ABOVEGROUND BIOMASS PARTITIONING IN RED CLOVER CANOPY

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Abstract

The aim of this study was to perform an exploratory analysis of red clover canopy structure in late phenological stages (80, 100 and 120 Days After Sowing – DAS), when structural complexity increases. The results showed that the modification of assimilatory surface heights occurred due to the phenological development and the light competition between phytomeres (a self-shading process). Spatial and temporal repartition of foliage biomass and caulinar biomass influenced the evolution of canopy architecture as a summing junction of the individual component forms. Heterogeneity of canopy structure increased with maturity. The average length of generative shoots was 41 cm at 80 DAS, 75 cm at 100 DAS, and 81 cm at 120 DAS. Laminae areas of vegetative shoots increased from 11.18 ± 5.04 (80 DAS) to 17.31 ± 8.19 cm^2 (120 DAS). For red clover aboveground structure, heterogeneity of individual plants can be expressed considering biomass partitioning, variations of leaf and stem parameters, modulations of phytomere characteristics, and modifications of branching pattern.

Key words: specific leaf area, leaf weight ratio, leaf area index, canopy growth, dry matter allocation

Red clover (*Trifolium pratense* L.) presents a high ecological plasticity and withstands more shading than other legumes. However, red clover cannot be relied on for long-term persistency. Persistence is the result of an interaction between the adaptation of the crop and its stress load (Taylor N.L., Quesenberry K.H., 1996). The clover vigor is prone to dwindle under ecophysiological and pathogenic factors such as interspecific competition, winter hazards, cutting frequency, pathogens, root rot phenomena, and environmental stresses (Dunea D., 2008).

Canopy structure and herbage growth are highly interdependent because structure results from growth pattern of individual plants within the canopy and it affects the rate at which resources are acquired by individual plants and the sward as a whole (Laca E.A., Lemaire G., 2000).

Changes in morphological ratios often describe the sources of variability in the population structure (Allirand J.M., 1998). Canopy - roots ratio is related to total biomass and leaves - stems ratio corresponds to aboveground biomass. The plant's architecture is independent of allocation trade-off and can be considered as a measure of how efficiently biomass is used (Schippers P., Olff H., 2000).

Red clover canopy cumulates the shape of each individual plant, which results from the ensemble of vegetative and generative shoots (basal and upper segments). The asymmetry of aerial axes distribution and morphology amplifies with the kinetic of growth and the intraspecific competition for light.

Competition for light is an instantaneous process of resource capture and the efficiency of resource capture is related to light interception and light use characteristics of the species (Kropff M.J., van Laar H.H., 1993). Vertical and horizontal distributions of assimilatory areas (mainly leaves) describe leaf area density (LAD), which is a spatial distribution parameter used in multi-layer mixed canopy models to calculate light profiles and absorption of light by species (Spitters C.J.T., Aerts R., 1983; Metselaar K., 1999). Leaf area index is the essential determinant of the solar radiation interception of pure stands (Nassiri M.M., 1998; Dunea D. et al., 2015).

Monitoring of morphological and spatial heterogeneity of plant canopies provides rigorous descriptions of architecture of the species that help to synthesize three-dimensional representations in accordance with the growth and development of real structural entities formed by spatial grouping of individual plants. The intraspecific competition for resources determines differentiated phenotypic responses of red clover plants considering the variability of aboveground dry matter partitioning

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in morphological components, dimensions of biometric components and leaf area parameters (specific leaf area – SLA, leaf weight ratio – LWR, leaf area ratio – LAR, and leaf area index – LAI).

The purpose of this study was to analyze the canopy structure of red clover pure crop during late phenological stages, when structural complexity increases. The aims were to characterize the spatial and morphological heterogeneity of individual plants within canopy and to describe architectural structures of red clover canopy to facilitate canopy reconstruction for visualization in a virtual environment.

MATERIAL AND METHODS

Violetta diploid cultivar (*figure 1*) was analyzed in a randomized block design with 6 replicates. The plots were sown on 1 May. A red clover plant needs a nutrition area of 10-16 cm^2. Consequently, to insure a dense sward, plant density was 1000 germinal seeds m^{-2}, respectively 20 kg util seeds ha^{-1}. Soil was heavy-river clay. The plants were given NPK fertilizer (12-10-18) at one rate (300 kg ha^{-1}) to avoid nutrient limiting growth.

Figure 1 **Canopy of Violetta diploid cultivar at 100 days after sowing**

Herbage measurements and characteristics

Measurements were performed at individual plant level and at population scale.

Four replicates of red clover were considered for detailed 3-D canopy structure measurements by analyzing small niches of population. Plots were sampled three times at intervals of three weeks (18 July, 8 August, and 29 August).

In results section, each sampling date will be referred as crop age, respectively 80 days, 100 days, and 120 days. During each sampling, the canopy increased in complexity due to the appearance and development of two overlaid structures, respectively generative axes and phytomeres (each axe being composed from a phytomere ensemble). A phytomere contains a petiole with corresponding lamina (three leaflets connected to petiole by petiolules), an axillar bud, and the subjacent internode. The complex of axillary buds enlarged as the plant grew to form the crown. Numerous axillary buds generated secondary axes.

Sampling was done using a 100 cm^2 quadrate collecting carefully ten entire neighbored plants. Biometrics measurements were divided in two units 1) vegetative stems and 2) generative stems to assess the aboveground architecture of these plants.

In addition, the results achieved at individual level were compared with a bulk harvest conducted at the same time using a 50 cm × 50 cm quadrate in two points of each replicate.

Clover laminae were individually measured with a Li-cor 3100 Leaf-Area Meter® (Li-Cor Inc., Lincoln, NE, USA) to obtain leaf area. Harvested material was separated in leaves, stems, reproductive organs, and senesced material, dried at 70 ºC for 24 hours, and weighted to fractionate dry matter allocation in morphological elements.

Light measurements

The light profile of Photosynthetically Active Radiation (PAR) was determined at each sampling using a Delta–T SunScan Canopy Analysis System (Delta-T Devices Ltd., Cambridge, UK) with beam fraction sensor at successive layers of 10 cm in the canopy from ground to top level. PAR measurements were repeated 10 times at each layer in different positions of the canopy to improve the accuracy of the light-extinction profile. The system provided estimations of LAI at various canopy heights, which were correlated with area meter results.

RESULTS AND DISCUSSION

At population level, the dry matter partitioning changed with the sequence of phenological phases towards maturity (*figure 2*).

Dry matter allocation showed the following patterns in the growth and development of red clover canopy at the population level:

- vegetative shoots: 19.07% (80 DAS), 5.01% (100 DAS) and 9.56% (120 DAS);
- leaves of vegetative shoots: 18.79% (80 DAS), 3.88% (100 DAS) and 6.82% (120 DAS);
- generative shoots: 39.29% (80 DAS), 56.71% (100 DAS) and 39.45% (120 DAS);
- leaves of generative shoots: 16.16% (80 DAS), 20.22% (100 DAS) and 12.83% (120 DAS);
- heads and buds: 6.67% (80 DAS), 10.20% (100 DAS) and 23.93% (120 DAS);
- senescent material: 0.01% (80 DAS), 3.94% (100 DAS) and 7.37% (120 DAS).

It was noticed that the leaf area weight of vegetative shoots diminished in favor of generative shoots foliage between 80 and 100 DAS, suggesting that the process of assimilation was transferred to the upper canopy segments.

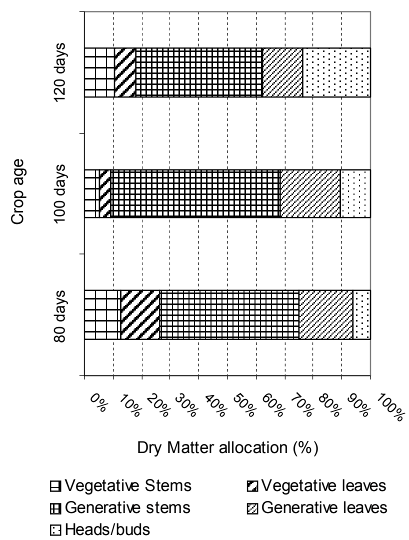


Figure 2 **Modifications of dry matter allocation (%) over time (days after sowing) in red clover (Violetta diploid cultivar)**

At 120 DAS, it was observed that the segment of the basal shoots regenerated replacing old or withered vegetative shoots with new ones. Developing of generative shoots was very strong being oriented towards the reproductive organs formation (flower heads). The weight of the heads achieved a percentage of 23.93% from the total aerial D.M. At the same time, the proportion of senescent material increased with growing stages becoming significant at 120 DAS.

A reduction of LAI was observed after 80 DAS during the growing cycle of Violetta diploid variety, although the corresponding D.M. presented higher values at 110 DAS (524.03 g D.M. m^{-2}) compared to 80 DAS (412.89 g D.M. m^{-2}). The "*plateau*" period characterized by the stopping of LAI growth occurred after 100 DAS (*fig. 3*). Canopy intercepted the maximum amount of incident solar radiation due to optimal spatial disposal and proper optical properties of leaves considering the leaf life span. Formation of new leaves, stems, and roots partially compensated the disappearance of morphological organs affected by senescence.

Foliar system properties have changed between 80 and 100 DAS due to the increasing of surface area and laminae mass (*figure 4*), which decreased the SLA, LWR and LAR, while mass stems increased significantly (*table 1*).

Measurements carried out in 5 points of each replicate to establish the average height of the canopy confirmed the homogeneity of this parameter in each of the three sampling dates. The difference between the replicates was not statistically significant. For example, at 120 DAS, the average height of canopy was 66.5 cm (CV% = 5.77), with a maximum of 73 cm and a minimum of 61 cm.

The red clover canopy cumulated the shape of each individual plant resulting from the intraspecific competition for resources, consisting of all vegetative and generative shoots.

At individual level, the asymmetric distribution of dimensions and morphology of axes was amplified due to the ontogenetic growth and development, and intraspecific competition for light. The main variables of the individual plant growth process in the functional group of the canopy were located both in the partitioning of dry matter in organs and in the variability of biometric characteristics.

Intraspecific competition was characterized by drawing profiles of neighboring plant dry matter accumulation (*figure 5*). D.M. partitioning in individual plants influenced significantly the biomass distribution of red clover canopy.

It was noticed that the reaching of maturity was differentiated in terms of D.M. partitioning in each area of ~100 cm^2 occupied by ten clover plants. The neighboring plants were relatively homogeneous at 80 DAS, after which differences increased. Consequently, at 120 DAS there were one or two vigorous plants characterized by a large number of heads and well spatially distributed generative shoots with assimilatory areas located to the middle and to the top of canopy that effectively captured the available incident light against the smaller neighboring plants.

Previous researches (Schippers P., Olff H., 2000; Schulte R.P.O, 2001) pointed out that the magnitude of intraspecific competition extends also to the root system and there is a direct correlation between aboveground biomass and root development.

Heterogeneity of biometric elements that form the canopy structure

Heterogeneity of canopy structure increased with maturity. The average length of generative shoots was 41 cm at 80 DAS and 75 cm at 100 DAS (*table 2*). The growth of generative stems significantly decreased after this period, at 120 days the average length being 81 cm.

The length of generative shoots increased due to the elongation of internodes (1, 3, 4 and 5 numbered from the base to top of the generative shoots). In general, the petioles located on inferior phytomeres showed longer lengths and steeper angles of insertion on the internode as compared to the upper ones. The flowering phenophase increased the canopy structural complexity especially in the upper height units.

Table 1

Leaf area parameters of red clover (Violetta diploid variety)

Indicator	Average	Std.Dev.	Average	Std.Dev.
Days after sowing	80		100	
Specific Leaf Area *(m^2 leaves· kg^{-1} D.M. leaves)*	34.59	1.44	27.27	0.001
Leaf Weight Ratio *(kg D.M. leaves /kg D.M. aboveground biomass)*	0.42	0.063	0.32	0.079
Leaf Area Ratio *(m^2 leaves· kg^{-1} total D.M.)*	14.51	1.82	8.75	2.17
Leaf Area Index	3.21	0.961	6.60	1.442

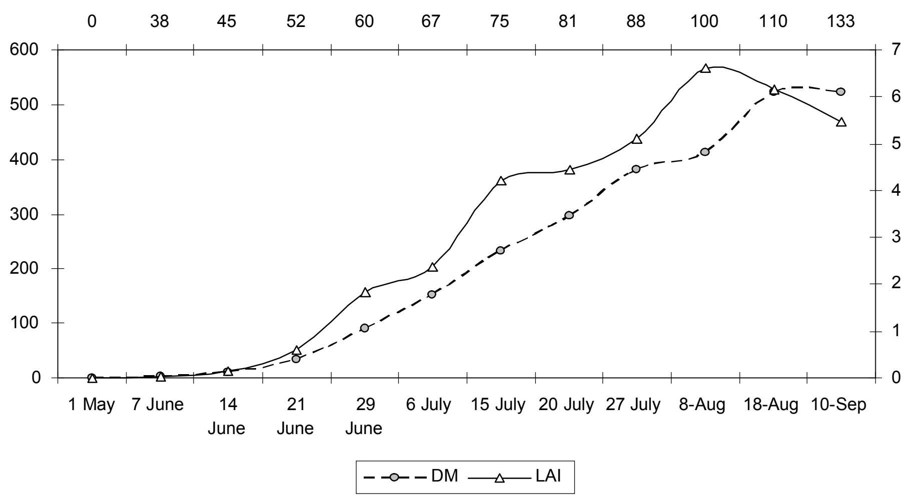
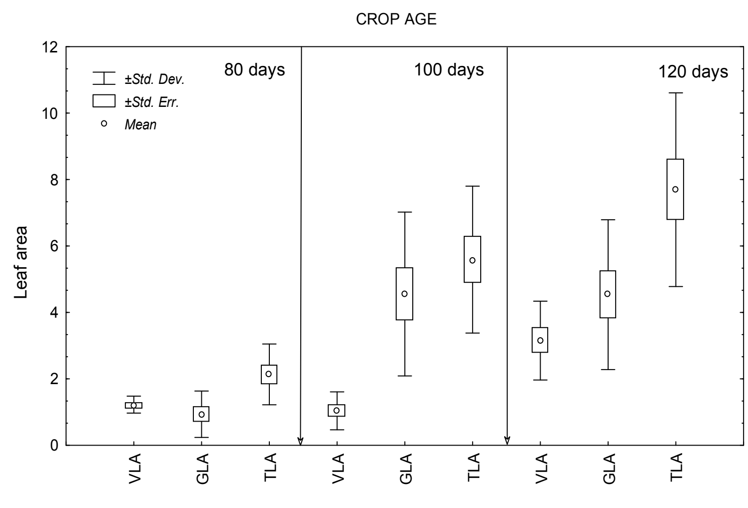


Figure 3 **Evolution of dry matter accumulation and leaf area index in the vegetation cycle of diploid red clover variety Violetta (left axis g D.M. m^{-2}; right axis m^{-2} leaves m^2)**



VLA – Leaf Area of vegetative stems [dm^2];
GLA – Leaf Area of generative stems [dm^2];
TLA – Total Leaf Area [dm^2].

Figure 4 **Variability of leaf parameters during vegetation cycle for specific canopy units**

Table 2
Biometrical dimensions of aboveground structure of red clover canopy (N = 120 plants).

Canopy unit	Average	Standard Error	Variance
80 DAS			
Vegetative stems (basal segment)			
Stem length (cm)	21.79 ± 5.80	0.749	33.68
Stem angle (^{o})	75.15 ± 7.15	0.923	51.18
Lamina area of vegetative stem (cm^2)	18.20 ± 5.40	0.698	29.23
Generative stems (upper segment)			
Internode 1 (cm)	7.30 ± 4.68	1.046	21.91
Internode 2 (cm)	15.38 ±5.01	1.213	25.01
Internode 3 (cm)	10.96 ±5.74	1.483	33.01
Internode 4 (cm)	7.29 ± 4.12	1.190	17.02
Petiole length (cm)	9.60 ± 5.68	0.734	32.32
Petiole angle (^{o})	63.58 ± 11.6	1.499	134.82
Lamina area of petiole (cm^2)	11.18 ± 5.04	0.651	25.41
100 DAS			
Vegetative stems (basal segment)			
Stem length (cm)	25.17 ± 10.2	1.415	104.18
Stem angle (^{o})	69.21 ± 9.22	1.278	85.03
Lamina area of vegetative stem (cm^2)	19.89 ± 8.23	1.142	67.85
Generative stems (upper segment)			
Internode 1 (cm)	10.29 ± 7.01	1.302	49.18
Internode 2 (cm)	15.29 ± 5.08	0.943	25.83
Internode 3 (cm)	16.30 ± 4.35	0.823	18.98
Internode 4 (cm)	12.03 ± 5.77	1.132	33.33
Internode 5 (cm)	10.31 ± 3.97	1.026	15.81
Petiole length (cm)	10.63 ± 7.00	0.623	49.01
Petiole angle (^{o})	17.31± 8.19	0.733	67.23

The stems length of vegetative shoots showed a difference of ~8 cm between the maximum values. Their leaf areas were relatively constant between 80 and 100 DAS, with a difference between the minimums of -1.14 cm^2 and maximums of 4.52 cm^2.

Laminae areas of vegetative shoots increased from 11.18 ± 5.04 (80 DAS) to 17.31 ± 8.19 cm^2 (120 DAS). Foliar parameters changed following the same pattern, respectively from a significant contribution of the basal segment to the total leaf area of the plant (80 DAS), to a higher percentage of the superior canopy units at 100 DAS. Later on, the contribution of generative shoots decreased due to the reproductive stage to form numerous heads, while the basal segment produced new stems and emerged new leaves (*figure 4*). Several differences among plants concerning the total and components weights were observed. The slope between maximum and minimum plant weight accentuated over time (*figure 5*).

In terms of biomass accumulation, such process could explain the growth kinetic variability of canopy components. A vigorous plant had a fast growth of upper segments and deployed numerous axes, and such, more assimilatory surfaces at the middle and top heights.

Consequently, higher allocated D.M. quantities in generative stems and their corresponding leaf areas were found for the vigorous plants.

The axillar components of phytomeres and the stem elongation showed particularly a fast growth. Therefore, the leaf area heterogeneity within red clover canopy was induced mainly by the structural complexity of the generative stems of the vigorous plants.

The senescent material in sward increased with time and leaf mass once the primary leaves begun to senesce. The self-shading process accelerated the rate of senescence and affected the leaf life span because of the canopy height augmentation towards maturity.

An exponential increment of the leaf area index between samplings was observed. In addition, an elevation shift effect of the leaf area density as a result of the crop age was observed. One reason could be that the growth process was oriented towards a reproductive strategy. This would favor the growth of generative shoots rather than the vegetative shoots.

Competition for resources was highlighted by the dry matter partitioning in ten neighboring plants (*figure 5*).

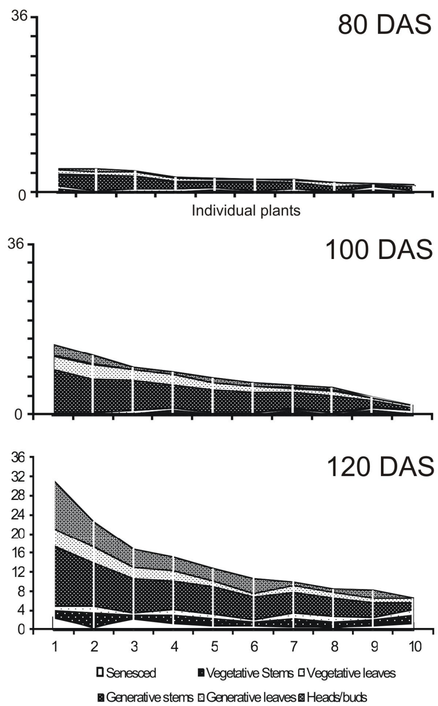


Figure 5 **Average values (n=120) of dry matter allocation (g D.M./plant) in morphological components in ten red clover neighboring plants (80, 100 and 120 Days After Sowing - DAS)**

At individual level, competitiveness depended on the deployment efficiency of morphological organs specialized to gain resources.

CONCLUSIONS

The present study established growth patterns of the multidimensional distribution of leaf area and structural elements of canopy in a red clover pure crop.

The results showed that the modification of assimilatory surface heights occurred due to the phenological development and the light competition between phytomeres (a self-shading process).

Spatial and temporal repartition of foliage biomass and caulinar biomass influenced the evolution of canopy architecture as a summing junction of the individual component forms.

For red clover aboveground structure, heterogeneity of individual plants can be expressed considering biomass partitioning, variations of leaf and stem parameters, modulations of phytomere characteristics, and modifications of branching pattern.

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