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How much N_2 is fixed by perennial clovers in Mediterranean sown pastures?

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Summary - This study investigated N_2 fixation by perennial clovers in an area of the Mediterranean basin (Ioannina, N.W. Greece). Pasture treatments were white clover (*Trifolium repens*) or red clover (*Trifolium pratense*) sown in binary mixtures with perennial ryegrass (*Lolium perenne*) and perennial ryegrass in monoculture. Production during two years after sowing was highest in red clover pastures and the yield of red clover was 2-6x that of white clover. N_2 fixation was measured using the ^{15}N dilution technique. The amount of N fixed in above ground tissues of white and red clover was 88 and 265 kg N ha^{-1} respectively, in the establishment year and 37-104 and 78-214 kg N ha^{-1} in the first productive year. N_2 fixed by the clovers will be an important N source for the associated non-legume species via transfer.

Key-words: white clover, red clover, N_2 fixation, Mediterranean, ^{15}N dilution technique

Résumé - *La fixation de l'azote par des trèfles pérennes a été étudiée dans la région de Ioannina en Grèce. Trois types de prairies ont été comparées:* Trifolium repens *ou* Trifolium pratense *associées à* Lolium perenne *ou le raygrass anglais en monoculture. Deux ans après semis, le trèfle violet a obtenu le meilleur rendement, 2 à 6 fois supérieur au trèfle blanc. La quantité d'azote fixée a été de 88 kg.ha-1 pour le trèfle blanc et 265 pour le trèfle violet l'année d'installation puis respectivement de 37 à 104 et de 78 à 214 au cours de la première année de production.*

Mots-clés: *trèfle blanc, trèfle violet, fixation d'azote*

Introduction

Sown perennial clover/grass pastures are relatively rare in the Mediterranean region, compared to other herbage resources. In suitable climatic conditions they could play a key role for animal nutrition. Legume-based pastures have been used successfully in temperate areas such as New Zealand, eliminating the need for N fertilizer inputs (Ledgard and Steele, 1992). The study of perennial pasture systems under Mediterranean conditions is a challenge in the context of achieving sustainable pastoral systems.

Measurements of N_2 fixation by perennial pasture legumes under Mediterranean conditions are very limited and usually indirect (Sulas *et al.*, 1998). This study used ^{15}N dilution to investigate the N_2 fixation performance of two perennial clovers, white clover and red clover in binary mixtures with perennial ryegrass for the establishment and first productive years under cutting and grazing.

Materials and Methods

The experimental area was located at Ioannina, North West Greece (400 m above sea level). The climate is continental Mediterranean with high annual precipitation (1014 mm) but dry summers. The soil is sandy clay with pH 6,8. During spring 1994 the plant mixtures (white clover cv. Grasslands Huia or red clover cv. Altaswede with perennial ryegrass) and perennial ryegrass alone (cv. Belida) were sown in replicated (n=3) plots. Additional treatments included perennial ryegrass monoculture which received 40, 80, 160 or 320 kg N ha^{-1} as ammonium nitrate each year. Clovers were not inoculated in this study. Previous research showed that local strains of effective rhizobia were present in the soil (Thanopoulos and Kefalogiannis, 1993).

In spring and autumn of 1994 and 1995, ^{15}N label was applied on microplots within the main plots for determining N_2 fixation. In 1994, all plots were mown and in 1995 they were sub-divided into mowing and grazing treatments. The latter were rotationally-grazed by sheep. Pastures were harvested post-grazing (exclosure cages used) and plant material from microplots was separated into its components, oven dried, milled and analyzed for ^{15}N. N_2 fixation was then calculated (e.g. Ledgard and Steele, 1992).

Results and Discussion

Red clover pastures were consistently the most productive (Figure 1). The proportion of red clover in pasture was always much higher than white clover, due to its rapid establishment, deeper root system and up-right morphology. Grazing treatments yielded less than the corresponding mowing treatments. This could be attributed to the more severe defoliation with grazing slowing re-growth rates in comparison with the standard height of the mowing treatment. The yield of ryegrass monoculture showed no significant effect from different N-fertilization rates. This can be attributed to the process of mineralization increasing soil N availability following the site preparation at establishment. Non-sown plants (mainly *Ranunculus sardous, Kickxia commutata, Plantago lanceolata, Daucus carota, Taraxacum officinale* and *Cynodon dactylon*) were an important component of pastures, especially in the establishment year. Standing senescent and dead matter was significant in 1995 due to the summer drought and to a rust infection of perennial ryegrass during the summer.

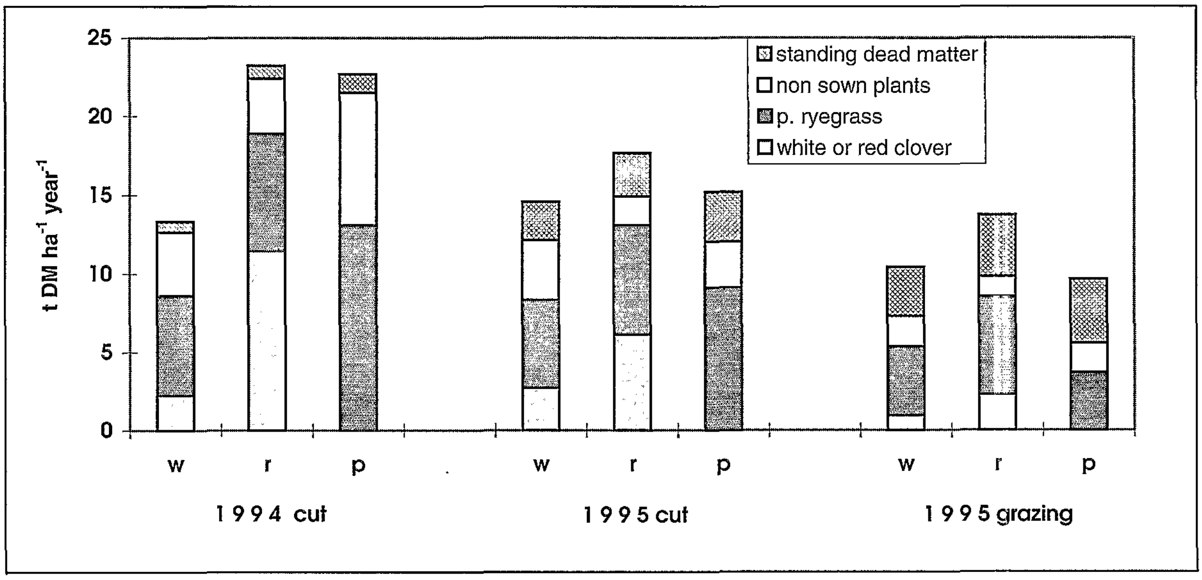

Figure 1. Total and component production of white clover + perennial ryegrass (w), red clover + p. ryegrass (r) and p. ryegrass only (p) pastures under cutting or grazing during 1994 and 1995 and nil N fertilization.

N derived from fixation (%Ndfa) tended to be higher in white clover than red clover, especially under cutting (Table 1). During the first production year the seasonal %Ndfa was in the following order (first number red clover and second white clover): Spring (92 and 97%) > autumn (83 and 89%) > summer (68 and 69) > (winter 43 and 60).

Table 1. Proportion of total N derived from fixation (%Ndfa) and amount of N_2 fixed in white or red clover shoots under cutting and grazing treatments in the establishment and first productive years.

Clover	*1994 Cutting* Ndfa (%)	N_2 fixed (kg ha^{-1})	1995 Cutting Ndfa (%)	N_2 fixed (kg ha^{-1})	1995 Grazing Ndfa (%)	N_2 fixed (kg ha^{-1})
White	69	88	74	104	91	37
Red	47	265	66	214	89	78
SED	16	83	6	19	5	22

Under continental Mediterranean climatic conditions, the amount of N fixed from atmospheric N_2 in above-ground herbage of perennial clovers was 88 to 265 kg N ha^{-1} in the establishment year and 37 to 214 kg N ha^{-1} in the first productive year. This is comparable to that in a summary of research in temperate environments at 20-310 kg N ha^{-1} yr^{-1} under mowing and 30-250 kg N ha^{-1} yr^{-1} under grazing (for clover shoots, reviewed by Ledgard, 1999). These estimates all ignore N_2 fixation associated with clover tissue below the cutting or grazing height, which has been estimated at 70% of that above cutting height (Jørgensen and Ledgard, 1997). Thus, total N_2 fixation in the current study would be approximately 150-450 kg N ha^{-1} in the establishment year and 60 to 360 kg N ha^{-1} in the first productive year. The fixed N in below cutting height tissue will be an important source of N transfer from clovers to ryegrass and non-sown plants via senescence and mineralization.

Conclusions

Under the specific conditions of this experiment, clovers fixed atmospheric N_2 at comparable levels to studies in temperate regions, despite the summer drought stress restricting production and N_2 fixation. This input of fixed N is an important source of N for the non-legume plants either sown or unsown. The management of sown pastures in the Mediterranean region should aim to retain a sufficient proportion of legumes in order to sustain production in the long-term through N transfer.

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