B.E. (1967). Seed size and seedling vigour in birdsfoot trefoil. *Canadian Journal of Plant Science* **47**: 603-609.

TWAMLEY, B.E. (1968). A management study of a birdsfoot trefoil strain trial. *Journal of the British Grassland Society* **23**: 322-325.

VAN KEUREN, R.W. AND DAVIS, R.R. (1968). Persistence of birdsfoot trefoil, *Lotus corniculatus* L. as influenced by plant growth habit and grazing management. *Agronomy Journal* **60**: 92-95.

VICKERY, P.J. (1981). Pasture growth under grazing. Chapter 4. In World Animal Science Vol. B1: *Grazing Animals.* Edited by F.H.W. Morley. Amsterdam, Elsevier. pp. 55-71. 405 p

VIGNOLIO, O., MACEIRA, N. AND FERNANDEZ O. (1994). Efectos del anegamiento en invierno y verano sobre el crecimiento y la supervivencia de *Lotus tenuis y Lotus corniculatus.* (Effects of winter and summer flooding on growth and survival of *Lotus tenuis y Lotus corniculatus*). *Ecología Austral* **4**: 19-28.

WAGHORN, G.C. AND SHELTON, I.D. (1997). Effect of condensed tannins in *Lotus corniculatus* on the nutritive value of pasture for sheep. *Journal of Agricultural Science,* Cambridge **128**: 365-372.

WAGHORN, G.C., DOUGLAS, G.B., NIEZEN, J.H., McNABB, W.C. AND FOOTE, A.G. (1998). Forages with condensed tannins – their management and nutritive value for ruminants. *Proceedings of the New Zealand Grassland Association* **60**: 89-98.

WANG, Y., DOUGLAS, G.B., WAGHORN, G.C., BARRY, T.N. AND FOOTE, A.G. (1996 a). Effect of condensed tannins in *Lotus corniculatus* upon lactation performance in ewes. *Journal of Agricultural Science,* Cambridge **126**: 353-362.

WANG, Y., DOUGLAS, G.B., WAGHORN, G.C., BARRY, T.N., FOOTE, A.G. AND PURCHAS, R.W. (1996 a). Effect of condensed tannins upon the performance of lambs grazing *Lotus corniculatus* and Lucerne (*Medicago sativa*). *Journal of Agriculture Science,* Cambridge **126**: 87-98.

WANG, Y., WAGHORN, G.C., MCNABB, W.C., BARRY, T.N., HEDLEY, M.J., AND SHELTON, I.D. (1996 b). Effect of condensed tannins in *Lotus corniculatus* upon the digestion of methionine and cysteine in the small intestine of sheep. *Journal of Agricultural Science,* Cambridge **127**: 413-421.

WATSON, R.N., SKIPP, R.A., AND BARRATT, I.P. (1989). Initiatives in pest and disease control in New Zealand towards improving legume production and persistence. In *Persistence of Forage Legumes.* Edited by G.C.Marten, A.G. Matches, R.F. Barnes, R.W. Brougham, R.J. Clements and G.W. Sheath. Proceedings of the Trilateral Workshop, Honolulu, Hawaii. July 1988. ISBN: 0-89118-098-2. 441-464.

WIDDUP, K.H., KEOGHAN, J.M., RYAN, D.L. AND CHAPMAN, H. (1987). Breeding *Lotus corniculatus* for South Island tussock country. *Proceedings of the New Zealand Grassland Association* **48**: 119-124.

3. CHANGES IN THE MORPHOLOGY, PRODUCTION AND POPULATION OF *Lotus corniculatus* L. cv. GRASSLANDS GOLDIE IN RESPONSE TO SEASONAL DEFOLIATION REGIMES ♣

♣ A restricted version of this chapter has been submitted to the *Proceedings of Agronomy Society of New Zealand* (Ayala *et al.*, 2000)

3.1 ABSTRACT

A field experiment was conducted from April to December 1997 at Massey University, Palmerston North, New Zealand to study responses in morphology, production and population of birdsfoot trefoil (BFT) (*Lotus corniculatus* L. cv. Grasslands Goldie) to seasonal defoliation strategies. A factorial design (2x3x2) was applied in a complete randomised block arrangement with four replicates on a three year old BFT stand with 94 plants/m^2. Treatments included two autumn managements (last cut April or June), and a combination of two defoliation intervals (20, 40 days) and three defoliation intensities (2, 6, 10 cm) during spring (September-December). Herbage mass, sward height, botanical composition, plant density and plant morphology parameters (primary and secondary shoots, root diameter, crown and root mass) were recorded. BFT spring production reached 3000 kg DM/ha with a mean regrowth rate of 34 kg DM/ha/day. Early autumn rest (final cut April) improved root carbohydrates content in early spring and increased BFT spring production 17%, but did not affect plant density or the main morphological parameters. Hard defoliation in spring (2 cm) reduced BFT production (17%) and plant population (21%) compared with the average of other defoliation intensities evaluated, and reduced root mass, crown mass, primary and total shoots/m^2, and root reserves. In general, height of defoliation had the greatest effects. Defoliation frequency did not affect forage production and plant density. Although Grasslands Goldie is a semi-prostrate BFT, intensive spring defoliation greatly reduced productivity and persistence, and late autumn utilisation diminished spring production.

Keywords: *Lotus corniculatus* L., seasonal management, forage production, plant morphology, persistence.

3.2 INTRODUCTION

Birdsfoot trefoil (*Lotus corniculatus* L.) (BFT) has received attention as an alternative legume species for New Zealand pastoral systems, primarily for its adaptability to less fertile dryland environments of hill and high country (Scott and Charlton, 1983; Chapman *et al.*, 1990; Charlton and Belgrave, 1992). It has a high nutritive value for milk (Woodward *et al.*, 1999), wool (Min *et al.*, 1998), and meat production (Douglas *et al.*, 1999) partly resulting from the presence of condensed tannins (John *et al.*, 1980; John and Lancashire, 1981). Grazing management strategies for BFT have received minor attention, and in many cases follow recommendations for lucerne (Scott and Charlton, 1983), despite it being recognised that BFT has a declining production and lack of persistence under intensive grazing (Bologna, 1996). The only BFT variety used in New Zealand is cv. Grasslands Goldie, a semi-prostrate type adapted to grazing (AgResearch Grasslands, 1995). Better persistence is reported for semi-prostrate types than for upright-growing types of BFT because of differences in the exposure of meristems to grazing (Van Keuren and Davis, 1968; Beuselinck *et al.*, 1984).

The objectives of this research were to determine the influence of the timing of cessation of defoliation in autumn and the frequency and intensity of spring defoliation on the productivity and persistence of BFT cv. Grasslands Goldie in a preliminary short term study.

3.3 MATERIALS AND METHODS

The experiment was conducted on a three year old *Lotus corniculatus* L. cv. Grasslands Goldie stand, at the Deer Research Unit, Massey University, Palmerston North, New Zealand (latitude 40°23'S), from April 1997 to December 1997. The soil type was a deep Tokomaru silt loam (Hewitt, 1992), with pH 5.7 and high fertility (Olsen P 24 mg/kg). During summer 1996/1997 and to the end of March 1997, the sward was grazed rotationally at intervals of 30-40 days, at moderate intensities with deer.

The treatments were applied in a 2x3x2 factorial combination, using a randomised complete block design with four replicates in plots of 3 x 6 m. Initially, there were two autumn managements with plots cut on 25 April (early autumn rest) or 25 April and 10 June (late autumn rest) with a residual height of 3 cm. Thereafter, the plots were rested until early spring, when a combination of three defoliation intensities (2, 6 or 10 cm) and two defoliation frequencies (20 or 40 days) were introduced. The 2 and 6 cm defoliation treatments started on 10 September, and 10 cm defoliation started on 30 September, when BFT Goldie achieved the minimum height required for treatments. The cutting sequence finished on 19 December for all treatments, to allow spelling for seed production.

Cuts to defined heights were made using a rotary lawn mower, removing herbage from plots. Plots were sprayed on 7 May with Nortron® (Ethofumesate, 1.4 l/ha a.i.) to control white clover, and on 24 June with Preside® (Flumetsulam, 24 g/ha a.i.) to control broadleaf weeds, applications being successful in both cases.

3.3.1 Measurements

3.3.1.1 Herbage production

Pre-harvest herbage mass was measured at each harvest date and post-harvest mass was recorded on three occasions, by cutting to ground level with an electric shearing hand-piece in two 500 x 200 mm quadrats per plot. Those quadrat areas were marked to avoid repeat sampling. Samples were washed to remove residues. Botanical composition was measured for each sample, by separating into BFT and other components (white clover, grasses and weeds), and then oven-drying at 60 °C for 48 hours. From each sample, 10 stems were randomly collected and dissected into leaves and stems, oven-dried and weighed. Sward height was evaluated in each quadrat, taking four readings at the top of the undisturbed BFT sward.

Herbage accumulation (Table 3-2) was calculated as Σ (pre-harvest herbage $\text{mass}_{(n+1)}$ – post-harvest herbage $\text{mass}_{(n)}$). Post-harvest herbage mass (kg DM/ha, y_{post}) when quadrat cuts were not taken was estimated from sward height (cm, x) using an equation constructed with measured post-harvest data.

3.3.1.2 Plant density, size and morphology

In July, September and December, a soil block of 250 x 250 x 250 mm was taken in each plot, and manually washed to remove soil and litter. Plants were counted, recording for each plant the number of primary and secondary shoots. Primary shoots were defined as the main shoots emerging from the crown, and secondary shoots as originating in the axils of each primary shoot (Plate 3-1). The diameter of the main root was measured at a section cut 10 mm below the level of insertion of primary shoots. Crown and roots (> 2 mm diameter) were oven-dried at 60 °C for 48 hours for dry weight. Crown was defined as the portion above the cut for root diameter measurement to ground level.

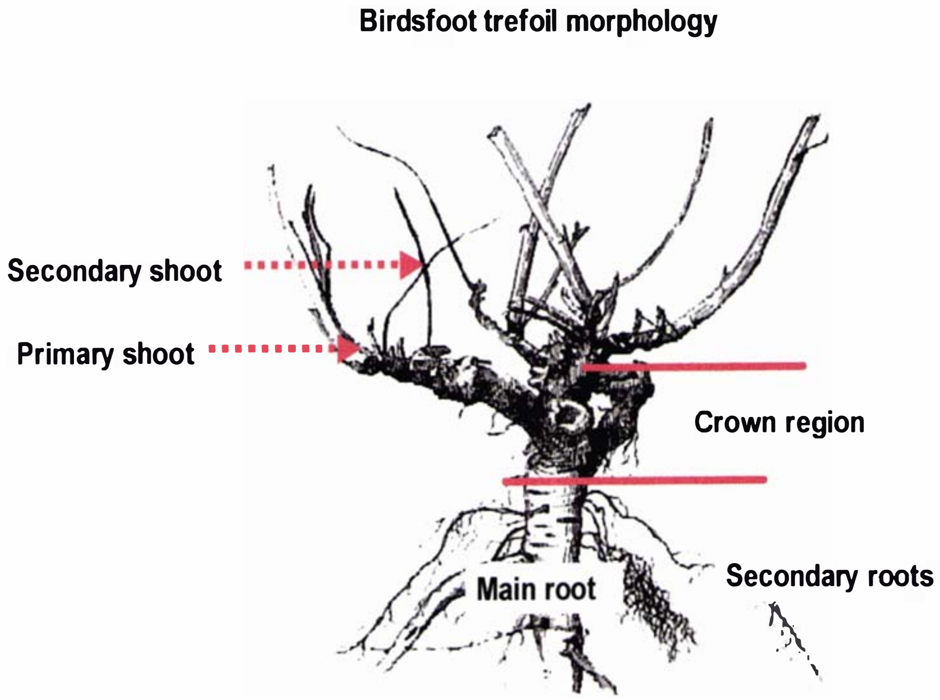


Plate 3-1. Birdsfoot trefoil plant showing the morphological parameters evaluated.

3.3.1.3 Root carbohydrate analysis

Root samples were collected in September 1997 (4 plants randomly selected from each autumn treatment) and December 1997 (1 plant/plot in each treatment). Root samples were washed, separated from crown and the main root, frozen and freeze-dried. Samples were ground to pass a 1mm sieve, making one sample per treatment bulked across replicates. From samples collected in December, only the early autumn (April) rest group with all combinations of frequency and intensity of defoliation were analysed, based on the reduced residual effects tested in other treatments at the end of the evaluation (December, 1997). Total available carbohydrates were extracted with perchloric acid and reaction with anthrone (modified from Clegg, 1956, see Appendix 1). The procedure included the extraction of sugars (glucose, fructose and sucrose) first, and the extraction of starch (amylose and amylopectin) in a second step (Southgate, 1991). In general, extraction with acid provides the same treatment contrasts as extraction using the enzyme method of Weinmann (1947). Carbohydrate percentages tend to be slightly higher than from the enzyme method, but involve less analytical time (Smith, 1962).

3.3.2 Statistical analysis

Data were analysed by SAS GLM procedures (SAS Institute, 1990), using a factorial model in a complete randomised block design for herbage accumulation, plant morphology and population parameters. Morphology and population parameters generated from sequential sampling were also analyzed using the 'repeated measures' option of SAS program. Carbohydrates reserves were not statistically analysed.

3.4 RESULTS

3.4.1 Climate conditions during experimental period

In general, rainfall was 18% less than the 60-year mean, with a dry winter in which rainfall was 55% of the average (Table 3-1). In spring, rainfall was 11% less than average. Soil temperatures were similar to the 60-year mean (11.1 °C), particularly during the growing season (AgResearch, Palmerston North).

Table 3-1. Monthly rainfall and soil temperature at 100 mm during the evaluation period and the 60-year average (Source: AgResearch, Palmerston North).

	Rainfall (mm)		Soil Temperature (°C)	
	1997	60-year average	1997	60-year average
April	145	81	12.5	13.2
May	24	89	11.6	10.1
June	60	97	8.1	7.7
July	32	89	6.4	6.7
August	60	89	7.3	7.6
September	79	75	9.1	9.9
October	78	88	12.4	12.5
November	57	78	15.5	15.1
December	103	94	17.0	17.3

3.4.2 Herbage production

Forage production of BFT was on average 3000±783 kg DM/ha during the spring period (10 September - 19 December). Herbage masses pre-grazing (kg DM/ha, y_{pre}) and post-grazing (kg DM/ha, y_{post}) were positively correlated with sward height (cm, x) by the equations $y_{pre} = 325.6 + 120.4x$ ($P<0.01$, $r^2 = 0.72$, n = 246) and $y_{post} = -150.3 + 139.4x$ ($P<0.01$, $r^2=0.77$, n =144), respectively.

Herbage accumulation was significantly affected by autumn management (P<0.01) and defoliation intensity in spring (P<0.05), but there were no significant effects of frequency of defoliation or interaction between main factors (Table 3-2). Early autumn rest (April) increased spring production by 17%, and forage production was also greater when managed under 6 cm stubble height than at 2 or 10 cm.

Table 3-2. The effect of defoliation management on BFT accumulation (kg DM/ha), growth rate (kg DM/ha/day) and contribution to total production (%) during spring.

Cutting treatments	BFT accumulation (kg DM/ha)	BFT growth rate (kg DM/ha/day)	BFT contribution (%)
Autumn defoliation			
Early rest	3290	37	65
Late rest	2730	31	56
SEM (n)	134 (24)	1.6 (24)	1.6 (24)
Significance	**	**	**
Spring frequency			
20 days	2830	32	58
40 days	3180	37	64
SEM (n)	134 (24)	1.6 (24)	1.6 (24)
Significance	NS	NS	*
Spring intensity			
2 cm	2690	28	49
6 cm	3400	35	62
10 cm	2920	38	71
SEM (n)	164 (16)	1.9 (16)	2.0 (16)
Significance	*	**	**

P<0.05; **P<0.01; NS, not significant; SEM, standard error of the mean; (n), number of observations for each treatment mean

Spring growth rate averaged 34 kg DM/ha/day, and was significantly influenced by autumn management ($P<0.01$) and defoliation intensity ($P<0.01$). An extended defoliation period during autumn reduced spring growth by 17%. Hard defoliation (2 cm) decreased growth by 24% compared with 6 or 10 cm defoliation height (Table 3-2). Despite the tendency for a high regrowth rate for the 10 cm treatment, total accumulation was lower than for the 6 cm height because of the delay to first harvest and consequent short accumulation period (80 days vs 100 days).

BFT contributed a mean of 61% of total dry mass in spring (Table 3-2), but the BFT proportion was reduced by delayed autumn rest (June), short defoliation frequency (20 day intervals), and hard defoliation (2 cm height). There were no significant interactions.

A significant interaction for frequency x intensity of defoliation ($P<0.01$) was detected for the ratio leaf/(leaf+stem). Under 20 day intervals between defoliation, the proportion of leaves decreased when defoliation intensity decreased from 2 cm to 10 cm residual height, but at 40 day intervals the reduction in the proportion of leaves was higher in 6 cm than the 10 cm height treatment (Table 3-3). Leaf proportion of plots cut at 2 cm and 20 day intervals was significantly higher than for the other treatments, while the three heights managed at 40 day intervals and the 10 cm height at 20 day intervals were not different, having a ratio leaf:stem near 1:1.

Table 3-3. Leaf/(Leaf+Stem) ratio (dry weight) in BFT under different defoliation frequencies and intensities in spring.

Frequency	Intensity	Leaf/(Leaf+Stem)	SEM	Observations
20 days	2 cm	0.69	0.017	52
20 days	6 cm	0.61	0.017	51
20 days	10 cm	0.48	0.021	36
40 days	2 cm	0.57	0.024	28
40 days	6 cm	0.46	0.025	28
40 days	10 cm	0.51	0.024	28

SEM, standard error of the mean

3.4.3 Plant density

During winter, stand reduction was 8% but independent of autumn defoliation management (P<0.85). Spring plant losses increased significantly (P<0.01) with defoliation intensity, with reductions of 2, 16 and 32% for the 10, 6 and 2 cm heights respectively, from September to December (Figure 3-1, Plate 3-2). A significant interaction for time x defoliation intensity (P<0.05) was detected. There were no effects from autumn management, spring defoliation frequency or other interactions between treatments on the sampling dates evaluated.

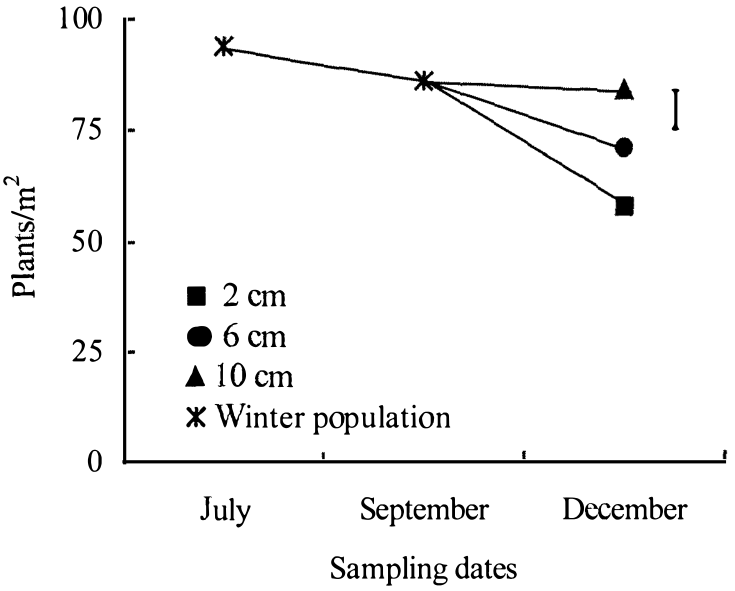


Figure 3-1. Treatment effects on seasonal changes in birdsfoot trefoil plant density. Vertical bar represents SEM, (n =16).

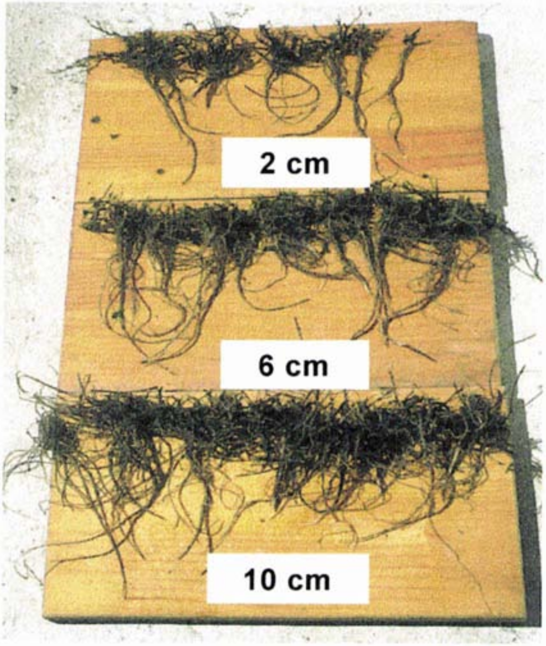


Plate 3-2. Number of plants of birdsfoot trefoil in December 1997 under three intensities of defoliation (2, 6 and 10 cm). Samples represent 250 x 250 mm quadrat.

3.4.4 Plant morphology

In general, morphological components of plants per unit area were not modified by autumn management or by spring frequency (Table 3-4) and no significant interaction effects were observed at the end of the trial. However, root mass/m^2 was decreased ($P<0.05$) by hard (2 cm) defoliation intensity (Table 3-4), largely due to a reduction in plant density. Crown mass/m^2 was reduced by 45% ($P<0.01$), and total belowground mass/m^2 by 41% in plants defoliated to 2 cm compared with the other defoliation intensities. Hard defoliation (2 cm) reduced primary shoots/m^2 by 49% compared with more lenient defoliation (Table 3-4). Also, secondary shoots were reduced significantly ($P<0.05$) under short defoliation intervals (20 days).

Table 3-4. Effects of defoliation treatments on plant morphology parameters/m^2 and plant density in December 1997 for birdsfoot trefoil cv. Grasslands Goldie.

Cutting treatments	**Root mass (g/m^2)**	**Crown mass (g/m^2)**	**Primary shoots (no./m^2)**	**Secondary shoots (no./m^2)**	**Plant Density (no./m^2)**
Autumn defoliation					
Early rest	40	84	285	661	69
Late rest	38	80	283	689	73
SEM (n)	3.3 (24)	6.0 (24)	25 (24)	49 (24)	4.7 (24)
Significance	NS	NS	NS	NS	NS
Spring frequency					
20 days	37	74	262	598	69
40 days	41	90	306	752	73
SEM (n)	3.3 (24)	6.0 (24)	25 (24)	49 (24)	4.7 (24)
Significance	NS	NS	NS	*	NS
Spring intensity					
2 cm	29	53	212	564	58
6 cm	43	97	312	692	71
10 cm	45	96	327	769	84
SEM (n)	4.1 (16)	7.3 (16)	31 (16)	59 (16)	5.7 (16)
Significance	*	**	*	NS	**

NS, not significant; *, $P<0.05$; **, $P<0.01$; SEM, standard error of the mean; (n), number of observations for each treatment mean

Autumn management affected root diameter in July (11 and 9 mm for April and June autumn rest, $P<0.05$, SEM 0.4), but not in early or late spring. Plants defoliated frequently and severely (each 20 days and at 2 cm height) showed a reduced root diameter in December, compared with plants defoliated frequently but at 10 cm height (8 mm vs. 9 mm, $P<0.05$, SEM 0.4). There were no differences in root diameter between defoliation intensities when defoliated at intervals of 40 days.

Individual crown and belowground masses were affected by spring defoliation frequency ($P<0.05$), and intensity ($P<0.01$), with both increasing under 40 day intervals or cutting at 6 cm height. Number of primary shoots declined over time ($P<0.01$), from 6 shoots/plant in July to 4 shoots/plant in December, but was not affected by management treatments.

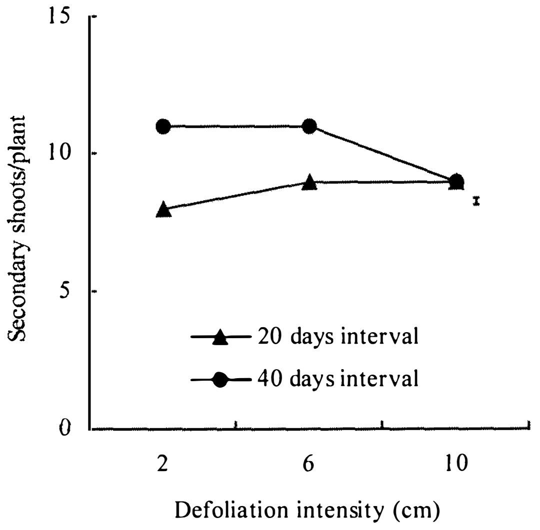


Figure 3-2. Effects of defoliation management on secondary shoots per plant of BFT in December, under two intervals and three intensities of defoliation. Vertical bar represents SEM, (n=8).

In December, a significant cutting frequency x intensity of defoliation interaction was detected for number of secondary shoots/plant ($P<0.05$), which declined progressively from 10 cm to 2 cm defoliation height in plants defoliated every 20 days, but were higher at 2 and 6 cm than 10 cm in plants defoliated every 40 days (Figure 3-2).

3.4.5 Root carbohydrate reserves

Results of root carbohydrate analysis should be treated with caution because of the lack of replication, but treatment contrasts were substantial. From exploratory analysis, root carbohydrate content in early spring (September) was four times greater for BFT plants that received an early rest in autumn (April) than in plants with a late autumn rest (June) (Figure 3-3). The free sugar fraction was most affected, being 6 times higher per root for early rest (April).

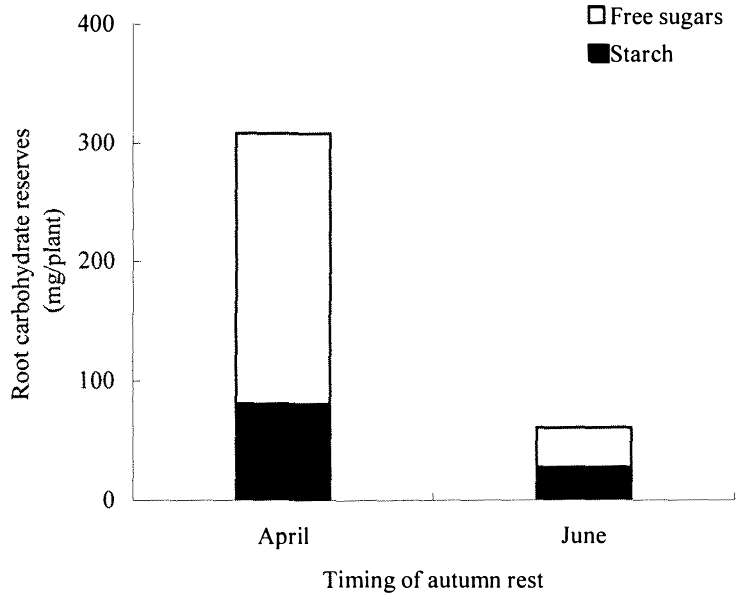


Figure 3-3. Carbohydrate reserves in BFT roots in early spring of plants receiving two contrasting autumn managements (early rest in April or late rest in June).

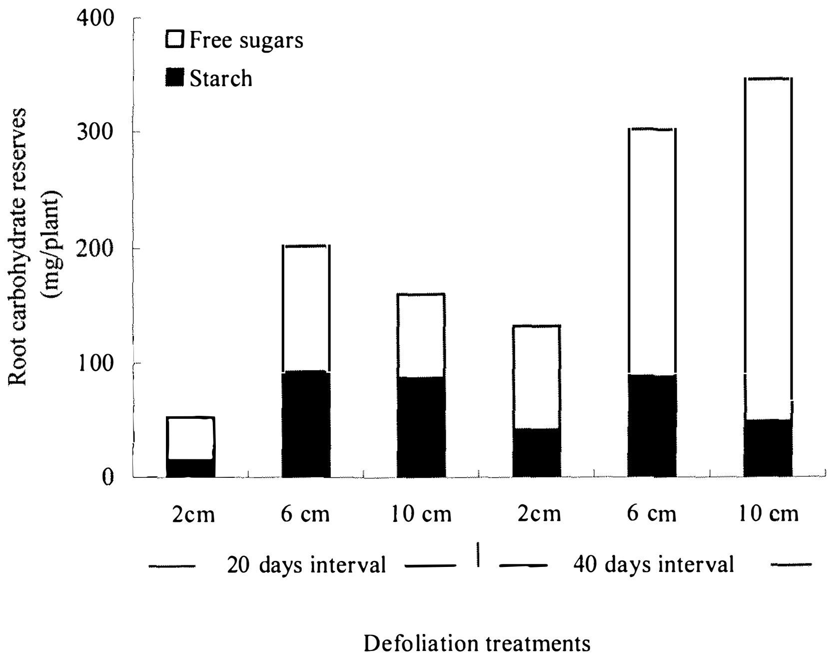


Figure 3-4. Carbohydrate reserves in BFT roots at the end of spring (December 1997) of plants managed under two intervals and three intensities of defoliation.

At the end of spring, total carbohydrate reserves were 19% higher on average for the 40 day than the 20 day defoliation interval, mainly by an increase in the content of free sugars (glucose, fructose, sucrose) (Figure 3-4). Plants defoliated at 2 cm height showed the lowest total carbohydrate content, free sugars and starch content, which was 2.7 times lower than the average of 6 and 10 cm height

3.5 DISCUSSION

The most significant result of this trial was the decline observed in plant density of BFT, from autumn to the end of spring. The intensity of spring defoliation affected plant survival, which was severely reduced under hard defoliation (Figure 3-1). Lesser defoliation intensities resulted in improved plant survival, and for the case of 10 cm defoliation height the initial spring population was effectively maintained. There were no effects of defoliation frequency or autumn management on plant survival. Winter losses were independent of autumn management, suggesting additional factors in that process.

The age of the BFT sward could partially explain plant losses, as a high incidence of crown and root diseases is reported for stands 2-3 years old in other regions (Altier, 1997). In this case, plants taken from the experimental area showed a high incidence of diseases (*Rhizoctonia, Fusarium*), when transferred to a warm and humid glasshouse environment (Chapter 4). Diseases can occur early and progress with the age of the plant (Leath, 1989), severe incidence being associated with warm and humid conditions (Greub and Wedin, 1971, Beuselinck, *et al.*, 1984; English, 1999). In Otago, New Zealand, Chapman *et al.*, (1990) reported losses of 50% of plants of a 3 year old BFT stand in winter-early spring, a complex of *Fusarium* species being the causal agent.

Intensive defoliation influenced a number of plant characteristics, resulting in a poor plant survival. Firstly, root diameter was reduced in plants defoliated severely each 20 days, suggesting a decline in carbohydrate reserve levels. Root carbohydrates analyses must be interpreted cautiously because they were not replicated, but hard defoliated plants (2 cm height) showed a content only 30% of values at other defoliation heights (Figure 3-4). Plants with low reserve levels and under stress are more susceptible to the effect of root diseases (Barta, 1978). Secondly, BFT plants defoliated at both 2 cm and 10 cm height had smaller crowns than plants defoliated at 6 cm. It can be suggested that in 10 cm height treatment a wide range of plant sizes could survive. However under intensive defoliation (2 or 6 cm height) only strong plants could survive, but the intensive defoliation depleted the crown of surviving plants at 2 cm height. In addition, the number of secondary shoots was reduced in plants defoliated intensively and frequently (2 cm – 20 days interval).

These findings at the individual plant level associated with the reduction in plant density resulted in declining root mass, crown mass and the number of primary stems per unit of area. The consequence of these associated changes was a reduction in spring forage production (Table 3-2), when intensive defoliation was applied. Lax defoliation is recognised to improve the development of shoots and yield (Cordeiro de Araujo and Jacques, 1974), and 7-10 cm of residual stubble height is generally recommended for BFT (Smith and Nelson, 1967). Better stand persistence under intensive grazing (2.5 cm) was observed only when the stand was defoliated three times during the year (Smith and Nelson, 1967). In the current study a reduction of 32% in plant density was accompanied by 24% reduction in spring forage production, for a stand with 86 plants/m^2. Results of Bologna (1996) managing a 1-2 year old stand of 40 plants/m^2 of BFT Grasslands Goldie, showed spring forage production ranged between 3.1-3.8 t DM/ha in environments of South Island of New Zealand, having only 13% of reduction in density if a stand was defoliated at 4 cm height every four weeks and no effects on density when defoliated at 6-8 week intervals. However, reduction in defoliation interval to 2 weeks resulted in a decline of 65% in plant density in approximately two years.

In the current trial (Figure 3-5), the total number of shoots/m^2 was closely associated with BFT contribution, achieving approximately 1100 shoots/m^2 with a stand density of 84 plants/m^2. Primary shoots contributed significantly to yield when population increased from 58 to 71 plants/m^2 by a change in defoliation height from 2 to 6 cm. However, plants tended to maintain the number of primary shoots when defoliation height increased from 6 to 10 cm height, and a further increase in the number of secondary shoots explained the increase in BFT contribution. Similar determinations were made by Volenec *et al.* (1987) for a series of lucerne cultivars, working with a density range between 11-172 plants/m^2; shoot density to obtain high dry matter yields ranged from 900-1000 shoots/m^2 approximately.

Thus, the combined effect of intensity and frequency of defoliation can alter stand density drastically, and the indications are that in short periods of time defoliation height has a stronger effect than frequency of defoliation. Plant density required for adequate forage production appears to be associated with the age of the stand, because it declines progressively with sward age. The short number of cutting cycles (one season), limited the effects of defoliation interval compared with results for long term trials (Bologna, 1996).

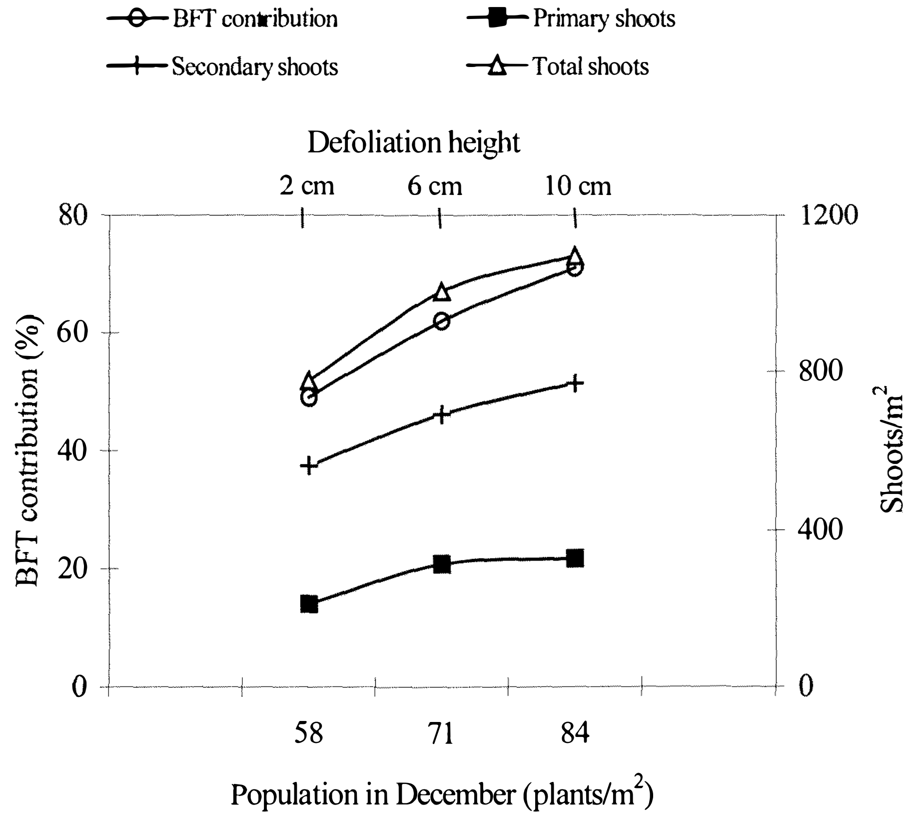


Figure 3-5. Influence of defoliation height on plant population, shoot density and herbage contribution to total pasture production in BFT cv. Grasslands Goldie in spring.

Late autumn defoliation reduced herbage accumulation and growth in spring, and reduced root reserves. These findings are in agreement with results of Assuero *et al.* (1990), who found the levels of carbohydrates reserves in late winter explained 48% of variation in regrowth of BFT during spring. Winter survival was not affected by autumn defoliation as reported for other crown forming species like lucerne (Keoghan, 1970) despite the reduction in carbohydrates root reserves. In the current study, it can be suggested that winter temperatures were not severe enough to produce the kind of effects reported for more extreme environments. The stress produced by autumn defoliation, and consequences to root reserves levels and root diseases, could explain the 8% reduction in plant density detected in this study. Moreover, weakened plants that survive winter could die in spring if a proper management is not applied, as is the case for chicory (Li, 1997), or for red clover under high temperatures (Kendall, 1958) or dry conditions (Smith, 1950), where reserves are deficient

BFT swards under intensive defoliation in autumn also could be less dense and competitive in spring, increasing the proportion of gaps for the establishment of other species, by a depletion in root reserves for winter active BFT types or by reduced competition by low growth of dormant types.

3.6 CONCLUSIONS

Recommended management to optimize forage production and plant persistence of BFT cv. Grasslands Goldie in spring should contemplate moderate defoliation intensities (6 cm), independently of defoliation intervals if defoliated for short periods. Management strategies based on high residual herbage mass after cutting and extended intervals between defoliations during spring will increase plant survival, but will decrease the number of grazing cycles possible in the growing season and result in lower quality forage. However, inappropriate management can reduce root mass, levels of root reserves and crown size of BFT plants, resulting in plants less vigorous and swards less dense and productive.

No evidence was found to suggest that autumn management has any particular influence on plant losses during winter in the conditions of North Island of New Zealand. However, early rest in autumn determined an early and high spring regrowth, based on a plant population with high carbohydrates root reserves.

3.7 REFERENCES

AGRESEARCH GRASSLANDS (1995). The Grasslands range of forage and conservation plants. AgResearch Grasslands, Palmerston North. New Zealand.

ALTIER, N. (1997). Enfermedades del Lotus en Uruguay. (Lotus diseases in Uruguay). *Serie Técnica* 93. INIA La Estanzuela, Uruguay. ISBN: 9974-38-083-9. 16 p

ASSUERO, S.G., ESCUDER, C.J., ANDRADE, F., FERNANDEZ, O. AND FERNANDEZ, H. (1990). Efecto de la intensidad de pastoreo sobre los carbohidratos solubles en raíces y el rebrote de *Lotus corniculatus* L. (Effect of grazing intensity on root reserves and regrowth of *Lotus corniculatus* L.) *Revista Argentina de Producción Animal* **10(6)**: 443-453.

BARTA, A.L. (1978). Effect of root temperature on dry matter distribution, carbohydrate accumulation, and acetylene reduction activity in alfalfa and birdsfoot trefoil. *Crop Science* **18**: 637-640.

BEUSELINCK, P.R., PETERS, E.J. AND McGRAW, R.L. (1984). Cultivar and management effects on stand persistence of birdsfoot trefoil. *Agronomy Journal* **76**: 490-492.

BOLOGNA, J.J. (1996). Studies on strategies for perennial legume persistence in lowland pastures. *Thesis of Master Agricultural Science.* Lincoln University, New Zealand. 220 p

CHAPMAN, H.M., LOWTHER, W.L. AND TRAINOR, K.D. (1990). Some factors limiting the success of *Lotus corniculatus* in hill and high country. *Proceedings of the New Zealand Grassland Association* **51**: 147-150.

CHARLTON, J.F.L. AND BELGRAVE, B.R. (1992). The range of pasture species in New Zealand and their use in different environments. *Proceedings of the New Zealand Grassland Association* **54**: 99-104.

CLEGG, K.M. (1956). The application of the Anthrone reagent to the estimation of starch in cereals. *Journal of the Science of Food and Agriculture* **7**: 40-44.

CORDEIRO DE ARAUJO, J. AND JACQUES, A.V.A. (1974). Características morfológicas e produçâo de materia seca de cornichâo (*Lotus corniculatus* L.) colhido em diferentes estádios de crescimento e a duas alturas de corte. (Morphology and production of dry matter of birdsfoot trefoil (*Lotus corniculatus* L.) at different growth stages and under two defoliation heights). *Revista da Sociedade Brasileira de Zootecnia*, Vol. **3(2)**: 138-147.

DOUGLAS, G.B., STIENEZEN, M., WAGHORN, G.C. AND FOOTE, A.G. (1999). Effect of condensed tannins in birdsfoot trefoil (*Lotus corniculatus*) and sulla (*Hedysarum coronarium*) on body weight, carcass fat depth, and wool growth of lambs in New Zealand. *New Zealand Journal of Agricultural Research* **42**: 55-64.

ENGLISH, J.T. (1999). Diseases of Lotus. In *Trefoil:The Science and Technology of Lotus*. CSSA Special Publication No. 28. Edited by P.R. Beuselinck. American Society of Agronomy, Inc.. Crop Science Society of America, Inc. Madison, USA. ISBN 89118-550-X. pp. 121-131.

GREUB, L.J. AND WEDIN, W.F. (1971). Leaf area, dry-matter accumulation, and carbohydrate reserves of alfalfa and birdsfoot trefoil under a three cut management. *Crop Science* **11**: 341-344.

HEWITT, A.E. (1992). New Zealand soil classification. DSIR Land Resources Scientific Report N° 19. Lower Hutt, New Zealand.

JOHN, A., ULYATT, M.J., JONES, W.T. AND SHELTON, I.D. (1980). Factors influencing nitrogen flow from the rumen. *Proceedings of the New Zealand Society of Animal Production* **40**: 226.

JOHN, A. AND LANCASHIRE, J.A. (1981). Aspects of the feeding and nutritive value of *Lotus* species. *Proceedings of the New Zealand Grassland Association* **42**: 152-159.

KENDALL, W.A. (1958). The persistence of red clover and carbohydrate concentration in the roots at various temperatures. *Agronomy Journal* **50**: 657-659.

KEOGHAN, J.M. (1970). The growth of lucerne following defoliation. *Thesis of Doctor of Philosophy*. Lincoln College, New Zealand. 383 p

LEATH, K.T. (1989). Diseases and forage stand persistence in the United States. In

Persistence of Forage Legumes. Edited by G.C Marten; A.G Matches; R.F. Barnes; R.W. Broughman; R.J. Clements; and G.W. Sheath. Second Printing 1989. ISBN 0-89118-098-2. pp. 465-479.

LI, G. (1997). Response of Chicory (*Chichorium intibus* L.) to defoliation. *Thesis of Doctor of Philosophy in Plant Science*. Massey University, New Zealand. 197 p

MIN, B.R., BARRY, T.N., MCNABB, W.C. AND KEMP, P.D. (1998). Effect of condensed tannins on the production of wool and its processing characteristics in sheep grazing *Lotus corniculatus*. *Australian Journal of Agricultural Research* **49**: 597-605.

SAS INSTITUTE INC. (1990). *SAS/STAT user's guide*. Version 6, 4th edition. SAS Institute Inc., Cary N.C.

SCOTT, D. AND CHARLTON, J.F.L. (1983). Birdsfoot trefoil (*Lotus corniculatus* L.) as a potential dryland herbage legume in New Zealand. *Proceedings of the New Zealand Grassland Association* **44**: 98-105.

SMITH, D. (1950). Seasonal fluctuations of root reserves in red clover (*Trifolium pratense* L.). *Plant Physiology* **25**: 702-710.SMITH, D. (1962). Carbohydrate root reserves in alfalfa, red clover and birdsfoot trefoil under several management schedules. *Crop Science* **2**: 75-78.

SMITH, D. AND NELSON, C.J. (1967). Growth of birdsfoot trefoil and alfalfa. I. Responses to height and frequency of cutting. *Crop Science* **7**: 130-133.

SOUTHGATE, D.A.T. (1991). *Determination of Food Carbohydrates*. AFRC Institute of Food Research, Norwich Laboratory, Norwich, UK. Elsevier Applied Science. ISBN 1-85166-652-4. Second edition. 232 p

VAN KEUREN, R.W. AND DAVIS, R.R. (1968). Persistence of birdsfoot trefoil, *Lotus corniculatus* L. as influenced by plant growth habit and grazing management. *Agronomy Journal* **60**: 92-95.

VOLENEC, J.J., CHERNEY, J.H. AND JOHNSON, K.D. (1987). Yield components, plant morphology, and forage quality of alfalfa as influenced by plant population. *Crop Science* **27**: 321-326.

WEINMANN, H. (1947). Determination of total available carbohydrates in plants. *Plant Physiology* **22**: 279-290.

WOODWARD, S.L. AULDIST, M.J., LABOYRIE, P.J. AND JANSEN, E.B.L. (1999). Effect of *Lotus corniculatus* and condensed tannins on milk yield and milk composition of dairy cows. *Proceedings of the New Zealand Society of Animal Production* **59**: 152-155.

4. EFFECTS OF DEFOLIATION INTENSITY ON GROWTH, BIOMASS DISTRIBUTION, AND MORPHOLOGICAL AND PHYSIOLOGICAL CHANGES OF BIRDSFOOT TREFOIL IN GLASSHOUSE CONDITIONS

4.1 ABSTRACT

The effects of defoliation intensity (2, 6, 10 cm cutting height and one undefoliated control) on biomass production and associated morphological and physiological changes of Birdsfoot trefoil (*Lotus corniculatus* L. Grasslands Goldie) were explored in a glasshouse experiment conducted from May to December 1997 at the Plant Growth Unit, Massey University, Palmerston North, New Zealand. Biomass production over 120 days under defoliation at 20 day intervals was 14, 36 and 44% of the undefoliated control for 2, 6 and 10 cm treatments respectively. Relative growth rate increased initially in plants defoliated to 2 cm, but after the third harvest (60 days) there were no differences between treatments. Below-ground biomass decreased over time under hard defoliation (2 cm). It remained unchanged under 6 cm cutting height and increased for 10 cm and the undefoliated control. The effects of defoliation intensity on plant components increased significantly over time, with secondary root mass, root diameter and the number of primary shoots being the most affected parameters. Herbage growth was positively correlated with crown size, primary roots mass and secondary roots mass, but not with root diameter. Linear correlation with herbage growth after 80 days of defoliation was $r^2=0.66$, $P<0.01$ for crown size, $r^2=0.38$, $P<0.05$ for primary roots mass and $r^2=0.67$, $P<0.01$ for secondary roots mass, $r^2>0.80$, $P<0.01$ for residual leaf area and $r^2=0.62$, $P<0.01$ for root carbohydrate reserves. It can be concluded that BFT plants under hard defoliation (2 cm) at 20 day intervals cannot produce biomass at adequate rates. This is despite the adjustments in relative growth rate, increase in leaf area ratio, specific leaf area or number of leaves per plant. Thus, plants reduce below-ground components severely, reducing the resources for regrowth and affecting plant survival. More lax defoliation regimes (6 or 10 cm) increased productivity and plant survival.

Keywords: Defoliation, biomass allocation, regrowth, plant components, carbohydrate reserves.

4.2 INTRODUCTION

The high sensitivity of *Lotus corniculatus* L. (BFT) to defoliation intensity has often been observed (Smith and Nelson, 1967; Greub and Wedin, 1971; Alison and Hoveland, 1989 a). Recovery after defoliation is associated with the amount, type and age of tissue removed, as well as environmental constraints, which determine resource supply (Richards, 1993). The stubble height after defoliation sets the remaining photosynthetic active leaf mass to manufacture new plant tissues (Silva, 1968). Moreover, the plant structure defines the residual herbage mass and the number of growing sites, suggesting reasons why more erect BFT cultivars are less persistent under severe defoliation (Frame *et al.*, 1998).

The amount of stored root reserves and their role in regrowth after defoliation has been extensively explored since Graber *et al.* (1927), with the emphasis being on crown-forming species where the taproot constitutes the main storage organ (Smith, 1962; Nelson and Smith, 1968; Cordeiro de Araujo and Jacques, 1974; Fankhauser and Volenec, 1989). In contrast to other crown-forming legumes, BFT has low levels of root carbohydrates during the active growing season, with the carbohydrate concentration not being substantially altered after successive defoliations as occurs in red clover or lucerne. Root reserves are significantly correlated with forage yield in BFT (Alison and Hoveland, 1989 b) and in lucerne (Feltner and Massengale, 1965), but root weight is not a good indicator of the amount of reserves in lucerne (Feltner and Massengale, 1965). Hodgkinson (1968) found that fine roots of lucerne were reduced after defoliation, limiting resource uptake.

There is little information in New Zealand literature on the physiology and morphology of BFT after defoliation. The more recent studies refer to morphological responses of the cultivar Grasslands Goldie to different frequencies of defoliation (Bologna, 1996). Previous field work in this thesis using BFT Grasslands Goldie (see Chapter 3), showed that severe defoliation during spring drastically reduced biomass production and plant density. Associated effects resulted in reductions in root mass and carbohydrate reserves, crown size and shoot density of BFT plants in swards defoliated at 2 cm stubble height. Plant adjustments after successive defoliation cycles in above and below-ground biomass, potential and relative growth rate, and production of new photosynthetic tissues are understood for some grasses (Oesterheld, 1992) and chicory (Li, 1997), but there is limited evidence for BFT. Thus, the objectives of this work were to provide detailed information on the degree of adaptability and plasticity of BFT cv. Grasslands Goldie plants to different intensities of defoliation, quantifying morphological and physiological plant responses.

4.3 MATERIALS AND METHODS

The experiment was conducted from 15 May to 30 December 1997 in a glasshouse of the Plant Growth Unit, Massey University, Palmerston North, New Zealand (latitude 40°23' S). Three-year-old adult plants of *Lotus corniculatus* cv. Goldie were collected from an established sward, selected for uniformity, and then transplanted to plastic grow-bags (10.8 litre in volume, 250 mm depth) with three plants per pot.

A standard long term medium (9 months release) was utilised, made up of bark and amended with dolomite (3 kg/m^3), agricultural lime (3 kg/m^3), iron sulphate (0.5 kg/m^3), and Osmocote plus® (NPK 16-3.5-10.8, 4 kg/m^3), as a fertiliser. Water was applied twice daily (5 mins each time) to each pot through an automatic irrigation system, which watered pots to field capacity (Plate 4-1 b). Temperatures were maintained between 16±3.2 and 24±4.6 ^{o}C (night/day), heating or ventilating when necessary. The average minimum and maximum temperatures were 14.4 and 32.3 ^{o}C, respectively from September to December.

After transplanting, the pots were maintained from May 15 to September 2 without treatments, giving a period of recovery for the development of secondary roots, and all pots were defoliated at 6 cm height on July 28 and August 1 (Plate 4-1 a).

A completely randomised block design with five replicates was used, with treatments being three defoliation intensities (2, 6 and 10 cm height), and a control treatment that was not defoliated after the initial cut at 6 cm height when evaluation started. Plants were defoliated every 20 days at the defined intensities, for 120 days from September 2 to December 30, 1997 (Table 4-1). Design included 5 internal replicates for each of four destructive harvests. These were conducted on day 0 (1 pot/block, see as pre-destructive harvest), and days 40, 80 and 120 (1 pot/treatment/block, see as destructive harvests one to three). The 65 pots under evaluation were grouped on independent tables per block, and pots in each block rotated weekly.

Table 4-1. Cutting and destructive harvests schedule for BFT pots from September to December 1997.

	2/9	21/9	11/10	1/11	21/11	11/12	30/12
Cuts							
2 cm	X	X	X	X	X	X	X
6 cm	X	X	X	X	X	X	X
10 cm	X	X	X	X	X	X	X
Control	X						
Destructive harvests							
2 cm	X		X		X		X
6 cm	X		X		X		X
10 cm	X		X		X		X
Control	X		X		X		X

General management included the control of slugs with Mesurol® pellets (20 g/kg of carbamate) at 10 g/m^2. Plants were sprayed to control aphids with Insectigas® (dichlorvos in liquid carbon dioxide aerosol) on 6 June. Aphids, white fly and thrips were controlled on 27 June applying 1 g/l of Orthene® 75 (750 g/kg acephate as a soluble powder) plus 1ml/litre of Attack® (25 g/l permethrin plus 475 g/l pirimiphos-methyl, emulsifiable concentrate). Aphid control was repeated on 14 July, 17 November, 5 December and 19 December with 1 ml/litre of Attack®.

Approximately 50% of the 200 pots initially prepared showed fungal disease symptoms (*Rhizoctonia* and *Fusarium*) in winter before the start of cutting treatments. The occurrence was randomly distributed and attributed to high temperature and humid conditions in the glasshouse (Plate 4-1 c). Severely affected plants were discharged, and the remaining pots were drenched with 1ml/l of fungicide Saprol® (190 g/l piperidine) to control root diseases on 13 June and 17 July. No further incidence of disease was apparent.

4.3.1 Measurements

At each cutting date, plant height, herbage mass, number and weight of leaves and stems and leaf area were measured. All mass data were measured on a dry weight basis from samples oven-dried at 100 °C for 24 hours.

At each destructive harvest, plant height and total above and below-ground masses were measured. The total above-ground mass was collected in two stages: first above the defoliation height applied (2, 6 or 10 cm height); and then the residual to ground level to estimate residual herbage mass and components for regrowth of remaining pots.

Primary and secondary shoots were counted, in accordance with definitions of section 3.3.1.2 of Chapter 3. A subsample of 20 stems was taken at random from each pot, and dissected into leaves and stems, number and weight of respective fractions and leaf area recorded. Leaf area per pot was measured using a LI-Cor LI-3100 leaf area meter (Lambda Instruments Co., Lincoln, NE, USA).

Roots were washed to remove media, with fine roots collected in a series of sieves after being washed. Below-ground mass was partitioned into primary roots, secondary roots and crown mass. Primary root mass included the taproot and part of more lignified structures (> 2 mm), that could be differentiated from fine and new developing root tissues. Primary root diameter was measured at a section 10 mm below the insertion of primary shoots.

Main root samples from each pot were collected after washing, kept frozen (-20 °C), then freeze-dried and ground to pass a 1 mm sieve and stored for carbohydrate analysis (see Appendix 1 and section 3.3.1.3 of Chapter 3).

4.3.2 Statistical analysis

Data were analysed with the SAS GLM program (SAS Institute, 1990), using a complete randomised block design comparing 4 treatments (2, 6 and 10 cm defoliation height and an undefoliated control). All data were initially tested for the assumptions of normality and homogeneity of variance. Data were transformed using natural logarithms to homogenise variance when assumptions were not valid, usually due to the large differences in magnitude of the values of the control treatment with respect to the other treatments. Parameters quantified repeatedly were analysed by a repeated measures model over time. Allometric relationships such as leaf area ratio (LAR), specific leaf area (SLA), weight per leaf and number of leaves per gram were calculated at each destructive harvest and also analysed by 'repeated measures analysis'. For morphological parameters measured at destructive harvests, a multivariate analysis of variance (MANOVA) was done to identify effects of defoliation intensity. The relationships between plant components (primary roots mass, secondary roots mass, crown mass, leaf area and root reserves) and herbage growth were tested using linear regression models, comparing data of destructive harvests and the following 20 days herbage growth.

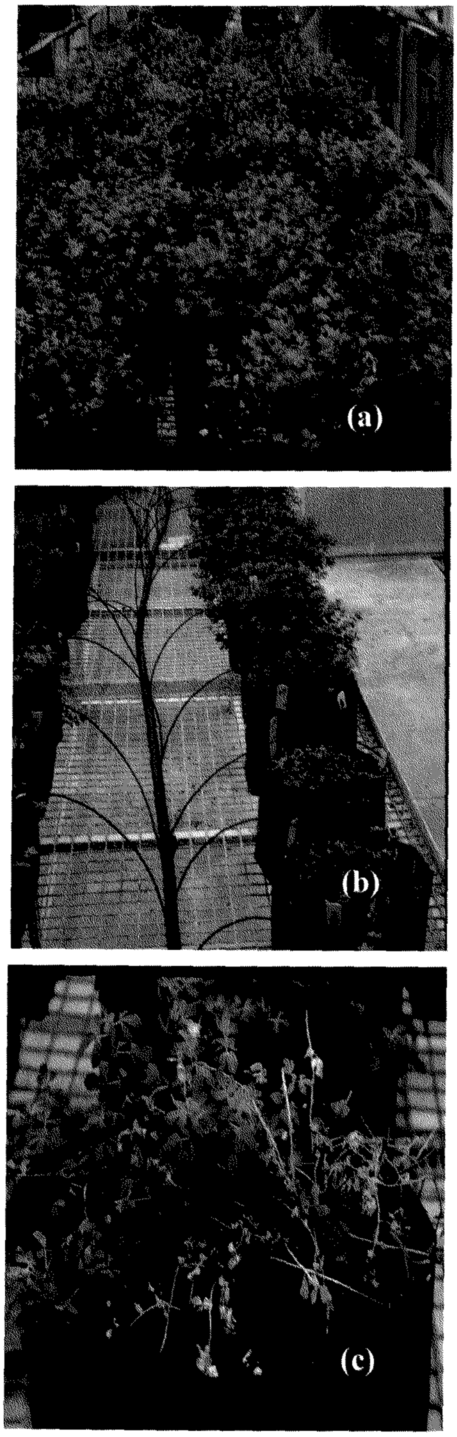


Plate 4-1. General view of BFT pots at the time of start (a) and during the trial (b) and disease symptoms on some BFT plants (c).

4.4 RESULTS

4.4.1 Growth analysis

The accumulation rate and the relative growth rate (RGR) of herbage harvested were calculated for each 20-day interval. The accumulation rate was expressed as: *g DM harvested/day* (Hodgson, 1979), and the RGR as: *g DM harvested per day/ g DM residual* (Hunt, 1978). The amount of herbage harvested at each 20-day defoliation was always in the order 10 cm > 6 cm > 2 cm height, and the overall treatment contrast was always significant ($P<0.01$) (Figure 4-1). However, the main contrast was between 10 cm and 6 plus 2 cm height at day 20, and between 10 cm plus 6 cm and 2 cm at days 100 and 120.

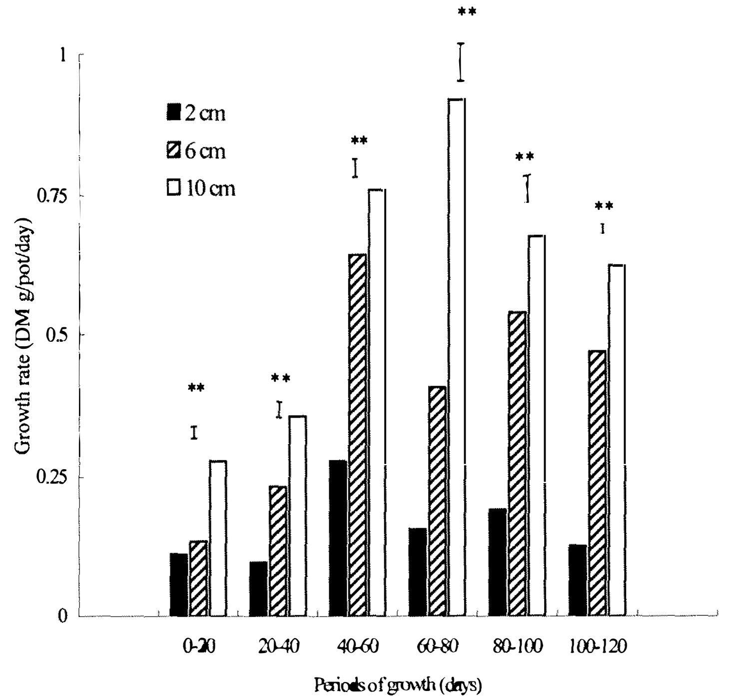


Figure 4-1. Accumulation rate of herbage harvested (DM g/pot/day) of BFT cv. Grasslands Goldie defoliated at three intensities in controlled conditions. Vertical bars represent SEM, (n= number of observations for each treatment mean, were 15, 10, 10, 5, 5 and 5 for 0-20, 20-40, 40-60, 60-80, 80-100 and 100-120 day intervals respectively).

The relative growth rate (RGR) increased when cutting intensity increased (P<0.01) in the first growth cycle (Figure 4-2). After that, only during the third growth cycle were differences in RGR significant, the RGR for 10 cm defoliation height being higher than the other treatments.

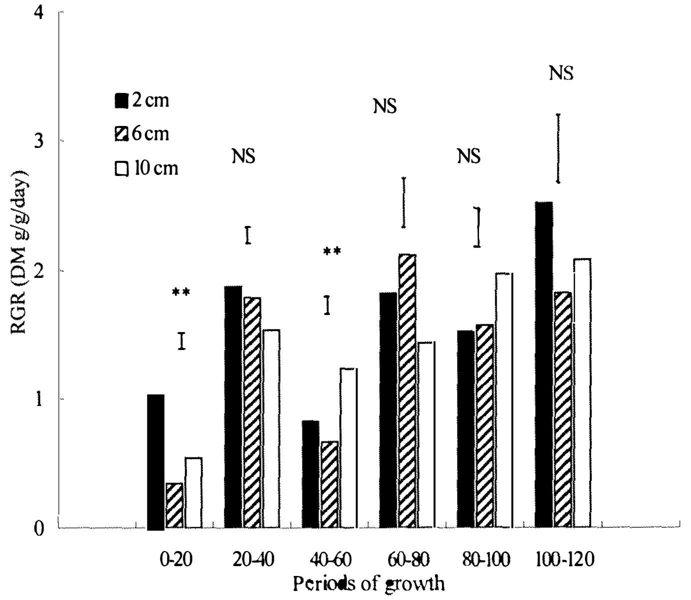


Figure 4-2. Relative growth rate (DM g/g/day) of BFT cv. Grasslands Goldie defoliated at three intensities in controlled conditions. Vertical bars represent SEM, (n= number of observations for each treatment mean, were 15, 10, 10, 5, 5 and 5 for 0-20, 20-40, 40-60, 60-80, 80-100 and 100-120 day intervals respectively).

4.4.2 Biomass production

Above-ground and below-ground biomass were analysed at 40, 80 and 120 days of growth when destructive harvests were conducted (Table 4-2). Data were transformed using natural logarithms, but actual values are also provided. *Cumulative above-ground growth* was defined as the herbage harvested over the respective defoliation height at each 20 days harvest and accumulated over time. *Below-ground mass* included the total root and crown biomass measured at each destructive harvest.

Cumulative above-ground growth at 40 days was significantly different between treatments ($P<0.01$), with greater biomass when residuals were high. The undefoliated treatment during the first 40 days did not differ significantly from 10 cm height. At 80 and 120 days there were significant differences ($P<0.01$) between treatments, with the control greater than 10, 6 and 2 cm height. Defoliation heights of 6 and 10 cm were not significantly different in biomass, and the hardest defoliated (2 cm) treatment had the lowest biomass. Total biomasses of defoliated treatments were 44, 36 and 14% of biomass produced by the control for 10, 6 and 2 cm height respectively. Over time, cumulative growth was significant ($P<0.01$) for all treatments in all cases (Table 4-2).

There were no significant changes over time in the above-ground biomass at time of destructive harvests for the defoliated treatments, but the control increasing significantly ($P<0.01$, Table 4-2) in biomass over time. At the first destructive harvest, the above-ground biomass was lower in 2 cm height than in the others. From the second to the third destructive harvest, the differences between treatments increasing in order of Control>10 cm>6 cm>2 cm.

The first destructive harvest produced significant differences between treatments in below-ground biomass ($P<0.01$), with a tendency for decrease when the intensity of defoliation was increased (Table 4-2). These differences between treatments were increased during the subsequent destructive harvests ($P<0.01$).

There was a significant interaction time x defoliation treatment ($P<0.01$), with the control significantly increasing below-ground biomass over time. The 10 cm height also increased biomass, but there were no differences between harvest two (80 days) and three (120 days). The 6 cm height maintained its biomass over the time, with no differences between destructive harvests. On the contrary, the 2 cm treatment reduced the below-ground biomass, particularly after the first destructive harvest (40 days), with no differences between destructive harvests two (80 days) and three (120 days).

The ratio of above/below biomass was highest on average over the experimental period for 6 cm defoliation.

Table 4-2. Cumulative above-ground growth, above-ground biomass at the time of destructive harvest and below-ground mass of BFT cv. Grasslands Goldie under different intensities of defoliation.

Cutting height	40 days		80 days		120 days		SEM/ Significance (time*treatment)
	Actual values	Log values	Actual values	Log values	Actual values	Log values	
Cumulative above-ground growth (g DM/pot)							
2 cm	4.1	1.319	20.9	3.030	37.8	3.619	
6 cm	6.7	1.879	42.9	3.734	94.0	4.535	
10 cm	12.6	2.531	54.4	3.990	115.3	4.743	0.0882
Control	13.3	2.556	87.4	4.456	263.8	5.566	**
SEM (n)		0.1353 (5)		0.0938 (5)		0.0634 (5)	
Significance		**		**		**	
Above-ground biomass at the time of destructive harvest (g DM/pot)							
2 cm	5.2	1.452	9.6	2.135	6.6	1.864	
6 cm	12.9	2.529	22.6	3.090	24.5	3.192	
10 cm	17.6	2.860	31.9	3.458	32.0	3.465	0.1409
Control	21.6	3.062	95.6	4.552	272.1	5.598	**
SEM (n)		0.1755 (5)		0.1625 (5)		0.0595 (5)	
Significance		**		**		**	
Below-ground mass (g DM/pot)							
2 cm	9.5	2.234	4.4	1.448	4.2	1.432	
6 cm	12.2	2.497	11.9	2.458	11.7	2.460	
10 cm	13.9	2.619	24.7	3.191	23.5	3.135	0.0802
Control	19.2	2.950	73.8	4.289	213.8	5.355	**
SEM (n)		0.0781 (5)		0.1027 (5)		0.0773 (5)	
Significance		**		**		**	

**, P<0.01; SEM, standard error of the mean; (n), number of observations for each treatment mean

4.4.3 Carbohydrate root reserves

There were significant treatment differences in the root carbohydrates content (Table 4-3). The undefoliated control accumulated starch and free sugars over time particularly after 80 days growth. Carbohydrate reserves in the control were higher than in defoliated plants. The starch content differed between defoliation intensities, with the differences increasing over time (Table 4-3).

Table 4-3. Content of starch and free sugars in roots of BFT cv. Grasslands Goldie under different intensities of defoliation over 120 days (actual values expressed in mg/plant and log values used for statistical analysis).

Cutting height	**Day 0**		**Day 40**		**Day 80**		**Day 120**		**SEM / Significance time*treatment**
	Actual values	Log values	Actual values	Log values	Actual values	Log values	Actual values	Log values	
					Starch				
2 cm			53	3.940	41	3.681	22	3.054	
6 cm	243±37	N/A	101	4.604	62	4.129	32	3.462	0.2230
10 cm			132	4.880	100	4.578	82	4.395	**
Control			160	5.066	475	6.133	3036	8.006	
SEM (n)				0.1255 (3)		0.1439 (3)		0.0750 (3)	
Significance				**		**		**	
					Free sugars				
2 cm			9	2.135	12	2.479	17	2.826	
6 cm	90±19	N/A	12	2.458	19	2.911	25	3.202	0.1786
10 cm			56	3.996	28	3.336	36	3.588	**
Control			23	3.101	64	4.111	2242	7.663	
SEM (n)				0.2015 (3)		0.1172 (3)		0.1300 (3)	
Significance				**		**		**	

**, $P<0.01$; at day 0 values represent the average±sd (n=3) for all treatments; N/A not statistically analysed; SEM, standard error of the mean; (n), number of observations for each treatment mean

Starch and free sugars rapidly declined after initial defoliation, and for defoliated treatments remained low and were not replenished during the active growth period. At all stages starch content decreased significantly on defoliated treatments ($P<0.01$), with increasing defoliation intensity. Also, free sugars content differed significantly at all stages, the control was the only treatment that increased starch reserves. At the first harvest, free sugars content of the control was lower than in the 10 cm height. Differences at the final destructive harvest (120 days) occurred between 10 cm and 2 cm defoliation height, the control being significantly higher than all defoliated treatments (Table 4-3).

4.4.4 Dynamics of plant components

Dynamics of below-ground and above-ground plant components were analysed over time during destructive harvests. Because of differences in magnitude of some components when compared with the control treatment, data were transformed to homogenise variance using natural logarithm values for statistical analysis.

4.4.4.1 Below-ground components

Analysis of below-ground components comprised crown mass, primary roots, secondary roots and root diameter. The original values and time trends are shown in Figures 4-3 to 4-5, followed by Table 4-4 for the statistical analysis over time based on transformed data.

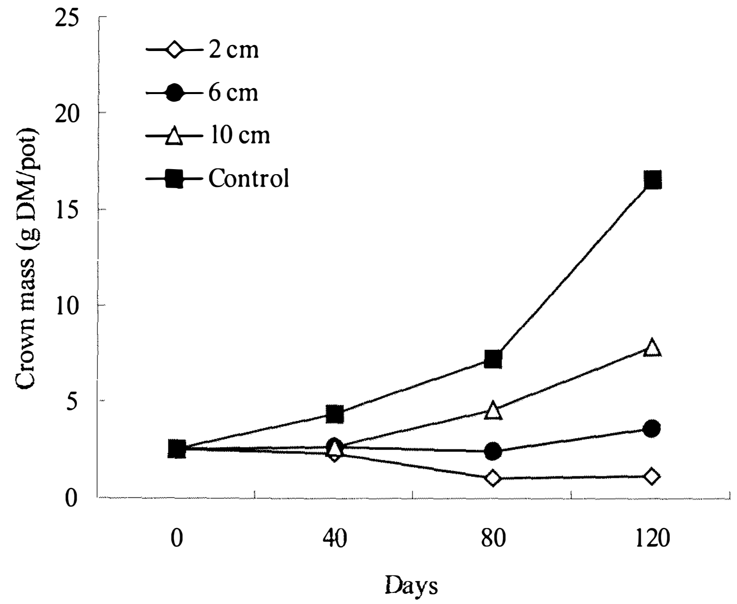
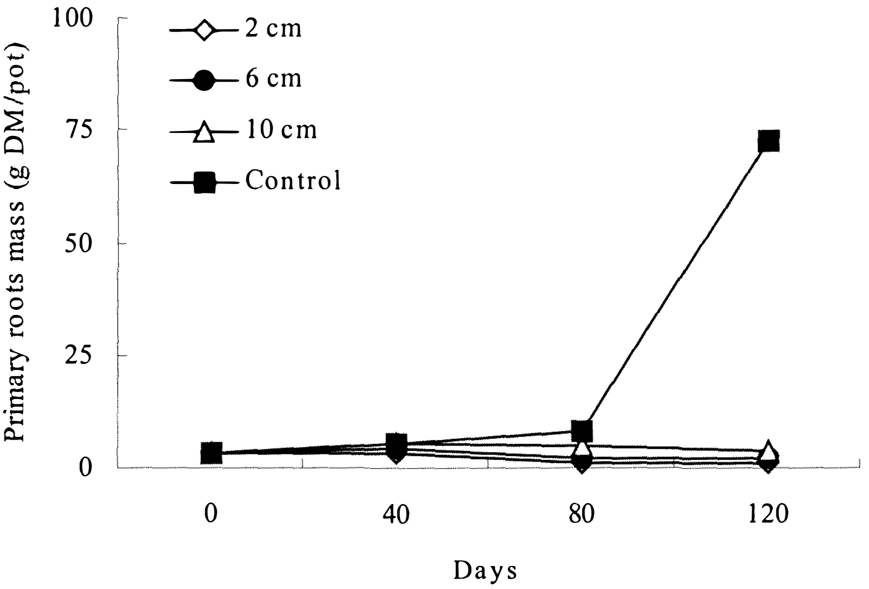


Figure 4-3. Crown mass (g DM/pot) of BFT cv. Grasslands Goldie at cutting heights of 2, 6 and 10 cm and for an undefoliated control over 120 days.

(a) Primary roots



(b) Secondary roots

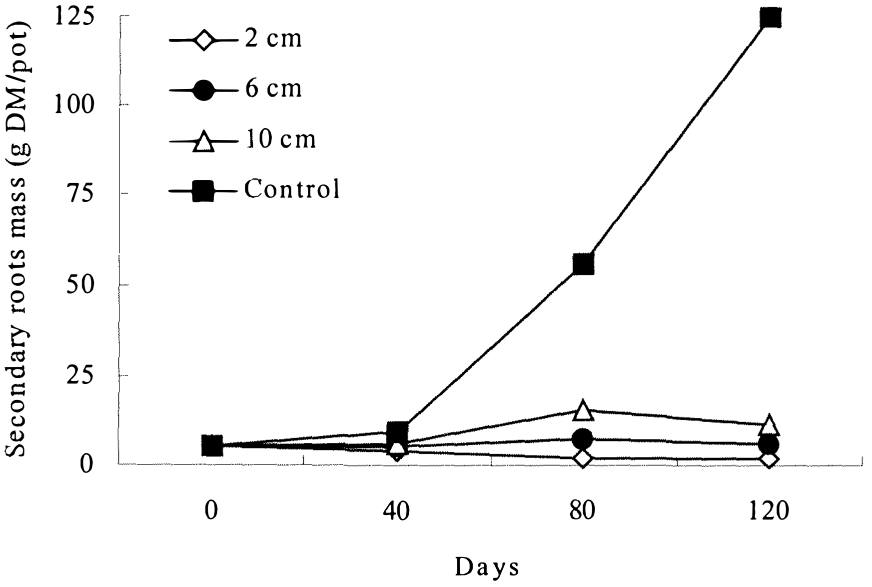


Figure 4-4. (a) Primary roots mass and (b) secondary roots mass (DM g/pot) of BFT cv. Grasslands Goldie at cutting heights of 2, 6 and 10 cm and for an undefoliated control over 120 days.

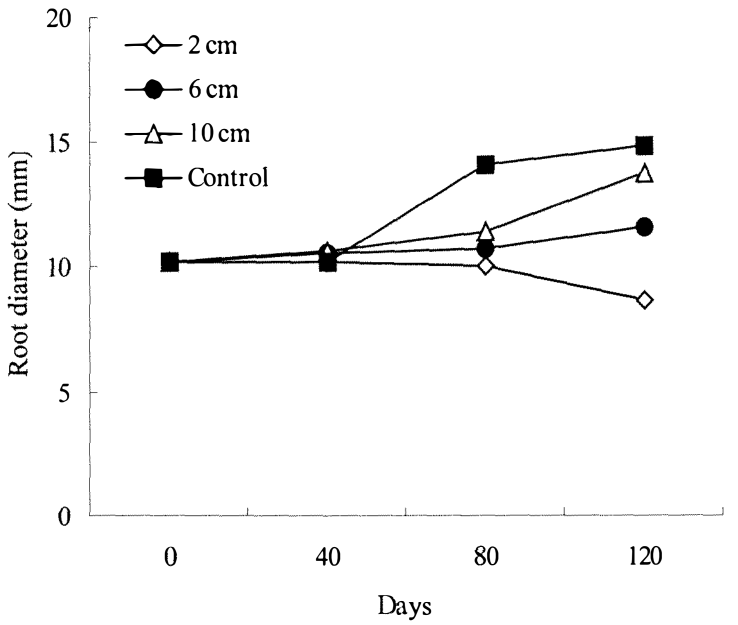


Figure 4-5. Root diameter (mm) of BFT cv. Grasslands Goldie at cutting heights of 2, 6 and 10 cm and for an undefoliated control over 120 days.

At 40 days, there were differences in crown mass ($P< 0.01$) between the control and the three cutting heights (Table 4-4); but no differences in crown mass between the cutting heights themselves. At 80 days, crown masses of the control and 10 cm height were significantly greater than for 2 cm height ($P<0.01$). The last destructive harvest (120 days) had differences ($P<0.01$) between all treatments, with a reduction in crown mass when the intensity of defoliation increased (Table 4-4).

Over time, there was a significant interaction of time x defoliation treatment ($P<0.01$) (Table 4-4). The control treatment significantly increased ($P<0.01$) crown mass over time, especially at the last destructive harvest (120 days), which differed from destructive harvests one (40 days) and two (80 days). For 10 cm defoliation height treatment, crown mass increased significantly over time. The 6 cm height treatment exhibited no differences in crown size over the experiment. The 2 cm treatment had a depletion in crown size after the first destructive harvest ($P<0.01$), but not between destructive harvests two (80 days) and three (120 days).

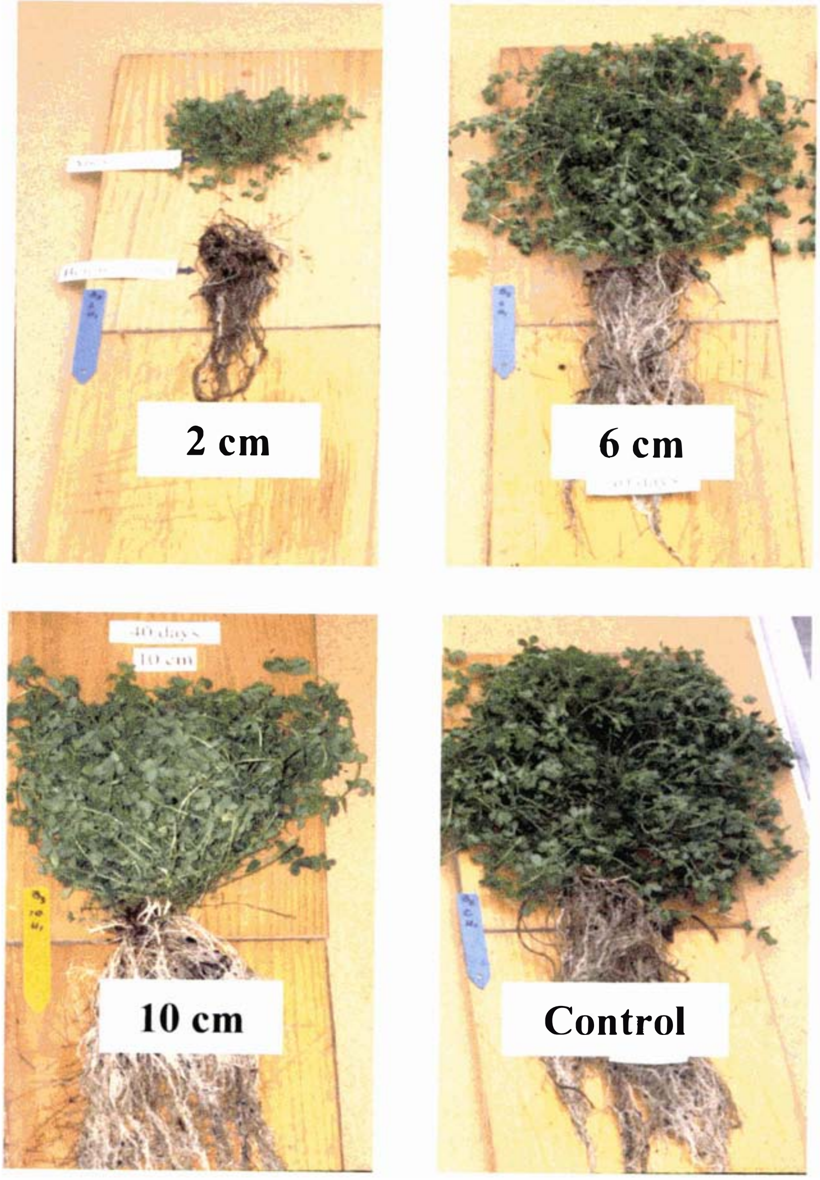


Plate 4-2. Above and below-ground biomass of BFT plants defoliated at different intensities and undefoliated control at 40 days destructive harvest.

Table 4-4. Crown mass, primary and secondary roots mass and root diameter (expressed in natural log (x+1) values) of BFT cv. Grasslands Goldie managed at cutting heights of 2, 6 and 10 cm and one undefoliated control treatment, over 120 days.

	40 days	80 days	120 days	SEM / Significance (time*defoliation treatment)
Crown mass				
2 cm	1.197	0.675	0.735	
6 cm	1.303	1.218	1.481	0.1103
10 cm	1.270	1.701	2.188	**
Control	1.674	2.052	2.855	
SEM (n)	0.0781 (5)	0.1448 (5)	0.1039 (5)	
Significance	**	**	**	
Primary roots mass				
2 cm	1.386	0.744	0.756	
6 cm	1.660	1.162	1.086	0.1445
10 cm	1.821	1.700	1.420	**
Control	1.791	2.037	4.276	
SEM (n)	0.1287 (5)	0.1482 (5)	0.1524 (5)	
Significance	NS	**	**	
Secondary roots mass				
2 cm	1.607	1.143	1.065	
6 cm	1.803	2.079	1.940	0.1108
10 cm	1.916	2.751	2.490	**
Control	2.337	4.199	4.822	
SEM (n)	0.0973 (5)	0.1413 (5)	0.0881 (5)	
Significance	**	**	**	
Root diameter				
2 cm	2.405	2.392	2.260	
6 cm	2.442	2.431	2.520	0.0609
10 cm	2.450	2.509	2.680	NS
Control	2.611	2.697	2.759	
SEM (n)	0.0435 (5)	0.0812 (5)	0.0594 (5)	
Significance	*	NS	**	

** $P < 0.01$; * $P < 0.05$; NS, not significant; SEM, standard error of the mean; (n), number of observations for each treatment mean

At 40 days there were no significant differences in primary root mass between the different defoliation treatments. However, at 80 days, there were significant differences ($P<0.01$), with a tendency for root mass to be decreased by severe defoliation (Table 4-4). The undefoliated control and 10 cm height treatment did not differ, but were significantly greater than the 6 and 2 cm heights. Also, primary root mass of 6 cm height treatment was significantly greater than 2 cm height. During the final destructive harvest (120 days), there were differences in main root mass ($P<0.01$) between treatments, the control having the higher mass and for the other treatments root mass decreased when intensity of defoliation increased. At 120 days, root mass of 10 cm treatment was significantly higher than that of the 2 cm treatment.

Over time, the sequence of destructive harvests showed a significant interaction of time x defoliation treatment ($P<0.01$), the undefoliated control increased primary root mass after the second destructive harvest (80 days) (Table 4-4). For the 10 cm height, the last destructive harvest (120 days) showed a reduction in root mass compared with the first destructive harvest (40 days). For 2 and 6 cm height, there were reductions in main root mass after first destructive harvest (40 days), but no significant differences between destructive harvests two (80 days) and three (120 days).

At all destructive harvests there were significant differences ($P<0.01$) in secondary root mass between treatments (Table 4-4). At the first destructive harvest (40 days) only the control had a higher mass of secondary roots than the other treatments. After 80 days, the control had the highest root mass, more than three times greater than lax 10 cm defoliation treatment (Figure 4-4 b). The mass of secondary roots diminished when intensity of defoliation increased, behaviour that was confirmed during the last destructive harvest (120 days).

There was a significant interaction time x defoliation treatment ($P<0.01$). The control treatment increased secondary root mass over the time, with significant differences between the three destructive harvests (Table 4-4). For the 10 cm defoliation height, secondary root mass increased between 40 and 80 days, but there were no differences between destructive harvests two (40 days) and three (80 days). For the 6 cm treatment, there were no differences over the time, contrasting with the reduction in secondary roots for the 2 cm treatment.

During the first and third destructive harvests there were differences in the root diameter of BFT plants under different defoliation intensities ($P<0.05$ and $P<0.01$, respectively). At 40 days, only the control differed from the other treatments, with diameter 2 mm greater than the best of the defoliated treatments (10 cm height) (Figure 4-5, Table 4-4). At 120 days, the control and 10 cm height were not different and had a greater root diameter than 2 cm height treatment. Also, the control had greater root diameter than 6 cm height, but 6 cm did not differ from 10 cm height (Table 4-4).

Over time, the undefoliated control did not show changes in root diameter (Table 4-4), whereas the 10 cm defoliation height increased root diameter when the first destructive harvest was compared with the third ($P<0.05$). If the pre-treatment harvest is considered, root diameter increased for 10 cm and control treatments. The root diameter of plants defoliated at 6 cm height remained unchanged over 120 days, but those defoliated at 2 cm height reduced in diameter, particularly at the last destructive harvest (120 days).

4.4.4.2 Above-ground components

Shoot numbers, leaf/(leaf+stem) ratio and allometric relationships (LAR, SLA, weight/leaf and leaves/gram) were analysed over time (see Tables 4-5 and 4-6).

The number of primary shoots was not affected at the first destructive harvest (40 days), but a significant decline ($P<0.01$) under hard defoliation (2 cm) was observed at 80 days (Table 4-5). At 120 days control plants had more primary shoots than all defoliated treatments ($P<0.01$). Between defoliated treatments, only 6 cm had a higher number of primary shoots than the 2 cm height. Over time, a significant interaction of time x defoliation treatment was observed ($P<0.01$). The undefoliated control increased primary shoots at the final destructive harvest (120 days), but 2 cm and 10 cm treatment reduced shoots per pot and the 6 cm treatment remained unchanged.

The number of secondary shoots was significantly higher in the control (P<0.05, Table 4-5) compared with 2 and 10 cm height, but defoliated treatments did not differ at first destructive harvest (40 days). During the second destructive harvest (80 days), the control had a higher number of secondary shoots than the 2 and 10 cm height, and the more lenient defoliation treatments (6 and 10 cm height) were higher than for the 2 cm defoliation height. At 120 days, the control remained higher than the 2 or 6 cm treatment. Between defoliated treatments, secondary shoots decreased significantly when the defoliation intensity increased. Over time, there was a significant interaction (P<0.01, Table 4-5), treatments decreased in shoot density after the initial increase at 40 days with the exception of 10 cm height that maintained shoots density over time.

The leaf/(leaf+stem) ratio was not affected by defoliation intensity, but it declined over time (P<0.05). The average was 0.62, 0.48 and 0.40 from the first to the third destructive harvests respectively (SEM 0.031) (data not shown).

The LAR at the first destructive harvest (40 days) showed differences (P<0.01) between treatments, the more lax the defoliation the higher LAR, and the control was not different from 10 or 6 cm height. At day 80, there were no differences between treatments, but at the final destructive harvest (120 days) there were differences (P<0.05). In this case, the control had the lowest LAR and, contrasting with first destructive harvest, 2 cm height had the highest value (Table 4-6). There was a significant interaction of time x defoliation treatment (P<0.05, Table 4-6), 2 and 6 cm increased LAR over time, but 10 cm and the control remained unchanged.

Table 4-5. Primary and secondary shoots per pot (actual and log values) of BFT cv. Grasslands Goldie managed at cutting heights of 2, 6 and 10 cm and one undefoliated control treatment, over 120 days.

	40 days		80 days		120 days		SEM / Significance (time x height)
	Actual values	Log values	Actual values	Log values	Actual values	Log values	
Primary shoots							
2 cm	14	2.62	5	1.39	8	2.09	
6 cm	13	2.57	10	2.23	15	2.59	0.142
10 cm	18	2.84	16	2.73	12	2.41	**
Control	11	2.35	15	2.53	30	3.34	
SEM (n)		0.113 (5)		0.202 (5)		0.152 (5)	
Significance		NS		**		**	
Secondary shoots							
2 cm	22	2.91	9	2.31	9	2.30	
6 cm	27	3.25	27	3.30	12	2.58	0.148
10 cm	18	2.88	21	3.09	20	3.00	**
Control	48	3.83	35	3.54	17	2.87	
SEM (n)		0.216 (5)		0.09 (5)		0.06 (5)	
Significance		*		**		**	

** P< 0.01; * P< 0.05; NS, not significant; SEM, standard error of the mean; (n), number of observations for each treatment mean

The SLA was only affected by defoliation height at the first destructive harvest (P<0.05, Table 4-6). The 10 cm and the control treatments showed higher SLA than 2 cm height and also 10 cm differed from 6 cm height. A significant interaction of time x defoliation treatment was observed (P<0.05); the defoliated treatments, but not the undefoliated control, increased in SLA from the first (40 days) to the third (120 days) destructive harvest.

The weight per leaf differed significantly (P<0.01) during the three destructive harvests (Table 4-6). In all cases, the control had heavier leaves than the defoliated treatments, and leaf weight decreased when the intensity of defoliation increased. There were no significant differences over time.

Table 4-6. Leaf area ratio (LAR), specific leaf area (SLA), weight per leaf (wt./leaf) and leaves/gram of Birdsfoot trefoil under different defoliation intensities over time.

	40 days	80 days	120 days	SEM / Significance (time x defoliation treatment)
LAR (mm^2/mg)				
2 cm	2.2	8.7	8.4	
6 cm	4.6	5.3	7.7	0.64
10 cm	6.2	7.3	6.6	**
Control	5.8	5.6	4.1	
SEM (n)	0.40 (5)	3.33 (5)	0.48 (5)	
Significance	**	NS	**	
SLA (mm^2/mg)				
2 cm	12.4	25.7	35.9	
6 cm	14.2	19.7	29.8	2.54
10 cm	19.3	28.8	26.2	*
Control	17.2	20.3	22.2	
SEM (n)	1.39 (5)	3.24 (5)	3.93 (5)	
Significance	*	NS	NS	
Wt./ leaf (mg)				
2 cm	2.3	3.4	2.4	(time)
6 cm	4.1	3.8	3.5	
10 cm	5.5	5.1	5.7	0.48
Control	6.9	7.1	8.2	NS
SEM (n)	0.41 (5)	0.48 (5)	0.49 (5)	
Significance	**	**	**	
No. Leaves/gram DM				
2 cm	267	165	195	(time)
6 cm	158	111	111	
10 cm	107	89	85	15.1
Control	96	77	43	**
SEM (n)	10.8 (5)	9.72 (5)	19.3 (5)	
Significance	**	**	**	

** $P < 0.01$; * $P < 0.05$; NS, not significant; SEM, standard error of the mean; (n), number of observations for each treatment mean. Note: when time*defoliation treatment interaction was not significant time effect was presented.

The number of leaves per gram differed significantly (P<0.01) between treatments, during the three destructive harvests. In all cases, 2 cm height had a higher number of leaves than the other treatments (Table 4-6). There was a significant time effect (P<0.01), treatments declining in leaf number per unit of DM from the first to the third destructive harvest (Table 4-6).

4.4.5 Relationships between herbage harvested and plant components

Herbage harvested after 20 days of growth, following defoliation after destructive harvests, was correlated with primary roots mass, secondary roots mass, crown size, root diameter and plant root reserves. Due to reduced association at early stages (first 20 days), results are presented for periods following defoliation on day 40 and 80. Also residual leaf area was correlated with herbage harvested, data including the three periods independently.

4.4.5.1 The influence of below-ground plant components

In general, association between parameters increased over time (Table 4-7). During the first period analysed, only primary roots showed a degree of association with herbage harvested, explaining 29% of growth variation. During the second period (days 80 to 100), the primary roots explained 38% of growth variation (Table 4-7).

In contrast, secondary roots mass explained 67% of herbage harvested variation in the second period, and changes in crown mass explained 66% during final sampling. However, root diameter did not show any degree of association at any stage (Table 4-7).

Table 4-7. Regressions between BFT herbage harvested (Y, g DM/pot) and below-ground parameters during two periods of growth after defoliation.

	Period of growth	
Independent variables (x)	**Day 40 to 60**	**Day 80 to 100**
Primary root mass (g/pot)	$Y= 1.622x+4.267$ $r^2=0.29$ $P<0.05$	$Y=1.584x+5.151$ $r^2=0.38$ $P<0.05$
Secondary root mass (g/pot)	$Y= 1.135x+5.441$ $r^2=0.14$ NS	$Y= 0.581x+4.577$ $r^2=0.67$ $P<0.01$
Crown mass (g/pot)	$Y= 1.035x+8.569$ $r^2=0.01$ NS	$Y= 2.098x+3.732$ $r^2=0.66$ $P<0.01$
Root diameter (mm/root)	$Y= 0.651x+4.408$ $r^2=0.02$ NS	$Y= 0.235x+6.890$ $r^2=0.01$ NS

4.4.5.2 Residual leaf area for regrowth

A linear regression was fitted for herbage harvested and residual leaf area during three periods of growth. In all cases, the increase in herbage harvested was associated with the increase in residual leaf area (Figure 4-6). Between 40 to 60 and 80 to 100 day periods, variations in residual leaf area explained more than 80% of variation in herbage harvested, in contrast with initial period (0 to 20 day) that only explained 46% of variation. Residual leaf area was on average 4 times and 6.1 times higher for 6 and 10 cm treatment respectively than for 2 cm treatment.

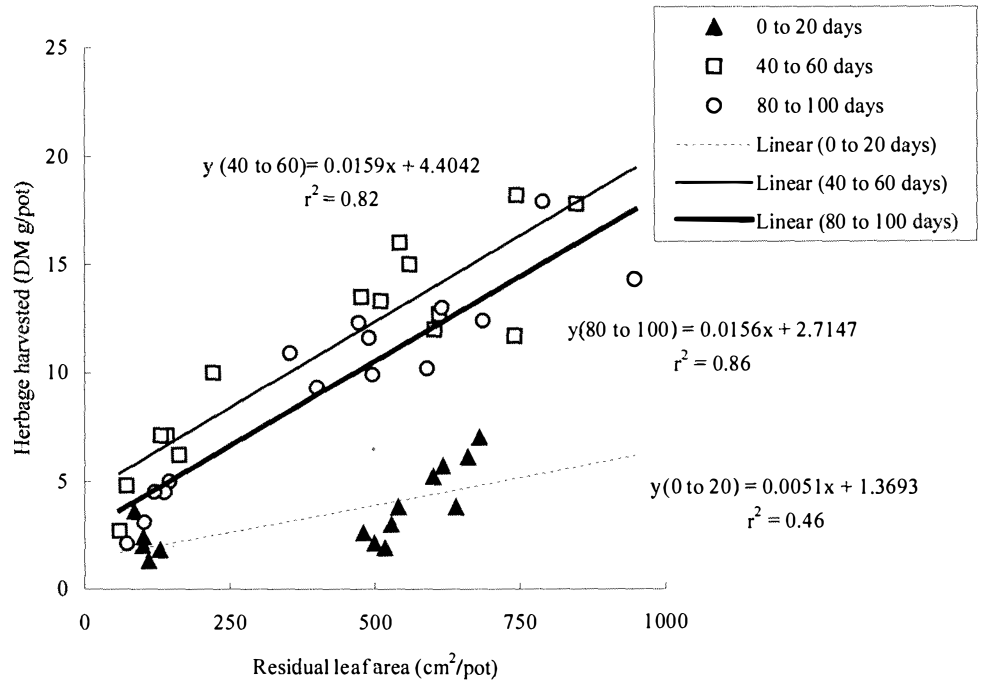


Figure 4-6. Regression between residual leaf area and herbage growth of BFT during three periods (day 0 to 20, 40 to 60 and 80 to 100).

4.4.5.3 Carbohydrate root reserves and regrowth

Total carbohydrate root reserves were significantly correlated with herbage harvested, regrowth increasing when plants had high root reserves (Figure 4-7).

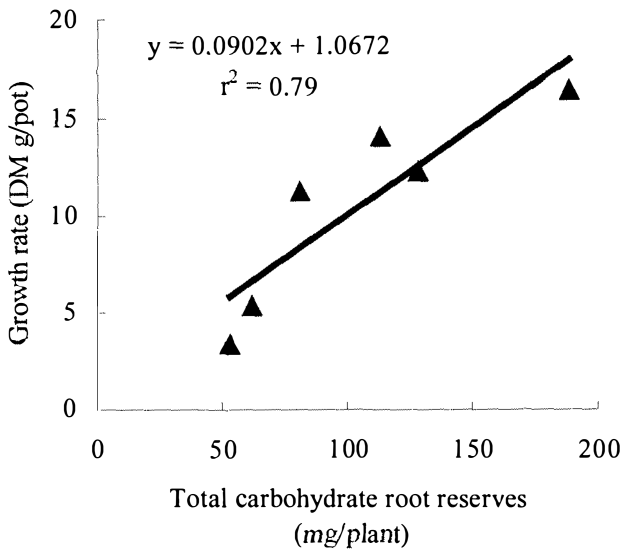


Figure 4-7. Regression between total carbohydrate root reserves and BFT herbage harvested of plants defoliated at 20 day intervals (data comprised average/treatment between day 40 to 60 and 80 to 100).

4.4.5.4 Effect of defoliation intensity on plant components

Information from a multivariate anova (MANOVA) performed on the main plant growth components is presented in Table 4-8. From the MANOVA, the first dimension for defoliation height explained 79, 99 and 99% of variation from destructive harvests one (40 days) to three (120 days), respectively. At 40 days, the significance test showed that defoliation height was not significant when all variables where considered together. At the second harvest (80 days), the significance test showed differences for defoliation height ($P<0.05$), and also root value increased over first harvest, and primary roots and root diameter were the parameters with highest eigenvectors. At the third harvest (120 days), there were significant differences for defoliation height ($P<0.01$), increasing the characteristic root value over previous harvests. Root diameter, secondary roots and primary shoots were the most affected components (Table 4-8). Thus, the effect of defoliation increased in significance over time.

Table 4-8. Multivariate analysis of variance (Manova test) performed on morphological components affected by defoliation height at three successive destructive harvests (40, 80 and 120 days).

Characteristic Root	Percent	Primary roots	Secondary roots	Crown weight	Root diameter	Primary shoots	Secondary shoots	Significance (Wilks' Lambda)
		(Eigenvectors)						
DESTRUCTIVE HARVEST 1 (40 days)								
3.1	78.8	-2.14	1.38	2.89	-1.05	5.94	0.53	NS
DESTRUCTIVE HARVEST 2 (80 days)								
108.2	99.2	3.54	1.41	1.22	2.21	-0.76	0.51	*
DESTRUCTIVE HARVEST 3 (120 days)								
218.5	99.1	-1.03	3.04	2.58	5.05	-3.34	1.23	**

** $P< 0.01$; * $P< 0.05$; NS, not significant.

4.4.6 Plant survival

Initially, 3 plants were allocated to each pot, and there were no significant differences in the number of surviving plants per pot between treatments at 40 and 80 days of evaluation. However, at final destructive harvest (120 days), the average of plants per pot was 2.8, 2.6, 2.0 and 1.4 for the undefoliated control, 10 cm, 6 cm and 2 cm defoliation height respectively (SEM 0.303, $P<0.05$).

4.5 DISCUSSION

The sensitivity of BFT Grasslands Goldie to defoliation intensity demonstrated in field conditions (Chapter 3) was also explored in this pot trial conducted in parallel. In this context, plasticity of BFT plants can be understood as "the ability to alter morphology and physiology in response to varying environmental conditions" (Brandshaw, 1965;

Schlichting, 1986; Grime *et al.*, 1986), to optimise resource capture for regrowth after defoliation. Understanding of these processes required a detailed set of measurements under controlled glasshouse conditions.

Plant biomass production and the pattern of distribution of components above and below ground were affected by defoliation intensity. The production of new tissues by BFT plants after defoliation was higher at lax than hard defoliation within and across time intervals. During the first cycle of defoliation, RGR increased in BFT plants defoliated hard (2 cm), but after three cuts differences between intensities disappeared and RGR remained unchanged over time (Figure 4-2). These results showed that initial plant adjustments to compensate the amount of biomass removed increased the relative regrowth rate (Oesterheld, 1992; Richards, 1993). However, there are limitations to the availability and allocation of resources to maintain this adjustment.

Intensively defoliated plants tended to allocate relatively more metabolites to the production of new leaf area, as observed in LAR and SLA relationships and in the number of leaves per unit of biomass (Table 4-6). Plants defoliated intensively tended to have a dense canopy of very small leaves (Table 4-6). This is a common phenomenon in temperate legumes, leaf size being highly sensitive to changes in defoliation patterns (Chapman and Lemaire, 1993). Treatment differences in residual leaf area explained differences in regrowth, the greater the residual leaf area the higher the regrowth (Figure 4-6).

Root carbohydrate reserves contributed to differences in growth rate. Starch declined following initial defoliation (Table 4-3), suggesting that these compounds were mobilised and used for successive growth, but reserves were not restored in the plants defoliated regularly. In contrast, control plants growing under undisturbed conditions stored starch and free sugars, particularly after 80 days growth accompanied by a substantial root development. Very low carbohydrate reserves in BFT during the active growth season were reported by Smith (1962), reserves not being fully replenished after successive defoliation. This is a clear difference from lucerne or red clover, where levels of accumulation and patterns of utilization of root reserves are clearer (Smith, 1962). In this trial, the frequent defoliation imposed (each 20 days) reduced the opportunity for replenishing reserves, as occurs when defoliation interval is extended from 21 to 42 days (Alison and Hoveland, 1989 b). Thus, regrowth was more dependant on residual leaf area than root reserves. Root reserves are considered important in legume survival, particularly in those cases when plants are under stress (Jung and Smith, 1961). The starch content and its conversion to soluble sugars are believed to contribute to increased winter hardiness of crown forming species (Bula *et al.*, 1956), despite the existence of other root constituents that can contribute to winter hardiness (Boyce and Volenec, 1992; Li *et al.*, 1996). Accumulation of carbohydrates in BFT is regulated by photoperiod and temperature, reserve accumulation patterns increasing under short days and low temperatures in autumn (Nelson and Smith, 1969; Greub and Wedin, 1971). Seasonal accumulation patterns of root reserves explain why BFT tolerates frequent but not close defoliation. Carbohydrates for regrowth depend primarily upon residual leaf area because root reserves are low (Nelson, 1995).

Production of above-ground biomass over 120 days of the undefoliated control was higher than any of the defoliated treatments, and the hard defoliated treatment the least productive. The cumulative effect of defoliation intensity over time was observed on above and below-ground plant components (Table 4-7), effects being significant from 80 days onwards. Below-ground biomass was reduced under repeated and severe defoliation (2 cm), moderately affected under 6 cm height, and was little affected or even increased on the 10 cm treatment, compared with plants growing undisturbed (see also Pierre and Jackobs, 1953). Crown size was negatively associated with the intensity of defoliation. It is suggested that reduction in crown size affects plant reserves, as reported by Greub and Wedin (1971). At the end of the experiment, the below-ground parameters most affected by defoliation treatment were root diameter and secondary root mass (Table 4-8). It is suggested that excessive defoliation during spring could severely affect plant survival, particularly in the following summer. An efficient root system is defined by the degree of exploration of soil (Barber, 1974 cited by Alison and Hoveland, 1989 b). These results showed an intense decline in secondary roots mass with increasing defoliation severity, suggesting that BFT plants severely defoliated reduced capacity to capture water and nutrients, and finally reduced the degree of plant survival.

Root diameter of BFT was affected by defoliation intensity. Alison and Hoveland (1989 b) observed similar effects of defoliation frequency in some BFT cultivars, but effects were not consistent. In this experiment, herbage growth was not correlated with root diameter of BFT Grasslands Goldie after 40 or 80 days. After 120 days, root diameter was the parameter that showed the closest association to defoliation intensities applied. However, root mass was a better predictor of herbage growth.

Overall, the intensive and repeated defoliation of BFT plants produced a general decline in below-ground mass that influenced regrowth after defoliation. Plants made some short term morphological adjustments but these were ineffective under hard defoliation partially attributed to the reduced potential for storage of root reserves during active growth season in comparison with other crown-forming species like alfalfa or red clover (Smith, 1962; Smith and Nelson, 1967). In fact, BFT shows a limited morphological and physiological plasticity to defoliation, as indicated by the increase in RGR of severely defoliated plants observed during the first cycle of regrowth. The absence of morphological responses does not mean plants lack plasticity, because stability can be achieved by altering only simple factors like growth rate (Schlichting, 1986). A high degree of morphological plasticity could be defined as an advantage if large amounts of resources are available, and also the degree of adaptation to stress conditions shows reduced changes in morphology and conservative utilization of resources (Grime *et al.*, 1986).

BFT plants were more dependent on residual leaf mass, and moderate to lax defoliation intensities showed advantages in herbage production and plant survival. Comparing 6 to 10 cm stubble height, 10 cm showed the best production and plant survival, though other factors like the frequency of defoliation should be considered. There was an increase in density of primary shoots on plants defoliated at 6 cm in comparison with those defoliated at 2 or 10 cm after 120 days (Table 4-5). This tendency was also observed in results presented in Chapter 3, where plants defoliated at 6 cm showed more primary shoots per plant at the end of the spring (see Table 3-4 to draw individual plant comparisons). The secondary shoots were increased at early stages (40 and 80 days) by 6 cm defoliation (Table 4-5), but at late stages studied (120 days) plants defoliated laxly (10 cm) showed more shoots than those defoliated more intensively. As reported for lucerne, shoots development is associated with the intensity of defoliation (Keoghan, 1970) and probably, based on these results, with the extension of the period under defoliation. The lax defoliation could determine a higher importance of secondary shoots by a high stubble height, reducing the potential of development of new shoots. The hard defoliation could stimulate the formation of new shoots, but plant reserves will be limiting shoots development if intensive defoliation is repeated over time. Moderate defoliation intensities could suggest a positive effect of defoliation in the formation of new shoots, as well as the importance of secondary shoots.

4.6 CONCLUSIONS

Vigour of BFT Grasslands Goldie plants was markedly reduced by regular close defoliation. Root reserves declined during the active growing season, regrowth being more dependent on residual leaf area. Root mass and carbohydrate reserves were also diminished. These factors limited regrowth potential, and eventually reduced plant survival. The evidence showed that Grasslands Goldie is sensitive to close defoliation, despite the reported semiprostrate plant habit. BFT plasticity in response to intensive defoliation comprised increase in RGR, LAR, SLA and production of new leaves to compensate the loss of tissues. However, these adjustments were not enough to compensate the amount of tissues removed by cutting. A stubble height of 10 cm after defoliation was the best combination for plant production and survival when plants were frequently defoliated. These results obtained in controlled glasshouse environment, were consistent with those observed in field conditions (Chapter 3), but provided a more detailed explanation of morphological and physiological processes of defoliated plants.

4.7 REFERENCES

ALISON, M.W. AND HOVELAND, C.S. (1989 a). Birdsfoot trefoil management. II. Yield, quality and stand evaluation. *Agronomy Journal* **81**: 745-749.

ALISON, M.W. AND HOVELAND, C.S. (1989 b). Birdsfoot trefoil management. I. Root growth and carbohydrate storage. *Agronomy Journal* **81**: 739-745.

BOLOGNA, J.J. (1996). Studies on strategies for perennial legume persistence in lowland pastures. *Thesis of Master of Agricultural Science.* Lincoln University, New Zealand. 220 p

BOYCE, P.J. AND VOLENEC, J.J. (1992). Taproot carbohydrate concentrations and stress tolerance of contrasting alfalfa genotypes. *Crop Science* **32**: 757-761.

BRANDSHAW, A.D. (1965). Evolutionary significance of phenotypic plasticity in plants. *Advances in Genetics* **13**: 115-155.

BULA, R.J., SMITH, D. AND HODGSON, H.J. (1956). Cold resistance and chemical composition in overwintering alfalfa, red clover and sweetclover. *Agronomy Journal* **46**: 397-401.

CHAPMAN, D.F. AND LEMAIRE, G. (1993). Morphogenetic and structural determinants of plant regrowth after defoliation. *Proceedings of the XVII International Grassland Congress*. New Zealand and Australia. pp. 95-104.

CORDEIRO DE ARAUJO, J. AND JACQUES, A.V.A. (1974). Influência do estádio de crescimento e da altura de corte sobre as reservas de glicidos e nitrogênio total de cornichao (*Lotus corniculatus* L.). (Effect of physiological stage and height of cutting on sugar reserves and nitrogen in birdsfoot trefoil). *Revista da Sociedade Brasileira de Zootecnia.* Vol. **3**(2): 123-137.

FANKHAUSER Jr., J.J. AND VOLENEC, J.J. (1989). Root vs. shoot effects on herbage regrowth and carbohydrate metabolism of alfalfa. *Crop Science* **29**:735-740.

FELTNER, K. AND MASSENGALE, M.A. (1965). Influence of temperature and harvest management on growth, level of carbohydrates in roots, and survival of alfalfa (*Medicago sativa* L.). *Crop Science* **5**: 585-588.

FRAME, J.; CHARLTON, J.F.L. AND LAIDLAW, A.S. (1998). Birdsfoot trefoil and greater lotus. In *Temperate Forage Legumes*. CAB International, Wallingford. ISBN 0-85-199-214-5. Chapter 6. pp. 245-271.

GRABER, L.F.; NELSON, N.T.; LUEKEL, W.A. AND ALBERT, W.B. (1927). Organic food reserves in relation to the growth of alfalfa and other perennial herbaceous plants. University of Wisconsin Agriculture Experimental Station. *Bulletin* **80.**

GREUB, L.J. AND WEDIN, W.F. (1971). Leaf area, dry matter accumulation and carbohydrate reserve levels of birdsfoot trefoil as influenced by cutting height. *Crop Science* **11**: 734-738.

GRIME, J.P., CRICK, J.C. AND RINCON, J.E. (1986). The ecological significance of plasticity. *Plasticity in Plants*. Edited by Jennings, D.H. and Trewavas, A.J. The company of Biologists Lmtd., Cambridge. pp.5-30.

HODGKINSON, K.C. (1968). Studies of the physiology of regeneration of lucerne (*Medicago sativa* L.). Review of Ph.D. Thesis University of New England. New South Wales 1967. *Journal of Australian Agricultural Science* **34**: 221.

HODGSON, J. (1979). Nomenclature and definitions in grazing studies. *Grass and Forage Science* **34**: 11-18.

JUNG, G.A. AND SMITH, D. (1961). Trends of cold resistance and chemical changes over winter in the roots and crowns of alfalfa and red clover. II. Changes in mineral constituents. *Agronomy Journal* **53**: 363-366.

LI, G. (1997). Response of Chicory (*Cichorium intybus* L.) to defoliation. *Thesis presented for the degree of Doctor of Philosophy in Plant Science*. Massey University, New Zealand. 197 p

NELSON, C.J. (1995). Photosynthesis and carbon metabolism. In *Forages*. Vol. 1. An Introduction to Grassland Agriculture. Edited by R.F. Barnes, D.A. Miller and C.J. Nelson. Fifth edition. pp. 31-43.

NELSON, C.J. AND SMITH, D. (1968). Growth of birdsfoot trefoil and alfalfa. III. Changes in carbohydrate reserves and growth analysis under field conditions. *Crop Science* **8**: 25-28.

NELSON, C.J. AND SMITH, D. (1969). Growth of birdsfoot trefoil and alfalfa. IV. Carbohydrate reserve levels and growth analysis under two temperature regimes. *Crop Science* **9**: 589-591.

OESTERHELD, M. (1992). Effect of defoliation intensity on above-ground and below-ground relative growth rates. *Oecologia* **92**: 313-316.

PIERRE, J.J. AND JACKOBS, J.A. (1953). The effect of cutting treatments on birdsfoot trefoil. *Agronomy Journal* **45**: 463-468.

RICHARDS, J.H. (1993). The physiology of plants recovering from defoliation. *Proceedings of the XVII International Grassland Congress.* New Zealand and Australia. pp. 85-94.

SILVA, J.P. (1968). Interrelations of leaf area and carbohydrate root reserves as determinants of the regrowth potential of alfalfa (*Medicago sativa* L.) Dissertation Abstracts 29, 1906.

SCHLICHTING, C.D. (1986). The evolution of phenotypic plasticity. *Annual Review of Ecological Systems* **17**: 667-693.

SMITH, D. (1962). Carbohydrate root reserves in alfalfa, red clover, and birdsfoot trefoil under several management schedules. *Crop Science* **2**: 75-78.

SMITH, D. AND NELSON, C.J. (1967). Growth of birdsfoot trefoil and alfalfa. I. Responses to height and frequency of cutting. *Crop Science* **7**: 130-133.

5. PERFORMANCE OF FOUR *Lotus corniculatus* L. CULTIVARS IN RESPONSE TO INTENSITY AND TIMING OF DEFOLIATION

5.1 ABSTRACT

A field experiment was conducted from May 1998 to April 2000 at INIA Treinta y Tres, Uruguay, South America to study the effects of intensity of defoliation and timing of initial defoliation on herbage production and morphological adaptations of four cultivars of *Lotus corniculatus* L. (birdsfoot trefoil, BFT). A factorial experiment (4x2x3) was applied in a complete randomised block design with four replicates. Tested factors included four BFT cultivars (San Gabriel, INIA Draco, Grasslands Goldie and Steadfast), two defoliation intensities (4, 8 cm height) and three times of initial defoliation during the establishment year (vegetative, 50% flowering and advanced maturity). During the second year, the original defoliation intensities treatments were each split further into two intensities (4 and 8 cm height). Plants were defoliated from mid spring to early autumn at intervals of 40 days each year, but cultivars were managed in two groups defined as *local* (San Gabriel and INIA Draco – winter active) and *introduced* (Grasslands Goldie and Steadfast – winter dormant). Herbage production, sward height, nutritive value, plant density and plant morphology were recorded. *Local cultivars* were 2.6 and 2.5 times more productive than *introduced cultivars* during Year 1 (6.3 vs. 2.4 t DM/ha) and Year 2 (4.7 vs. 1.8 t DM/ha) respectively, with production advantage throughout the year. All cultivars showed adequate standards of forage quality, varying from 590-720 g/kg DM for digestibility of organic matter, 25-39 g/kg DM for nitrogen, 230-400 g/kg DM for acid detergent fibre and 22-31 g/kg DM for condensed tannins. Plant density decreased drastically during the second year as a consequence of severe drought conditions. There were substantial differences in morphology between cultivars, especially in root and crown size and shoots per plant. Production was greater when defoliated at 4 than 8 cm height for all cultivars. The timing of initial defoliation affected annual production on Year 1, but only minor effects were observed in Year 2.

Keywords: *Lotus corniculatus* L. cultivars, defoliation management, forage production, nutritive value, plant morphology, plant population.

5.2 INTRODUCTION

Birdsfoot trefoil (BFT) is a widely used forage legume in extensive and intensive farming systems of Uruguay, growing alone or in mixtures with grasses and other legumes. Its success is recognised in a wide range of environments and soil conditions, for forage and seed production. Lack of persistence is reported, due to inadequate defoliation strategies, and high incidence of root and crown diseases with production and population declining from the third year of use (Altier, 1988; Rebuffo and Altier, 1996).

Despite these limitations, BFT is an important summer feed alternative in pastoral areas where especially feed quality and sometimes feed quantity are limited. Thus, potential growth, levels of accumulation and associated nutritive value of BFT are key issues in production systems.

The objectives of this research were to explore the degree of adaptability, productivity and nutritive value of contrasting BFT cultivars in the eastern region of Uruguay, and to investigate more effective defoliation strategies for the species. The effects of management decisions like timing and intensity of defoliation in the year of establishment on herbage production, and on the morphology and physiology of BFT plants, were examined.

Four BFT cultivars with contrasting characteristics were selected to explore defoliation responses, two commercial cultivars used in Uruguay (San Gabriel and INIA Draco), one from New Zealand (Grasslands Goldie) and one from the United States (Steadfast).

Introduced from Brazil around 50 years ago (Gardner *et al.*, 1968), BFT San Gabriel is the oldest cultivar used in Uruguay. It shows a high degree of adaptability to the local ecological conditions. BFT Ganador, an Uruguayan selection, is another recommended cultivar (Carámbula *et al.*, 1996), but productivity of these two cultivars has reduced drastically by diseases in areas where they have been used extensively (Altier, 1997). Formoso (1993) reported values of 4, 10, 7 and 5 t DM/ha from the first to fourth year of production, working with BFT San Gabriel. Digestibility and nitrogen values are high in early spring, 730 and 35 g/kg of DM respectively, but decline over the growing season to 610 and 30 g/kg of DM respectively in early autumn (Formoso, 1993).

Research efforts in Uruguay are oriented to increase productive persistence by selecting new cultivars resistant to disease complexes that can substitute traditional cultivars. Recently, the cultivar INIA Draco was developed, following two cycles of selection of field persistent plants from parental material of BFT Ganador and a local population from La Estanzuela, Uruguay, with the objective to extend pasture life to 3-4 years and expand the range of cultivars available (Rebuffo and Altier, 1996). Advantages in the forage production of BFT INIA Draco of 8, 12, 42 and 74% over BFT San Gabriel were observed from the first to fourth year of pasture respectively (Rebuffo and Altier, 1996). Crown size, density of stems and leaf proportion are improved characteristics of INIA Draco. In general, Uruguayan cultivars have intermediate to erect growth habits, are early flowering and are winter active (Formoso, 1993; Rebuffo and Altier, 1996). The nutritive value of BFT cultivars need to be more studied, in particular for those recently developed. Also, there are no references about tannin content in the conditions of Uruguay, levels of concentration of these compounds having significance in animal performance.

BFT Grasslands Goldie is described as a semi-prostrate cultivar adapted to grazing (AgResearch, 1995), with winter dormancy in the conditions of Uruguay (Juan Bologna, personal communication). Steadfast is a prostrate cultivar, with small to medium sized leaves and stems (Norberg, 1999), and is the first BFT cultivar with the ability to spread by rhizomes. It was developed from the mating of accessions from Morocco with commercial cultivars Norcen and AU-Dewey and germplasm MU-81 from USA (Beuselinck and Steiner, 1995). Early reports showed forage production of 3.5 t DM/ha/year in Iowa (Norber, 1999), which was lower than the traditional BFT cultivars planted that produced between 4.4-4.9 t DM/ha. There are no reports on the performance of Steadfast in Uruguay.

5.3 MATERIALS AND METHODS

The study was sited at Palo a Pique Research Unit, INIA, Treinta y Tres, Uruguay (latitude 33° 54’ S, longitude 54° 38’ W), on a fine, thermic, mixed, vertic Argiudoll (ARS-USDA classification, Fernando García, personal communication) moderately fertile, poor in phosphorus content and acid (Table 5-1).

Table 5-1. Soil characteristics at experimental site in Palo a Pique Research Unit. (Source: Laboratory of Soils, INIA La Estanzuela, Uruguay).

Soil testing dates	Soil depth (cm)	pH (H_2O)	Organic carbon (%)	P (Bray I) (µg P/g soil)	K (mequiv/100g soil)
April 1998	0 – 7.5	5.4	2.6	3.0	0.3
	7.5 - 15	5.5	1.6	2.9	0.2
March 1999	0 – 7.5	5.5	2.5	6.5	0.3
	7.5 - 15	5.6	1.4	2.3	0.2

The trial was sown manually over a conventional seedbed on May 8 1998, and evaluated to April 2000. The BFT cultivars established were San Gabriel (Brazil), INIA Draco (Uruguay), Grasslands Goldie (New Zealand) and Steadfast (USA). The seeding rate of 12 kg/ha was based on that of INIA Draco and corrected for germination and purity and adjusted for seed weight to sow the same number of viable seeds per cultivar (870 viable seeds/m^2). Seed was inoculated with *Rhizobium* and pelleted. Superphosphate (N-P_{total} -$P_{soluble}$-K; 0-21-23-0) was applied at 26 kg P/ha at seeding time, followed by 26 kg P/ha in March 1999.

A factorial experiment (2x3x4) was used with treatments laid out in plots of 5 x 2.5 m in a complete randomised block design with four replicates (Plate 5-1). Treatments were a combination of two intensities of defoliation (4 or 8 cm cutting height) and three initial defoliation times in the year of establishment (defined as vegetative, 50% flowering and advanced maturity), applied to the four BFT cultivars previously described.

Because of differences in the growth pattern of the cultivars, they were managed in two independent groups. The first group comprised the cultivars with winter activity (San Gabriel and INIA Draco, defined as *local cultivars*). Their cutting sequence started on November 4 (vegetative), December 15 (50% flowering) and January 25 (advanced maturity). The second group was the winter dormant cultivars (Grasslands Goldie and Steadfast, defined as *introduced cultivars*), for which defoliation commenced one month later on December 4 (vegetative), January 13 (50% flowering) and February 22 (advanced maturity). After the initial cut, defoliation was at intervals of 40 days until it ceased in April 1999 (April 5 and 15 for *introduced* and *local* cultivars, respectively), during the first year (Table 5-2). After that, the trial was uncut until October 4, 1999 for *local cultivars*, and November 15, 1999 for *introduced cultivars*. At this time, original plots were split into sub-plots 2.5 m x 2.5 m, and two defoliation intensities (4 cm and 8 cm) were applied over the original defoliation intensities in a standard defoliation routine each 40 days to April 2000. The initial defoliation time treatments were not repeated during the second cutting season (Table 5-2).

Table 5-2. Cutting schedule of BFT plots sown on May 1998.

Year	Cultivars	Initial time (year 1)	Dates of cutting					
			4 Nov	15 Dec	25 Jan	8 Mar	15 Apr	
Year 1 (1998-99)	Local	Vegetative	x	x	x	x	x	
		50% Flowering		x	x	x	x	
		Advanced maturity			x	x	x	
				4 Dec	13 Jan	22 Feb	5 Apr	
	Introduced	Vegetative		x	x	x	x	
		50% Flowering			x	x	x	
		Advanced maturity				x	x	
			4 Oct	15 Nov	24 Dec	1 Feb	10 Mar	20 Apr
Year 2 (1999-00)	Local		x	x	x	x	x	x
	Introduced			x	x	x	x	x

When specifically referred, the seasons over the year were defined as autumn (from March to May), winter (from June to August), spring (from September to November) and summer (December to February).

5.3.1 Measurements

5.3.1.1 Forage production

Forage production was measured by cutting to defined sward heights a 1 m x 5 m strip for initial plots and 1 m x 2.5 m for split plots, using a reciprocating blade machine. Ten sward heights were recorded in each plot before cutting, using a ruler. Forage samples were weighed fresh in the field and subsamples taken for dry matter and botanical composition. One subsample was oven-dried at 60 ^{o}C for 48 hours for dry matter and forage quality determinations, and another subsample was separated into components (Birdsfoot trefoil, grasses and weeds). Samples of the botanical fractions were oven–dried to determine dry matter content.

Prior to cutting, a 100 mm x 100 mm quadrat was cut to ground level, and separated into strata mass above and below cutting height treatment (4 or 8 cm) to estimate total leaf area and leaf fractions above and below cutting height. Leaves were dissected from stems, and area measurements made using a LI-Cor LI-3100 Leaf Area Meter (Lambda Instruments Co, Lincoln, NE, USA). Leaf/stem ratio of each sample was estimated after oven-drying dissected leaves and stems.

5.3.1.2 Forage quality

Determinations of *in vitro* organic matter digestibility (OMD) (Tilley and Terry, 1963), nitrogen by Micro-Kjeldahl and acid detergent fibre (ADF) (Goering and Van Soest, 1970) were made for the three initial defoliation times in the two groups of cultivars. After first time defoliation, quality parameters were monitored regularly at 40 day intervals during the cutting season. The accumulation of digestible organic matter

(DOMA= herbage dry matter accumulation, corrected for ash content, multiplied by organic matter digestibility) was calculated for the different physiological and presented for San Gabriel and INIA Draco. Determinations were made at the Laboratory of Nutrition of INIA La Estanzuela, Uruguay.

5.3.1.3 Condensed tannins

Two forage samples of each cultivar were hand clipped at the vegetative stage (September, 1999), stored at –20 °C, dried and ground to pass through a 1 mm sieve. Condensed tannins (CT) fractions (acetone/water-extractable, protein-bound and fibre-bound) were determined using the butanol-HCL method of Terrill *et al.* (1992). Analyses for extractable and protein-bound fractions were made on two sub-samples, and for fibre-bound fraction in one sample per treatment. CT determinations were conducted at the Institute of Food Nutrition and Human Health, Massey University, Palmerston North (Juan Mieres, personal communication).

5.3.1.4 Plant density and morphology

Plant density of BFT was counted in July 1998, March 1999, October 1999 and March 2000 by collecting a soil block 250x250x250 mm in each plot. Plants were washed to remove soil, and counted. Then, 5 BFT plants were randomly taken for morphological measurements. In each plant, the number of primary shoots, secondary shoots, rhizomes, new developing shoots, primary root length and diameter were recorded. Crown and roots (> 2 mm diameter) were oven-dried at 60 °C for 48 hours for dry weight.

5.3.1.5 Plant architecture

In December 1999, the vertical distribution of plant tissues within the BFT sward canopy after 30 days of regrowth was analysed, using an inclined point quadrat (Warren Wilson, 1963), placed at 32.5^{0} to the horizontal. Ten stations per treatment were recorded, and in each case contacts included living structures (leaves and stems), dead structures (leaves and stems) and reproductive structures (flowers and pods) of BFT and other species. Information was expressed by the number of contacts per 4 cm of sward height, and set out graphically to determine the distribution of different components within the sward.

5.3.2 Statistical analysis

Data were analysed using the statistical package SAS (SAS, 1990). The analysis of annual herbage production was based on a factorial design with 4 replicates for Year 1, with cultivars, defoliation intensity and time of initial defoliation as the main factors. In Year 2, the factor defoliation intensity in Year 1 was split in two defoliation intensities for Year 2, with defoliation height of Year 1 as the main plot, and defoliation height of Year 2 as the split-plot factor.

The differences between cultivars determined that they were managed in two independent pairs. Thus, accumulation dates, growth rates and forage quality parameters were recorded at different dates for each pair. The statistical analysis of these parameters was made for each group independently (*local* or *introduced cultivars*), using a ‘repeated measures’ option of SAS GLM procedures. Total condensed tannins, protein-bound and extractable fractions were analysed using a complete randomised design with two replicates, but fibre-bound fraction was not statistically analysed because analyses were not replicated.

Variation in plant density over time was examined by ‘repeated measures’ analysis, and morphology parameters at the end of each year as independent dates, in both cases for all cultivars simultaneously. Also, morphological parameters were all analysed simultaneously by canonical discriminant analysis using the CANDISC procedure of SAS programme (SAS, 1990).

Plate 5-1. General view of BFT cultivars under different intensities and timing of defoliation in the year of establishment (Year 1).

5.4 RESULTS

5.4.1 Climate conditions during the experimental period

Rainfall was 24% higher during 1998 and 29% less during 1999 than the 8-year average. During winter rainfall was 30% higher than average, but during spring and summer it was lower, especially in 1999 with rainfall 52 and 69% less than average respectively (Table 5-3). From the end of spring 1999 to summer 2000, soil temperatures were higher than average (Table 5-3).

Table 5-3. Monthly rainfall and soil temperature at 50 mm depth during the evaluation period and the 8-year average (Source: INIA, Treinta y Tres, Uruguay).

	Rainfall (mm)				Soil Temperature (°C)			
	1998	1999	2000	8-year average	1998	1999	2000	8-year average
January	147	48	66	97	24.6	27.2	30.1	27.9
February	58	14	128	103	25.7	27.1	27.6	26.5
March	97	139	120	104	22.6	26.9	25.1	24.9
April	288	87		137	20.2	18.7		19.9
May	128	46		100	15.8	15.0		15.9
June	168	259		123	12.6	12.1		12.5
July	213	61		109	13.5	12.3		12.0
August	99	68		101	14.0	13.8		13.9
September	57	64		84	16.5	16.9		16.3
October	63	40		90	21.5	19.8		20.2
November	71	19		81	23.9	24.4		24.3
December	133	26		104	25.5	27.7		27.2
Total	1523	870	--	1232	--	--	--	--

Soil water balance was positive during autumn and winter 1998 and part of winter 1999 (June and July). During the rest of the evaluation period water balance was negative, severely so from October 1999 to February 2000 (Figure 5-1). From November 1999 to January 2000, the water deficit was between 130 and 147 mm.

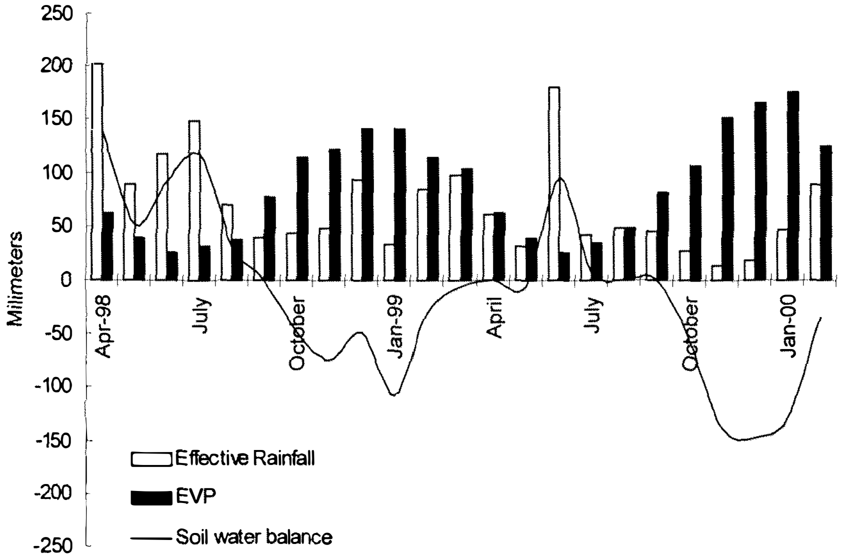


Figure 5-1. Effective rainfall, evapotranspiration (EVP) and soil water balance for the period April 1998-February 2000 in Palo a Pique, Research Unit (Raúl Bermúdez and Jose Terra, personal communication).

5.4.2 Herbage production to first harvest

Three initial defoliation times were defined (vegetative, 50% flowering and advanced maturity), that differed between the two pairs of cultivars, because of the different periods of forage accumulation from sowing to first harvest. *Local cultivars* had a faster initial growth than *introduced materials*, so were defoliated earlier. Analysis was therefore done for BFT fraction, because other components made a minimum contribution, and for each group independently.

Table 5-4. Herbage harvested (kg DM/ha) and plant height at harvest (cm) of *local* BFT cultivars from sowing (8/5/98) to three different stages of development in the establishment year.

		Vegetative 4 November (180 days)	50% Flowering 15 December (221 days)	Advanced maturity 25 January (262 days)
Herbage harvested (kg DM/ha)				
Cultivars				
	INIA Draco	700	2370	3360
	San Gabriel	1630	2690	3360
	SEM (n)	105 (8)	56 (8)	132 (8)
	Significance	**	**	NS
Defoliation height				
	4 cm	1380	2850	3630
	8 cm	950	2210	3090
	SEM (n)	105 (8)	56 (8)	132 (8)
	Significance	*	**	*
Plant height at harvest (cm)				
	INIA Draco	20	33	37
	San Gabriel	29	38	43
	SEM (n)	1.3 (8)	0.4 (8)	0.6 (8)
	Significance	**	**	**

**, P<0.01; *, P<0.05; NS, not significant; SEM, standard error of the mean; (n), number of observations for each treatment mean

For *local cultivars*, there was a significant time x cultivar effect (P<0.01), accumulation increasing over time. There were significant differences between cultivars and defoliation height (Table 5-4), but no significant interaction between these variables. San Gabriel accumulated significantly (P<0.01) more dry matter than INIA Draco up to November and December, but by January herbage harvested for the two cultivars was identical. The height of defoliation determined levels of dry matter harvested, in all cases 4 cm height producing more than 8 cm height. San Gabriel showed significantly (P<0.01) greater plant height than INIA Draco at all dates. (Table 5-4). Maximum plant heights at first harvest in January (262 days after sowing) were 43 and 37 cm for San Gabriel and INIA Draco, respectively.

Table 5-5. Herbage harvested (kg DM/ha) and plant height at harvest (cm) of *introduced* BFT cultivars from sowing (8/5/98) to three different stages of development in the establishment year.

		Vegetative 4 December (210 days)	50% Flowering 13 January (250 days)	Advanced maturity 22 February (290 days)
Herbage harvested (kg DM/ha)				
Cultivars				
	Grasslands Goldie	760	1720	1850
	Steadfast	1060	1580	1950
	SEM (n)	75 (8)	119 (8)	120 (8)
	Significance	*	NS	NS
Defoliation height				
	4 cm	1290	2130	2660
	8 cm	530	1180	1130
	SEM (n)	75 (8)	119 (8)	120 (8)
	Significance	**	**	**
Plant height at harvest (cm)				
	Grasslands Goldie	22	30	32
	Steadfast	20	25	26
	SEM (n)	0.6 (8)	0.5 (8)	0.9 (8)
	Significance	NS	**	**

**, P<0.01; *, P<0.05; NS, not significant, SEM, standard error of the mean; (n), number of observations for each treatment mean

For *introduced cultivars*, there was a significant interaction time x height of defoliation effect (P<0.01). Only in early December, there was a significant difference (P<0.05) in herbage harvested in favour of Steadfast (Table 5-5). The height of defoliation affected herbage harvested (P<0.01), in all cases the yield was higher when defoliated at 4 cm rather 8 cm. Plant height showed significant differences in January and February (P<0.01 in both cases), Grasslands Goldie being taller than Steadfast (Table 5-5). Maximum plant heights at final harvest (290 days from sowing) were 32 and 26 cm for Grasslands Goldie and Steadfast respectively.

5.4.3 Annual herbage production

The annual herbage production of Year 1 (1998-1999) and Year 2 (1999-2000) was analysed for each pair of cultivars (*local and introduced*), because of differences in growth patterns. Comparisons between the two pairs are also presented.

Year 1

The adequate plant establishment limited the incidence of other species, mainly some annual grasses (*Vulpia australis* and *Gaudinina fragilis*). The contribution of these species for *local cultivars* was 0.13 t DM/ha, and for *introduced cultivars* 0.25 t DM/ha. There were no differences between cultivars in each group, so only results for the BFT contribution are presented. The overall herbage production of BFT from sowing in May 1998 to April 1999 (Year 1) was 4.3 t DM/ha. The average production of the winter active BFT (*local cultivars*) was 2.6 times higher than the average of winter latency BFT (*introduced cultivars*) (6.3 vs 2.4 t DM/ha for *local* and *introduced cultivars* respectively, SEM 0.16, $P<0.01$). There was a significant interaction ($P<0.05$ in both groups) cultivar x initial defoliation time (Figure 5-2).

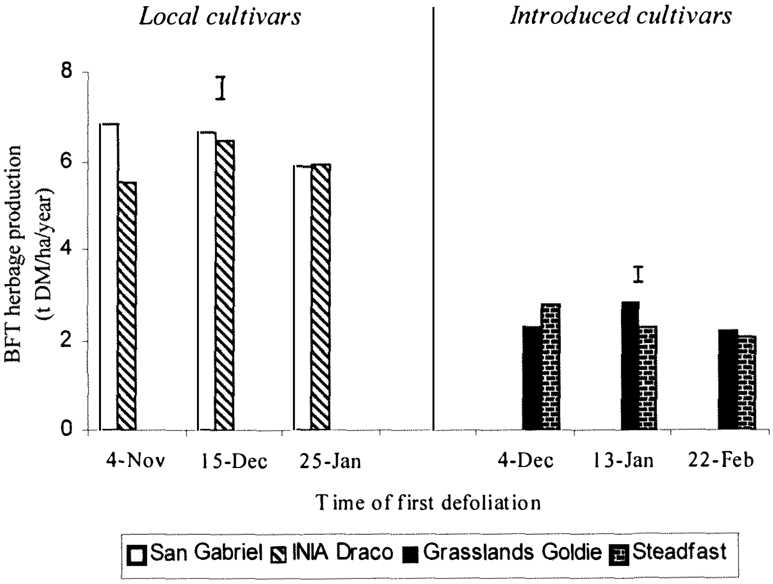


Figure 5-2. Annual herbage production (t DM/ha/year) of BFT cultivars affected by timing of initial defoliation during the Year 1. Vertical bars represent SEM for each group of cultivars, (n=8).

Between *local cultivars*, the highest production was achieved by San Gabriel when cutting started in November or December, and by INIA Draco when cutting started in December (Figure 5-2). For *introduced cultivars*, Steadfast showed the highest production when the initial cut was done in December and Grasslands Goldie when cutting sequence started on January (Figure 5-2).

For *local cultivars,* production was increased 23% when defoliated at 4 cm rather than 8 cm height (6.9 and 5.6 t DM/ha/year for 4 and 8 cm height respectively, SEM 0.15, $P<0.01$). The results for *introduced cultivars* showed a 100% increase when defoliated at 4 rather 8 cm height (3.2 and 1.6 t DM/ha/year for 4 and 8 cm height respectively, SEM 0.10, $P<0.01$).

Year 2

The average of herbage production from April 1999 to April 2000 (Year 2) was 3.3 t DM/ha/year. As reported in Year 1 contribution of other species was low, 0.1 and 0.36 t DM/ha in average for *local* and *introduced cultivars* respectively, information is presented for BFT fraction only. The production of *local cultivars* was 2.5 times ($P<0.01$) greater than *introduced cultivars* (Table 5-6).

Between *local cultivars*, San Gabriel and INIA Draco did not differ in production, the average being 4.7 t DM/ha/year. There were no residual effects of timing of initial defoliation in Year 1, but there was a significant interaction ($P<0.01$) defoliation height Year 1 x defoliation height Year 2 (Table 5-6). Plots defoliated at 4 cm in Year 1 had higher production than plots defoliated at 8 cm, and plots intensively defoliated at 4 cm over 2 years were more productive than the other combinations tested. There were no differences between treatments defoliated in the 4 cm-8 cm and 8 cm-4 cm sequences respectively (Table 5-6).

There were significant differences ($P<0.01$) between *introduced cultivars*, Grasslands Goldie being more productive than Steadfast (2.0 and 1.7 t DM/ha/year respectively, SEM 0.06). Also a significant interaction ($P<0.01$) defoliation height Year 1 x defoliation height Year 2 was observed (Table 5-6). The same tendency was observed for *local cultivars*, plots defoliated intensively in Year 1 or in both years were more productive than the other treatments (Table 5-6). There were no residual effects from timing of defoliation in Year 1 or other interaction effects.

Table 5-6. The effect of defoliation intensity applied in Year 1 and 2 on annual herbage production (t DM/ha/year) in Year 2 of two groups of BFT cultivars (*local* and *introduced*).

Defoliation intensity		*Local cultivars* (t DM/ha/year)	*Introduced cultivars* (t DM/ha/year)
Year 1	Year 2		
4 cm	4 cm	5.3	2.4
	8 cm	4.9	1.9
8 cm	4 cm	4.6	1.8
	8 cm	4.1	1.5
SEM (n)		0.12 (24)	0.08 (24)
Significance		**	**

**, P<0.01; SEM, standard error of the mean; (n) number of observations for each treatment mean

Plots were not defoliated from autumn to early spring, but cultivars differed in autumn-winter activity. The first cut in spring of Year 2 included all accumulated production from the previous autumn, herbage production being significantly higher for *local cultivars* than *introduced cultivars* (3.1 and 0.4 t DM/ha respectively, SEM 0.05, P<0.01). INIA Draco and San Gabriel produced 65 and 68% of annual production from autumn to spring, respectively. On the contrary, *introduced cultivars* Grasslands Goldie and Steadfast only produced 23 and 29% of annual production in the same period.

5.4.4 Growth rates

The growth rates were analysed for each year during the active growth periods (spring and summer), under defoliation intervals of 40 days. Contrasting growth patterns determined the management in the two groups of cultivars (*local and introduced*) previously described. Differences in timing of defoliation and length of evaluation periods made it necessary to analyse the information at three levels: individually by cultivar, by pair of cultivars and by the number of regrowth periods, particularly in Year 1.

Year 1

First, a preliminary analysis of the general growth pattern by cultivar (Figure 5-3 a-d) showed maximum growth rates in early summer declining over the season ($P<0.001$ in all cases). In the majority of periods, there were higher growth rates on plots when cut at 4 cm than at 8 cm height. BFT Steadfast and Grasslands Goldie had a shorter and less productive growth season than INIA Draco and San Gabriel. The growth rates for Grasslands Goldie and Steadfast in early autumn were poor, less than 10 kg DM/ha/day (Figure 5-3 c, d), contrasting with rates over 20 kg DM/ha/day for San Gabriel and INIA Draco (Figure 5-3 a, b).

There were no differences in growth rate between *local cultivars*, maximum growth rate being achieved ($P<0.01$) in December-January, and the more intense defoliation (4 cm) increased growth rate ($P<0.01$) (Figure 5-4). For *introduced cultivars,* there was a significant interaction cultivar x height of defoliation at the last cutting ($P<0.01$), Grasslands Goldie producing more at 4 cm than at 8 cm height or Steadfast at 4 cm, and these higher than Steadfast at 8 cm (Figure 5-5). However, the low growth rates measured at this time minimise the importance of these differences.

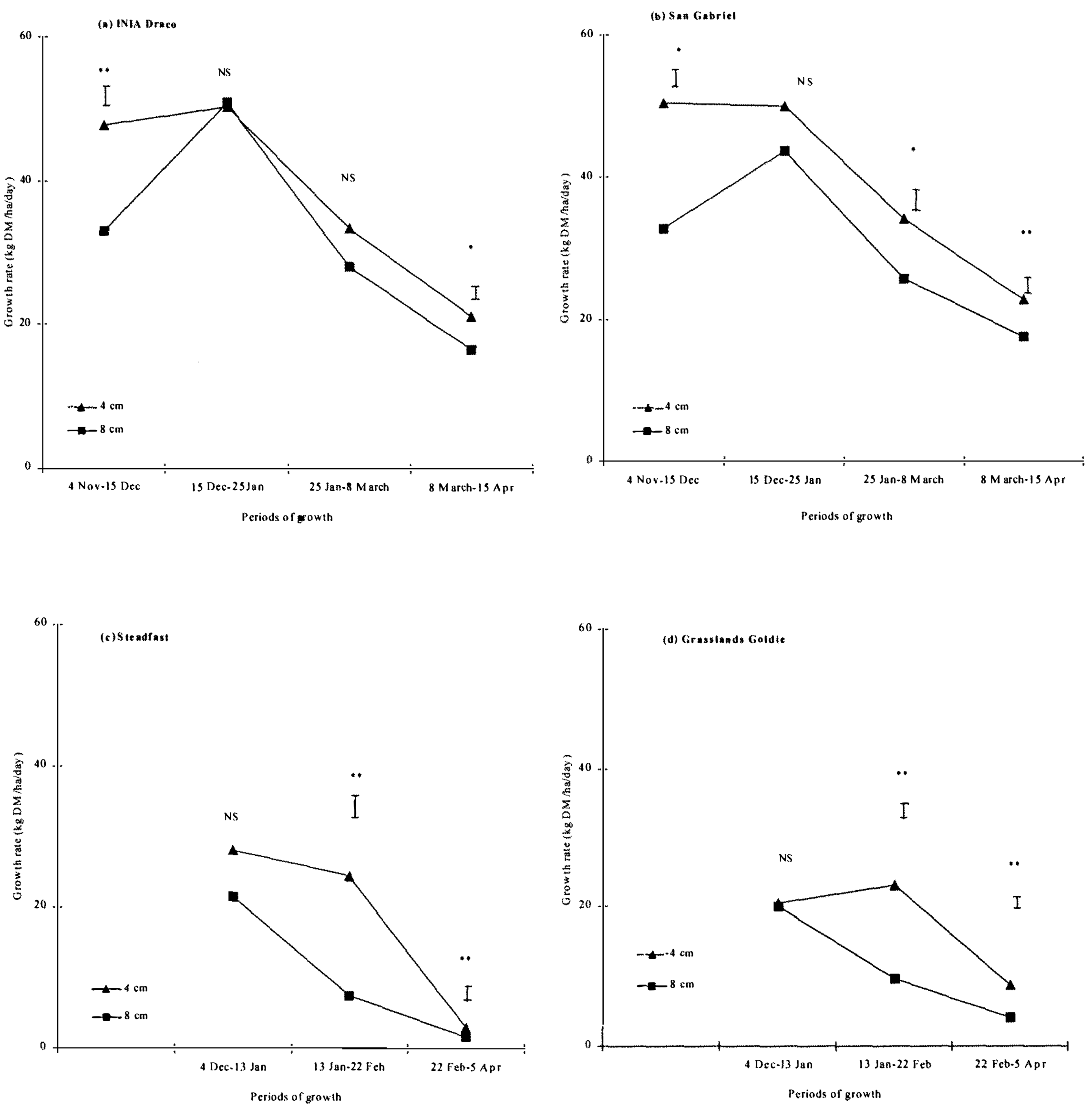


Figure 5-3. Seasonal variation in growth rates (kg DM/ha/day) of BFT cultivars (a) INIA Draco, (b) San Gabriel, (c) Steadfast and (d) Grasslands Goldie under two defoliation intensities. Vertical bars represent SEM; **, ($P<0.01$); *, ($P<0.05$) and NS, (differences not significant) for corresponding growth periods. The number of observations (n) for each treatment mean was 4 (4 Nov-15 Dec), 8 (15 Dec-25 Jan),12 (25 Jan-8 March) and 12 (8 March-15 Apr) for INIA Draco and San Gabriel. For Steadfast and Grasslands Goldie (n) was 4 (4 Dec-13 Jan), 8 (13 Jan-22 Feb) and 12 (22 Feb-5 Apr).

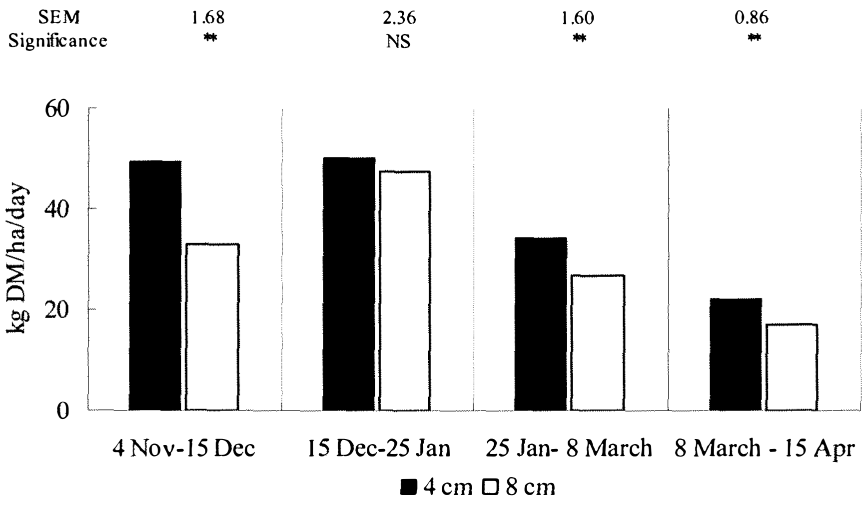


Figure 5-4. Effect of height of defoliation on growth rates of *local* BFT *cultivars* from November 1998 to April 1999. (, P<0.01; NS, not significant; SEM, standard error of the mean, n = 8 (4 Nov-15 Dec), n=16 (15 Dec-25 Jan), n=24 (25 Jan-8 March and 8 March-15 Apr)).**

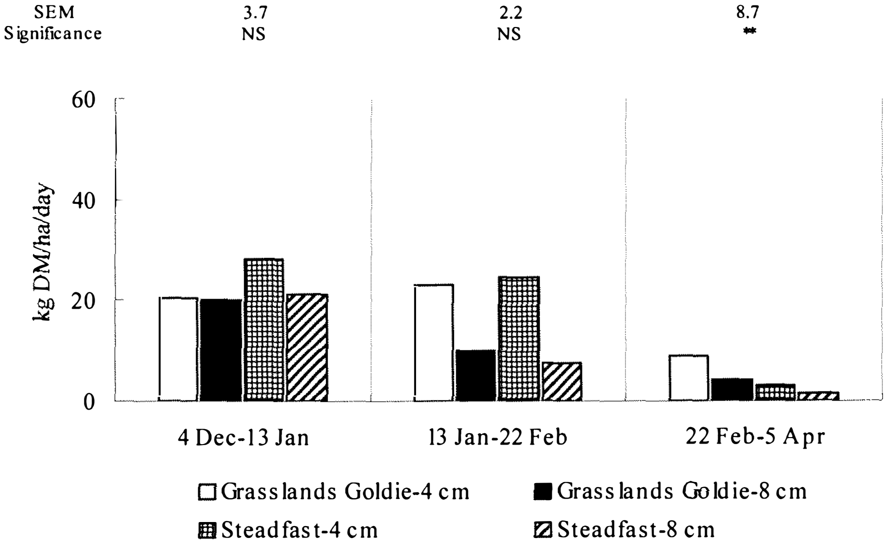


Figure 5-5. Growth rate of *introduced* BFT *cultivars* defoliated at two different heights from December 1998 to April 1999. (, P<0.01; NS, not significant; SEM, standard error of the mean, n=4 (4 Dec-13 Jan), n=8 (13 Jan-22 Feb), n=12 (22 Feb-5 Apr)).**

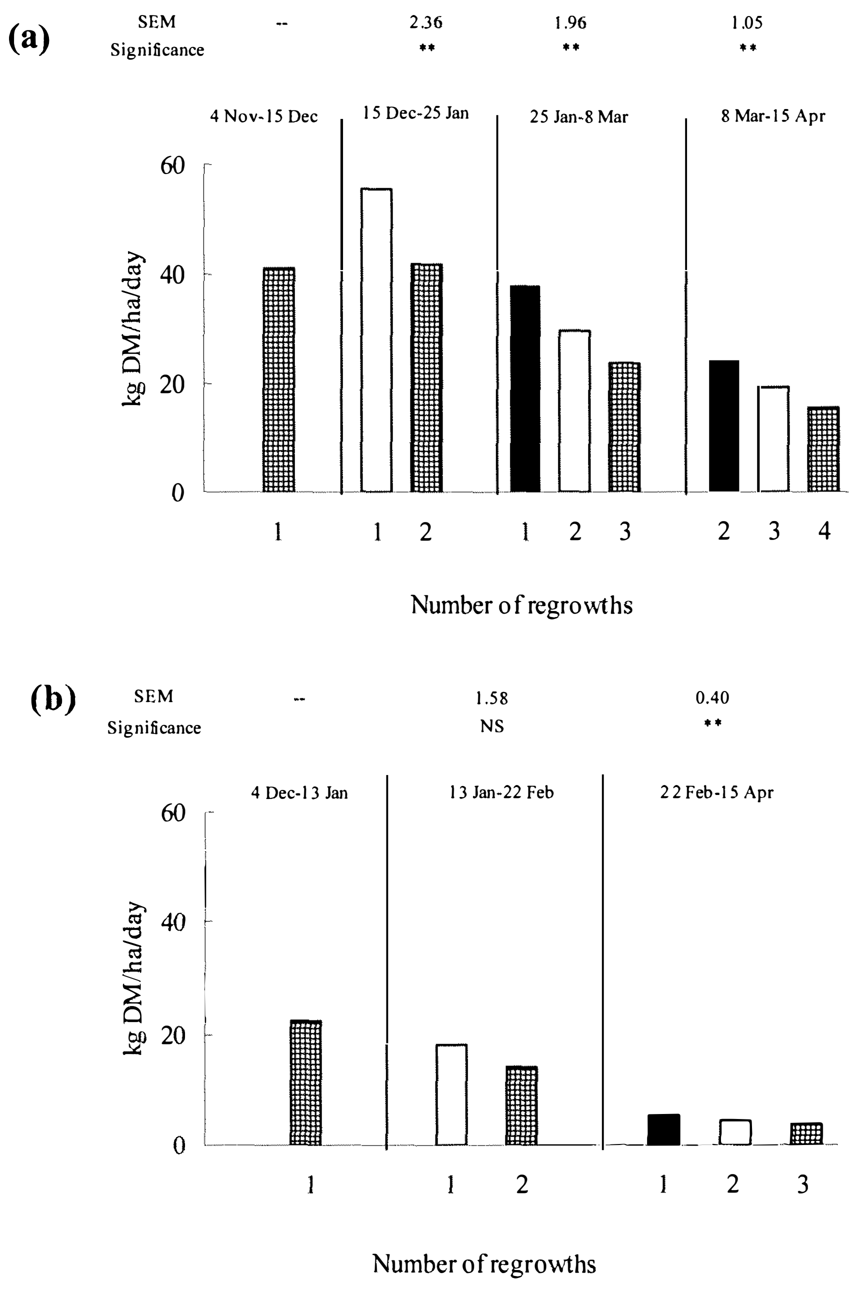


Figure 5-6. Growth rates of (a) *local cultivars and* (b) *introduced cultivars* of BFT, influenced by the number of previous defoliation during spring and summer. (, P<0.01; NS, differences not significant for corresponding growth periods; SEM, standard error of the mean, n=16). Bars with same colour represent the same treatment across periods.**

Because of different times of initial defoliation, there were plots with different numbers of cuts at the same time. The effect of number of cuts on regrowth is presented in Figures 5-6 a and b for *local* and *introduced cultivars,* respectively. Between *local cultivars*, there were significant differences ($P<0.01$ in all cases) in regrowth rates when the number of cuts increased from one to two, one to three or two to four regrowths (Figure 5-6 a). The number of successive defoliations affected regrowth rates of *introduced cultivars,* only for the period February-April (Figure 5-6 b), first regrowth being more productive than the second or third regrowth ($P<0.01$).

Year 2

The dry conditions of Year 2 limited the growth rates of all cultivars during spring-summer. There were significant differences ($P<0.01$) between *local cultivars* in the overall growth rate, in favour of INIA Draco. Also, these materials showed higher growth rates ($P<0.01$) when defoliated at 4 cm rather than 8 cm. There was a residual effect of the timing of first defoliation ($P<0.05$) in Year 1, with high growth rates in plots defoliated latest in Year 1 (Table 5-7). There were no interaction effects.

There were significant differences ($P<0.01$) between *introduced cultivars*, Grasslands Goldie having the best performance. As occurred with *local cultivars*, defoliation intensity in Year 2 affected ($P<0.01$) growth rate of *introduced materials*, 4 cm being better than 8 cm height (Table 5-7).

The analysis of the different growth periods showed in general that the best growth conditions occurred from October to December, when the intensity of drought was not extreme. During summer, the growth rates declined and there were no significant differences between main treatments.

Table 5-7. Effects of Year 1 and Year 2 treatments on overall growth rates (kg DM/ha/day) during spring-summer of Year 2 in two pairs of BFT cultivars.

Local cultivars	Growth rate (kg /ha/day)	*Introduced cultivars*	Growth rate (kg /ha/day)
Cultivars		*Cultivars*	
INIA Draco	10.7	Grasslands Goldie	10.7
San Gabriel	9.4	Steadfast	7.9
SEM (n)	0.34 (48)	SEM (n)	0.42 (48)
Significance	**	Significance	**
Defoliation intensity (Year 2)		*Defoliation intensity (Year 2)*	
4 cm	11.9	4 cm	10.6
8 cm	8.2	8 cm	8.0
SEM (n)	0.34 (48)	SEM (n)	0.42 (48)
Significance	**	Significance	**
Timing effect (residual effect Year 1)			
November	9.4		
December	9.8		
January	11.0 (32)		
SEM (n)	0.42		
Significance	*		

**, $P<0.01$; *, $P<0.05$; SEM, standard error of the mean; (n) number of observations for each treatment mean

5.4.5 Sward structure characteristics

Sward characteristics were described at two levels. Firstly, the residual forage after cutting (section 5.4.5.1), including the residual herbage mass, leaf area and leaf:stem ratio of defoliated swards. Also, a description of plant architecture, where a point quadrat technique was used to describe the tissues distribution of BFT in the sward, is provided in section 5.4.5.2.

5.4.5.1 Residual forage.

Local cultivars did not show differences in residual herbage mass, residual leaf area for regrowth or leaf/(leaf+stem) ratio (Table 5-8). But the height of defoliation determined a lower residual herbage mass ($P<0.01$) and lower LAI ($P<0.01$) when swards were defoliated at 4 cm than 8 cm height. Defoliation height did not affect residual leaf/(leaf+stem) ratio. There were no differences in the parameters when cutting was done at different growth stages.

Table 5-8. Residual herbage mass, residual leaf area index (LAI) and leaf/(leaf+stem) ratio after cutting of *local cultivars* managed at two defoliation heights and three times of initial defoliation.

Variables	Residual herbage mass (DM kg/ha)	Residual LAI (cm^2 leaves/cm^2 soil)	Residual Leaf/(leaf+stem) Ratio
Cultivars			
INIA Draco	920	0.09	0.09
San Gabriel	980	0.07	0.08
SEM (n)	51 (24)	0.010 (24)	0.010 (24)
Significance	NS	NS	NS
Defoliation height			
4 cm	660	0.04	0.08
8 cm	1240	0.13	0.09
SEM (n)	51 (24)	0.010 (24)	0.010 (24)
Significance	**	**	NS
Time of initial cutting			
Vegetative	920	0.08	0.10
50% Flowering	930	0.08	0.09
Advanced maturity	1020	0.007	0.08
SEM (n)	58 (16)	0.032 (16)	0.011 (16)
Significance	NS	NS	NS

**, $P<0.01$; *, $P<0.05$; NS, not significant; SEM, standard error of the mean; (n) number of observations for each treatment mean

There were no differences in residual herbage mass between *introduced cultivars*, but Steadfast had a higher residual leaf area index ($P<0.05$) than Grasslands Goldie (Table 5-9). The height of defoliation significantly affected the residual herbage mass ($P<0.01$) and the residual leaf area index ($P<0.01$), 8 cm being higher than 4 cm height in both cases, and the residual ratio leaf/(leaf+stem) ($P<0.01$), 4 cm being higher than 8 cm height.

Table 5-9. Residual herbage mass, residual leaf area index (LAI) and leaf/(leaf+stem) ratio of *introduced cultivars* managed at two defoliation heights and three times of initial defoliation.

Variables	Residual herbage mass (DM kg/ba)	Residual LAI (cm^2 leaves/cm^2 soil)	Residual Leaf/(leaf+stem) Ratio
Cultivars			
Grasslands Goldie	1190	0.25	0.19
Steadfast	1240	0.35	0.21
SEM (n)	59 (24)	0.035 (24)	0.024 (24)
Significance	NS	*	NS
Defoliation height			
4 cm	1010	0.16	0.14
8 cm	1420	0.43	0.26
SEM (n)	59 (24)	0.035 (24)	0.022 (24)
Significance	**	**	**
Time of initial cutting			
Vegetative	1200	0.36	0.31
50% Flowering	1210	0.27	0.19
Advanced maturity	1270	0.19	0.0.10
SEM (n)	54 (16)	0.032 (16)	0.024 (16)
Significance	NS	**	**

**, $P<0.01$; *, $P<0.05$; NS, not significant; SEM, standard error of the mean; (n) number of observations for each treatment mean

5.4.5.2 Plant architecture

The vertical distribution of plant components in the sward strata at December 1999 is presented in Figures 5-7 and 5-8, for *local and introduced cultivars,* respectively. A higher proportion of dead material was distributed between 0 and 12 cm height for *introduced cultivars* in comparison with *local* ones. Annual grasses (*Vulpia australis* and *Gaudinia fragilis*) represented the main components of the dead fraction.

Local cultivars tended to be taller than *introduced cultivars,* independently of defoliation intensity applied. A higher proportion of dead stems was observed in *local cultivars* than in *introduced cultivars* between 0-12 cm height, the proportion being higher for the 8 cm defoliation height treatment. Live stems were better distributed across strata in *local cultivars*, and for *introduced cultivars* stems were mainly in the first 20 cm strata.

The proportion of leaves between 0-4 cm was low in all cases, and between 4-8 cm only Steadfast defoliated at 4 cm height showed a high frequency of leaves (Figure 5-8 c). The pattern of leaf distribution in Steadfast changed with the intensity of defoliation, intensive defoliation increasing the distribution of leaves in low strata.

Reproductive structures (flowers and pods) were placed from 20 cm approximately to the top of sward, *introduced cultivars* having a high proportion of flowers than *local cultivars* that were mainly in pod stage.

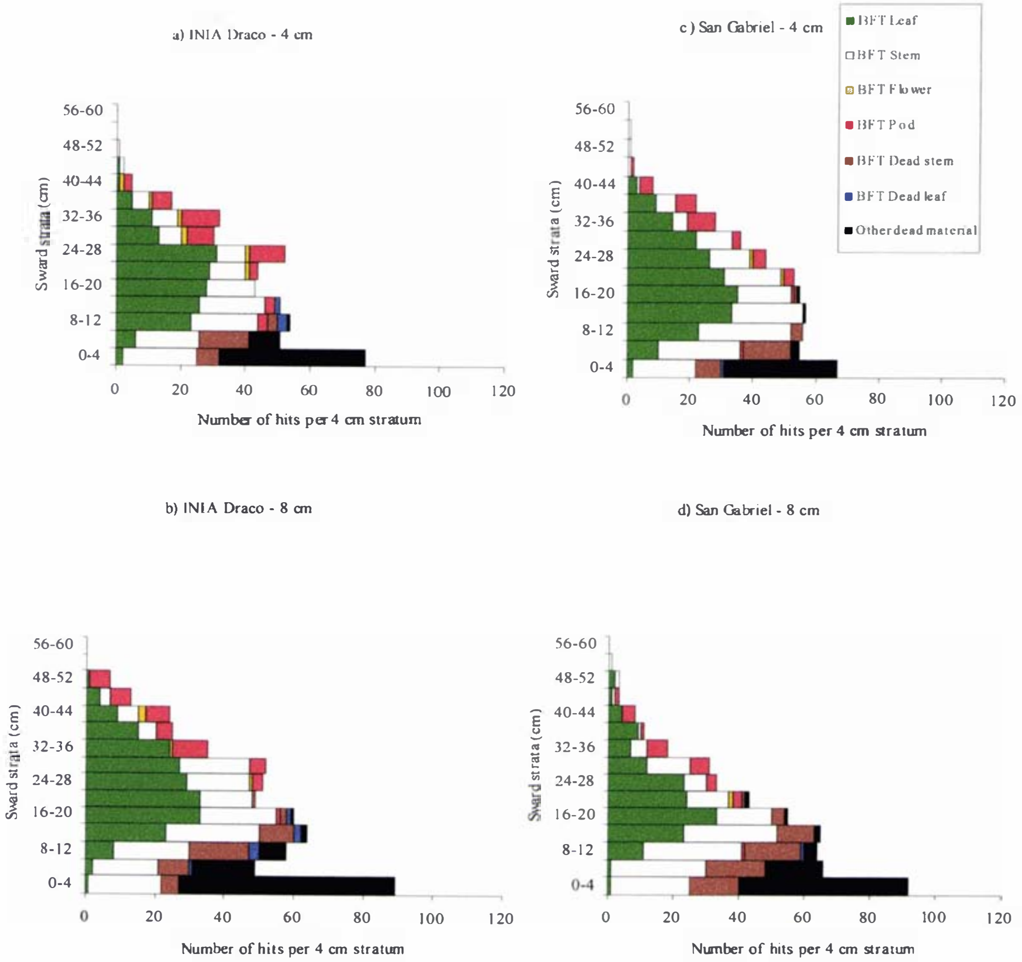


Figure 5-7. Vertical distribution of tissues in BFT swards strata for *local cultivars* under two defoliation heights in December 1999, determined from inclined point quadrat contacts.

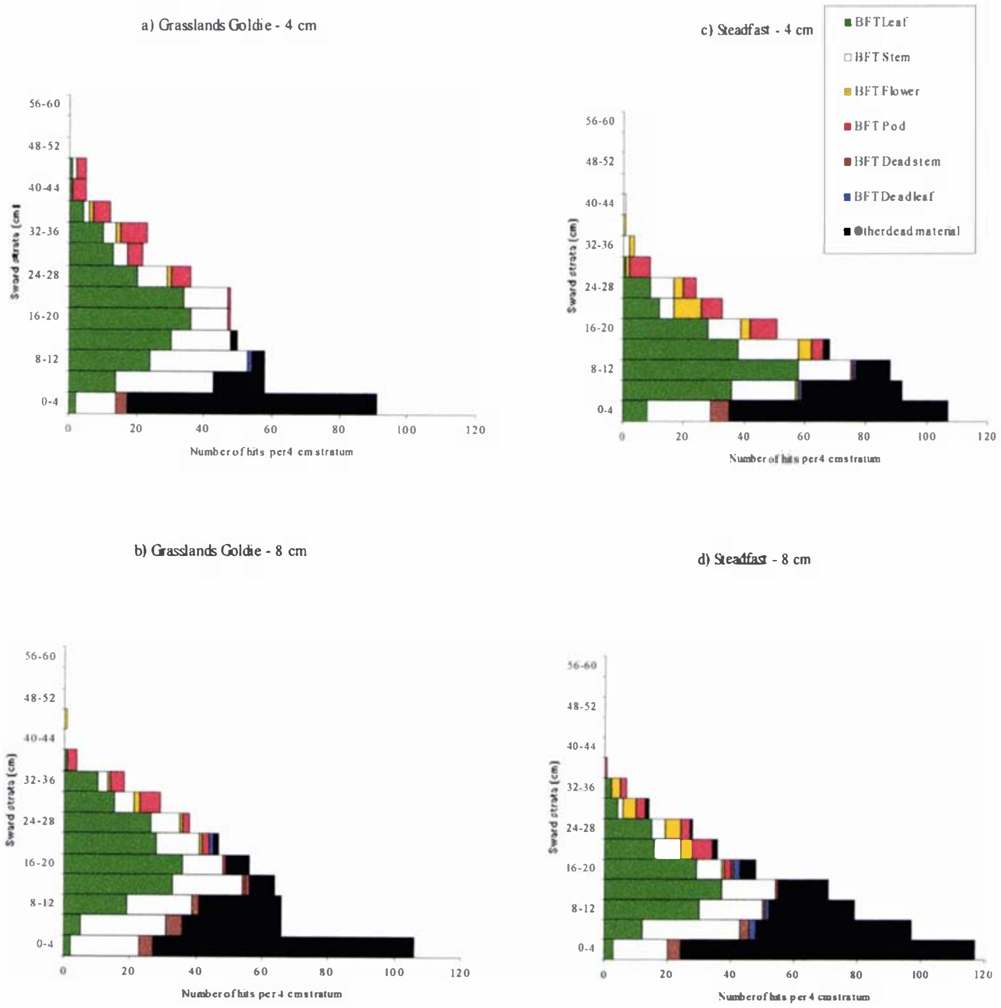


Figure 5-8. Vertical distribution of tissues in BFT swards strata for *introduced cultivars* under two defoliation heights in December 1999, determined from inclined point quadrat contacts.

5.4.6 Nutritive value

Nutritive value (*in vitro* organic matter digestibility, nitrogen and acid detergent fibre) of BFT cultivars was compared at the different dates of first harvest and reported as the nutritive value of accumulated BFT forage. Also, nutritive value was monitored at 40 days defoliation intervals, after first cut in November or December for *local* and *introduced* cultivars respectively. Because of differences in growth patterns, *local* and *introduced cultivars* were compared independently. A significant interaction effect time x cultivar x defoliation height was observed in some of tested parameters, thus information presented in Tables 5-10 to 5-13 shows this interaction for all cases and when it is not significant, the time effect is also shown.

Also in this section is reported the condensed tannin content of cultivars during the spring of Year 2, when cultivars were at the vegetative stage.

5.4.6.1 Nutritive value of accumulated BFT forage

Local cultivars

A significant interaction time x cultivar x defoliation height was observed in OMD (Table 5-10). In November, Draco had higher OMD than San Gabriel, independently of defoliation height, but in December San Gabriel defoliated at 8 cm showed higher digestibility than the others. In January and after 262 days of herbage accumulation, there was a general decline in forage quality and no differences between treatments (Table 5-10).

There was only a time effect (P<0.01) for the nitrogen content of *local cultivars*, nitrogen declining over time in all cases (Table 5-10). The ADF fraction showed differences over time (P<0.01), increasing when the accumulation period increased. There were no differences between cultivars or defoliation heights studied (Table 5-10).

Table 5-10. Nutritive value of *local* BFT *cultivars* for the first harvest from sowing to three different periods of dry matter accumulation for plots defoliated at two different heights.

	Sampling dates/ (length of accumulation from sowing)			SEM (time*cultivar*height) [SEM (time)]
	November 4 (180 days)	December 15 (221 days)	January 25 (262 days)	
***In vitro* OMD (g/kg)**				
INIA Draco - 4 cm	663	625	538	
INIA Draco - 8 cm	670	651	562	10.8 **
San Gabriel – 4 cm	626	619	495	
San Gabriel – 8 cm	647	683	515	
SEM (n)	2.8 (4)	4.3 (4)	4.8 (4)	
Significance	*	**	NS	
Nitrogen (g/kg)				
INIA Draco - 4 cm	39	27	19	
INIA Draco - 8 cm	39	29	23	0.9 NS
San Gabriel – 4 cm	30	25	19	[0.6 **]
San Gabriel - 8 cm	33	28	23	
SEM (4)	0.1 (4)	0.5 (4)	0.9 (4)	
Significance	NS	NS	NS	
ADF (g/kg)				
INIA Draco - 4 cm	267	328	432	
INIA Draco - 8 cm	218	228	383	11.2 NS
San Gabriel - 4 cm	291	310	444	[8.4 **]
San Gabriel - 8 cm	219	227	412	
SEM (4)	5.3 (4)	5.7 (4)	12.4 (4)	
Significance	NS	NS	NS	

OMD, organic matter digestibility; ADF, acid detergent fibre; SEM, standard error of the mean; (n) number of observations for each treatment mean; **, P<0.01; *, P<0.05; NS, not significant; [SEM(time)], presented when SEM (time*cultivar*height) not significant.

Introduced cultivars

A significant interaction time x cultivar x defoliation height (P<0.01) was observed for OMD (Table 5-11). In early December, Steadfast defoliated at 4 cm showed the highest OMD, but the lowest if defoliated at 8 cm. Grasslands Goldie did not show differences in OMD as a consequence of defoliation height. In January, there were no differences between treatments, but Grasslands Goldie showed higher OMD than Steadfast in February, and each cultivar had higher OMD if defoliated at 8 cm than 4 cm.

Table 5-11. Nutritive value of *introduced* BFT *cultivars* for the first harvest from sowing to three different periods of dry matter accumulation for plots defoliated at two different heights.

	Sampling dates/(length of accumulation from sowing)			SEM (time*cultivar*height) [SEM (time)]
	December 4 (210 days)	January 13 (250 days)	February 22 (290 days)	
***In vitro* OMD (g/kg)**				
Grasslands Goldie - 4 cm	663	615	521	
Grasslands Goldie - 8 cm	666	642	542	11.5 **
Steadfast – 4 cm	680	549	484	
Steadfast – 8 cm	645	563	513	
SEM (n)	3.6 (4)	6.3 (4)	1.4 (4)	
Significance	**	NS	*	
Nitrogen (g/kg)				
Grasslands Goldie - 4 cm	33	25	24	
Grasslands Goldie - 8 cm	39	28	26	1.7 **
Steadfast - 4 cm	37	24	23	
Steadfast - 8 cm	35	25	24	
SEM (n)	0.6 (4)	0.7 (4)	0.2 (4)	
Significance	**	NS	**	
ADF (g/kg)				
Grasslands Goldie - 4 cm	295	379	457	
Grasslands Goldie - 8 cm	219	322	377	8.0 NS
Steadfast - 4 cm	213	403	472	[4.3 **]
Steadfast - 8 cm	224	411	448	
SEM (n)	3.1 (4)	5.8 (4)	3.3 (4)	
Significance	**	**	**	

OMD, organic matter digestibility; ADF, acid detergent fibre; SEM, standard error of the mean; (n) number of observations for each treatment mean; **, $P<0.01$; *, $P<0.05$; NS, not significant; [SEM(time)], presented when SEM (time*cultivar*height) not significant.

There was a significant interaction time x cultivar x height of defoliation for the nitrogen content, the decline being significant ($P<0.01$) with the more extended period of accumulation. The ADF fraction increased over time ($P<0.01$). In December, Grasslands Goldie defoliated at 4 cm had highest ADF content, but in January the ADF of Steadfast was higher than in Grasslands Goldie. For the final date, Steadfast defoliated at 4 cm had the highest proportion of fibre, Grasslands Goldie defoliated at 4 cm and Steadfast defoliated at 8 cm were intermediate, and Grasslands Goldie cut at 8 cm lowest (Table 5-11).

5.4.6.2 Nutritive value parameters under regular defoliation

For *local cultivars*, there were four periods of regrowth from November to April during Year 1 (see Table 5-2). A significant interaction time x cultivar x defoliation height ($P<0.01$ in all cases) was observed for OMD, nitrogen and ADF content (Table 5-12).

The OMD of San Gabriel was higher ($P<0.01$) than INIA Draco for the first period, but at the end INIA Draco defoliated at 8 cm height had higher OMD than San Gabriel defoliated at 4 or 8 cm and INIA Draco at 4 cm. There was a tendency for OMD to be lower in January and March sampling and high in December or April (Table 5-12).

The content of nitrogen increased during the last sampling (April), with no differences between cultivars or defoliation heights. Differences between cultivars were detected in December, San Gabriel having higher content than INIA Draco independently of defoliation intensity (Table 5-12).

Levels of ADF for San Gabriel increased from December to January, and then were maintained over time (Table 5-12). However, INIA Draco increased ADF levels from December to March, but reduced ADF in the April sampling. Comparatively, INIA Draco had higher ADF concentration, except in April where San Gabriel was higher for the average of defoliation heights than INIA Draco. Differences in ADF concentration caused by defoliation intensity were clearly detected in April, 4 cm being higher than 8 cm independently of cultivars.

Table 5-12. Nutritive value of *local* BFT *cultivars* managed at two defoliation heights over 40 day intervals from November 1998 to April 1999.

	Periods of accumulation				
	4 Nov-15 Dec	15 Dec-25 Jan	25 Jan-8 Mar	8 Mar-15 Apr	SEM (time*cultivar*height)
***In vitro* OMD (g/kg)**					
INIA Draco – 4 cm	638	589	585	647	
INIA Draco – 8 cm	618	614	640	717	18.5 **
San Gabriel – 4 cm	670	639	639	677	
San Gabriel – 8 cm	662	622	626	695	
SEM (n)	5.7 (4)	7.0 (4)	2.4 (4)	8.2 (4)	
Significance	**	NS	**	**	
Nitrogen (g/kg)					
INIA Draco – 4 cm	28	25	27	35	
INIA Draco – 8 cm	25	25	29	38	1.5 **
San Gabriel – 4 cm	31	27	29	37	
San Gabriel – 8 cm	30	26	27	37	
SEM (n)	0.2 (4)	0.6 (4)	0.5 (4)	0.1 (4)	
Significance	**	NS	**	NS	
ADF (g/kg)					
INIA Draco – 4 cm	315	365	405	338	
INIA Draco – 8 cm	310	367	348	249	17.8 **
San Gabriel – 4 cm	258	313	322	322	
San Gabriel – 8 cm	230	328	309	291	
SEM (n)	5.1 (4)	8.1 (4)	6.3 (4)	9.0 (4)	
Significance	**	**	**	**	

OMD, organic matter digestibility; ADF, acid detergent fibre; SEM, standard error of the mean; (n) number of observations for each treatment mean; **, P<0.01, NS; not significant.

For *introduced cultivars*, there were three periods of regrowth from December to April of Year 1 (see Table 5-2). A significant interaction time x cultivar x defoliation height was observed for OMD (P<0.01) and ADF content (P<0.01) (Table 5-13).

OMD increased over time for Steadfast (4 and 8 cm height) and Grasslands Goldie- 4 cm, but Grasslands Goldie-8 cm showed higher OMD than others in January, mantained its value in February and then declined in April. In January, there were large differences between cultivars, Steadfast having lower OMD than Grasslands Goldie. The OMD of Grasslands Goldie-8 cm fell sharply from February to April.

The ADF content of Grasslands Goldie did not differ between sampling dates, but for Steadfast-4 cm concentration was highest in February and for Steadfast-8 cm in January (Table 5-13).

Table 5-13. Nutritive value of *introduced* BFT *cultivars* managed at two defoliation height and 40 day intervals from December 1998 to April 1999.

	Periods of accumulation			SEM (time*cultivar*height) [SEM (time)]
	4 Dec-13 Jan	13 Jan-22 Feb	22 Feb-5 Apr	
***In vitro* OMD (g/kg)**				
Grasslands Goldie - 4 cm	643	677	675	
Grasslands Goldie - 8 cm	660	660	619	21.4 **
Steadfast - 4 cm	626	653	671	
Steadfast - 8 cm	605	665	657	
SEM (n)	2.6 (4)	5.4 (4)	5.7 (4)	
Significance	**	NS	**	
Nitrogen (g/kg)				
Grasslands Goldie - 4 cm	28	36	37	
Grasslands Goldie - 8 cm	28	32	32	0.4 NS
Steadfast - 4 cm	30	36	39	[0.4 **]
Steadfast - 8 cm	32	35	36	
SEM (n)	0.7 (4)	0.4 (4)	0.5 (4)	
Significance	**	**	**	
ADF (g/kg)				
Grasslands Goldie - 4 cm	319	323	317	
Grasslands Goldie - 8 cm	330	346	325	
Steadfast - 4 cm	297	342	304	22.1 **
Steadfast - 8 cm	336	305	295	
SEM (n)	5.5 (4)	6.7 (4)	7.6 (4)	
Significance	**	**	NS	

OMD, organic matter digestibility; ADF, acid detergent fibre; SEM, standard error of the mean; (n) number of observations for each treatment mean; **, P<0.01; NS, not significant; [SEM(time)], presented when SEM (time*cultivar*height) not significant

The nitrogen content increased over time (P<0.01), Steadfast having higher content than Grasslands Goldie in January. Grasslands Goldie defoliated at 8 cm tended to have lower nitrogen content during February and April than the other treatments. When significant, differences by defoliation height tended to show higher nitrogen concentration in plots defoliated at 4 cm than at 8 cm. Steadfast-8 cm had higher nitrogen concentration than Steadfast-4 cm only in January (Table 5-13).

5.4.6.3 Condensed tannin content

A preliminary analysis of tannin content was done comparing the cultivars under study at the vegetative stage in September 1999. Total CT concentrations showed differences ($P<0.05$) between cultivars, San Gabriel having the highest content, Grasslands Goldie and INIA Draco intermediate and Steadfast the lowest. Over 86% of CT was bound (Table 5-14), with the largest component being protein-bound. San Gabriel showed a tendency ($P=0.097$) to have a higher protein-bound fraction than Steadfast.

Table 5-14. Condensed tannin (CT, g/kg DM) contents of four BFT cultivars at vegetative stage in early spring.

Cultivars	Extractable CT	Protein-bound CT	Fibre-bound CT	Total CT	Bound CT (% total)
San Gabriel	4.2	25.1	1.4	30.7	86.3
INIA Draco	4.3	20.7	0.9	25.9	83.4
Grasslands Goldie	2.5	19.7	1.8	24.0	89.6
Steadfast	3.4	16.8	1.4	21.6	84.2
SEM (n)	0.58 (2)	1.48 (2)	N/A	0.97 (2)	--
Significance	NS	(0.097)		*	

*, $P<0.05$; NS, not significant; N/A, not replicates for statistical analysis; SEM, standard error of the mean; (n) number of observations for each treatment mean

5.4.7 Plant density

Plant density was evaluated at four stages of the experiment from July 1998 to March 2000. The initial sowing rate was 875 viable seeds/m^2. After 110 days from sowing (July 1998), overall plant density was 273 plants/m^2, increasing to 409 plants/m^2 in March 2000, as a consequence of further late germination during spring. The establishment showed significant differences ($P<0.01$), San Gabriel having the highest density and Grasslands Goldie the lowest (Figure 5-9). Over the following sampling dates, significant differences between cultivars ($P<0.01$ in all cases) were observed, San Gabriel and Steadfast always having the denser stands. During the second year, there was a general decline in density in all cultivars. In October 1999, plant density was 18% of that measured in March 1999, and in March 2000 the density recorded was only 44% of the plants surviving in October 1999. The final plant density was 33 plants/m^2. There was a significant time x cultivar effect ($P<0.01$) on the average of the plant density over the period (Figure 5-9), because the increase in population from July 1998-March 1999 by late germination after July 1998 was higher in Steadfast than others. A higher proportion of hard seeds was found in Steadfast than the others in germination tests done previously.

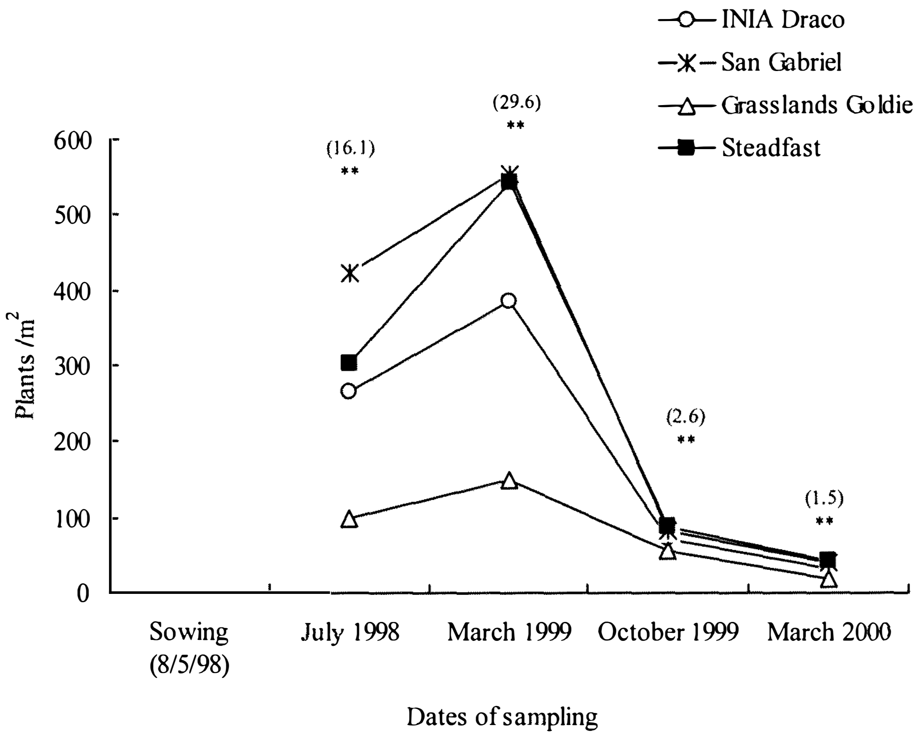


Figure 5-9. Changes in plant density of four BFT cultivars over the period July 1998 – March 2000. Numbers in brackets indicate the SEM between treatments at corresponding dates, (n=24).

5.4.8 Plant morphology

Firstly, individual plant morphology parameters were analysed at an early stage of plant development (110 days after sowing) to determine characteristics of initial growth of cultivars. After that, plant morphology parameters at the end of each cutting season were analysed individually (anova analysis) and integrated (canonical discriminant analysis). Parameters tested included the number of primary, secondary and new developing shoots per plant, root diameter, crown and root mass, and total aboveground mass per plant.

Initial development

BFT San Gabriel was the cultivar that significantly exhibited ($P<0.05$) an early development in plant height, followed by INIA Draco and Steadfast, and Grasslands Goldie the shortest (Plate 5-2 a). Also, San Gabriel had ($P<0.05$) the highest accumulation of biomass above ground, Grasslands Goldie and Steadfast having the lowest accumulation (Table 5-15). There were no differences in root length, stem number and below-ground mass per plant at this stage of development. However, the above/below-ground ratio was higher for *local cultivars* than *introduced* ones (Table 5-15). San Gabriel had the high ratio (3.9) and Grasslands Goldie the lowest (1.9).

Table 5-15. Morphological parameters of four BFT cultivars 110 days after a sowing made on May 8, 1998.

Cultivars	Plant height (cm)	Root length (cm)	Primary stems (no./plant)	Above-ground mass (g/plant)	Below-ground mass (g/plant)
San Gabriel	8	6	4	0.089	0.023
INIA Draco	5	6	4	0.062	0.020
Grasslands Goldie	3	6	5	0.035	0.018
Steadfast	5	6	5	0.044	0.020
SEM (n)	0.4 (24)	0.4 (24)	0.3 (24)	0.0056 (24)	0.0022 (24)
Significance	*	NS	NS	*	NS

*, $P<0.05$; NS, not significant; SEM, standard error of the mean; (n) number of observations for each treatment mean

The development of rhizomes was explored in the cultivar Steadfast (Plate 5-2 b). During the first year, there were no plants with rhizomes. Rhizome development was observed in 11% of a sample of 240 plants in April 1999, increasing to 17% in October 1999. Well-developed rhizomes exceeded 50 mm in length, the maximum number being 3 per plant.

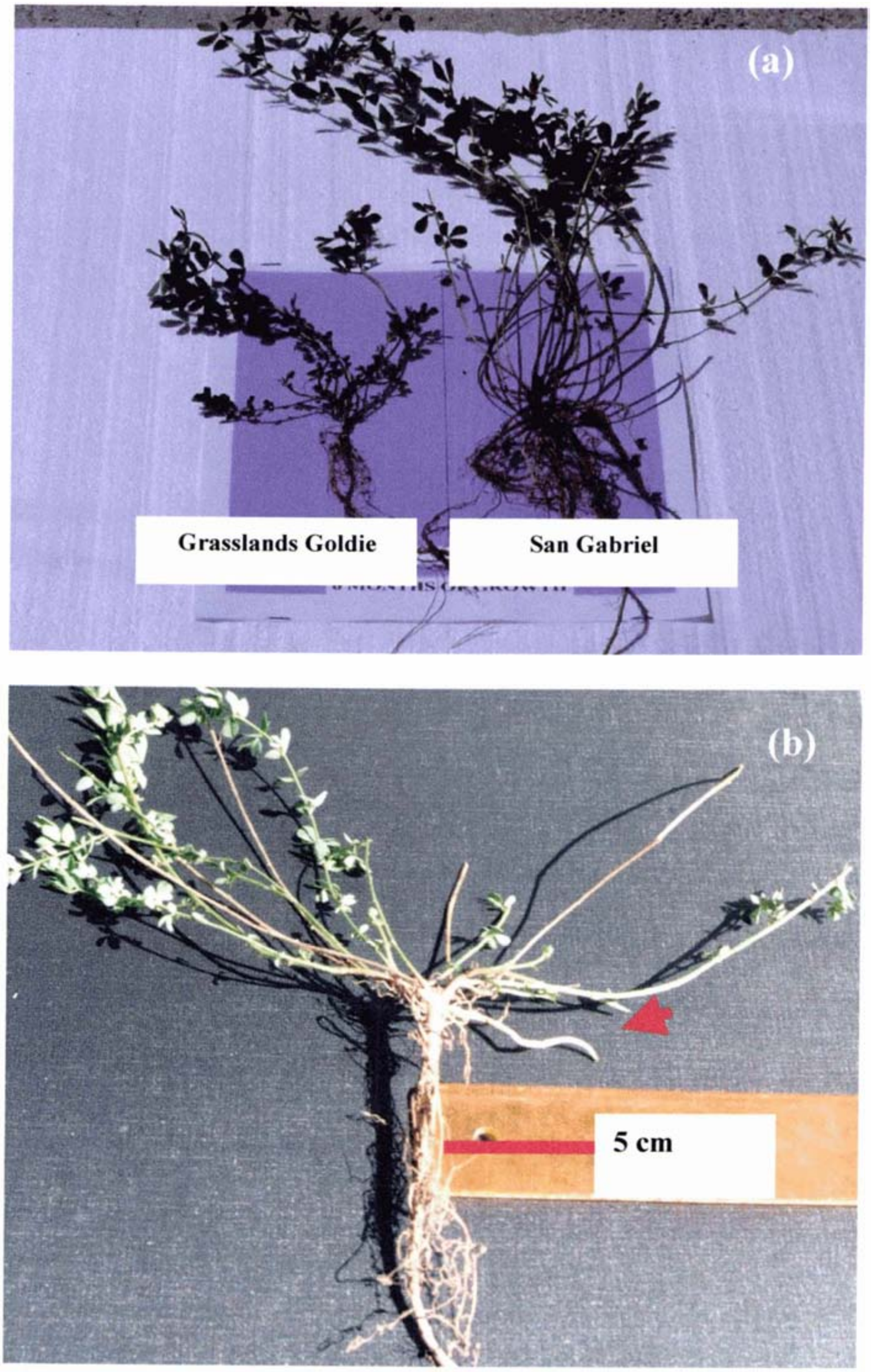


Plate 5-2. BFT plants (6 months old) of San Gabriel and Grasslands Goldie (a), and one year old plant of Steadfast showing the development of rhizomes (b).

Morphological parameters under defoliation

A significant interaction cultivar x time x height of defoliation was observed in some of morphological parameters tested at the end of each of the two years. Tables 5-16 and 5-17 show treatment values for each year, with some discussion of main effects, particularly differences between cultivars.

Year 1

A the end of the first year, the cultivar x time x height of defoliation interaction was significant (P<0.01) for root and crown weight (Table 5-16). There was a strong influence of cultivars for root weight, Grasslands Goldie showing the greatest root mass (0.12, 0.11, 0.69 and 0.28 g/plant for INIA Draco, San Gabriel, Grasslands Goldie and Steadfast respectively; SEM 0.020; P<0.01).

There were differences in crown weight between cultivars, Grasslands Goldie having the greatest and Steadfast the lowest crown mass (0.36, 0.24, 0.17 and 0.06 g/plant for INIA Draco, San Gabriel, Grasslands Goldie and Steadfast respectively; SEM 0.012; P<0.01).

Root diameter differed only between cultivars, the mean value for Grasslands Goldie being higher than the other cultivars, and also INIA Draco differed from Steadfast (6, 5, 4, and 4 mm for Grasslands Goldie, INIA Draco, San Gabriel and Steadfast; SEM 0.20; P<0.01). Also the number of primary shoots differed significantly (P<0.01) between cultivars, being higher in Grasslands Goldie (7 shoots/plant) than the others cultivars (3 to 4 shoots/plant, SEM 0.020; P<0.01).

Table 5-16. Individual plant morphology parameters in BFT cultivars affected by the intensity and timing of defoliation at the end of the first year (March 1999).

Cultivar x height x timing			Root weight (g/plant)	Crown weight (g/plant)	Root diameter (mm)	Primary shoots (no./plant)	Secondary shoots (no./plant)	New shoots (no./plant)
INIA Draco	4	1	0.17	0.33	5	4	8	4
	4	2	0.12	0.31	4	4	5	4
	4	3	0.13	0.43	5	4	7	3
	8	1	0.09	0.25	4	3	8	3
	8	2	0.12	0.54	4	4	8	3
	8	3	0.09	0.31	4	4	7	3
San Gabriel	4	1	0.12	0.23	4	3	5	2
	4	2	0.08	0.21	4	3	3	1
	4	3	0.09	0.30	4	4	5	2
	8	1	0.15	0.21	4	3	5	2
	8	2	0.13	0.22	4	3	5	2
	8	3	0.09	0.30	4	3	4	2
Grasslands Goldie	4	1	0.66	0.17	5	8	14	4
	4	2	0.88	0.23	7	7	14	2
	4	3	0.59	0.11	6	6	12	2
	8	1	0.58	0.14	5	7	8	2
	8	2	0.59	0.18	6	7	10	2
	8	3	0.83	0.21	6	8	10	2
Steadfast	4	1	0.18	0.06	3	3	6	2
	4	2	0.29	0.07	4	5	7	1
	4	3	0.22	0.04	3	3	6	1
	8	1	0.23	0.05	3	4	7	1
	8	2	0.29	0.06	4	4	7	1
	8	3	0.48	0.08	4	4	8	1
SEM			0.071	0.029	0.5	0.9	1.7	0.6
Significance (cultivar x height x timing)			**	**	NS	NS	NS	NS

**, $P<0.01$; NS, not significant; SEM, standard error of the mean; (n=4); time (1, 2 and 3), correspond approximately to vegetative, 50% flowering and advanced maturity stages respectively

There was a significant interaction cultivar x defoliation height ($P<0.05$) for the number of secondary shoots per plant. Grasslands Goldie reduced the number of shoots/plant from 13 to 9 when defoliated at 8 cm rather than 4 cm, but for other cultivars shoots/plant remained unchanged with defoliation intensity.

The number of dead secondary shoots was analysed as a measure of potential differences in the production of new growth sites between cultivars or management variables. Dead shoots per plant were affected by defoliation height ($P<0.01$), initial time of defoliation ($P<0.01$), and cultivars ($P<0.01$). Grasslands Goldie had 6 dead shoots per plant, higher than the rest of the cultivars. Plants that were first defoliated earlier had more dead shoots that plants defoliated late (5 and 3 shoots per plant respectively). Also, plants defoliated at 4 cm height had more dead shoots that those defoliated at 8 cm (4 and 3 shoots per plant respectively).

The number of new developing shoots differed significantly (P<0.01) between cultivars, INIA Draco having the highest number of new shoots per plant and Steadfast the lowest (3, 2, 2 and 1 for INIA Draco, San Gabriel, Grasslands Goldie and Steadfast respectively; SEM 0.23).

Year 2

The interaction cultivar x defoliation height x time was significant (P<0.01) for root weight, crown weight and root diameter (Table 5-17). For root and crown weight, there were significant differences between cultivars at the end of the experiment. The root weight was highest in Grasslands Goldie, and San Gabriel and in INIA Draco lowest (0.48, 0.20, 0.08 and 0.07 g/plant for Grasslands Goldie, Steadfast, INIA Draco and San Gabriel respectively; SEM 0.017; P<0.01). But INIA Draco had the biggest crown mass (0.25, 0.12, 0.10 and 0.04 g/plant for INIA Draco, Grasslands Goldie, San Gabriel and Steadfast respectively; SEM 0.007; P<0.01). Also, there were differences between cultivars in root diameter (4, 3, 3 and 2 mm for Grasslands Goldie, INIA Draco, Steadfast and San Gabriel respectively, SEM 0.1; P<0.01).

Numbers of primary shoots differed between cultivars (5, 3, 3 and 1 shoots/plant for Grasslands Goldie, Steadsfast, INIA Draco and San Gabriel respectively; SEM 0.24; P<0.01). There was a significant interaction cultivar x defoliation height (P<0.05) in the number of active secondary shoots. Grasslands Goldie declined from 9 to 6 secondary shoots per plant when defoliated at 8 cm instead of 4 cm, but the other cultivars were not affected. The number of new shoots differed between varieties, (2 new shoots/plant for INIA Draco and Grasslands Goldie and 1 shoot/plant in San Gabriel and Steadsfast; SEM 0.16; P<0.01). Also, there were differences in the number of dead secondary shoots between cultivars (P<0.01), and defoliation heights (P<0.01). Grasslands Goldie showed 4 dead secondary shoots/plant, two more than the other cultivars, and also when it was defoliated at 4 cm had more than at 8 cm height (3 and 2 shoots/plant respectively).

Table 5-17. Individual plant morphology parameters in BFT cultivars affected by the intensity and timing of defoliation at the end of the second year (March 2000).

Cultivar x height x timing			Root weight (g/plant)	Crown weight (g/plant)	Root diameter (mm)	Primary shoots (no./plant)	Secondary shoots (no./plant)	New shoots (no./plant)
INIA Draco	4	1	0.12	0.23	3	3	5	3
	4	2	0.08	0.21	3	2	4	3
	4	3	0.09	0.30	4	3	5	2
	8	1	0.07	0.18	3	2	6	2
	8	2	0.08	0.38	3	3	6	2
	8	3	0.07	0.21	3	3	5	2
San Gabriel	4	1	0.05	0.09	2	2	3	1
	4	2	0.03	0.09	2	2	2	1
	4	3	0.03	0.12	2	2	3	2
	8	1	0.07	0.09	2	2	4	2
	8	2	0.21	0.09	2	2	3	2
	8	3	0.04	0.09	2	2	3	2
Grasslands Goldie	4	1	0.46	0.12	4	6	10	3
	4	2	0.62	0.16	5	5	10	2
	4	3	0.41	0.07	4	4	8	1
	8	1	0.41	0.10	4	5	6	1
	8	2	0.42	0.12	4	5	7	1
	8	3	0.58	0.14	4	5	7	2
Steadfast	4	1	0.12	0.04	2	2	4	1
	4	2	0.20	0.05	3	4	5	1
	4	3	0.15	0.03	2	2	4	1
	8	1	0.16	0.04	2	3	5	1
	8	2	0.21	0.04	3	3	5	1
	8	3	0.33	0.06	3	3	5	1
SEM			0.041	0.018	0.3	0.6	1.2	0.4
Significance (cultivar x height x timing)			**	**	**	NS	NS	NS

**, P<0.01; NS, not significant; SEM, standard error of the mean; (n=4); time (1, 2 and 3), correspond approximately to vegetative, 50% flowering and advanced maturity stages respectively applied during 1999.

The ratio above/below-ground biomass was consistently lower for *introduced cultivars* compared with *local* ones at all stages (average being 0.9-1.0 and 1.2-1.4 for *introduced* and *local cultivars* respectively).

5.4.8.1 Integrated morphology analysis

The total canonical structure based on individual plot values (Figure 5-10 a) explained 97% of the variation for Year 1 and 92% for Year 2 (Figure 5-10 b). For canonical variate 1, the root weight and crown weight were the most significant parameters, and for canonical variate 2 the above-ground mass per plant, root diameter and primary shoots were major contributors, in both years.

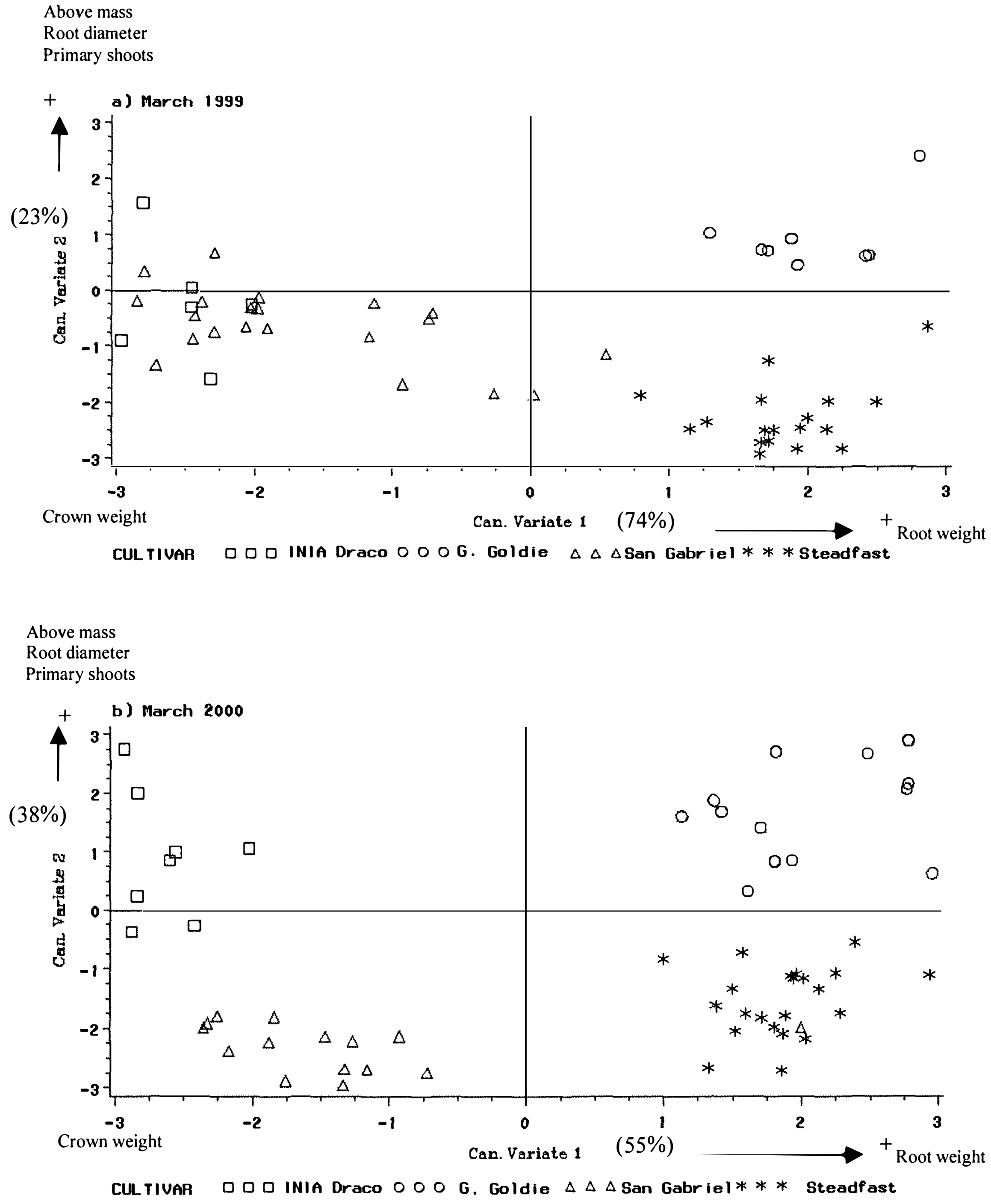


Figure 5-10. Canonical analysis for morphology parameters in the Year 1 (a) and Year 2 (b).

Plants of cultivar Grasslands Goldie had strong roots, and more primary shoots than the other cultivars in Year 1, and together with Steadfast, had higher root weight than San Gabriel and INIA Draco. At this stage, there were few contrasts between *local cultivars*.

In Year 2, the four cultivars appeared more clearly differentiated (Figure 5-10 b). Grasslands Goldie showing high root mass, root diameter, shoots per plant and above-ground mass per plant. Steadfast presented high root mass. But *local cultivars* appear more clearly differentiated, San Gabriel showed lower above-ground mass, number of primary shoots and root diameter than INIA Draco, but both cultivars exhibited lower root and crown mass than *introduced cultivars*.

5.5 DISCUSSION

The main results of this experiment are grouped for discussion, including firstly the productivity and adaptability of cultivars, secondly growth, plant type and defoliation management, thirdly the quality of BFT forage and finally the option for forage accumulation of BFT during late spring and summer.

5.5.1 Productivity and adaptation of BFT to the eastern region of Uruguay

Annual herbage production differed significantly between cultivars, being between 2.5 to 2.6 times higher for *local cultivars* than *introduced cultivars* (Figure 5-2, Table 5-4), differences occurring over all seasons. Annual production of *local cultivars* was comparable with results reported by Formoso (1993) with the cultivar San Gabriel, particularly during the first year where environmental conditions were not limiting for growth. Some differences in production can be attributed to the degree of winter activity between the two groups of cultivars; traditional cultivars used in Uruguay all being winter active. The active growth period for *introduced cultivars* occurred from November to early April. The importance of autumn-winter contribution to annual production in this study was overemphasised, because drought conditions determined a poor spring-summer contribution in Year 2. The cultivar Steadfast was developed in Missouri, USA (latitude 38° 4' N) and Grasslands Goldie in New Zealand (higher than latitude 36° S), contrasting with *local cultivars* developed in an area lower than 34° 5' latitude. High latitudes have shorter growing season, with longer daylenght and cooler temperatures (Alison and Hoveland, 1989) than those in Uruguay.

When winter active cultivars from South America were sown in New Zealand, autumn growth was associated with latitude, performance increasing when sown in low latitudes of New Zealand, North Island (Charlton *et al.*, 1978; Widupp *et al.*, 1987). In addition, frost damage in autumn and spring affected performance. Some results with alfalfa showed that when winter active cultivars were grazed in winter, spring production was lower than dormant cultivars, because of a significant reduction in root reserves (White and Lucas, 1990), demanding a differential management policy. The reported risks of inappropriate winter defoliation in lucerne may also apply to BFT, particularly in environments with cold winters. Also, the avoidance of grazing in early spring allows plants to restore root reserves, improving spring growth (White and Lucas, 1990).

Herbage production increased when plots were defoliated at 4 cm instead of 8 cm height, behaviour observed during the two years. These results agree with those of Pierre and Jackobs (1953) and Duell and Gausman (1957) comparing defoliation intensities of 2.5 versus 10 cm height and 2.5 versus 7.5 cm height respectively. Both studies reported that the magnitude of differences during the second year in favour of the 2.5 cm treatment was lower than during the first year, a consequence of a severe decline in stand density in all treatments. Pierre and Jakcobs (1953) mentioned higher production from lax defoliation at the third year of evaluation. Work by Nelson and Smith (1968) and Greub and Wedin (1971) showed a more vigorous regrowth when this originated from shoots close to crown. Additionally, it can be suggested that intervals between successive defoliation (40 days) were appropriate, as was the extended rest interval between mid-autumn to spring at the end of Year 1, for plants to replenish reserves. Intensive defoliation (4 cm) was not so severe as previously reported by Pierre and Jackobs (1953) and Duell and Gausman (1957) or results of Chapter 3 of this thesis where herbage production increased if more lax defoliation applied (2< 6 < 10 cm height).

The establishment of Grasslands Goldie was the lowest, the cultivar achieving a reduced population, that could have incidence on DM and morphological results reported. The slow establishment of *introduced cultivars* suggested that earlier sowing dates could allow a better establishment. On the other hand, early spring sowing could be preferable to late autumn sowing (J. Bologna, personal communication), because the low establishment and poor competitive capacity could produce a poor stand. However, final establishment of plants sown in spring will be associated with the absence of drought conditions in summer (Carámbula *et al.*, 1994). In the conditions of the trial, the competition from annual grasses was higher for *introduced* than *local cultivars* because of prostrate growth form of the former from autumn-mid spring. In Uruguay, *local* BFT *cultivars* are tolerant to sowing dates in late autumn, comparatively more than white clover (Carámbula *et al.*, 1994), but an important additional establishment from late germination seed was observed in this trial in spring (Figure 5-9).

5.5.2 Growth, plant type and defoliation management

Growth rates were highest between December – January for both *local* and *introduced cultivars*, then declined to autumn (Figure 5-3). *Local cultivars* registered maximum rates between 45-50 kg DM/ha/day during the first year. These results agreed with those reported by Formoso (1993), for a series of 17 experiments between 1963-1990 conducted at la Estanzuela, Uruguay working with BFT San Gabriel. For *introduced cultivars,* maximum growth rates were 21 and 28 kg DM/ha/day for Grasslands Goldie and Steadfast respectively during the first year. The extreme drought conditions registered during the second year (Figure 5-1) reduced growth rates and plant density significantly in all cultivars. This decline can not be attributed to disease incidence, because only minor symptoms were detected on roots when plant morphology measurements were done. Also the trial was established on an area of native grasses without history of BFT or other crops.

The intensity of defoliation affected growth rates, which were always greater for 4 cm than 8 cm defoliation, as was discussed for total herbage production. *Introduced cultivars* showed higher residual leaf area than *local* ones, in particular Steadfast. Residual leaf area in *local cultivars* was extremely low for regrowth, which did not increase much under lax defoliation (8 cm), regrowth being more dependent on plant reserves than residual leaf area. In addition, there were few leaves in lower strata (Figures 5-7 and 5-8) because of drought conditions. Tolerance to drought of *local cultivars* can be considered higher than introduced ones if productivity is observed, but in terms of survival of plants the decline in density during the second year was similar in all cultivars.

These findings suggest that environmental constraints and adaptability rather than post defoliation residues determined growth responses of different cultivars. The residual leaf area for *local cultivars* did not appear to exert a strong influence, despite the erect growth habit. The levels of root reserves could be determining these effects, and defoliation intervals of 40 days applied during spring-summer and permitting rest from autumn to early spring may have allowed plants to rebuild reserves for successive regrowths. When defoliation intensity was a significant factor influencing plant morphology, the number of shoots tended to be higher in plants defoliated hard (4 cm height), suggesting that plants reacted by providing more new regrowth sites. Regrowth rates declined with successive defoliation (Figure 5-6), an effect which can be attributed to the decline in root reserves levels for plants progressively defoliated.

Introduced cultivars showed a lower ratio of above/below-ground biomass compared with *local cultivars* (1.9-2.2 vs. 3.1-3.8 respectively). High ratios for above/below-ground mass found in *local cultivars* indicate that they could be sensitive to hard defoliation, more than *introduced cultivars* (Table 5-15). Although this is an arbitrary value, a more conservative strategy and reduced risk to plant survival could be attributed to plants with a low above/below-ground ratio As well, the more prostrate plant structure of *introduced cultivars* could have advantages in terms of regrowth from a higher residual leaf area. Thus, the environmental constraints may have a stronger influence on the performance of *introduced cultivars* than the morphological structure for growth, *local cultivars* showing characteristics less tolerant to hard defoliation. The development of INIA Draco cultivar, with improved conditions of crown size, shoots and leaf density (Rebuffo and Altier, 1996) was corroborated in the experiment. However, this did not result in productive advantages during the first two years of the BFT pasture. It is possible that potential advantages may be expressed later, and the disease pressure in the area of the trial was not severe enough to test the better degree of resistance of INIA Draco over San Gabriel.

The presence of rhizomes has been suggested as a useful character to increase BFT persistence particularly in those environments where diseases pressure is high (Li and Beuselinck, 1996). Only a portion of plants of BFT Steadfast expressed this character, rhizome development occurred in autumn when daylength and temperature declined. Steadfast does not appear to be a suitable option for Uruguayan environments, but the potential advantages of the presence of rhizomes could be achieved if this character is transferred to cultivars with winter activity like San Gabriel or Draco, increasing persistence without losses in productivity.

5.5.3 Forage quality

In general, the results showed a high nutritive value of herbage of all BFT cultivars evaluated, in many cases comparable to lucerne, a recognised high quality forage (Spedding and Diekmahns, 1972, cited by Frame *et al.*, 1998).

When BFT was defoliated regularly, organic matter digestibility varied from 590 to 700 g/kg for *local cultivars* and from 600 to 680 g/kg for introduced ones. The decline that is reported in other trials to end of summer (Formoso, 1993) did not occur in this trial, and OMD increased from summer to early autumn. The drought that affected the trial during summer reduced the proportion of leaves in lower strata, reducing OMD, and the increase in rainfall at the end of first summer promoted new regrowth, increasing OMD.

In other conditions, drought increased digestibility by an improved leaf:stem ratio and delaying maturity (Petersen *et al.*, 1992 cited by Frame *et al.*, 1998).

Plant nitrogen content was high, reflecting the high nutritive value reported for BFT. For *local cultivars* when managed under regular defoliation, nitrogen ranged between 25 and 38 g/kg DM and for *introduced cultivars* between 30 and 36 g/kg DM. These results can be compared with those obtained by Acuña (1995) for a series of winter active cultivars from South America (including San Gabriel) where nitrogen ranged between 26 and 32 g/kg DM, or by Formoso (1993) for San Gabriel between 29 and 37 g/kg DM. Changes in nitrogen over time were not consistent with those reported by Formoso (1993), who showed a steady and significant decline over the summer period. In the current study, drought conditions during the first summer were followed by rain in early autumn, allowing a regrowth of cultivars and consequently increasing the nitrogen content of cut forage.

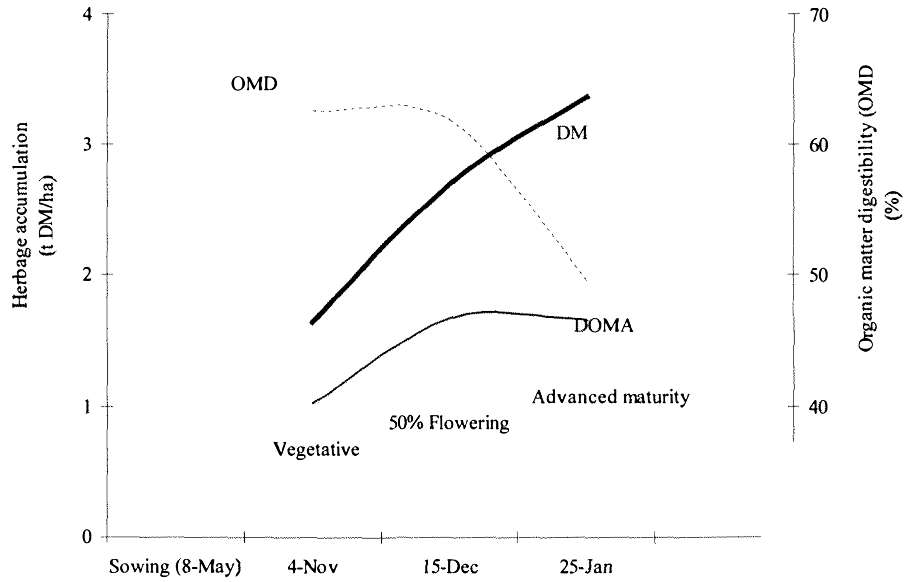
ADF content ranged from 230 to 400 g/kg DM in *local cultivars* and from 290 to 350 g/kg DM in *introduced cultivars*. Acuña (1995) reported variations between 240 and 380 g/kg DM for a group of South American accessions.

Condensed tannins results (Table 5-14) need to be carefully interpreted because of the limited number of samples involved in the analyses. The total CT content varied from 22 to 31 g/kg DM, concentrations within the recommended range of 20-40 g/kg DM that produce beneficial effects on bloat protection, intake levels and fibre digestibility compared with higher concentrations in the diet (Barry, 1989; Waghorn *et al.*, 1990). San Gabriel showed the highest content, the bound-protein fraction being the largest component.

5.5.4 Forage accumulation

The availability of quality forage in summer is crucial for many systems, especially for young animal categories that demand good forage quality. In conditions of Uruguay, C_4 grasses are the main components of native swards, thus the addition of a high quality protein bank of BFT can play a strategic role. Stockpiling BFT forage to late spring or summer is possible, but losses in forage mass and nutritive value determine the levels and periods of accumulation. In this trial, the use of an early spring growth cultivar like BFT San Gabriel showed advantages for late spring accumulation. But the rapid decline in ordigestibility in San Gabriel after flowering (Figure 5-11 a) suggests that INIA Draco could be recommended as a better cultivar for mid summer (Figure 5-11 b). In San Gabriel, periods of accumulation after 50% flowering showed no advantages in the levels of digestible organic matter accumulated (Figure 5-11 a). In general, stems decline in organic matter digestibility much faster than leaves do and the increase in stem proportion at mature stages produces a large influence on total digestibility (Buxton *et al.*, 1985). The low levels of forage accumulation of *introduced cultivars* (Table 5-6) in comparison with *local cultivars* meant that there was no advantage to be gained in digestible organic matter production from an extended period of growth in spring. However, these results relate to first year swards, and patterns of accumulation may be different in older swards.

(a) San Gabriel



(b) INIA Draco

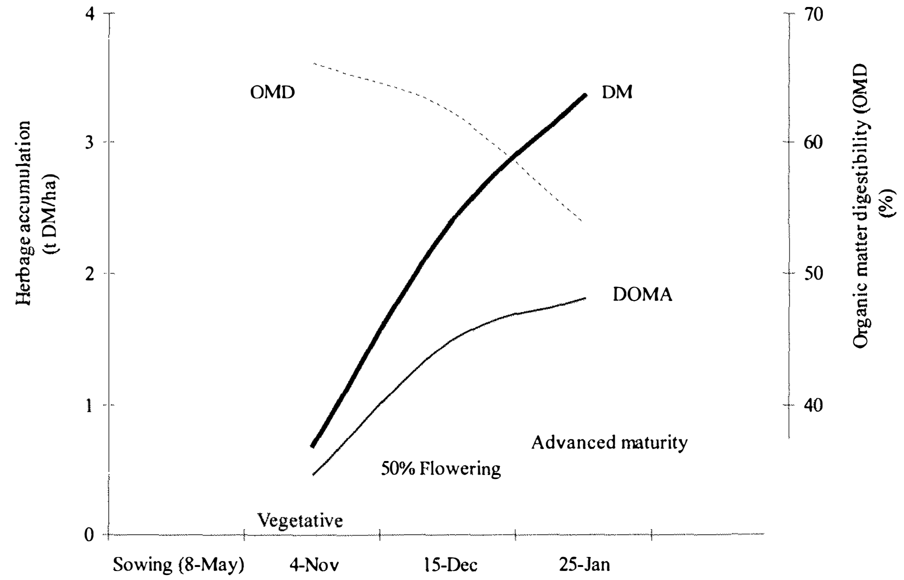


Figure 5-11. Changes in dry matter harvested (DM), digestible organic matter harvested (DOMA), and organic matter digestibility (OMD) of two BFT cultivars (a) San Gabriel and (b) INIA Draco from sowing to three physiological stages.

5.6 CONCLUSIONS

The levels of productivity achieved by tested BFT cultivars in the eastern region of Uruguay showed that *local cultivars* are more adapted and productive than introductions from USA and New Zealand, production being consistently higher over the evaluation. There were differences in the degree of winter activity of cultivars. *Local cultivars* show good growth during late autumn and winter in contrast with introductions that are winter dormant. In addition, winter active cultivars are more productive early in spring, having a more extended period of utilisation around the year. Thus, BFT introductions that can show an adequate degree of performance need to be winter active. Between *local cultivars*, San Gabriel showed an earlier spring production than INIA Draco.

Local cultivars are semi-erect to erect BFT types, in contrast with *introduced cultivars* that are semi-prostrate types. The *introduced cultivars* showed a high concentration of forage mass in low strata, with a high residual leaf area remaining for regrowth. It is suggested that the *introduced cultivars* might have had a better balance between the distribution of plant tissues above and below-ground, in contrast with *local cultivars* where the high ratio of above-ground mass to below-ground mass introducing the risk of poor persistence. This advantage on plant morphology of *introduced cultivars* could not be expressed because of the winter dormancy that these materials showed in the conditions of Uruguay.

The more intensive defoliation increased forage production during the two years, with no significant decline in plant density. These results suggest that for the case of prostrate cultivars, the remaining leaf area in lower strata could maintain regrowth rates and increase the number of new sites for regrowth by more shoots. In the case of erect types that presented a reduced leaf area independent of cutting height evaluated (4 or 8 cm), the extended periods of rest (autumn to spring) together with extended intervals (40 days) between cutting allowed plants to rebuild levels of root reserves to an effective regrowth under intense defoliation. The initial time of defoliation during the year of establishment did not affect persistence of BFT.

In general, all cultivars suffered a strong decline in plant density during the second year, which was attributed to drought conditions rather than disease incidence.

The nutritive value of BFT can be considered adequate in all cultivars in terms of digestibility, protein content, fibre and condensed tannins. When BFT is considered for stockpiling during late spring to mid summer, *local cultivars* are more suitable than introduced by the high levels of forage accumulation. Because the differences in spring growth, San Gabriel appears more adapted to accumulation in late spring and Draco for mid summer, whilst retaining high nutritive value.

5.7 REFERENCES

ACUÑA, H. (1995). Comparación de variedades de tres especies del género Lotus (*Lotus corniculatus* L., *Lotus uliginosus* Cav. y *Lotus tenuis* Wald et Kit.) en suelos de aptitud arrocera. (Comparison of three species of Lotus genus (*Lotus corniculatus* L., *Lotus uliginosus* Cav. y *Lotus tenuis* Wald et Kit.) in soils adapted for rice production). INIA Chile. *Revista Agricultura Técnica* **58**(1): 7-14. ISSN: 0365-2807.

AGRESEARCH GRASSLANDS (1995). The Grasslands range of forage and conservation plants. AgResearch Grasslands, Palmerston North. New Zealand.

ALISON, M.W. AND HOVELAND C.S. (1989). Birdsfoot trefoil management. II. Yield, quality and stand evaluation. *Agronomy Journal* **81**(5): 745-749.

ALTIER, N. (1988). Enfermedades de plantas forrajeras. (Diseases in forage plants). In Jornada de Forrajeras (mimeo). Resumen de trabajos. CIAAB, Estación Experimental La Estanzuela. Colonia, Uruguay. pp. 4-10.

BARRY, T.N. (1989). Condensed tannins: their role in ruminant protein and carbohydrate digestion and possible effects upon the rumen ecology. *The role of* *protozoa and fungi in ruminant digestion.* Eds. Nolan, J.V.; Leng, R.A. and Deneyer, D.I.. Pernanbul Books, Armidale , Australia. pp. 153-169.

BEUSELINCK, P.R. AND STEINER, J.J. (1995). Registration of ARS-2620 birdsfoot trefoil. Electronic address: /ttic/tektran/data/000006/33/ 0000063374. Html.

BUXTON, D.R., HORNSTEIN, J.S., WEDIN, W.F. AND MARTEN, G.C. (1985). Forage quality in stratified canopies of alfalfa, birdsfoot trefoil, and red clover. *Crop Science* **25**: 273-279.

CARAMBULA, M., BERMUDEZ, R. AND AYALA, W. (1996). Características del *Lotus pedunculatus* Maku. (Characteristics of *Lotus pedunculatus* Maku). Producción Animal. Serie Actividades de Difusión No. 110. INIA Treinta y Tres. pp. 33-43.

CARAMBULA, M., CARRIQUIRY, E. AND AYALA, W. (1994). Siembra de Mejoramientos en Cobertura. (Establishment of oversown pastures). In Boletín de Divulgación No. 46. 1994. INIA. ISBN: 9974-38-015-4. 20 pg. Junio 1994.

CHARLTON, J.F.L.; WILSON, E.R.L. AND ROSS, M.D.(1978). Plant introduction trials. Performance of *Lotus corniculatus* introductions as spaced plants in Manawatu. *New Zealand Journal of Experimental Agriculture* **6**:201-206.

DUELL, R.W. AND GAUSMAN, H.W. (1957). The effect of differential cutting on the yield, persistence, protein and mineral content of birdsfoot trefoil. *Agronomy Journal* **49**: 318-319.

FORMOSO, F. (1993). *Lotus corniculatus.* I. Performance forrajera y características agronómicas asociadas. (Productive performance and agronomic characteristics). *Serie Técnica* No. 37. INIA Uruguay. ISBN: 9974-556-69-4 . 20 p.

FRAME, J.; CHARLTON, J.F.L. AND LAIDLAW, A.S. (1998). Birdsfoot trefoil and greater lotus. Chapter 3 and 6. In *Temperate Forage Legumes.* CAB International, Wallingford. ISBN 0-85-199-214-5. pp. 245-271.

GARDNER, A.L.; CENTENO, G.A.; DE LUCIA, G.R. AND ALBURQUERQUE, H.E. (1968). Comportamiento de once variedades de *Lotus corniculatus* en La Estanzuela. (Behaviour of eleven cultivars of *Lotus corniculatus* at La Estanzuela). MGA. CIABB. Boletín Técnico No. 8. 23 p.

GOERING, H.K. AND VAN SOEST, P.J. (1970). Forage fiber analysis. U.S.D.A. – A.R.S. Agricultural Handbook No. 379.

GREUB, L.J. AND WEDIN, W.F. (1971). Leaf area, dry-matter accumulation, and carbohydrate reserve levels of birdsfoot trefoil as influenced by cutting height. *Crop Science* **11**: 734-738.

LI, B. AND BEUSELINCK, P.R. (1996). Plant genetic resources. Rhizomatous *Lotus corniculatus* L.: Morphology and anatomy of rhizomes. Crop Science 36: 407-411.

NELSON, C.J.; AND SMITH, D. (1968). Growth of birdsfoot trefoil and alfalfa. II. Morphological development and dry matter distribution. *Crop Science* **8**: 21-25.

NORBERG, S. (1999). Steadfast birdsfoot trefoil. The first rhizomatous trefoil cultivar. Missouri Agricultural Experiment Station. MU College of Agriculture, Food and Nutrition Resources. Hundley-Whaley Farm. Field day Report Internet address: www.aes.missouri.edu/hundwhal/fielday/page14.stm

PIERRE, J.J. AND JACKOBS, J.A. (1953). The effect of cutting treatments on birdsfoot trefoil. *Agronomy Journal* **45**: 463-468.

REBUFFO, M. and ALTIER, N. (1996). *Lotus corniculatus* L. LE 65-56 (INIA Draco, a posteriori). Boletín Interno . (*Lotus corniculatus* L. LE 65-56. Internal report). Programa Pasturas. INIA La Estanzuela, Uruguay. 6 p.

SAS INSTITUTE (1990). *SAS/STAT User's Guide*, Version 6. Cary, NC: SAS Institute.

SMITH, D. AND NELSON, C.J. (1967). Growth of birdsfoot trefoil and alfalfa. I. Responses to height and frequency of cutting. *Crop Science* **7**: 130-133.

TERRILL, T.H., ROWAN, A.M., DOUGLAS, G.B. AND BARRY, T.N. (1992). Determination of extractable and bound condensed tannin concentrations in forage plants, protein concentrate meals and cereal grains. *Journal of the Science of Food and Agriculture* **58**: 321-329.

TILLEY, J.M.A. AND TERRY, R.A. (1963). A two-stage technique for the in vitro digestion of forage crops. *Journal of the British Grasslands Society* **18**: 104-111.

WAGHORN, G.C., JONES, W.T., SHELTON, I.D. AND McNABB, W.C. (1990). Condensed tannins and the nutritive value of herbage. *Proceedings of the New Zealand Grassland Association* **51**: 171-176.

WARREN WILSON, J. (1963). Estimation of foliage denseness and foliage angle by inclined point quadrat. *Australian Journal of Botany* **11**: 95-105.

WHITE, J.G.H. AND LUCAS, W.J. (1990) Management of lucerne in the cool season. *Proceedings of the New Zealand Grassland Association* **52**: 41-43.

WIDDUP, K.H.; KEOGHAN, J.M.; RYAN, D.L. AND CHAPMAN H. (1987). Breeding *Lotus corniculatus* for South Island tussock country. *Proceedings of the New Zealand Grassland Association* **48**: 119-124.

6. FORAGE PRODUCTION AND PERSISTENCE OF BIRDSFOOT TREFOIL (*Lotus corniculatus* L.) IN MIXTURE WITH WHITE CLOVER IN RESPONSE TO DIFFERENT STRATEGIES AND INTENSITIES OF DEFOLIATION♣

♣ Part of the results in sections 6.4.5 to 6.4.7 have been accepted for publication in *Proceedings of XIX International Grassland Congress*, Sao Paulo, Brazil (Ayala *et al.*, 2001).

6.1 ABSTRACT

The effects of grazing management on herbage production, plant density, seed production, seed bank size and seedling emergence of *Lotus corniculatus* L. San Gabriel (BFT) and *Trifolium repens* cv. Zapicán (WC) oversown swards were evaluated from April 1998 to March 2000 at INIA Treinta y Tres, Uruguay, South America. A complete randomized block design with 4 replicate blocks was used, in which 4 grazing strategies (grazing all year (S1), summer spelling for seed production (S2), winter rest plus summer spelling (S3) and autumn rest plus summer spelling (S4)), were combined with two defoliation intensities (4 and 10 cm height postgrazing residuals). Plots of 110 m^2 were grazed monthly by sheep.

The herbage accumulation varied from 7.7 to 10 t DM/ha/year, legume contribution being 52-54% of total. The total herbage accumulation was improved by autumn rest (S4) during the two years and by winter rest (S3) in Year 2. Lax grazing (10 cm) increased total accumulation 18-21% over intensive grazing (4 cm). BFT contribution (2.2 to 2.3 t DM/ha/year) was affected by grazing management on Year 1 and 2, increased if swards were lax defoliated (10 cm) or managed under extended rest period, particularly an autumn rest (S4). WC contribution declined from 3.0 t DM/ha in Year 1 to 1.9 t DM/ha in Year 2, affected by extreme drought conditions. WC contribution was increased by autumn rest (S4) and lax defoliation (10 cm).

BFT plant density was unaffected by grazing management in Year 1, but it was reduced by intensive defoliation (4 cm) and by grazing strategies S1 and S2 between May and December of Year 2. BFT stand was reduced 45% during the second summer and WC growing points almost disappeared, irrespective of previous grazing management. The seed production of BFT and WC in Year 2 was 13% and 2 % of production obtained in Year 1 respectively. Summer spelling for seed production improved seed yield, especially in BFT. Severe defoliation (4 cm) reduced seed inputs drastically (46% in BFT and 64% in WC). BFT seed production was improved by winter rest (S3) reaching

11110 viable seeds/m^2. In WC, seed production was improved by autumn rest (S4), producing 11360 viable seeds/m^2. Potential seedling emergence from the soil seed bank, between June and December, was 44% and 35% in BFT and WC, respectively. Seedling emergence of BFT increased under high seed production levels (S3), and also under intensive grazing during autumn and winter of 1999. There were no effects on WC seedling emergence. Emergence from the soil seed bank was 5-13% and 4-7% in BFT and WC respectively. The soil seed bank can preserve seedling recruitment rates in the short term, but maintenance of species balance will depend on spelling management for seeding.

The results of this study indicate that grazing management of BFT/WC mixtures to increase annual herbage accumulation should consider a rest during autumn and lax grazing (10 cm). To promote seed production of legumes for future recruitment a summer seed spelling period of 2 months is recommended in years with a decline in stand density. Seedling recruitment is associated with soil seed reserves, but it has a low efficiency under sward competition, demanding additional management strategies to increase recruitment of new individuals.

Keywords: *Lotus corniculatus, Trifolium repens,* herbage production, persistence, seed production, soil seed reserves, seedling emergence.

6.2 INTRODUCTION

The oversowing of *Lotus corniculatus/Trifolium repens* mixtures (BFT/WC) in native grasslands of the eastern region of Uruguay can improve forage production from 3.4 t DM/ha to 8.6 t DM/ha, increase organic matter digestibility of forage from 520 to 650 g/kg and nitrogen content from 21 to 29 g/kg (Ayala and Carámbula, 1995). Recommended management strategies to improve winter forage availability involve an early autumn deferment for periods of 60-80 days for BFT/WC mixtures (Carámbula and Ayala, 1995). Rotational grazing, using rest periods around 50 days between successive defoliations in autumn and winter, and 30 days in spring and summer encouraged productive potential and persistence of BFT/WC mixtures. Experimental results show liveweight gains between 390 to 550 kg/ha/year for five years under mixed grazing with lambs and steers, multiplying by 6 times the levels of productivity of rangeland (Ayala and Carámbula, 1995).

The possibility to reduce establishment failures, complementary growth cycles of BFT and WC, and the potential advantages of condensed tannins of BFT to the nutritive value of mixed forages (Waghorn and Shelton, 1997) are some of the proposed advantages for the use of mixed legume pastures. However, these advantages tend to be misleading when management decisions are considered. Different fertilization requirements (Ayala and Bermúdez, 1992; Carámbula *et al.*, 1994), and different defoliation or rest period requirements (Carámbula and Ayala, 1995), make it difficult to develop management strategies in order to maintain an adequate balance between BFT and WC in sward composition.

The effects of late autumn and winter defoliation could be considered as detrimental for BFT plant survival, and the advantages of autumn deferment for subsequent forage accumulation have already been demonstrated (Ayala and Carámbula, 1995). Effects of disease incidence on BFT is an additional factor for stand reductions (Altier, 1997), making it necessary to encourage seed production to incorporate new individuals into the plant population.

In general, BFT is considered as short-lived perennial legume, and strategies to improve persistence using traditional BFT plant types are based on the recruitment of new individuals from the soil seed bank. BFT and WC seeds are small and are produced from late spring to summer. Roberts and Bodrell (1985) determined that seedlings of BFT and WC emerged mainly in spring, emergence patterns being reduced after 3 years in BFT but maintaining a more regular emergence flux in WC. BFT did not show surviving viable seeds after 5 years, contrasting with WC in which some seeds remained viable after 5 years (Roberts and Boddrell, 1985). Variations in patterns and magnitude of seedling emergence between species suggest that the balance between species in the sward can be easily altered. The final stage constitutes the seedling establishment phase, requiring that seeds be in a proper physiological state for germination, and under optimal environmental conditions to contribute positively to the dynamics of the plant population of interest (Buhler *et al.*, 1997).

Aspects related to dynamics of BFT and WC populations are being partially investigated in grasslands systems of Uruguay. Recent studies on BFT population dynamics showed that, by managing recruitment processes, BFT densities could be maintained in association with native grasses (Olmos, 1996). On the other hand, Arana and Piñeiro (1999), working with WC, determined soil seed banks between 2600 to 12000 seeds/m^2, seedling emergence of 200 seedlings/m^2, but levels of seedling establishment were limited by a low seedling survival during summer. Thus, seedling recruitment was not an effective mechanism to increase WC persistence. Also, the poor persistence of WC (3 to 4 years for conventional sowing) is associated with high death of stolons in summer, resulting from high temperatures and water deficit (Carámbula, 1977 cited by Arana and Piñeiro, 1999).

The objectives of this study were to evaluate the effect of different grazing strategies and intensities on the production and persistence of BFT in mixed grass/legume swards and quantify the effects of autumn or winter rest on production and plant survival of BFT. A more detailed understanding of nutritive value of BFT/WC mixtures is expected to elaborate more detailed grazing plans in the conditions of Uruguay. The potential of seed production of the species under grazing and the role of the soil seed bank for natural reseeding were explored. Knowledge of these processes will lead to the development of more refined management for BFT/WC mixtures in the conditions of Uruguay.

6.3 MATERIALS AND METHODS

The study was carried out at Palo a Pique Research Unit, INIA, Treinta y Tres, Uruguay (latitude 33° 54' S, longitude 54° 38' W), from April 1998 to March 2000. The pasture was a mixture of *Lotus corniculatus* cv. San Gabriel (8 kg/ha) and *Trifolium repens* cv. Zapicán (4.5 kg/ha) associated with native grasses (mainly perennial C_4 grasses) and established in May 1996 by oversowing. The fertilization history was 26 kg P/ha in 1996, 17 kg P/ha in 1997 and 13 kg P/ha in 1998 and 1999 using superphospate ($N-P_{total}-P_{soluble}-K$; 0-21-23-0). The soil type was a fine, thermic, mixed, vertic Argiudoll (ARS-USDA classification, Fernando García, personal communication) with moderate fertility, and the soil characteristics reported in Table 6-1. During summer 1998, pasture was rested for seed production and was grazed in early March with cattle. After grazing, the experimental area was cut uniformly at 10 cm height with a lawn mower.

Table 6-1. Soil nutrients levels at experimental site in Palo a Pique Research Unit. (Source: Soils Laboratory, INIA La Estanzuela, Uruguay).

Soil testing dates	Soil depth (cm)	pH (H_2O)	Organic carbon (%)	P (Bray I) (µg P/g soil)	K (me/100g soil)
April 1998	0 - 7.5	5.4	2.9	6.6	0.4
	7.5 – 15	5.6	1.5	1.7	0.1
March 1999	0 - 7.5	5.3	2.6	6.1	0.3
	7.5 – 15	5.6	1.3	2.2	0.1

In April 1998, four defoliation strategies and two defoliation intensities were combined in a complete randomised block design with four replicates in plots of 110 m^2.

Defoliation strategies (Figure 6-1) were grazing all year (S1), summer spelling for seed production (S2), winter rest plus summer spelling (S3) and autumn rest plus summer spelling (S4). Defoliation intensities were specified as post-grazing sward height of 4 or 10 cm.

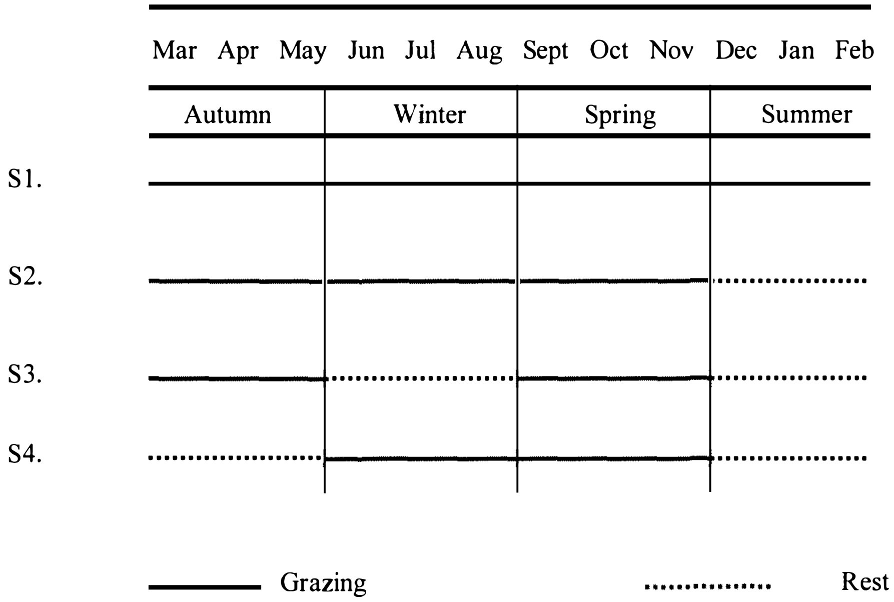


Figure 6-1. Description of grazing strategies applied on the birdsfoot trefoil-white clover mixture from April 1998 to March 2000. Each strategy was defoliated at 4 and 10 cm height.

During grazing cycles, plots were grazed monthly using mature Corriedale ewes (15-20/paddock) for short periods of time (< 12 hours). Animals were removed overnight to reduce faeces accumulation and rest areas. Sward height pre and post-grazing was controlled by taking 50 readings/plot (Plate 6-1). Because sheep tend to graze hard around fences, no samples were taken inside a boundary of one meter around plots. Initial sward heights were established on 1 April 1998 using a lawn mower, and plots were mown again in March 1999 after grazing to trim rejected mature grasses (mainly *Paspalum dilatatum* and *Sporobolus indicus*).

When specifically referred, the seasons over the year were defined as autumn (from March to May), winter (from June to August), spring (from September to November) and summer (December to February).

6.3.1 Measurements

Forage production and quality

Pre-grazing and post-grazing herbage mass were measured by cutting to ground level with an electric shearing hand-piece two 500 x 200 mm quadrats per plot. Samples were washed to remove residues. Those quadrat areas were marked to avoid repeat sampling. Four values of sward height in each cut quadrat were recorded using a ruler.

The paired forage samples were individually weighed and then bulked for analysis. A subsample was oven-dried at 60 ^{o}C for 48 hours for dry matter determination, and another subsample was separated by hand into components (birdsfoot trefoil, white clover, grasses and weeds), which were then oven-dried. The dead material was included in each respective category with the green material.

Forage quality assessment of pre-grazing herbage mass (bulk samples) included in vitro organic matter digestibility (OMD) (Tilley and Terry, 1963), nitrogen by Micro-Kjeldahl and acid detergent fibre (ADF) (Goering and Van Soest, 1970).

Plant population

The plant population of BFT was recorded in May, September and December each year, and at the end of the evaluation (March 2000). Counts were made in two fixed quadrats per plot (1 x 0.1 m each). All BFT plants with 5 or more true leaves were recorded as adults or established plants. On two occasions (December 1999 and March 2000) growing points of white clover were monitored in each quadrat.

Plant morphology

In May and December of 1998 and 1999, five BFT plants were dug out to 250 mm depth in each plot, and manually washed to remove soil and other components. In each plant, the number of primary shoots and secondary shoots was recorded (see Chapter 3 for definitions). The diameter of the main root was measured at a section cut 10 mm below the level of insertion of primary shoots. Crown and roots (> 2 mm diameter) were oven-dried at 60 °C for 48 hours for dry weight.

Seed production

Seed production was monitored from December 1998 to February 1999 (Year 1998-1999) and from December 1999 to February 2000 (Year 1999-2000), in two fixed quadrats per plot of 0.1 m^2 each. BFT mature pods and WC mature heads were collected regularly over the season, dried and threshed. Measurements included number of inflorescences, seed yield, seed number and 1000 seed weight and germination tests (ISTA, 1985).

Soil seed reserves

In April 1998, March 1999 and March 2000, six soil cores per plot (22.9 cm^2 x 5 cm depth) were taken randomly, and legume seeds recovered by a process that included hand crumbling, sieving, air flow and addition of ethylene-cloride (C_2Cl_4) (Prestes, 1995; Appendix 2). Seeds were then hand sorted, weighed, counted and germination tests performed (ISTA, 1985). Preliminary studies showed that in samples deeper than 5 cm the seed density was extremely low and the possibility of germination negligible.

Seedling emergence

From June to December 1998, potential seedling emergence was checked regularly from four soil cores collected per plot (22.9 cm^2 x 5 cm depth) and placed in a complete randomised design in an adjacent area maintained free of ground cover. Germinated seeds were removed weekly. From March to August 1999, seedling emergence was also examined weekly in one fixed quadrat (0.1 m^2) per plot.

6.3.2 Statistical analysis

All data presented were evaluated using the analysis of variance of the General Linear Model (GLM) of SAS (SAS Institute Inc., 1990) for a balanced randomised complete block design. Forage production and quality data were grouped by season for analysis and presentation. Plant population, plant morphology and seed production patterns were analysed using a 'repeated measures analysis'.

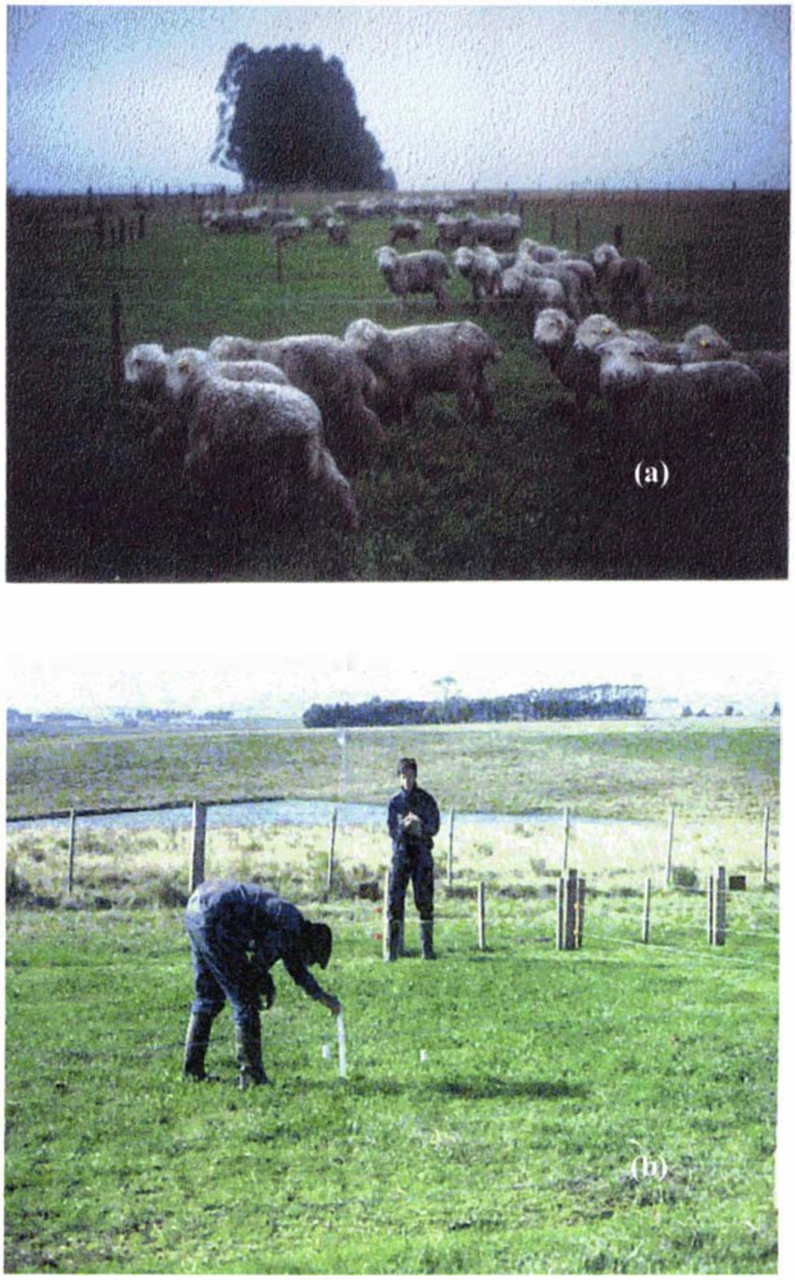


Plate 6-1. (a) Sheep grazing birdsfoot trefoil/white clover oversown mixture in the experimental site at Palo a Pique Research Station, Treinta y Tres, Uruguay. (b) Postgrazing sward heights were recorded to maintain contrasting defoliation intensities of 4 and 10 cm.

6.4 RESULTS

6.4.1 Climate conditions during experiment

Climate conditions during the experiment showed contrasting patterns for the two years, particularly in rainfall occurrence, the year 1999-2000 being extremely dry. Monthly rainfall, soil temperatures and soil water balance of the experimental site are reported in Chapter 5 of this thesis (see Table 5-3 and Figure 5-1).

6.4.2 Herbage production and quality

The herbage measurements were done between May 1998 and March 2000. Year 1 included the herbage production of 11 months from May 1998 to March 1999, and Year 2 included herbage production between March 1999 and March 2000. Pre and post-grazing sward heights, herbage accumulation (defined as $\sum(pregrazing_{(n+1)} - postgrazing_{(n)})$) and herbage quality parameters are reported by season.

6.4.2.1 Pregrazing and postgrazing sward height

Pregrazing sward heights varied between and across seasons, reflecting differences in growth or accumulation periods (Table 6-2). Postgrazing sward heights showed that contrasts between defoliation intensities were maintained over the experimental period (Table 6-2).

Table 6-2. Seasonal average heights (cm) of pre and post-grazing herbage (showed as pre and post) and standard deviation values in brackets for all treatment combinations.

		S1- 4 CM	S1-10 CM	S2-4 CM	S2-10 CM	S3-4 CM	S3-10 CM	S4-4 CM	S4-10 CM
Autumn 1998	Pre	10 (3.1)	14 (3.0)	9 (2.8)	13 (2.8)	10 (3.2)	14 (3.0)	21 (4.1)	22 (4.0)
	Post	4 (1.5)	10 (2.0)	4 (1.6)	10 (2.0)	4 (1.4)	10 (1.9)	4 (2.1)	9 (1.9)
Winter 1998	Pre	9 (4.3)	13 (4.8)	9 (4.1)	12 (4.4)	10 (6.0)	17 (7.4)	10 (3.9)	13 (5.0)
	Post	4 (1.3)	9 (2.1)	4 (1.3)	9 (1.9)	4 (1.1)	10 (1.6)	4 (1.5)	9 (1.9)
Spring 1998	Pre	10 (5.1)	14 (5.2)	10 (5.7)	15 (6.8)	10 (4.8)	16 (5.7)	11 (4.4)	15 (5.5)
	Post	5 (2.5)	10 (2.7)	5 (3.2)	10 (2.7)	5 (2.0)	10 (2.4)	5 (2.3)	10 (2.9)
Summer 1999	Pre	11 (4.1)	13 (4.9)	23 (5.1)	23 (5.2)	21 (4.9)	23 (5.8)	23 (6.2)	24 (7.1)
	Post	5 (2.6)	11 (3.3)	4 (0.9)	10 (1.1)	4 (0.8)	10 (0.9)	4 (1.0)	10 (0.9)
Autumn 1999	Pre	8 (2.5)	13 (3.1)	9 (2.5)	14 (2.9)	8 (2.2)	14 (2.9)	11 (2.8)	15 (3.6)
	Post	5 (1.3)	10 (1.8)	5 (0.9)	10 (4.2)	5 (1.0)	10 (1.6)	4 (1.6)	10 (1.8)
Winter 1999	Pre	7 (2.3)	12 (4.4)	7 (2.0)	12 (4.4)	13 (4.1)	19 (5.2)	8 (3.0)	14 (5.8)
	Post	4 (1.2)	10 (2.3)	4 (1.3)	10 (1.2)	5 (1.1)	10 (1.2)	4 (1.3)	10 (2.0)
Spring 1999	Pre	9 (2.7)	15 (5.3)	8 (2.8)	15 (3.8)	10 (2.7)	16 (6.1)	10 (2.7)	15 (3.9)
	Post	4 (1.1)	10 (1.5)	4 (1.4)	10 (1.4)	4 (1.2)	10 (1.3)	4 (1.3)	10 (1.5)
Summer 2000	Pre	7 (2.1)	13 (2.6)	15 (5.6)	17 (6.0)	14 (5.1)	15 (4.9)	13 (5.1)	16 (6.0)
	Post	4 (1.2)	10 (1.7)	4 (1.0)	10 (3.6)	4 (2.5)	10 (1.2)	4 (1.2)	10 (2.3)

Each height value reported includes the average of 50 records/plot in four blocks repeated three times over the season, excepting periods of rest or spelling when information is reported once at the end of the season. Autumn 1998 included only two measurements because evaluation started in May.

6.4.2.2 Annual and seasonal herbage accumulation

The average of herbage accumulation in Year 1 reached 10015 kg DM/ha, BFT and WC contributing 22% and 30% of the total, respectively. Grasses were 44% of the total, and *Paspalum notatum, Paspalum dilatatum, Sporobolus indicus, Chloris spp., Vulpia australis, Gaudinia fragilis and Lolium multiflorum* were the main contributing species. Weeds were less than 5%, with *Eringium horridum* the most frequent. The general average of seasonal accumulation was 18, 13, 41 and 28% for autumn, winter, spring and summer respectively. The autumn contribution only included two months (April and May) because the trial started in April 1998. There were significant differences in total herbage accumulation in Year 1, between strategies (P<0.01) and intensities of defoliation (P<0.01), but there were no interaction effects between main factors (Table 6-3). The S4 strategy accumulated more forage than the others, and S1 was the least productive. The lax grazed plots (10 cm) produced 18% more forage than the intensively grazed plots (4 cm).

Table 6-3. Annual and seasonal herbage accumulation (kg DM/ha) of an oversown birdsfoot trefoil/white clover mixture managed under different strategies and intensities of grazing during the third and fourth year after establishment.

	Year 1					Year 2				
	Autumn	Winter	Spring	Summer	Total	Autumn	Winter	Spring	Summer	Total
S1 - 4 cm	1575	920	2505	2495	7505	1635	1040	2270	620	5570
S1 – 10 cm	1555	1225	2655	3585	9025	1625	1320	3105	875	6920
S2 - 4 cm	1625	1285	3075	2380	8360	2020	1355	2990	635	7005
S2 – 10 cm	1960	1570	3475	3025	10025	2475	1930	3670	565	8645
S3 - 4 cm	1585	1055	4465	2330	9430	2025	1475	3245	545	7285
S3 – 10 cm	1985	1080	6095	3175	12345	2490	1755	4480	535	9265
S4 - 4 cm	1780	1565	4545	2825	10715	2160	1800	3245	505	7710
S4 – 10 cm	2155	1805	6240	2512	12715	2170	1765	4720	575	9230
SEM (S x I)	102	102	163	198	270	155	81	103	80	195
Signif. (S x I)	NS	NS	**	**	NS	NS	**	**	NS	NS
Signif. (S)	NS	**	**	NS	**	**	**	**	NS	**
Signif. (I)	**	NS	**	**	**	NS	*	**	NS	**

**, P<0.01; NS, not significant, SEM (S x I), standard error of the mean of the interaction strategy x intensity of defoliation; n=4; Signif., significance; S, strategy of defoliation; I, intensity of defoliation

Autumn herbage accumulation in Year 1 was affected only by grazing intensity (P<0.01, Table 6-3), production being 14% higher in plots grazed at 10 cm height. In contrast, herbage accumulation during winter of Year 1 was affected only by grazing strategy, the autumn rest strategy (S4) being the most productive. A significant interaction strategy x intensity of defoliation was observed (P<0.01) in spring of Year 1 (Table 6-3). Strategies S3 and S4 showed an increase in accumulation when intensity of grazing changed from 4 to 10 cm but accumulation remained unchanged in S1 and S2. In summer of Year 1, there was an interaction of strategy x intensity (P<0.01, Table 6-3), strategies increasing accumulation if lax defoliated with the exception of S4 that was not affected by defoliation intensity.

In Year 2, the average of herbage accumulation was 7705 kg DM/ha. The yield was composed of 25, 29, 40 and 6% of BFT, WC, grasses and weeds respectively. There was a decline in WC and grasses production that affected total accumulation. In contrast, BFT accumulation was similar to that in Year 1, reflecting a high tolerance of BFT to drought. Herbage accumulation in Year 2 was affected by strategies (P<0.01) and intensities of defoliation (P<0.01), as occurred in Year 1 (Table 6-3). The general average of seasonal distribution was 27, 20, 45 and 7% for autumn, winter, spring and summer respectively. There were no interaction effects for total production (Table 6-3). Herbage accumulation was improved by rest period independently of the timing, ranking being S3 and S4> S2>S1. The lax grazing (10 cm) remained more productive (21% higher) than the intensive grazing (4 cm) as was observed in Year 1.

The herbage accumulation in autumn of Year 2 was significantly affected ($P<0.01$) by grazing strategies, the more intensive system (S1) reduced accumulation by 27% when compared with strategies that included any kind of rest during the year (S2, S3 or S4). Winter accumulation was affected significantly by the interaction strategy x intensity ($P<0.01$, Table 6-3), and herbage accumulation increased under lax defoliation for S1, S2 and S3 strategies, but S4 was not affected by defoliation intensity. In spring, a significant interaction was observed for strategy x intensity of defoliation ($P<0.01$, Table 6-3). Plots grazed laxly (10 cm) had an accumulation 26% higher than those defoliated more intensively, and strategies that had more extended rest were more productive (S3 and S4>S2>S1). There were no significant treatment effects on summer production (Table 6-3).

6.4.2.3 Species contribution

The seasonal and annual accumulation of BFT, WC and grasses during the two years is reported in Tables 6-4 to 6-6. Weeds made a minor contribution to total herbage accumulation between 5-6% during the two years and data are not presented.

Birdsfoot trefoil

BFT accumulation was similar in the two years (2165 and 2270 kg DM/ha for Year 1 and Year 2 respectively). Consistently during the two years, there was a significant interaction strategy x intensity of defoliation on total BFT accumulation ($P<0.01$, Table 6-4). In Year 1, herbage accumulation increased if BFT grazed at 10 cm height instead of 4 cm in all systems excepting S3. In Year 2, herbage accumulation was improved 30% if swards were grazed at 10 cm rather than at 4 cm height, and also by those grazing strategies that had more extended rest during the year. Autumn rest was more productive than winter rest (S4>S3>S2>S1).

In Year 1, there was a significant interaction strategy x intensity of defoliation in all seasons (P<0.01 in all cases, Table 6-4). In autumn, there was no improved accumulation by lax defoliation in S1, in contrast with other strategies. The same pattern was registered in winter for S3, in spring for S2 and in summer for S4 (Table 6-4). Summer contribution was improved in S1 over the other strategies, because the effect of seeding rest in the other strategies determined losses of BFT herbage.

Table 6-4. Annual and seasonal herbage accumulation of birdsfoot trefoil (kg DM/ha) in a birdsfoot trefoil/white clover mixture managed under different strategies and intensities of grazing during the third and fourth year after establishment.

	Year 1					Year 2				
	Autumn	Winter	Spring	Summer	Total	Autumn	Winter	Spring	Summer	Total
S1 - 4 cm	375	90	380	1035	1885	375	85	265	135	860
S1 - 10 cm	240	310	560	1695	2805	495	285	755	285	1820
S2 - 4 cm	415	160	440	440	1450	565	275	480	155	1475
S2 - 10 cm	600	455	590	605	2245	1000	485	1115	205	2810
S3 - 4 cm	570	300	745	895	2505	925	665	785	120	2490
S3 - 10 cm	570	145	695	975	2385	1205	350	1095	160	2810
S4 - 4 cm	480	300	470	570	1820	940	590	905	220	2660
S4 - 10 cm	575	420	660	565	2220	1110	440	1535	155	3240
SEM (S x I)	32	20	27	59	63	88	22	44	29	96
Signif. (S x I)	**	**	**	**	**	NS	**	**	**	**
Signif. (S)	NS	NS	NS	**	NS	**	**	**	NS	**
Signif. (I)	NS	NS	NS	NS	NS	**	NS	**	NS	**

**, P<0.01; NS, not significant; SEM (S x I), standard error of the mean of the interaction strategy x intensity of defoliation; n=4; Signif., significance; S, strategy of defoliation; I, intensity of defoliation

During autumn of Year 2, main effects were significant (P<0.01, in both cases), accumulation improved when swards were defoliated laxly or received an extended rest in the previous year. During winter, the interaction strategy x intensity was significant (P<0.01), with strategies increasing accumulation if lax defoliated, with the exception of S4 (Table 6-4).

In spring the interaction strategy x intensity of defoliation was significant (P<0.01, Table 6-4), and main effects were also significant (P<0.01). Accumulation increased 46% if lax defoliated (10 cm) over the intensive defoliation (4 cm), and in less intensive grazed strategies (S4>S3>S2>S1). In summer, there was a substantial reduction in accumulation by drought. The interaction strategy x intensity was significant (P<0.01), the S1 treatment increasing accumulation if lax grazed, but the other strategies did not differ if defoliated at 4 or 10 cm height.

White clover

The total accumulation of WC was higher in Year 1 than in Year 2 (2970 and 1925 kg DM/ha for Year 1 and Year 2 respectively). There was a significant interaction strategy x intensity of defoliation in the two years (P<0.01, Table 6-5). General patterns over time showed that the intensity was a significant variable from the first spring to the end of the experiment, and consistently there were differences between systems in spring and summer of each year. In Year 1, strategies increased total accumulation if defoliated at 10 cm rather than at 4 cm, but S2 accumulation was not affected by defoliation intensity. Effects of main factors were also significant, showing an increase of 31% in WC accumulation if grazed at 10 cm height. The autumn rest improved WC contribution over the other treatments, the contribution being reduced by the more extended grazing over the year.

Table 6-5. Annual and seasonal herbage accumulation of white clover (kg DM/ha) in a birdsfoot trefoil/white clover mixture managed under different strategies and intensities of grazing during the third and fourth year after establishment.

	Year 1					Year 2				
	Autumn	Winter	Spring	Summer	Total	Autumn	Winter	Spring	Summer	Total
S1 - 4 cm	275	350	680	315	1620	315	415	615	0	1345
S1 - 10 cm	380	450	825	460	2110	395	505	725	30	1650
S2 - 4 cm	440	645	1000	140	2220	420	440	705	0	1565
S2 - 10 cm	440	590	985	245	2260	485	860	1110	0	2460
S3 - 4 cm	420	550	1525	135	2630	190	320	780	0	1290
S3 - 10 cm	550	515	3280	280	4630	575	705	1500	0	2785
S4 - 4 cm	660	530	2045	125	3400	380	545	625	0	1545
S4 - 10 cm	800	790	3030	295	4920	530	760	1470	0	2760
SEM (S x I)	64	72	70	26	121	28	38	45	2	58
Signif. (S x I)	NS	NS	**	NS	**	**	**	**	**	**
Signif. (S)	**	NS	**	**	**	NS	NS	*	**	**
Signif. (I)	NS	NS	**	**	**	**	**	**	**	**

**, P<0.01; NS, not significant; SEM (S x I), standard error of the mean of the interaction strategy x intensity of defoliation; n=4; Signif., significance; S, strategy of defoliation; I, intensity of defoliation

In autumn of Year 1, there was an effect of grazing strategies on WC accumulation ($P<0.01$), the autumn rest increased WC contribution over the other strategies. In winter there were no treatment effects on WC accumulation. During spring, the interaction strategy x intensity was significant ($P<0.01$, Table 6-5). The accumulation of WC in S1 and S2 strategies was not affected by grazing intensity, but in S3 and S4 increased if plots were grazed at 10 cm height. In summer of Year 1, there were significant effects of the strategy and intensity of defoliation ($P<0.01$ in both cases). Accumulation of WC was higher in the treatment S1 compared with those that received a rest for spelling, and WC increased when plots were defoliated at 10 cm height.

In Year 2, the interaction strategy x intensity was significant in autumn ($P<0.01$, Table 6-6) and the effect of intensity was also significant ($P<0.01$). Strategies S1 and S2 did not differ in accumulation by effect of defoliation intensity, but S3 and S4 increased accumulation if defoliated at 10 cm rather than at 4 cm height. During winter, the interaction strategy x intensity was significant ($P<0.01$, Table 6-6). Strategies S2, S3 and S4 increased accumulation when grazed at 10 cm rather than 4 cm, but S1 was not affected by changes in defoliation intensity. The tendency described in winter was observed in spring (Table 6-5). In summer of Year 2, there was a decline in WC contribution, only a minimum presence of WC was observed in S1-10 cm during early sampling in summer (Table 6-5).

Grasses

Grasses were less affected than legumes by grazing management. Total herbage accumulation from grasses in Year 1 was only affected by grazing strategies (SEM 133, $P<0.05$), ranking being S4>S2, S3>S1. In Year 2, there were no significant effects on total production (Table 6-6).

Table 6-6. Annual and seasonal herbage accumulation of grasses (kg DM/ha) in a birdsfoot trefoil/white clover mixture managed under different strategies and intensities of grazing during the third and fourth year after establishment.

	Year 1					Year 2				
	Autumn	Winter	Spring	Summer	Total	Autumn	Winter	Spring	Summer	Total
S1 - 4 cm	895	460	1335	1015	3705	855	500	1305	440	3100
S1 - 10 cm	905	430	1205	1295	3840	635	465	1560	505	3170
S2 - 4 cm	705	435	1510	1645	4295	815	485	1505	405	3215
S2 - 10 cm	845	395	1770	2020	5035	745	370	1365	280	2760
S3 - 4 cm	530	180	2030	1200	3940	620	435	1490	380	2920
S3 - 10 cm	755	385	1970	1815	4925	515	665	1695	345	3220
S4 - 4 cm	545	650	1820	2000	5015	795	485	1635	250	3165
S4 - 10 cm	685	480	2465	1590	5225	470	415	1565	395	2845
SEM (S x I)	72	68	109	146	188	106	58	103	71	145
Signif. (S x I)	NS	NS	**	*	NS	NS	*	NS	NS	NS
Signif. (S)	NS	NS	*	NS	*	NS	NS	NS	NS	NS
Signif. (I)	NS	NS	NS	NS	NS	*	NS	NS	NS	NS

**, $P<0.01$; *, $P<0.05$; NS, not significant; SEM (S x I), standard error of the mean of the interaction strategy x intensity of defoliation; n=4; Signif., significance; S, strategy of defoliation; I, intensity of defoliation

In spring and summer of Year 1 there was a significant interaction strategy x intensity of grazing (Table 6-6). In spring, only S4 increased accumulation if defoliated at 10 cm rather than at 4 cm height (Table 6-6). During summer, accumulation of S4 decreased if lax defoliated (10 cm), in contrast with other strategies that increased accumulation under more lax defoliation. However, the results need to be carefully interpreted, because the accumulation included three months of rest, with the implications of maturity, and death of herbage.

6.4.2.4 Herbage quality

The *in vitro* organic matter digestibility (OMD), nitrogen content and acid detergent fibre (ADF) content were analysed by season in pre-grazing herbage samples. During Year 1, there were significant differences in OMD (SEM 5.5, 2.8, 2.5 and 7.3 for autumn, winter, spring and summer respectively, $P<0.01$ in all seasons) between grazing strategies. The highest OMD levels in autumn were observed for S4, in winter for S3, in spring for S4 and in summer for S1. The intensity of defoliation affected OMD during autumn (SEM 3.9, $P<0.01$), winter (SEM 6.2, $P<0.05$) and spring (SEM 1.8, $P<0.01$), but not in summer. The OMD when significant, was always higher in 4 cm than 10 cm height. There were no interaction effects strategy x intensity of defoliation for OMD in Year 1 (Table 6-7).

In Year 2, a significant interaction strategy x intensity of defoliation was observed for OMD in winter and spring (P<0.01 in both cases). In the two seasons OMD increased if plots were grazed intensively, with the exception of S4 in winter and S3 in spring, where OMD was unaffected by changes in defoliation intensity. In autumn, there was a strategy of defoliation effect (P<0.01), S4 having higher OMD than the other strategies, as was observed in the previous year. During summer there were no significant effects (Table 6-7).

Table 6-7. Seasonal averages of *in vitro* organic matter digestibility (g/kg DM) of a birdsfoot trefoil/white clover oversown mixture, during two years.

	Year 1				Year 2			
	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer
S1 - 4 cm	580	679	534	471	566	666	510	497
S1 - 10 cm	552	666	519	443	561	633	472	496
S2 - 4 cm	574	673	554	372	559	661	528	509
S2 - 10 cm	554	672	524	336	556	613	507	512
S3 - 4 cm	576	700	559	344	550	638	502	504
S3 - 10 cm	574	693	532	353	556	656	511	496
S4 - 4 cm	634	677	576	370	617	626	509	506
S4 - 10 cm	591	661	541	376	607	635	531	503
SEM (S x I)	7.8	5.0	3.6	10.4	6.3	4.0	6.1	8.0
Signif. (S x I)	NS	NS	NS	NS	NS	**	**	NS
Signif. (S)	**	**	**	**	**	**	**	NS
Signif. (I)	**	*	**	NS	NS	**	NS	NS

**, P<0.01; *, P<0.05; NS, not significant; SEM (S x I), standard error of the mean of the interaction strategy x intensity of defoliation; n=4; Signif., significance; S, strategy of defoliation; I, intensity of defoliation

The nitrogen content in Year 1 was affected by grazing strategies in spring (SEM 0.4, P<0.05) and in summer (SEM 0.3, P<0.01). In spring, the nitrogen content in S4 was higher than in S3 and S1. In summer, treatment S1 showed the highest nitrogen content. In autumn, there was a significant effect of defoliation intensity (SEM 0.3, P<0.05), plots defoliated at 4 cm had higher nitrogen content than those defoliated at 10 cm height. In winter, there were no differences in nitrogen content between treatments (Table 6-8).

Table 6-8. Seasonal averages of nitrogen content (g/kg DM) of a birdsfoot trefoil/white clover oversown mixture, during two years.

	Year 1				Year 2			
	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer
S1 - 4 cm	30	35	27	22	21	25	14	14
S1 - 10 cm	30	35	28	21	22	25	14	14
S2 - 4 cm	31	35	29	16	22	26	14	15
S2 - 10 cm	30	29	28	15	22	24	14	14
S3 - 4 cm	30	32	28	14	22	23	16	14
S3 - 10 cm	30	33	28	15	22	24	15	14
S4 - 4 cm	32	36	30	13	27	27	15	14
S4 - 10 cm	29	35	29	14	27	28	14	14
SEM (S x I)	0.7	1.9	0.6	0.4	0.6	0.6	0.5	0.6
Signif. (S x I)	NS	NS	NS	NS	NS	NS	NS	NS
Signif. (S)	NS	NS	*	**	**	**	NS	NS
Signif. (I)	*	NS	NS	NS	NS	NS	NS	NS

**, $P<0.01$; *, $P<0.05$; NS, not significant; SEM (S x I), standard error of the mean of the interaction strategy x intensity of defoliation; n=4; Signif., significance; S, strategy of defoliation; I, intensity of defoliation

In Year 2, nitrogen content was affected by strategies of defoliation during autumn (SEM 4.4, $P<0.01$) and winter (SEM 2.9, $P<0.01$). In autumn, the S4 treatment had the highest Nitrogen content. In winter, S3 and S1 had higher nitrogen content than S2 and S4.

During Year 1, the ADF content did not differ between treatments in autumn, but it was affected by defoliation intensity in winter ($P<0.01$, SEM 2.1), the higher fibre content being observed in plots lax defoliated (10 cm). In spring, there was a significant interaction strategy x intensity of defoliation ($P<0.05$, Table 6-9), the S3 strategy increased ADF if defoliated at 10 cm rather than 4 cm but the other strategies remained unchanged. In summer, there was a significant effect of defoliation strategy (SEM 7.3, $P<0.01$), S1 having the lowest ADF content.

In autumn of Year 2, there was a significant effect of defoliation strategy (SEM 5.2, $P<0.01$), the ranking being S4< S1, S2 and S3. In winter there was a significant interaction strategy x intensity of defoliation ($P<0.01$, Table 6-9). Lax defoliation increased ADF in strategies S2 and S3, but decreased in S4. In spring, the ADF content was affected by defoliation intensity (SEM 2.9, $P<0.05$), increasing in swards lax defoliated. Finally in summer of Year 2, ADF was significantly affected by defoliation intensity (SEM 3.2, $P<0.05$), ADF fraction increasing in lax defoliated swards (10 cm) as reported in spring.

Table 6-9. Seasonal averages of acid detergent fibre (g/kg DM) of a birdsfoot trefoil/white clover oversown mixture, during two years.

	Year 1				Year 2			
	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer
S1 - 4 cm	352	331	340	438	385	331	399	411
S1 - 10 cm	364	341	343	465	395	339	426	423
S2 - 4 cm	350	331	343	549	393	308	406	417
S2 - 10 cm	366	339	356	535	388	362	422	434
S3 - 4 cm	352	328	331	521	384	372	392	411
S3 - 10 cm	347	340	356	517.	386	355	420	423
S4 - 4 cm	347	322	341	500	325	327	397	415
S4 - 10 cm	363	344	337	498	325	346	416	420
SEM (S x I)	7.7	4.1	5.0	8.9	7.3	4.7	5.8	6.4
Signif. (S x I)	NS	NS	*	NS	NS	**	NS	NS
Signif. (S)	NS	NS	NS	**	**	**	NS	NS
Signif. (I)	NS	**	*	NS	NS	**	**	*

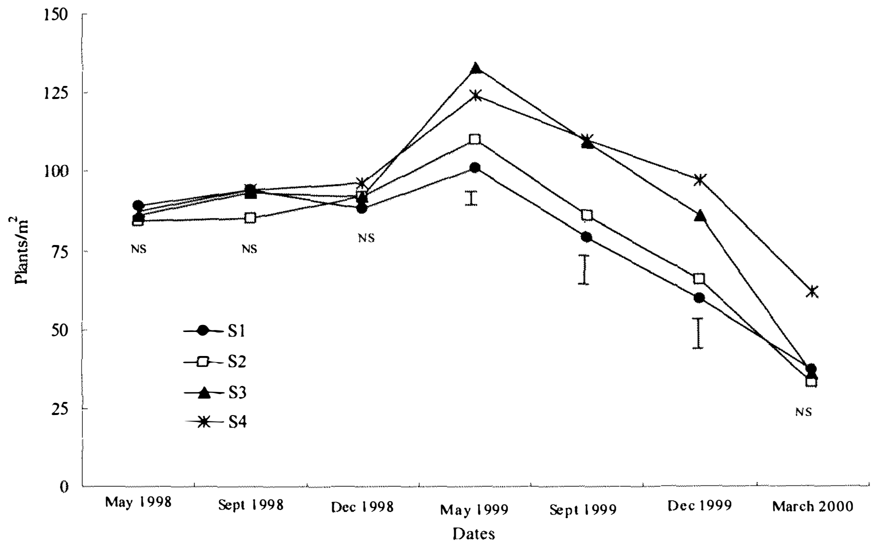
**, $P<0.01$; *, $P<0.05$; NS, not significant; SEM (S x I), standard error of the mean of the interaction strategy x intensity of defoliation; n=4; Signif., significance; S, strategy of defoliation; I, intensity of defoliation

6.4.3 Plant density

Initially in May 1998, plant density of BFT was uniform with an average of 86 plants/m^2. Over time, a significant interaction time x defoliation strategy ($P<0.05$) was observed (Figure 6-2 a), but there were no other interaction effects.

Plant density of BFT increased on average 34% from May 1998 to May 1999, but decreased 54% from May 1999 to March 2000. During the first year, differences were registered in May 1999. Plant density in strategies S3 and S4 was 20% higher on average than in strategies S1 and S2 (Figure 6-2 a), and 10% higher in swards grazed at 10 cm rather than 4 cm (Figure 6-2 b).

(a) Defoliation strategies



(b) Defoliation intensities

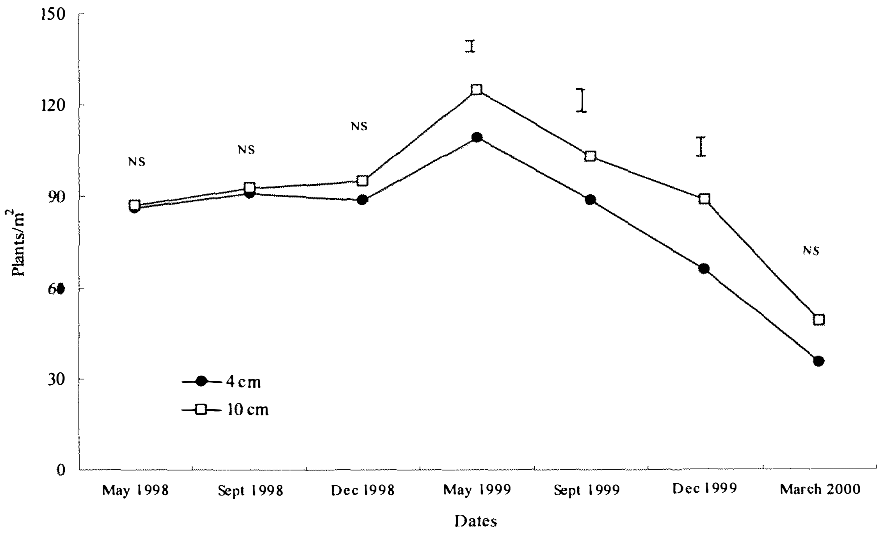


Figure 6-2. Changes in BFT density (adult plants) under (a) four defoliation strategies and (b) two defoliation intensities from April 1998-March 2000. Vertical bars indicate SEM ($n_{strategies}$=16, $n_{intensities}$=32), and NS not significant differences at corresponding sampling dates.

Plant density was significantly affected (in May P<0.01, in September P<0.01 and in December P<0.05) by defoliation strategies, S3 and S4 having higher density than S1 and S2. (Figure 6-2 a); and by defoliation intensity (in May P<0.01, September P<0.05 and December 1999 P<0.01respectively) (Figure 6-2 b), the 10 cm height having higher plant density than 4 cm. The rate of plant loss was high during spring 1999 and summer 2000, coinciding with drought conditions. Population declined 45% in summer 2000, leaving a final density of 42 plants/m^2. There were no differences in final density between treatments (Figure 6-2), resulting in an open and heterogeneous sward.

During the drought period growing points of white clover were monitored. In December 1999, there were significant differences between defoliation intensities (29 and 11 growing points/m^2 for 10 and 4 cm height respectively, SEM 6.7, $P<0.05$). Surviving growing points were located mainly in areas covered by native grasses or dead material. In March 2000, there were no growing points of WC in any of the treatments.

6.4.4 Plant morphology

Information about the number of primary shoots, secondary shoots, crown mass, root mass and root diameter in BFT plants was analysed from May 1998 to December 1999, and is presented in the sections 6.4.4.1 to 6.4.4.5.

6.4.4.1 Primary shoots

Over time, there was a decline in the general average from 6 to 4 primary shoots per plant. There was a significant interaction time x defoliation strategy ($P<0.01$, Table 6-10), but not for defoliation intensity or strategy x intensity over time. There were differences between defoliation strategies in May and December 1998, but not during 1999. From May 1998, the autumn rest (S4) increased the number of shoots/plant over the other treatments (Table 6-10). In December 1998, defoliation strategies that had a previous rest during the year (S3 and S4) had higher shoot density than systems without rest (S1 and S2) (Table 6-10).

Table 6-10. Number of primary shoots/plant of BFT under different strategies and intensities of defoliation from April 1998 to December 1999.

	May 1998	December 1998	May 1999	December 1999	SEM /Significance
Defoliation strategies					
S1	5	4	3	4	
S2	6	4	4	4	SEM (time x strategy)
S3	5	5	4	4	0.9 **
S4	8	6	4	4	
SEM (n)	0.6 (8)	0.3 (8)	0.5 (8)	0.5 (8)	
Significance	**	**	NS	NS	
Defoliation intensities					
4 cm	6	5	3	4	SEM (time)
10 cm	6	5	4	4	0.5 NS
SEM (n)	0.4 (16)	0.2 (16)	0.3 (16)	0.4 (16)	
Significance	NS	NS	NS	NS	

**, P<0.01; NS, not significant; SEM, standard error of the mean; (n) number of observations for each treatment mean

6.4.4.2 Secondary shoots

A significant time x strategy x intensity of defoliation interaction (P<0.01) was observed for the number of secondary shoots (Table 6-11), the average of shoots per plant decreasing from 13 to 8 during the experimental period. Within sampling dates, only during the first sampling was the interaction strategy x intensity significant (P<0.05, Table 6-11), secondary shoots increasing if plants received a rest (S4); the other treatments did not differ if defoliated at 4 or 10 cm height. In December 1998, the height of defoliation affected secondary shoots per plant (SEM 1.0, P<0.05), plant lax defoliated (10 cm) having more secondary shoots than those hard defoliated (4 cm). The final sampling showed significant differences by defoliation intensity (SEM 0.6, P<0.01), plants lax defoliated maintaining a high density of secondary shoots.

Table 6-11. Evolution of secondary shoots (no./plant) of BFT plants under a combination of four defoliation strategies and two defoliation intensities from April 1998 to December 1999.

	May 1998	December 1998	May 1999	December 1999	SEM /Significance
Defoliation Strategy x intensity					
S1-4 cm	13	6	6	7	SEM (time x strategy x intensity) 1.4 **
S1-10 cm	11	11	6	10	
S2-4 cm	12	9	7	6	
S2-10 cm	14	14	8	9	
S3-4 cm	13	12	6	6	
S3-10 cm	12	11	6	11	
S4-4 cm	12	9	5	7	
S4-10 cm	23	20	10	9	
SEM (n)	2.1 (4)	2.0 (4)	1.4 (4)	1.3 (4)	
Significance	*	NS	NS	NS	

**, P<0.01 ; *, P<0.05; NS, not significant; SEM, standard error of the mean; (n), number of observations for each treatment mean

6.4.4.3 Crown mass

There was a significant interaction time x defoliation intensity effect (P<0.05), but no effect of defoliation strategy or strategy x intensity over time (Table 6-12). A clear tendency to reduce crown mass of plants grazed at 4 cm height in contrast with those grazed at 10 cm was observed during the last three sampling dates (Table 6-12). Differences in crown mass between defoliation strategies were found during the three first dates (Table 6-12), S4 having bigger crowns than the others. The average for all sampling dates showed that plants of S4 strategy had crowns 53% bigger than the average of the other strategies.

Table 6-12. Evolution of crown mass (g/plant) of BFT plants under four defoliation strategies and two defoliation intensities from April 1998 to December 1999.

	May 1998	December 1998	May 1999	December 1999	SEM /Significance
Defoliation strategies					
S1	0.8	0.5	0.8	0.8	SEM (time x strategy) 0.12 NS
S2	1.0	0.7	0.9	0.9	
S3	0.9	0.9	1.0	1.0	
S4	1.3	1.2	1.4	1.2	
SEM (n)	0.13 (8)	0.08 (8)	0.10 (8)	0.16 (8)	
Significance	*	**	**	NS	
Defoliation intensities					
4 cm	1.0	0.6	0.9	0.7	SEM (time x intensity) 0.08 *
10 cm	1.1	1.0	1.2	1.3	
SEM (n)	0.09 (16)	0.05 (16)	0.07 (16)	0.11 (16)	
Significance	NS	**	**	**	

**, $P<0.01$; *, $P<0.05$; NS, not significant; SEM, standard error of the mean; (n), number of observations for each treatment mean

6.4.4.4 Root mass

Significant interactions time x defoliation strategy ($P<0.05$) and time x defoliation intensity ($P<0.05$) were observed for root mass (Table 6-13), but there was no interaction between main effects over time. Defoliation strategies affected root mass significantly during the first three sampling dates (Table 6-13). The more intensive strategies (S1 and S2) had less root mass per plant compared with more lax strategies, especially S4. Plants grazed at 10 cm had consistently greater root mass than those grazed at 4 cm height (Table 6-13), and the differences were significant for last three sets of dates studied.

Table 6-13. Evolution of root mass (g/plant) of BFT plants under four defoliation strategies and two defoliation intensities from April 1998 to December 1999.

	May 1998	December 1998	May 1999	December 1999	SEM /Significance
Defoliation strategies					
S1	1.1	0.9	1.1	0.8	SEM (time x strategy)
S2	1.2	1.2	1.3	0.9	0.13 *
S3	1.2	1.4	1.6	0.9	
S4	2.2	1.8	2.0	1.0	
SEM (n)	0.21 (8)	0.14 (8)	0.5 (8)	0.13 (8)	
Significance	**	**	**	NS	
Defoliation intensities					SEM (time x intensity)
4 cm	1.2	0.9	1.1	0.7	0.09 *
10 cm	1.6	1.7	1.9	1.1	
SEM (n)	0.15 (16)	0.09 (16)	0.12 (16)	0.09 (16)	
Significance	NS	**	**	**	

**, P<0.01; NS, not significant; SEM, standard error of the mean; (n) number of observations for each treatment mean

6.4.4.5 Root diameter

A significant interaction time x defoliation intensity (P<0.05) was observed for root diameter, values for BFT plants defoliated at 10 cm height being greater than those for plants defoliated at 4 cm (Table 6-14). A fast decline in root diameter of plants defoliated at 4 cm occurred between first and second sampling, differences maintained during the rest of the period excepting the final evaluation (Table 6-14).

There were differences among defoliation strategies in December 1998 (P<0.01, Table 6-14). Root diameter of BFT plants in strategies that received a previous rest (S3 and S4) was higher than in strategies without any rest to this time (S1 and S2).

Table 6-14. Evolution of root diameter (mm) of BFT plants under four defoliation strategies and two defoliation intensities from April 1998 to December 1999.

	May 1998	December 1998	May 1999	December 1999	SEM /Significance
Defoliation strategies					
S1	10	8	6	7	SEM (time x strategy) 2.8 NS
S2	11	8	7	8	
S3	10	10	8	7	
S4	12	10	8	7	
SEM (n)	0.6 (8)	0.3 (8)	0.5 (8)	0.5 (8)	
Significance	NS	**	NS	NS	
Defoliation intensities					
4 cm	11	8	6	7	
10 cm	11	10	8	8	
SEM (n)	0.4 (16)	0.2 (16)	0.3 (16)	0.4 (16)	SEM (time x intensity) 0.5 *
Significance	NS	**	*	NS	

**, P<0.01; *, P<0.05; NS, not significant; SEM, standard error of the mean; (n), number of observations for each treatment mean

6.4.5 Seed production

Seed production differed significantly between years. Seed yield of BFT in Year 2 was only 13% of the yield in Year 1 (SEM 0.679, P<0.01, Table 6-15). Seed production of WC was also seriously reduced during the second year, yield being only 2% of that achieved in the previous year (SEM 0.334, P<0.01, Table 6-15). There were no interaction effects, Table 6-15 shows main effects in each species (strategy and intensity of defoliation); analysis was done by year independently due to disparities in seed yield.

BFT seed production was significantly affected (P<0.01) by grazing strategies, yield for the unspelled treatment (S1) being only 6% of the summer spell treatment (S2). Winter rest improved BFT seed production over the other rest treatments (Table 6-15). Management effects on WC were not significant, though again seed production was lower in S1 than in the other treatments. Defoliation intensity did not affect seed production in BFT, but intense defoliation (4 cm) reduced seed production in WC to 32% of yield in swards defoliated at 10 cm (Table 6-15).

Table 6-15. Annual seed production (g/m^2) of birdsfoot trefoil (BFT) and white clover (WC) in mixture under different strategies and intensities of defoliation during two years.

	Year 1		Year 2	
	BFT	WC	BFT	WC
Defoliation strategy				
S1	0.6	2.5	0.2	0.004
S2	9.7	4.3	0.9	0.024
S3	15.0	4.2	1.0	0.000
S4	9.5	6.4	2.4	0.024
SEM (n)	1.26 (16)	0.73 (16)	0.39 (16)	0.0070 (16)
Significance	**	NS	NS	NS
Defoliation intensity				
4 cm	6.9	2.0	1.0	0.006
10 cm	10.5	6.6	1.2	0.020
SEM (n)	0.89 (32)	0.52 (32)	0.28 (32)	0.0049 (32)
Significance	NS	**	NS	NS
General mean	8.7	4.3	1.1	0.01

**, $P<0.01$; NS, not significant; SEM, standard error of the mean; (n), number of observations for each treatment mean

During the second year, the seed production of BFT and WC was not affected by either grazing strategy or the intensity of defoliation (Table 6-15). In general, the SEM values reported in Table 6-15 are high compared with treatment means during the two years, a consequence of the heterogeneity in species distribution in a three year old oversown pasture.

6.4.5.1 Seed yield components

The number of inflorescences/m^2 in BFT was affected ($P<0.01$) by defoliation strategies in Year 1, the unspelled treatment (S1) having only 9% of the inflorescences of the spelled treatment (S2), and the winter rest further increased the number of the inflorescences of BFT. In Year 2, there were no effects of defoliation strategy on BFT inflorescences. Defoliation intensity did not affect the inflorescences/m^2 in either Year 1 or Year 2 (Table 6-16). In WC, the number of inflorescences was affected ($P<0.05$) by defoliation intensity only in Year 1; under intensive defoliation (4 cm) the inflorescences were 32% of those recorded in 10 cm defoliation height treatment.

Table 6-16. Inflorescences/m^2 (I), viable seeds/m^2 (S) and 1000 seed weight (W) (g) of BFT/WC mixture under different strategies and intensities of defoliation, evaluated during two years.

	Year 1998-1999						*Year 1999-2000*					
	BFT			WC			BFT			WC		
	I	S	W	I	S	W	I	S	W	I	S	W
Defoliation Strategy												
S1	30	410	1.204	135	4850	0.555	20	150	1.119	2	5	0.618
S2	345	8995	1.180	215	7585	0.559	70	710	1.211	5	35	0.616
S3	540	11105	1.244	345	7135	0.542	100	805	1.206	2	0	---
S4	340	7020	1.189	310	11360	0.551	200	1800	1.246	6	30	0.478
SEM (n=16)	46.1	1594	0.0734	96.3	1432	0.0369	29.2	300	0.0547	1.5	9	0.0271
Signif.	**	**	NS	NS	NS	NS	NS	**	NS	NS	*	NS
Defoliation Intensity												
4 cm	260	4850	1.239	120	4100	0.553	90	765	1.182	1	10	0.531
10 cm	365	8920	1.176	380	11365	0.551	105	965	1.228	6	30	0.573
SEM (n=32)	32.6	1127	0.0388	68.1	1013	0.0265	20.7	197	0.0325	1.0	7	0.0192
Signif.	NS	*	NS	*	**	NS	NS	NS	NS	*	*	NS

**, $P<0.01$; $P<0.05$; NS, not significant; SEM, standard error of the mean; (n), number of observations for each treatment mean

The number of viable seeds produced was affected by defoliation strategy in BFT during the two years ($P<0.01$ in both cases). The unspelled treatment (S1) had the lowest number of viable seeds, numbers increasing with winter rest in Year 1 or autumn rest in Year 2. The intensity of defoliation affected the number of viable seeds ($P<0.05$) in Year 1, viable seeds in the intensely defoliated swards being 54% of viable seeds produced when defoliated at 10 cm height.

The number of viable seeds in WC was unaffected during Year 1 by defoliation strategies, but there were differences ($P<0.05$) in Year 2 despite the low seed production. The spell treatment increased viable seeds, and the autumn rest resulted in further improvement (Table 6-16). Defoliation intensity significantly affected viable seeds production in the two years ($P<0.01$ and $P<0.05$ respectively), in both cases seed numbers being greater in swards defoliated at 10 cm than at 4 cm.

The 1000 seed weight of BFT and WC was not affected by defoliation treatments, and did not differ significantly between years. General means for 1000 seed weight were 1.20 and 0.56 g for BFT and WC respectively.

6.4.5.2 Patterns of seed production

In 1998-1999, mature BFT seed collection began on 22 January and continued during February. Over the season, significant interactions time x defoliation strategy ($P<0.01$) and time x defoliation intensity ($P<0.01$) were observed (Figure 6-3 a,b). The spelled treatments (S2, S3 and S4) started to produce mature seeds earlier than the unspelled treatment (S1). In early February, the winter rest treatment (S3) was significantly more productive than the others and the unspelled treatment (S1) the poorest seed producer. At the last sampling in late February, defoliation strategies still affected seed production, the unspelled treatment (S1) producing less than the other treatments. During the first year, the effect of defoliation intensity on BFT seed production was observed only in the first seed collection. At that time the intensively defoliated plots (4 cm) produced only 14% of the seed produced by lax defoliated treatments (Figure 6-3 b)

During 1999-2000, BFT seed production was poor compared with the previous year. There were effects of defoliation strategies only in early January (Figure 6-3 a), when the autumn rest (S4) produced more than the other treatments. There were no effects of defoliation intensity during the season (Figure 6-3 b). Seed production started earlier in WC than in BFT during the 1998-1999 season. The first records of mature seeds were on 24 December (Figure 6-4). Significant interactions time x defoliation strategy ($P<0.05$) and time x defoliation intensity ($P<0.01$) were observed during 1998-1999.

There were significant effects of defoliation strategy on WC seed production in early January and early February (P<0.05, in both cases) (Figure 6-4 a). The unspelled treatment (S1) produced less than the spelled treatment (S2). The intensity of defoliation affected WC seed production from late December to early February (Figure 6-4 b), in all cases 10 cm defoliation height producing more seed than 4 cm height. During 1999-2000, there were no effects of defoliation strategies and defoliation intensities, seed production was scarce and concentrated during the sampling in early January (Figure 6-4).

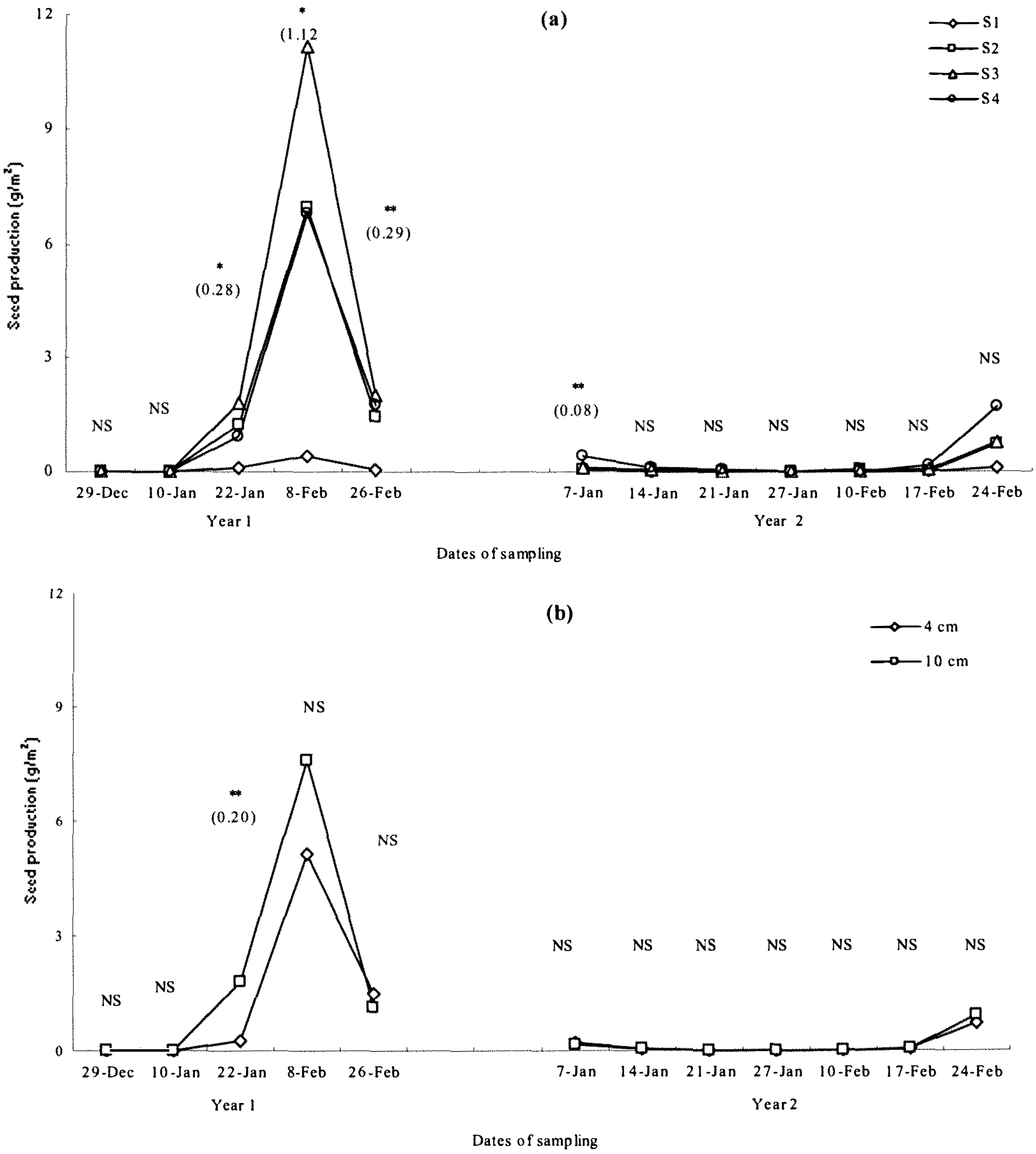


Figure 6-3. Patterns of seed production in BFT (g/m^2) over two summer seasons affected by defoliation strategies (a) and by defoliation intensities (b). **, $P<0.01$; *, $P<0.05$; NS, not significant; numbers in brackets, SEM ($n_a=16$, $n_b=32$).

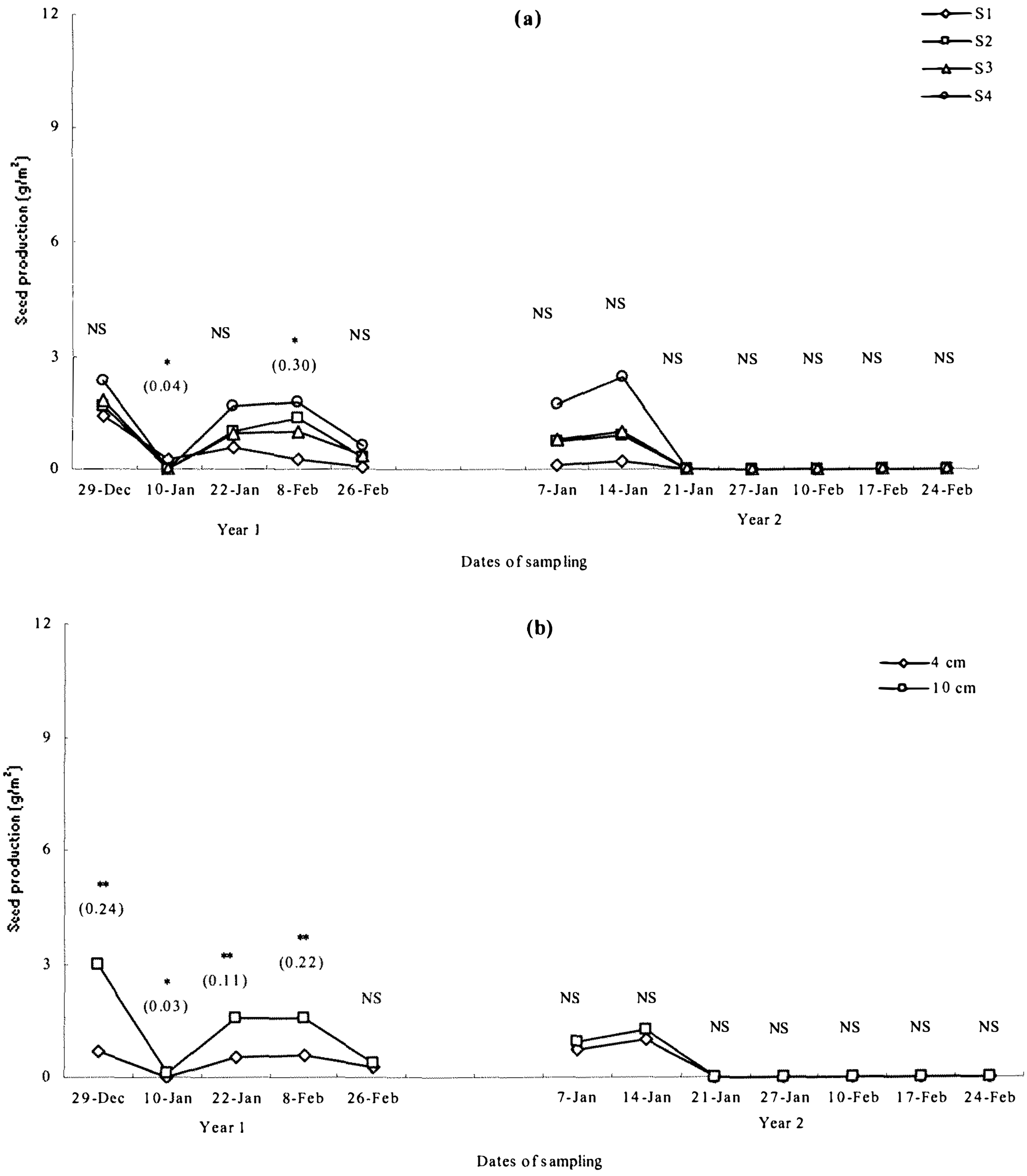


Figure 6-4. Patterns of seed production in WC (g/m^2) over two summer seasons affected by defoliation strategies (a) and by defoliation intensities (b). **, $P<0.01$; *, $P<0.05$; NS, not significant; numbers in brackets, SEM ($n_a=16$, $n_b=32$).

6.4.6 Soil seed reserves

In April 1998, initial soil reserves were 4340±1015 and 2570±1149 seeds/m^2 for BFT and WC respectively. Thousand seed weight was 1.171± 0.010 g for BFT and 0.580±0.011 g for WC, with 64% of hard seeds in BFT and 78% in WC.

BFT soil reserves in March 1999 were significantly affected by both grazing strategies and grazing intensity (Table 6-17), being 10% less than in April 1998 in S1 and 59, 78 and 66% greater in treatments S2, S3 and S4. Treatment contrasts for WC were similar, though in all treatments reserves were substantially higher in 1999 than 1998. Seed reserves were greater following lax (10 cm) than severe (4 cm) defoliation in both BFT and WC, in both cases increasing seed reserves over the initial sampling (1998). In March 1999, BFT 1000 seed weight was not affected by either grazing strategy or severity, and in WC was affected only by grazing severity (Table 6-17).

BFT soil reserves in March 2000 were affected by defoliation strategy ($P<0.01$) and by defoliation intensity ($P<0.01$). There was an effect of spell period on seed reserves (Table 6-17), but there were no differences between spell treatments. Intensive defoliation (4 cm) reduced reserves compared with lax defoliation (10 cm). A significant interaction time x defoliation height (SEM 639, $P<0.05$) was observed, soil seed reserves declined from initial values when the unspelled treatment (S1) or intensive defoliation (4 cm) were applied.

WC reserves in March 2000 were affected by defoliation strategy ($P<0.05$) and defoliation intensity ($P<0.01$) (Table 6-17). There was no effect of spell treatment (S1=S2), but the autumn rest improved seed reserves over the unspelled treatment. The intensive defoliation (4 cm) showed lower WC reserves than lax defoliation (10 cm) (Table 6-17). Over time, there was a significant interaction time x defoliation height (SEM 1033, $P<0.05$). WC seed reserves increased at the end of first year, then declined but values remained higher than initial values (April 1998) in all cases.

At the end of the evaluation in March 2000, thousand seed weight of BFT and WC were not affected by defoliation strategy or intensity (Table 6-17). Also, there were no significant effects over time in either BFT or WC 1000 seed weight.

Table 6-17. Soil seed reserves and 1000 seed weight parameters in mixed birdsfoot trefoil (BFT) and white clover (WC) swards under different defoliation strategies and intensities, during two years.

	March 1999				March 2000			
	BFT Seeds/m^2 (no./ m^2)	BFT 1000 seed weight (g)	WC Seeds/m^2 (no./ m^2)	WC 1000 seed weight (g)	BFT Seeds/m^2 (no./ m^2)	BFT 1000 seed weight (g)	WC Seeds/m^2 (no./ m^2)	WC 1000 seed weight (g)
Defoliation strategy								
S1	3895	1.217	7670	0.551	1810	1.176	3920	0.527
S2	6890	1.235	8780	0.556	3965	1.229	4550	0.567
S3	7725	1.234	8980	0.547	3450	1.251	6620	0.560
S4	7225	1.262	14070	0.559	4520	1.299	7495	0.556
SEM (n)	968 (48)	0.029 (8)	1682 (48)	0.007 (8)	512 (48)	0.0337 (8)	989 (48)	0.0158 (8)
Significance	*	NS	*	NS	**	NS	*	NS
Defoliation Intensity								
4 cm	5050	1.258	6165	0.544	2395	1.237	3600	0.557
10 cm	7815	1.215	13580	0.562	4475	1.241	7690	0.548
SEM (n)	684 (96)	0.021 (16)	1190 (96)	0.005 (16)	361(96)	0.0238 (16)	699 (96)	0.0111 (16)
Significance	**	NS	**	*	**	NS	**	NS

**, $P<0.01$; *, $P<0.05$; NS, not significant; SEM, standard error of the mean; (n), number of observations for each treatment mean

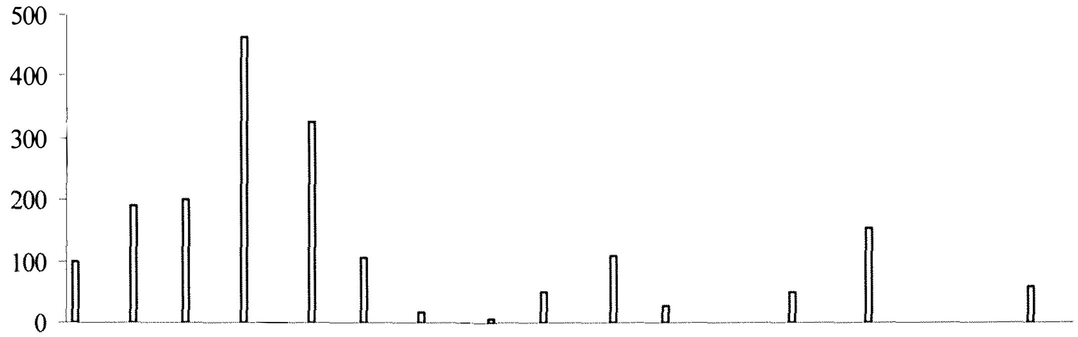
6.4.7 Seedling emergence

The two species followed similar emergence patterns in controlled field conditions without any sward competition between June and December 1998 (Figure 6-5, Plate 6-2), achieving 1860 and 880 emerged seedlings/m^2 for BFT and WC respectively, corresponding with 44 and 35% of potential emergence from the 1998 seed bank.

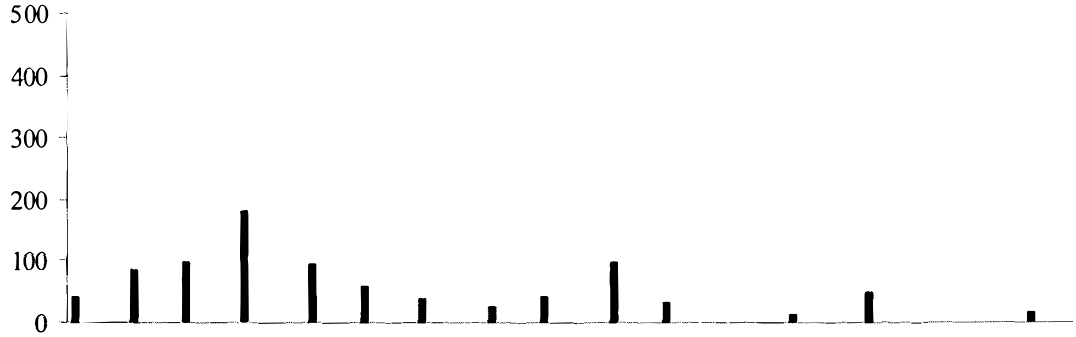
During winter, there was 76 and 71% of total emergence for BFT and WC respectively. In spring, emergence was low in the two species, pulses of seedling emergence occurring after periods of rain, combinations of rain and drought and peaks of variation in soil temperature. However, there was a general decline in seedling emergence to the end of spring.

Plate 6-2. Seedling emergence was checked regularly from soil cores placed in an adjacent area to experimental site and maintained free of ground cover.

(a) Emerged BFT seedlings/m^2/week



(b) Emerged WC seedlings/m^2/week



(c) Climatic parameters

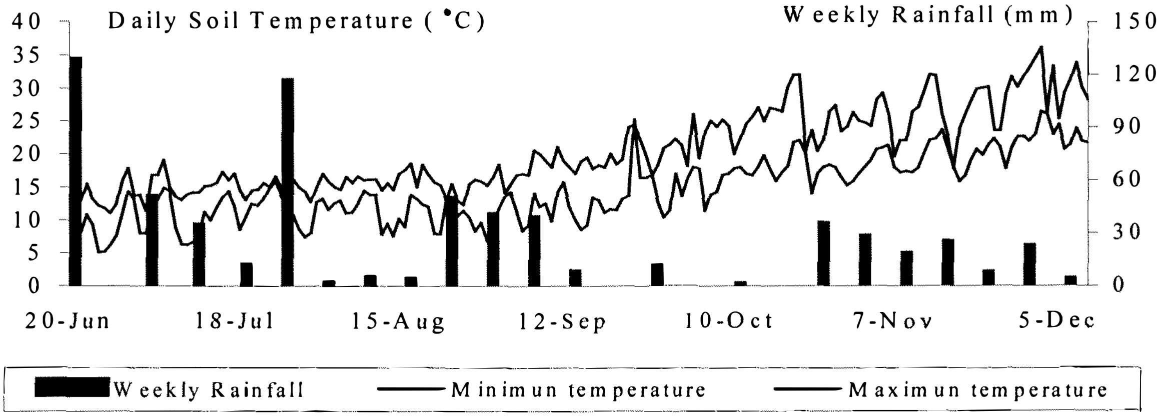


Figure 6-5. Seedling emergence patterns of (a) birdsfoot trefoil (BFT) and (b) white clover (WC) and (c) climatic parameters, evaluated on field from June to December 1998.

From March to August 1999, seedling emergence under sward competition was substantially lower than values shown in Figure 6-5, varying from 5-13% in BFT and 4-7% in WC of 1999 seed reserves (Table 6-18). BFT seedling emergence showed significant effects of defoliation strategy, with particularly high emergence in S3, mainly associated with a high seed input during spelling. A reduction of sward competition by intense defoliation (4 cm) promoted an increase of 71% in BFT seedling emergence compared with more lax defoliation (10 cm). In contrast, WC did not show any treatment effect on seedling emergence, though a high percentage of recruitment was observed under severe defoliation for both BFT and WC (Table 6-18).

Table 6-18. Seedling emergence (no./m^2) and percentage of emergence from soil seed reserves of birdsfoot trefoil (BFT) and white clover (WC) under different strategies and intensities of defoliation from March – August 1999.

	Seedling emergence March 1999-August 1999 (no./m^2)		Percentage of emergence from March 1999 soil seed reserves (%)	
	BFT	WC	BFT	WC
Defoliation strategy				
S1	320	325	8	4
S2	415	420	6	5
S3	825	650	11	7
S4	460	640	6	5
SEM (n)	126 (8)	120 (8)	--	--
Significance	*	NS	--	--
Defoliation intensity				
4 cm	640	450	13	7
10 cm	375	570	5	4
SEM (n)	89 (16)	85 (16)	--	--
Significance	*	NS	--	--

*, $P<0.05$; NS, not significant; SEM, standard error of the mean; (n), number of observations for each treatment mean

6.5 DISCUSSION

The results showed a high degree of variation between years due to changing climatic conditions. The herbage production of Year 2 was 77% of that obtained in Year 1. The seed production of BFT and WC in Year 2 was 13% and 2% of production obtained in Year 1 respectively. BFT plant density declined 45% during the second year and active growing points of WC almost disappeared at the end of the second year.

Species that grow in pastoral areas of Uruguay are affected by irregular droughts and wet periods. These conditions determine that grazing management should be oriented to obtain a high grazing efficiency without putting at risk the survival of species introduced into native communities. Comparatively, BFT has a high degree of tolerance to drought conditions compared with WC, by the presence of a deeper taproot system (Seaney and Henson, 1970) that contrasts with the shallow root system of WC. The drought conditions and high soil temperatures recorded during the second summer caused a 45% reduction in BFT stand and a massive death of WC plants. The death of stolons in WC is affected by these factors (Belaygue *et al.*, 1996; Woodfield and Caradus, 1996), which are reported as critical during summer in Uruguay (Carámbula, 1977). The average soil temperature (registered at 5 cm depth in soil without vegetation cover) was 30.9, 33.4 and 30.4 ^{o}C for December, January and February respectively with maximum values of 36, 37 and 34 ^{o}C for the respective months.

To increase annual herbage accumulation, the autumn rest was an effective strategy during the two years and winter rest only in the second year. Lax grazing (10 cm) increased the herbage accumulation between 18 and 21% in comparison with intensive defoliation (4 cm). BFT contribution was affected by defoliation management during the two years, accumulation increasing by lax defoliation (10 cm) and by strategies with extended rest periods (S4), effects that showed a similar tendency in WC during the two years. There were no advantages to the accumulation of BFT in spring from winter rest. The benefits produced by autumn rest were in accordance with results reported in Chapter 3. The inclusion of an early autumn rest in BFT swards promotes herbage production in spring. These results suggest that BFT/WC mixtures should receive a rest period in autumn to increase annual productivity, and this will be discussed in detail in the final discussion (Chapter 7). Previous work at INIA Treinta y Tres with BFT/WC oversown mixtures showed that rest periods between 60 and 80 days were enough to enhance herbage production without excessive losses of quality (Carámbula and Ayala, 1995).

The advantages of autumn rest to herbage accumulation were associated with an improvement in morphology of BFT plants. Primary plant branching of BFT increased in the autumn rest treatment during the first year, but a decline over time occurred for all grazing strategies. The intensive grazing (4 cm) reduced plant branching in BFT, and differences were observed in December of both years. Also, secondary branches were reduced by intensive grazing, as occurred in swards under close cutting (Chapter 3).

The root mass, crown size and root diameter of plants intensively grazed (4 cm) tended to be reduced if compared with those plants lax grazed (10 cm), tendencies that are in agreement with those presented in Chapters 3 and 4 when a range between 2 to 10 cm of defoliation were contrasted. Autumn rest strategy determined that BFT plants had stronger crowns than in the other treatments. In addition, this was associated with differences in root mass, S4 being more rooted than S1 and S2. At the end of the evaluation these differences disappeared, probably by a reduction in stand density. The rest in autumn (S4) or winter (S3) increased root diameter of BFT plants in comparison with treatments that did not receive rest (S1 and S2).

Although frequency of defoliation was not studied in this trial, the results obtained suggest that strategies that included grazing all year-round with monthly intervals between grazing were excessive, adversely affecting herbage production and persistence and differences being higher during the second year (Table 6-4, Figure 6-2). In comparison, results reported on Chapter 3 showed that the frequency of defoliation was not a significant factor if studied during a short period (spring in that case). Thus, management shows a cumulative effect, suggesting that BFT can only tolerate inappropriate defoliation for short periods. Climatic conditions exerted the strongest influence on the final results. Bologna (1996) showed a negative effect of defoliation intervals shorter than 4 weeks on BFT production. Several studies reported that BFT management needs to be a compromise between frequency and intensity of defoliation, combinations of frequent and close grazing being inappropriate (Smith and Nelson, 1967; Greub and Wedin, 1971 a; 1971 b). The favourable effects of rotational grazing rather than continuous grazing in BFT were early observed by Van Keuren and Davis (1968).

A high seed yield was obtained under favourable climatic conditions, achieving an average of 11 and 10 times the sowing rate used for BFT and WC respectively. The spelling period increased BFT seed production 16 times, the winter rest further increasing seed production. The seed production of WC was not affected by grazing strategies. WC tolerated more intensive defoliation than BFT for seed production. Seed production of an unspelled treatment was 5 times higher than the seeding rate used in WC.

Conditions for flowering are controlled by daylength and temperature. In WC, higher temperatures and longer days (>12 hours) at the end of spring favour flowering (Hill *et al.*, 1999), and BFT requires a minimum daylength between 14 to 14.5 hours (McKee, 1963). In this experiment, WC showed an earlier seed production period than BFT, related to these factors.

The summer spelling period in the experiment extended from early December to late February. Results from Year 1 showed that seed yield between December and early February was more than 82 and 90% of total yield in BFT and WC respectively. Despite the indeterminate growth habit of BFT and successive fluxes of flowering, Li and Hill (1988) showed that more than 70% of inflorescences are produced in a short period (25 days). In WC, seed production is not the only mechanism involved in reproduction, vegetative reproduction being a more important alternative. Based on these arguments, it can suggested that 60-70 days from December is a long enough spelling period for BFT/WC mixtures. In practice, this allows a period of intensive grazing of mature forage at the end of summer to clean swards for recruitment of new seedlings in autumn.

The size of a soil seed bank is the result of previous inputs by reproduction of parental plants and eventually reflects the inputs by sown or dispersed seed (Pearson and Ison, 1997). The quantification of reserves showed densities from 1800 to 7800 seeds/m^2 of BFT and from 2500 to 14000 seeds/m^2 of WC. Arana and Piñeiro (1999) working with WC Zapicán in Uruguay, determined annual inputs to the soil seed bank between 2600 and 12000 seeds/m^2. Bologna (1996) reported soil seed reserves of BFT Grasslands Goldie between 19000 to 28000 seeds/m^2, in environments of South Island of New Zealand. In the case of *Lotus pedunculatus* Maku, seed banks larger than 6000 seeds/m^2 are required for persistent swards in high latitudes (>32°S) of Australia (Blumenthal and McGraw, 1999).

Bologna (1996) found 15% of soil seed reserves in the first 2 cm of soil strata of swards defoliated at 2 week intervals, in contrast with 60% of those defoliated at 8 week intervals. Differences were attributed to trampling by sheep, particularly in wet conditions or by intensive grazing. In this experiment, the fraction of seeds under 5 cm depth was minimal, and the probability that these seeds would produce viable seedlings was low.

The average of seedling emergence was 5-13% in BFT and 4-7% in WC of soil seed reserves. Seedling emergence appears associated with seed inputs produced by summer spelling. The soil seed bank can act as a buffer maintaining relative rate of emergence if annual seed input is reduced, as occurred in intensive grazing schemes in Year 1 or when seed input declined in Year 2 due to poor climatic conditions. Emergence was also improved by intensive defoliation (4 cm), results that agree with those of Bologna (1996) who found in BFT an increase in seedling recruitment under frequent defoliation, recruitment being lower than 10% for an environment of South Island of New Zealand. A fraction between 35 and 42% of seeds, depending on the species, was activated if sward competition was eliminated, exposing seeds to fluctuating temperature and humidity to break down dormancy.

These facts suggest that the low efficiency of the seedling recruitment process needs to be augmented by additional management strategies especially in autumn. The intensive defoliation reduces sward competition, creating gaps (Pearson and Ison, 1997) for seedling establishment. These requirements need to be compatible with previous recommendations in terms of advantages of autumn rest to improve herbage accumulation. It can be suggested that in those years when stand density needs to be improved, extended autumn rest should be avoided, giving opportunity for new seedlings establishment. Based on the potential seedling emergence results, in those cases when plant frequency of species of interest is low and adequate densities of seed are present in the soil, more extreme intensities of sward disturbance (eg. the application of herbicides, intensive grazing or soil disturb) could be practised to accelerate recruitment.

6.6 CONCLUSIONS

Evidence from this trial suggests that management to improve herbage accumulation of BFT/WC mixtures requires a rest period in autumn, grazing swards at lax intensities when monthly intervals are used between grazing cycles. Summer spelling for 60-70 days starting in December is necessary to increase soil seed reserves, management that is further enhanced by autumn rest or winter rest to improve WC or BFT seed production respectively. However, an extended seed spelling period is recommended in years where stand density or seed soil reserves decline. Recruitment of new individuals from the soil seed bank has a low efficiency, demanding high seed reserves to have more chance to incorporate new individuals or eventually the application of alternative practices to activate the dormant soil seed bank fraction and increase the absolute values for the recruitment of new individuals.

6.7 REFERENCES

ALTIER, N. (1997). Enfermedades del Lotus en Uruguay. (Lotus diseases in Uruguay). INIA La Estanzuela, Uruguay. *Serie Técnica* No. **93**. ISBN: 9974-38-083-9. 16 pg.

ARANA, S. AND PINEIRO, G. (1999). Déficit hídrico y manejo: su influencia en la demografía y producción del trébol blanco. (Water deficit and management: the influence in the demography and production of white clover). *Tesis Facultad de Agronomía.* Montevideo Uruguay.

AYALA W. AND BERMUDEZ R. (1992). Fertilización Fosfatada de Pasturas. (Phosphatic fertilization of pastures). **In** Mejoramientos Extensivos en la Región Este, pg 49-59. Resultados Experimentales 1992. INIA Treinta y Tres. Estación Experimental del Este. Octubre 1992.

AYALA W. AND CARAMBULA M. (1995). Mejoramientos Extensivos en la Región Este: Manejo y Utilización. (Oversown pastures in the Eastern Region: Management and Utilization). In *Seminario de Actualización Técnica sobre Producción y Manejo de Pasturas.* pgs. XVIII-1 a XVIII-5. 17 al 19 de Octubre, 1995. Tacuarembó. Uruguay.

BUHLER, D. D. HARTZLER, R. G. FORCELLA, F. (1997). Implications of weed seed bank dynamics to weed management. *Weed Science* **45**: 329-336.

BELAYGUE, C., WERY, J., COWAN, A.A. AND TARDIEU, F. (1996). Contribution of leaf expansion, rate of leaf appearance, and stolon branching to growth of plant leaf area under water deficit in white cover. *Crop Science* **36**: 1240-1246.

BLUMENTHAL, M.J. AND McGRAW, R.L. (1999). Lotus adaptation, use and management. *Trefoil: The science and technology of Lotus.* CSSA Special Publication Number 28. Ed. by P.R. Beuselinck. American Society of Agronomy, Inc. Crop Science Society of America, Inc. Madison, Wisconsin, USA. pp. 97-119.

BOLOGNA, J.J. (1996). Studies on strategies for perennial legume persistence in lowland pastures. *Thesis of Master of Agricultural Science.* Lincoln University. New Zealand. 220 p.

CARAMBULA, M. (1977). *Manejo de pasturas sembradas.* (Management of cultivated pastures). Editorial Hemisferio Sur. 374 pp.

CARAMBULA, M. AND AYALA, W. (1995). Algunas Pautas de Manejo de Mejoramientos Extensivos. (Management recommendations for oversown pastures). In *Mejoramientos Extensivos: Manejo y Utilización.* Area Producción Animal. Serie de Actividades de Difusión No. 75. INIA Treinta y Tres. pgs. 12-18. Treinta y Tres. Octubre, 1995.

CARAMBULA, M., CARRIQUIRY, E. AND AYALA, W. (1994). Siembra de Mejoramientos en Cobertura. (Establishment of oversown pastures). In *Boletín de Divulgación* No. 46. 1994. INIA. ISBN: 9974-38-015-4. 20 pg. Junio 1994.

GREUB, L.J. AND WEDIN, W.F. (1971 a). Leaf area, dry-matter accumulation, and carbohydrate reserve levels of birdsfoot trefoil as influenced by cutting height. *Crop Science* **11**: 734-738.

GREUB, L.J. AND WEDIN, W.F. (1971 b). Leaf area, dry-matter accumulation, and carbohydrate reserves of alfalfa and birdsfoot trefoil a three-cut management. *Crop Science* **11**: 341-344.

GOERING, H.K. AND VAN SOEST, P.J. (1970). Forage Fibre Analysis. U.S.D.A. – A.R.S. Agricultural Handbook No. 379.

HILL, M.J.; HAMPTON, J.G. AND ROWARTH, J.S. (1999). Herbage seeds. Chapter 16. *New Zealand Pasture and Crop Science.* Edited by J. White and J. Hodgson. Oxford, University Press, Auckland. ISBN 0-19-558375-2. pp. 249-262.

ISTA. (1985). International Rules for Seed Testing 1985. *Proceedings of the International Seed Testing Association* **13**: 299-520.

LI, Q. AND HILL, M.J. (1988). An examination of different shoot age groups and their contribution to the protracted flowering pattern in birdsfoot trefoil (*Lotus corniculatus* L.). *Journal of Applied Seed Production* **6**: 54-62.

McKEE, G.W. (1963). Influence of daylength on flowering and plant distribution in birdsfoot trefoil. *Crop Science* **3**:205-208.

PEARSON, C.J. AND ISON, R.L. (1997). Generation. Chapter 3. *Agronomy of Grasslands Systems.* 2nd Edition.. Cambridge University Press. pp 37-59.

PRESTES, N.E. (1995). Sobressemeadura do cornichão (*Lotus corniculatus* L.) cv. San Gabriel em pastagem natural – diferemento e adubação. (Sowing of birdsfoot trefoil (Lotus corniculatus L.) in grasslands – stockpilling and fertilization.)

Dissertação de Mestrado em Zootecnia (Plantas Forrageiras), Facultade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brasil. 118 p

ROBERTS, H.A. AND BODDRELL, J.E. (1985). Seed survival and seasonal pattern of seedling emergence in some Leguminosae. *Annals of Applied Biology* **106**: 125-132.

SAS INSTITUTE (1990). *SAS/STAT User's Guide*, Version 6. Cary, NC: SAS Institute.

SEANEY, R.R. AND HENSON, P.R. (1970). Birdsfoot trefoil. *Advances in Agronomy* **22**: 119-157.

SMITH, D. AND NELSON, C.J. (1967). Growth of birdsfoot trefoil and alfalfa. I. Responses to height and frequency of cutting. *Crop Science* **7**: 130-133.

TILLEY, J.M.A. AND TERRY, R.A. (1963). A two-stage technique for the in vitro digestion of forage crops. *Journal of the British Grassland Society* **18**: 104-111.

VAN KEUREN, R.W. AND DAVIS, R.R. (1968). Persistence of birdsfoot trefoil, *Lotus corniculatus* L. as influenced by plant growth habit and grazing management. *Agronomy Journal* **60**: 92-95.

WAGHORN, G.C. AND SHELTON, I.D. (1997). Effect of condensed tannins in *Lotus corniculatus* on the nutritive value of pasture for sheep. *Journal of Agricultural Science,* Cambridge **128**: 365-372.

WOODFIELD, D.R. AND CARADUS, J.R. (1996). Factors influencing white clover persistence in New Zealand pastures. *Proceedings of the New Zealand Grassland Association* **58**: 229-235.

7. INTEGRATING DISCUSSION

7.1 INTRODUCTION

Birdsfoot trefoil (BFT) is recognised as a valuable feed source in many areas around the world, with special contribution in marginal areas, where limitations in tolerance of low fertility, low pH or drought limit the productivity of other legumes commonly used. Two major reasons for renewing interest in BFT nowadays are its value for low input systems and its high feed value particularly due to the presence of condensed tannins (Section 2.2.4.5). Despite extended research over decades, the weakness of BFT under intensive grazing and the incidence of crown-rot diseases are the main unsolved problems that limit long term persistence.

The development of controlled management strategies to improve productive persistence of BFT in pastoral systems of New Zealand and Uruguay provided the focus for this work. A series of four field and glasshouse experiments (Table 7-1) were conducted in Palmerston North, New Zealand (latitude 40°23' S) and Treinta y Tres, Uruguay (latitude 33°54' S) with the objective to determine appropriate defoliation strategies for different BFT cultivars, quantifying morphological and physiological plant adaptations under defoliation and analysing population dynamics and strategies to improve BFT persistence. Despite the importance attributable to disease incidence, this was not a specific objective in these experiments.

These objectives were explored over four BFT cultivars with contrasting plant structure. Defoliation strategies included a range of intensities between 2 to 10 cm and 4 to 10 cm under cutting and grazing, respectively (Table 7-1). Defoliation intervals of 20 and 40 days were contrasted in spring, and 20, 30 or 40 day intervals were applied in those cases when this was not studied as a variable. Timing of initial cutting in the year of establishment varied from vegetative to late mature stages, and swards evaluated varied from one to four years old, pure BFT (Experiment 1 to 3) or mixed with white clover and native grasses (Experiment 4).

Table 7-1. Description of experiments conducted in this project.

Experiment	Location	Defoliation variables	Pasture type/age	Period
1. (Chapter 3)	DRU, Massey University, New Zealand	Intensity (2, 6 and 10 cm) Frequency (20 and 40 days) with or without autumn rest	Pure, 3 years	April 1997 – December 1997
2. (Chapter 4)	PGU, Massey University, New Zealand	Intensity (2, 6 and 10 cm) Frequency (20 days)	Plants, 3 years	September 1997 – December 1997
3. (Chapter 5)	Palo a Pique, INIA, Uruguay	Intensity (4 and 8 cm) Frequency (40 days) Timing (vegetative, flowering , maturity) Cultivars (4)	Pure, 1-2 years	May 1998- April 2000
4. (Chapter 6)	Palo a Pique, INIA, Uruguay	Intensity (4 and 8 cm) Frequency (30 days) Grazing strategies (4)	Mixed, 3-4 years	April 1998- March 2000

DRU, Deer Research Unit; PGU, Plant Growth Unit; INIA, National Institute of Agricultural Research

The integrating discussion, which follows, is structured in sections:

(i) Evaluation of the characteristics, adaptability and performance of the four genotypes studied and the relevance of this information to future genotype developments.

(ii) Analysis of the effect of defoliation management on herbage production and plant survival, with particular reference to the intensity (main factor studied), frequency and timing of defoliation.

(iii) Assessment of the factors affecting sward persistence, with particular reference to elements of seed production and seedling recruitment which contribute to the maintenance of plant population density.

(iv) Development of a series of practical recommendations for seasonal and general management of BFT swards, drawing together the conclusions from the preceding sections.

7.2 BIRDSFOOT TREFOIL GENOTYPES

A wide range of variation in behaviour and production was observed between genotypes studied. Plant types varied from semi-erect and erect (San Gabriel and INIA Draco) to semi-prostrate types (Grasslands Goldie and Steadfast). Morphological parameters and biomass distribution varied over the experiments, genotypes reacting differently to defoliation. Shoot density is a desirable character in BFT to improve herbage production (Figure 3-5), intensive defoliation reducing the number of shoots per plant. Grasslands Goldie showed a higher density of primary and secondary shoots than the other genotypes (Section 5.4.8). The production of shoots could be associated with crown size, under the hypothesis that bigger crowns could develop more new shoots. However, the effects of defoliation on the rate of replacement of shoots are still unclear and should be more exhaustively studied.

The general effects of defoliation on the rate of replacement of intensively defoliated BFT plants (Tables 3-4 and 4-4, and Sections 5.4.8 and 6.4.4), suggest than those plants with a low ratio of above/below-ground biomass, a strong root system and a bigger crown could be more tolerant of severe defoliation, with the risk to be less productive. The role of root reserves could be enhanced in plants with well-developed root systems. The selection of genotypes with these improved characteristics could help to overcome persistence problems (Nora Altier, personal communication). However, the results showed that the performance of cultivars was primarily limited by environmental rather than morphological constraints.

Herbage production showed a wide range of variation between cultivars when compared in Uruguay, annual production of local cultivars being more than two times higher than introduced cultivars (Figure 5-2). The degree of winter activity of BFT genotypes at low latitudes (Uruguay) resulted in important differences in herbage production. New Zealand latitudes range from 33° to 47° S approximately, in contrast with Uruguay with latitudes between 30° to 35° S. Genotypes currently used in Uruguay (San Gabriel and INIA Draco) showed a degree of activity in winter and early spring that contributed to substantial advantages in production and periods of utilisation in the year over introduced cultivars. Winter dormant genotypes, in which production is concentrated from end of spring to late summer, are not recommended at low latitudes due to a short growing season. In New Zealand, there is available only one commercial BFT cultivar (Grasslands Goldie), adapted to grow at higher latitudes and under more extreme winter conditions like those occurring in environments of the South Island of New Zealand. However, in the North Island of New Zealand, at lower latitudes and under warmer conditions, there is scope to evaluate the potential of winter active genotypes. Despite the limited area of BFT sown in New Zealand, the study of other BFT cultivars adapted to specific environments could contribute to increase the interest in this species.

The novel development of a rhizomatous characteristic in cv. Steadfast offers opportunity to shortcut some of the reported problems that affect plant persistence in crown-forming plants, as has recently been demonstrated in red clover (Hyslop *et al.*, 1999). Steadfast showed a low potential of productivity (Figure 5-2, Table 5-6), probably due to the winter dormant characteristic, and the rhizomatous character is not an attribute of all individuals in the population. Nevertheless, breeding programmes could introduce the rhizome characteristic in other genotypes, like winter active types, opening more opportunities for BFT survival. The use of prostrate material, offering a certain degree of adaptation to intensive defoliation (Figure 5-8 c, d), requires more extensive evaluation with focus on the development of genotypes more tolerant to intensive grazing.

The options of BFT cultivars available for Uruguay cover reasonably well the requirements of adaptability, productivity and persistence. The breeding programme conducted by INIA is focusing on aspects of disease resistance and improved morphological characteristics at the same time, as was demonstrated with the recent release of INIA Draco.

In conclusion, the results obtained in this area are providing evidence about the importance of selecting genotypes for specific environments. More detailed information about the production, nutritive value and morphology of alternative cultivars is required, in particular for cultivars of recent release like INIA Draco and Steadfast where available information is limited.

7.3 DEFOLIATION MANAGEMENT, PRODUCTION AND PLANT SURVIVAL

The intensity, frequency and seasonal timing of defoliation are considered as the main factors involved in the development of defoliation strategies of BFT. The main emphasis in the current study was on the defoliation height and the timing of defoliation, defoliation interval being only partially explored because recent studies in New Zealand on the cultivar Grasslands Goldie have increased and clarified the information available (Bologna, 1996).

Extensive research over the last fifty years on the intensity of defoliation (Table 7-2) has shown that the effects of defoliation intensity are related to growth habit, upright plant types being more sensitive to intensive defoliation (Pierre and Jackobs, 1953). The available information in some cases showed advantages in the amount of herbage harvested when more intensive defoliation was applied (Table 7-2), in particular under extended periods of accumulation between defoliations (Cordeiro de Araujo and Jacques, 1974). However, productive persistence can not be maintained over time under intensive defoliation, production and stand density being reduced when an intensive defoliation strategy is applied for long periods of time. Thus, the general consensus is that *BFT can be defoliated frequently but not at high intensities* (Alison and Hoveland, 1989). The relatively greater importance of residual leaf area than root reserves for regrowth supports these recommendations (Alison and Hoveland, 1989).

Table 7-2. Summary of published research with emphasis in defoliation intensity on birdsfoot trefoil.

Authors	Defoliation treatments	Herbage production results
Pierre and Jackobs, 1953	2.5, 5 and 10 cm height	Close, frequent and late fall defoliation reduced BFT yield. Cultivars responded differentially
Duell and Gausman, 1957	2.5, 7.5 cm height 20-day intervals Pre-bloom to seed dehiscence stages	Herbage yield was 2.5>7.5 cm in Year 1, differences being reduced in Year 2
Twanley, 1968	5, 15 cm height	Herbage yield was greater for 5 cm height
Smith and Nelson, 1967	2.5, 7.6 and 15.2 cm height 3 to 6 times of defoliation during growing season	Herbage yield in Year 1 was 2.5>7.5>15.2 cm height, excepting 5 and 6 cuts treatments. During second year a higher stubble was needed to maintain yield independently of frequency
Greub and Wedin, 1971	3.8, 7.6 and 11.4 cm height	Herbage yield of 7.6 and 11.4 cm > 3.8 cm
Cordeiro de Araujo and Jacques, 1974	3, 6 cm height vegetative to flowering stages	Defoliation at more mature stages increased yield. Herbage yield of 3 cm > 6 cm if only 1 cut is applied, but 6 cm >3 cm if plants defoliated more than once
Alison and Hoveland, 1989	5 and 10 cm height 21, 28 and 42-day intervals 3, 5 and 10 cm height 21-days interval	Harvests at intervals of 21 days and at 3 cm height reduced herbage yield drastically

Results observed in the current project showed that in three of four studies, herbage production was greater at lax (6-10 cm) than hard (2-4 cm) defoliation (Figure 7-1). Reductions in herbage production and plant survival are the main consequences of intensive defoliation (Table 3-2, Figure 3-1, Table 4-2), for a semi-prostrate cultivar, suggesting a priori a strong effect for erect BFT types. There was a general decline in below-ground BFT plant parameters, resulting in a reduction in shoot density per plant and consequently in herbage yield (Figure 3-5). The reductions observed in root number and mass indicate that BFT plants subjected to intensive defoliation are limited in the capacity to capture resources (nutrients and water) for successful regrowth. Despite the recognised good production in summer and tolerance to drought conditions of BFT, these factors have influence in the survival of BFT in summer, as was confirmed by results obtained (Figure 6-2). Despite the short time nature of Experiments 1 and 2, and acknowledging this as a limitation from which to draw conclusions, the results were consistent in showing that plant survival and production declined quickly. These results, which were subsequently confirmed under grazing conditions (Chapter 6), emphasise the risks of defoliation below 4 cm irrespective of defoliation frequency.

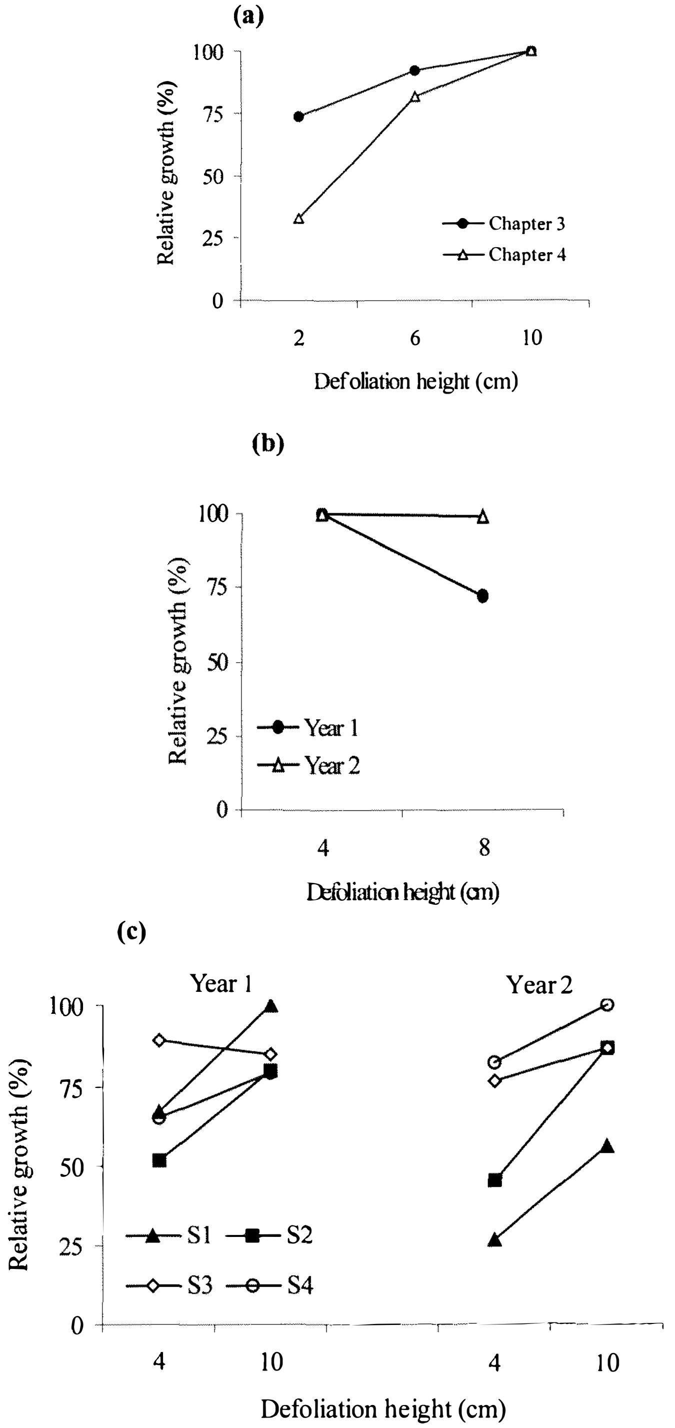


Figure 7-1. Results related to the effect defoliation intensity in BFT growth from information presented in (a) Chapters 3 and 4, (b) Chapter 5 and (c) Chapter 6.

In Experiment 3 herbage production increased by intensive defoliation (4 cm) if compared with more lax defoliation (8 cm) (Figure 7-1 b). Similar results were observed by Duell and Gausman (1957), Smith and Nelson (1967), Twanley (1967) and Cordeiro de Araujo and Jacques (1974), questioning previous evidence on the importance of lax defoliation. However, these results may be explained as follows. First, in all these studies, the interval of defoliation was relatively long (40 days), and/or swards received an extended rest from autumn to spring allowing plants to accumulate reserves and re-structure root systems for the next growing season. Secondly, it is apparent from Figures 5-7 and 5-8 (Section 5.4.5.2, Chapter 5), that there is a concentration of plant dry matter (mainly stems and shoots) in the low strata of the sward canopy in both erect and prostrate genotypes, but that almost all the entire leaf is carried higher in the canopy than 8 cm from soil level. Thus, evidence from plant structure suggests that residual leaf area is likely to be relatively insensitive to variation in height of defoliation. This suggestion is reinforced by the evidence from Experiment 3 (Table 5-8), where the amount of leaf remaining after cutting (Table 5-8) was higher for 8 cm than for 4 cm height, but the absolute values could be considered too low to promote a fast regrowth (0.13 and 0.43 of LAI for upright and semi-prostrate types respectively). In conclusion, these results demonstrate that the advantages of intensive defoliation (4 cm) are reduced over time or, as reported in other cases, lax defoliation (8 cm) is preferable if long-term experiments are analysed.

The impact of intensity of defoliation on BFT production is clearly influenced by defoliation interval, and by the duration of the treatment. For example, measurements over a full year demonstrated that when a monthly sequence of defoliation was applied, BFT declined in production, and advantages of lax defoliation (10 cm) increased over time (Year 2) (Figure 6-4). Rest periods in autumn or winter increased herbage production (Figure 7-1 c). Thus, the intensity of defoliation should be considered in association with defoliation intervals, in terms of defined grazing cycles. Defoliation intervals should be adjusted in accordance with seasonal patterns of growth. During active growth in spring, there were no advantages of extended (40 days) over short (20 days) intervals. The combination lax defoliation-extended interval was less productive than more intensive combinations in a short term experiment (Chapter 3) because of the increase in dry matter losses in the lax defoliation treatment.

Long term evaluations show that defoliation intervals between 30-42 days have advantages over short intervals (Bologna, 1996) when swards are defoliated to 4 cm height. When intensities of 4 and 10 cm where compared under 30-day intervals the advantages to the lax defoliation became more appreciable during the second year of defoliation (Figure 6-4), differences between intensities increasing for systems that received between 9 and 12 grazing periods in contrast with those that received 6 grazing periods.

The results of Experiments 1 and 2 (Sections 3.4.4 and 4.4.4), suggested that shoot population per plant was greater in plants defoliated to 6 cm than in those defoliated to height of 2 or 10 cm. As in lucerne (Keoghan, 1970), shoot population has been shown to have a dominant influence on plant growth (Figure 3-5). After defoliation, plants may compensate for the amount of herbage removed by increasing relative growth and developing new growth sites (stems), processes that have only a short term significance in BFT (Chapter 4). Intensive defoliation repeated over time will deplete the potential for plants to produce new shoots, but conversely lax defoliation will reduce the potential for development of new primary shoots by enhancing secondary shoots development. The finding that 6 cm stubble can increase shoot numbers per plant could be explained if defoliation is severe enough to promote the development of new primary shoots, and stubble height is enough to contribute with sites for the development of new secondary shoots if apical dominance is broken by cutting or grazing.

The practical significance of these effects will be influenced by the relative importance attached to concepts of production and plant survival. Altier (1997) observed that individual BFT plants can survive for only 2-3 years. BFT swards defoliated frequently (20 days) and close (2-4 cm) can not persist for long (Figure 3-1), and also the strategy S1 (grazing all year each 30 days) adversely affected productive parameters and plant survival. Persistent pastures can be achieved if adequate rest periods are allowed, but this may imply losses of production and quality. In extensive and low input systems, attention may be focused on “productive persistence” as a major objective, and depression in herbage production and utilisation may be acceptable consequences.

The timing of defoliation in the year of establishment did not affect persistence of BFT, but there were consequences to the amount and quality of herbage harvested. Delay in initial defoliation increased herbage accumulation, in accordance with previous reports (Table 7-2). However, herbage quality declined with the extension of accumulation (Tables 5-10 and 5-11). Digestible organic matter harvested did not increase after the flowering stage (mid-December) when a cultivar with an early spring growth was used (San Gabriel), but increased to advanced maturity (late January) for a later spring growth cultivar (INIA Draco). There were no substantial losses in quality under extended stockpiling (Tables 5-10 and 5-11), conferring more flexibility of management opportunity. The nutritive value of BFT, analysed for different management strategies, seasons and cultivars was in general good, reinforcing the high feeding value ascribed to BFT (Formoso, 1993).

In general, decline in BFT population was observed in the short term (Figure 3-1) under close defoliation, but less intensive defoliation (8-10 cm) required more time to express the same pattern (Figure 6-2 b). Unfortunately the decline in plant density as a result of drought conditions reduced the significance of the long term comparisons on Experiments 3 and 4 (Figures 5-9 and 6-2). It must be assumed that the combined effect of grazing and disease incidence will increase over time, resulting in an inevitable decline in stand density. Thus, the philosophy of management is how to model the "assumed and inevitable" decline in stand density (losses/outputs) with the potential inputs or gains of new individuals, and the defoliation intensity defining the rate of stand decline. These concepts provide the link to the following section, in terms of identifying the best alternatives to provide natural accessions of new plants to help to maintain a productive population.

7.4 THE ROLE OF THE SOIL SEED BANK ON POPULATION DYNAMICS AND PERSISTENCE OF BIRDSFOOT TREFOIL

The short-lived nature of BFT plants, early described by Pierre and Jackobs (1953), was confirmed in the four experiments of this project for different genotypes, plant ages, management conditions and environments, and indicate the need to develop strategies to replace the losses of individual plants in BFT swards. Stand decline is not only attributable to management practices (Table 3-1) and disease incidence (Altier, 1997). Adverse climatic conditions (Figure 5-9 and Figure 6-2) can produce drastic reductions in plant population density, in some cases independently of the most conservative management applied. Actually, the expectations of enhanced plant survival by breeding are no longer than three or four years (Rebuffo and Altier, 1996). The soil seed bank is the primary and only source of plant regeneration for traditional BFT types, before introducing pasture renewal alternatives. Understanding seed bank dynamics can help to define management strategies on BFT swards.

Results achieved in birdsfoot trefoil/white clover mixtures showed maximum seed inputs of 11100 viable seeds/m^2 of BFT following summer spelling, declining under drought conditions to less than 1800 seeds/m^2 of BFT. These disparities in seed inputs reinforce the importance of the buffer role of the seed bank for the maintenance of plant populations. BFT seed inputs are improved by summer spelling, and winter rest or lax grazing. The reproductive structures in BFT are disposed at the top of the sward, being easily eliminated by grazing, thus the spelling period is necessary to allow a complete sequence from flowering to seed maturity.

Based on the climatic variation reported (Table 5-3, Figure 5-1), the development of the soil seed bank should be promoted from early pasture stages, probably from the year of establishment, to reduce risks of population loss under adverse climate conditions. The soil seed reserves are variable, depending on the success of summer spelling over years. Seed reserves between 4000 to 7500 seeds/m^2, after a year of low seed input, were observed in swards four years old (Table 6-17). Despite the large values reported, soil seed dynamics are quite complex, and not all seeds are in condition to produce seedlings immediately because of dormancy mechanisms (Harper, 1977; Pearson and Ison, 1997). Preliminary results from this trial (Table 6-18), as well as other studies on legume seedling recruitment (Miller *et al.*, 1964; Bologna, 1996 and Arana and Piñeiro, 1999 among others) confirm the low efficiency of the emergence-recruitment processes. Low rates of seedling survival are also reported in the establishment of oversown legumes, sward competition, nodulation failures and low N_2 fixation being described as the most limiting factors (Lowther *et al.*, 1989), as well as climate conditions (Fraser et al., 1994). Additionally, low seedling vigour in BFT is a characteristic that limits rapid establishment (Twanley, 1967), but can be improved by breeding (Twanley, 1967; Frame *et al.*, 1998). BFT seed weight was not altered by defoliation management (Table 6-16), but an increase in seed weight is achieved by early closing (Bologna, 1996).

Under sward competition, recruitment from the soil seed bank is frequently lower than 10% (Carámbula *et al.*, 1994), a situation that demands management practices to increase seedling recruitment and the maintenance of a high soil seed bank to increase potential recruitment in absolute values. More detailed information is required for the conditions of Uruguay about patterns of seedling emergence and survival. Low winter temperatures may inhibit seedling survival, but on the other hand can contribute to break down dormancy of hard seeds. However, seedlings that emerge in spring have a limited survival in summer under drought conditions that can affect new plants with an undeveloped root system, as occurred in environments of South Island, New Zealand (Bologna, 1996).

There is a partial understanding of the soil seed bank dynamics and natural reseeding processes for oversown birdsfoot trefoil/white clover mixtures. Knowledge of processes that occur in the soil seed bank, and patterns of seedling emergence and seedling survival, will contribute to determine strategies for spelling frequency between years, required seed reserves and management to increase the efficiency of recruitment. On the mixed swards studied (Chapter 6), the manipulation of spelling processes could give opportunities to manipulate the balance between species of interest. The percentages of potential seedling emergence (35-44%) over a period of approximately 6 months, under reduced sward competition, demonstrated the potential of the soil seed bank for pasture renewal in low density stands. If there is a reasonable density of seeds in soil reserves of species of interest, reductions of competition by grazing, application of herbicides, soil disturbance or eventually fire could promote the emergence of desirable seedlings increasing stand density.

In conclusion, the levels of seed production in BFT reported in this study are enough to develop large soil seed reserves. However, the establishment of new plants was limited by a low efficiency of seedling recruitment. These results focus the discussion about the value of a seed bank for plant recruitment to maintain sward productivity and sward persistence. In fact, the management of a BFT stand to increase soil seed reserves by summer spelling is in conflict with the requirements to achieve the maximum productivity and efficiency of utilisation in a short period of time. The emerging questions of this strategy relate to the balance between the economic benefits of increasing pasture life by adjustment of grazing to enhance natural recruitment, or conversely establishing more intensive systems where pasture renewal is considered as a component of the management package. Results obtained were relevant in terms of the provision of information for extensive systems of Uruguay. The available information in this area is limited (Olmos, 1996; Arana and Piñeiro, 1999), and this thesis is basically one of the first reports to quantify reproductive processes for birdsfoot trefoil/white clover mixtures in Uruguay. This important issue was discussed in Chapter 6, and will not be considered further here.

Clarification of these options requires more intensive research. The dynamics of soil seed reserves, patterns of emergence, and seed inputs and outputs are crucial to elaborate strategies to extend the productive life of BFT swards. In addition, reproduction and dynamics of BFT plants under grazing need to be quantified to ensure that recruitment processes constitute a viable option to maintain replacement rates of plants. The developing knowledge in this area will contribute to the production of predictive models of plant and seed dynamics (Emery *et al.*, 1999), including the effects of environmental factors and grazing.

7.5 PRACTICAL MANAGEMENT RECOMMENDATIONS

Based on available knowledge of BFT management (Chapter 2) and findings reported in this work (Chapter 3 to 6), a series of recommendations can be formulated to increase the productive persistence of birdsfoot trefoil pastures.

7.5.1 Seasonal management

Autumn

Autumn is considered a critical time for BFT, because many of the decisions made in autumn can have a carryover effect on production and survival in the following seasons, particularly in winter and spring. Autumn is the time where BFT plants rebuild root reserves (Nelson and Smith, 1968) which contribute to improved winter plant survival and early spring regrowth. Under the conditions evaluated, winter survival was not affected by autumn defoliation (Figure 3-1), probably because winter temperatures in the experimental sites were not so low as to affect plant survival. However, improved spring regrowth was observed if BFT received an early autumn rest (Table 3-2).

In autumn, BFT plants develop new shoots from the crown, and these contribute to spring growth (Bologna, 1996), as occurred with BFT plants that received an autumn rest in Year 1 of management of the BFT/WC mixture in Experiment 4 (Tables 6-10 and 6-11). The deferment of defoliation of BFT from early to mid-autumn constitutes an adequate practice in terms of the transfer of herbage of high quality to winter, without effects on the persistence of BFT in BFT/WC mixtures. When stand density is reduced, intensive defoliation in early autumn will improve the recruitment of new BFT plants from the soil seed bank.

Recommendation:
Management of established BFT stands should avoid intensive (4 cm) and late defoliation in autumn (June).

Winter

Winter management will be influenced by the type of cultivars in use and the place of use. For Uruguay and when BFT is used in mixtures with white clover, winter rest showed advantages for annual herbage production of BFT and seed production in the following summer. When BFT is used as a pure stand, the recommendation is for utilisation in spring-summer, with a rest during winter. This scheme is followed in New

Zealand for winter dormant cultivars. Findings on winter active lucerne cultivars in New Zealand showed that despite the advantages in winter yield of these materials, winter grazing resulted in significant reductions in spring growth compared with winter dormant types (White and Lucas, 1990). For winter active cultivars growing at low latitudes (Uruguay), the avoidance of winter grazing did not show as great advantages as those resulting from autumn rest.

Recommendation:

Rest periods in winter will allow increased annual herbage production and seed yields of BFT swards, and reduce the effects of intensity of defoliation.

Spring

During the active growth period in spring, it is not recommended to use long defoliation intervals. Otherwise, if extended intervals (40 days) and lax intensities (10 cm) are combined, herbage losses will increase, particularly in low sward strata (Chapter 3), and a short grazing season will result (approximately two grazing periods in the season). Short intervals (20- 30 days) demonstrated advantages in herbage production if lax defoliation (10 cm) was applied. In those swards that received a previous autumn rest, early and high spring growth could be expected, in comparison with swards more intensively grazed in autumn.

Recommendation:

A range between 6-10 cm defoliation height in combination with 20-30 days defoliation intervals should achieve high growth rates, high efficiency of utilisation of herbage produced, avoiding herbage losses and without risks in BFT persistence.

Summer

BFT can be managed under two contrasting criteria in this season. First, dense and pure BFT swards can contribute with a high production because summer is a period of active growth and adequate levels of forage quality. In those regions where BFT has a restricted grazing season (spring-summer), swards have an extended rest period of around 6 months in the year, thus plants can replenish adequately root reserves and below-ground biomass for the next growing season and are more tolerant of defoliation in summer.

However, in those environments where a year-round defoliation is practised, in mixed swards and relatively old or less dense stands, a period of 60-70 days for seed spelling starting in early December is a recommended alternative to increase persistence. Also, lax defoliation increases summer herbage production.

Recommendation:
The maintenance of BFT swards in summer should be associated with moderate defoliation intensities (≥ 6 cm) or even the application of rest periods to encourage seed production and to promote seed bank reserves for the maintenance and stability of stand.

7.5.2 General management

The seasonal recommendations formulated above need to be integrated and prioritised when an annual grazing plan is defined for BFT. In this context, defoliation management should avoid late autumn grazing (May-June) to encourage plants to build root reserves and shoot development. In winter, defoliation can be practised for winter active cultivars without excessive risks in sward persistence, particularly when BFT is in mixtures. Frequent (20 days) and moderate intensities (6-10 cm) are proposed for spring to achieve adequate utilisation. In the rest of the year, frequency should be between 30-40 days approximately. For summer, management should be adjusted in response to stand density and soil seed reserves. In the case of poor stands or reduced soil seed reserves a spelling period for 60-70 days from early December is recommended.

7.6 CONCLUSIONS

The most important conclusions from the current research program about defoliation management to improve productivity and persistence of birdsfoot trefoil cultivars are summarised as follows, providing suggestions for future research in areas where this is required.

i) Intensive defoliation should be avoided, because it will result in a decline in herbage production and plant survival even over short periods of time. The reduction in shoot density by intense defoliation contributes to reductions in herbage production, root mass and crown size. A more detailed understanding is required of the physiological mechanisms and genetic variability involved in the production of new shoots, and the influence of defoliation on the development of primary and secondary shoots.

ii) BFT plants have a limited and short term plasticity in response to defoliation, observed by the increase in relative growth rate, leaf area ratio, specific leaf area and number of leaves per plant.

iii) Autumn rest of BFT swards contributes to improve herbage and seed production, and may also influence plant survival in more extreme winter conditions.

iv) In spring, defoliation management should be based on intensities between 6-10 cm height and 20-30 days intervals.

v) There is a high degree of morphological variation between BFT cultivars. The evidence suggests that crown size and root mass could be variables to be used in breeding programmes to select cultivars with improved persistence. The presence of rhizomes is a desirable character in BFT to increase persistence, but it would be valuable to introduce this character in winter active BFT cultivars in order to combine improved herbage production potential and stand survival.

vi) Levels of seed production in BFT are not generally limiting to the development of adequate soil seed reserves. Seed spelling in summer and a winter rest improved seed BFT yields.

vii) The limited efficiency in seedling emergence under grazing conditions raises questions about the real value of the soil seed bank. These findings indicate the need for increased research in this area, to develop strategies to promote a more effective recruitment process.

viii) BFT has a recognised place among legume options for direct grazing in pastoral systems of Uruguay. Its relevance is based on extended adaptability to soil conditions and tolerance of low soil phosphate status. However, the additional advantages that BFT herbage confers to animal production by the presence of condensed tannins are not fully understood and applied. The value of BFT in pure swards or in mixtures with white clover, achieving advantages in herbage utilisation, bloat control and anthelmintic effects, justify further studies on management strategies.

ix) In New Zealand, BFT constitutes an alternative legume species, and the only BFT cultivar is a winter dormant type with a defined grazing season during spring-summer. The limited tolerance to intensive defoliation and competitive capacity limit the grazing season, in comparison with other alternative species. BFT is grazed for around 6 months of the year, with the remaining 6 months under rest. The reduced period of utilisation and poor tolerance to intensive grazing and poor herbage production in comparison with traditional species, means that farmers do not find BFT a good option to increase profitability. The development of cultivars more competitive and tolerant to grazing could improve interest in BFT for New Zealand farming. The potential of winter active cultivars in some environments should be tested.

7.7 REFERENCES

ALISON, M.W. AND HOVELAND, C.S. (1989 b). Root and herbage growth response of birdsfoot trefoil entries to subsoil acidity. *Agronomy Journal* **81**: 677-680.

ALTIER, N. (1997). Enfermedades del Lotus en Uruguay. (Lotus diseases in Uruguay). INIA La Estanzuela, Uruguay. *Serie Técnica* No. **93**. ISBN: 9974-38-083-9. 16 pg.

ARANA, S. AND PINEIRO, G. (1999). Déficit hídrico y manejo: su influencia en la demografía y producción del trébol blanco. (Water deficit and management: the influence in the demography and production of white clover). Tesis Facultad de Agronomía. Montevideo Uruguay.

BOLOGNA, J.J. (1996). Studies on strategies for perennial legume persistence in lowland pastures. *Thesis of Master of Agricultural Science.* Lincoln University. New Zealand. 220 p

CARAMBULA, M., CARRIQUIRY, E. AND AYALA, W. (1994). Siembra de Mejoramientos en Cobertura. (Establishment of oversown pastures). In *Boletín de Divulgación* No. 46. 1994. INIA. ISBN: 9974-38-015-4. 20 pg. Junio 1994.

CORDEIRO DE ARAUJO, J. AND JACQUES, A. (1974). Características morfológicas e producào de matéria seca de cornichào (*Lotus corniculatus* L.) colhido em diferentes estádios de crescimento e a duas alturas de corte. (Morphology and production of dry matter of birdsfoot trefoil (*Lotus corniculatus* L.) at different growth stages and under two defoliation heights). *Revista da Sociedade Brasileira de Zootecnia.* Vol. **3**(2): 138-147.

DUELL, R.W. AND GAUSMAN, H.W. (1957). The effect of differential cutting on the yield, persistence, protein and mineral content of birdsfoot trefoil. *Agronomy Journal* **49**: 318-319.

EMERY, K.M., BEUSELINCK, P., AND ENGLISH, J.T. (1999). Evaluation of the population dynamics of the forage legume *Lotus corniculatus* using matrix population models. *New Phytologist* **144**: 549-560.

FORMOSO, F. (1993). *Lotus corniculatus.* I. Performance forrajera y características agronómicas asociadas. (Productive performance and agronomic characteristics). *Serie Técnica* No. 37. INIA Uruguay. ISBN: 9974-556-69-4 20 pg.

FRAME, J., CHARLTON, J.F.L. AND LAIDLAW, A.S. (1998). Birdsfoot Trefoil and Greater Lotus. Chapter 6. In *Temperate Forage Legumes.* CAB International, Wallingford. ISBN 0-85-199-214-5. pp. 245-271.

FRASER, W.J., OGDEN, S.C., WOODMAN, R.F. AND LOWTHER, W.L. (1994). Role of re-seeding and seedling recruitment for sustainable *Lotus corniculatus* based pastures in dry hill and high country. *Proceedings of the New Zealand Grassland Association* **56**: 139-142.

GREUB, L.J. AND WEDIN, W.F. (1971). Leaf area, dry-matter accumulation, and carbohydrate reserve levels of birdsfoot trefoil as influenced by cutting height. *Crop Science* **11**: 734-738.

HARPER, J.L. (1977). *Population Biology of Plants.* Academic press. New York. p. 84.

HYSLOP, M.G., KEMP, P.D. . AND HODGSON, J. (1999). Vegetatively reproductive red clovers (*Trifolium pratense* L.): An overview. *Proceedings of the New Zealand Grassland Association* **61**: 121-126.

KEOGHAN, J.M. (1970). The growth of lucerne following defoliation. *Thesis of Doctor of Philosophy.* Lincoln College, New Zealand. 383 p

LOWTHER, W.L., HOGLUND, J.H. AND MACFARLANE, M.J. (1989). Aspects that limit the survival of legume seedlings. In *Persistence of Forage Legumes.* Edited by G.C.Marten, A.G. Matches, R.F. Barnes, R.W. Brougham, R.J. Clements and G.W. Sheath. Proceedings of the Trilateral Workshop, Honolulu, Hawaii. July 1988. ISBN: 0-89118-098-2. pp. 265-275.

MILLER, J.D., KREITLOW, K.W., DRAKE, C.R. AND HENSON, P.R. (1964). Stand longevity studies with birdsfoot trefoil. *Agronomy Journal* **56**: 137-139.

NELSON, C.J. AND SMITH, D. (1968). Growth of birdsfoot trefoil and alfalfa. III. Changes in carbohydrate reserves and growth analysis under field conditions. *Crop Science* **8**: 25-28.

PEARSON, C.J. AND ISON, R.L. (1997). Generation. Chapter 3. *Agronomy of Grasslands Systems.* 2nd Edition. Cambridge University Press. pp. 37-59.

PIERRE, J.J. AND JACKOBS, J.A. (1953). The effect of cutting treatments on birdsfoot trefoil. *Agronomy Journal* **45**: 463-468.

REBUFFO, M. and ALTIER, N. (1996). *Lotus corniculatus* L. LE 65-56 (INIA Draco, a posteriori). Boletín Interno. (*Lotus corniculatus* L. LE 65-56. Internal report). Programa Pasturas. INIA La Estanzuela, Uruguay. 6 pg.

SMITH, D. AND NELSON, C.J. (1967). Growth of Birdsfoot Trefoil and Alfalfa. I. Responses to height and frequency of cutting. *Crop Science* **7**: 130-133.

TWAMLEY, B.E. (1967). Seed size and seedling vigour in birdsfoot trefoil. *Canadian Journal of Plant Science* **47**: 603-609.

TWAMLEY, B.E. (1968). A management study of a birdsfoot trefoil strain trial. *Journal of the British Grassland Society* **23**: 322-325.

WHITE, J.G.H. AND LUCAS, W.J. (1990). Management of lucerne in the cool season. *Proceedings of the New Zealand Grassland Association* **52**: 41-43.

APPENDIX I

METHOD TO MEASURE TOTAL AVAILABLE CARBOHYDRATES

Extraction with perchloric acid and reaction with anthrone

Clegg (1956) adopted the method of McCready *et al.*, (1950) including the extraction of simple sugars with aqueous ethanol and starch with perchloric acid from the residue.

Reagents

Ethanol: 80% (v/v)

Perchloric acid: 52% (v/v)

Anthrone reagent: 1 ml of anthrone (9,10-dihydro-9-oxoanthracene) and 10 g of thiourea was dissolved in one litre of H_2SO_4 (76%) and stored at 0-4 °C. The colour of reagent increases with the time. Reagent can be used for two weeks (Southgate, 1991).

Glucose standards: A standard glucose solution was diluted to give a series of standards (6.25, 12.5, 25, 50, 100, 200, 400 and 800 µg/ml).

Procedures

a. Extraction of sugars (glucose, fructose and sucrose)

0.1 g of fine dried ground sample was put in a 50 ml centrifuge tube, with two drops of 80% (v/v) ethanol to moisten the sample, then add 2.5 ml of water and stir thoroughly. Add 12.5 ml of hot 80% (v/v) ethanol, stir for 5 min and centrifuge at 5000 rpm for 10 min. Decant the supernatant and store, then repeat the extraction adding 15 ml of hot 80% (v/v) ethanol. Decant the supernatant and combine with the first extraction and then remove the ethanol by evaporation at reduced pressure. Filter the mixture and store in a 10 ml volumetric flask adding water to make the volume 10 ml. Dilute the solution to 1/50 dilution, taking 0.2 ml of sample and adding 9.8 of water.

b. Extraction of starch (amylose and amylopectin)

Add 2.5 ml of water to the pellet after the ethanol extraction. Then 3.5 ml of perchloric acid (52%, v/v) and stirring the mixture for 5 min. After that, it was stirred intermittently for 15 min, following the addition of 10 ml of water and centrifuge at 5000 rpm for 10 min. Decant the supernatant and store in a 50 ml volumetric flask. Re-extract the residue as before and combine the supernatant with the first extraction adding water to make the final volume 50 ml. Filter for anthrone analysis.

c. Analysis of extracts

For blank and each standard solution add 0.25 ml of solution and then 5 ml of anthrone reagent in tubes with rubber stoppers. For the samples take 0.25 ml of solution, adding 5 ml of anthrone in tubes with rubber stoppers. The content of tubes are mixed and heated for 12 min in a boiling water-bath. Then, tubes are placed in dark for 25 min and finally the absorbance of the solution is measured at 620 nm.

APPENDIX II

METHOD TO EVALUATE SOIL SEED RESERVES

The method used is based on direct counting of seeds in soil, following a technique used by Prestes (1995).

Procedure

1. *Collection of samples.*

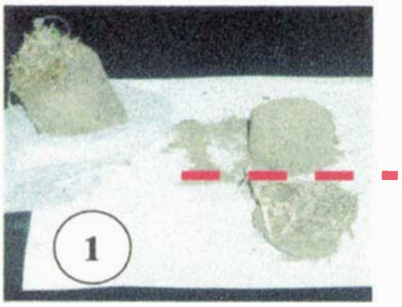
Soil cores (22.9 cm^2 x 5 cm depth) are taken randomly in the field.

2. *Laboratory analysis*

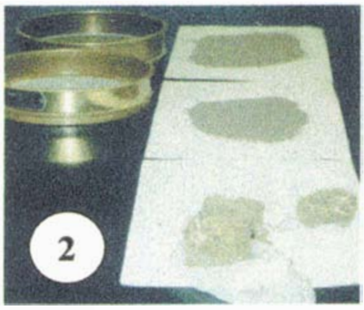
The sequence of procedures is represented in Figure 1.

1. Samples are hand crumbled
2. Then, samples are sieving in a series of standard sieves from 4.76 to 0.5 mm aperture
3. The remaining material is passed through on air flow to eliminate tiny particles
4. The material is disposed in a becker of 250 ml, adding ethylene cloride (C_2Cl_4). This is a high density solvent (1.6) separating organic from inorganic material. Finally, from this supernatant material seeds are hand sorted, separating seeds of *Lotus corniculatus* and *Trifolium repens* and discarding others.

After separation, seeds are counted, weighed and germination tests performed.

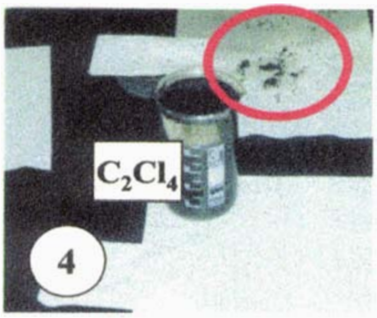


Hand crumbled

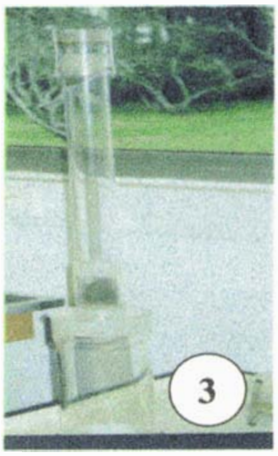


U.S. Standard sieve series
No. 4 - 4,76 mm - Tyler eq. 4 mesh
No. 11 - 2 mm - Tyler eq. 10 mesh
No. 35 - 0.5 mm - Tyler eq. 32 mesh

Hand sorting



Becker 250 ml, High density solvent
Promote separation of organic from
inorganic material



Air Flow

Plate 1. Soil seed bank analysis