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The Productivity and Quality of Alfalfa (*Medicago sativa* L.) in Romanian Forest Steppe

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Abstract

Alternative use of alfalfa, for various purposes, including the production of biofuels or food supplement for human alimentation, is a study topic still in its early stages of research. Studying and understanding the biology of alfalfa and the factors with a major influence on it are very important activities. The productivity and quality of alfalfa are two indicators that help determine, in addition to economic value, the way in which alfalfa can be used. Evolution of alfalfa yield and quality depends on many factors, such as the growth stage of alfalfa plants at harvesting. It was observed over three years of vegetation the influence of alfalfa plant growth stage at harvest on plant height, leaves/stems ratio, production of leaves, stems and whole plant (DM - dry matter) per hectare and on quality indicators (CP - crude protein, NDF - neutral detergent fiber and ADF - acid detergent fiber). The results showed that, with the advancement of phenological phases, from early bud stage to complete flowering, the total biomass output raised from 2.79 Mg·ha^{-1} to 4.60 Mg·ha^{-1}, the neutral detergent fiber raised from 48.4-50.6% to 62.0-67.7%, while crude protein content decreased from 21.2-24.0% to 13.3-16.5%. The parameter values were correlated with alfalfa growth stage during the harvesting (significant at the 0.05 and 0.01 probability levels).

Keywords: biology, crude protein, harvest time, growth stage, leaves, lucerne, stems

Introduction

Alfalfa (*Medicago sativa* L.) is one of the most valuable crops due to high yields obtained and its remarkable chemical composition. Although alfalfa crop fields have the appearance of a uniform mass consisting of stems, leaves, flowers and petioles, each of these parts differ in terms of chemical composition (Tyrolová and Výborná, 2008; Vasileva, 2013; Vyas *et al.*, 2009).

Chemical composition varies according to the cultivated variety, pedoclimatic conditions of the area, the technology used, the number of cuts (Orloff and Putnam, 2007; Petkova and Panayotova, 2007). In addition to the essential amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine) which are found in a balanced proportion, one finds in alfalfa a number of vitamins (A, C, D, K, B_1, B_2, B_6, B_{12}) and other valuable compounds such as: saponins, coumarins, flavones, isoflavones, cumestani, anthocyanins etc. (Christopher and Jorgensen, 1987; Zanin, 1998; Vyas *et al.*, 2009). As a result, alfalfa crop has many uses. Thus, alongside its basic function as a source of feed, the plant is used for the production of biofuels, as a nutritional supplement (powder, concentrated juice), or as seedling, in human nutrition and for the production of industrial enzymes, such as lignin peroxidase, alpha-amylase, cellulase and phytase, or biodegradable polymers (Dale, 1983; Hanson *et al.*, 1988; Martin and Jung, 2010; Saruul *et al.*, 2002).

Among the elements of alfalfa cultivation technology, harvesting time is key factor with great influence on the relationship between quantity and quality. The phenophase of the alfalfa plants at harvesting determines the chemical composition of alfalfa. An important indicator in determining the chemical composition of alfalfa, is the leaf/stem ratio, which also depends on the time of harvesting. The aim is to have the highest possible percentage of leaves, since the leaves have a higher content in proteins, minerals and vitamins than the stems (Goliński and Golińska, 2008; Ketterings *et al.*, 2008; Lamb *et al.*, 2003, 2007; Rimi *et al.*, 2010; Samuil *et al.*, 2012; Tyrolová and Výborná, 2008; Vasileva, 2013).

Improving the technology of growing alfalfa and thorough analysis of the relationship between biomass productivity and quality, in the stationary conditions from Northeast Moldavian Forest Steppe is an activity of topical and high interest.

In the attempt to find answers to these problems, this study aimed to determine the nutritive value of alfalfa harvested at different growth stages. This study provides a detailed picture of the quality of the leaves, stems and whole plant alfalfa in six growth stages at harvest, from early bud to full bloom.

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Table 1. Influence of growth stage at harvest on the alfalfa productivity parameters

3 years average (2011, 2012 and 2013)					
Experimental plot	Plant height (cm)	Dry matter production (Mg·ha^{-1})			Leaves/ stems ratio
		Leaves	Stems	Whole plant	
a_1 - early bud (control)	36.2	1.04	1.76	2.79	0.59
a_2 - mid bud	42.2**	1.22**	2.44**	3.66**	0.50°°
a_3 - late bud	62.1**	1.35**	2.79**	4.14**	0.48°°
a_4 - early bloom	66.4**	1.32**	3.11**	4.43**	0.42°°
a_5 - 10% bloom	70.3**	1.11	3.45**	4.56**	0.32°°
a_6 - full bloom	71.0**	0.97	3.63**	4.60**	0.27°°
$LSD_{0.05}$	*0.4*	*0.08*	*0.11*	*0.16*	*0.01*
$LSD_{0.01}$	*0.5*	*0.10*	*0.15*	*0.22*	*0.02*

°(-) and *(+) - Significant at the 0.05 probability level;
°°(-) and **(+) - Significant at the 0.01 probability level;

Materials and methods

The research was conducted between 2011-2013, at Ezăreni Farm, Iaşi (47°05' - 47°10' North latitude and 27°28' - 27°33' East longitude). The soil was a cambic chernozem, characterized by pH 6.73, 40.3% clay, humus 2.32%, 0.164% total nitrogen, P-Al 18 ppm, 210 ppm K-Al.

The biological material used was the alfalfa variety 'Sandra' (F 660-94), registered in 2003 at National Agricultural Research and Development Institute (NARDI) Fundulea, Bucharest (Schitea *et al*., 2007). Harvesting was carried out with a behind tractor mower, at a height of 7 cm above the ground.

The study period represented the second, third and fourth year of alfalfa (*Medicago sativa* L.) vegetation and the analyses were noted at the first cut. It was observed the influence of alfalfa plant growth stage at harvest on plant height, leaves/stems ratio, production of leaves, stems and whole plant (DM - dry matter) per hectare and on quality indicators (CP - crude protein, NDF - neutral detergent fiber and ADF - acid detergent fiber).

The experiment was laid by randomized block method, with a harvested area of 10 m^2 (2 x 5 m) in three replicates. Graduations of the studied factor were represented by the growth stage at harvest: early bud (a_1) - one node with visible buds, mid bud (a_2) - two nodes with visible buds, late bud (a_3) - ≥3 nodes with visible buds; early bloom (a_4) - one node with one open flower, 10% bloom (a_5) - at least 10 open flowers on 100 stems, full bloom (a_6) - ≥2 nodes with open flowers; growth stages were determined as described by Kalu and Fick (1981), Ball (1998), Barnes (2007), Mueller and Teuber (2007).

Plant height was determined at harvest by measuring plants from the same location in each parcel, in 3 repetitions. The leaves/stems ratio was determined by separating the petiole, leaflets, buds and flowers from the stem, weighing them separately and establishing the ratios for these quantities, from a sample of 2.5 kg green alfalfa plants. Production was determined by weighing the yield obtained from a harvested surface of 10 m^2, which was afterwards transformed per hectare. The dry matter was determined by drying the vegetal material in an oven at 103 °C for 3 hours. The leaves and stems productions were calculated based on the leaves/stems ratio.

The CP content was determined by multiplying the total nitrogen content (determined with the Kjeldahl method) with the factor 6.25. The NDF and ADF contents were determined by the Van Soest method.

The data were interpreted statistically by analysis of variance and calculation of least square difference (LSD). Also, equation correlations were calculated (quadratic regression significance) between harvesting time and the production of leaves, stems, whole plants, as well as their NDF, ADF and CP content.

Results and discussions

The research conducted in this experiment indicated that the plant height increased during the development of the plant with a trend of stabilization. Starting with the beginning of flowering, harvesting time has a positive, significant influence at the 0.01 probability level (Table 1). The results of this study had similar trend with the conclusions of Alibés *et al.* (1991), Layug *et al.* (1996), Nishikawa (1965), Pecetti *et al.* (2001), Rimi *et al.* (2010), Shroyer *et al.* (1984), in studies conducted on the influence of the harvesting time on plant height.

The leaves/stems ratio is an important quality indicator, because it influences the quality of alfalfa. The aim is to have the highest possible percentage of leaves, since the leaves, as shown by numerous authors (Lamb *et al.*, 2007; Orloff and Putnam, 2007; Petkova and Panayotova, 2007; Popovic *et al.*, 2001) have a CP content at least double compared to that from the stems. For this reason, breeding specialists work towards creating varieties with a larger number of internodes and a reduced height. Thus, for this purpose were created varieties with a higher percentage of leaves (varieties with shorter internodes, with a greater number of leaves floors, or pentafoliate leaves varieties). Rotili *et al.* (2001), Lamb *et al.* (2006), Petkova and Panayotova (2007), Tyrolová and Výborná (2008), highlighted these issues in their studies. The leaves/stems ratio varies according to numerous factors: the variety grown, fertilizer application, number of cuts, climatic conditions etc., but the obtained results showed that the phenological phase when alfalfa is harvested also has a predominant influence on this indicator. These is in agreements with the data obtained in other researches also (Hall *et al.*, 2000; Katić *et al.*, 2009; Lamb *et al.*, 2003; Mehrdad *et al.*, 2004; Sheaffer *et al.*, 2000).

With the advancement of vegetation, the DM production for the whole plant and the stems are constantly growing. The DM production in leaves increased until the end of budding - early flowering stage, after which it started to decrease. More precisely, with the emergence of the first flowers, the leaves from the lower floors of the stem etiolate, dried and fall off. The phenomenon directly impacts on the quality of production. Thus, the later the plants are harvested after the start of flowering, the lower the leaf production and the poorer the quality of the hay inferior. In this case, the leaves were represented by the petiole, leaflets, buds and flowers separated from the stem.

Table 2. Influence of growth stage on alfalfa quality parameters at harvest

Experimental plot	Leaves CP	Leaves NDF	Leaves ADF	Stems CP	Stems NDF	Stems ADF	Whole plant CP	Whole plant NDF	Whole plant ADF
2011									
	% from DM			% from DM			% from DM		
a_1 - early bud (control)	32.4	26.5	21.2	17.4	60.8	53.6	24.0	49.0	43.8
a_2 - mid bud	32.5*	26.2°°	21.3*	15.8°°	62.3**	54.4**	23.1°°	51.3**	45.4**
a_3 - late bud	32.3°	27.5**	21.6**	14.7°°	65.1**	55.8**	21.2°°	52.7**	46.1**
a_4 - early bloom	30.6°°	28.3**	22.2**	13.5°°	67.2**	56.9**	18.9°°	54.9**	48.0**
a_5 - 10% bloom	30.0°°	28.2**	22.0**	12.6°°	71.9**	59.1**	18.1°°	61.3**	50.1**
a_6 - full bloom	29.4°°	29.2**	22.7**	11.8°°	73.8**	60.2**	16.5°°	63.3**	51.1**
$LSD_{0.05}$	*0.1*	*0.2*	*0.1*	*0.1*	*0.1*	*0.2*	*0.1*	*0.1*	*0.1*
$LSD_{0.01}$	*0.2*	*0.3*	*0.3*	*0.2*	*0.2*	*0.3*	*0.2*	*0.2*	*0.2*
2012									
	% from DM			% from DM			% from DM		
a_1 - early bud (control)	32.5	28.3	22.6	14.4	62.5	55.1	21.9	48.4	41.7
a_2 - mid bud	32.4	29.1**	23.3**	13.4°°	64.6**	56.4**	20.4°°	51.4**	44.0**
a_3 - late bud	31.7°°	30.0**	23.5**	12.2°°	68.2**	58.5**	19.3°°	54.2**	45.7**
a_4 - early bloom	30.5°°	30.3**	23.6**	11.4°°	70.8**	59.9**	18.0°°	56.6**	47.2**
a_5 - 10% bloom	29.9°°	31.1**	24.0**	10.4°°	72.6**	59.9**	16.3°°	60.0**	49.2**
a_6 - full bloom	29.5°°	31.3**	24.3**	10.0°°	73.7**	60.3**	15.4°°	62.0**	50.1**
$LSD_{0.05}$	*0.2*	*0.1*	*0.3*	*0.2*	*0.2*	*0.3*	*0.1*	*0.1*	*0.1*
$LSD_{0.01}$	*0.3*	*0.2*	*0.4*	*0.3*	*0.3*	*0.4*	*0.2*	*0.2*	*0.2*
2013									
	% from DM			% from DM			% from DM		
a_1 - early bud (control)	33.2	27.5	21.7	14.3	63.8	57.9	21.2	50.6	44.7
a_2 - mid bud	32.7°°	28.8**	22.8**	12.7°°	66.5**	59.5**	19.5°°	53.8**	47.5**
a_3 - late bud	31.4°°	30.3**	23.3**	11.7°°	69.8**	62.0**	17.7°°	57.6**	50.3**
a_4 - early bloom	29.9°°	30.9**	24.3**	10.7°°	72.5**	64.1**	16.7°°	60.1**	52.4**
a_5 - 10% bloom	29.0°°	31.7**	25.0**	9.7°°	76.2**	65.9**	14.5°°	65.5**	55.7**
a_6 - full bloom	28.4°°	32.7**	25.5**	9.1°°	78.1**	66.6**	13.3°°	67.7**	57.5**
$LSD_{0.05}$	*0.3*	*0.2*	*0.2*	*0.3*	*0.4*	*0.4*	*0.2*	*0.4*	*0.3*
$LSD_{0.01}$	*0.4*	*0.3*	*0.3*	*0.4*	*0.5*	*0.6*	*0.3*	*0.6*	*0.4*

CP - crude protein; NDF - neutral detergent fiber; ADF - acid detergent fiber; DM - dry matter
°(-) and *(+) - Significant at the 0.05 probability level
°°(-) and **(+) - Significant at the 0.01 probability level

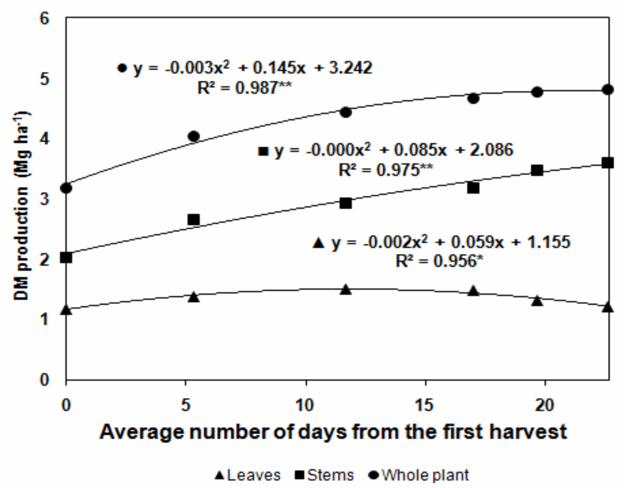

Fig. 1. Correlations between growth stage at harvest and alfalfa DM production; 2011, 2012 and 2013 average
* - Significant at the 0.05 probability level
** - Significant at the 0.01 probability level

In the field, the percentage of leaves and stems remained constant until the beginning of flowering, after which the leaves from the lower floors of the stem begin to fall. Thus, at full flowering stage, there were only a few levels of leaves towards the top of the stem.

The proportion of buds and flowers grows constantly from the early bud stage until full flowering. Thus, if the plants are harvested at full flowering, the production will consist only of stems, inflorescences and only a few floors of leaves. Orloff *et al.* (1997), Sheaffer *et al.* (2000), Thompson *et al.* (2000), Jung and Engels (2002), Overman and Scholtz (2005), showed the same tendency.

The correlation between harvesting time and the DM yields of the whole plant, stems and leaves (Fig. 1) was statistically assured (significant at the 0.01 probability level for the production of whole plants and stems and significant at the 0.05 probability level for the production of leaves, in the case of used equations). The results of the present experiment were comparable with similar data in the specialized literature (Homolka *et al.*, 2008; Overman and Scholtz, 2005; Tyrolová and Výborná, 2008; Waddington and Steppuhn, 1988).

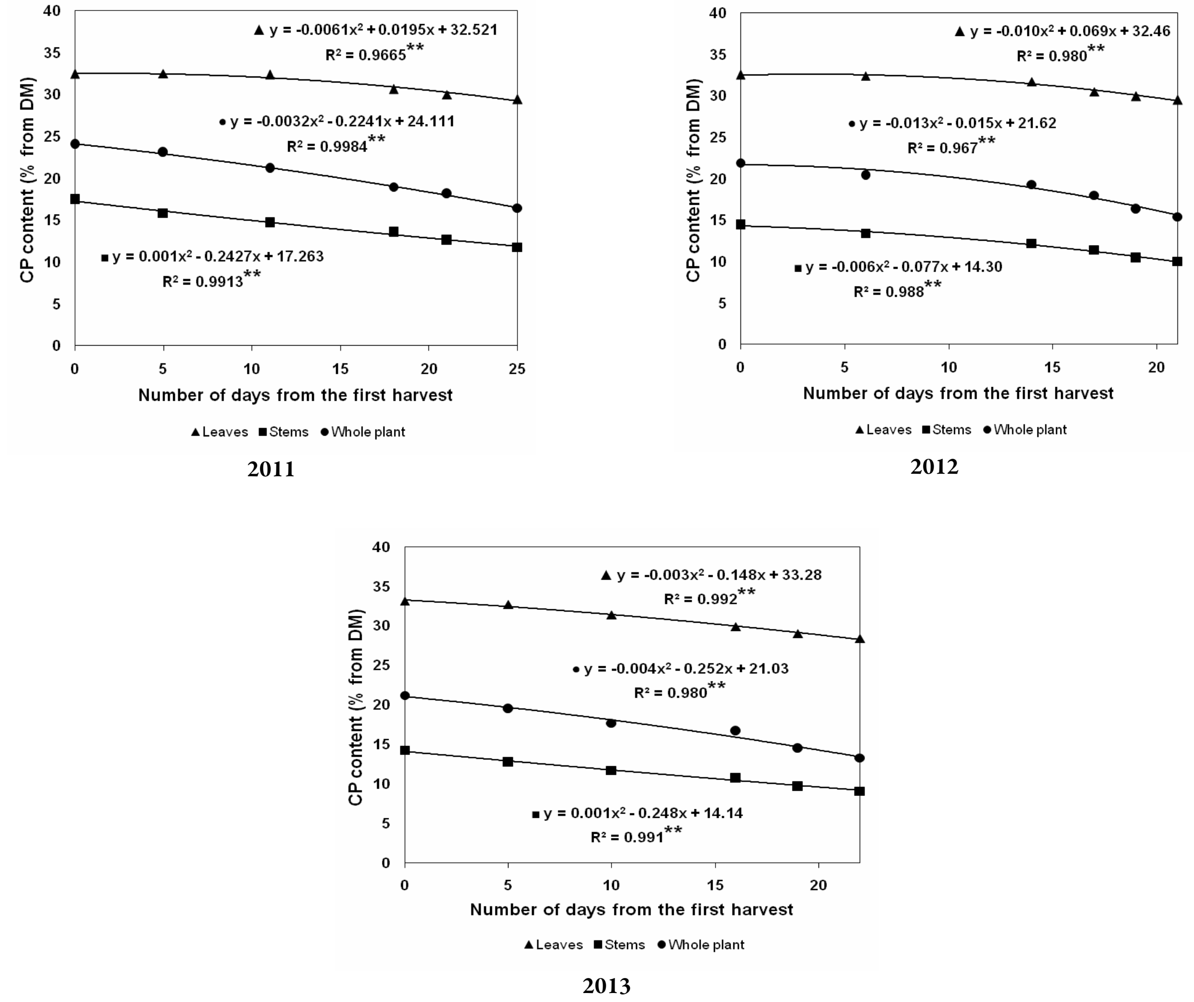

Fig. 2. Correlations between growth stage at harvest and alfalfa CP content
* - Significant at the 0.05 probability level
** - Significant at the 0.01 probability level

With the advancement of vegetation, the content of crude protein in plants, leaves and stems of alfalfa constantly decreased (Table 2). This aspect was also highlighted by other studies (Alibés *et al.*, 1991; Kaiser and Combs, 1989; Marković *et al.*, 2007; Sheaffer, 1990; Sheaffer *et al*, 1995; Thompson *et al.* 2000; Yu *et al.*, 2003).

In the present study, the correlation between the phenological phase at harvesting and the valuable components content in the plants, leaves and stems was negative and distinct significant (Fig. 2). As phenophases succeed one another, the PB content in the plants was increasingly influenced by the PB content in stems, since their share in the biomass output was increasingly higher (Table 2).

The analysis of harvesting time influence on the NDF and ADF content in whole plants, leaves and stems of alfalfa indicated that as plants age, the value of these indicators increase. The correlation between the harvesting phenological phase and the NDF and ADF contents in alfalfa plants, leaves and stems was positive, significant and distinct significant (Figs. 3 and 4). As phenological phases succeed one another, the NDF and ADF content in plants was increasingly influenced by the content of the stems, since the percentage of stems in the obtained fodder was higher. The results confirmed the conclusions of other researchers (Alibés *et al.*, 1991; Canbolat *et al.*, 2006; Dolores *et al.*, 1996; Kaiser and Combs, 1989; Lacefield, 2004; Pop *et al.*, 2009; Sheaffer, 1990).

While the NDF (Fig. 3) and ADF (Fig. 4) content in leaves barely changed with vegetation advancement, the percentage of both NDF and ADF in the stems and the whole plant increased constantly, due to a rising quantity of stems. Most research (Hakl *et al.*, 2010; Mehrdad *et al.*, 2004; Mueller, 1994; Overman and Scholtz, 2005; Thompson *et al.*, 2000) showed that the rising percentage of stems has an increasingly higher influence on the NDF and ADF content in alfalfa plants.

Choosing the adequate harvesting time is an essential condition not only for a high yield, but also for a good quality. The moment when alfalfa is harvested influences the plant frost resistance, the accumulation of reserve substances in the crown and the intensity of regeneration after each cut and during next spring. All of these influence productivity and longevity of alfalfa crops (Al-Hamdani and Todd, 1989; Meyer and Helm, 1994; Orloff and Carlson, 1997; Shroyer *et al.*, 1984). Several studies recommend that at least one of the cuts should be harvested in a more advanced stage of vegetation (30-50% blooming), to enable the plant to accumulate reserve substances necessary for regeneration. Based on the results from this research, it might be also suggested this would be in the benefit of a quality production.

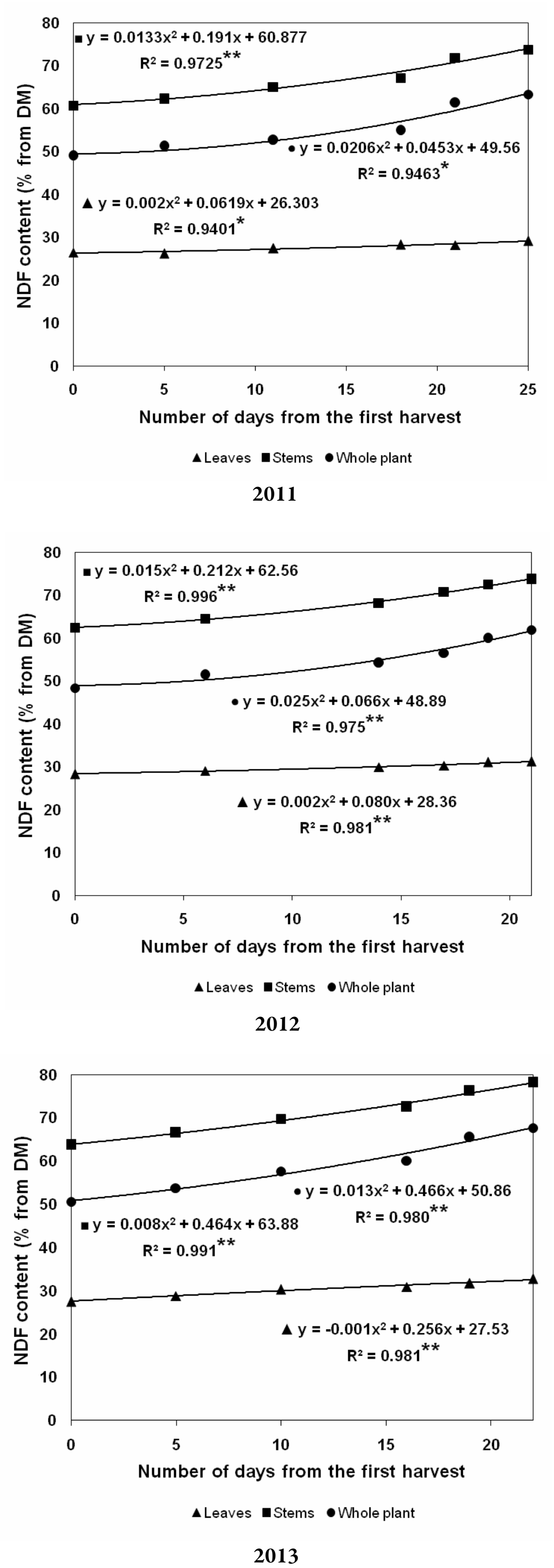

Fig. 3. Correlations between growth stage at harvest and alfalfa NDF content

* - Significant at the 0.05 probability level

** - Significant at the 0.01 probability level

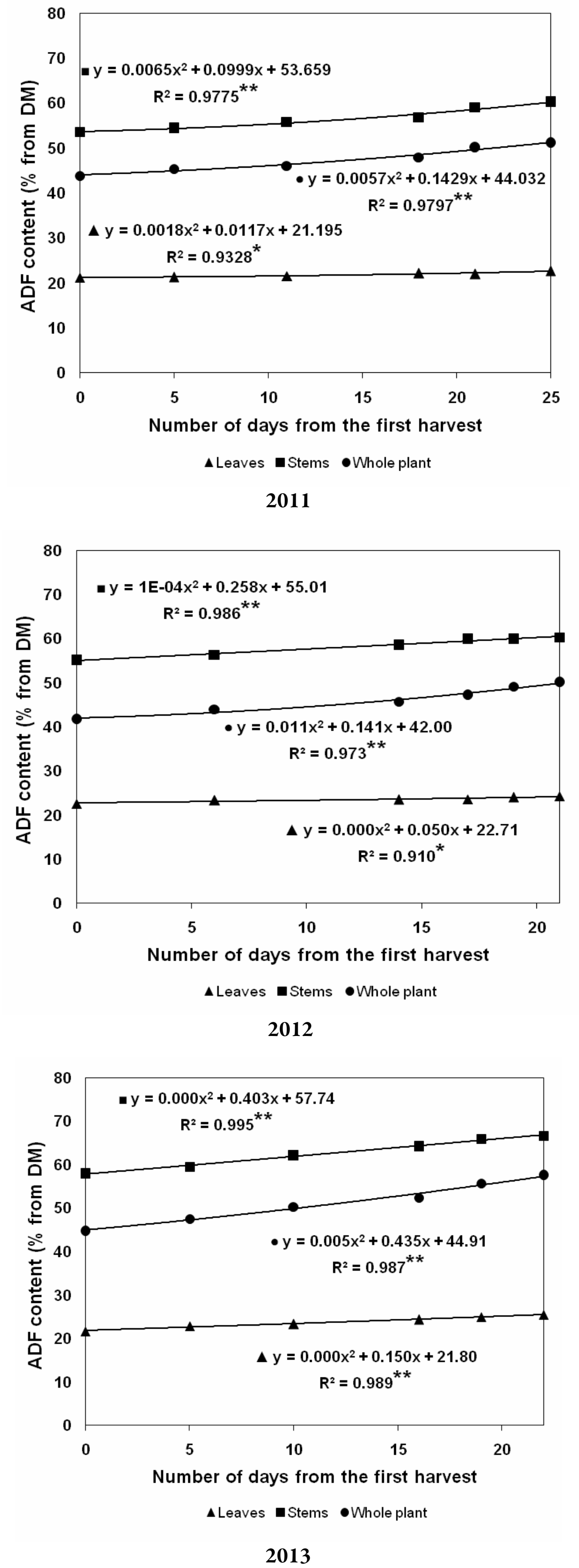

Fig. 4. Correlations between growth stage at harvest and alfalfa ADF content

* - Significant at the 0.05 probability level

** - Significant at the 0.01 probability level

Conclusions

In alfalfa forage crop (*Medicago sativa* L.), with the advancement of growth stages, from early bud stage until the early bloom stage, the production of stems, leaves and whole plant increased continuously. With the emergence of the first flowers, the leaves production decreased, while the total biomass output tends to stabilize. In all of these cases, DM yields obtained from the whole plant, stems and leaves were correlated with the harvesting time. The phenological phase when alfalfa is harvested represents the most decisive factor influencing the quality of production. There was a significant negative correlation between the harvesting time and the leaves/stems ratio and with the advancement of vegetation the content of crude protein in whole plants, leaves and stems of alfalfa continued to decrease. As phenophases succeed each other, CP plant content increased. The growth stage at harvest positively influenced the NDF and ADF content in alfalfa plants, leaves and stems. The plants content of NDF and ADF was increasingly influenced by the content of stems within these indicators, due to the increase of stems proportion in the alfalfa production. It is recommend that at least one of the cuts should be harvested in a more advanced stage of vegetation (30-50% blooming), to enable the plant to accumulate reserve substances necessary for regeneration.

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