Ecophysiologica Responses of White Clover - Hybrid Ryegrass Mixture to Foliar Fertilisation

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Abstract

The rationale of the study was to check whether the association of hybrid ryegrass with white clover owing a moderate competition capacity, and optimal foliar fertilization will provide high and stable yields by reducing conventional fertilization rates. The experiment was carried out during two years of cropping with six growth cycles in a randomized block design with three replicates in Targoviste Piedmont Plain, South of Romania. In the first year, the plots received no nitrogen fertilisers in order to allow the biological potential development. In the second year, foliar fertilisation was applied six times in the fertilised variants during the vegetation season. The comparison between the six growth cycles showed that the biological potential of white clover in pure stand without foliar fertilisation was higher in the first cropping year than in the second one. Application of foliar fertilisation treatments enhanced the competition capacity of the hybrid ryegrass in the first production cycle of the second year. In white clover pure stand, the fertilisation increased the assimilatory areas from 3.6 to 4.8 (p<0.05). The contribution of hybrid ryegrass to the cumulative Leaf Area Index of species in the binary mixture was significant (4.7 no fertilisation; 4.9 foliar fertilisation), which revealed a low competition capacity of white clover in mixture (0.69 no fertilisation; 1.03 foliar fertilisation). In white clover-hybrid ryegrass mixtures, foliar fertilisation showed benefits in the first two growth cycles according to the phenophase particularities and weather conditions.

Keywords: leaf measurements, grass/clover stands, herbage yield, plant competition, radiation use efficiency

Introduction

In pure stand, the high yielding species of grasses such as hybrid ryegrass, orchard grass or tall fescue consume important quantities of nitrogen along with other nutrients. The use of conventional fertilizers ensures a proper balance for obtaining the envisaged forage yield gain and for maintaining the equilibrium of nutrients in soil (Zheng et al., 2013). However, the use of substantial fertilizer inputs in intensive forage systems has often determined negative impacts on the groundwater (Stuart et al., 2011). The application of foliar fertilizers on plants’ foliage reduces the risk of water pollution with nitrates due to the small quantities used in optimal fertilization schemes, as well as through the consumption stimulation of excess nutrients existing in the soil (Crews and Peoples, 2004). These fertilizers should be used only as a complement to obtain forage yield gains and should not be used exclusively to avoid soil depletion or degradation in short time (Dunca and Dincă, 2014).

Nitrogen (N) influences plant growth by increasing leaf area and hence carbon assimilation (Cui et al., 2014). The process depends on the enzymes activity in chloroplasts. Chloroplasts contain up to 75% of the leaf tissues’ nitrogen in plants with C3 photosynthesis (Chapin et al., 1987). Nitrogen fertilisation in high supplies determines a negative effect on clover species, because this element positively influences the crop performances of perennial grasses enhancing their light interception and absorption (Gatti et al., 2013). Annicchiarico and Tomasoni (2010) found that, without N fertilisation, mixtures out-yielded white clover and grass pure stands. With N fertilisation at double rate to pure grass, yields from mixtures were greater than from clover and comparable to Italian ryegrass. A decrease in the leaf nitrogen levels from top to bottom of the canopy was observed in many forage crops (Xiaohong et al., 2011). The modifications of light interception profile positively correlate with this phenomenon. Leaf area index is the essential determinant of the solar radiation interception of pure stands (Fletcher et al., 2013). Nitrogen availability has a substantial effect on the light use efficiency of perennial grasses in phytosociological associations (Dunea and Motcă, 2007; Den Hollander et al., 2007; Lantinga et al., 1999).

The rationale of the study was to check whether the association of hybrid ryegrass (Lolium hybridum Hausskn.) with a legume species owing a moderate competition capacity i.e. white clover (Trifolium repens L.), and optimal foliar...
fertilization will provide high and stable yields by reducing conventional fertilization rates. The role of white clover in the mixture was to fix the symbiotic nitrogen favouring the fast growth and development of hybrid ryegrass in the first growth cycle of the vegetation season. Clover contribution to the total biomass yield increases in the next cycles during two years of cropping. Further expected benefits are the increasing of overall forage quality and digestibility (Phelan et al., 2013; Malinowski et al., 2012).

The research sought to characterize and optimize the agronomic performance during two years of cropping, considered economically viable, of the following forage systems: white clover pure stand, hybrid ryegrass pure stand, and white clover-hybrid ryegrass binary mixture (50%:50%). The focus of the study was to establish the effects of complex foliar fertiliser application (N\(_5\)P\(_2\)K\(_3\)+3MgO) on the relevant ecophysiological characteristics (e.g., radiation use efficiency, leaf area index, dry matter etc.) of the species in the second vegetation season on the soil and weather conditions of the Targoviste Piedmont Plain, South of Romania. The experiment was conducted during 2004-2005, the second cropping year presenting the highest precipitations amount recorded between 1961 and 2014. The present study quantified the effect of these unusual weather conditions on growth characteristics of species.

**Materials and methods**

The experiment was carried out on plots in a randomized block design with three replicates in Targoviste Piedmont Plain – South of Romania (i.e. Dobra village: N 44° 46' 99.5, E 25° 34' 05.45, 179-m a.s.l) during two cropping years (2004-2005) due to the fact that hybrid ryegrass is a biennial species. The soil was pseudogleic brown alluvial (pH – 5.6; humus – 3.51; Nitrogen index – 2.50; mobile P\(_2\)O\(_5\) – 3.9 ppm; and mobile K\(_2\)O – 117 ppm, in the ocric horizon).

The annual average temperature (9.3 °C) in the second year (i.e. 2005) was the lowest of the 1995-2005 interval (10.29 °C multiannual average). Available Photosynthetically Active Radiation (PAR) during the vegetation season of 2005 was lower than 2004 due to the accentuated nebulosity occurred in May, June, and July (Fig. 1). The weather data of the 2004 vegetation season showed a rainfall regime close 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 annual rainfall sum of 2005 exceeded 1000 mm, being the highest amount recorded between 1961 and 2014. The multiannual average of rainfalls recorded in Targoviste Plain between 1995 and 2004 was 474.69 mm. Rainfalls recorded in 2005 vegetation season were significant as follows: 308.8 mm in March-May, 283.7 mm in June-July and 448.7 mm in August-September (Fig. 2).

The 'Karina' white clover cultivar was tested in pure culture and in binary mixture (50:50) with the hybrid ryegrass ('Zefir' – tetraploid cultivar) in a randomized block design with four replicates. 'Karina' is a diploid cultivar with rich foliage having broad leaflets; it is persistent in pure stand and resistant to diseases and pests. 'Zefir' is a Romanian cultivar which optimally combines the production and quality characteristics of Italian ryegrass with the lateness, grazing suitability, winter resistance, and persistence characteristics of perennial ryegrass.

The plots were sown to ensure an optimal density (20 kg ha\(^{-1}\)) on April 24, 2004 in plots of 3 m \(\times\) 5 m. Three forage cuttings were performed each year; C1, C2, and C3 were the abbreviations for the growth cycles in 2004 until cutting dates, respectively C4, C5, and C6 for the year 2005 (Fig. 1).

In the first year, the plots received no nitrogen fertiliser to allow the biological potential development. In the second year, the foliar fertilisation was applied six times in the fertilised (Ft) variants during the vegetation season (Fig. 1). The selected formula for foliar fertilisation was N\(_5\)P\(_2\)K\(_3\)+3MgO using an approved commercial product. The selection criteria took into account the physicochemical properties of the soil (low phosphorous supply), the possibility of fractional application of fertiliser, and the facilitation of foliar nutrients and microelements absorption resulting from the quasi-horizontal position of the foliage in the clover canopy. The chemical composition of the fertiliser was Nitrogen (N) – 15%; Nitrate-N (NO\(_3\)) – 11.3%; Ammonia-N (NH\(_4\)) - 3.7%; P\(_2\)O\(_5\) – 5%; K\(_2\)O – 30%; MgO – 3%; B – 0.025; Mo - 0.004; and chelates: Cu EDTA – 0.010; Fe EDTA – 0.070; Mn EDTA – 0.040; Zn EDTA – 0.075.

Homogeneous foliage spraysings (2.5 kg ha\(^{-1}\)) were performed at 49, 70 and 91 DAR (Days After Regrowth) in C4; 119 and 147 DAR in C5, respectively 189 DAR in C6 (Fig. 1).

The eco-physiological aspects of the extra-radicular absorption were carefully analysed in order to adapt the method of foliar fertilisation to uniform and to heterogeneous canopies. The uniform distribution of the foliar fertiliser within the canopy was dependent on the leaf area distribution, the height and density of the assimilatory surfaces, respectively. In the mixture, the hybrid ryegrass exceeded the white clover plants' height early in C4. The height difference between the two species resulted in difficulties when applying foliar fertiliser at 91 DAR (third treatment), which we compensated by spraying plants on two height units: over the top of mixed canopy (65 cm), and at the average height of white clover (34 cm). The C5 and C6 growth cycles did not encounter such impediments, as the periods of hybrid ryegrass exceeding clover height were much shorter.

Fig. 1. Daily average temperatures and accumulated heat units (°C) in 2005, and monthly average of PAR (MJ m\(^{-2}\)) in 2004 (circles) and 2005 (triangles); C4, C5 and C6 – cutting moments in 2005; N\(_5\)P\(_2\)K\(_3\)+3MgO foliar fertilisation application (arrows).
Results and discussions

The experimental results provided detailed information regarding the dynamics of changes in the evolution of dry matter accumulation for each growth cycle, and hybrid ryegrass and white clover LAIs, required in the description of the interspecific competition for light in the mixed canopy. The descriptive characterization regarding the dry matter accumulation of each growth cycle (C4, C5 and C6) of the second vegetation season allowed the estimation of the intrinsic interrelations between foliar fertilisation treatments and eco-physiological responses of the species in pure stand and in mixture.

Influence of foliar fertilisation on the bioconversion efficiency of the species

The white clover-hybrid ryegrass mixture showed higher yield increments, compared to both pure stand fertilised variants and the ones with no fertilisation treatment. The distribution of dry matter production efficiency on growth cycles in white clover pure stand without fertilisation (Mt) was 367 (C4), 198 (C5), and 115 g DM m⁻² (C6) – 6.8 t DM ha⁻¹ y⁻¹, while the foliar fertilised variants (Ft) recorded 426 (C4), 258 (C5) and 149 g DM m⁻² (C6) – 8.3 t DM ha⁻¹ y⁻¹. In the Mt mixture, total DM accumulation was 750 (C4), 424 (C5), and 167 g DM m⁻² (C6) – 13.4 t DM ha⁻¹ y⁻¹, while Ft variants produced 966 (C4), 578 (C5), and 180 g DM m⁻² (C6) – 17.2 t DM ha⁻¹ y⁻¹ (Table 1). The first two cuttings (C4 + C5) provided 83.14 to 89.57% from the total annual yield of dry matter in the second year, which corresponded to the percentages presented by Frame et al. (1998), i.e. 80-90% at the harvests in June and early August.

Forage yield of white clover in pure stand

The first growth cycle (C4) in the second year of the experiment lasted approximately 105 days from the regrowth of species in spring to the cutting moment (March 13 – June 25, 2005) and was less productive than C1 cycle for both Mt and Ft variants. The allocation of white clover dry matter in the aboveground morphological components in pure stand showed a distinct significant increase of the foliage mass as a result of N₂P₂K₃+3MgO foliar fertilisation treatment (+53.4 g DM m⁻² leaves – P<0.01), but the stems, stolons and petioles quantity remained almost constant. In the Mt variant, white clover produced 246.48 g DM m⁻² stems, petioles and stolons, 114.6 g DM m⁻² leaves and 6 g DM m⁻² heads and buds. The sequential fertilisation improved the increasing of the biomass for all the components, as follows: 248.4 g DM m⁻² stems, petioles and stolons, 168 g DM m⁻² leaves and 9.6 g DM m⁻² heads and buds.

Table 1. Dry matter accumulation in the experiment performed in Targoviste Piedmont Plain, South of Romania

| Variant                      | White clover | White clover + Hybrid ryegrass | White clover Mt | White clover Ft | White clover + Hybrid ryegrass Mt | White clover + Hybrid ryegrass Ft |
|------------------------------|--------------|--------------------------------|-----------------|----------------|-----------------------------------|----------------------------------|
| Year                         | 2004         | 2005                           |                 |                |                                   |                                  |
| 1st cutting (g m⁻²)          | 486.4        | 650.8                          | 367             | 426           | 750                               | 966                              |
| 2nd cutting (g m⁻²)          | 195.8        | 564.9                          | 198             | 258           | 424                               | 578                              |
| 3rd cutting (g m⁻²)          | 108          | 257.7                          | 115             | 149           | 167                               | 180                              |
| Total DM (t ha⁻¹ y⁻¹)        | 7.9          | 14.7                           | 6.8             | 8.3           | 13.4                              | 17.2                             |

Mt – not fertilised
Fr – foliar fertilisation (N₂P₂K₃+3MgO)

Fig. 2. Monthly average of precipitations (mm) in the vegetation seasons and on multiannual scale in Targoviste Piedmont Plain, South of Romania

Samples were collected once every two weeks using a 50 x 50 cm frame from 2 representative points on the diagonal of each plot. The harvested material was separated into morphological components in pure stands and for each species in mixtures. The samples were dried at 105 °C for 24 h and weighted using a precision balance. The leaf area index (LAI) was established by using a Li-cor 3100 Leaf Area Meter⁶ (Li-Cor Inc., Lincoln, NE, USA). At each cutting, the harvested green mass was weighed from each control plot immediately after harvest.

On-site microclimate monitoring was performed by using a WS-GPI Delta-T Devices Automatic Weather Station, which provided PAR, air and soil temperature, rainfall parameters, and relative humidity. The data were associated with the meteorological data obtained from Targoviste meteorological station (WMO id 153750) to compare with the local weather conditions of Targoviste Piedmont Plain.

The determined morphophysiological variables were the following: dry matter accumulation (DM), relative growth rate (RGR), leaf area index (LAI), leaf area ratio (LAR), specific leaf area (SLA), leaf weight ratio (LWR), net assimilation rate (NAR), and radiation use efficiency (RUE).

The application of the multiple comparison tests (Tukey Honestly Significant Differences) allowed the statistical significance estimation of the fertilisation scheme, cropping system, and weather factors influence on the eco-physiological characteristics of the white clover in pure stand and in mixture with hybrid ryegrass, and the response to foliar fertilisation.
Table 2. Effect of foliar fertilisation (N = P = K = +3MgO) on dry matter accumulation of white clover and hybrid ryegrass in the binary mixture (50:50)

| Indicator                      | Unit       | C4  | C5  | C6  |
|-------------------------------|------------|-----|-----|-----|
| Total Dry Weight - Mt Cn      | (g DM m⁻²) | 750 | 424.06 | 166.8 |
| Total Dry Weight - Ft Cn      | (g DM m⁻²) | 965.76 | 578 | 179.8 |
| Dry Weight Gain               | (g DM m⁻²) | 215.76** | 153.94*** | 13 |
| C₄, Ft - C₅, Mt               | (%)        | 28.77 | 36.3 | 7.2 |
| Total Dry Weight - Cn         | (g DM m⁻²) | 650.8 | 564.9 | 275.7 |
| C₄, Mt - C₅, Difference       | (g DM m⁻²) | 99.2* | -140.84*** | -108.9** |
| C₄, Ft - C₅, Difference       | (g DM m⁻²) | 305.96*** | 13.1 | -95.9* |

Mt – not fertilised; Ft – foliar fertilised; C₄ = C₅; C₆ = growth cycles of the second cropping year; C₄ = C₁; C₂; C₃; Multiple comparison using Tukey HSD test (*** p<0.001; ** p<0.01; * p<0.05), DL – difference limit.

The effect of foliar fertilisation on the total yield obtained per unit area (m²) showed the absence of statistical significance regarding the increment of 58.92 g DM compared to the Mt variant in the edaphic and ecoclimatic conditions of the trial experiments. The comparison between the first growth cycles (C₄ and C₁) showed that the biological potential of white clover was higher in the first cropping year than in the second one for this specific period; its phenotypic expression was the high dry weight quantity as compared to Mt (p<0.05) and Ft variants.

In the second growth cycle (C₅; June 25 – August 23, 2005 – 59 days after C₄), the effect of foliar fertilisation application on DM accumulation was distinctly significant (p<0.01) for the Mt-Ft difference (+61.82 g DM m⁻² - DL 1% = ± 59.42), gaining 23.75%.

The retrospective analysis of foliage productivity showed significant differences (p<0.05) between C₅ (Ft) and C₂ (Mt) recorded in the white clover pure stands (+64.42 g DM m⁻²) and not significant between C₅ (Mt) and C₂ (Mt).

In the final growth cycle (C₆), which lasted 39 days after C₅ cutting (August 23 – September 30), a single application of foliar fertiliser was performed two weeks before the last cutting. The white clover response to the foliar fertilisation (p<0.001) provided an additional 33.8 g DM m⁻² (DL 1% = ± 17.34), representing 29.47% gain compared to the unfertilised variants.

The evolution of the cumulative leaf growth followed a sigmoidal shaped curve, the rate of the process decreasing in intensity towards maturity. Leaf number, leaf areas and positions on the plant determined modifications of the LAI. Leaf area distribution in the mixture was asymmetric, following a triangular shape, white clover having the maximum leaf area positioned in the lower third of the total height of the canopy (25-31%), and ryegrass between 38 and 45%.

Foliar fertilisation applied in the C₄ growth cycle increased the assimilatory areas of the white clover in pure stand (p<0.05). The fertilised variants showed higher LARs (+1.29 m² g⁻¹; DL 5% = ± 1.22), compared to the unfertilised variants. The leaf area ratio (LAR), which is a morphological indicator of the plant leafiness, presented a statistical significant decrease (-1.54 m² g⁻¹; DL 1% = ± 1.39), which suggested an increase in the weight of leaves, positively correlated with the leaf area development as a favourable response to the fertilisation system. LWR had a difference of 0.02 (p>0.05) between the Mt and Ft variants (Table 3), Consequently, white clover showed positive responses of the foliage characteristics to the foliar fertilisation stimuli in the first growth cycle of the second cropping year.
In the white clover-hybrid ryegrass mixture, white clover did not show the same clear increase in the leaf area observed in pure stand, as a response to foliar fertilisation. The contribution of hybrid ryegrass to the cumulative LAI of species in the binary mixture was significant (4.7 in Mt; 4.97 in Ft), which revealed a low competition capacity of white clover in association (0.69 in Mt; 1.03 in Ft). However, at 105 DAR in pure stand, white clover had higher LAIs of 3.63 in Mt and 4.87 in Ft variant.

Other experiment (Den Hollander et al., 2007) reported LWR values of 0.44-0.45 at 75 days after sowing (DAS), LAI values between 4.2 and 4.6 at 82 DAS, and SLA values of 3125-3282 for two white clover cultivars (large leaved; Aran, and medium leaved; Riesling) in pure stands. These results were in agreement with our findings.

The effects of foliar fertilisation on the Radiation Use Efficiency

The RUE values emphasize the particularities of solar energy bioconversion to forage yield (Monteith, 1977). Pure stands of white clover showed a mean RUE value of 1.03 g DM MJ⁻¹ m⁻², and with foliar fertiliser treatments, this indicator increased to 1.20 g DM MJ⁻¹ m⁻² at the first cutting of the second year. The same effect of fertilisation was also observed at the second growth cycle, RUE increasing by 0.28 g DM MJ⁻¹ m⁻² (DL 1% = ± 0.27), i.e. from 0.92 to 1.2 g DM MJ⁻¹ m⁻². In the third cycle, the increase was 0.26 (DL 1% = ± 0.16), i.e. from 0.88 to 1.14 (p<0.01).

The experimentally confirmed response of white clover to foliar fertilisation stimuli allowed the assessment of the foliar fertilisers’ effect on RUE and thus the biological efficiency of the species in uniform canopies. The application of foliar fertilisation in the second and third growth cycles appeared to have a significant influence on the increasing of white clover bioconversion of energy; this suggested that, from the feasibility point of view, this type of fertiliser should be applied in the last two regrowth cycles. In the mixed canopy, the efficiency of PAR utilization was particularly dependent on the dynamics of species’ participation to the total yield per area unit in various phenophases of the vegetation season. The superior competition capacity of the hybrid ryegrass was the most influencing factor in the foliar fertilised variants during the C4 cycle. The interspecific competition inhibited the growth and development of white clover, while hybrid ryegrass reached higher RUEs of 1.71 (Mt) and 2.15 g DM MJ⁻¹ (Ft). Its capacity diminished considerably in the next two growth cycles.

The results observed in the mixture for white clover (0.4 not fertilised, and 0.57 g DM MJ⁻¹ foliar fertilised) and for hybrid ryegrass corresponded to the previous observations. Nassiri (1998) found that the RUEs of the ‘Alice’ variety were 0.53 (without nitrogen - N) and 0.55 g DM MJ⁻¹ (with 150 kg N ha⁻¹); for the perennial ryegrass (Lolium perenne L), they were 1.95 (without N) and 2.14 g DM MJ⁻¹ (N). In the mixture with ‘Gwenda’ variety, perennial ryegrass showed 1.86 (without N) and 2.02 g DM MJ⁻¹ (N) and white clover cultivated RUEs of 0.54 (N) and 0.41 g DM MJ⁻¹ (without N). In pure stand, RUEs (g DM MJ⁻¹) were as follows: 1.02 – ‘Alice’ variety, 0.96 – ‘Gwenda’ variety, 1.13 (without N) and 1.68 (N) for ryegrass.

In Targoviste Piedmont Plain, the overall cumulative RUE of both species per unit area was higher than in the pure stands of species for both Mt and Ft variants. The radiative resources were captured and absorbed in a more efficient and complementary manner. Foliar fertilisation resulted in very significant increases (p<0.001) of the RUE values from 2.11 to 2.72 g DM MJ⁻¹ in C4 (DL 0.1% = ± 0.47) and in C5 from 1.97 to 2.69 g DM MJ⁻¹ (DL 0.1% = ± 0.41) and not significant in C6, from 1.28 to 1.38 g DM MJ⁻¹ (DL 5% = ± 0.58).

Conclusions

One of the methods that can maintain or further increase the presence and participation of white clover to the forage yield formation in mixtures with perennial grasses is the foliar fertilisation applied according to the phenophases, weather conditions and regrowth period requirements.

The adaptation of the foliar fertiliser application in the canopies must be performed using a simplified balance, both to the requirements of white clover and hybrid ryegrass in different phases of vegetation, and to the weather conditions influencing the ammonium nitrification and nitrate solubilisation. The fractional application of fertilisers allowed a better combination between mineral and organic elements, and a compensation of the application cost by using optimal amounts and spraying timings.

The comparison between the growth cycles of the two cropping years showed that the biological potential of white clover in pure stand without fertilisation was higher in the first cropping year at the first cutting (4.8 t DM ha⁻¹) that the corresponding cycle of the next vegetation season (4.2 t DM ha⁻¹), but the annual yield (7.9 t DM ha⁻¹), was lower than in the second year (8.3 t DM ha⁻¹). The dry matter accumulation of the binary mixture showed a higher total production per unit area (17.2 t DM ha⁻¹) as compared with the first year of cropping (14.7 t DM ha⁻¹). The phenotypic expression of foliar fertilisation was the higher total dry weight quantity and superior LAI though the clover content was diminished.

Foliar fertilisation treatments enhanced significantly the competition capacity of hybrid ryegrass in the first production cycle of the second year. In white clover pure stand, such fertiliser type was efficient in the last two regrowth cycles. In the white clover-hybrid ryegrass mixture, foliar fertilisation brought benefits in the first two growth cycles, according to the phenophase particularities and weather conditions.

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