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# PERSISTENCE ASSESSMENT OF RED CLOVER (*Trifolium pratense* L.) IN TÂRGOVIŞTE PLAIN

# EVALUAREA PERSISTENŢEI TRIFOIULUI ROŞU (*Trifolium pratense* L.) ÎN CÂMPIA PIEMONTANĂ A TÂRGOVIŞTEI

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*Since the most important deficiency of forage stands is the inability to maintain adequate legume participation in mixture, it is the purpose of this paper to examine persistence in red clover in Târgovişte Plain eco-climatic conditions, together with the factors that affect it. Six red clover cultivars (Napoca-Tetra, Dacia Tetra, Vesna – tetraploids; Flora, Roxana, Start – diploids) and one white clover diploid cultivar (Karina) were used in pure culture and in mixture (50:50) with hybrid ryegrass (Zefir – tetraploid) in a randomized block design with three replicates. Ground cover assessment in early spring was a suggestive indicator of the stand persistence to define the stability and sustainability boundaries of a reliable intensive system. In the beginning of the third year of cropping, ground cover was 54.33% for tetraploid cultivars (CV = 43.25%), and 67% for diploid cultivars (CV = 6.83%) in pure stands. Same ground cover average of 27% was established both for tetraploid cultivars (CV = 36.47%), and for diploid cultivars (CV = 16.97%) in mixtures with hybrid ryegrass.*

**Key words**: red clover, white clover, hybrid ryegrass, ground cover, persistence

## Introduction

Perennial plant through its biological nature, red clover is widely cultivated in diverse ecological conditions and in a variety of cropping systems. Red clover has continuously diversified under natural and artificial selections from annual cultivars to 4-5 years perennial cultivars. It often behaves as a biennial plant, being generally considered to be lacking in persistence. Persistence of red clover may be defined as the ability to maintain a stand over a long period of time without depending upon reseeding or volunteering (Taylor and Quesenberry, 1996).

The longevity or red clover *life span* has been the subject along with that of other legumes of considerable speculation (Smith and Kretschmer, 1988) primarily concerned with means of increasing or maintaining legumes in mixtures with grasses for hay, silage or pasture.

Since the most important deficiency of forage stands is the inability to maintain adequate legume participation in mixture, it is the purpose of this paper to examine persistence in red clover in Târgovişte Plain eco-climatic conditions, together with the factors that affect it. The overall aim of this work was to perform a detailed analysis of red clover in pure culture and as a component of a grass-clover mixture. Red clover soled crop (three diploids and three tetraploid cultivars), and hybrid ryegrass intercropping 50-50% were used in order to define the stability and sustainability boundaries of a reliable intensive system that might increase the persistence traits. Experimental trials were performed between 2004 and 2006 in Târgovişte Plain (Dobra) - Romania.

***Persistence characterization***

It is axiomatic that to be persistent, a crop must be well adapted to its environment. Red clover persistence is the result of an interaction between its adaptation and its stress load (Taylor and Quesenberry, 1996). Stress load may be defined as any factor, whether physiogenic or pathogenic, that affects the growth and development of the species, and consequently the maintenance of a closed canopy. Environmental stresses such as interspecific or/and intraspecific competition, winter hazards, management practices, and pathogens, are seen as reducing the vigor of the forage legume plants. Weakened plants are then susceptible to damage from other pathogens which increases *root rot* phenomenon that leads to shortened persistence through senescence occurrence and finally, to lose the forage stand.

Persistence, then, is determined by the species eco-physiological characteristics, the perennial ecotypes traits, the environmental influence and the grassland applied technologies. The most negative factors that diminish the clover persistence are as follows: improper harvesting performed before pre-bloom stage, late autumn harvesting, low temperatures and spring drought, and root rot phenomenon. It has been suggested that root rots in forage legumes may be an unavoidable disease (Rufelt, 1982).

Persistence will lead to optimal plant density forage crops, which might be also positively influenced through phosphorous and potassium fertilization and through the avoidance of late autumn cutting or grazing (C. Bărbulescu *et al.*, 1991). In the context of environmental protection, the maximization of the economical benefits in clover-grass agro-ecosystems depends on the precise estimation of the factors that insures the clover persistence in the next cropping years. Such factors that provide the rational use of the clover crops need to take into account the seeding date, the cutting frequencies and periods (harvest management), the rainfall distribution within vegetation cycle, cultivars' traits and the insurance of optimal carbohydrates reserves before winter occurs (Dunea D., 2006). A detailed overview of the red clover persistence and approaches to the development of greater persistence can be found in Taylor and Quesenberry, 1996.

***Previous cultivar trials for persistence assessment***

Occasionally, stands of red clover are reported to last 9 or more years, but invariably, when seed from such a field is sown in cultivar trials, the clover persists only 3 to 4 years (Fergus and Hollowell, 1960). A cultivar trial was performed in Belgium in two locations with different eco-climatic conditions using 8 red clover cultivars (5 *Ackerklee* and 3 *Mattenklee* cultivars) in mixtures with grasses (Lehmann *et al.*, 1998). Experimental results provided a persistence ranking of the cultivars confirming that red clover Swiss types (*Mattenklee* cultivars) such as Ruttinova, Temara and Vanessa were the most persistent and productive (dry matter, energy and protein content). In the first cropping year (1996), the *Mattenklee* cultivars had a slower growth rate as compared to *Ackerklee* types. However, the ground cover was superior in the next years, Ruttinova cultivar being the most persistent in both locations. After 3 years of cropping (1999), the ground cover was 64% compared to 38% (average of *Ackerklee* types) in Corroy-le-Grand location, and 40% compared to 11% in Michamps site. Some *Ackerklee* cultivars (Barfiola, Verdi, Diper and Kvarta) almost lost their stands due to senescence in Michamps.

Due to their superior characteristics, *Mattenklee* cultivars need to be tested for phenological plasticity in the clover-growing regions of Romania to improve the existent national cultivar list. In Cluj-Napoca, a cultivar trial was performed using 45 diploid and tetraploid cultivars to test the variability of the morphological and physiological characteristics and their implications on the persistence, the forage yield and the forage quality. Based on the experimental results, it was recommended to use cultivars from Center and Western Europe (*subvar. intermedium*) as genitors for green fodder production amelioration. Renova and Ruttinova (*Mattenklee* cultivars) have been remarked from this category for superior precocity and regeneration after cutting (Muntean L. and E. Tămaş, 2005).

Any breeding program that increases adaptation in red clover should increase persistence within the limits imposed by plant's life cycle. Persistence improvement in red clover depends on the individual selection considering the root rot vectors resistance. The most efficient method is to expose the plants to the environmental conditions and to select for survival. Breeding programs to increase persistence have been successful leading to clover cultivars that live usually up to 3 or 4 years in most countries, and occasionally longer if the stress load is not severe (Anderson *et al.*, 1974). Examples that may be cited include Vesna cultivar from DLF Trifolium, Merkur in Sweden, the cultivars Hokuski and Tarsetsu in Japan, Arlington, Marathon in the northern red clover belt and Kenland and Kenstar in the southern part of the belt in U.S., as well as the new Mattenklee Swiss cultivars such as Milvus, Formica and Corvus. New cultivars must be distinct, homogeneous, with increased stability and superior agronomical and technological values (Moga I. *et al.*, 1996). The valuable characteristics of the red clover (productivity, forage quality and ecological plasticity) are induced by its morphology and eco-physiology, which are correlated with the genetics of persistence, and with the environmental conditions.

## Materials and Methods

The experiment was carried out on plots in Târgovişte Plain (Dobra: N 44°46¹.905, E 25°43¹.045, 179-m altitude) in 2004. Six red clover cultivars (*Napoca-Tetra*, *Dacia Tetra*, *Vesna* – tetraploids; *Flora*, *Roxana*, *Start* – diploids) were used in pure culture and in mixture (50:50) with hybrid ryegrass (*Zefir* – tetraploid) in a randomized block design with three replicates. One white clover diploid cultivar (*Karina*) was also used in pure culture and in mixture. The plots were sown on April 24, 2004. Climatic data of the 2004 vegetation season showed a rainfall regime closed to the normal of the region (205.4 mm in March-May, 213.7 mm in June-July and 111.4 mm in August-September). In comparison with 2004, the next year annual rainfall sum exceeded 1000 mm. The multi-annual average recorded in Targoviste Plain between 1995 and 2004 was 474.69 mm. Rainfalls of the 2005 vegetation season were significantly higher than in 2004: 308.8 mm in March-May, 283.7 mm in June-July and 448.7 mm in August-September. Annual average temperature (9.3°C) in 2005 was the lowest in entire 1995-2005 interval. The soil was pseudogleic brown alluvial. In the first year, the plants were given nitrogen fertilizer in all experimental variants at one rate (40 kg N $ha^{-1}$) to avoid nutrient limiting growth. In the second year (2005), foliar fertilization ($N_{15}P_5K_{30}$+3MgO) was applied six times during the vegetation season. Starter doses of simple super-phosphate (16-22 % $P_2O_5$) were given in all variants at one rate. Three forage cuttings were performed each year (in 2004 and in 2005).

## Results and Discussions

Many European researchers have reported superior persistence and forage productivity for tetraploid cultivars in comparison with diploids (Tomaszewski, 1989; Jonsson, 1985; Savatti, 1990 etc. cited by Taylor and Quesenberry, 1996). Experimental results from U.S. have pointed out that diploids performed better than tetraploids considering abovementioned parameters (Taylor and Wiseman, 1985). However, both genotypes detain valuable characteristics, expressing their biological efficiency potential depending on the eco-climatic and soil conditions of the crop location. From this point of view, both diploid and tetraploid forms were included in this experiment to assess their persistence in Târgovişte Foothill Plain. In grass-clover mixtures, a closed and persistent stand with sufficient legume participation might be obtained when the *dual stability thresholds* (optimal light, water and mineral elements resources requirements) are identified for both components: accompanying grass or grasses – red clover. Crop practices must be adapted according to these identified thresholds (Dunea D., 2002). Ground cover assessment in early spring was a good and suggestive indicator of the clover stand persistence, being expressed as percentage (%). Red clover cultivars showed contrasting behaviors concerning persistence both in pure cultures (fig.1) and in mixtures with hybrid ryegrass.

Fig. 1 Persistence reduction of the red clover pure cultures in Târgovişte Plain eco-climatic conditions – second year of *Vesna* cultivar cropping, spring, 2005 (left); third year of *Napoca-Tetra* cultivar cropping, spring, 2006 (right).

Tetraploids showed in pure cultures a higher variation of the ground cover (*CV* = 29.14%) recorded after two winters, from an average of 48% in Napoca-Tetra cultivar stands to 86% in Vesna cultivar.

Fig. 2 Relative stability of the ground cover in red clover-hybrid ryegrass mixture assessed in spring (19.03.2006) – third year of cropping.

In terms of persistence, diploid cultivars were relatively constant throughout the same period (*CV* = 4.74%) presenting an average of 74.83% compared to 65.66% ground cover in tetraploids (table 1).

White clover (Karina cultivar) showed better persistence characteristics than red clover with 88.5% ground cover after two years of cropping. This species did not present significant fluctuations of ground cover and the stand reduction that affected all red clover cultivars (fig.3).

Fig. 3 Persistence stability in the white clover pure culture in Târgovişte Plain eco-climatic conditions – second year of *Karina* cultivar cropping, spring, 2005 (left); third year of *Karina* cultivar cropping, spring, 2006 (right).

In the 2005-2006 winter, some major factors that have contributed to the red clover persistence reduction were the very low temperatures and the lacking of snow cover in these periods. Winter killing occurred in the less adapted cultivars (Napoca-Tetra, Dacia-Tetra and Roxana).

In the beginning of the third year of cropping (spring 2006), ground cover recorded 54.33% for tetraploid cultivars (*CV* = 43.25%), and 67% for diploid cultivars (*CV* = 6.83%) – fig.4.

Table 1 Ground cover (%) evolution of the red clover cultivars and white clover in pure cultures in spring (2005 and 2006)

| Cultivar | Napoca-Tetra | Dacia Tetra | Vesna | Roxana | Flora | Start | Karina *T.repens* |
|---|---|---|---|---|---|---|---|
| ***Ploidy*** | ***4n*** | ***4n*** | ***4n*** | ***2n*** | ***2n*** | ***2n*** | ***2n*** |
| $2^{nd}$ year | 65 | 72 | 94 | 79 | 85 | 84 | 96 |
| $3^{rd}$ year | 31 | 54 | 78 | 63 | 66 | 72 | 81 |
| Average | 48 | 63 | 86 | 71 | 75.5 | 78 | 88.5 |
| Group average | **65.66** | | | **74.83** | | | **88.5** |

Tetraploids showed similar persistence fluctuations in clover-grass mixtures (50:50 participation ratio), accentuated by the high concurrence capacity for resources of the hybrid ryegrass (Zefir tetraploid cultivar) – table 2. Hybrid ryegrass contributed to a closed stand insuring the relative stability of the ground cover (fig.2). Grass species has occupied the empty spaces that were observed in red clover pure cultures. Vesna cultivar was the most persistent within clover cultivars, showing an average of 45% ground cover in the beginning of the third cropping year. Start cultivar was the most persistent within diploid group with an average of 31%. The less persistent cultivar was Napoca-Tetra with 21.5% ground cover – fig.5. In the beginning of the third cropping year (spring 2006), the same ground cover average of 27% was recorded both for tetraploid cultivars ($CV$ = 36.47%), and for diploid cultivars ($CV$ = 16.97%).

Hybrid ryegrass was very competitive in mixture with white clover determining a drastic reduction of the clover component in the mixed canopy (near to 18% ground cover).

Table 2. Ground cover (%) evolution of the red clover cultivars and white clover in mixture (50:50) with hybrid ryegrass (*Zefir* tetraploid cultivar) in spring (2005 and 2006).

| Cultivar | Napoca-Tetra | Dacia Tetra | Vesna | Roxana | Flora | Start | Karina *T.repens* |
|---|---|---|---|---|---|---|---|
| ***Ploidy*** | ***4n*** | ***4n*** | ***4n*** | ***2n*** | ***2n*** | ***2n*** | ***2n*** |
| $2^{nd}$ year | 24 | 39 | 52 | 41 | 44 | 48 | 22 |
| $3^{rd}$ year | 19 | 24 | 38 | 22 | 28 | 31 | 14 |
| Average | 21.5 | 31.5 | 45 | 31.5 | 36 | 39.5 | 18 |
| Group average | **32.66** | | | **35.66** | | | **18** |

Consequently, the mixtures showed better ground cover than the red clover pure stands, with a superior contribution of the hybrid ryegrass that insured a closed stand and maintained clover component in acceptable limits.

A red clover cultivars' ranking was assessed by combining their persistence characteristics observed in pure cultures and in mixtures, as follows:


*Ranking of the red clover cultivars based on the multi-annual persistence in pure cultures and hybrid ryegrass mixtures in Târgovişte Plain eco-climatic conditions*

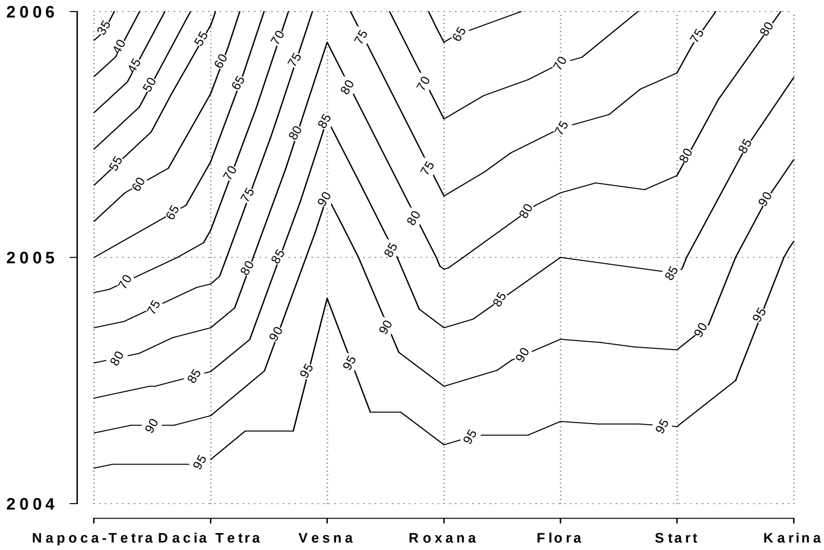


Fig. 4 Ground cover (%) evolution of the clover species in pure cultures in Târgovişte Plain eco-climatic conditions (2004-2006) – *Karina: white clover cultivar.*

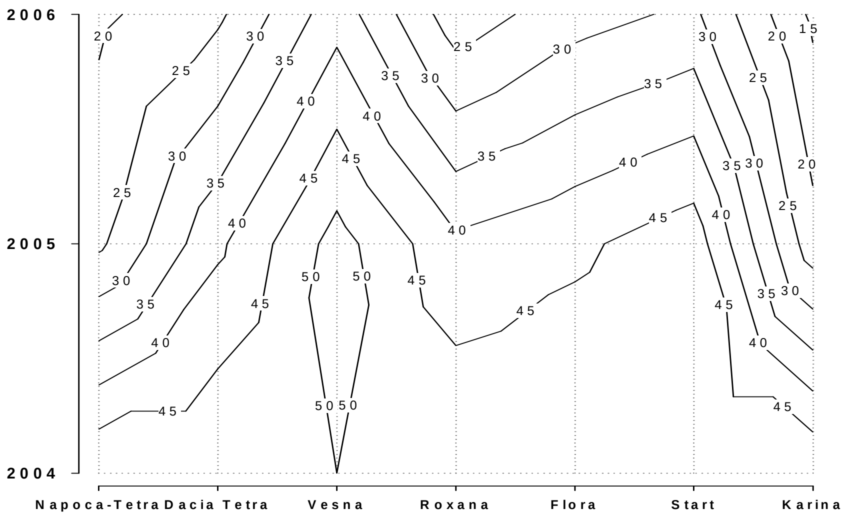


Fig. 5 Ground cover (%) evolution of the clover species in binary mixtures with hybrid ryegrass (50:50) in Târgovişte Plain eco-climatic conditions (2004-2006) – *Karina: white clover cultivar.*

## Conclusions

The main objective of this work was to assess the persistence of red clover (*Trifolium pratense* L.) and white clover (*Trifolium repens* L.) in two cropping systems: pure cultures and mixtures with hybrid ryegrass (*Lolium hybridum* Hausskn.) during three years of growth in Târgovişte Plain eco-climatic conditions.

In red clover, diploid cultivars showed increased persistence characteristics, homogeneity between cultivars and a good adaptation to the environment both in pure stands and in mixtures. Ground cover stability was less evident in tetraploids.
White clover was more efficient in pure culture. Its association with hybrid ryegrass did not provide positive aspects that might recommend such forage system. The most persistent cultivar was Vesna in both variants. Start cultivar showed the best multi-annual persistence from the diploid group. Napoca-Tetra was the most affected cultivar in terms of ground cover and winter killing, being one of the oldest varieties used in Romania.

Romanian Flora diploid cultivar presented good performances from the persistence point of view. This behavior recommends Flora cultivar for use in developing forage systems in similar eco-climatic regions with Târgovişte Plain.

Consequences for overall productivity and quality of the fodder should be also known for the selection of the most appropriate grass species and cultivar to be added to the system. The quest is on to comprise intrinsic elements of red clover-grass forage system in a holistic approach in order to characterize the *genotype - environment - management* interaction within such forage system.

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