

EFFECTS OF SEEDING PATTERN AND HARVEST DATE OF PERSIAN CLOVER AND ANNUAL RYEGRASS ON HAY YIELD AND QUALITY IN A MEDITERRANEAN ENVIRONMENT

Yasar Tuncer Kavut*

Ege University, Faculty of Agriculture, Department of Field Crops, Izmir, TURKEY

*Corresponding author: tuncer.kavut@ege.edu.tr

Received: 11.12.2018

ABSTRACT

This study was carried out at Experimental fields of Ege University Faculty of Agriculture Bornova/Izmir/Turkey, during the years of 2012-2014. Three different harvest dates (Early Spring, Mid Spring and Late Spring) and mixture rates (100+0%, 80+20%, 60+40%, 40+60%, 20+80% and 0+100%, respectively) of Persian clover (*Trifolium resupinatum*) with annual ryegrass (*Lolium multiflorum*) were tested. The experiment was arranged in a split block design with three replications. As the average of 2 years results, highest yield were recorded in sole Persian clover sown as catch crop and harvested in late spring under Mediterranean climatic conditions. The data also indicated that pure Persian cover sowing provided the highest green matter, dry matter and crude protein yields, being 36.04 t ha⁻¹, 5.61 t ha⁻¹ and 89 kg ha⁻¹, respectively. Legume ratio, NDF and ADF concentrations increased by advancing harvest dates.

Keywords: Harvest date, *Lolium multiflorum*, mixture rate, *Trifolium resupinatum*, yield and forage quality.

INTRODUCTION

Legume crops have a benefit over many other crops in that it has the ability to fix its own nitrogen. Legumes also can increase soil-organic carbon and phosphorus and suppress weed growth by competing for water and nutrients (Ashwort et al., 2012). In agricultural ecosystems, grass-legume mixtures have the potential to increase productivity, improve soil structure and prevent soil erosion and nitrate leaching (Peyraud et al., 2009) and increased environmental awareness over the past few decades has challenged farmers to pursue optimal C and N management in field rotation (Kramberger et al., 2013). Legumes can be fitted into diverse cropping systems and thrive under a range of agro climatic condition, and these crops are generally included in crop rotation to help maintaining soil fertility and productivity (Priyadharshini and Seran, 2010). Intercropping legume with cereal is an extensively applied planting pattern in crop cultivation and additionally, the availability of forage legumes allows ruminant production to be integrated into the farming system and relieve pressure on overgrazed and seasonally available rangeland (Caballero et al., 1992; Zhang et al., 2015). Thompson and Stout (1997) showed that Persian clover is a valuable addition to barley-ryegrass mixtures; it reduces fertilizer needs, improves mid-season yield and improves forage nutritive value. The relative proportion of the component crops in mixture is an important factor determining yield, quality and production efficiency of a grass-legume mixture (Willey and Osiru, 1972).

High proportion of legumes in mixture is undesirable since these normally have a low dry matter content (Gilliland and Johnston, 1992). The yield of mixtures primarily depends on the yield of grass component, while only to a small degree on legume species. Increasing the share of legume seeds in the sowing norm increase, their share in the yield, but the yield of grass and the total yield of mixtures generally decrease (Staniak et al., 2014). Vasilakoglou and Dhima (2008) found that in Mediterranean short-season growing environments as alternative of berseem clover sole crop or common vetch-cereal intercrops (at 750-113 seeds m², respectively) for high forage and protein yield with more balanced nutritive concentration. Albayrak et al. (2004) found the highest dry matter and crude protein yield in the mixture including 70% hairy vetch + 30% triticale and when the legume rate increased in the mixture the green herbage yield also increased. Etebari and Tansi (1994) reported that when intercropping was used, yield parameters increased indicating that the current environmental conditions were used more efficiently. They also stated that it is important to choose the cultivars which yield best under given habitat conditions.

The optimum harvest date of legume-cereal mixture types is crucial for the potential of each rotation system and it has special importance in crop plantation and management in grass-legume mixtures since it affects growth and crop traits at the various development stages (Kavut et al., 2014; Acar et al., 2017). Yavuz (2017)

reported that when cutting stages progressed from the beginning of flowering to pod binding period, forage yields increased, but quality characteristics decreased. Turk and Albayrak (2012) also stated that, crude protein content decreased with the advancing growth while dry matter yield, crude protein yield, ADF and NDF contents increased.

The aim of this study was to evaluate the influence of grass + legume mixture rates and different sowing dates on the herbage yield and some yield components of Persian clover and annual ryegrass mixture under the conditions of Mediterranean ecology.

MATERIALS AND METHODS

The research was conducted at Bornova experimental field in Ege University, Izmir, Turkey under Mediterranean climate conditions during the 2012-2013 and 2013-2014 winter seasons. Some meteorological characteristics of the region during the experimental period are presented in Table 1. The soil was silty-clay loam with the following characteristics; pH: 7.8 and organic matter 1.1%.

Table 1. Monthly average temperatures and total precipitations at Bornova-Turkey location

Months	Monthly Temperature (°C)			Monthly Precipitation (mm)		
	2012-2013	2013-2014	LA	2012-2013	2013-2014	LA
October	21.7	17.2	18.9	22.1	94.1	46.2
November	16.4	15.0	14.1	56.9	129.1	97.3
December	10.7	8.5	10.6	218.2	9.1	147.5
January	9.4	11.7	8.8	252.5	149.9	118.6
February	11.2	11.7	9.4	187.0	14.8	103.8
March	14.0	13.2	11.7	56.8	106.4	75.3
April	17.3	17.0	15.9	30.2	132.2	48.3
May	22.7	20.8	20.9	43.7	15.3	26.9
Mean/Total	15.4	14.4	13.8	867.4	650.9	663.9

LA: Long year average

The field experiment was conducted on three different harvest dates (HD-I: Early Spring; the last week of March; HD-II: Mid Spring; second week of April and HD-III: Late Spring; last week of April) and various mixture rates (mixture of annual ryegrass + Persian clover ; 100:0, 80:20, 60:40, 40:60, 20:80 and 0:100, respectively) was tested. *Lolium multiflorum* cv. Hellen with *Trifolium resupinatum* cv. Demet-82 were used.

Germination of all seed materials was tested and accordingly sowing densities were adjusted. Seeding rates of pure annual ryegrass and Persian clover were 30 and 50 kg ha⁻¹, respectively. Seed amounts of species per hectare were based on the seed amounts of their pure sowing. Seeds were planted by hand on September 11th 2012 and September 10th 2013.

The field experimental design was a split plot (harvest dates main plot and mixtures sub plots) with three replications. Each sub-plot was consisted of 14 rows with 20 cm apart and 4 m length (11.2 m²). Nitrogen and phosphorus fertilizers (N:P:K, 20:20:0), at 50 kg ha⁻¹ N and 50 kg ha⁻¹ P₂O₅, were applied into the soil before sowing.

None of the legumes seeds were inoculated with *Rhizobium* bacteria, which existed naturally in the soil. Since there were no significant problems of pests, diseases or weeds in the study, no chemical was applied.

The plots (plants in monoculture and in mixtures) were harvested on March 25th and March 24th for early spring, April 15th and April 14th for mid spring and April 29th and April 29th for late spring in 2013 and 2014, respectively.

At each harvest period, the plots were cut to a 5 cm stubble height with manual shears, and separated by hand for the determination of fresh weight of each species. All plots were conserved for hay production after our experimental cuttings. Harvested fresh forage were weighed and dried to a constant weight at 65 °C during 48 h. The crude protein content was calculated by multiplying the Kjeldahl N concentration by 6.25. The neutral detergent fibre and acid detergent fibre concentrations were measured to Ankom Technology.

Statistical analyses were done by using TOTEMSTAT Statistical program (Acikgoz et al., 2004). The treatment means were compared by LSD test described by Steel and Torrie (1980).

RESULTS AND DISCUSSION

All of the parameters were significantly influenced by different harvest dates, mixture ratios and two factor interaction (Table 2). The results were discussed based on 2 years average in Table 3.

Table 2. Mean squares obtained from combined analysis over years.

Source of Variation	DF	FHY	DMY	CPC	CPY	NDF	ADF
Replication (R)	2	0.207ns	0.001ns	0.008ns	0.014ns	3.233ns	6.504ns
Harvest Date (HD)	2	536.666**	1228.184**	401.911**	641.173**	210.471**	100.101**
Mixture Ratios (MR)	5	327.110**	104.489**	1854.662**	369.562**	92.463**	17.245**
MRxHD	10	18.240**	30.982**	2.510**	26.547**	3.235**	0.814**
CV (%)		% 6.03	% 5.83	% 1.85	% 6.58	% 2.82	% 4.84

Degrees of freedom, CV: Coefficient of variation, ns: Not significant, *P>0.05, FHY: Fresh herbage yield, DMY: Dry matter yield, CPC: Crude protein content, CPY: Crude protein yield, NDF: Neutral detergent fiber, ADF: Acid detergent fiber

Table 3. Means of forage yield and some yield components of Persian clover and annual ryegrass mixtures at different harvest dates (means of two years)

Mixture Ratios (MR)	Fresh Herbage Yield (t ha ⁻¹)				Dry Matter Yield (t ha ⁻¹)			
	Harvest Date (HD)				Harvest Date			
	HD-I	HD-II	HD-III	Mean	HD-I	HD-II	HD-III	Mean
<i>Persian Clover:Annual Ryegrass</i>								
100:0	26.57e	32.71d	48.85a	36.04	3.10i	5.27e	8.46a	5.61
80:20	22.45fg	26.53e	37.77b	28.92	3.12i	4.72f	7.14c	4.99
60:40	21.01g	23.96f	34.95c	26.64	2.98ij	4.46f	7.24c	4.89
40:60	18.88h	21.09g	32.73d	24.23	2.79j	4.16g	7.06c	4.67
20:80	15.17i	17.52h	26.75e	19.81	2.45k	3.52h	5.91d	3.96
0:100	13.94i	15.12i	34.39d	21.15	2.33k	3.15i	7.88b	4.45
Mean	19.67	22.82	35.91		2.80	4.21	7.28	
LSD (5%)	MR= 0.94	HD= 1.46	MRxHD= 1.63		MR= 0.16	HD= 0.26	MRxHD= 0.28	

Mixture Ratios	Crude protein content (%)				Crude protein yield (kg ha ⁻¹)			
	Harvest Date				Harvest Date			
	HD-I	HD-II	HD-III	Mean	HD-I	HD-II	HD-III	Mean
<i>Persian Clover:Annual Ryegrass</i>								
100:0	17.37a	16.05b	14.70d	16.04	54g	85c	125a	89
80:20	15.15c	14.16f	12.91i	14.07	47h	67e	92b	69
60:40	14.77d	13.65g	12.56j	13.66	44hi	61f	91b	66
40:60	14.38e	13.32h	12.17k	13.29	40ij	55g	86c	61
20:80	13.59g	12.47j	11.28l	12.45	33kl	44hi	67e	48
0:100	12.60j	11.49l	10.10m	11.40	29l	36jk	79d	48
Mean	14.64	13.52	12.29		41	58	90	
LSD (5%)	MR= 0.11	HD= 0.23	MRxHD= 0.18		MR= 2	HD= 4	MRxHD= 4	

Mixture Ratios	NDF content (%)				ADF content (%)			
	Harvest Date				Harvest Date			
	HD-I	HD-II	HD-III	Mean	HD-I	HD-II	HD-III	Mean
<i>Persian Clover:Annual Ryegrass</i>								
100:0	38.04k	42.02hi	45.10fg	41.72	25.38h	27.51gh	34.92bcd	29.27
80:20	39.26jk	43.81g	47.02de	43.37	29.99fg	30.85f	36.24abc	32.36
60:40	39.92j	44.81fg	47.82cd	44.18	30.68f	31.80ef	36.99abc	33.16
40:60	40.48ij	45.94ef	48.74c	45.05	30.24fg	32.18def	37.66ab	33.36
20:80	41.59hi	47.85cd	51.19b	46.88	31.29f	32.73def	37.78b	33.93
0:100	42.08h	49.25c	52.93a	48.08	31.61f	34.40cde	38.62a	34.88
Mean	40.22	45.61	48.80		29.86	31.58	37.04	
LSD (5%)	MR= 0.70	HD= 1.17	MRxHD= 1.21		MR= 1.34	HD= 1.47	MRxHD= 2.33	

Fresh herbage yield (t ha⁻¹)

The effects of mixture ratio, harvest date and two factor interaction were significant on fresh herbage yield for Persian clover + annual ryegrass mixture (Table 3). Highest fresh herbage yield was obtained from 100% Persian clover with 48.85 t ha⁻¹ at third harvest date (HD-III), in two years average (Table 3). However, the lowest

forage yield was found in 100% annual ryegrass plots, at first harvest date with 13.94 t ha⁻¹.

In our experiment, total fresh herbage yield increased by advancing harvest date. When the ratio of Persian clover in mixture increased, fresh herbage yield also increased in per unit area.

One of the most important factors effecting total fresh forage yield is the ratio of legume in the mixture. Fresh biomass production of pure legumes was higher than monocrop *Lolium multiflorum* plots due to larger habitus of the Persian clover. From the sowing ratios of 100:0 to 0:100 Persian clover:annual ryegrass mixtures, mean legume contribution to forage yield naturally dropped and it was also recorded that *Trifolium resupinatum* had a higher growth rhythm than *Lolium multiflorum* (Haynes, 1980). Hunady and Hochman (2014) reported that if the humidity is higher and therefore the growth of pea is intensive, then there is a risk of overlaying cereals by legume plants and thereby stopping further growth steps of cereals. However, Albayrak et al. (2004) found that forage yield increased with increasing legume ratios in mixtures with cereal and the highest fresh herbage yield was obtained from pure legume plots. Parallel with some previous studies in Mediterranean regions, our study showed similar results (Kokten et al., 2009; Budakli and Celik, 2014).

Two year averages showed that maximum fresh herbage yield was recorded at HD-III stage (35.91 t ha) followed by HD-II stage (22.82 t ha⁻¹) against the lowest at HD-I (19.67 t ha⁻¹). Harvest time and fertilizer application as a part of management strategies can affect dry matter yield and chemical composition and nutritive values of forage, thus these factors need to be considered when making management decisions. Harvest date, in another word, maturity stage of crop is the most important factor affecting forage quality. Plants continually changes in forage yield and quality as they mature. Physiologically plants cell wall content increases (Geren, 2014) and indigestible lignin accumulates which results in decreasing forage quality. Similar observations were reported by Anwar et al. (2010) and Geren (2014). This result were suggest that delaying harvesting time through HD-I stage might be useful for the highest yield.

Dry matter yield (t ha⁻¹)

Mixture ratio, harvest date and their interactions had significant effects on the dry matter yield of legume + grass mixture (Table 3). The highest dry matter yield (8.46 t ha⁻¹) was obtained from 100% *Trifolium resupinatum* at third harvest date, higher than other different mixture ratios in two years average (Table 3). However, the lowest dry matter yield (2.33 t ha⁻¹) was found in 100% *Lolium multiflorum* plots at first harvest date.

Dry matter yield linearly increased at later harvest time in the study and decreased in unit area as grass ratio increased in the mixtures.

Dry matter content of forage crops is one of the dependable criteria of biomass production, and high rate of dry matter content is mostly indicate a better adaptability and yield performance (Kavut and Avcioglu, 2015). Although the grass had higher dry matter content than legumes, the amount of dry matter yield produced per unit land area increased with increased legume ratio in the mixture due to the high fresh herbage yield of legumes in our study (Table 3). Similar results were reported by

Haynes (1980) indicating that legume have a higher temperature optimum than ryegrass and, thus a different seasonal high growth rate. On the other hand, Vasilakoglou and Dhima (2008) and Rahetlah et al., (2013) observed that dry matter yields decreased as the seed rate of legume in mixture increased. This finding indicated that dry matter accumulation was significantly different in all mixture ratios.

Despite the lower dry matter content of legume at the advanced maturity stage, the amount of dry matter yield produced per unit land area increased with delayed harvesting due to the high fresh herbage yield per land area. Thus, a better alternative might be to grow these legumes in pure stands. Two year averages showed that average dry matter yields of different harvest dates were 2.80, 4.21 and 7.28 t ha⁻¹, respectively. The data indicated that dry matter yield had similar trend as was observed in fresh forage yield. During plant maturation from HD-I to HD-III stage, dry matter content tended to increase. Similar information was declared by Anwar et al. (2010). The accumulated dry matter is largely dependent on the climatic conditions of the experimental areas (De Ruiter and Hanson, 2004). However, the effect was likely minimal since changes in nutrient density between the HD-I and HD-II stages appear to be relatively small. Turk and Albayrak (2012) reported that the DM yields linearly increased at later harvest stages and delaying of HD-III stage harvesting time might be useful to increase the dry matter yield.

Crude protein content (%)

The effects of mixture ratio, harvest date and two factor interaction were significant on crude protein content for Persian clover + annual ryegrass mixture (Table 3). Highest fresh herbage yield was obtained from 100% Persian clover with 17.37% at first harvest date (HD-I), in two years average (Table 3). However, the lowest crude protein content was found in 100% annual ryegrass plots, at third harvest date (HD-III) with 10.10%.

The content of crude protein is one of the most important parameter for forage quality evaluation (Ansar et al., 2010). The crude protein concentration of sole legume crop ranged between 10.10–17.37% in the study and the significantly higher crude protein concentration was observed in the pure legume plots compared to grass-legume mixtures. Pure *Lolium multiflorum* stands produced herbage with the lowest crude protein content. When the ratio of legume plant in the mixture increased, so did the crude protein content of that mixture also, and on the contrary as proportion of the grass increased in the mixture crude protein content significantly decreases (Karagic et al., 2011). The mean crude protein content for grass + legume mixtures observed in this study were found to be similar with the results of Lithourgidis et al. (2007); Kramany et al. (2012) and Kusvuran et al. (2014).

As seen in Table 3, the crude protein content decreased with advanced of the cutting stages, and the lowest crude protein content (12.29%) was obtained at third cutting

stage, whereas, crude protein content was the highest (14.64%) at first cutting stage.

All mixtures containing high rate of legumes has higher crude protein content in all cutting times. Similar results were obtained by Collar and Aksland (2001); Rebolé et al. (2004); Turk and Albayrak (2012) and they found that crude protein concentrations were higher for early harvest dates.

Crude protein yield (kg ha⁻¹)

The effects of mixture ratio, harvest date and two factor interactions were significant on crude protein yield for Persian clover + annual ryegrass mixture (Table 3). Monoculture Persian clover at third harvest date had the highest crude protein yield (125 kg ha⁻¹), in contrast, annual ryegrass monoculture had the lowest yield at first harvest date (29 kg ha⁻¹) in our study. As the growth stage of plants progressed, the crude protein content in the plant decreased, but the dry matter yield and crude protein yield also increased by progressing late harvest time, while crude protein yield also increased in unit area as legume ratio increased in the mixtures.

The total crude protein yield of intercrops was related to crude protein content in intercrops yield (Šarūnaitė et al., 2010). Sadeghpour et al. (2014) reported that the mixtures of barley with annual medics had higher crude protein yield than pure crop, and it can be concluded that the tested mixture rates and pure legume are significantly different in term of protein content depending on their genetic properties. Our findings are consistent with the results of many researchers reporting that the crude protein yield increases as the ratio of legumes in the mixture increases (Vasilakoglou and Dhima, 2008; Kusvuran et al., 2014).

Two year averages showed that average crude protein yields among harvest dates were 41, 58 and 90 kg ha⁻¹, respectively. As the crop developed from HD-I to HD-III stage, crude protein content decreased. Uzun and Asık (2011) reported that crude protein contents in legumes were higher than in cereals and declined as plants matured from the vegetative stage through the reproductive stages particularly after flowering. However, despite the lower crude protein content of grass-legume mixture at advanced maturity stage, the amount of protein produced per unit area increased with delayed harvesting due to the higher dry matter yield per unit area in the present study. These results also confirm by the findings of Turk and Albayrak (2012).

NDF content (%)

Mixture ratio, harvest date and their interactions had significant effects on the NDF content of legume + grass mixture (Table 3). The highest NDF content (52.93 %) was obtained from 100% *Lolium multiflorum* plots at third harvest date, higher than other different mixture ratios in two years average (Table 3). However, the lowest NDF content (38.04 %) was found in 100% *Trifolium resupinatum* plots at first harvest date.

The concentrations of neutral detergent fiber and acid detergent fiber which are affected by intercropping (Lithourgidis et al, 2007) are another important quality characteristic for forage. Grasses have higher cell wall concentrations and a more rapid accumulation of lignin and cellulose than legumes and thus a more rapid decline in digestibility with maturity (Tan and Mentese, 2003). The increasing legume proportion in forage is usually associated with an increase of cell contents and a decrease of cell walls and has a positive effect on its nutritive value (Haj-Ayed et al., 2000). Contreras-Gova et al. (2006) reported that forage quality in terms of NDF concentration was improved by wheat-clover intercropping compared with cereal pure crop. Pure legume crop performed better than the other treatments with regard to NDF content in this study. Generally, pure Persian clover and *annual ryegrass+Persian clover* mixtures had better performance than the pure annual ryegrass. NDF concentrations were affected positively by the increasing legume ratio in the mixture. Many research workers emphasized that pure cereal cropping is not a good option to feed livestock due to the lower quality and biomass yield. (Aasen et al., 2004; Karagic et al., 2011 and Kusvuran et al., 2014).

The two year average demonstrated that average NDF content among harvest dates were 40.22, 45.61 and 48.80%, respectively. In our study NDF contents of pure annual ryegrass and annual ryegrass mixtures with legume increased with delaying harvesting stage. It was also true for pure Persian clover sowings. Rebolé et al. (2004) reported that NDF and ADF contents increased from flowering to seed filling stages. Similar results in NDF content were stated by Turk et al. (2015) who declared that; the more delayed the date of harvest, the more increased NDF content was.

ADF (%)

Mixture ratio, harvest date and their interactions had significant effects on the ADF content of legume + grass mixture (Table 3). The highest ADF content (38.62 %) was obtained from pure annual ryegrass plots at third harvest date, higher than other different mixture ratios in two years average (Table 3). However, the lowest ADF content (25.38 %) was found in pure Persian clover plots at first harvest date.

ADF primarily represents cellulose and lignin and is often used to calculate digestibility of forage. In this study pure legume plant had lower ADF content than annual ryegrass and annual ryegrass mixtures with legume. ADF concentrations decreased as the legume proportion increased in the mixtures and similar results have also been reported by Karagic et al. (2011); Sadeghpour et al. (2014); Kusvuran et al. (2014).

Two year averages showed that average ADF content of different three harvest stages were 29.86%, 31.58% and 37.04%, respectively. ADF contents of pure and mixture sowing of plants increased from HD-I to HD-III stage. NDF and ADF rates continuously increased with delayed harvesting time on the country of crude protein. Similar results have been reported by some researchers studied

similar mixtures of cereals and legumes (Rebolé et al., 2004; Turk and Albayrak, 2012).

CONCLUSIONS

Average of 2 years results indicated that harvesting at late stages caused decreased in forage quality. Advanced harvest dates increased fresh herbage yield, dry matter yield, crude protein yield. It was increased ADF and NDF content of Persian clover- annual ryegrass mixtures but decreased crude protein content. The data also indicated that pure Persian clover sowing provided the highest green herbage, dry matter and crude protein yields, being 48.85 t ha⁻¹, 8.46 t ha⁻¹ and 125 kg ha⁻¹, respectively. We concluded that to get high hay yield under catch crop forage production in a Mediterranean environment, sole Persian clover can be sown in autumn and cut on the second half period of April or in late spring.

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