Yield and forage quality of red clover (*Trifolium pratense* L.) cultivars in the lowland and the mountain regions

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ABSTRACT

Six *Trifolium pratense* L. cultivars, five diploid and one tetraploid, were grown in the lowland region (123 m altitude) and the mountain region (650 m altitude). Dry matter (d.m.) and green mass (g.m.) yield, stem height and leaf proportion were assessed from each of the three growing seasons. Chemical composition was assessed from the average samples of all cuts in the second year of the experiment. Greater green mass (54.14 t/ha) and dry matter yield (9.86 t/ha), stem height (0.61 m), crude protein (157.6 g/kg), crude fiber (222.9 g/kg), crude ash (100.68 g/kg) and crude fat (30.09 g/kg) content were observed in the mountain region, compared to the lowland region (45.61 t/ha g.m. 8.92 t/ha d.m. 0.59 stem height, 156.38 g/kg crude protein, 216.6 g/kg crude fiber, 94.85 g/kg crude ash, 24.98 g/kg crude fat). The greater leaf proportion (47.2%) and nitrogen free extract content (420.13 g/kg) were observed in the lowland region compared to the mountain region (42.95% and 402.99 g/kg, respectively). A significant location × cultivar interaction was found for all investigated parameters except for leaf proportion.

Keywords: *Trifolium pratense* L.; yield; leaf proportion; stem height; chemical composition

Red clover (*Trifolium pratense* L.) is one of the most important herbage legume in temperate agriculture. Its success has been due to its adaptation to a wide range of soil and environmental conditions, molecular nitrogen (N_2)-fixing ability and high nutritive value for ruminants. The cause of yield variation and quality differences of *Trifolium pratense* L. cultivars in various environmental conditions are different climatic characteristics of each individual area, primarily precipitation and temperatures, different ploidity degree of individual cultivars and a different growth rhythm (Užík and Mištinová 1978b, Graman 1988). While comparing the yield of *Trifolium pratense* L. cultivars at different altitudes it has been determined that the red clover yield primarily depends on humidity within the research area. Therefore, higher yields of *Trifolium pratense* L. were achieved at altitudes of 500–700 m in comparison with altitudes of 160–360 m (Užík and Mištinová 1978a). While Gospodarczik (1985), Ježikova and Michalec (1988), Gospodarczik and Nowak (1989) have recorded greater *Trifolium pratense* L. yield in the lowland region and altitudes 400–500 m, in comparison with altitudes above 700 m. With increasing altitude, the feeding quality of forage improved due to a decreased content of crude fibers, lignin and cellulose (Caputa 1966). Knežević et al. (1997) have determined higher degradability of *Trifolium pratense* L. cultivars cultivated in the mountain region (650 m altitude), than those cultivated in the lowland (123 m altitude). Our objectives were: a) to evaluate the productivity and quality of six *Trifolium pratense* L. cultivars grown in the lowland and the mountain region, b) to determine interaction between cultivars and locations regarding characteristics investigated.

MATERIAL AND METHODS

The experiment was conducted in north-western Croatia, during three years (1995–1998), at two Faculty of Agriculture experimental fields: Zagreb – alluvium-colluvium's brown soil (123 m altitude – lowland, 836 mm average annual precipitation, 10.3°C mean annual temperature) and Medvednica – brown acid soil (650 m altitude – mountain region, 1230 mm average annual precipitation, 6.6°C mean annual temperature). Five diploid *Trifolium pratense* L. cultivars (Croatia, K-17, Reichersberger, Marino, Viola) and one tetraploid cultivar (Nada) were sown on May 1995. At the time of seeding the experimental areas received N, P and K at the rate of 40, 130 and 130 kg/ha, respectively. No further N was applied and the fertilizer application over next two years was 130 kg P/ha and 130 kg K/ha. The seeding rate for all cultivars was 18 kg/ha. The experimental design was a randomised complete block with six replications. The plot size was 5 × 2.5 m with an effective harvest area of 4 × 1.5 m after allowing for discarded borders. The plots were cut three times in the first year, four times in the second, and once in the third year at a height of 4 cm, when red clover plants reached 10% flowering. Before each

cut, a random sample of 20 stems was collected from each plot for stem height determination. At each harvest the fresh herbage from each plot was weighed in the field and a sample of 1 kg was randomly taken and stored in a polythene bag. In the laboratory a 500 g subsample was weighed out and dried to constant weight in an oven controlled at 105°C and amount of dry matter (d.m.) harvested was determined. A further 500 g subsample of the fresh herbage was separated into leaves and stems + flowers to determine the leaf proportion in the fresh herbage. From each plot and experimental field average samples of all cuts in 1996 were made to determine content of crude protein, crude fiber, crude ash, crude fat and nitrogen free extract (samples dried at 60°C for 48 hours).

Mean monthly temperatures and precipitations at two experimental fields (Zagreb and Medvednica) are presented in Table 1.

RESULTS AND DISCUSSION

The cultivar reaction to different agro-ecological conditions is interpreted as a manifestation of the modification effects of the elevation factor, the most significant of which are the weather conditions (temperature and rainfall) (Graman 1988). Cultivars grown in the mountain region had higher green mass yield (18.7%) and dry matter yield (10.5%) compared to the lowland region ($P < 0.01$, Table 3). The yield increase may be explained by moderate temperatures, higher annual precipitation, and better moisture distribution over the growth season at the mountain region. Greater yield (more than 30%) at the mountain region in comparison with the lowland region has been reported by Užík (1975), Užík and Mištinová (1978a), Graman (1988). There were no significant differences between cultivars in the lowland region in green mass and dry matter yield, but in the mountain region significant differences in green mass and dry matter yield were found. The greatest green mass yield occurred with cv. Nada (62.36 t/ha) and greatest dry matter yield with cvs. Nada (10.76 t/ha) and Croatia (10.22 t/ha). A significant location × cultivar interaction was found for green mass and dry matter yield (Table 2), which indicates that cultivars responded differently under various ecological conditions. Thus, all cultivars except cv. K-17 had a higher green mass yield in the mountain region compared to the lowland, whereas greater dry matter yield in the mountain region compared to the lowland region were achieved with cvs. Croatia, Nada and Reichersberger. Higher altitude mostly increased the productivity of tetraploid cv. Nada, which had a 36% higher green mass yield and a 26% higher dry matter yield in the mountain region

Table 1. Precipitation and temperature data (April–October) in the lowland region (Zagreb) and the highland region (Medvednica), for the study period (1995–1997), with long-term (1963–1992) average

Month	Precipitation (mm)							Mean temperature (°C)						
	Zagreb				Medvednica			Zagreb				Medvednica		
	1995	1996	1997	1963–1992	1995	1996	1997	1963–1992	1995	1996	1997	1963–1992	1995	1996	1997	1963–1992
4.	85.9	49.6	67.2	59.0	135.5	116.2	83.2	87.2	10.9	8.2	12.8	10.5	5.8	2.6	7.0	5.5
5.	76.2	94.9	72.1	75.0	132.3	114.5	102.8	102.4	15.5	17.1	17.2	15.3	9.8	11.3	11.6	10.5
6.	94.2	62.6	87.1	98.0	191.3	58.4	90.3	153.8	18.1	20.4	20.1	18.6	12.2	14.6	14.4	13.2
7.	88.3	69.3	80.7	79.0	166.0	63.2	102.1	97.1	23.0	19.4	20.6	20.4	17.5	14.0	14.9	16.0
8.	172.4	14.3	57.8	96.0	269.4	183.9	72.6	109.9	19.6	20.0	20.6	19.5	14.5	14.6	15.6	15.8
9.	161.4	175.8	21.9	78.0	235.4	234.4	27.9	108.1	15.1	13.3	16.5	15.8	10.1	8.0	12.2	12.4
10.	5.6	60.7	51.4	74.0	3.7	93.3	40.0	120.6	12.1	11.7	9.7	10.4	9.6	7.1	5.2	7.4

Table 2. Combined analysis of variance for green mass yield, dry matter yield, stem hight and leaf proportion of *Trifolium pratense* L. cultivars (Zagreb and Medvednica, 1995–1997)

Source of variation	Green mass yield	Dry matter yield	Stem hight	Leaf proportion
Location (L)	**	**	*	**
Growing season (Y)	**	**	**	**
L × Y	**	*	*	**
Cultivar (C)	**	*	**	**
L × C	**	*	*	ns
Y × C	ns	**	**	**
L × Y × C	*	ns	**	ns

*significant at the 0.05 level, **significant at the 0.01 level

compared to the lowland region. In more humid climate conditions tetraploid red clover cultivars had 10–60% higher green mass yield compared to diploid cultivars (Wolffhardt 1972, Polak et al. 1973, Zosimovič et al. 1975). The differences between diploid and tetraploid cultivars in dry matter yield are less then 10% due to the higher content of water in poliploid cultivars (Wolffhardt 1972, 1973). Under dry conditions or unfavourable distribution of precipitation, tetraploid cultivars do not even reach the level of diploid cultivars, which may be explained by their greater moisture demand (Užík 1975, Užík and Mištinová 1978a, Graman 1988). This research confirms previous results, because the highest variation in yield occurred with tetraploid cv. Nada. During the season with optimal humidity and moderate temperatures (1st cut) the highest green mass and dry matter yield was achieved with cv. Nada. In the first half of June 1996 the extremely warm and dry weather caused slow re-growth of this cultivar and the lowest yield among all explored cultivars (no data).

The mean stem height was significantly higher in the mountain region ($P < 0.05$, Table 3). Differences in stem height between cultivars occurred within each location. Cvs. Nada, Croatia and Viola had the greatest stem height in the lowland region (Table 3), while there were no differences between

Table 3. Average green mass yield, dry matter yield, stem hight and leaf proportion of red clover (*Trifolium pratense* L.) cultivars (Zagreb and Medvednica, 1995–1997)

	Green mass yield (t/ha)		Dry matter yield (t/ha)		Stem hight (m)		Leaf proportion (%)	
	Zagreb	Medvednica	Zagreb	Medvednica	Zagreb	Medvednica	Zagreb	Medvednica
Croatia	47.04	56.99	9.19	10.22	0.597	0.623	47.74	42.43
Nada	46.67	62.36	8.54	10.76	0.570	0.626	51.50	49.98
Reichersberger	43.65	52.87	8.71	9.70	0.582	0.608	46.36	41.55
K-17	48.36	52.34	9.56	9.85	0.619	0.621	43.43	39.49
Marino	43.56	49.09	8.66	9.11	0.575	0.597	46.60	42.48
Viola	44.38	51.22	8.86	9.53	0.597	0.601	47.70	41.78
Mean	45.61	54.14**	8.92	9.86**	0.590	0.613*	47.22**	42.95
LSD(0.05)[a]	4.05		0.78		0.0237		ns	
LSD(0.05)[b]	5.06		0.88		0.0265			

[a]*LSD* values for comparing means within locations, [b]*LSD* values for comparing means across locations, ns = not significant for location × cultivar, *significant at 0.05 probability level by *F*-test, **significant at 0.01 probability level by *F*-test

other cultivars and cvs. Croatia and Viola. In the mountain region the largest stem heights were found with cvs. Nada, Croatia and Viola, among which there were no significant differences. A significant location × cultivar interaction ($P < 0.05$, Table 2) indicated that all cultivars responded with greater stem height in the mountain region (Table 3). Cv. Nada had the greatest response on increased altitude (9.7% higher stem at the highland) due to more humid climate of that region. This result is consistent with the findings of Užík and Mištinová (1978b) and Graman and Sakova (1988) who reported that tetraploid cultivars have increased stem height but only in the conditions of the favourable soil humidity.

The digestibility of *Trifolium pratense* L. primary growth declines with advancing maturity in an approximately linear fashion and is typically related to the declining leaf proportion (declining leaf/stem ratio) (Frame et al. 1998). The mean leaf proportion of all cultivars was higher in the lowland region (47.22%) compared to the mountain region (42.95%) ($P < 0.01$, Table 3). This confirms the results of Užík (1975), Užík and Mištinová (1978b) who also found higher leaf proportion in the lowland (160 m altitude) for all investigated red clover cultivars. Increased leaf proportion in the lowland has been probably due to smaller plant heights, which shifts leaf/stem ratio in favour of leaf. The largest mean leaf proportion during all growing seasons and locations was observed for tetraploid cv. Nada (50.74%) and the smallest for cv. K-17 (41.46%), which accords with results of Užík (1975) who reported about 10–30% higher leaf proportion for tetraploid cultivars compared with diploid in a humid year, and 6–10% in a dry year. A cultivar × location interaction was not significant for leaf proportion (Table 2), which indicates that the cultivars do not respond differently under various ecological conditions.

It is well recognized that the chemical composition of herbage species is influenced by factors such as the stage of growth, season of year, plant part sampled, soil-nutrient status and soil pH. The differences between cultivars in chemical composition were observed within and between locations ($P < 0.01$). A significant location × cultivar interaction ($P < 0.01$) for all forage quality parameters indicated that environmental conditions had a substantial influence on cultivar performance (Table 4). All cultivars had a higher crude fat content, a higher or equal crude ash and lower nitrogen free extract (except cv. Marino) in the mountain region compared to those in the lowland region. Cvs. Nada, Reichersberger and Viola had a higher crude protein in the highland region compared to the lowland region, whereas cvs. Nada and Marino had a lower crude fiber

Table 4. Chemical composition of red clover (*Trifolium pratense* L.) cultivars (Zagreb and Medvednica, 1996)

	Crude ash (g/kg)		Crude protein (g/kg)		Crude fat (g/kg)		Crude fiber (g/kg)		Nitrogen free extract (g/kg)	
	Zagreb	Medvednica	Zagreb	Medvednica	Zagreb	Medvednica	Zagreb	Medvednica	Zagreb	Medvednica
Croatia	95.42	104.03	164.50	159.38	24.43	29.77	192.93	209.28	440.03	409.88
Nada	101.92	101.60	154.68	158.98	27.08	34.33	221.87	213.83	406.77	404.73
Reichersberger	96.75	102.40	157.02	161.55	27.42	29.92	216.97	234.38	415.35	384.67
K-17	92.48	92.32	161.57	158.23	26.02	28.00	207.60	225.48	431.83	415.02
Marino	92.40	101.58	159.33	150.92	21.52	28.10	240.35	222.42	405.50	411.25
Viola	90.15	102.15	141.20	156.65	23.38	30.45	220.03	232.23	421.28	392.42
Mean	94.85	100.68**	156.38	157.62**	24.98	30.09**	216.63	222.94**	420.13**	402.99
LSD(0.01)[a]	0.517		0.398		0.248		0.195		0.209	
LSD(0.01)[b]	0.517		0.398		0.250		0.210		0.218	
Location (L)	**		**		**		**		**	
Cultivar (C)	**		**		**		**		**	
L × C	**		**		**		**		**	

[a]*LSD* values for comparing means within locations, [b]*LSD* values for comparing means across locations, **significant at 0.01 probability level by *F*-test

content in the highland compared to the lowland region (Table 4). The average of the following parameters were significantly higher ($P < 0.01$) in the mountain region compared to the lowland region: crude ash (6.2%), crude protein (0.8%), crude fat (20.4%) and crude fiber (3%). Only nitrogen free extract was found to be lower (4%) in the mountain region compared to the lowland region. Graman (1988) found a 13–18% decrease for crude protein and a 13–21% decrease for nitrogen free extract of red clover grown at 600–680 m altitude in comparison with red clover grown at 380 m altitude with even a more expressive decrease at an altitude of 780–800 m. At both locations the highest crude protein was found with cv. Croatia (164.5 g/kg) and the lowest with cv. Viola (141.2 g/kg). The highest crude fiber content at both locations was found with cv. Marino (240.35 g/kg) and the lowest with cv. Croatia (192.93 g/kg).

Achieved results indicate the importance of choosing red clover cultivars for various environmental conditions due to the significant cultivar × location interaction for all parameters examined (except for leaf proportion). The yield achieved and the quality of red clover cultivars (primarily high crude protein content, low crude fiber content and high leaf proportion) demonstrate that cv. Nada and cv. Croatia have the best potential for growth under agro-ecological conditions that are similar to the conditions as found in this research.

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ABSTRAKT

Výnos a kvalita píce odrůd jetele lučního (*Trifolium pratense* L.) v nížinných a horských oblastech

Pět diploidních a jedna tetraploidní odrůda *Trifolium pratense* L. byly pěstovány v nížinné (123 m n. m.) a v horské (650 m n. m.) oblasti. Byl hodnocen výnos sušiny a zelené hmoty, výška lodyh a podíl listů v průběhu tří let vegetace. Ve druhém roce vegetace bylo hodnoceno chemické složení píce v průměrném vzorku za všechny seče. Byl zaznamenán vyšší výnos zelené hmoty (54,14 t/ha), sušiny (9,86 t/ha), výška lodyh (0,61 m), obsah celkového proteinu (157,6 g/kg), celkové vlákniny (222,9 g/kg), celkových popelovin (100,68 g/kg) a celkového tuku (30,09 g/kg) v horské oblasti ve srovnání s oblastí nížinnou (45,61 t/ha zelená hmota, 8,92 t/ha sušina, 0,59 m výška lodyh, 156,38 g/kg

celkový protein, 216,6 g/kg celková vláknina, 94,85 g/kg celkové popeloviny, 24,98 g/kg celkový tuk) a naopak vyšší podíl listů (47,2%) a obsah bezdusíkatých látek výtažkových (420,13 g/kg) v nížinné oblasti ve srovnání s oblastí horskou (42,95% a 402,99 g/kg). Průkazné interakce mezi faktorem polohy a odrůdy byly nalezeny u všech sledovaných parametrů vyjma podílu listů.

Klíčová slova: *Trifolium pratense* L.; výnos; podíl listů; výška lodyh; chemické složení

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