UNIVERSIDADE FEDERAL DO PARANÁ

TIAGO CELSO BALDISSERA

O AMBIENTE LUMINOSO: DO IMPACTO NO CRESCIMENTO E DESENVOLVIMENTO EM NÍVEL DE PLANTA FORRAGEIRA A DOSSÉIS EM SISTEMAS INTEGRADOS DE PRODUÇÃO AGROPECUÁRIA

CURITIBA
2014

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Tese apresentada ao Curso de Pós-Graduação em Agronomia, Área de concentração em Produção Vegetal, Setor de Ciências Agrárias, Universidade Federal do Paraná, como requisito parcial para obtenção do título de Doutor em Ciências.

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CURITIBA
2014

UNIVERSIDADE FEDERAL DO PARANÁ
SETOR DE CIÊNCIAS AGRÁRIAS
PROGRAMA DE PÓS-GRADUAÇÃO EM
AGRONOMIA - PRODUÇÃO VEGETAL

PARECER

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em Agronomia - Produção Vegetal, reuniram-se para realizar a argüição da Tese de DOUTORADO, apresentada pelo candidato **TIAGO CELSO BALDISSERA**, sob o título **"O AMBIENTE LUMINOSO: IMPACTO NO CRESCIMENTO E DESENVOLVIMENTO EM NÍVEL DE PLANTA FORRAGEIRA A DOSSÉIS EM SISTEMAS INTEGRADOS DE PRODUÇÃO AGROPECUÁRIA"**, para obtenção do grau de Doutor em Ciências do Programa de Pós-Graduação em Agronomia - Produção Vegetal do Setor de Ciências Agrárias da Universidade Federal do Paraná.

Após haver analisado o referido trabalho e argüido o candidato são de parecer pela "**APROVAÇÃO**" da Tese.

Curitiba, 21 de Março de 2014.

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RESUMO

Os sistemas de produção devem atender a demandas quantitativas e qualitativas na produção de alimentos. Contudo, devem também contemplar exigências de sustentabilidade. Nos arranjos produtivos existem diversas formas e estratégias de cultivo, dentre elas a integração de cultivos numa mesma área e ao mesmo tempo. Entretanto, as diferentes espécies competem pelos recursos do ambiente, dentre eles a luz, que é considerada um dos principais fatores que interferem na arquitetura das plantas e na dinâmica do dossel vegetal. Deste modo, o objetivo central deste trabalho foi de estudar os processos de crescimento e desenvolvimento de espécies forrageiras em ambientes com alterações das condições de luz. Os primeiros dois capítulos da tese avaliam o efeito das árvores, em integração lavoura-pecuária, sobre o crescimento e desenvolvimento de: *Axonopus catharinensis*, *Brachiaria brizantha* cv. Marandu, *Megathyrsus maximus* cv. Aruana, *Hemarthria altissima* cv. Flórida, *Cynodon* spp. hibrido Tifton 85 e *Paspalum notatum* cv. Pensacola. O terceiro capítulo aborda o efeito da luz azul no crescimento e desenvolvimento de genótipos de alfafa (*Medicago sativa*). O quarto capítulo avalia os efeitos da competição por luz em estandes puros e mistos de alfafa com festuca, verificando quais processos mais interferem na expansão da área foliar da alfafa. Foi possível concluir que os mecanismos de resposta ao efeito de árvores em interação com nitrogênio são espécie-dependentes e apresentam consequências para o manejo do pasto em sistemas integrados com árvores. O efeito da luz azul foi mais significativo nas alterações das características morfológicas quando o genótipo de alfafa tinha hábito de crescimento ereto, que apresenta características de mecanismo de escape a sombra. As diferenças na área foliar total de plantas de alfafa é dependente principalmente da ramificação lateral dos ramos principais e do número de ramos, mais do que do tamanho específico de cada folha.

Palavras chave: integração lavoura-pecuária; manejo de pastagens; dossel forrageiro; competição; interceptação luminosa

ABSTRACT

Production systems should meet the quantity and quality demands on food production. However, should include the maintenance of production sustainability requirements. There are several ways and strategies for production systems, some types of them is the consortium of species in the same area and in the same temporal scale. However, different species compete for environmental resources, including light, which is considered one of the main factors that affect plant architecture and dynamics of plant canopy, and may have consequences for production and also for the management strategies. Thus, the aim of this study was to evaluate the growth and development of forage species in different light environmental conditions. The first two chapters of this thesis evaluates the effect of trees and nitrogen in an integrated crop-livestock system, on the growth and development of tropical C_4 grasses: *Axonopus catharinensis*, *Brachiaria brizantha* cv. Marandu, *Megathyrsus maximus* cv. Aruana, *Hemarthria altíssima* cv. Flórida, *Cynodon* spp. hibrido Tifton 85 e *Paspalum notatun* cv. Pensacola. The third chapter discusses the effect of blue light on the growth and development of contrasting genotypes of alfalfa (*Medicago sativa*). The fourth chapter evaluates the effects of competition for light in pure and mixed stands of alfalfa with grass, and which processes more interfere in the expansion of alfalfa leaf area. It was possible to conclude that there is species dependence for the responses of growth and development due to the effect of shading by trees and nitrogen, with consequences for the management of these species in a integrated system with trees. The blue light effect resulted in more significant changes of the morphological characteristics on the genotype of erect growth habit, showing the trend that this genotype has characteristics to escape shade. The effect of light competition in pure stand of alfalfa is greater than in consortium with grass, differences in leaf area of alfalfa is dependent mainly on lateral branching and number of shoots, harder than the leaf size.

Key words: integrated crop-livestock system; pasture management; forage canopy; competition; light interception

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SUMÁRIO

1. INTRODUÇÃO

Sistemas intensivos de produção requerem altos níveis de energia na forma de trabalho e insumos. Contudo, muitos desses sistemas apresentam respostas incompatíveis com as emergentes demandas por sustentabilidade.

O uso de sistemas integrados de produção agrícola e pecuária[1] constituem a melhor alternativa para atingir a sustentabilidade, segundo a FAO (Food and Agriculture Organization of the United Nations – 2010). A característica diferencial é que estes sistemas de produção são planejados para explorar sinergismos e propriedades emergentes frutos de interações nos compartimentos solo-planta-animal-atmosfera de áreas que integram atividades de produção agrícola e pecuária (Moraes et al., 2012).

Entre as principais peculiaridades que conferem esse predicado aos sistemas integrados estão: redução da degradação química, física e biológica do solo; aumento da atividade microbiológica e taxa de mineralização e reestruturação do solo; aumento da matéria orgânica do solo; equilíbrio no ciclo de pragas e doenças; redução de uso de agrotóxicos; maior ciclagem de nitrogênio e outros nutrientes; aumento do índice de conforto térmico animal; melhor retenção da umidade solo; proteção contra erosão; sequestro de carbono atmosférico; aumento da biodiversidade e da resiliência dos agroecossistemas (Pagiola et al, 2007; Bernardino e Garcia, 2009; Balbino, 2011; Moraes et al., 2014).

Sendo assim, o aproveitamento das interações em sistemas de produção integrados é chave para obtenção de sucesso, tendo como resultado final maior sustentabilidade e produtividade total por unidade de área (Nair, 2011). Nesse sentido, as interações devem ser planejadas em diferentes escalas espaço-temporais e abranger a exploração de cultivos agrícolas e produção animal na mesma área de forma concomitante ou sequencial, entre áreas distintas ou em sucessão (Moraes et al., 2012).

Porém, é necessário o conhecimento e entendimento dos efeitos das interações entre os fatores bióticos e abióticos envolvidos e, também, considerar sua dinâmica e as características peculiares de cada ambiente, analisando-os de forma sistêmica. Quando as plantas estão crescendo em comunidade, experimentam ambiente luminoso heterogêneo em termos de quantidade e qualidade de luz. A luz é considerada um dos principais fatores que interferem

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[1] Nesta Tese adotou-se a terminologia Sistemas Integrados de Produção Agropecuária (Moraes et al., 2012) para designar sistemas que conjugam os componentes pecuária e lavoura, o primeiro sendo obrigatório e o segundo podendo se constituir de diferentes cultivos, árvores inclusive. São concebidos para explorar sinergismos e propriedades emergentes e conhecidos comumente como Integração Lavoura-Pecuária. Diferem dos sistemas Silvipastoris e Agrosilvipastoris.

na arquitetura das plantas e dinâmica do dossel vegetal, podendo trazer conseqüências para a produção e também para o manejo das pastagens.

Por exemplo, no caso de sistemas integrados com a presença do componente arbóreo, o ambiente luminoso no interior do sub-bosque é continuamente modificado. São relatadas reduções na produção de biomassa e alterações na qualidade da forragem com a redução da intensidade luminosa, pois o sombreamento imposto a pastagem é considerado o fator isolado que mais reduz o desempenho produtivo do componente forrageiro (Lin et al., 1999; Feldhake et al., 2009). Associado aos efeitos do sombreamento, a ocupação de nichos ecológicos similares que são disputados pelas diversas espécies envolvidas pode gerar diferentes níveis de competição entre plantas, caso não sejam adequadamente planejados.

Muitos trabalhos desenvolvidos a partir de 1980 já se concentravam na busca de informações sobre interceptação e uso da radiação em sistemas silvipastoris (Rao et al., 1998). Alterações na quantidade de radiação solar incidente em sub-bosques silvipastoris têm sido estudadas por vários grupos de pesquisa no mundo (Bergez et al., 1997; Knowles, 1999; Silva-Pando et al., 2002; Burner e Belesky, 2004; Feldhake et al., 2009; Lacorte e Esquivel, 2009; Varella et al., 2010).

Em termos qualitativos, a radiação que atinge o estrato herbáceo do sub-bosque, após a absorção ou reflexão pela copa e tronco das árvores, também é alterada, pois há absorção preferencial das porções vermelha e azul do espectro solar pelo dossel arbóreo. Assim, a radiação incidente no sub-bosque apresenta maior proporção de comprimentos de onda cor-de-laranja, amarelos, verdes e vermelho distante. Essas alterações qualitativas no espectro da radiação que atinge o estrato herbáceo são as principais responsáveis pelas respostas morfofisiológicas das plantas crescendo em sub-bosques, em comparação com o crescimento em ambiente aberto (Cruz, 1997; Healey et al., 1998; Varella et al., 2010). Sob esse cenário, a plasticidade e / ou adaptação morfofisiológica das plantas assumem papel fundamental na persistência das espécies neste ambiente.

Portanto, a escolha das espécies forrageiras que irão compor os sub-bosques em sistemas integrados com componente arbóreo é fundamental, pois aquelas espécies serão submetidas a condições de luminosidade reduzida e desfolha freqüente, tendo que manter produção e valor nutritivo para que sejam viáveis agronômica e economicamente.

A composição genética e a flexibilidade fenotípica irão determinar a capacidade das espécies em se adaptar ao estresse oriundo do processo de competição. Dentre algumas das respostas gerais das plantas a alterações da quantidade e da qualidade da luz estão os efeitos que maximizam a captação da luz, a otimização da estrutura em relação parte aérea:raiz, aumento no comprimento dos colmos, além de alterações na morfologia e anatomia das folhas (aumento da área da folha, maior área foliar específica). Todas essas alterações podem levar, por exemplo, a mudanças na composição da comunidade vegetal, ou também diminuição da persistência das pastagens, com reflexos no manejo e na produtividade.

Está tese está organizada em capítulos que tratam, de diferentes formas, o objetivo geral de avaliar o efeito das mudanças do ambiente luminoso sobre o crescimento e o desenvolvimento de espécies forrageiras.

Os objetivos específicos referentes a cada capítulo são:

- Capítulo 1: Verificar como às árvores, em sistema integrado, afetam a estrutura do dossel forrageiro de gramíneas C_4 tropicais;
- Capítulo 2: Avaliar a dinâmica dos processos morfogênicos e de crescimento de gramíneas C_4 tropicais sob árvores em sistema integrado;
- Capítulo 3: Mensurar o efeito da luz azul na morfologia e no crescimento da alfafa;
- Capítulo 4: Determinar quais os processos morfogênicos mais afetados e que influenciam a área foliar total da alfafa em estandes puros ou em consórcio com gramínea.

2. CAPÍTULO 1

Trees canopy and N supply effect on sward height of tropical C_4 grasses[1]

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[1] Elaborado de acordo com as normas da Revista Agroforestry Systems.

Trees canopy and N supply effect on sward height of tropical C_4 grasses

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Abstract

A study was conducted over two years to determine the influence of shading provided by trees (*Eucalyptus dunnii*) canopy and nitrogen availability (0 and 300 kg N ha^{-1} $year^{-1}$) on pasture sward height at 95% light interception (LI), since this is a valuable strategy of defoliation frequency to deal with the variability of herbage accumulation throughout the year, particularly with C_4 grass pastures. Six perennial tropical forage species were compared. Plots were cut at 95% LI, and the residual kept was 50% of the sward height at 95% of LI. The effect of trees caused increases in stem and leaf size, and decreases in tiller density and leaf stem ratios. Therefore, species growing in the system with trees showed taller sward heights, except *Paspalum notatum* and *Megathyrsus maximus* that did not show differences between treatments, particularly in the first year of evaluations. As sward height at 95% of LI was variable as a function of shading and nitrogen fertilization, and showing species-dependency, caution is deserved to management targets based on LI. Results suggest that in integrated crop-livestock systems with trees the sward height would be higher for species that are influenced by shading or nitrogen.

Key words: management; light; integrated crop-livestock systems; shade avoidance syndrome

1. Introduction

The global features are in a transition state with regards to land use and natural resources, turning attention to production systems that meet quantitative and qualitative standards for food production and energy generation, without excluding the environment preservation (Malézieux et al. 2009). In this context, the integrated crop-livestock systems (ICLS) appear to be an interesting alternative to enhance productivity and provide environmental services (O´Mara, 2012; Sanderson et al. 2013).

The renewed interest in ICLS is primarily because they provide opportunities for the diversification of rotations, perenniality, nutrient recycling, and greater energy use efficiency (Entz et al. 2005). So, since middle 80's, these production systems are receiving increasing attention as a sustainable land-management option worldwide (Nair et al. 2011). Due to its ecological, economic, and social attributes, ICLS can positively change the biophysical and socio-economic dynamics of farming systems (Keulen and Schiere 2004), becoming more efficient systems than monocrops (Nair, 2011).

ICLS are systems that can intentionally integrate trees, forage crops, and livestock into a structural practice of planned interactions (Clason and Sharrow, 2000). These integrated systems can promote biodiversity, for example, via organic matter provided by pastures (Lemaire et al. 2003), and especially on no-till systems (Carvalho et al. 2011).

An important aspect associated with the incorporation of tree species in pastures (or vice-versa) is microclimate changes imposed by trees canopy, which can affect plant growth and, consequently, the sward dynamics. For instance, the light quantity (i.e. photon flux density) and quality (e.g. changes in red: far-red ratios) is dependent of trees canopy (Beaudet et al. 2011). On ICLS with trees, the light environment is continuously changed by the tree component and, in general, reductions on light intensity are related to changes on dry matter production and nutritive value of forage (Varella et al. 2010). In sustainable ICLS, the success in the integration of herbaceous and woody components depends on the use of adapted forage genotypes that show good yield performance and persistence under shading (Nair, 1993). In general, the lower is the incoming radiation level in systems with trees, the lower is forage production (Feldhake and Belesky, 2009; Paciullo et al. 2008; Devkota et al. 2009; Soares et al. 2009).

Nowadays, methods and models to estimate plant growth in monospecific cropping systems are well developed (Robertson et al. 2002; Fourcaud et al. 2008), but its suitability for multispecies systems is unclear. Sward height and leaf area index (LAI) are the most commonly variables used as tools for grassland management, due to their high correlation with forage production and sward structure (Laca and Lemaire, 2000; Hammer et al. 2002). Plant growth is primarily conditioned by leaf area, which largely determines light interception and transpiration in plants, and the consequent net photosynthesis assimilations (Monteith, 1977). Therefore, sward height (or LAI) can be used as a cutting criterion, since it reflects the canopy light interception (LI) (Mesquita et al. 2010).

Several recent studies in Brazil with C_4 grass species showed high correlation between LI and sward height for grasses growing in full sun (Fagundes et al. 1999; Carnevalli et al. 2006; Trindade et al. 2007). The maximum leaf accumulation had been observed at 95% LI, which allows high herbage intake rate and animal production (Trindade et al. 2007, Zanini et al. 2012). Consequently, sward management targets had been proposed based on sward heights corresponding to the 95% LI *momentum*. However, at shading conditions, plants can show mechanisms to tolerate to, or escape from, a reduced light condition (Ballaré and Casal, 2000; Valladares and Niinemets, 2008). These mechanisms can promote different responses, as higher sward height due to the stem elongation (Belesky et al. 2011). Further, changes in tiller dynamics (i.e. reduction in the number of tillers per plant), in the leaf expansion rate, and in specific leaf area can also occur (Smith and Whitelam, 1997; Ballaré et al. 1997; Kebrom and Brutnell, 2007; Stamm e Kumar, 2010).

Moreover, since nitrogen (N) interferes directly in the capture and use of light (Lemaire et al. 2007), the N deficit can magnify the responses of plants to shade, altering their capacity to tolerate low light (Valladares and Niinemets, 2008). Therefore, due to these plant responses that modulate plant growth as a function of shade or nitrogen (Jones et al. 1984; Brisson et al. 2008), the relationship between sward height and LI can be modified. Hence, these relationships need to be measured accurately when light is a limited resource, in order to contribute to refining management practices for ICLS with trees.

Additionally, few studies in ICLS had evaluated forage crops growth by using IL as a criterion of defoliation in order to support management targets. In most rotational stocking systems, standard pre-defined resting periods are usually adopted (e.g. Paciullo et al. 2008), in disagreement with the dynamics of plant physiology and growth. So, decreased pasture production and persistence, as well as reduction of forage quality, can occur.

We investigate the hypothesis that changes in sward structure due to the interactive effect of trees and N supply can change the relation between LI and sward height, and, consequently, the leaf canopy height at the target 95% LI. Therefore, we compare the interactive effect of shading from *Eucalyptus dunnii* trees and two nitrogen levels, upon the sward height at the 95% of LI, for six C_4 tropical forage species.

2. Materials and Methods

2.1 Site characteristics

The experimental site was located at the Agronomic Institute of Paraná (IAPAR), Ponta Grossa-PR (25°07’22’’S, 50°03’01’’W), at 880 m altitude. The climate is Cfb according to Köppen classification, with no dry season, annual precipitation of 1400 mm, more frequent during spring-summer and scarce in autumn. The soil is an Oxisoil, and texture is around 30% of clay. The average values of chemical soil analysis during the experiment period were: $P = 4.23$ mg dm^{-3}; $C = 22.2$ g dm^{-3}; $pH = 5.14$; $Al = 0.025$ $cmol_c$ dm^{-3}; $H + Al = 4.23$ $cmol_c$ dm^{-3}; $Ca = 2.95$ $cmol_c$ dm^{-3}; $Mg = 2.15$ $cmol_c$ dm^{-3}; $K = 0.16$ $cmol_c$ dm^{-3}.

2.2 Establishment of the experiment and treatments

Six perennial C_4 grasses mostly used in Brazil were studied (*Axonopus catharinensis* (Ac), *Brachiaria brizantha* cv. Marandu (Mb), *Megathirsus maximus* cv. Aruana (Mm), *Hemarthria altissima* cv. Flórida (Ha), *Cynodon spp.* hybrid Tifton 85 (Cc) and *Paspalum notatum* cv. Pensacola (Pn)). Most of them hold characteristics recommended to face shade conditions (see Soares et al. 2009).

Eucalyptus dunnii were planted in 2007, fitting to an east – west orientation, following the contour, in a double row arrangement using 3m between plants within rows and 4 m between rows, spaced 20 m apart (3x4x20 m). The initial population was 267 trees ha^{-1}. In the winter – autumn 2011 a thinning management was done and reduced the population to 155 trees ha^{-1}.

Forage species were planted in pure stands from January 2010: plots of 4.5 m² (1,5 x 3 m) in full sun (no tree integration) *vs.* 100 m² (5 x 20 m) in the shaded area. The trees shading condition will be referred as the Integrated Crop-Livestock System treatment (ICLS). For all species, a standardization cut was performed at 10 cm above soil level in the beginning of the experimental period.

Treatments were arranged in a randomized block design, with three replicates. Two system types, ICLS (i.e. shaded) *vs.* full sun, and two nitrogen levels (0 and 300 kg ha^{-1} $year^{-1}$) were defined as treatments. Nitrogen was applied as urea in the beginning of the growing season (early spring). Each year, in early spring, calcareous, P_2O_5 and K_2O were supplied according to soil analysis to ensure these nutrients did not limit plant growth. Soil water content (%) was measured using the HFM2010 - HidroFarm® in the 20 cm top soil layer for 2012 and 2013 every ~15 days.

2.3 Plant measurements

The light interception (LI) and sward height were measured weekly using a ceptometer (AccuPAR LP-80) and a sward stick, respectively. At the ICLS, measures with ceptometer were assessed at five positions, i.e. 2, 4, 10, 16 and 18 m from one of the trees rows to compose the mean of the plot. Concerning sward height, 20 measures per plot were performed. In the full sun, 3 and 10 measurements were performed with ceptometer and sward stick, respectively. The pastures were mechanically harvested when its canopy reached 95% of LI (cutting frequency). The stubble height corresponded to a 50% reduction in the cutting height (cutting intensity). Residues were removed from the site.

Two functional plant traits, sheath length (SL) and mean leaves length (LL) per tiller, were measured in summer 2012. Ten and 25 tillers were randomly collected in each plot of the full sun and ICLS treatments, respectively, then traits measures were taken in the laboratory.

The tillers density was assessed in summer 2012 and 2013. Tiller population was performed by counting tillers number in a 0,0625 m^2 square and using 5 and 1 sample units per plot for ICLS and full sun, respectively.

The leaf:stem ratio was measured in spring and summer 2012, samples were taken in a 0,0625 m^2 square at soil level when the canopy reached 95% of LI. Samples were manually separated in leaves and stems, so they were dried at 65 ºC until constant weight.

2.4 Meteorological measurements and thermal time calculation

Photosynthetic photon flux density (PPFD - μmol cm^{-2} s^{-1}) in full sun and in the ICLS was measured using a ceptometer (AccuPAR LP-80) for the summer (beginning of the year) 2011 and 2013. The measurements were taken in the same positions described in item 2.2, every 30 min from 8:00 to 18:00 o'clock. From December 2011 to July 2012, the PPFD was measured using bars containing five cells of amorphous silicon in parallel of 15 x 15 cm, connected to a datalogger (CR1000; Campbell Scientific® Ltda). The data were collected every 30 s, and mean values were calculated and stored every 5 min. Hence, light reduction in the ICLS could be calculated as the difference between sensors at both systems.

Air temperature (Tm) was collected and stored every 5 min in 3 individual dataloggers (HOBBO U10 - 001 - Onset®) placed at positions 2, 10 and 18 m from one of the trees rows in the ICLS, and one datalogger in full sun.

2.5 Statistical analyses

Statistical analyses were performed using R software (R Development Core Team, 2014). Analyses of covariance (ANCOVA, glm procedure) were performed using the Tukey method for multiple mean comparison tests in post-ANOVA/ANCOVA. Data were transformed when necessary to reach the normality of residues. Transformations were performed using the procedure Box Cox (package MASS). Species were analyzed separately, since the response of sward height in function of LI is specie-dependent. Year, season, nitrogen and system effects on sward height were analyzed at the cutting date (i.e. 95% of LI). Data analyzed using ANCOVA analysis was performed using LI as a covariant variable. This type of analyses was used because for ICLS it was the LI average, in distinction to different distances from the tree row, which was used to set the moment of cut. The actual LI ranged from 91 to 99.5 %. Only interactions that explained more than 6.5% of the variance were discussed. Regression analyses were performed between sward height and LI for the longer growing season (i.e. summer). This analysis was performed with data obtained in the first year. Regression curves were fitted for each species in each system, then analyses of covariance (ANCOVA, lm procedure) were used to compare regression curves.

3. Results

3.1 Environment and trees canopy

The mean daily temperature during the experimental period was 1 ºC warmer in full sun than ICLS (Figure 1). Year 2 was 0.8 ºC warmer than year 1, except during the summer period (December-March), which was 0.6 ºC colder than first year. The mean of maximum temperatures was 1.7 ºC higher in full sun, however, the maximum absolute temperature recorded was 36.1 ºC in ICLS and 34.9 ºC in full sun. The mean of minimal temperatures was 0.2 colder in ICLS than full sun, but the minimum absolute temperature recorded was -2.9 ºC in full sun and -1.4 ºC in the ICLS. These lower minimal temperatures are probably due to frozen, which resulted in differences for the beginning of regrowth in the spring between systems. For instance, in ICLS, pastures reached 95% of LI almost one month earlier than full sun (data not showed).

Soil moisture (%) was measured from December 2011 until June 2013, and it was significantly ($P<0.05$) lower in the ICLS than full sun (Figure 2). However, in the driest period (November 2012) ICLS area presented a higher percentage of soil moisture (16.7 ± 2.69%) than full sun (9.37 ± 1.46%).

The percentage of shade increased along the experimental period, from ~ 40 % in the spring 2011, the beginning of the experiment, to ~ 59 % in the end of summer 2013, due to trees growth. In the summer of the first year, trees presented a height of 17.58 ± 2.4 m and 21.50 ± 3.24 cm of diameter at the breast height. One year later, trees reached 22.57 ± 2.6 m of height and 27.38 ± 3.04 cm of diameter.

3.2 Sward height

3.2.1 Sward height at the cutting date

Outputs of the ANCOVA for sward height at the cutting date are shown in Table 1. ANCOVA reveled that for almost all species, the system and seasonal variations had the greatest effects on sward surface height (Table 1) in terms of variance explained (VE). For all species the sward height was higher in the summer and spring and lower in the autumn (Table 2). The highest differences in sward height between systems were observed for *H. altissima* (+23 cm on ICLS conditions, Table 2). Only *cultivar P. notatum* was not affected by integrated crop-livestock system (ICLS) (P > 0.07).

After these variables, the factor year was an important source of variation, mainly for *B. brizantha* (VE = 13%). For this species, the sward height increased 4.6 cm in the second year. N supply effect was significant for *M. maximus, H. altissima* and *Cynodon* spp., accounting for a maximum of 23% of total variance. Sward height was higher in N0 than N300 (Table 2), and *M. maximus* was the species with the highest increase (+8.4 ± cm) due N fertilizer application.

Some significant interactions were found between the factors analyzed (Table 1). The most important interactions were between Year x Season, for *M. maximus* and *P. notatum*, and between system x year x season for *A. catharinensis, H. altissima* and *Cynodon* spp.. Means for the interaction Year x Season are showed in Table 3. *For M. maximus,* while in the first year the sward height was higher during the summer, in the second year highest height value was observed during the spring. For *P. notatum* this interaction was significant due differences in order of magnitude in all seasons with an increase in height values from the first to the second year (Table 3). The interaction system x nitrogen is not showed because presented values than 6.5% in terms of V.E.

3.2.2 Sward height x Light interception

A significant linear regression was observed between sward height and LI for all species and independent of the system (Figure 3). Since no differences between slopes (P > 0.15) were observed in ANCOVA for ICLS vs. full sun, the distances between intercepts could be compared. It means that the higher sward height in ICLS for some species is independent of LI level (Figure 3). Height values obtained from regressions

(Figure 3) were similar to the means found using only the data at the cutting date. The relative increase of sward height was 37, 36, 32 and 22 % for *H. altissima*, *Cynodon* spp., *B. brizantha* and *A. catharinensis,* respectively. The relationship between sward height and LI of *M. maximus* and *P. notatum* was similar (i.e. no differences in slopes and intercepts) in ICLS and full sun.

3.3 Plant traits

Leaf length (LL) increased for *A. catharinensis*, *B. brizantha*, *H. altissima* and *P. notatum* in ICLS when compared with full sun (Table 4). Nitrogen fertilization had also a significant effect on leaf length, i.e. it increased on N300 treatment for all species, except for *P. notatum* (Table 4).

Sheath length also increased in ICLS, except with *M. maximus* (Table 4). Further, plants without N fertilization (i.e. N0) exhibited longer sheaths (Table 4), except *P. notatum*.

Nitrogen supply had the strongest effect on tiller density for all species ($P < 0.01$), except for species *H. altissima* ($P = 0.54$). The N input (i.e N300) increased the number of tillers (Table 4). In relationship to the systems, a reduction on tiller density was observed in ICLS only for *H. altissima* (< 34%) and *Cynodon* spp. (< 47%, Table 4).

Leaf:Stem ratio was mainly affected by season (in terms of V.E.). For all species, the leaf:stem ratio was higher in spring compared to the summer period (data not showed). *B. brizantha, P. notatum* and *Cynodon* spp. showed higher leaf:stem ratio in the full sun (Table 4). The opposite was observed for *M. maximus* and *P. notatum*, i.e.

leaf:stem ratio was higher in ICLS (Table 4). N supply tended to increase leaf:stem ratio, except for *Cynodon* spp. (Table 4).

4. Discussion

Our hypothesis that changes in sward structure due to the interactive effect of trees and N supply can change the relation between LI and sward height was confirmed by our controlled experiment. Further, important variations on leaf canopy height at 95% LI, mainly across seasons, were observed. Therefore, in order to maintain 95% as a target LI level, grassland managers should cut or graze each species at different height, for example, for systems with trees, in conditions of nitrogen limitation and across seasons (i.e. for swards being vegetative or reproductive).

4.1 Alterations in plant morphology

For the species studied here, changes in plant morphology due the treatments resulted in changes in sward height at 95% LI (Table 4). For instance, shading increased the sward height of most species and reduced the tiller density of *H. altissima* and *Cynodon* spp. (Table 2). These are key characteristics of shade avoidance plants, due to changes in red:far red light. Plants tend to avoid the new tillers production in order to maintain the allocation of photoassimilates to the existents tillers (Casal, 2000; Wherley et al. 2005; Evers et al. 2007, Belesky et al. 2011). The effect of light on stems by the extension of internodes is well demonstrated in the literature for species that presents shade avoidance strategies (Casal, 2000; Valladares and Niinemets, 2008; Zhu et al.

2014). Navas and Garnier (2002) also showed that this effect is independent of other stresses (i.e. water or nutrient).

The increase on sward height due to an increase in leaf size with shading is controversy, since other morphological characteristics of leaves can be associated to an increase in the light capture (Lin et al. 2001), such as leaf angle (Fernández et al. 2004, Peri et al. 2007a). For instance, *P. notatum* did not showed differences in sward height due to the ICLS, despite an increase in leaf length. On the other hand, *A. catharinensis*, *B. brizantha* and *H. altissima* showed higher sward height and longer leaves (Table 4) in ICLS. This shade effect on leaf length could be a plant strategy in order to increase light capture (Dale, 1988).

Leaf length and tiller density increased for all species with N fertilizer application, except *P. notatum,* and *H. altissima*, respectively (Table 4). For leaf length, this pattern is expected (Lemaire and Chapmman, 1996), since N increases the leaf expansion rate (Gastal et al. 1992). However, according to Sbrissia and Silva (2001), sward height is maintained constant despite an increase in leaf size with an increase in N availability, since heavier leaves alter the leaf angle in the sward structure.

Further, diverse authors (Simon and Lemaire, 1987; Duru and Ducrocq, 2000; Singer, 2002, Gatti et al. 2013) showed that the bigger importance of N is on leaf appearance and expansion. Tiller dynamic is much more variable in function of light and pasture management (Kephart and Buxton, 1993; Sbrissia et al., 2010).

4.2 The differences in sward height per se

There was an increase in the sward height in function of year, mainly for species cultivated in the ICLS. This effect can be explained by the decrease of light reaching on forage sward (~ 40% in summer of 2012 to ~ 59% in the end of summer 2013). The magnitude of these differences can be increased throughout the years if the shading effect increases. Lin et al. (2001) showed, with various C_3 and C_4 forages species, an increase in sward height with the increase of shade. In this way it is important the management of trees in order to reduce the variability on forage growth and development over time.

Seasonal effects were important in the sward height at 95% LI (Table 1). In general, there was a decrease from spring and summer to autumn, which could be in turn explained by stem formation due to plant maturity developmental stage, since during the fall all species were in vegetative stage (data not shown). A similar pattern in sward height between seasons was observed by Giacomini et al. (2009) with *B. brizantha* and by Medinilla-Salinas et al. (2013) with *M. maximus.* However, they did not attribute these differences on sward height to plant maturity.

The relative increase in sward height from full sun to SS was 52, 50, 43, 24 and 7% for *H. altissima*, *Cynodon* spp., *B. brizantha*, *A. catharinensis* and *M. maximus* respectively. Gobbi et al. (2009) showed that reductions on light availability increase the height of *B. brizantha* cv. Basilisk. The same pattern was found for *Dactylis glomerata* (Peri et al. 2007b), and with a diverse range of C_3 and C_4 species (Lin et al. 2001). For *M. maximus*, Medinilla-Salinas et al. (2013) showed that plants growing without trees were 12.5% taller than in the shaded condition. However, they measured the plants in a fixed period of regrowth. In a shaded condition, plants can exhibit lower growth rates (Valladares and Niinemets, 2008), and this can lead to differences in sward height.

According to Mesquita et al. (2010), N affects only the time and not the height that swards reaches 95% of LI, due to the acceleration on appearance and tissue expansion of plants with higher amounts of nitrogen (Gastal and Nelson, 1994; Duru and Ducrocq, 2000; Alexandrino et al. 2005; Paiva et al. 2012). However, a decrease in sward height at 95% LI for *A. catharinensis, M. maximus* and *H. altissima* (Table 2) with N fertilization was observed, which in turn could be explained by changes in plant morphology as the increase in sheath length for plants without N nutrition (Table 4).

Since no significant differences were observed in slopes for the regression analysis for *A. catharinensis, B. brizantha, H. altissima* and *Cynodon* spp. between sward height and light interception (Figura 3), the increase in sward height was independent of the level of LI. It suggests that an early signal of changes in light quality is perceived by plants (Ballaré et al. 1987; Aphalo et al. 1999), before the pasture canopy closure (i.e. 95% of LI). Then, changes in the understory occurred probably due to changes in light quantity, but also in light quality due to the trees canopy (Varella et al. 2010; Beaudet et al. 2011). This results can interferes directly in the pasture management due to the changes in plant morphology related to alterations in light quality. For example, *B. brizantha* and *Cynodon* spp. presented lower values of leaf:stem ratio in ICLS, which means higher levels of stems in the sward structure.

In full sun canopies, it has been showed that an increase in sward height leads to a decrease in the leaf:stem ratio (Fonseca et al. 2012), which is directly correlated with the light competition in the canopy. When LI levels are higher than 95%, there is a faster increase in stem elongation. In this way, our results can help to target the pre-grazing sward height in function of shade. However, advances are still necessary about the post-grazing height. In this work, it was used 50% of the initial height for the cutting intensity, because follows the pattern of animal behavior. The level of cutting intensity also has interference on sward structure (Silveira et al. 2010). Belesky et al. (2011) showed that the long-term of tiller production was compromised for the higher cutting intensity in shaded condition. In this way, studies of leaf lifespan, forage quality and animal behavior (Fonseca et al. 2013) can help to define better management strategies for cutting intensities.

To sum up, the response of pasture sward height as a function of shading and nitrogen fertilization are variable depending on the grass species evaluated. The management using LI in integrated systems can be used, but the cutting height can be higher for species that are influenced by shading and by nitrogen.

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Table 1 – Proportion of variance explained (VE) and statistical significance of F ratios from analysis of covariance for sward height and for each C$_4$ forage species. Ac – *Axonopus catharinesnis*, Bb – *Brachiaria brizantha*, Mm – *Megathyrsus maximus*, Ha – *Hemarthria altissima*, Cc – *Cynodon* spp., Pn – *Paspalum notatum*.

	Ac		Bb		Mm		Ha		Cc		Pn	
	F	%	F	%	F	%	F	%	F	%	F	%
LI	3.6^{ns}	1.6	57.7^{***}	11.7	0.6^{ns}	0.3	12.3^{***}	3.9	5.9^{*}	1.7	0.01^{ns}	0.01
System	56.6^{***}	25.3	186.9^{***}	38.0	6.1^{*}	2.4	127.0^{***}	39.9	135.0^{***}	38.1	3.42^{ns}	2.63
Year	22.9^{***}	10.2	44.1^{***}	9.0	7.5^{**}	3.0	13.9^{***}	4.4	22.1^{***}	6.3	19.6^{***}	15.1
N	11.0^{**}	4.9	1.7^{ns}	0.3	22.7^{***}	9.0	10.9^{**}	3.4	6.6^{*}	1.9	0.27^{ns}	0.21
Season	11.1^{***}	9.9	25.3^{***}	10.3	43.3^{***}	34.1	18.8^{***}	11.8	33.9^{***}	19.2	22.2^{***}	34.3
Block	2.2^{ns}	4.0	5.4^{***}	4.4	3.0^{*}	4.7	1.6^{ns}	2.1	0.1^{ns}	0.2	0.72^{ns}	2.21
System x Year	-	-	4.8^{*}	1.0	-	-	6.8^{*}	2.2	-	-	-	-
Year x Season	-	-	-	-	8.5^{***}	6.8	-	-	-	-	9.8^{***}	15.1
N x Season	-	-	-	-	-	-	-	-	6.0^{**}	3.4	-	-
System x Year x N	-	-	3.46^{*}	2.1	-	-	-	-	-	-	-	-
System x Year x Season	2.5^{*}	7.9	-	-	-	-	4.2^{***}	8.0	4.3^{***}	8.6	-	-

*P<0.05; **P<0.01; ***P<0.001; ns, not significant.

Table 2 – Sward height means (cm) and standard error (se) at cutting date within each year, season, nitrogen level and system for the six C_4 forage species. See Table 1 for species codes.

	Ac	se	Bb	se	Mm	se	Ha	se	Cc	se	Pn	Se
Year 1	38.7 b	1.0	25.4 b	0.5	52.4 b	1.2	51.1 b	1.3	36.0 b	0.8	29.2 b	1.1
Year 2	43.2 a	1.4	30.0 a	0.5	57.8 a	1.3	59.5 a	1.3	41.1 a	1.6	36.8 a	1.1
Spring	40.8 ab	2.0	30.2 a	0.6	62.9 a	1.6	50.6 b	1.7	37.6 b	1.8	32.5 b	1.8
Summer	44.9 a	1.0	28.7 a	0.5	59.0 a	1.2	63.0 a	1.5	44.7 a	1.0	38.3 a	1.0
Autumn	37.1 b	1.0	24.3 b	0.7	43.5 b	1.6	52.5 b	1.7	33.6 b	1.6	28.3 b	1.2
N-	43.5 a	1.5	28.3 a	0.5	59.3 a	1.3	58.5 a	1.4	36.9 a	1.6	33.4 a	1.3
N+	38.4 b	0.7	27.2 a	0.5	50.9 b	1.1	52.1 b	1.2	40.2 a	0.9	32.6 a	0.9
Full Sun	36.5 b	1.2	22.8 b	0.5	53.2 b	1.1	43.8 b	1.2	30.8 b	1.0	33.7 a	0.9
ICLS	45.4 a	1.1	32.6 a	0.5	57.1 a	1.3	66.8 a	1.5	46.3 a	1.4	32.4 a	1.2

Means with the same letter for each variable analyzed are not significantly different according to the Tukey test (P>0.05).

Table 3 - Sward height means (cm) and standard error (se) for *M. maximus* (Mm) and *P. notatum* (Pn). Data show Year x Season interaction.

	Mm	se	Pn	se
Spring x Year 1	57.6 b	2.22	31.05 cd	2.47
Spring x Year 2	68.2 a	2.46	37.09 ab	2.35
Summer x Year1	61.1 b	1.61	35.99 bc	1.31
Summer x Year2	56.9 b	1.92	42.02 a	1.61
Autumn x Year1	38.6 d	2.31	25.81 d	1.73
Autumn x Year2	48.3 c	2.44	31.84 bc	1.81

Means with the same letter in the column are not significantly different according to the Tukey test (P > 0.05).

Table 4 – Means and standard error (se) of Leaf Length (LL – cm), Sheath Length (SL – cm), Tiller Density (TD) and leaf:stem ratio for six C_4 forage species within each system and nitrogen treatment. See Table 1 for species codes.

	Ac	se	Bb	Se	Mm	se	Ha	se	Cc	se	Pn	Se
LL Full Sun	22.1 b	2.3	18.0 b	0.6	26.1 a	2.1	14.9 b	0.9	14.9 a	1.2	34.8 b	1.3
LL ICLS	26.2 a	0.9	22.7 a	1.1	27.3 a	1.5	20.0 a	0.7	15.0 a	0.5	42.3 a	1.1
LL N0	22.1 b	0.9	19.4 b	1.2	24.7 b	0.6	18.1 b	0.6	13.4 b	0.4	41.5 a	1.4
LL N300	28.4 a	1.0	24.4 a	1.2	28.9 a	2.1	20.2 a	1.1	16.6 a	0.7	40.5 a	1.5
SL Full Sun	17.8 b	0.7	12.4 b	1.0	25.3 a	1.4	37.6 b	2.1	20.8 b	0.7	11.2 b	0.3
SL ICLS	28.5 a	0.8	16.8 a	0.4	24.4 a	0.7	50.1 a	1.3	31.3 a	0.7	13.1 a	0.1
SL N0	30.7 a	1.2	18.2 a	0.5	25.8 a	0.8	54.5 a	1.8	32.3 a	1.0	12.1 b	0.2
SL N300	21.5 b	0.6	13.9 b	0.6	23.8 b	1.0	41.6 b	1.4	26.8 b	0.6	13.6 a	0.2
TD Full sun	808 a	46	1141 a	46	1351 a	150	952 a	161	3074 a	648	-	-
TD ICLS	856 a	25	1117 a	56	1435 a	68	707 b	45	1643 b	138	-	-
TD N0	786 b	37	1022 b	63	1221 b	76	721 a	79	1256 b	159	-	-
TD N300	911 a	18	1220 a	64	1620 a	72	775 a	56	2508 a	239	-	-
Leaf:Stem Full Sun	0.90 a	0.12	2.4 a	0.3	0.65 b	0.12	0.34 a	0.05	0.87 a	0.12	1.7 b	0.2
Leaf:Stem ICLS	0.94 a	0.06	1.8 b	0.1	0.91 a	0.06	0.31 a	0.02	0.56 b	0.03	3.7 a	0.5
Leaf:Stem N0	0.74 b	0.04	1.9 a	0.1	0.73 b	0.06	0.26 b	0.02	0.55 a	0.05	4.5 a	0.7
Leaf:Stem N300	1.04 a	0.08	1.9 a	0.2	0.94 a	0.08	0.35 a	0.02	0.67 a	0.05	2.5 b	0.4

Means with the same letter for each variable analyzed in function of systems and nitrogen effects are not significantly different according to the Tukey test ($P > 0.05$). ICLS, integrated crop-livestock system.

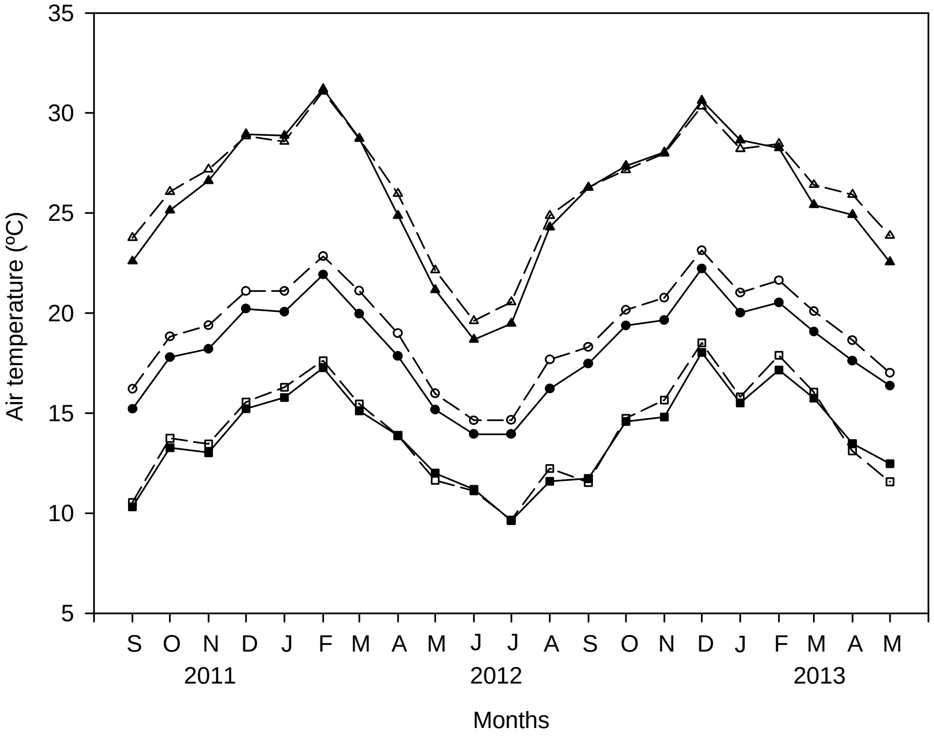

Figure 1 – Mean (circle), maximum (triangle) and minimal (square) average monthly air temperatures from September 2011 to May 2013. Open symbols and short dash lines for full sun and closed symbols solid lines for integrated crop-livestock system.

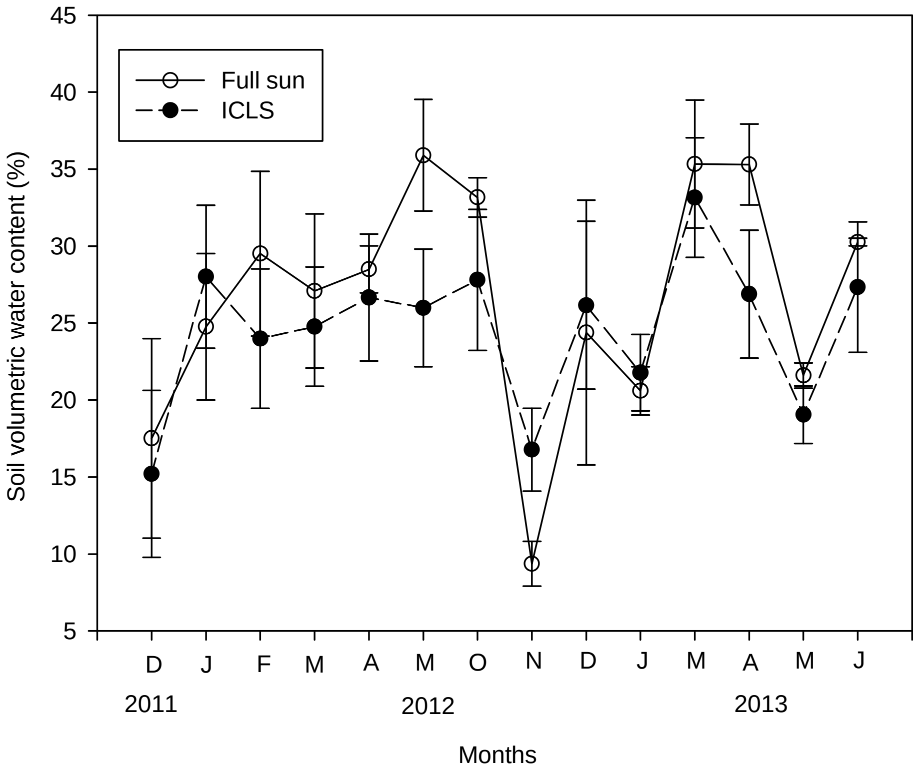

Figure 2 – Monthly mean soil volumetric water content in the top 200 mm (measured every 15 days).

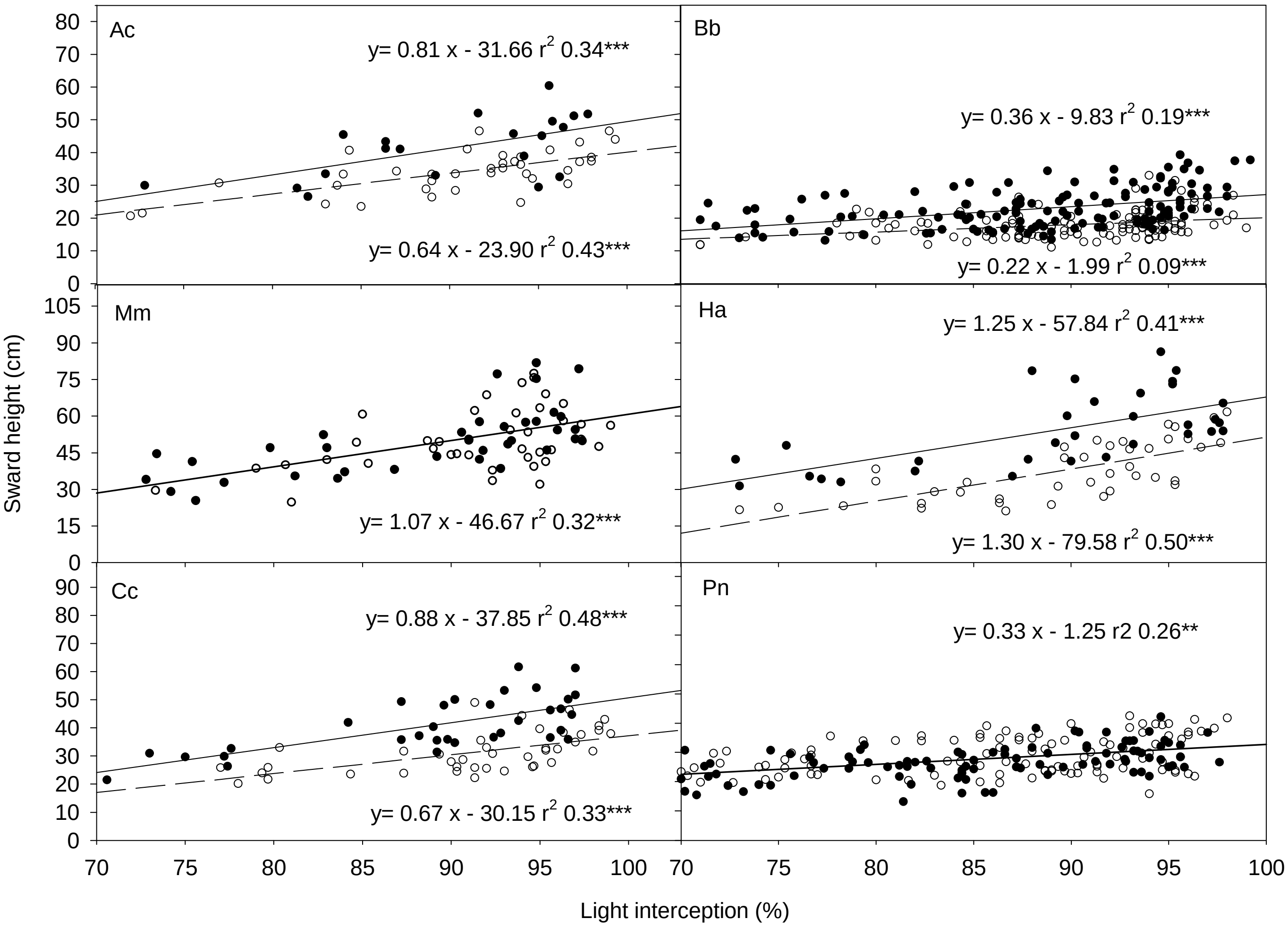

Figure 3 – Relationship between sward height (cm) and light interception (%) for six C_4 forage species at the integrated crop-livestock system (closed symbols and solid lines) and full sun (open symbols and short dash lines). Ac – *Axonopus catharinesnis*, Bb – *Brachiaria brizantha*, Mm – *Megathyrsus maximus*, Ha – *Hemarthria altissima*, Cc – *Cynodon* spp., Pn – *Paspalum notatum*. ANCOVA results are presented in each panel: two lines in the case of no interaction and difference between intercepts between the categorical independent variable (i.e. sward height), and a single line in the case of no significant effect of the continuous variable. (*P<0.05; **P<0.01; ***P<0.001; ns, not significant).

3. CAPÍTULO 2

Morphogenesis and growth dynamics of tropical forage species according to shade and nitrogen[1]

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[1] Elaborado de acordo com as normas da Revista Agroforestry Systems

Morphogenesis and growth dynamics of tropical forage species according to shade and nitrogen

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Abstract

Monocultures of six C_4 tropical forage species that are widely used in Brazilian livestock were compared in an experimental field with two systems, i.e. open pasture *vs*. under tree canopy (i.e. an integrated crop-livestock system, ICLS), and two levels of N supply (zero vs. 300 kg of N ha^{-1} $year^{-1}$). Our aim was to determine the interactive shading effect from trees (i.e. *Eucalyptus dunnii*) canopy, and nitrogen supply on morphogenetic characteristics and growth parameters of six C_4 grass species in order to assess their potential for use as a component of ICLS. 95% of light interception was used as a cutting frequency and 50% of initial height as cutting intensity. The shading effect from trees canopy was specie-dependent. For instance, *B. brizantha*, *M. maximus* and *Cynodon* spp. had a decrease on leaf growth rates in ICLS with trees, while the opposite was observed with *A. catharinensis. H. altissima* maintained the same leaf growth rates in the two systems, i.e. with and without trees. However, even for species that showed decreases on values of morphogenetic variables (e.g. phyllochron) and leaf growth rates, they maintained a satisfactory development and growth in ICLS. The N supply helps the maintenance of a satisfactory development and growth of forages species in the ICLS.

Key words: integrated crop-livestock systems; management; light interception; competition

1. Introduction

Since intensive production systems require high amounts of energy by labor and inputs, their results become incompatible with the emergent demands that aim to ensure the sustainability of agroecosystems (Carvalho and Moraes, 2011). Integrated crop-livestock systems (ICLS) appears, therefore, as a good option to increase the overall land productivity and/or its sustainability by making best use of the environmental resources and, at the same time, by generating environmental services (O´Mara, 2012; Sanderson et al. 2013). Further, several reports have demonstrated the value of including trees in these systems because the integrated timber and livestock production can generate higher profits, which provide farmers with a means of surviving markets that fluctuate through time (Zhai et al. 2006).

However, in the ICLS, that can intentionally integrates trees, forage crops, and livestock into a structural practice of planned interactions (Clason and Sharrow, 2000), the pasture component is affected by changes in the understory, mainly due changes in light quantity and quality. The amount of light in an ICLS depends on tree species, tree density and tree management. Further, the response to shading depends on the forage species and on soil fertility, especially nitrogen (Paciullo et al. 2011). Therefore, a better understanding about plant responses to interactive shading and N availability and the consequent growth dynamics in ICLS is essential to enhance our ability to forecast management practices and biomass production in order to ensure pasture persistence and a sustainable production (Hodgson and Silva, 2000).

Morphogenetic processes, like the rate of appearance of new organs, control the growth and development of plants in a specific environment. These processes determine sward structural characteristics, as the leaf area index which allows light interception by the sward, and consequently, the biomass production (Lemaire and Chapman, 1996). However, management practices, like cutting frequency and intensity and fertilization, interferes on the plant morphogenesis (Duru and Ducrocq, 2000, Peri et al. 2007b; Sbrissia et al. 2010). Further, in a system with trees, shade appears like another management effect, as it is possible to manage in a certain way the shade produced by trees (Combes et al. 2002).

When forage grass plants are growing in association with trees, grass tolerance depends on the physiological and morphological adaptations for irradiance reduction. Different hypothesis on the suite of characteristics responsible for species' shade tolerance have been recognized on the literature as, for example, carbon gain *vs*. stress tolerance hypothesis (see Valladares & Niinemets, 2008 for a review). However, most studies reporting these differing views about shade tolerance were done on woody species (Seidlova et al. 2009). Further, Valladares & Niinemets (2008) argued that shade tolerance could depend both on the efficiency of carbon gain in low light and on the tolerance of stresses interacting with light availability. Therefore, different plant features can be associated to shading tolerance and N availability, with different consequences for plant growth dynamic; hence, they need to be identified for a broader range of forage species.

For instance, shading effect can has a strong influence on morphogenetic variables by, for example, reductions on leaf appearance, leaf elongation rate and tiller appearance (Gautier et al. 1999; Granier and Tardieu, 1999; Bos et al. 2000, Casal, 2000), which in turn affect the pasture biomass accumulation. Under moderate shading, some grass species have the capacity to maintain growth at satisfactory levels (Paciullo et al. 2008; Soares et al. 2009; Pachas et al. 2013). However, in general, the lower is the incoming radiation level in systems with trees, the lower is the forage production (Feldhake and Belesky, 2009; Paciullo et al. 2008; Devkota et al. 2009; Soares et al. 2009). Therefore, detailed studies of tissue dynamic can provide new insights for changes in sward state variables in response to variation in shading and in N level.

The objective of this study was to compare interactive effect of shading, provided by *Eucalyptus dunnii*, and two nitrogen levels on the morphogenetics variables, sward structural characters and growth and senescence fluxes of six C_4 tropical forage species.

2. Materials and Methods

2.1 Site characteristics

The experimental site was located at the Agronomic Institute of Paraná (IAPAR), Ponta Grossa-PR (25°07’22’’S, 50°03’01’’W), at 880 m altitude. The climate is Cfb according to Köppen classification, with no dry season, annual precipitation of 1,400 mm, more frequent during spring-summer and scarce in autumn. The soil is an Oxisoil, and texture is around 30% of clay. The average values of chemical soil analysis during the experiment period were: P = 4.23 mg dm^{-3}; C = 22.2 g dm^{-3}; pH = 5.14; Al = 0.025 $cmol_c\ dm^{-3}$; H + Al = 4.23 $cmol_c\ dm^{-3}$; Ca = 2.95 $cmol_c\ dm^{-3}$; Mg = 2.15 $cmol_c\ dm^{-3}$; K = 0.16 $cmol_c\ dm^{-3}$.

2.2 Establishment of the experiment and treatments

Six perennial C_4 grasses widely used in Brazilian livestock were studied (*Axonopus catharinensis* (Ac), *Brachiaria brizantha* cv. Marandu (Mb), *Megathirsus maximus* cv. Aruana (Mm), *Hemarthria altissima* cv. Flórida (Ha), *Cynodon spp.* hybrid Tifton 85 (Cc) and *Paspalum notatum* cv. Pensacola (Pn)). Most of them hold characteristics recommended to face shade conditions (see Soares et al. 2009).

Eucalyptus dunnii' trees were planted in 2007, fitting to an east – west orientation, following the contour, in a double row arrangement using 3m between plants within rows and 4 m between rows, spaced 20 m apart (3x4x20 m). The initial population was 267 trees ha^{-1}. In the winter – autumn 2011 a thinning management was done and reduced the population to 155 trees ha^{-1}.

Forage species were planted in pure stands from January 2010: plots of 4.5 m² (1,5 x 3 m) in full sun (no trees integration) *vs.* 100 m² (5 x 20 m) in the shaded area. The trees shading condition will be referred as the Integrated Crop-Livestock System treatment (ICLS). Plant measurements were done in December of 2011 and 2012.

Treatments were arranged in a randomized block design, with three replicates. Two system types, ICLS (i.e. shaded) *vs.* full sun, and two nitrogen levels (0 and 300 kg ha^{-1} year^{-1}) were defined as treatments. Nitrogen was applied as urea in the beginning of the growing season (in early spring). Each year, in early spring, calcareous, P_2O_5 and K_2O were supplied according to soil analysis to ensure that these nutrients did not limit plant regrowth. Soil water content (%) was measured in the top 20 cm soil layer, using the equipment HFM2010 - HidroFarm during the morphogenesis analysis.

The pastures were mechanically harvested when its canopy reached 95% of LI (cutting frequency). The stubble height corresponded to a 50% reduction in the cutting height (cutting intensity). Residues were removed from the site. The light interception in each plot was monitored every week, using a ceptometer (AccuPAR LP 80).

2.3 Leaf and Plant measurements

Measurements at leaf and plant level were done in 25 and 10 tillers per plot in the full sun and ICLS, respectively. Only tillers on vegetative stage were marked. For both treatments, marked tillers were located along 2 transects in full sun and 5 in ICLS, distanced 50 cm between each tiller. The distances of transects in ICLS corresponded to 2,4,10,16 and 18 m from one of the trees rank. Every 2-3 days, during 20 days, the following measurements were done: the lengths of green and senescing (green parts only) laminae were measured from the ligule for mature leaves (i.e. those with a visible ligule) and from the ligule of the last mature leaf for growing leaves; the length of the pseudostem of vegetative tillers or stem of reproductive tillers (measured from the ligule of the last mature leaf or the flag leaf to the ground); the appearance of new leaves.

These measurements were used to calculate the following parameters per plant part: phyllochron (Phyl – calculated as the inverse of the linear regression between number of leaves in function of thermal-time); leaf elongation rate (LER – cm tiller^{-1} ºCd^{-1}); duration of leaf elongation (DLE - ºCd); leaf length (LL – cm); leaf lifespan (LLS - ºCd); stem elongation rate (SER – cm tiller^{-1} ºCd^{-1}); leaf blade senescence rate (LSR – cm tiller^{-1} ºCd^{-1}); number of green leaves (NGL – leaves tiller^{-1}); final leaf length (LL – cm).

For each measurement period, destructive samples were collected, at random and within each plot, to estimate weight per unit length (g DM cm^{-1}) of each plant part.

In the end of each measurement period, the tiller density (TD – tiller m^{2}) per unit ground area was determined by counting in 1 or 5 samples of 0,0625 m^{2} per plot in full sun and ICLS, respectively.

A growth flux (GF – kg ha^{-1} day^{-1}) was calculated by the following equation:

$$GF = LER \times SWEL \times Tm \times NEL \times TD \times 10 \qquad \text{Eq. 1}$$

Where: SWEL is the specific weight of expanding leaves (g^{-1} cm^{-1}); Tm (ºC) is the daily mean temperature of the experimental period; NEL is the number of expanding leaves per tiller; and the factor 10 is in order to transform in kg ha^{-1}. Senescence flux (SF – kg ha^{-1} day^{-1}) was calculated by the following equation:

$$SF = LSR \times SWML \times Tm \times TD \times 10 \qquad \text{Eq. 2}$$

Where: SWML is the specific weight of mature leaves (g^{-1} cm^{-1}); Tm (ºC) is the daily mean temperature of the experimental period; and the factor 10 is in order to transform in kg ha^{-1}. The tissue turnover fluxes were calculated per population unit and then per area (ha), by multiplying the mean tissue fluxes per population unit by the mean density of these units (i.e. TD).

2.4 Meteorological measurements and thermal time calculation

Photosynthetic photon flux density (PPFD µmol cm^{-2} s^{-1}) in full sun and in the ICLS was measured at the same positions described in item *2.3*. For the summer 2012, the PPFD was measured using bars containing five cells of amorphous silicon in parallel of 15 x 15 cm, connected to a datalogger (CR1000; Campbell Scientific® Ltda). The data were collected every 30 s, and mean values were calculated and stored every 5 min. Hence, light reduction in the ICLS could be calculated as the difference between sensors at both systems. For the summer 2013, measurements were taken using a ceptometer (AccuPAR® LP-80), every 30 min from 8:00 to 18:00 o’clock.

Air temperature (Tm) was collected and stored every 5 min in 3 individuals dataloggers (Hobbo) located at 2, 10 and 18 m from one of the trees rows in the ICLS, and one datalogger in full sun. Thermal-time was calculated for integrated and full sun systems from the daily integration of air temperatures minus the base temperature (T_b):

$$TT = \int_0^h \max[0;(T_m - T_b)]dt \qquad \text{Eq. 3}$$

where *TT* is thermal time expressed in cumulative degree days (°Cd, Bonhomme, 2000). *TT* is expressed in degree-days, calculated as the sum of the mean daily temperature minus the base temperature (T_b). During this study, the T_b value used was 10ºC for all species.

2.5 Statistical analyses

Statistical analyses were performed using R software (R Development Core Team, 2014). Analyses of variance (ANOVA, glm procedure) were performed using the Tukey method for multiple mean comparison tests in post-ANOVA. Data were transformed when necessary to reach the normality of residues. Transformations were performed using the procedure Box Cox (package MASS).

3. Results

3.1 Environmental and tree measurements

The mean daily temperature during the experimental period was 20.2 ºC in ICLS, created by eucalyptus trees, and 21.0 ºC in full sun treatment in the first year. During the second year, temperatures increased 2 ºC. Although there was an increase on temperature from the first to second year, the differences between ICLS and full sun treatments remained similar (Figure 1).

Soil moisture percentage was also monitored during the experimental period. Soil moisture was lower in the first year (15±5.42 % and 18±6.48 % in ICLS and full sun, respectively) when compared to the second year (26±5.46 % and 24±8.60 % in ICLS and full sun, respectively).

Mean trees height ranged from 17.6 ± 2.4 m in summer 2012, with a diameter at breast height of 21.5 ± 3.24 cm, until 22.6 ± 2.6 m at the end of summer 2013 (27.4 ± 3.04 cm of diameter), which gave tree canopy closures (i.e. % of photosynthetic photon flux reduction) of ~40 % and ~59 %, respectively.

3.2 Morphogenical and structural characteristics

Outputs of the ANOVA for morphogenical and structural characteristics and growth fluxes are show in Table 1. ANOVA reveled that species had the greatest effect on all variables (Table 1), in terms of variance explained (VE). Means per species are showed in Figure 2. There were significant effects of system, N and year for almost all variables. Further, interactions of second and third order were also observed (see Table 1). Therefore, interactions between species x system (Figure 2) and species x N levels (Figure 3) are showed, since they were significant for almost all variables analyzed. Further, means for the interactions species x year are available in supplementary material.

The faster species on leaf appearance (i.e. lower Phyllochron) was *H. altissima*, and the slowest were *M. maximus* and *B. brizantha* (Figure 2). On average, N fertilization decreased Phyl. (132 ± 4.99 ºCd and 109 ± 3.86 ºCd at N0 and N300, respectively). Forage species showed a significant higher Phyllochron (Phyl.) in ICLS (Figure 2). However, *H. altissima* had a higher Phyl. in full sun, and *Cynodon* spp. not showed differences between systems (Figure 2).

Leaf elongation rate (LER) ranged between 0.14 ± 0.007 cm tiller^{-1} ºCd^{-1} for *B. brizantha* to 0.24 ± 0.01 cm tiller^{-1} ºCd^{-1} for *H. altissima* (Figure 2). Only *H. altissima* had a significant higher LER in ICLS (Figure 2). The N fertilization increased LER, except for *M. maximus* and *H. altissima,* which not showed significant differences between N levels (Figure 3). *H. altissima* displayed the highest duration of leaf elongation (DLE), while similar values were observed between the others species (Figure 2). Further, this species and *Cynodon* spp. were not affected by system (Figure 2). N input decreased the DLE (211 ± 6.87 ºCd and 195 ± 5.87 ºCd at N0 and N300, respectively). *B. brizantha* had leaves with longer lifespan than the other species (Figure 2). Only *A. catharinesnis* had significant differences on LLS in function of system (Figure 2). Further, N fertilization decreases the LLS of *B. brizantha* and *Cynodon* spp. (Figure 3).

The stem elongation rate (SER) was higher for *A. catharinensis*, *H. altissima* and *Cynodon* spp. (Figure 2). ICLS significantly increases the SER for *B. brizantha, H. altissima* and *Cynodon* spp., and decreases the SER for *M. maximus* (Figure 2). Only *A. catharinensis* did not showed differences between systems. The N effect was significant (Table 1). On average, N fertilization increases the SER (0.020 ± 0.001 cm tiller^{-1} ºCd^{-1} and 0.024 ± 0.001 cm tiller^{-1} ºCd^{-1} at N0 and N300, respectively).

H. altissima displayed 8.5 ± 0.22 green leaves per tiller (NGL), while *M. maximus* displayed a maximum of 3.63 ± 0.08 leaves per tiller (Figure 2). *M. maximus* and *Cynodon* spp. were the species that not exhibited differences for NGL in function of system. A higher NGL was observed in full sun only for *A. catharinensis*, the others species showed higher NGL in ICLS. However, the magnitude of these differences was small. For instance, *A. catharinensis* had 4.9 ± 0.28 leaves in full sun and 5.5 ± 0.17 NGL in ICLS, i.e. the difference was only 0.6 leaves. Higher differences were observed for *H. altissima* (+1.33 ± 0.41 leaves in full sun, Figure 2). The N fertilization increases the NGL, mainly for *A. catharinensis* and *H. altissima* (Figure 3).

The species evaluated increased significantly the leaf length (LL) in ICLS, except *M. maximus* and *Cynodon* spp. (Figure 2). *M. maximus* has longer leaves compared to the other species, independently of the system (Figure 2), and also it was the only species to reduce LL due N fertilization (Figure 3).

Lower tiller density (TD) was observed in ICLS for *H. altissima* and mainly for *Cynodon* spp. (Figure 2). N fertilization significantly increased the number of tillers for all species (Table 2). This increase ranged between +154 tiller for *A. catharinesnis* and +982 tillers for *Cynodon* spp..

3.3 Explanatory variables

Leaf senescence rate (LSR) and specific leaf weight (SLW) were used for growth and senescence fluxes' calculations.

LSR was lower in full sun for *H. altissima* (Figure 4). The N fertilization decreased LSR for *A. catharinensis*, *M. maximus* and *Cynodon* spp. (Figure 4). SLW was significant different only for *B. brizantha* and *M. maximus* due to the system, these species showed a higher SLW in the full sun (Figure 4). The N input decreased SLW for *B. brizantha* and increased for *M. maximus* and *Cynodon* spp., the other species did not show significant differences (Figure 4).

3.4 Growth and senescence fluxes

The growth flux (GF) ranged between 46 ± 7.8 kg DM ha^{-1} day^{-1} for *H. altissima* and 105 ± 5.9 kg DM ha^{-1} for *Cynodon* spp. (Figure 5). The GF was higher in the full sun, but *A. catharinensis* showed the opposite response, with a higher GF in ICLS. No significant differences were observed with *H. altissima* (Figure 5). N300 treatment increased GF for all species. Further, the magnitude of these responses was very different, varying from +11.8 kg ha^{-1} day^{-1} for *H. altissima* and +88.3 kg ha^{-1} day^{-1} for *Cynodon* spp. that was the species with highest growth flux (GF). The SF was significantly lower in the ICLS for *M. maximus* and *Cynodon* spp (Figure 5). For N fertilization, differences were observed for *A. catharinensis* with a lower SF in the treatment N300 while *B. brizantha* had a higher SF in the treatment N300 (Figure 5).

3.5 *Year effect on variables*

Significant differences between years were observed for Phyl., LER, DLE, LSR, SER and growth and senescence fluxes (Table 1). On average, higher values were observed in the first year than in the second (Table 2), except for LER, SER and GF.

In general, significant year x species interactions showed significant effects for some species, while the others remained with similar values between years. For instance, only *B. brizantha* had lower NGL in the first year when compared to the second year, and only *H. altissima* showed shorter leaves in the second year (see supplementary material). With Phyl. an opposite response was found. Phyl. was higher for all species in the first year, except for *A. catharinensis* (90 ± 16.1 and 131 ± 5.22 ºCd for the first and second year, respectively).

4. Discussion

Phyllochron (Phyl.) was higher in ICLS, except for *Cynodon* spp. and *H. altissima* that not showed differences. Further, this last species had the fast leaf appearance rate (i.e.

lower Phyl.), showing values higher than those reported by Oliveira et al. (2000). The effect of light on Phyl. is divergent on the literature. While some authors indicate that the competition for light increases the Phyl. (Duru et al. 1993; Gatti et al. 2013), others results appoints for a reduction (Gautier et al. 1999). A possibility for the increase found for three species in our study (Figure 2) is a probably lower carbon production and allocation in ICLS, which in turn affected the Phyl. (Gautier et al. 1999).

Some authors reported that the leaf elongation rate (LER) is directly correlated with the amount of radiation (i.e. photosynthetic photon flux density - PPFD), and linked with photoassimilates production (Granier and Tardieu, 1999; Bos et al. 2000). For instance, Paciullo et al. (2008), observed a higher LER for *Brachiaria decumbens* Stapf. growing in a system with 50% of shade. In our study, the effect of shade by trees on LER was observed only for *H. altissima,* with an increase on LER in ICLS (Figure 2) when compared to full sun. Further, *H. altissima* was the only specie with higher leaf senescence rate in the ICLS (Figure 4). According to Hirosaka (2005), since higher senescence can provide a faster N remobilization from leaves, this response could be correlated to a better N condition in shade for the expanding leaves, despite no significant effects of N inputs for this species (Figure 3) in both light conditions. N has a markedly effect on LER, mainly by an acceleration on cell division (Gastal and Nelson, 1994; Duru and Ducrocq, 2000; Alexandrino et al. 2005)

The N input decreases DLE. Further, the duration of leaf elongation was higher in ICLS, except for *H. altissima* and *Cynodon spp.* These results are directly correlated with the LER increase/decrease by N and shade, since these characteristics are correlated (Gastal and Nelson, 1999; Duru and Ducrocq, 2000; Vos et al. 2005; Corré, 1983; Tardieu et al. 1999; Cookson and Granier, 2006).

Small differences were found in the total number of green leaves in function of treatments (Figures 2, 3) and year (Figure 6). This characteristic is relatively constant within a species, but with large variations between species (Lemaire and Chapman, 1996). Only *A. catharinensis* showed a significant increase on LLS in ICLS (Figure 2).

LLS is the product of phyllochron and the number of mature leaves. As the number of leaves had a small change in ICLS, the differences observed on LLS could be related to the lower leaf appearance rate (i.e. higher phyllochron) for this species in ICLS. This response could be explained by an adaptive process, i.e. plants increase the leaf lifespan in order to increase the time for doing photosynthesis, since photosynthetic rates are lower with a light restriction. Also, this response can be just correlated to the carbon balance and nutrient utilization (e.g. N remobilization) (Chabot and Hicks, 1982). The use of LLS is important as a management tool, because can be used to define the cutting frequency. Lower LLS means a more frequent cutting regime, otherwise higher losses in pasture can occur due to senescence (Lemaire and Chapman, 1996). Hence, in relationship to the results observed with LLS, cut frequency can be similar for all species independently of the system, except for *A. catharinensis*, which can be cut lees frequently in ICLS.

Final leaf length (LL) is a function of LER and DLE. Therefore, a higher LL for *H. altissima* was probably due a higher LER in ICLS (Figure 2), and the higher LL for *A. catharinensis* and *B. brizantha* can be explained by the higher DLE (Figure 2). An increase in leaf size due to shade can be a plant strategy in order to increase light capture (Givnish, 1988).

H. altissima and *Cynodon* spp. showed lower tiller density (TD) in the ICLS (Figure 2). TD is an important mechanism to optimize sward leaf area and production (Matthew et al. 2000). The reduction of light and specially changes in red:far red can avoid the production of new tillers in order to maintain the allocation of photoassimilates to the existents tillers (Casal, 2000). The effect of N is the opposite of the light, an increase in N fertilization tends to increase the number of tillers (Cruz and Boval, 1999).

Stem elongation rate (SER) is an important morphogenical process that has a large interference on the structure of tropical grass species (Sbrissia and Silva, 2001). Species showed a higher SER in the ICLS, except *M. maximus* and *A. catharinesnis* (Figure 2). The effect of light on stem elongation is one of the most typical responses of shade avoidance plants, due to reductions on light quantity and changes in light quality, plants increase internodes to reach a better light condition (Ballaré et al. 1987; Ballaré et al. 1997; Casal, 2000).

While shade tended to increase Phyl., N input decreased the Phyl for all species in a similar way, since no significant interactions between N x system were found for this variable. The N effect on the increase of leaf appearance is well reported for tropical species (Garcez Neto et al. 2002; Alexandrino et al. 2004; Pereira et al. 2011). The N input reduced LLS for all species (Figure 3), similar results were found for two species of *Brachiaria* by da Silva et al. (2009), and for *Dactylis glomerata* by Duru and Ducrocq (2000).

LER and DLE were lower in the second year, except *A. catharinensis* (Figure 6). However, SER was higher in the second year, but not for *A. catharinensi* species (Figure 6). These results could be explained by a probable water stress (Granier and Tardieu, 1999), as the first year was dryer compared to the second year (see section 3.1), or also due to the increase (see section 3.1) in the shade by trees canopy (Lin et al. 2001). Granier and Tardieu (1999) reported that water stress not affect DLE.

4.1 Growth and senescence fluxes

The growth flux (GF) was higher in the full sun for *B. brizantha*, *M. maximus* and *Cynodon* spp.. Only *A. altissima* showed higher GF in the ICLS and only *H. altissima* did not showed significant differences between systems (Figure 5). It was expect a reduction for all species in the GF in function of reductions in the light quantity by the trees canopy. However, the responses were species-dependent, i.e. probably due different species strategies in response to shade (Givnish, 1988; Cruz, 1997).

There was an effect of increase on GF due to N input for all species (Figure 5), this is an expected response, since N had an impact on LER, DLE and TD as reported before.

In the second year all species presented a higher GF (Figure 6). Although the reduction on PPFD reaching the forage canopy due to the trees canopy (see section 3.1), in the second year, the main daily temperature for the experimental period was higher compared to the first year (Figure 1), also first year was drier than second year (see section 3.1). These factors (i.e. water and temperature) could explain the differences between years for GF, beside the tree growth.

The senescence flux (SF) was significant different for *M. maximus* and *Cynodon* spp., with lower values in the ICLS (Figure 5). A parameter that could explain these variations in SF is the leaf senescence rate (LSR), however, only *H. altissima* showed differences in the LSR.

The N input reduced senescence flux for *A. catharinensis* and increased for *B. brizantha* (Figure 5), the effect of N is expect to reduce the senescence flux, as the plant necessities a lower remobilization of N from old leaves to produce new leaves (Hikosaka, 2005).

4.2 Relations between morphogenenis and growth fluxes for each species

In general, there was not an exclusive morphogenetic or structural characteristic that better explain differences in growth flux due to the system or nitrogen, but there is a specie dependency.

A. catharinensis showed higher GF in the ICLS. Pachas et al. (2013) showed that the growth rate of *A. catharinenis* was higher in a silvopastoral system (38% of PPFD reduction) than full sun. These results could be explained by the higher LER and higher DLE in the ICLS (Figure 2).

B. brizantha is considered as tolerate to moderate levels of shade (Paciullo et al. 2011). Here, we observed a reduction of 28% on GF in the ICLS. *M. maximus* and *Cynodon* spp. showed also reductions in the GF in ICLS, both of -41%. These results could be explained by a higher phyllochron in the ICLS (Figure 2) and by a lower specific leaf weigh (SLW) in this system. Results of Paciullo et al. (2007) shows a reduction on leaves biomass production with 65% of shade, and no differences with 35% of shade. In the work of Araújo et al. 2011, *B. brizantha* showed high morphogenic rates with higher intervals of cutting frequency in a study with coconut trees. For *Cynodon* spp. the reduction is also attributed to the drastic reduction on tiller density (Figure 2), as the other parameters (i.e. Phy. and SLW) did not showed significant variations from full sun to ICLS.

No differences were found for *H. altissima* in the GF between treatments. This species had higher rates for all morphogenical parameters evaluated and longer leaves in ICLS (Figure 2). However, there was a great reduction in the number of tillers (Figure 2). It is supposed that the higher rates in ICLS compensated the reduction on tillers density and maintained similar GF. *H. altissima* had also a negative balance (i.e. SF >

GF, see Figure 5) in both systems. Hence, a management with higher cutting frequency (i.e. lower than 95% of LI) will be probably necessary for this species in order to avoid losses by senescence.

To sum up, the effect of trees in the growth of tropical forage is specie-dependent, while *B. brizantha*, *M. maximus* and *Cynodon* spp. had a decrease on growth rates in ICLS, *H. altissima* did not changed and *A. catharinensis* increased leaf growth rates. However, even the species that showed decreases on leaves growth rates; they maintained a satisfactory development and growth at the ICLS. Also, the balance of GF and SF was positive, except for *H. altissima* that had a negative balance for both systems (-14.1 and -25.2 kg ha^{-1} day^{-1} in full sun and ICLS respectively, Figure 5). Further, the N supply contributes to the maintenance of a satisfactory development and growth of forages species in ICLS.

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Table 1 - Percentage of variance explained (VE) and statistical significance from the ANOVA for phyllochron (Phyl.), leaf elongation rate (LER), duration of leaf elongation (DLE), leaf lifespan (LLS), leaf senescence rate (LSR), stem elongation rate (SER), number of green leaves (NGL), specific leaf weight (SLW), leaf length (LL), tiller density (TD), growth flux (GF) and senescence flux (SF).

	Specie	System	N	Year	Sp x System	Sp x N	Sp x Year	N x Year	Sp x N x Year	Sp x System x Year
Morphogenical characteristics										
Phyl.	45.16^{***}	0.85^{*}	3.73^{***}	1.00^{**}	2.83^{***}	-	2.89^{***}	-	-	-
LER	15.77^{***}	2.43^{***}	6.77^{***}	5.79^{***}	7.85^{***}	2.41^{**}	-	1.01^{*}	-	3.49^{*}
DLE	13.03^{***}	3.55^{***}	1.18^{*}	2.85^{***}	2.75^{*}	-	5.50^{***}	-	-	-
LLS	16.61^{***}	2.19^{**}	1.03^{*}	0.33^{ns}	2.14^{*}	3.28^{**}	2.55^{*}	-	5.17^{***}	-
LSR	31.29^{***}	1.09^{**}	4.49^{***}	1.51^{**}	3.61^{***}	5.15^{***}	2.92^{***}	4.25^{***}	3.14^{***}	-
SER	21.98^{***}	1.26^{**}	1.20^{**}	9.19^{***}	7.68^{***}	-	6.31^{***}	-	-	-
Structural characteristics										
NGL	75.49^{***}	0.03^{ns}	1.50^{***}	0.02^{ns}	1.23^{***}	1.21^{***}	0.75^{*}	-	1.46^{***}	-
SLW	74.87^{***}	3.01^{***}	0.37^{**}	0.02^{ns}	3.70^{***}	4.66^{***}	-	-	-	-
LL	42.73^{***}	3.32^{***}	0.41^{ns}	0.21^{ns}	2.20^{**}	4.24^{***}	1.50^{*}	0.57^{*}	2.34^{**}	-
TD	42.96^{***}	4.39^{***}	10.49^{***}	0.01^{ns}	7.57^{***}	3.42^{***}	-	-	-	1.70^{**}
Growth and senescence fluxes										
GF	20.77^{***}	1.88^{***}	15.84^{***}	5.18^{***}	4.69^{***}	3.98^{***}	-	-	-	2.52^{*}
SF	13.09^{***}	0.36^{ns}	0.04^{ns}	1.43^{*}	2.96^{*}	3.36^{**}	2.59^{*}	2.91^{***}	4.28^{**}	-

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ns, not significant.

Table 2 – Means and standard error (se) for morphogenetic characteristics and growth and senescence fluxes for the years 2011 and 2012.

	2011	se	2012	se
Leaf Elongation rate (cm tiller^{-1} ºCd^{-1})	0.16 b	0.007	0.20 a	0.005
Duration of leaf elongation (ºCd)	220.5 a	8.47	189.2 b	4.28
Leaf senescence rate (cm tiller^{-1} ºCd^{-1})	0.07 a	0.006	0.06 b	0.003
Stem elongation rate	0.017 b	0.001	0.027 a	0.001
Growth flux (kg ha^{-1} day^{-1})	78.2 b	4.68	97.4 a	3.93

Means with the same letter in the line are not significantly different according to the Tukey test ($P > 0.05$).

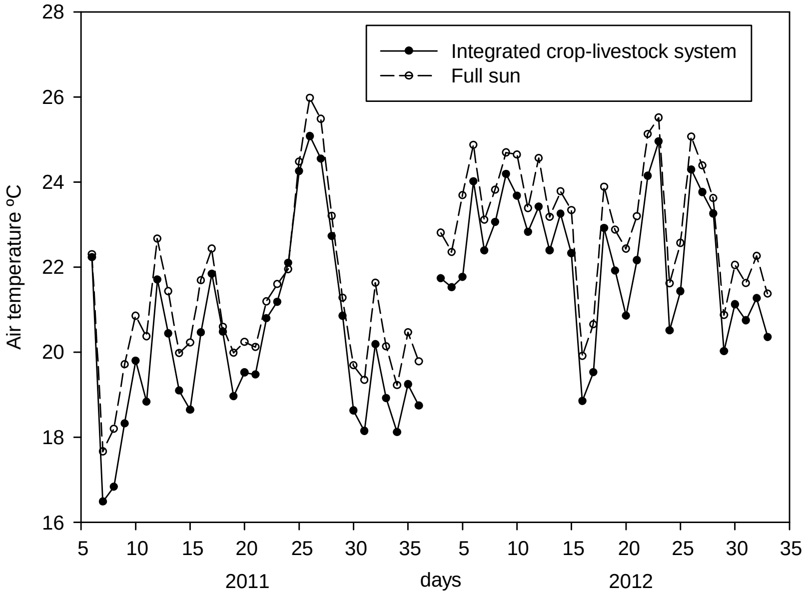


Figure 1 – Daily Mean air temperatures during 2011 and 2012 for the experimental period.

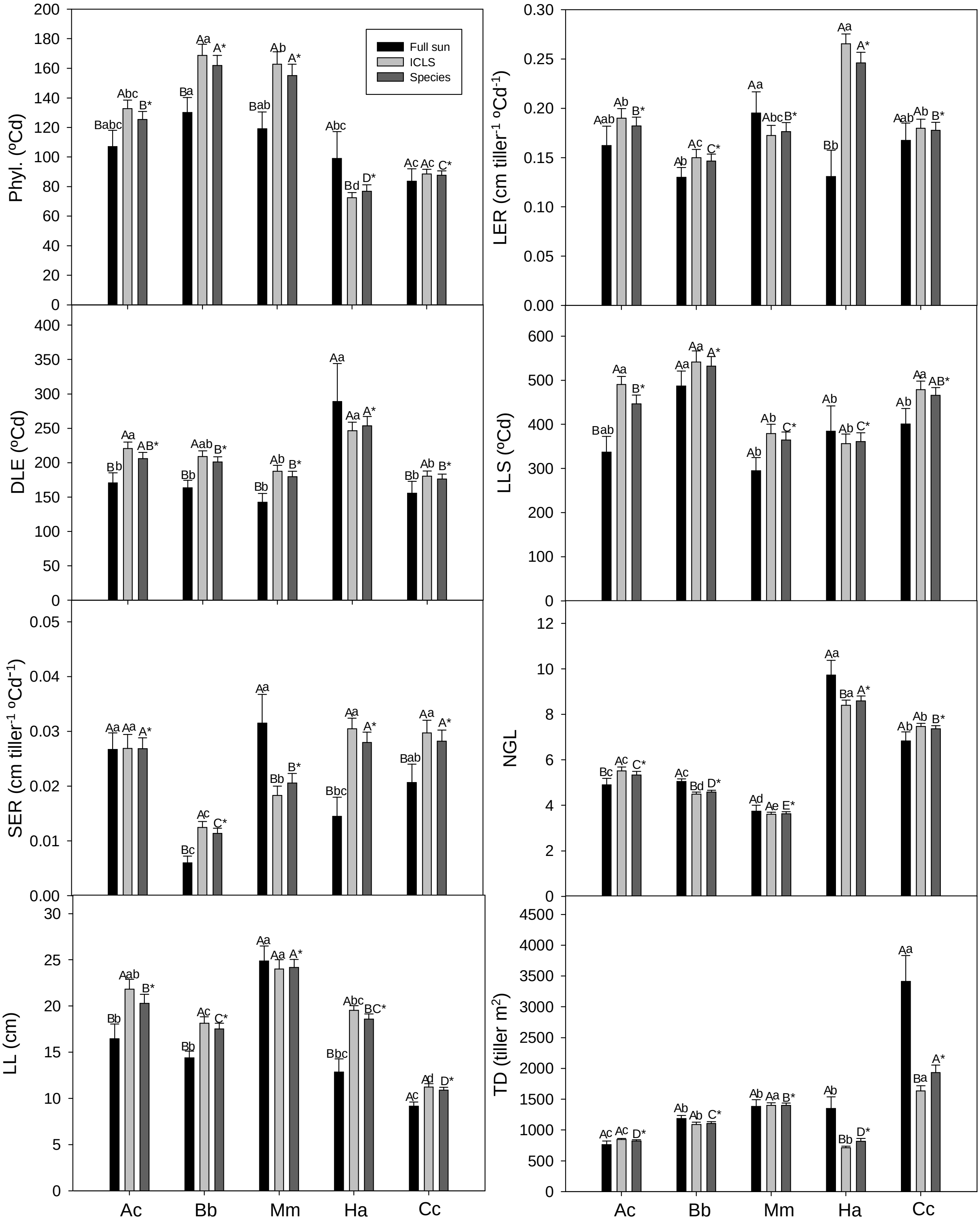


Figure 2 – Means for the morphogenical and structural parameters for each species and also within each system (i.e. data shown the species x system interaction). Means with the same capital letters compares systems, means with small letters compares species within each system and means with capital letters with * compares species according to the Tukey test (P >0.05). Bars indicate the standard error of the mean. Species code: *Axonopus catharinensis* (Ac)*;* Bb – *B. brizantha*; Mm – *Megathyrsus maximus* Ha – *Hemarthria altissima*; and Cc – *Cynodon* spp. Variables code: phyllochron (Phyl.); leaf elongation rate (LER); duration of leaf elongation (DLE), leaf lifespan (LLS; leaf

senescence rate (LSR); stem elongation rate (SER); number of green leaves (NGL); leaf length (LL); tiller density (TD).

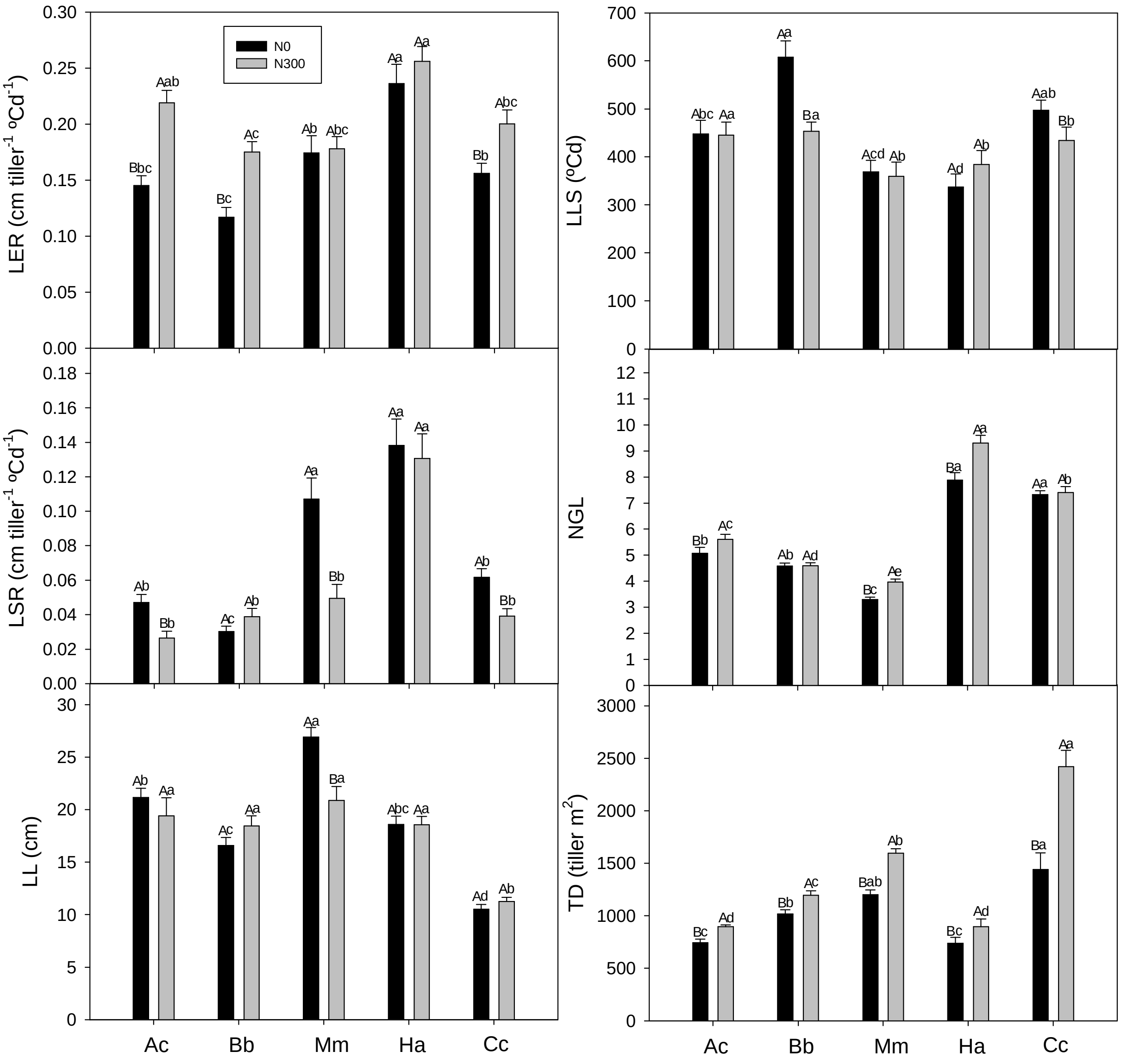


Fig. 3 - Means for the morphogenical and structural characteristics for the interaction species x nitrogen. Means with the same capital letters compares systems, means with small letters compares species within each system according to the Tukey test ($P > 0.05$). Bars indicate the standard error of the mean. Species code: *Axonopus catharinensis* (Ac)*;* Bb – *B. brizantha*; Mm – *Megathyrsus maximus* Ha – *Hemarthria altissima*; and Cc – *Cynodon* spp. Variables code: leaf elongation rate (LER); leaf lifespan (LLS); leaf senescence rate (LSR); number of green leaves (NGL); leaf length (LL); tiller density (TD).

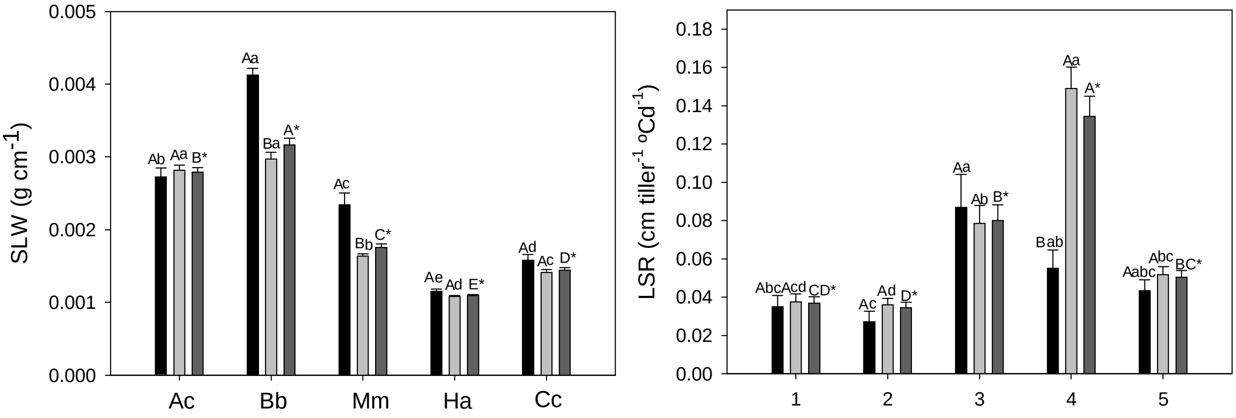


Fig. 4 – Means for the morphogenical and structural parameters for each species and also within each system (i.e. data shown the species x system interaction). Means with the same capital letters compares systems, means with small letters compares species within each system and means with capital letters with * compares species according to the Tukey test (P >0.05). Bars indicate the standard error of the mean. Species code: *Axonopus catharinensis* (Ac)*;* Bb – *B. brizantha*; Mm – *Megathyrsus maximus* Ha – *Hemarthria altissima*; and Cc – *Cynodon* spp. Variables code: specific leaf weight (SLW); leaf senescence rate (LSR).

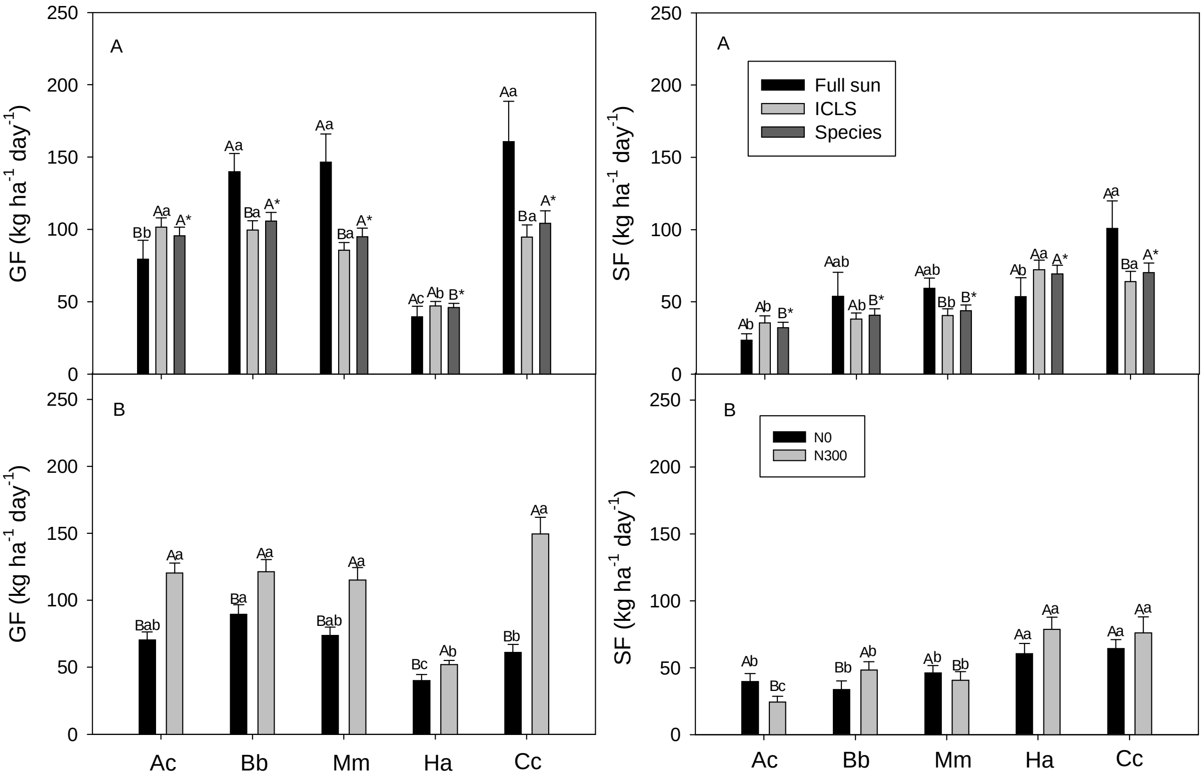
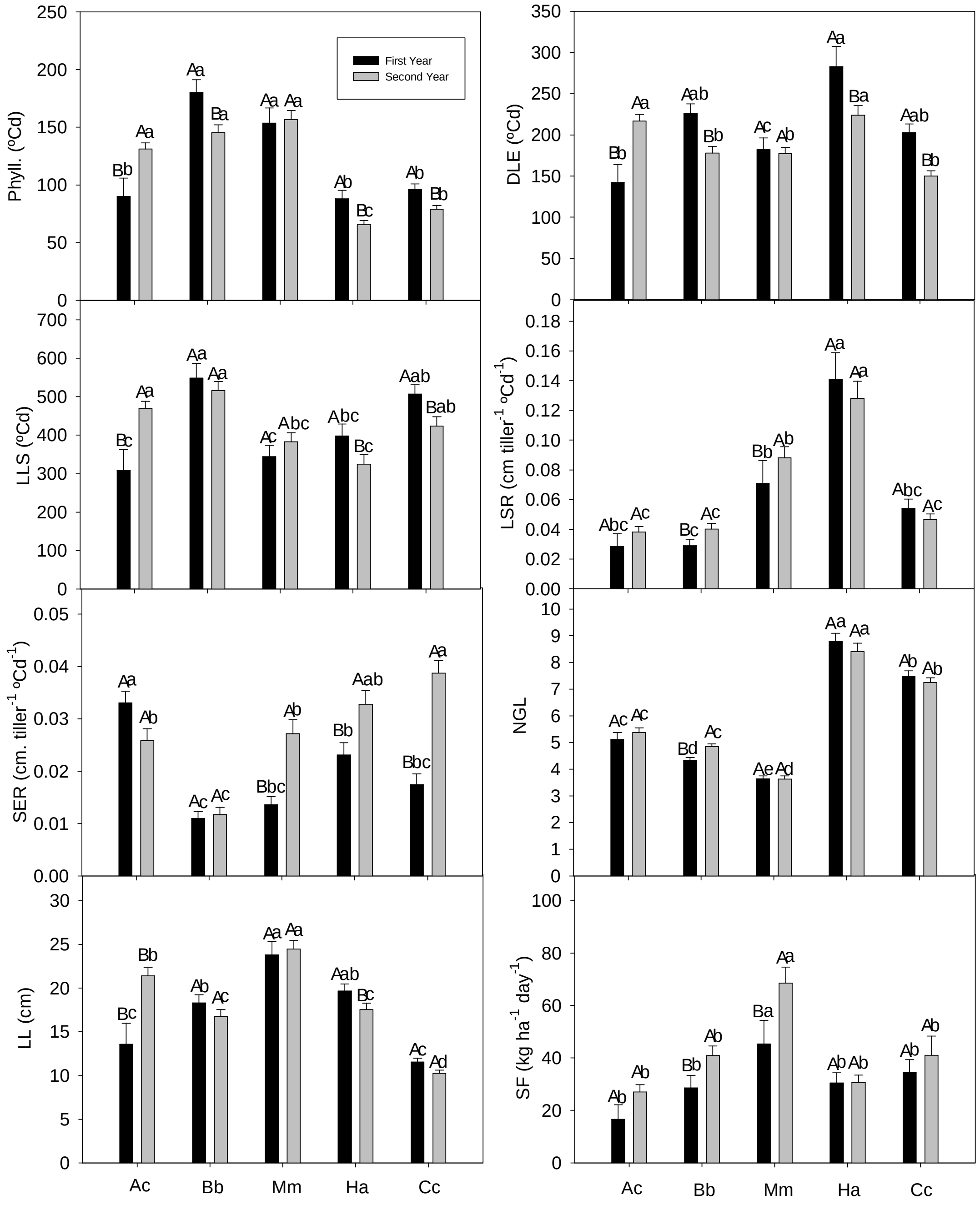


Fig. 5 – A) Means for the growth and senescence fluxes for each species and also within each system (i.e. data shown the species x system interaction).. B) Means for growth and senescence fluxes for the interaction species x nitrogen. Means for the morphogenical and structural parameters for each species and also within each system (i.e. data shown the species x system interaction). Means with the same capital letters compares systems, means with small letters compares species within each system and means with capital letters with * compares species according to the Tukey test (P >0.05). Bars indicate the standard error of the mean. Species code: *Axonopus catharinensis* (Ac)*;* Bb – *B. brizantha*; Mm – *Megathyrsus maximus* Ha – *Hemarthria altissima*; and Cc – *Cynodon* spp.



Supplementary data Fig S1 – Means for morphogenical and structural characteristics and the interaction species x year condition. Means with the same capital letters compares light condition, means with small letters compares species within each light condition according to the Tukey test (P >0.05). Species code: *Axonopus catharinensis* (Ac)*;* Bb – *B. brizantha*; Mm – *Megathyrsus maximus* Ha – *Hemarthria altissima*; and Cc – *Cynodon* spp. Variables code: phyllochron (Phyl.); duration of leaf elongation (DLE); leaf lifespan (LLS); leaf senescence rate (LSR); stem elongation rate (SER); number of green leaves (NGL); leaf length (LL); senescence flux (SF).

4. CAPÍTULO 3

Effect of blue light on two alfalfa morphotypes contrasting on their growth habits[1]

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[1] Elaborado de acordo com as normas da Revista Agronomy journal.

Effect of blue light on two alfalfa morphotypes contrasting on their growth habits

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Abstract

Light is considered the major resource driving plant architecture and vegetation dynamic. Different species and even different genotypes of the same species can exhibit contrasted strategies to capture and use light. These strategies in the context of light competition could determine plant's capacity to grow in monoculture or/and in mixtures. Than, understand morphological responses to blue light could be particularly helpful for that. The present study aimed to determine the effect of blue less light on the development and growth of two morphologically contrasted genotypes of *Medicago sativa*, that exhibit contrasted growth habits, an erected and other a prostrated morphotype. During the experimental period, 60 clones of each genotype were grown under 380 µmol m^{-2} s^{-1} of photosynthetic activated radiation (PAR) and 14h photoperiod. Two light conditions were simulated in growth cabinets: low blue light level (B-) and neutral light (B+). The differences in the response to blue light of several morphological parameters (internodes and petiole length, leaf area) between the two genotypes strongly suggest difference in the strategy for light foraging with genotype B4 (erect) presenting more trends to escape and genotype D3 (prostated) to tolerate shade.

Key words: competition; crop mixtures; light quality; pasture canopy; shade-avoidance

1. Introduction

When plants are growing in a canopy, they experience a heterogeneous light environment in terms of light quantity and also light quality (Varlet-Grancher et al., 1993a; Ballaré et al., 1997, Holmes and Smith, 1977; Werger et al., 2002). This is mainly due to interactions of light with plant organs characterized by the capacity to absorb, transmit and reflect solar radiations within the range of 350nm to 800nm (Smith, 1982).

Both differences in the photon flux density (400-700nm) and changes in light quality (i.e. Blue, Red and Far red wavebands) induce morphogenetic responses that affect the capacity of plants to capture light (Tardieu et al., 1999; Lötscher and Nösberger, 1997; Gautier et al., 2000; Cookson and Granier, 2006). However, light quality is considered as principal cue driving plant architecture and vegetation dynamic (Ballaré et al., 1997; Kasperbauer and Hunt, 1992).

Specifically, blue light in the range of 350-500 nm regulates important aspects of plant development and growth (Varlet-Grancher et al., 1993b). In shade-avoidance plants, the effects of blue light is well known to trigger a variety of photomorphogenic responses such changes in stem and leaf length due to the increase in internodes lengths and leaf elongation rate (Gautier and Varlet-Grancher, 1996; Ballaré and Casal, 2000). Indeed, blue light can interfere on plant function, due to the influence on gas exchange through stomatal functioning (Zeiger et al., 1987; Talbott et al., 2002; Barillot et al., 2010). However, some studies suggest that amplitude and capacity of plants to respond to blue light depends on the stage of development and species (Casal and Smith, 1988; Drumm-Herrel and Mohr, 1991; Mitchell and Woodward, 1988).

The enhancement of stem and leaf elongation are due to decrease of blue light within a plant canopy. The changes in blue light are sensed mainly by two types of receptors, cryptochromes and the phototropins (Christie and Briggs, 2001; Lin, 2002) that allow plants to characterize their nearby environment and particularly the intensity of light competition.

It is well known that light is a major factor of competition between individuals in growing stands as grasslands, monocultures or in intercropping systems (Wedin and

Tilman 1993; Ballaré et al., 1997; Lemaire et al., 2005; Baldissera et al., 2014). Depending on plant density, genetic heterogeneity (even intra-specific) and the constraints imposed by the operating mode in these systems (plants density or diversity/cut/pasture), the lighting conditions of individual plants and light partitioning between species vary significantly. Consequently, the changes in mass of individuals as well the proportion of each species and its contribution the overall leaf area index in the in the stand are related to experienced light conditions (Barilllot et al., 2011). Thus, different species and even different genotypes of the same species can exhibit contrasted strategies to capture and use light. These strategies in the context of light competition could determine plant's capacity to grow in monoculture or/and in mixture and the morphological responses to blue light could be particularly helpful for that.

The present study aimed to determined the effect of blue less light on the development and growth of two morphologically contrasted genotypes of *Medicago sativa*, that exhibit contrasted growth habits, an erected and a prostrated morphotype respectively.

2. Material and Methods

The experiments were performed in the laboratory at the INRA Lusignan research station France. Two morphologically contrasted genotypes of *Medicago sativa* were used in this experiment: B4 and D3 an erected and a prostrated morphotype, respectively. 60 clones of each genotype were prepared in October 2011. During December 2011 clones were transferred from nursery to greenhouse into 2L individual plastic pots filled with sand. Clones were maintained in the greenhouse at 15°C during 2 months under additional light sources and were watered 8 times a day. Plants were then transferred into two growth cabinets for three weeks of pre-treatment at 17°C night and day. During this period clones were grown under 380 µmol m^{-2} s^{-1} of photosynthetic activated radiation (PAR) and 14h photoperiod provided by 6 metallic iodure lamps (HQI 400W, Osram, France) at 80 % of relative humidity and were automatically watered 8 times a day with a complete nutrient solution containing 1.9 mol m^{-3} KNO_3, 0.55 mol m^{-3} $Ca(NO_3)_2$, 2.5 mol m^{-3} NH_4NO_3, 0.5 mol m^{-3} $CaCl_2$, 0.1 mol m^{-3} NaCl, 0.5 mol m^{-3} $MgSO_4$, 0.4 mol m^{-3} KH_2PO_4, 0.3 K2HPO4, 25 10^{-3} mol m^{-3} H_3BO_3, 2 10^{-3} mol m^{-3} $MnSO_4$, 2 10^{-3} mol m^{-3} $ZnSO_4$, 0.5 10^{-3} mol m^{-3} $CuSO_4$, 0.5 10^{-3} mol m^{-3} H_2MoO_4 and 20 10^{-3} mol m^{-3} Fe-HEDTA. Finally, 40 clones of each genotype were selected on the base of the total number of stems and theirs length. Clones were then cut at 8cm from the collet (collar) and were randomly distributed under two light treatments in two others growth cabinets (T°C=17°C, 14h photoperiod, 80% HR, full nutrient solution). Plants were grown under these conditions until the beginning of flowering (around 2 months) and during this period depending on their developmental stage they received from 80 to 520 ml per day of full nutrient solution.

2.1 Light treatment

Two light conditions were simulated in the growth cabinets: low blue light level (B-) and neutral light (B+). The low blue light level (5 µmol m^{-2} s^{-1}) was thus obtained with a Lee Filter HT 015 (Lee Filter, Hampshire, England) associated to 9 metallic iodure lamps (HQI 400W, Osram, France) which supplied high PAR levels. The neutral light was obtained by using the Lee 216 filter associated to 6 metallic iodure lamps. This filter provided a neutral shade as it identically lowered the energy from all wavelengths of the incident light (around 25%). The distance between plants and light sources +

filters was adjusted in both growth cabinets in order to provide photon flux with the same photosynthetic efficiency in B+ (PAR= 407 µmol/m2/s; YPF= 379 µmol/m2/s) and B- (PAR= 438 µmol/m2/s ; YPF= 383 µmol/m2/s) treatments.

2.2 Morphological measurements

The measurements were done around two months after the beginning of the light treatments. On each clone a main vigorous growing shoot (main axis) was identified and it length measured with a ruler. The number of nodes on this shoot as well the number of associated leaves was reported. For each node rank of this shoot the length of internodes and petioles were measured with a ruler. Other shoots were cut at 8 cm from collet, their number reported and total leaf area measured (once leaves separated from shoots), using a planimeter (LI-3100, LI-Cor, inc, Lincoln, NE, USA).

For the end of regrowth (i.e. after first cut), plant biomass was determined. Plants were separated in above ground and roots components for total above ground and roots biomass analysis. Roots were carefully removed from the pot and washed. To measure dry mass, all samples were dried in the oven for 48h at 60°C.

From the measurements of the main axis it were calculated specific leaf area (SLA – $cm^2 g^{-1}$), specific stem weight (SSW – $g^{-1} cm^{-1}$), leaf:stem ratio of the main axis (leaves dry weight / stem dry weight). For the biomass measurements from the regrowth period it was calculated shoot:roots ratio (above ground dry weight / roots dry weight).

2.3 Statistical analyses

Statistical analysis were performed using R software (R Development Core Team, 2014). For each node position on the mains axis, T test (t.test procedure) was used to compare internode and petiole lengths between light treatments for each genotype. The effects of light treatment, genotype and their interaction on shoot number, total leaf area, specific leaf area, leaves dry weight of main axis, stem dry weight, of the main axis, specific stem weight, leaves:stem ratio, total plant dry weight, above ground dry weight and above ground:roots dry weight ratio were tested by analysis of variance (ANOVA, aov procedure), and Tukey (HSD.test package AGRICOLAE) method for multiple mean comparison tests in post-ANOVA. Data were transformed when necessary to reach the normality of residues, transformations were performed using the procedure Box Cox (package MASS).

3 Results

3.1 Internodes and petioles lengths

Irrespective of light condition, there were differences in morphological plant development as described for internode and petiole appearance probability and evaluated for each genotype and light treatment (Figure 1). All plants presented at least three nodes, except for genotype D3 growing without blue light. 80% of the plants showed seven nodes, and the biggest plants reached 14 nodes.

There was a significant ($P < 0.04$) increase of internode lengths from the 2nd to the 6th for genotype B4 under blue less (B-) light, whereas no significant ($P > 0.29$) changes were observed for D3 genotype (Figure 2). For genotype B4, the length of petioles was ranged from the maximum of 50 mm and 30 mm in B- and B+ treatments, respectively to around 7mm for both treatments. The genotype D3 exhibited lower values, ranging for both light treatments from 7mm to 33mm.

For the petiole lengths there was minor effect for genotype B4 compared to the responses on internode lengths (Figure 3) and significant difference between light treatments occurred only in the 2nd ($P = 0.02$) and the 4th node ($P = 0.005$). As occurred for internodes length, B- light treatment did not have effect on petioles lengths ($P > 0.09$) in genotype D3(Figure 3).

3.2 Number of shoots

The total number of shoots was not affected by the blue light treatment, differences were observed only for genotype (Table 1). The genotype D3 showed a higher average number of shoots (6.45 shoots) compared to genotype B4 (5.33 shoots) (Table 2).

3.3 Plant leaf area and specific leaf area

The ANOVA outputs showed a significant genotype x blue light treatment interaction for the leaf area of main axis (Table 1). This interaction was due to the increase in leaf area for the genotype B4 in the absence of blue light. This genotype exhibited 107 cm^2 of leaf area on the main axis under B- conditions whereas only 73 cm^2 were measured under B+ conditions. Genotype D3 did not change main axis leaf area under B- treatment (49 cm^2) and exhibited 64 cm^2 on main shoot in the B+ treatment that was similar to genotype B4 (Table 3). Leaf area of main axis for D3 genotype was slightly higher in B+ but was not significantly different between light treatments. Consequently, the specific leaf area was affected only by light treatment (Table 1), showing an increase under B- light conditions (Table 2).

3.4 Dry weight and its partition

The dry weight of leaves and stems were evaluated for the main axis in the first growth period. The biomass of leaves from the main axis was also higher when exposed to the treatment with blue light, and genotype B4 showed higher leaves dry weight compared to genotype D3 (Table 2). For stems, ANOVA results showed an interaction between light treatment x genotype (Table 1). This interaction was mainly due to the increase of stem dry weight for plants of genotype B4 growing in B-.

Under B+ treatment the stem weight was similar for both genotypes whereas it was significantly different under B- treatment (Table 3).The differences in stem weight can be explained by the specific stem weight (SSW) and stem height (Table 1). Genotype B4 showed similar SSW (Table 3), but had higher internodes lengths (Figure 2) in the treatment B-, producing longer stems. For genotype D3, there was no difference in lengths (Figure 2), however the SSW was lower for plants growing without blue light.

For the regrowth period (i.e. after first cut) it was analyzed the total plant biomass and its partition between above ground and roots biomass. Total dry weights of plants were higher with the B+, and there was no difference between genotypes (Table 2).

For above ground and roots biomass the same pattern of total biomass occurred, where it was affected only by light treatment, with an increase on dry weight for the plants growing under blue light (B+).

4. Discussion

The objective of our study was to analyze the effect of blue less light on the development and growth of two morphologically contrasted genotypes of *Medicago sativa*. The differences in the response to blue light of several morphological parameters (internodes and petiole length, leaf area) between the two genotypes strongly suggest difference in the strategy for light foraging with genotype B4 (erect) presenting more trends to escape and genotype D3 (prostated) to tolerate shade (Givnish, 1988).

4.1 Effects of blue light on Elongation and Leaf area

The effect of light quality (Red:Far Red) on stem elongation is well reported in the literature, plants trying to escape shade tend to increase stems to reach a better light condition (Morgan and Smith, 1981; Morgan 1982, Child and Smith, 1987). Our results demonstrate that the isolated effect of blue light also has an impact on the stem elongation (Figure 2). In contrast, petiole elongation was slight affected by blue light treatment on genotype B4 and genotype D3 was not affected at all (Figue 3). The effect of light quality in petiole elongation is expected to be higher in plants that exhibit a prostrated pattern of growth, however this was not the case in this work. For white clover petiole elongation and inclination is affected by blue light (Gautier et al., 1997, Christophe et al., 2006). Low blue light change petiole inclination and helps to position leaves in the upper layers of the canopy interfering in light capture. Gautier et al. (2001) also showed that in the case of white clover, the response of petioles was independent of the plant position. For the estoloniferous species *Potentilla reptans*, the response to shade occurred more in a vertical way, due to the increase of petioles lengths, rather than internodes lengths (Huber and Stuefer, 1997).

The increase in the leaf area of main stem occurred only for genotype B4 in the absence of blue light (Table 3). Plant leaf area on a stem is dependent of the total number of leaves and leaf size. According to Gautier et al. (1997), modifications in light quality can leads to increases of leaf size. However, Baldissera et al. (2014) showed that in alfalfa competing for light, there was a reduction in final plant leaf area due to plant density, and this reduction was referred to plant development (i.e. branches appearance, number of shoots), harder than the size of leaves.

Blue light did not affected total leaf area, but changed specific leaf area, that was higher for both genotypes (Table 1). In general, plants growing in a reduced light condition exhibit thinner leaves compared to high light condition, also various authors showed that the response of SLA is highly correlated with photosynthetic photon flux density (Tucker et al., 1987; Dale, 1988; Niinemets and Kull, 1998; King, 2003, Petritan et al., 2009), also the response of SLA can be linked with the species tolerance to shade. However, some investigations show that more shade-tolerant species tend to show a higher SLA with the decrease in light availability (Niinemets and Kull, 1997; Barthod and Epron, 2005; Klooster et al., 2007). But others did not confirm this pattern (DeLucia et al., 1998; Stancioiu and O’Hara, 2006).

Due to the results found on total leaf area of the main axis (Table 3), it could be hypothesized that blue light may does not interfere in leaves appearance, but only on leaf morphology as it has been shown for other forage species (Gautier and Varlet-Grancher, 1996)

4.2 Effects of blue light on biomass partitioning between leaves and stems

The decrease in leaves dry weight (Table 2) corroborates with the results obtained by Lin et al. (2001), for fifteen forage species including alfalfa, these authors also showed an increase in total leaf area, decreases on leaves dry weight are directly correlated with SLA (see section 4.1)

On the other hand, stem weight of the main axes was higher for the genotype B4 for the treatment B- (Table 4), whereas the SSW did not changed, than the increase on stem weight can be correlated with the increase in internodes lengths (Figure 2), resulting in longer stems. In the case of genotype D3, the stem weight of main axis tended to be lower under B- treatment, mainly due to the lower SSW (Table 4), as there were no changes in internodes lengths (Figure 2). The modulation of carbon allocation to leaves and stems depends on species and light conditions (Samarakoon et al., 1990) and often result in distinct leaf:stem ratios between genotypes. Finally in our experiment, D3 genotype did not changed leaf:stem in response to light treatments whereas genotype B4 reduced its ratio under B- treatment. This higher leaf:stem ratio can be positive response, because it trigger a better forage quality (Lin et al., 2001).

This differences in biomass allocation to leaves and stems also agrees with distinct strategies between genotypes to capture light, that was already commented in the topic 4.1, and the results showed in our work for the blue light effect are similar from those reported for changes in red:far-red or in the reduction of photosynthesis photon fluxes density (Corré 1983; Keiller and Smith, 1989; Fortnum and Kasperbauer 1992, Kasperbauer and Hunt 1992 a,b). While genotype B4 (erect habit of growth) tended to elevate leaves by increase of stem height, and changing biomass allocation to stems, genotype D3 (prostrated habit of growth), maintained allocation to leaves and reduced for stems, than we could hypothesize this is correlated with the plagiotropic pattern of growth of this genotype.

4.3 Effects of blue light on total plant biomass and root-shoot ratio

Total biomass production was reduced by the lack of blue light, also total above ground and roots biomass followed the same pattern, resulting in no differences for the ratio above ground:roots dry weight (Table 2). The reduction on biomass can be an effect of blue light on stomata opening (Smith, 1982; Fankhauser and Chory, 1997; Kinoshita et al., 2001; Talbott et al., 2002; Barillot et al., 2010). Blue light stimulated stomata opening, when stomata are closed, it prevents the CO_2 passage to the interior of the cells, resulting in a decrease of photosynthesis. Barillot et al. (2010) showed that under a decrease level of blue light, there is a instantaneous closure of stomata, and a gradual reopening after 20 min, but leading to a new steady-state, never reaching the initial state.

4. 4 summarizing

To sum up, morphological and growth process are affected on alfalfa by blue light, however contrasting genotypes in their habit of growth showed distinct responses to light and distinct strategies to capture light. These findings should help in define management strategies, mainly for consortiums. Baldissera et al. (2014) evaluated the same erected morphotype, and showed that when growing in a consortium with tall fescue (*Festuca arundineacea*), alfalfa tended to overlap the graminea species.

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Table 1 – F-ratios and statistical significance of ANOVAs of plant traits in function of blue light and contrasted genotypes of *Medicago sativa.*

	Treatment	Genotype	Treatment x Genotype
Shoot number	n.s.	11.47**	n.s.
Leaf area of main axis	n.s.	7.9**	6.86*
Specific leaf area	30.77***	n.s.	n.s.
Leaves dry weight of the main axis	4.89*	4.91*	n.s.
Stem dry weight of the main axis	n.s.	9.02**	7.65*
Leaf:stem ratio	10.68**	5.30*	8.12**
Specific stem weight	8.70**	15.26***	5.17*
Total dry weight	23.37***	n.s.	n.s.
Above ground weight	17.99***	n.s.	n.s.
Roots dry weight	31.17***	n.s.	n.s.
Above ground:roots ratio	n.s	n.s	n.s

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; n.s., not significant.

Table 2 – Plant traits in function of blue light (B- less bluelight; B+ neutral blue light) and contrasted genotypes of *Medicago sativa* (B4 prostrate; D3 erect).

	B-	B+	B4	D3
Shoot number	5.7 (0.24) a	5.9 (0.26) a	5.3 (0.20) b	6.4 (0.26) a
Specific leaf area (cm^2 g^{-1})	329.1 (10.9) a	238.3 (11.84) b	284.4 (13.1) a	280.1 (14.6) a
Leaves dry weight of the main axis (g)	231.31 b	312.63 a	315.21 a	22.51 b
Total dry weight (g)	6402.8 (347.5) b	10297.8 (654.3) a	8860.7 (561.5) a	7953.4 (673.5) a
Above ground dry weight (g)	3687.5 (209.7) b	5715.2 (384.9) a	4996.2 (328.1) a	4341.0 (361.1) a
Root dry weight (g)	2691.8 (147.2) b	4421.4 (254.3) a	3801.7 (230.5) a	3360.5 (272.6) a
Ratio Shoot:Root dry weight (g)	1.3 (0.04) a	1.31 (0.03) a	1.3 (0.03) a	1.3 (0.04) a

Means with the same letters for light treatment and for genotype treatment are not significantly different according to the Tukey test (P . 0.05). standard error of the mean in parenthesis.

Table 3 – Leaf area of main (cm^2) axis in function of blue light (B- less bluelight; B+ neutral blue light) and contrasted genotypes of *Medicago sativa* (B4 prostrate; D3 erect).

	B4	D3
B-	107.36 (14.0) Aa	49.08 (7.0) Ab
B+	72.81 (10.1) Ba	64.63 (7.0) Aa

Means with the same capital letters in the column and small letters in the lines are not significantly different according to the Tukey test (P . 0.05). standard error of the mean in parenthesis.

Table 4 - Stem dry weight of the main axis (g) and specific stem weight (g. cm^{-1}) in function of blue light (B- less bluelight; B+ neutral blue light) and contrasted genotypes of *Medicago sativa* (B4 prostrate; D3 erect).

Stem weight of main axis	B4	D3
B-	310.5 (46.0) Aa	111.88 (21.7) Ab
B+	210.61 (31.3) Ba	200.52 (32.5) Aa
Specific stem weight	B4	D3
B-	0.844 (0.07) Aa	0.44 (0.05) Ab
B+	0.881 (0.05) Aa	0.772 (0.08) Ba

Means with the same capital letters in the column and small letters in the lines are not significantly different according to the Tukey test (P . 0.05).

Table 5 – Leaf:stem in function of blue light (B- less bluelight; B+ neutral blue light) and contrasted genotypes of *Medicago sativa* (B4 prostrate; D3 erect).

	B4	D3
B-	1.260 (0.10) Bb	1.671 (0.09) Aa
B+	1.814 (0.12) Aa	1.757 (0.16) Aa

Means with the same capital letters in the column and small letters in the lines are not significantly different according to the Tukey test (P . 0.05).

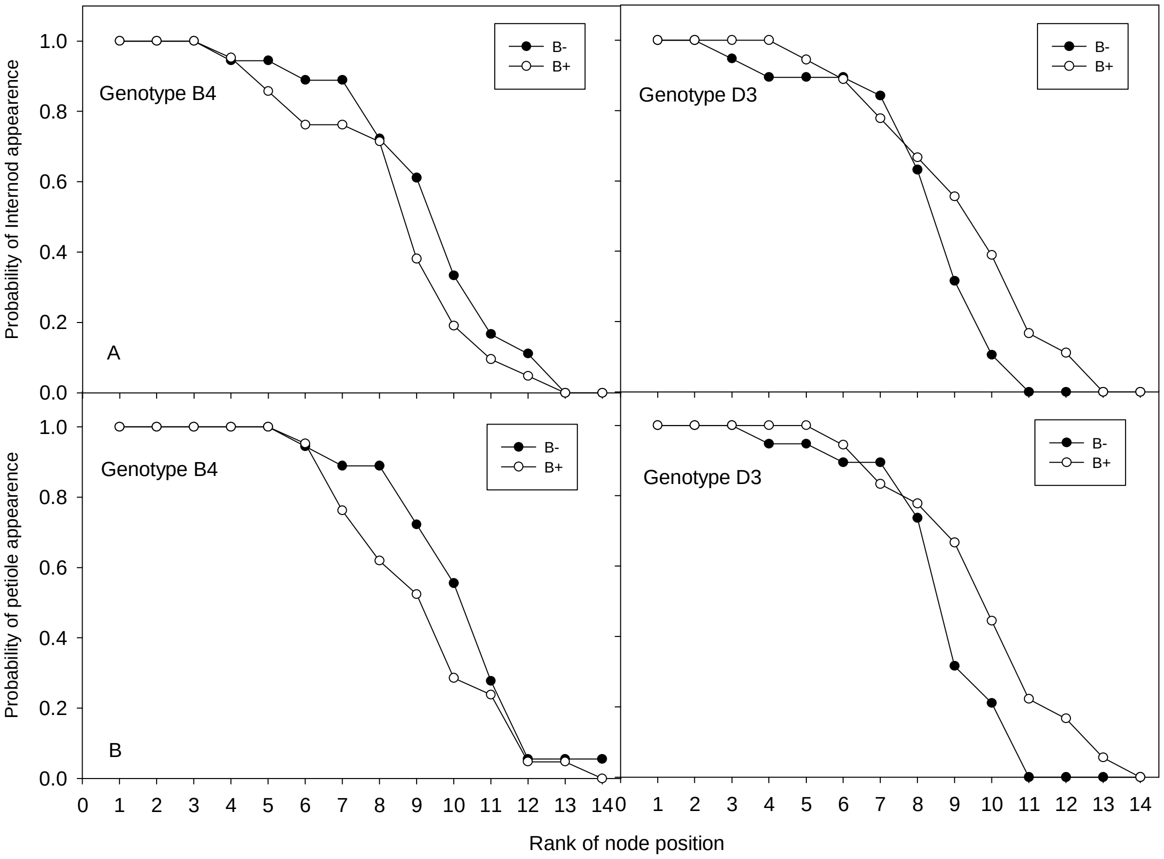

Figure 1 – Probability of internode and petiole appearance in respect to each node position in the main axis of contrasted genotypes of *Medicago sativa*. (B- less bluelight; B+ neutral blue light). (B4 prostrate; D3 erect).

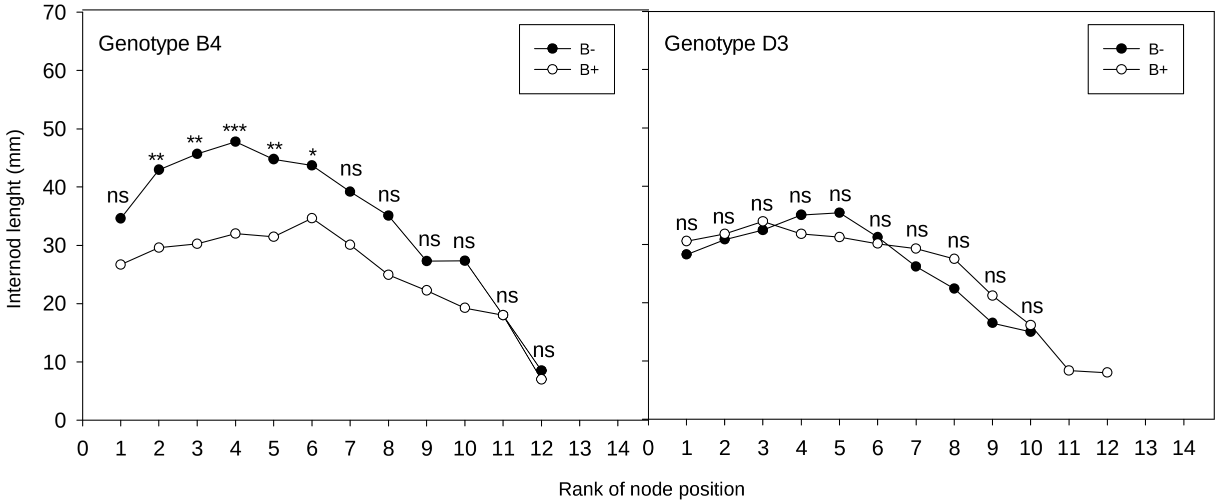

Figure 2 – Internod lengths in respect to each node position in the main axis. of contrasted genotypes of *Medicago sativa*. (B- less bluelight; B+ neutral blue light). (B4 prostrate; D3 erect) (*P< 0.05; **P < 0.01; ***P<0.001; ns, not significant).

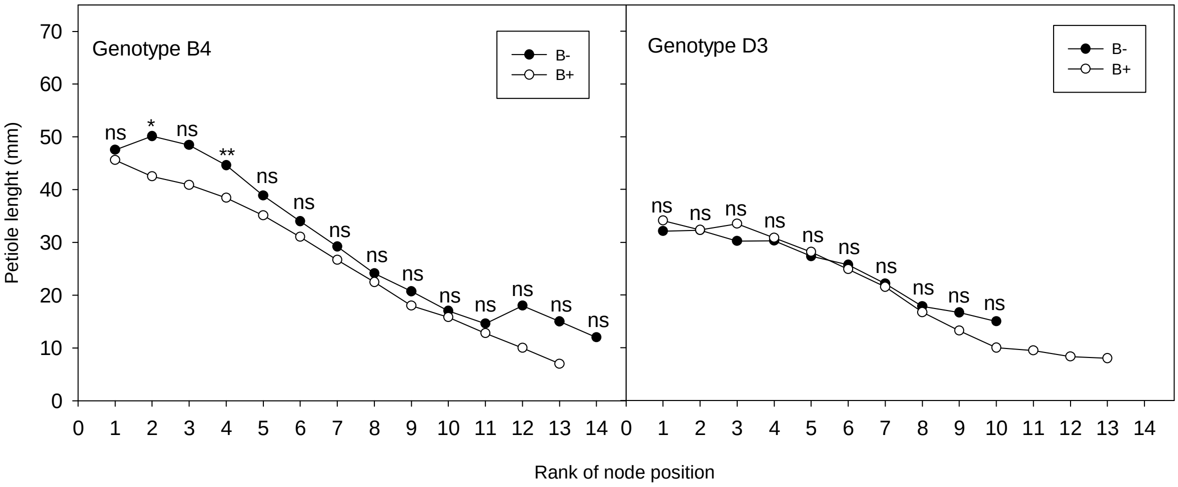

Figure 3 - Petiole lengths in respect to each node position in the main axis of contrasted genotypes of *Medicago sativa*. (B- less bluelight; B+ neutral blue light). (B4 prostrate; D3 erect) (*P< 0.05; **P < 0.01; ***P<0.001; ns, not significant).

5. CAPÍTULO 4

Plant development controls leaf area expansion in alfalfa plants competing for light

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Plant development controls leaf area expansion in alfalfa plants competing for light

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- *Background and Aims* The growth of crops in a mixture is more variable and difficult to predict than that in pure stands. Light partitioning and crop leaf area expansion play prominent roles in explaining this variability. However, in many crops commonly grown in mixtures, including the forage species alfalfa, the sensitivity and relative importance of the physiological responses involved in the light modulation of leaf area expansion are still to be established. This study was designed to assess the relative sensitivity of primary shoot development, branching and individual leaf expansion in alfalfa in response to light availability.
- *Methods* Two experiments were carried out. The first studied isolated plants to assess the potential development of different shoot types and growth periods. The second consisted of manipulating the intensity of competition for light using a range of canopies in pure and mixed stands at two densities so as to evaluate the relative effects on shoot development, leaf growth, and plant and shoot demography.
- *Key Results* Shoot development in the absence of light competition was deterministic (constant phyllochrons of 32·5 °Cd and 48·2 °Cd for primary axes and branches, branching probability of 1, constant delay of 1·75 phyllochron before axillary bud burst) and identical irrespective of shoot type and growth/regrowth periods. During light competition experiments, changes in plant development explained most of the plant leaf area variations, with average leaf size contributing to a lesser extent. Branch development and the number of shoots per plant were the leaf area components most affected by light availability. Primary axis development and plant demography were only affected in situations of severe light competition.
- *Conclusions* Plant leaf area components differed with regard to their sensitivity to light competition. The potential shoot development model presented in this study could serve as a framework to integrate light responses in alfalfa crop models.

Key words: *Medicago sativa*, leaf area, light competition, branching, shoot, development, leaf growth, morphogenesis, model.

INTRODUCTION

Leaf area largely determines light interception and transpiration in plants (Monteith, 1977). An increase in crop leaf area over time depends on variables at different levels of organization: plant density at the population level; the number of shoots per plant and shoot development at the organism level; and ultimately individual leaf expansion at the organ level (Monteith and Elston, 1983; Ong and Baker, 1985; Varlet-Grancher and Gautier, 1995; Lafarge, 1998). However, all these variables are seldom considered in crop models. Plant density (crops) and shoot/tiller density (perennial forages such as grasses or alfalfa) are often taken as fixed input parameters in the most detailed representations based on an average plant/shoot leaf area expansion (e.g. CERES, Jones *et al.*, 1984; APSIM, Robertson *et al.*, 2002). In many cases, the potential leaf area index (LAI) of a crop is even described directly as a function of time, encompassing the effects of all these variables in the parameters of a growth function (Gosse *et al.*, 1984; Goudriaan and Monteith, 1990; Brisson *et al.*, 2003). Combined with plant responses that modulate leaf area expansion as a function of drought, temperature or nitrogen (Jones *et al.*, 1984; Brisson *et al.* 2008), these approaches have yielded good estimates of light interception and biomass production in many crops (Boote *et al.*, 1996; Hammer *et al.*, 2002).

In crop mixtures, on the other hand, success has been more limited and much work is still required to predict the LAI dynamics of different species when they interact (Malezieux *et al.*, 2009). Indeed, crop leaf area expansion may differ markedly, at both the plant and canopy levels, between pure and mixed crops (Sinoquet and Cruz, 1993; Nassiri, 1998). One critical point is usually to explain the effects of competition on the population dynamics of plants or shoots (Kiniry and Williams, 1995; Brisson *et al.*, 2004; Soussana and Oliveira Machado, 2000). In particular, account needs to be taken of the effects of light, as this factor is often ignored with respect to leaf area expansion in pure crops (Jones *et al.*, 1984; Brisson *et al.*, 2008).

Light can affect morphogenesis and leaf area expansion, through trophic effects related to the photosynthetic photon flux density (PPFD), and light quality effects (Lötscher and Nösberger, 1997; Tardieu *et al.*, 1999; Gautier *et al.*, 2000; Cookson *et al.*, 2006). At the population level, competition for light commonly induces a size structure of plant populations at high densities (Obeid *et al.*, 1967; Weiner and Thomas, 1986; Gosse *et al.*, 1988) or in populations of subordinate species in mixtures (Weiner, 1985; Louarn *et al.*, 2012*a*). Self-thinning may occur under severe light competition and modify plant density (Kays and Harper, 1974; Westoby and Howell, 1982), preferentially affecting smaller plants in the population, in which the carbon balance may become negative (Dewar, 1993; Louarn *et al.*, 2012*b*). At the plant level, total plant leaf area is the consequence of the organogenesis process, controlled by the functioning of meristems and individual leaf growth. The effects of light competition on plant development, and on a reduction of branching in particular, are well established (Deregibus *et al.*, 1985; Kasperbauer and Karlen, 1986; Gautier *et al.*, 1999). However, they are difficult to predict and intimately related to the distribution of light into the canopy and to the location of plant meristems (Holmes and Smith, 1977; Ballaré *et al.*, 1997; Gautier *et al.*, 2000; Evers *et al.*, 2006; Stamm and Kumar, 2010). At the organ level, a reduction in PPFD causes no effect on leaf expansion during the rapid growth period, while the leaf is autotrophic, but considerably reduces the relative expansion rate during early leaf development (Tardieu *et al.*, 1999). On the other hand, modifications to light quality can result in increased leaf size (Varlet-Grancher and Gautier, 1995). In many crops commonly grown in mixtures, including the forage species alfalfa (*Medicago sativa* L.), the relative sensitivity of these plant responses to light availability, as well as their relative importance to LAI modulation, still need to be established.

Alfalfa–grass mixtures are among the most widespread forage crops in many temperate areas (Fick *et al.*, 1988; Basigalup, 2007). In such communities, alfalfa leaf area expansion has been shown to be the main attribute that explains light interception by the legume component and its biomass production at both the canopy and plant scales (Barillot *et al.*, 2011; Louarn *et al.*, 2012*a*, *b*). Alfalfa leaf area components display systematic variations across regrowth cycles in response to temperature and photoperiod (Brown *et al.*, 2005). Light responses are mostly inferred from density experiments in pure stands, where a trade-off between plant density and the number of shoots per plant is usually reported (Cowett and Sprague, 1962, 1963; Mattera *et al.*, 2013), leading to relatively steady shoot density. In mixtures, however, the persistence of the legume is an issue, and such substitutive relationships are not ensured (Jones and Carter, 1989). Density-dependent mortality can lead to a permanent decline of the legume proportion in favour of the companion species. In alfalfa, density-dependent mortality may be related to the plant's ability to maintain shoots with a consistent leaf area (Louarn *et al.*, 2012b). The aspects of plant leaf area (PLA) regulation most affected by light competition may therefore be important traits that could be improved to promote legume persistence in mixtures.

The main objective of this study was therefore to assess the relative sensitivity of the morphogenetic processes of alfalfa involved in PLA expansion (namely primary shoot development, branching and leaf expansion) in response to light availability. The intensity of competition for light was manipulated using a range of canopies in pure stands and in a mixture with tall fescue (*Festuca arundinacea* Schreb.) at two densities. Growth and regrowth phases were studied during the vegetative period of development. Shoot development in the various situations was analysed with respect to a potential shoot development model established for plants in the absence of competition (isolated plants under non-limiting supplies of water and nutrients).

MATERIALS AND METHODS

Plant materials and growing conditions

The experiments were performed outdoors between April and August in 2009, 2010 and 2012 at the INRA Lusignan research station in France. Alfalfa (*Medicago sativa* L.) cultivar 'Orca' (large stems, erect cultivar) was used for all the studies. The seeds were pre-germinated in the dark at 25 °C for 48 h before being transplanted into pots. All the plants were then grown in individual 1 L pots (5 × 52 cm cylindrical pots at high density; 8 × 21 cm cylindrical pots at low density) filled with a medium that comprised sterile potting mix, sand and brown soil (1:1:1, v/v/v). The pots were ferti-irrigated three times a day with a complete nutrient solution. The nitrogen concentration of the solution (8 mM) was non-limiting and prevented the nodulation of alfalfa roots.

Experiment 1. In 2009 and 2012, a total of 20 isolated plants (i.e. 0·25 m distance between the pots in all directions) were grown to assess the potential development of alfalfa shoots in the absence of light competition. The plants were followed during the initial growth phase from seeds, and during the first regrowth phase (cut back at the mid-bloom stage, to 5 cm above soil level).

Experiment 2. In 2009 and 2010, plants were grown in stands to assess the impact of light competition on shoot and whole-plant growth and development. The plants were cultivated at two densities: low density (LD; 15 cm between plants, 50 plants m^{-2}, about 2 m^2 stand) and high density (HD; 5 cm between plants, 460 plants m^{-2-}, about 1 m^2 stand). In 2009, the plots consisted of pure alfalfa stands whereas in 2010 they consisted of 50/50 mixtures of alfalfa (*M. sativa* 'Orca') and tall fescue (*Festuca arundinacea* Schreb. 'Noria'). Grass plants were also grown in individual pots and ferti-irrigated, so that were competing with alfalfa for light only. In all the stands, the pots were arranged according to a hexagonal lattice, making all plants equidistant within the stand (Harper, 1961; Boffey and Veevers, 1977). In addition, each individual in the mixed stands was surrounded by three of its own species and three of the other species. The plants in each stand were followed during the initial growth phase from seeds, and during the first two regrowth phases (cut at the mid-bloom stage, to 5 cm above soil level).

Meteorological measurements and thermal time calculations. Air temperature (T_m), PPFD and air humidity (RH) data were collected every 30 s, and mean values were calculated and stored every 3600 s in a Datalogger (CR10 Wiring Panel; Campbell Scientific Ltd, Shepshed, Leics., UK). Supplementary Data Table S1 presents the daily average, mean and maximum values for temperature, PPFD and vapour pressure deficit (VPD) observed during the different regrowth periods in expts 1 and 2.

Thermal time was calculated from the daily integration of air temperatures minus the base temperature (T_b):

$$TT = \int_0^h \max[0; (T_m - T_b)]dt \qquad (1)$$

where TT is thermal time expressed in cumulative degree-days (°Cd; Bonhomme, 2000).

TT is expressed in degree-days, calculated as the sum of the mean daily temperature minus the base temperature (T_b).

During this study, the T_b value used depended on the daily average temperature and changed according to the relationships proposed by Brown *et al.* (2005). Above 15 °C, T_b equalled 5 °C. In between 1 and 15 °C, T_b increased linearly so that T_b was 1 °C at 1 °C and 5 °C at 15 °C.

Nine PPFD sensors were distributed at ground level in each of the studied stands. The ratio of the PPFD between their averaged value and incoming light value enabled calculation of the canopy light interception efficiency (LIE).

Plant measurements

Plant and shoot development. The number of shoots per plant was counted at 5–7 d intervals on 20 plants in expt 1 and on 50 and 30 plants at HD and LD, respectively, in expt 2. Several types of shoots can be distinguished in alfalfa. During the initial growth phase, a primary axis (PA) develops from the seed, and secondary shoots subsequently develop from the axillary buds of cotyledons and PA leaves (Fick *et al.*, 1988). After cutting, two types of shoots can be distinguished as a function of their origin; namely type 2 shoots (T2 for primary axes emerging from buds on the taproot crown) and type 1 shoots (T1 from axillary buds on the stem bases remaining after the previous harvest) during regrowth periods (Leach, 1968; Gosse *et al.*, 1988). The PA, T1 and T2 may present differences in their potential ontogeny (Gosse *et al.*, 1988). A distinction was therefore made between them for our shoot measurements. In expt 1, the numbers of visible leaves per shoot were recorded at 5–7 d intervals for 20 plants on the PA during the initial growth phase, and on two shoots per plant (one T1 and one T2) during regrowth (Supplementary Data Fig. S1). Unfolded leaves were included and counted according to the scoring scale proposed by Maitre *et al.* (1985). The number of leaves was determined on the main axis (primary leaves) and on each branch (distinction between secondary and tertiary axes) from the second node onwards (corresponding to the first trifoliate leaf on the PA). Branches were referred to as a function of their position on the primary axis, branch B1 corresponding to the axil of the first trifoliate leaf. In expt 2, the same measurements were performed on all the shoots of 20 plants at HD and on 10 plants at LD, the two stages of development corresponding to the dates of magnetic digitization of the plants (see the following section). The rate of leaf appearance was calculated for each axis by linear regression between the time in °Cd and the number of visible leaves. The phyllochron was calculated as the reciprocal of the rate of leaf appearance (RLA^{-1}). The thermal time of budburst of a secondary branch was calculated as the intercept of the line $y = 0$ with the linear regression between branch leaf number and thermal time accumulated since emergence. A probability P_i of axillary bud burst was calculated for each of the ith node positions on the primary axis by dividing the number of branches actually observed at this position throughout the shoot population studied by the total number of shoots.

Leaf size and plant leaf area. In expt 2, the topological relationships between phytomers and the length of the central leaflet of each phytomer were recorded by 3D magnetic digitization (3Space Fastrak, Polhemus Inc., Colchester, VT, USA) on samples of 20 plants at HD and 10 plants at LD. The pots were moved indoors just before digitization and plants were measured using 3A software (Adam *et al.*, 1999). Two measurement periods were fixed every year, one in the course of the first growth phase (PSG and MSG for the pure stand and mixed stand, respectively), and one at the end of the second regrowth (PSR and MSR for pure stand and mixed stand, respectively). Lateral leaflets sizes (length and width) and central leaflet width were recorded on a sub-sample of phytomers corresponding to the primary leaves of the most developed shoot on each plant. Allometric relationships were built specifically for each plant using these data, considering (1) central leaflet length as a predictor of lateral leaflet length and (2) leaflet length and phytomer position along the stem as predictors of leaflet width. This enabled an estimate of the length and width of the remaining leaflets on each leaf. The precision of leaflet length and width estimated by digitization were checked against manual measurements with a ruler, and proved accurate with no bias [$y = 0{\cdot}98x - 0{\cdot}01$; $r^2 = 0{\cdot}94$; $n = 204$; root mean square error (RMSE) = 0·23 cm].

The sub-sample of leaflets used for simultaneous length and width determinations was scanned (Konica Minolta C352/C300, Konica Minolta Sensing, Osaka, Japan) and their surface measured was by image analysis (ImageJ software, http://rsbweb.nih.gov/ij/). An allometric relationship common to all leaflets was found between the leaflet surface and the product of its length and width (surface = 0·732 × length × width, $r^2 = 0{\cdot}99$; $n = 62$; RMSE = 0·14 cm^2). This relationship was applied to all the leaflets of the digitized plants. The total surface of each plant (PLA) was then calculated as the sum of the surfaces of all its leaflets.

Plant mass distributions and size structure of the study populations. At each harvest during expt 1 (30 plants in 2012) and expt 2 (100 plants at HD and 60 plants at LD), individual above-ground parts of alfalfa plants were collected, dried (48 h at 60 °C) and weighed. The dry mass distribution of plants in each situation was used to assess the degree of inequality of resource partitioning among individuals in the population. In addition, 100 seeds were weighed to estimate an initial value of size inequality. We used the Gini coefficient (G), which is a measure of the relative mean difference (i.e. the arithmetic average of differences between all pairs of individuals; Sen, 1973; Weiner and Solbrig, 1984), to perform this estimate:

$$G = \sum_{i=1}^{n}\sum_{j=1}^{n} |x_i - x_j| / (2n^2\bar{x}) \qquad (2)$$

The G values thus calculated were then multiplied by $n/(n-1)$ to give unbiased values (G'). These G' values ranged from 0 (all individuals sharing resources equally) to 1 (all resources captured by a single individual). As light was the only contested resource in these experiments, G' was used as an indicator of competition intensity among plants in the different populations studied.

Statistical analyses

Statistical analyses were performed using R software (R Development Core Team, 2012). Analyses of variance (ANOVA, *aov* procedure) were used to test for significant differences between means. Analyses of covariance (ANCOVA, *lm* procedure) were used to test for the effects of continuous and categorical variables simultaneously and to compare the slopes and intercepts of linear relationships. Data transformation (root square transformation) was applied prior to ANCOVA analyses for variables related through a quadratic relationship (e.g. total number of leaves on branches as a function of time or primary axis development). Two-sample Kolmogorov–Smirnov (KS) tests (*ks.test* procedure) were used to compare the distributions of shoot size between the density treatments. The null hypothesis of identical distributions was rejected for P-values $<0{\cdot}05$.

RESULTS

Potential development of an alfalfa shoot

Primary axis development. Primary axis development reached up to 19·6 ± 0·82 leaves in expt 1–2009 and 25·2 ± 2·06 in expt 1–2012 during the initial growth phase. During the regrowth phase, the number of expanded leaves at the time of the cut was 14·5 ± 0·92 in T2 and 12·3 ± 0·57 in T1. The appearance of leaves on the different shoots during the growth and regrowth phases was linear as a function of TT (Fig. 1). The ANCOVA analyses did not demonstrate any significant differences in the rate of development regarding the different shoot types and growth periods ($P > 0{\cdot}1053$, PA in expt 1 and expt 2 for the growth phase; T2 and T1 in expt 1 for the regrowth phase). The phyllochron was 32·54 ± 0·40 °Cd and did not change shortly after the beginning of flowering.

Probability of branching, delay of budburst and development on secondary axes. Figure 2 shows the increase in the total number of secondary leaves in relation to TT, demonstrating that branch development had a marked effect on the total number of leaves accumulating on a shoot. For instance, the number of secondary leaves was more than five times higher than that of primary leaves at the bloom stage in both years on the shoots of isolated plants. This total number of secondary leaves resulted from the sum of all secondary axes developing at the axil of primary leaves. To determine the number of secondary axes at a given time, we examined the probability and time delay of branching at each position on the primary axis (Fig. 3). At all positions, branching was systematic after a delay (branching probability reached 1), in both the growth and regrowth cycles. The ANCOVAs revealed that the rate of appearance of new branches was the same as that of primary leaf appearance ($P < 0{\cdot}0001$) and that there was a constant delay of 1·75 ± 0·15 phyllochrons between primary leaf appearance and its axillary bud burst, irrespective of the position on the shoot (Fig. 3B). The results were the same during both the growth and regrowth cycles (not shown).

Secondary axes thus appeared sequentially on the shoots, from B1, located in the node of the first trifoliate leaf, to B*n*, on the *n*th primary leaf. The appearance of leaves on secondary axes was also linear as a function of TT (r^2 ranging from 0·85 to 0·90). The rate of development was compared between branches at different positions during expt 1 in 2009 and 2012 (Supplementary Data Fig. S2). The rates of development of branches were always lower than those of the PA (51·68 ± 4·57 °Cd, ANCOVA, $P < 0{\cdot}0001$). No significant differences between the phyllochron of the different branches were found (ANCOVA, $P > 0{\cdot}06$; on average 48·2 ± 3·38 °Cd), except for B1, which developed more rapidly than all the other branches ($P < 0{\cdot}02$). No significant effects of years were found (ANCOVA, $P \geq 0{\cdot}17$).

Modelling of potential shoot development. The results presented above suggest that the potential development of an alfalfa shoot subjected to low competition for light is fairly deterministic (constant phyllochrons, branching probability of 1) and identical irrespective of the origin of its apical meristem (from the seed, T2 taproot bud or T1 axillary bud) and of the year (expt 1 in 2009 and 2012). For the two first branching orders, potential development could be deduced from the phyllochron of the primary axis, a time delay for secondary axis budburst and a

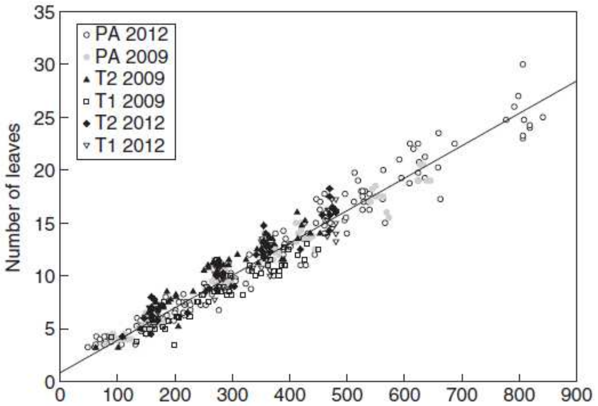

FIG. 1. Number of visible primary leaves as a function of thermal time expressed in cumulative degree-days from first leaf appearance during the growth and regrowth phases of expt 1. The regression was estimated for all data on the plot ($y = 0{\cdot}0301x + 1{\cdot}09$, $n = 433$, $r^2 = 0{\cdot}94$). PA, primary axis; T1, type 1 axis; T2, type 2 axis.

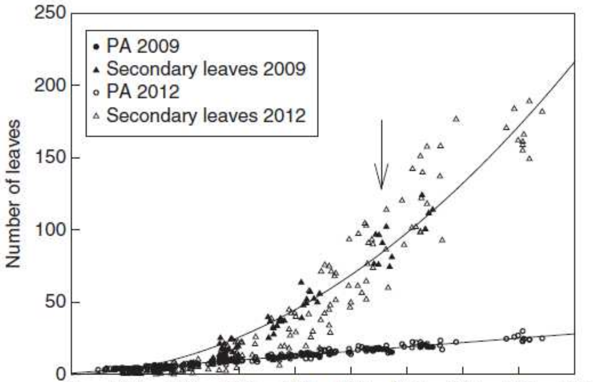

FIG. 2. Numbers of primary and total number of secondary leaves as a function of thermal time expressed in cumulative degree-days from first leaf appearance during the growth phases of expt 1. The regressions were estimated for all data on the plot ($y = 0{\cdot}0003x^2 - 0{\cdot}0275x$, $n = 207$, $r^2 = 0{\cdot}91$ for secondary leaves; same fit as Fig. 1 for primary leaves). The arrow indicates the early bloom stage. PA, primary axis.

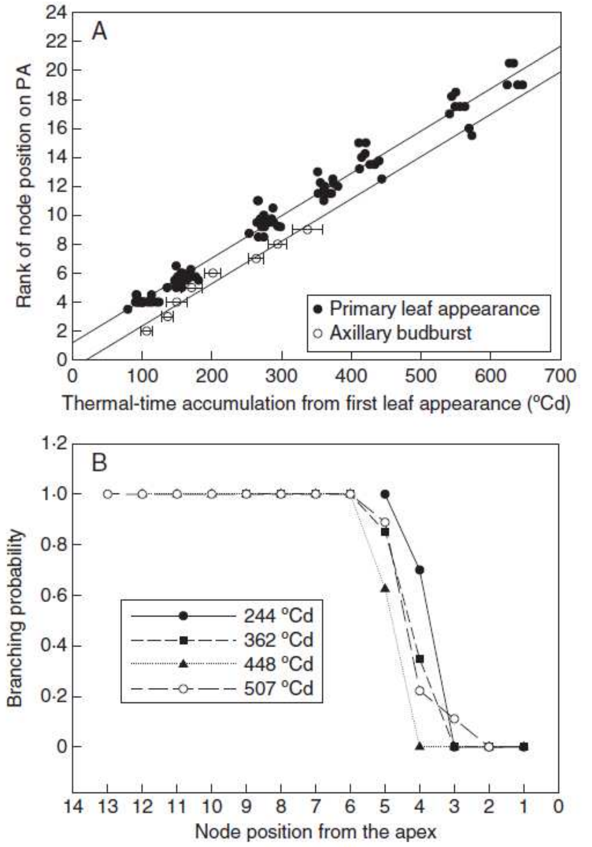

FIG. 3. (A) Thermal time of primary leaf appearance and axillary budburst according to node position on the primary axis (PA) and (B) branching probability with respect to nodal position from the apex at four developmental stages for shoots during the growth phase of expt 1. The regressions were estimated for all data on the plot ($y = 0{\cdot}029x - 0{\cdot}5682, n = 8, r^2 = 0{\cdot}96$ for branch budburst; same fit as Fig. 1 for primary leaves).

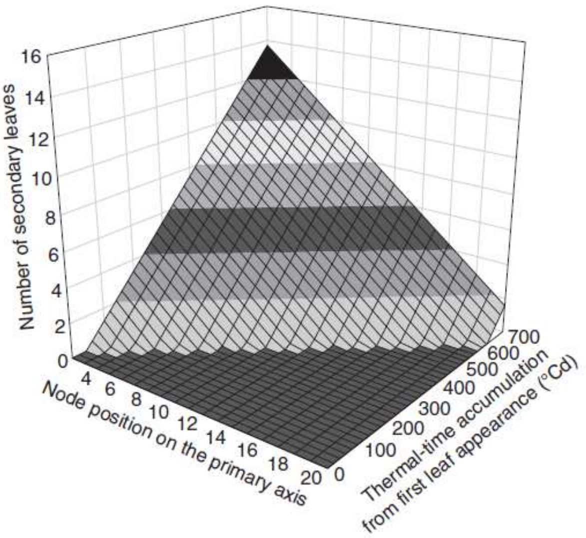

FIG. 4. Number of secondary leaves as a function of branch position and thermal time as predicted by the potential shoot development model [eqns (3–6)].

TABLE 1. *Gini coefficient values for plants in pure stands and mixed stands at high density (HD) and low density (LD) in expt 2*

	Seeds	PSG	MSG	MSR	PSR
LD	0·12	0·17	0·21	0·25	0·26
HD	0·12	0·21	0·23	0·34	0·52

PSG, primary stand growth; MSG, mixed stand growth; PSR, primary stand regrowth; MSR, mixed stand regrowth.

single phyllochron for secondary axes. These variables could be used to summarize spatial and temporal variations in leaf appearance on a shoot under non-limiting growing conditions. A simple model of primary and secondary axis development is thus proposed:

$$N_{\mathrm{I}} = a_1 TT + b_1 \tag{3}$$

$$\mathrm{TT}_{\mathrm{bud}}(i) = (i + d_{\mathrm{b}} - b_1)/a_1 \tag{4}$$

$$N_{\mathrm{II}}(i) = a_2[\mathrm{TT} - \mathrm{TT}_{\mathrm{bud}}(i)] \text{if TT} > \mathrm{TT}_{\mathrm{bud}}(i), \text{ else } 0 \tag{5}$$

$$N_{\mathrm{tot}} = N_I + \sum_{i=1}^{i=n} N_{\mathrm{II}}(i) \tag{6}$$

where a_1 represents the phyllochron of the primary axis, a_2 the phyllochron of secondary axes, b_1 accounts for pre-formed leaves at shoot emergence, d_{b} represents the delay of budburst of branches (expressed in number of new leaves which appeared on the primary axis since bearing node appearance), $\mathrm{TT}_{\mathrm{bud}}(i)$ represents the time of budburst in °Cd of the *i*th secondary branch, and N_{I} and N_{II} stand for the numbers of primary and secondary leaves, respectively. Such a model can predict over (thermal) time the maximum number of primary and secondary leaves, and their topological distribution. Figure 4 shows the changes to the secondary leaf profile as a function of TT as predicted by this model, taking $a_1 = 32$ °Cd, $a_2 = 48$ °Cd and $d_{\mathrm{b}} = 1{\cdot}75$.

In the following, we used this potential shoot development model, derived from independent experiments with limited light competition, as a neutral benchmark to assess the developmental effects induced by light competition in a series of competition experiments.

Impact of light competition on development and plant leaf area expansion

Size structure and competition intensity in the different alfalfa populations studied. The competition intensity was characterized through estimates of light interception efficiencies at the whole-stand scale (Supplementary Data Fig. S3) and with unbiased Gini coefficients (G') at the plant population scale (Table 1). Irrespective of the treatment, canopy closure (LIE >0·95) was faster during the regrowth than during the initial growth phase. Canopy closure was also systematically faster at HD than at

LD, and for pure stands with respect to mixtures. In mixtures, however, LIE cannot indicate the share of light intercepted by each plant population. G' was thus used to account for the degree of inequality in resource capture within the different populations studied. Isolated plants had a relatively low G' value (e.g. $G' = 0{\cdot}10$ in expt 1–2012) which was close to that of the initial seed population ($G' = 0{\cdot}12$). This confirmed that competition for light in these populations resulted in a weak impact of larger plants on the growth of smaller plants. The range of G' values observed for alfalfa in the competition studies (Table 1) covered competition intensities from situations close to those characterized for isolated plants (e.g. $G' = 0{\cdot}17$ for LD–PSG, no mortality) to populations with severe competition experiencing self-thinning (e.g. $G' = 0{\cdot}52$ for HD–PSR, 13 % of plant mortality during the last regrowth). As for LIE, the G' coefficient increased in line with both density and time (growth and regrowth differences), indicating that these two factors tended to increase competition intensity. The G' coefficient also increased more markedly in pure stands than in mixed stands ($0{\cdot}52 > 0{\cdot}31$ at HD). Overall, contrasted situations relative to competition for light were thus actually generated by the study treatments in alfalfa populations. An approximate ranking observed for the two densities was PSG < MSG < MSR < PSR.

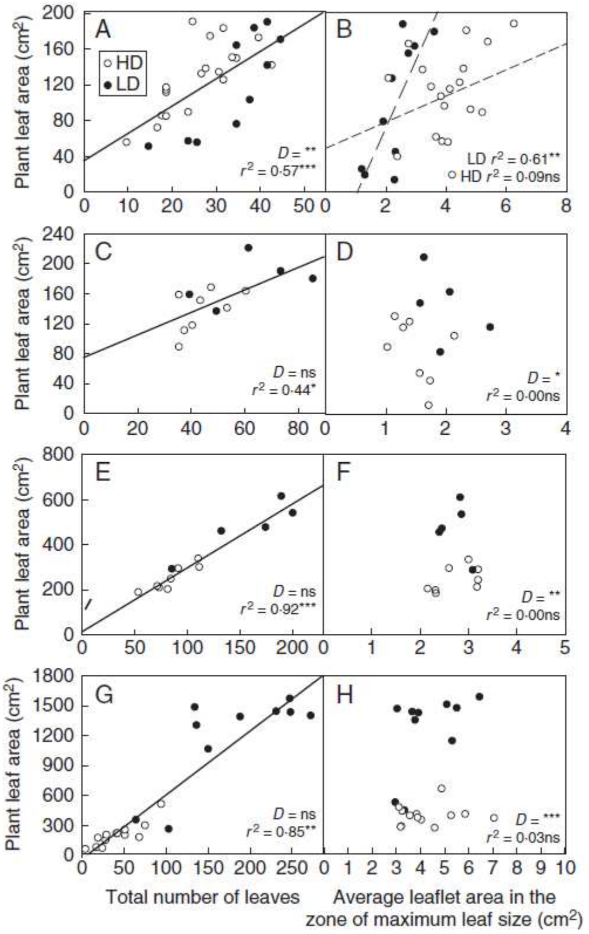

FIG. 5. Relationships between plant leaf area and leaf number (left), and plant leaf area and maximum leaf size (right) in the four situations studied during exp 2: (A, B) PSG, pure stand growth; (C, D) MSG, mixed stand growth; (E, F) MSR, mixed stand regrowth; and (G, H) PSR, pure stand regrowth. High (HD) and low density (LD) are as indicated in the key in (A). ANCOVA results are presented in each panel: two lines in the case of an interaction between the categorical independent variable (i.e. density) and the continuous variable, no line in the case of a non-significant effect of the continuous variable, and a single line in the case of a significant effect of the continuous variable without any interaction. In the absence of interaction, D indicates the level of significance of the categorical independent variable ($*P < 0{\cdot}05$; $**P < 0{\cdot}01$; $***P < 0{\cdot}001$; ns, not significant). r^2 indicates the coefficient of determination of the regression in the case of a significant effect of the continuous variable.

Relative impacts on plant development and leaf growth. The impact of light competition on the developmental and growth processes controlling PLA was assessed (Fig. 5). Four situations were distinguished for which all the plants experienced the same environment (sowing date, cutting date, air temperature, VPD, etc.) except for their light interception (modulated by overall density and neighbouring plants). In each situation, marked variations in individual PLA values were shown (variations ranging from 4- to 10-fold between individual PLAs in the sample of digitized plants). The part of PLA variations explained by plant development (as reflected by the total number of leaves) and leaf growth (as reflected by the average leaflet area of fully expanded leaves in the zone of maximum primary leaf size, between nodes ranking 8–10) was examined by means of ANCOVA and regression analysiss in each situation. The ANCOVA revealed that the total number of leaves did indeed explain a significant part of PLA variations in all the situations studied. Plants displaying a larger number of leaves also had a higher PLA. A single relationship between PLA and leaf number was found in three out of four cases (MSG, MSR and PSR) which explained individual PLA variations both within and between the density treatments. The part of variance explained by plant development was high ($r^2 > 0{\cdot}44$) and increased over time ($r^2 > 0{\cdot}85$ in the regrowth cycles). In contrast, PLA variations were usually loosely associated with maximum leaf size. No significant relationship was found between PLA and maximum leaf size in the MSG, MSR and PSR situations, either within or between density treatments (ANCOVA, $P > 0{\cdot}08$), indicating that plants with larger leaves were not necessarily those with the largest total leaf area, and vice versa. For PSG, ANCOVA revealed an interaction between density and maximum leaf size, the relationship between PLA and leaf size only being significant at LD.

Important plant to plant variations in maximum leaf size were observed in all the situations and densities. A significant

TABLE 2. *Average leaflet area in the zone of maximum primary leaf size (node rankings 8–10) in the different populations and growth phases of expt 2*

Stands	Density	Growth	Regrowth
Pure stand	HD	$4{\cdot}07^{a}$	$4{\cdot}31^{a}$
	LD	$2{\cdot}28^{bc}$	$4{\cdot}33^{a}$
Mixed stand	HD	$1{\cdot}60^{c}$	$2{\cdot}75^{b}$
	LD	$1{\cdot}99^{bc}$	$2{\cdot}73^{b}$

Means with the same letter are not significantly different according to the Duncan test ($P > 0{\cdot}05$).

HD, high density; LD, low density.

interaction effect on maximum leaf size was found between density treatments and the overall growing conditions that prevailed during the period of study (ANOVA, $P < 0{\cdot}001$; Table 2). Once again, the density effect was significant for PSG only.

PLA variations as explained by shoot number and shoot development. We further broke development down into shoot production and shoot development (as reflected by the average number of leaves per shoot) to understand how each component was affected by light competition (Fig. 6). Irrespective of density, PLA was tightly related to the shoot number in regrowth cycles (ANCOVA, $P < 0{\cdot}001$ in MSR and PSR). Over 75 % of PLA variance could be explained by shoot number during these periods. In the initial growth phase, on the other hand, most plants had very similar shoot numbers (2–4) and PLA was either not significantly (PSG) or weakly (MSG) related to shoot numbers. Furthermore, shoot development was significantly related to PLA in all the situations studied. As compared with shoot numbers, shoot development explained larger parts of PLA variance during the initial growth phase, but contributed to a lesser extent during later regrowth phases (except for HD–MSR).

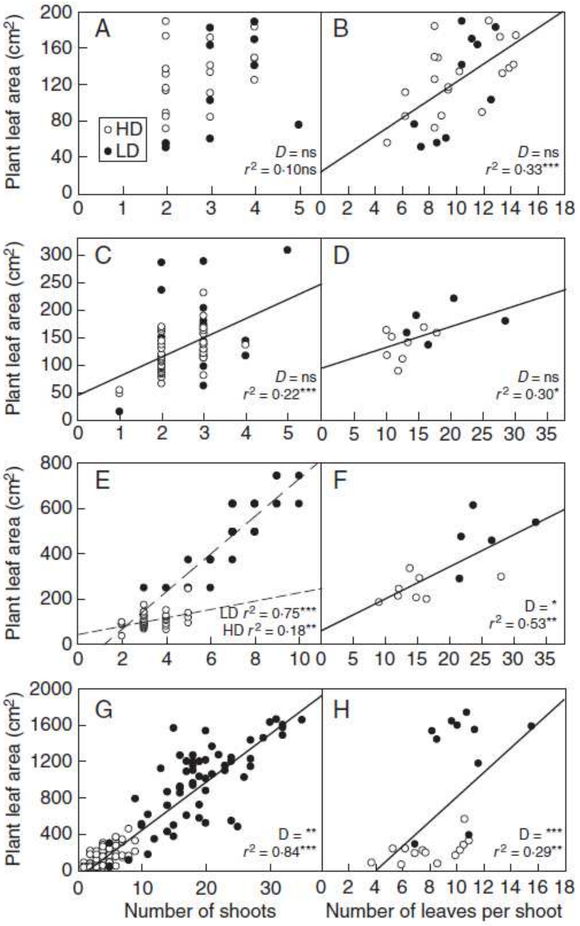

FIG. 6. Relationships between plant leaf area and the shoot number (left), and plant leaf area and the number of leaves per shoot (right) in the four situations studied during exp. 2: (A, B) PSG, pure stand growth; (C, D) MRG, mixed stand growth; (E, F) MSR, mixed stand regrowth; and (G, H) PSR, pure stand regrowth. High (HD) and low density (LD) are as indicated in the key in (A). ANCOVA results are presented in each panel: two lines in the case of an interaction between the categorical independent variable (i.e. density) and the continuous variable, no line in the case of a non-significant effect of the continuous variable, and a single line in the case of a significant effect of the continuous variable without any interaction. In the absence of interaction, *D* indicates the level of significance of the categorical independent variable ($*P < 0{\cdot}05$; $**P < 0{\cdot}01$; $***P < 0{\cdot}001$; ns, not significant). r^2 indicates the coefficient of determination of the regression in the case of a significant effect of the continuous variable.

Shoot developmental responses to light competition. At the shoot level, potential shoot development and branching are strongly dependent on primary axis development. Figure 7 presents the relationships between primary axis development and the number of leaves on secondary branches. As predicted by the potential shoot development model, the total number of secondary leaves increased with primary axis development according to a power law superior to 1 (more than linearly) in all the situations studied and under all density treatments. However, a reduction in the total number of secondary leaves for a given number of primary leaves was seen on all shoots subjected to light competition when compared with potential shoot development. A significant reduction in branch development was also observed between the LD and HD treatments, in all situations (ANCOVA, $P < 0{\cdot}04$). The profiles of secondary axis development are presented in Fig. 8 at HD and LD for shoots having reached a similar primary axis development. Except for LD–PSG, significant departures from the potential shoot development pattern were observed in all the situations studied. The reductions in branch development as compared with the potential pattern were usually small at the top of the canopy for the youngest branches (up to three leaves) and were maximum for the oldest branches. Branches in the various canopies studied apparently ceased their development after a time which varied in line with both the competition intensities (regrowth < growth cycles; pure < mixed stands).

Distribution of shoot size and organogenesis of primary axes. Not all shoots within a plant or within a density treatment displayed synchronous development. The final number of primary leaves could thus vary, which in turn could affect some of the average shoot characteristics mentioned above. Figure 9 presents the distribution of final shoot size (as reflected by the number of emerged primary leaves) in the different situations and densities studied. Final shoot size did vary in all the situations. However, remarkably, shoot size distributions were not affected by density in three of the four situations (PSG, MSG and MSR; KS tests *P*-values $>0{\cdot}24$), suggesting that factors other than light competition might have caused these developmental variations. In PSR, however, significant differences were observed between the primary development of shoots in the HD and LD populations (KS tests, *P*-values $<0{\cdot}001$). At LD, the mode of the distribution showed that the highest proportion of shoots were of a large size (12–14 primary leaves). At HD, only a small proportion of the shoots growing during the same period were able to reach this stage of development, suggesting that many primary axes had ceased or reduced their development as compared with LD.

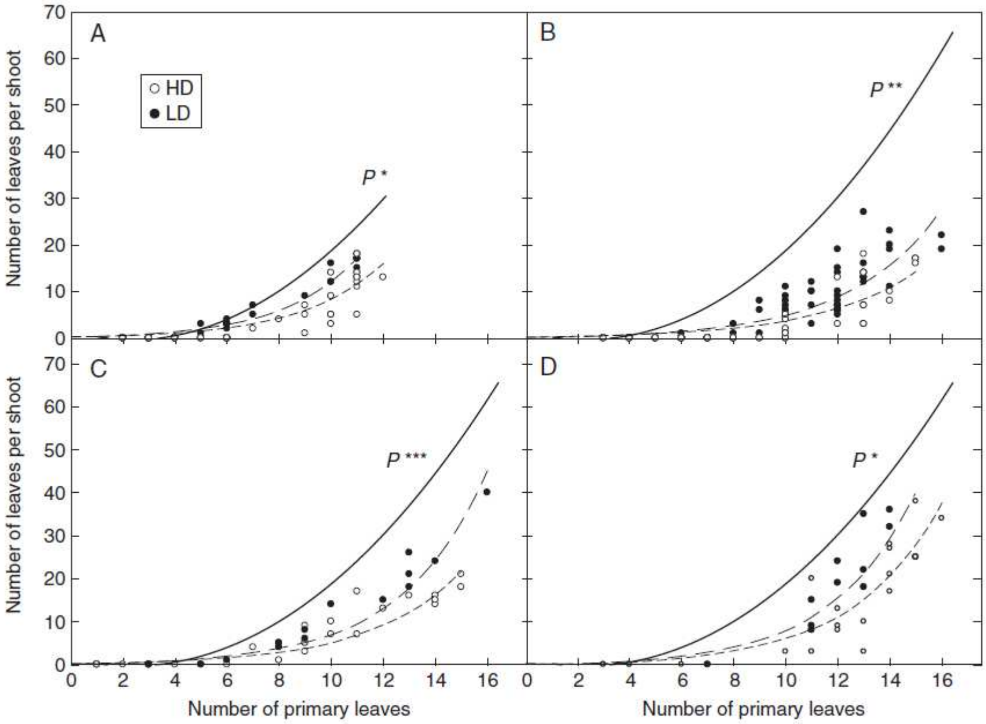

FIG. 7. Number of secondary leaves per shoot as a function of primary axis development in the four situations studied during expt. 2: (A) PSG, pure stand growth; (B) PSR, pure stand regrowth; (C) MSG, mixed stand growth; and (D) MSR, mixed stand regrowth. High (HD) and low density (LD) are as indicated in the key in (A). The solid line represents the relationship predicted by the potential shoot development model [eqns (3–6)]. ANCOVA results comparing HD and LD relationships are presented in each panel: two dashed lines are plotted in the case of an interaction between the categorical independent variable (i.e. density) and the continuous variable. *P*-values indicate the level of significance of the interactions ($*P < 0{\cdot}05$; $**P < 0{\cdot}01$; $***P < 0{\cdot}001$; ns, not significant).

DISCUSSION

Potential shoot development was a deterministic function of thermal time in the range of temperature and photoperiod tested

Our results suggest that the potential development of an alfalfa shoot is deterministic when expressed as a function of thermal time and identical irrespective of its apical meristem origin (from the seed, from T2 taproot buds or T1 axillary buds) and of the year (expt 1 and expt 2 on isolated plants). Our study covered the whole vegetative period and extended to mid-bloom. We showed that four characteristics could summarize the potential organogenesis of a whole shoot: primary axis phyllochron; systematic branching of all axillary buds; delayed bud burst; and a constant phyllochron for secondary branches. Similar patterns of whole-shoot development and branching have previously been reported in several species with indeterminate growth (Belaygue *et al.*, 1996; Seleznyova *et al.*, 2002; Lebon *et al.*, 2004; Louarn *et al.*, 2007; Moreau *et al.*, 2007). The stability of primary axis development (constant rate of leaf appearance) was also characteristic in these studies. In alfalfa, however, it has remained a matter of debate. Various studies have reported a linear relationship between N_I and TT during the vegetative growth period (although there have been controversies regarding the T_b value used to calculate TT; Wolf and Blaser, 1971; Sharratt *et al.*, 1989; Brown and Moot, 2004; Brown *et al.*, 2005; Teixeira *et al.*, 2011). However, unlike our results, which showed similar phyllochron values in the growth and regrowth phases [32 °Cd, close to the phyllochrons reported by Brown *et al.* (2005) or Teixeira *et al.* (2011) in the field and Allirand (1998) or Pearson and Hunt (1972) under controlled environments for isolated plants], several studies have supported the idea of a primary axis phyllochron that is significantly higher during the initial growth phase (Robertson *et al.*, 2002; Teixeira *et al.*, 2011). Our results demonstrated that no ontogeny-related difference could explain the slower rate of leaf appearance in the field. In contrast to field experiments, we controlled nitrogen nutrition using an N-rich nutrient solution. We can thus hypothesize that a part of the differences previously reported might be related to a degree of nitrogen limitation during the initial growth phase when nodules hosting rhizobia, and allowing atmospheric nitrogen fixation, were forming (Voisin *et al.*, 2003). Differences in phyllochron related to the photoperiod have also been reported, in particular between growth periods during increasing and decreasing photoperiods (Brown *et al.*, 2005, 2006). Our experiments were all performed close to the summer solstice (the photoperiod between 14 h and 16 h), with both growth and regrowth phases starting when photoperiods were still increasing. No significant photoperiodic effect is expected under such conditions, in contrast to field conditions with autumn sowing (Brown *et al.*, 2005). This could also explain some discrepancies with the literature.

The development of branches had a great importance in controlling the potential number of leaves of alfalfa shoots subjected

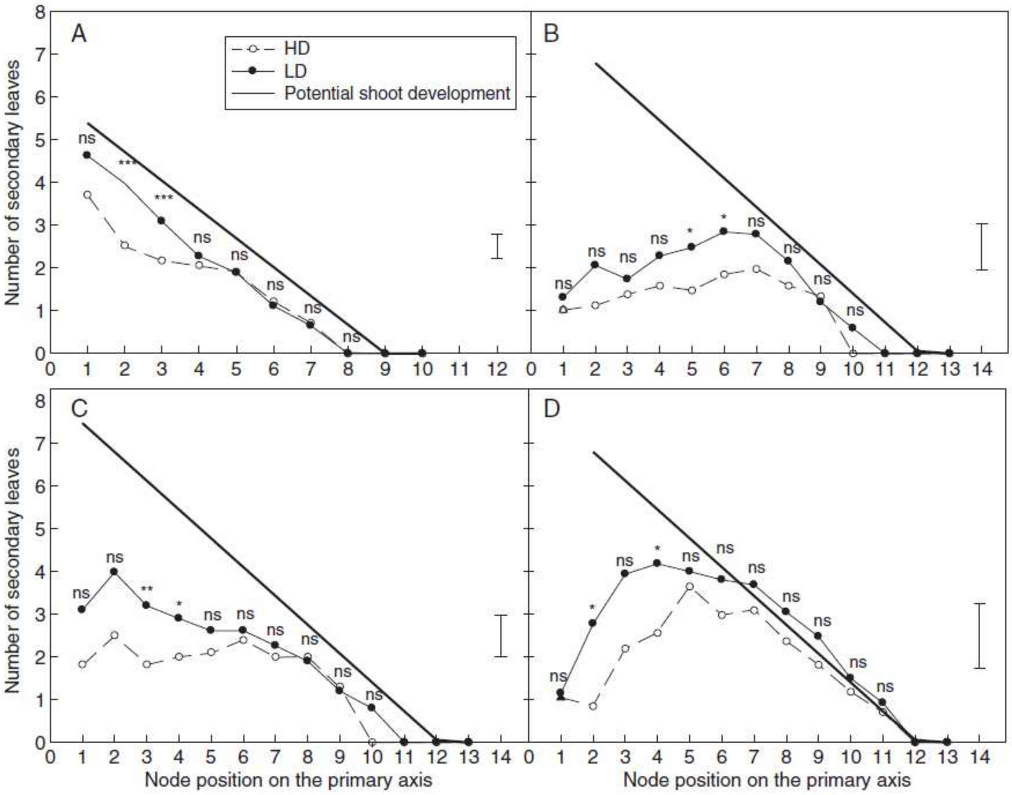

FIG. 8. Number of secondary leaves at each node position on high (HD) and low density (LD) plants in the four situations studied during expt 2: (A) PSG, pure stand growth; (B) PSR, pure stand regrowth; (C) MSG, mixed stand growth; and (D) MSR, mixed stand regrowth. Shoots were selected at a given stage of development in each situation (shoots with 12–14 primary leaves). The dotted line represents the number of secondary leaves predicted by the potential shoot development model [eqns (3–6)]. *P*-values indicate the results of the *t*-test for a comparison between LD and HD at each node position ($*P < 0{\cdot}05$; $**P < 0{\cdot}01$; $***P < 0{\cdot}001$; ns, not significant).

to a low level of light competition. Under these conditions, every leaf axil produced a branch after a constant delay. Such a systematic branching had previously been reported on *Medicago truncatula* (Moreau *et al.*, 2007) or grapevine (Lebon *et al.*, 2004; Louarn *et al.*, 2007) and assisted greatly in simplifying the formalism to model potential shoot development using a simple deterministic approach. The rate of development of branches was also constant over time, but the phyllochron was lower than for the primary axis, with a reduction of around 30 % for B1 and 50 % for other branches. Allirand (1998) and Moreau *et al.* (2007) also reported a reduced rate of development of branches when compared with the primary axis. These authors also demonstrated changes in the phyllochron of branches as a function of their position on the main stem. However, their study mainly covered the reproductive growth period of *M. truncatula*. The development of flowers along the primary axis is likely to be a factor that reduces the phyllochron of branches in alfalfa as well (Brown *et al.*, 2005).

Plant development tunes leaf area expansion in alfalfa competing for light

Competition for light tends to be the principal factor leading to size inequalities and size structure in even-aged plant populations of productive habitats (Weiner, 1985; Weiner and Thomas, 1986; Schwinning and Weiner, 1998). Louarn *et al.* (2012*a*, *b*) showed that PLA was the most important variable in alfalfa to explain light partitioning and individual plant productivity in pure and mixed stands. However, little was known about the relative importance of physiological processes governing PLA to cope with changes in light competition intensity.

Tissue expansion and final individual leaf size have been shown to play key roles in the light response of many crops (Dosio *et al.*, 2003; Chenu *et al.*, 2005). Lower levels of PPFDs tend to decrease the leaf expansion rate but to increase the duration of leaf expansion (Corré *et al.*, 1983; Tardieu *et al.*, 1999; Chenu *et al.*, 2005; Cookson and Granier, 2006). In this study, differences between HD and LD populations in the final size of leaves were observed only at the lowest levels of competition intensity studied (PSG, with $G' < 0{\cdot}21$). More shaded plants had larger leaves. In all other situations, no density effect could be demonstrated. Marked variations in maximum leaf size were observed between plants within a treatment, irrespective of density, dates or total PLA (2- to 3-fold variations). The same range of variation was also found in isolated plants (not shown). This variability could be related to the fact that alfalfa cultivars are a population of genetically distinct plants, thus containing significant genetic and phenotypic variability

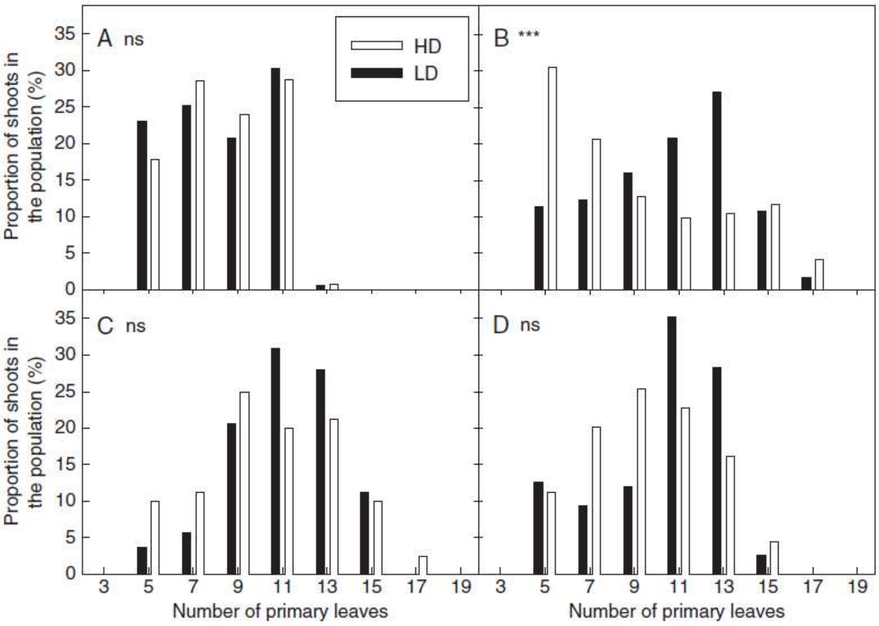

FIG. 9. Comparison between primary shoot size distributions at high (HD) and low density (LD) in the four situations studied during expt 2: (A) PSG, pure stand growth; (B) PSR, pure stand regrowth; (C) MSG, mixed stand growth; and (D) MSR, mixed stand regrowth. *P*-values indicate the level of significance of the Kolmogorov–Smirnov test (**P* < 0·05; ***P* < 0·01; ****P* < 0·001; ns, not significant).

(Julier *et al.*, 2000). Leaf size variability did not generally reflect variations in total plant area. A significant relationship was only found for LD–PSG, a situation where competition intensity was very low and where most plants were close to their potential development (i.e. did not differ in their total number of leaves).

Organogenesis, resulting from meristem activities, is the other physiological process that plays a key role in regulating PLA. It has been shown to control PLA expansion in many indeterminate woody plants and herbaceous species (Pagès *et al.*, 1993; Belaygue *et al.*, 1996; Turc and Lecoeur, 1997; Seleznyova *et al.*, 2002) and to affect PLA regulation in response to environmental constraints (Christophe *et al.*, 2006; Lebon *et al.*, 2006; Pallas *et al.*, 2011). Our results definitely confirmed a strong role for plant development in regulation of the PLA of alfalfa in response to light availability, which was far stronger than any effect on tissue expansion. The total number of leaves accounted for variations in PLA both within and between density treatments. It explained >85 % of PLA variance in all situations after the first regrowth.

Developmental processes differ in their response to competition intensity

The total number of leaves on a plant can be described as resulting from several intertwined developmental processes: the initiation of new shoots at the level of the crown; primary development; and finally the degree of secondary branching for each shoot. Our findings suggest that these developmental processes were not affected to the same extent or could display differences in the timing of their response when subjected to different light competition intensities. The development of secondary axes appeared as the only variable that was affected in all the stands studied. Significant reductions were systematically observed as compared with isolated plants. The higher the intensity of competition, the more marked was the reduction in branch development. Only the branches at the base of the shoots were affected. Because alfalfa cv. Orca presents an upward canopy development, the zones where secondary development ceased corresponded to canopy heights with reduced light transmission (Louarn *et al.*, 2012*a*) and correlatively modified light quality (Escobar-Gutiérrez *et al.*, 2009). The greater reduction observed at HD, vs. LD, probably resulted from more rapid canopy closure and earlier effects of local light quantity/quality on the organogenesis of branches. Differences in the shape of secondary branch profiles between the pure and mixed stands for a given growth period may also have been induced by different light distributions in these two situations. Similar light quantity and quality effects on the branching of vegetative shoots have been reported on various species (Ballaré *et al.*, 1997; Smith and Whitelam, 1997; Ballaré and Casal, 2000; Christophe *et al.*, 2006). In alfalfa, Brown *et al.* (2005) showed that shorter branches at the bottom of the shoot resulted from an earlier cessation of their development and not from a delay in the outgrowth of axillary buds.

The regulation of branch organogenesis is critical with regard to the PLA response to stress in species where the number of branching shoots is fixed (e.g. determined by pruning rules: Lebon *et al.*, 2006; Pallas *et al.*, 2008). In our study, however, the part of PLA variance explained by average shoot development remained limited (r^2 ranging from 0·29 to 0·55, Fig. 7).

This may have been due (1) to the fact that an important variation of primary shoot development was observed within each treatment and the effect of which was only imperfectly captured by an average shoot (the relationship between primary and secondary development not being linear), and (2) to the variation in shoot numbers per plant that might explain a significant proportion of PLA variance. Changes in the shoot number did explain a significant part of PLA variance in three out of four situations (except for PSG, the lowest level of competition intensity), its importance relative to shoot development increasing with time. As previously reported (Fick *et al.*, 1988; Kephart *et al.*, 1992), increased densities resulted in a reduced number of shoots per plant. However, the relationship between competition intensity and average shoot number was not straightforward (e.g. very similar G' values between HD–MSG, LD–MSR and LD–PSR produced markedly different average shoot numbers: 2·5, 6·1 and 20·3, respectively). The branching of the initial primary axis (and thus the number of buds initiated at the crown level) is likely to limit the possibility of achieving a large number of shoots per plant during the early stages. Conversely, important plant to plant variations in shoot numbers reported at a later stage may have derived from the cumulative effects of branching on the number of buds, as well as from the effects of the light environment on the dynamics of shoot population (Teixeira *et al.*, 2007).

Finally, primary axis development was also involved in the plant response to light competition, but only at the highest competition intensities recorded. At HD–PSR, most of the shoots had a very limited final size (4–6 leaves), suggesting that many primary axes ceased to develop during regrowth. Possibly the smaller primary axes might stop developing in the shade of taller ones, in line with phenomena already discussed in the case of branches. The delayed response of primary axes to competition intensity could in part be explained by differences in the growth behaviour of primary and secondary axes (primary axes grow vertically, at a higher elongation rate than branches) and local light conditions at the apex (Louarn *et al.*, 2012*b*). Similarly, the difference in the fate of primary axes of T1 and T2 reported in dense stands (Gosse *et al.*, 1988) could simply result from shorter nodes and reduced growth of T1 stems (not shown), even if they present the same potential of development under favourable light conditions.

To sum up, developmental processes controlled plant and crop leaf area expansion in alfalfa and differed in their response to light competition intensity. These findings should help in defining relevant traits (such as rapid plant/shoot branching) that could be favoured in the definition of mixtures to prevent legume density-dependent mortality and mixture failure. Moreover, as a result of light competition, alfalfa leaf area dynamics and light interception efficiencies varied greatly among the treatments studied. The deterministic pattern of shoot development identified should aid in the development of more versatile models of leaf area expansion. In particular, it may be possible to overcome the limitations of fixed patterns of crop leaf area dynamics (Gosse *et al.*, 1984; Goudriaan and Monteith, 1990) to deal with the question of light partitioning in heterogeneous intercropping systems by introducing formalisms that account specifically for the light effects on the key developmental variables involved in the response to light availability.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of the following. Table S1: environmental conditions experienced for the different growth periods studied during the two experiments. Figure S1: diagrams of the arrangement of the main axis, secondary and tertiary axes on a seedling plant (initial growth cycle) and the types of main axes emerging either from the taproot or from the axil of a leaf just below the cutting height of a mature plant during a regrowth cycle. Figure S2: number of leaves on branches as a function of thermal time accumulation expressed in cumulative degree-days from shoot emergence during the growth phases of expt 1. Figure S3: dynamics of canopy light interception efficiency measured in the different pure and mixed stands studied.

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Table S1. Environmental conditions experienced for the different growth periods studied during the two experiments. Tm, PPFD and VPD refer to daily average temperature (°C), daily average photon flux density (µmole PAR.m^{-2}) and daily average vapour pressure deficit (kPa), respectively. Values in parenthesis are for minimum and maximum values over the period.

	Year	Growth period	Tm		PPFD	VPD	
Exp 1	2009	1 (Growth)	14.5	(7.2 - 23.6)	1018	1.3	(0.9 - 2)
		2 (Regrowth)	18.9	(14.3 - 25.1)	1064	1.4	(1.1 - 1.9)
	2012	1 (Growth)	16.0	(9.9 - 22.7)	934	1.4	(0.7 - 2)
		2 (Regrowth)	18.4	(15.4 - 26.5)	981	1.5	(1.1 - 2.1)
Exp 2	2009	1 (Growth)	14.5	(7.2 - 23.6)	1018	1.3	(0.9 - 2)
		2 (Regrowth)	18.9	(14.3 - 25.1)	1064	1.4	(1.1 - 1.9)
	2010	1 (Growth)	15.1	(7.8 - 22.5)	919	1.4	(0.8 - 1.8)
		2 (Regrowth)	21.0	(13.1 - 26.5)	1136	1.7	(1.1 - 2.2)

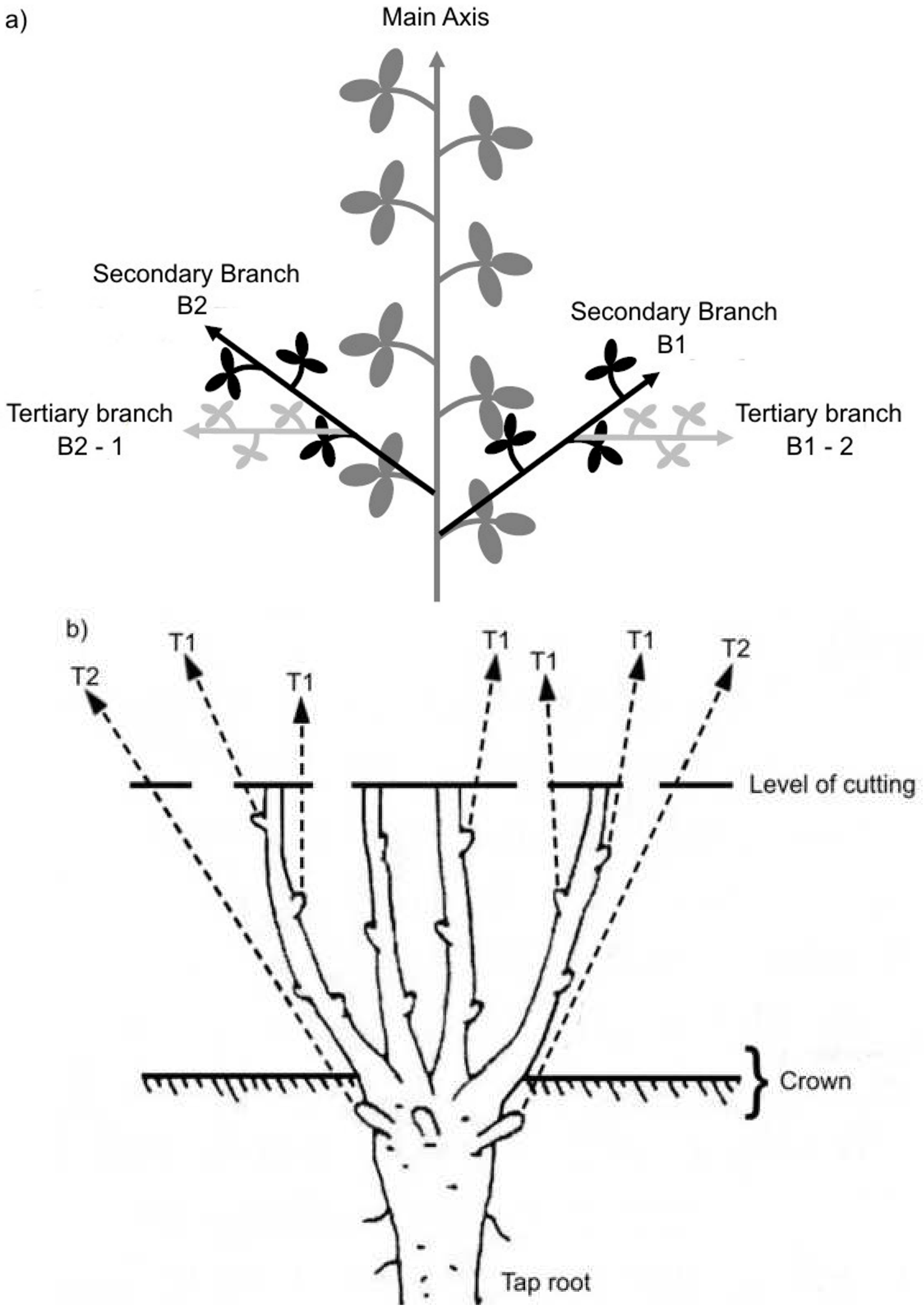

Fig. S1. Diagrams of a) the arrangement of the main axis, secondary and tertiary axes on a seedling plant (initial growth cycle) and b) the types of main axes emerging either from the taproot (T2) or from the axil of a leaf just below the cutting height (T1) of a mature plant during a regrowth cycle. Redrawn from Moreau et al. (2007) and Gosse et al.(1988).

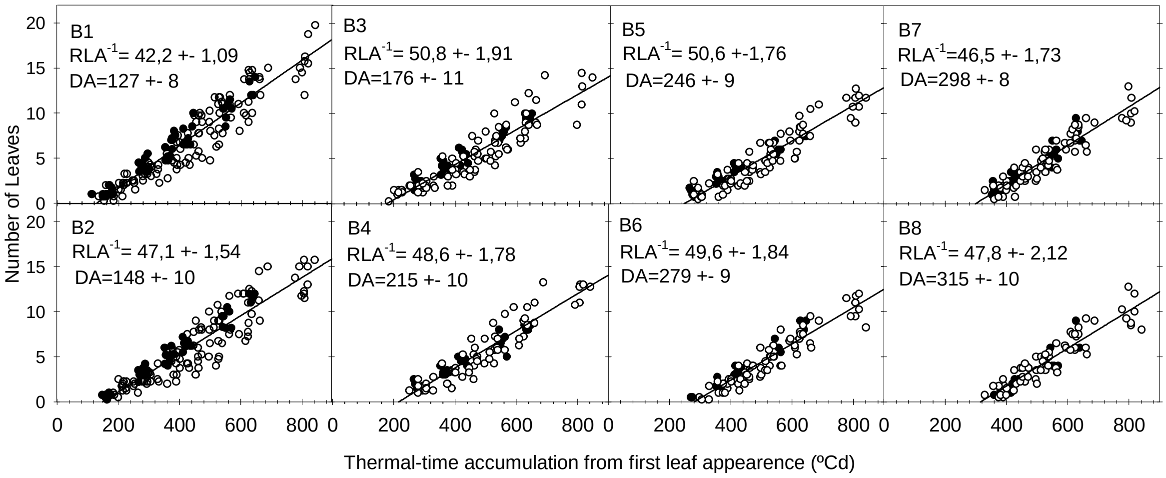

Fig. S2. Number of leaves on branches as a function of thermal-time accumulation expressed in cumulative degree-days from shoot emergence during the growth phases of Exp. 1. Open and closed symbols indicate 2012 and 2009 data, respectively. Date of branch appearance (DA) and phyllochron (RLa-1) estimated from linear regressions are indicated in each panel.

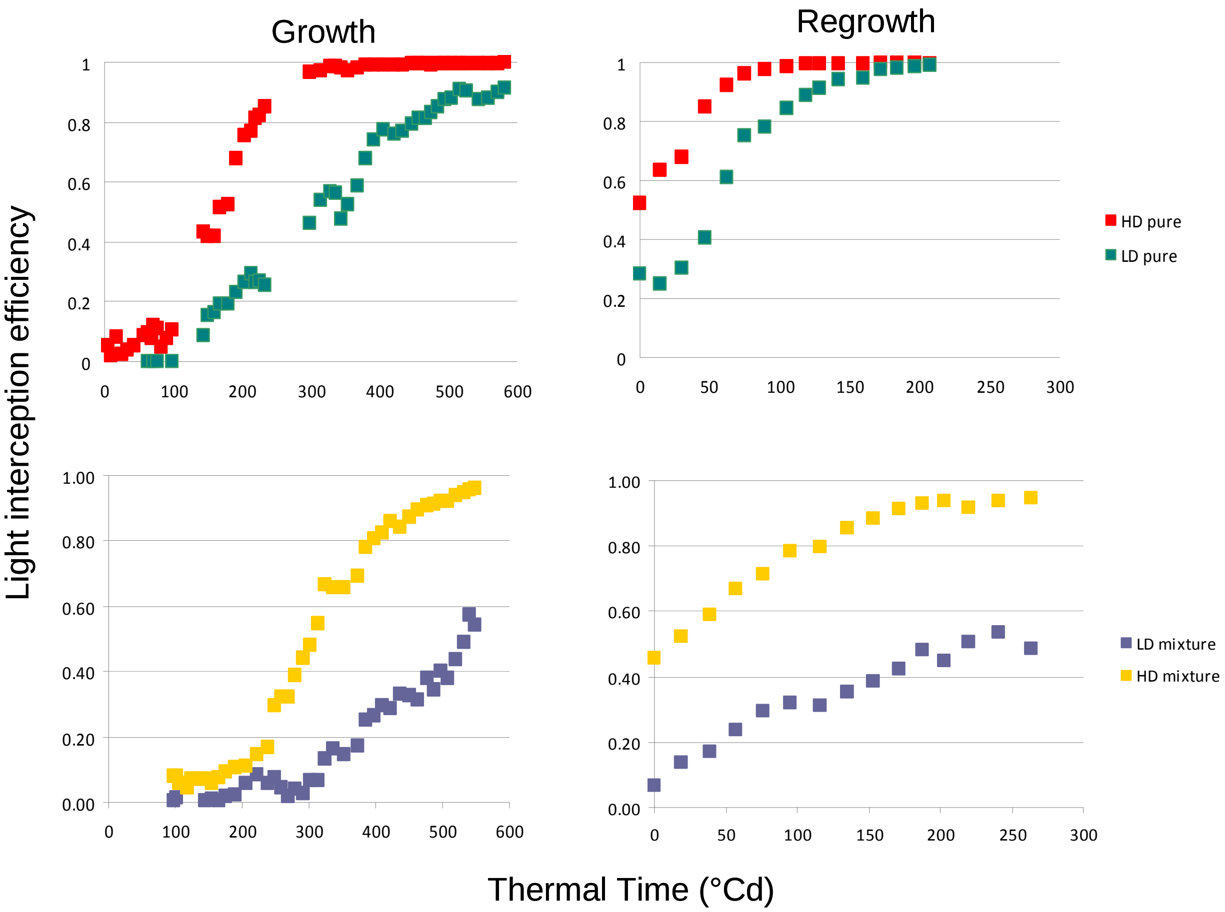

Fig. S3. Dynamics of canopy light interception efficiency measured in the different pure and mixture stands studied.

6. CONSIDERAÇÕES FINAIS

Os resultados observados nos diferentes trabalhos desenvolvidos demonstram que existe grande espécie dependência em função dos efeitos da luz, havendo até distintas respostas em função de diferentes genótipos de uma mesma espécie, como é o caso da alfafa. Tal fator dificulta a predição do comportamento das espécies e, consequentemente, o manejo em função de alterações da luz. Contudo, é possível apontar alguns mecanismos de alteração das plantas que podem ser utilizados como alvo da pesquisa e deste modo facilitar a avaliação do comportamento de cada espécie em relação a luz.

Um dos mecanismos de resposta das plantas comumente observado nos resultados aqui apresentados, e que já havia sido reportado inúmeras vezes na literatura, é a elongação do colmo em função das alterações na quantidade e/ou qualidade da luz.

Nos trabalhos realizados com alfafa, os resultados obtidos resultaram em importantes informações, as quais ajudam na construção de modelos de interceptação da luz pelo dossel vegetal e, portanto, a predição da produção. Tais resultados também têm enorme importância para o manejo da alfafa em consórcio com gramíneas. Por exemplo,...

No caso das espécies tropicais C_4 avaliadas sobre o efeito de árvores, *A. catharinensis*, *B. brizantha*, *H. altíssima* e *Cynodon* cpp. demonstraram claramente que as alturas atingidas aos 95% de interceptação luminosa são maiores do que aquelas obtidas a pleno sol. Para as espécies *M. maximus* e *P. notatum* não ocorreram variações importantes. Demonstrando assim a variabilidade comentada acima para diferentes espécies, ainda as dificuldades de utilizar o critério de altura como estratégia de manejo nesses sistemas.

Neste sentido, poderia se sugerir que a pesquisa avance na busca de facilitar o uso diretamente da medição da interceptação luminosa, como controle da entrada de animais em pastejo e não do uso da altura.

Outro aspecto importante do manejo a ser abordado é a intensidade de corte. No trabalho com as espécies arborizadas, foi utilizada uma intensidade de 50% de corte da altura em que a pastagem atingia 95% de interceptação luminosa, contudo este corte foi realizado mecanicamente. É necessário deste modo, aliar o comportamento animal nos estudos de intensidade de manejo de espécies em sistemas integrados com árvores.

Ainda, é necessário entender como o efeito da sombra das árvores vai interferir no comportamento animal. Sugere-se que, principalmente em lugares com altas intensidades de radiação, os animais iriam pastejar mais nos lugares com maior sombra ao longo do dia, isso poderia resultar em diferenças na estrutura do dossel da pastagem ao longo do gradiente de sombra.

Nas metodologias propostas para avaliações das espécies em sistema integrado com árvores, existe grande importância em separar os diferentes fatores que interferem no crescimento e no desenvolvimento das plantas. Neste estudo, tentou-se objetivar o efeito do sombreamento. Contudo, não é possível fazer a completa separação dos efeitos de água, por exemplo. Inicialmente era pretendida a irrigação das áreas com árvores e a pleno sol, mas não foi possível realizar em função de custos e logística dos experimentos.

Por fim, informações a respeito do manejo de espécies forrageiras nos mais variados tipos de consórcio são de extrema importância, pois auxiliam a técnicos e produtores na tomada de decisões e também são determinantes para aumentar a adesão destes tipos de sistemas.

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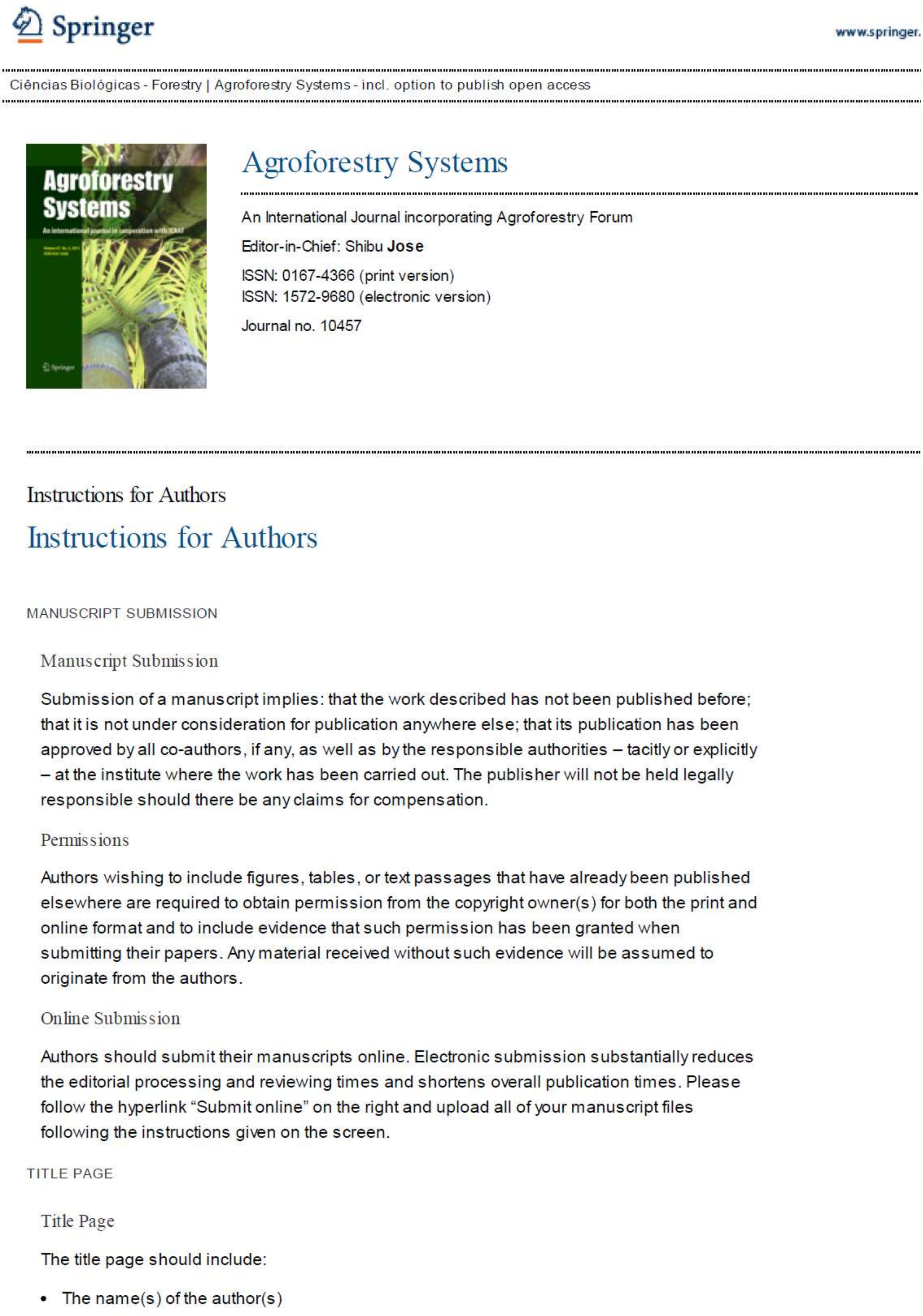
8. ANEXOS



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Please provide an abstract of 150 to 250 words. The abstract should not contain any undefined abbreviations or unspecified references.

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Please provide 4 to 6 keywords which can be used for indexing purposes.

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Acknowledgments of people, grants, funds, etc. should be placed in a separate section before the reference list. The names of funding organizations should be written in full.

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SCIENTIFIC STYLE

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Citation

Cite references in the text by name and year in parentheses. Some examples:

- Negotiation research spans many disciplines (Thompson 1990).
- This result was later contradicted by Becker and Seligman (1996).
- This effect has been widely studied (Abbott 1991; Barakat et al. 1995; Kelso and Smith 1998; Medvec et al. 1999).

Reference list

The list of references should only include works that are cited in the text and that have been published or accepted for publication. Personal communications and unpublished works should only be mentioned in the text. Do not use footnotes or endnotes as a substitute for a reference list.

Reference list entries should be alphabetized by the last names of the first author of each work.

- Journal article

  Gamelin FX, Baquet G, Berthoin S, Thevenet D, Nourry C, Nottin S, Bosquet L (2009) Effect of high intensity intermittent training on heart rate variability in prepubescent children. Eur J Appl Physiol 105:731-738. doi: 10.1007/s00421-008-0955-8

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  Smith J, Jones M Jr, Houghton L et al (1999) Future of health insurance. N Engl J Med 965:325–329
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- Book

  South J, Blass B (2001) The future of modern genomics. Blackwell, London
- Book chapter

  Brown B, Aaron M (2001) The politics of nature. In: Smith J (ed) The rise of modern genomics, 3rd edn. Wiley, New York, pp 230-257
- Online document

  Cartwright J (2007) Big stars have weather too. IOP Publishing PhysicsWeb. http://physicsweb.org/articles/news/11/6/16/1. Accessed 26 June 2007
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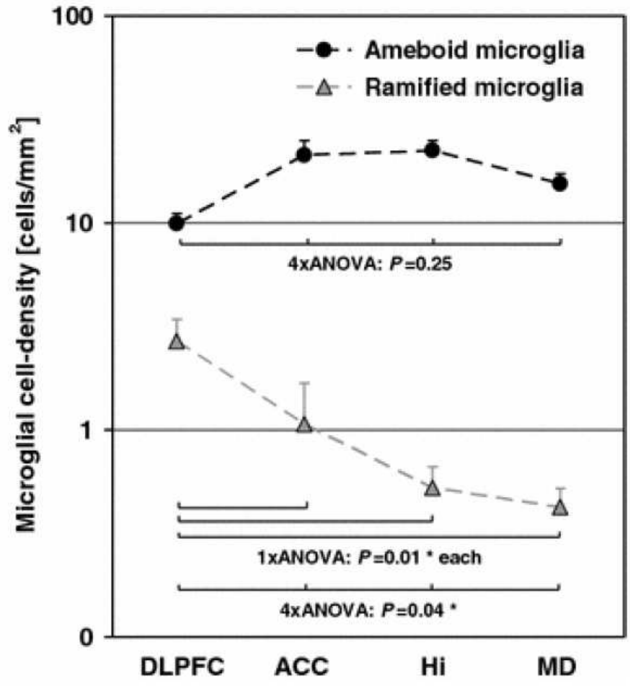

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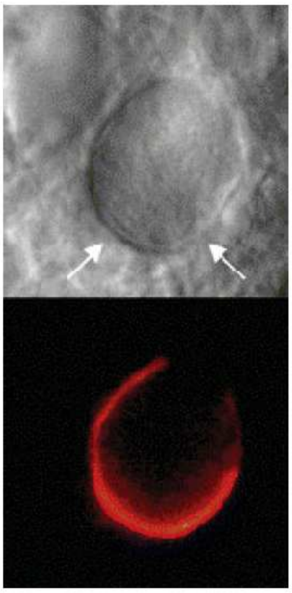

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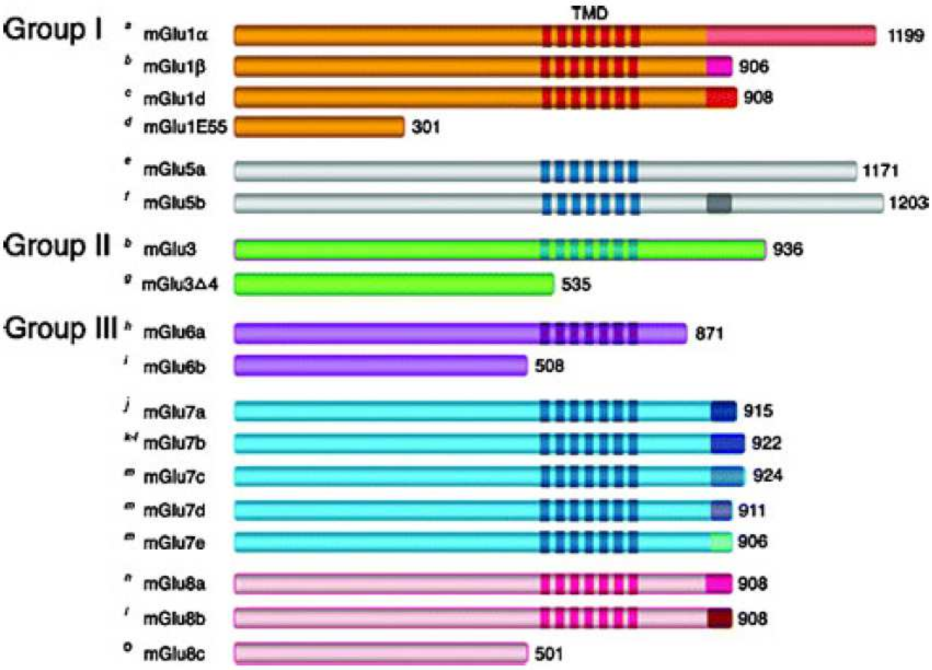

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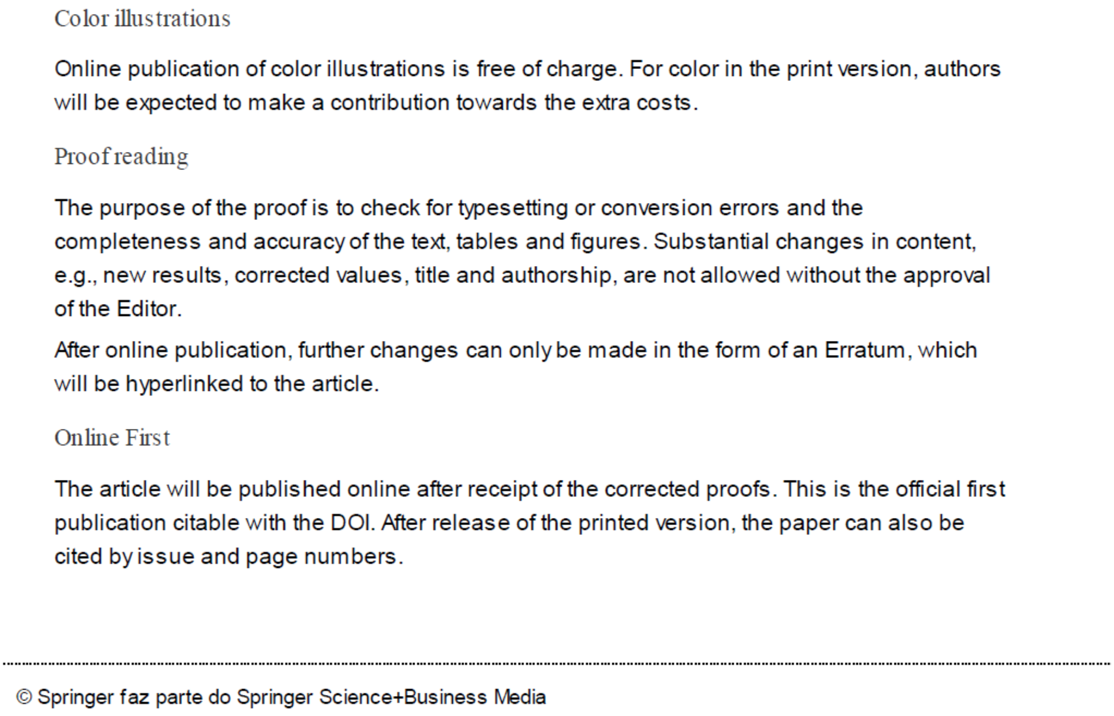

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Spelling: *Webster's New Collegiate Dictionary*.
Soil: For soil series names see the "Official Soil Series Descriptions" websitefrom the USDA-NRCS (http://soils.usda.gov/technical/classification/osd/index.html). Amendments to the U.S. system of soil taxonomy (Soil Survey Staff, 1975) have been issued in the *National Soil Survey Handbook* (NRCS, 1982-1996) and in *Keys to Soil Taxonomy* (Soil Survey Staff, 1996). Updated versions of these and other resources are available at http://soils.usda.gov/. The *Glossary of Soil Science Terms* (SSSA, 2001) is available online (https://www.soils.org/publications/soils-glossary). It contains definitions of more than 1800 terms, a procedural guide for tillage terminology, an outline of the U.S. soil classification system, and the designations for soil horizons and layers.
Scientific Names of Plants: *A Checklist of Names for 3000 vascular plants of Economic Importance* (USDA Agric. Handb. 505, see also the USDA Germplasm Resources Information Network database, http://www.ars-grin.gov/npgs/searchgrin.html).
Chemical Names of Pesticides: *Farm Chemicals Handbook* (Meister Publishing, revised yearly).
Fungal Nomenclature: *Fungi on Plants and Plant Products in the United States* (APS Press).

Journal Abbreviations: Chemical Abstracts Service Source Index (American Chemical Society, revised yearly).

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