Effect of defoliation management and plant arrangement on yield and N_2 fixation of berseem-annual ryegrass mixture

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Abstract

The research was carried out in a Mediterranean semi-arid environment on berseem clover, annual ryegrass and their mixture to study the effect of defoliation management [date of first cut (FC) 85, 119, 140, 169 days after sowing] and different plant arrangements (sowing the two components in alternate rows or in the same row) on yields, N content, N_2 fixation and N transfer. The experimental design was a split-plot with four replications. The ^{15}N isotope dilution technique was used (8 kg N ha^{-1} as ammonium sulphate at 10 atom% ^{15}N excess) to evaluate the N_2 fixation. Total seasonal DM yield was, on average, significantly higher for FC119 and FC140 (approx. 12.3 t ha^{-1}) than for FC85 and FC169 (approx. 10.6 t ha^{-1}). Plant arrangement did not significantly influence total yield of the mixture. However, the legume yield was higher (+20%; $P<0.0001$) in the same row than in alternate rows arrangement. N content of ryegrass was significantly higher in the mixtures than in pure stand and in the 'same row' plant arrangement than in the 'alternate rows'. Intercropped berseem always had a significant higher % of Ndfa than the monocropped one (on average 74.7% and 57.7% respectively). The apparent transfer of fixed N from berseem to ryegrass was not detected in either plant arrangement.

Keywords: Berseem clover, annual ryegrass, nitrogen fixation

Introduction

The traditional forage systems of semiarid rain-fed Mediterranean areas are based mainly on grazing land (pasture and rangeland) and annual forage crops (vetch, oat and berseem clover in either pure or mixed stands). Many authors consider the annual grass-legume mixture system an effective alternative to pure stands due to several advantages over monocultures. These include improvement in quantity and quality of forage production, improved forage yield stability and seasonal distribution and more efficient use of the resources, which gives a reduction in costly inputs (Carr *et al*., 2004; Fukai and Trenbath, 1993; Mooso and Wedin, 1990; Sengul, 2002). Nitrogen economy and the success of the grass-legume intercrop in low input systems depend on the effectiveness of N_2 fixation and on the current N transfer from legume to the non-legume companion (Jørgensen *et al*., 1999). However, little data is available about the effects of crop management on symbiotic nitrogen fixation by annual forage legumes in Mediterranean environments and on the contribution of their N_2 fixed to the N supply of a non-legume grown in mixture.

The main aim of this study, carried out under field conditions in a semi-arid Mediterranean environment, was to estimate the effect of different defoliation managements on dry matter yield and symbiotic nitrogen fixation by berseem clover in pure stand and in mixture with annual ryegrass and to quantify the N transfer from legume to grass.

Materials and Methods

The research was carried out in 2003/04 at Pietranera farm (Agrigento, Italy; 37°30′N, 13°31′E; 178 m a.s.l.) on a deep, well-structured soil (0.38 clay, 0.25 silt, 0.37 sand; pH in water 8.4; 1.27% organic matter and 0.85‰ total N). The previous crop was wheat. The soil was ploughed at a depth of 25 cm in summer and harrowed in autumn. Before harrowing, all plots received 69 kg P_2O_5 ha^{-1}. The experiment was set up in a split-plot design with 4 replications. Main plots consisted of berseem (*Trifolium alexandrinum* L. cv Lilibeo) in pure stand (B), annual ryegrass (*Lolium multiflorum* Lam subsp. *Wersterwoldicum* cv Elunaria) in pure stand (R) and their mixture arranged either in alternate rows (BR_{AR}) or in the same row (BR_{SR}). Sub-plot treatments were defoliation management: 1) FC85, first cut at 85 days after sowing (DAS), and subsequent cuts at 119, 140, 169 and 198 DAS; 2) FC119 first cut at 119 DAS, and again at 140, 169 and 198 DAS; 3) FC140, first cut at 140 DAS and again at 169 and 198 DAS; 4) FC169, first cut at 169 DAS and again at 198 DAS. Sub-plot size was 4.0 m x 2.6 m (13 rows spaced every 0.2 m, length of row 4 m). Plots were hand-sown on 16 December 2003 using 1300 germinable seeds m^{-2} for pure stands and a 0.5:0.5 ratio for mixed stands. All plots were hand-weeded. The ^{15}N isotope dilution technique was used to estimate nitrogen fixation by clover. ^{15}N fertilizer [$(NH_4)_2SO_4$ at 10 atom% ^{15}N excess] was applied to a microplot (2.88 m^2) in the central area of each plot, following the procedure described by Høgh-Jensen and Schjoerring (1994); the total amount of labelled fertilizer (8 kg N ha^{-1}) was split at crop emergence and after defoliations. On the same dates, the rest of the plot outside the ^{15}N-labelled area received an equal amount of ammonium sulphate.

At each harvest, plants were cut at 5 cm stubble height and the total fresh and dry weights were recorded. A forage sample, from the central area of the microplot (0.80 m^2), was separated into botanical components (B, R), dried to a constant weight at 60 °C, ground to a fine powder (sieved through a 0.1 mm mesh) and analysed by the Iso-Analytical laboratory (EA-IRMS technique) for ^{15}N enrichment and total N content.

Data on ^{15}N enrichment of biomass was used to calculate the percentage of clover nitrogen derived from symbiotic N_2 fixation (%Ndfa) according to Fried and Middleboe (1977). The fraction in the associated grass of atmospherically derived nitrogen transferred from clover (%Ntrans) was calculated using the formula reported by Ta and Faris (1987).

The GLM procedures of the Statistical Analysis System (SAS) were used to analyse the variance (version 8.1, SAS Institute, Cary, NC). The Least Significant Difference (LSD at $P \leq 0.05$) was used to make comparisons among the treatments.

Results and Discussion

Total rainfall during the growing season was higher than normal (690 mm *vs* 550 of the long-period average) and well distributed and resulted in high DM yields.

On average, berseem in pure stand and mixtures yielded about 20% more DM than ryegrass in pure stand ($P<0.01$) (Table 1). The response to defoliation management varied greatly according to the sward type (interaction significant for $P<0.0001$). Total DM yield of ryegrass in pure stand increased as first cut was delayed (and number of defoliations was reduced). On the contrary, berseem increased the total DM production when it was subjected to several defoliations (i.e. bringing forward the date of first cut). However, when the first cut was done very early (T1) the total DM yield was decreased as the crop did not accumulate adequate reserves for prompt regrowth at the first defoliation, and the interval between successive cuts was not sufficient to recover the initial gap. In mixed stands the effect of defoliation management on total DM production resulted intermediate compared to the two pure stands and no significant differences were observed between the two plant arrangements. However, compared to sowing berseem and ryegrass in alternate rows, the legume content on the total DM increased significantly ($P<0.0001$) when sowing the two components in the same row.

In the mixed stands, also the frequency of defoliation had a significant effect on the proportion of the two species. Berseem content on dry matter basis of the harvested herbage showed a trend similar to that observed for the DM yield of the pure stand.

Table 1. Total above-ground dry matter yield (kg ha^{-1}) of berseem clover (B), ryegrass (R) in pure stand and their mixtures ($BR_{(AR)}$ alternate rows; $BR_{(SR)}$ same row) for the defoliation treatments (first cut at 85, 119, 140, 169 DAS)

Defoliation treatment	Dry matter yield (kg ha^{-1})				
	B	$BR_{(AR)}$	$BR_{(SR)}$	R	Mean^{+}
FC85	11822	11489 (65.5)§	11607 (77.9)	7692	10653 b
FC119	14180	13310 (86.7)	13826 (89.2)	8365	12420 a
FC140	12739	12417 (70.7)	12893 (84.3)	11026	12269 a
FC169	9394	10383 (38.5)	9829 (63.0)	12413	10505 b
Mean^{+}	12034 a	11900 a	12039 a	9874 b	
Interaction	P<0.0001; $LSD_{0.05}$ = 899				

$^{+}$ Mean followed by the same letter are not different at P≤0.05; ‡ ns = not significant
§In brackets the proportion of berseem (%) on total DM production

Berseem content was always higher than that of ryegrass except when the first cut was delayed up to 179 DAS (T4) and plants of both species were arranged in alternate rows. According to Fukai and Trenbath (1993), such results highlight the importance of crop husbandry practices and management on relative competitive abilities and hence on the yields of the component crops.

When first cut was delayed, the mean N content in the above-ground biomass of both pure stands decreased (Table 2). N content of ryegrass was always significantly higher in the mixtures than in pure stand, as observed by several authors studying grass-legume intercrops (e.g. Høgh-Jensen and Schjoerring, 1994; Jørgensen *et al*., 1999). In ryegrass mixed with clover, the 'same row' plant arrangement gave higher N contents compared to the 'alternate rows'. This result was not due to a higher N uptake from the soil (data not shown) but may be related to the lower DM production recorded for ryegrass in 'same row' arrangement.

The stronger competitive ability for N of ryegrass in mixed stand did not affect the N content in berseem and, in fact, no significant differences were observed either between pure and mixed stand or between the two different plant arrangements. The depletion of soil N, due to the ryegrass uptake, forced the legume to increase the N_2 fixation to meet its own needs. In the berseem grown in mixed stand, the amount of N_2 fixation was, on average, significantly higher than in pure stand, and slight differences (but significant at P<0.05) were observed between the plant arrangements (Table 3). For berseem in pure stand, %Ndfa decreased significantly when the first cut was delayed from early March (T1; 66.9%) to late April (T3; 49.7%) and increased again when the first cut was done at the end of May (T4; 58.8%). As well as a delay of the first cut, a progressive reduction of %Ndfa was observed in the mixed stands even though the variations were lower compared to those of pure stand.

Table 2. N concentration (g kg^{-1} DM) of above-ground biomass of berseem clover and annual ryegrass in pure stand and in mixtures (AR alternate rows; SR same row) for the defoliation treatments (first cut at 85, 119, 140, 169 DAS)

Defoliation treatment	Berseem				Ryegrass			
	Pure stand	Mixture		Mean+	Pure stand	Mixture		Mean+
		AR	SR			AR	SR	
FC85	35.4	33.6	32.4	33.8 a	21.3	30.0	32.9	28.1 a
FC119	30.5	29.9	30.2	30.2 b	19.3	32.6	35.9	29.3 a
FC140	28.2	26.7	28.0	27.6 c	16.4	26.0	34.2	25.5 b
FC169	24.8	23.2	22.4	23.5 d	11.6	16.9	23.2	17.3 c
Mean+	29.7	28.4	28.3		17.2c	26.4b	31.6 a	
Interaction	*ns*‡				P=0.0012; $LSD_{0.05}$ = 2.53			

+ Mean followed by the same letter are not different at P≤0.05. ‡ ns = not significant

Table 3. Percentage of N_2 fixation (%Ndfa) in above ground biomass of berseem and atom % ^{15}N excess in above ground biomass of ryegrass in pure stand and in mixtures (AR alternate rows; SR same row) for the defoliation treatments (first cut at 85, 119, 140, 169 DAS)

Defoliation treatment	%Ndfa				Atom % ^{15}N excess			
	Berseem				Ryegrass			
	Pure stand	Mixture		Mean+	Pure stand	Mixture		Mean+
		AR	SR			AR	SR	
FC85	66.9	85.7	82.9	78.5 a	0.1624	0.1979	0.2343	0.1982 b
FC119	55.4	75.9	70.6	67.3 b	0.1330	0.2715	0.3365	0.2470 a
FC140	49.7	74.3	69.6	64.5 b	0.1558	0.1784	0.2383	0.1909 b
FC169	58.8	70.2	68.6	65.9 b	0.1580	0.1581	0.1846	0.1669 b
Mean+	57.7 c	76.5 a	72.9 b		0.1523 c	0.2015 b	0.2484 a	
Interaction	P = 0.0212; $LSD_{0.05}$ = 5.7				P = 0.084; $LSD_{0.05}$ = 0.06061			

+ Mean followed by the same letter are not different at P≤0.05. ‡ ns = not significant

Atom % ^{15}N excess of ryegrass was significantly higher in mixed stands than in pure stand (Table 3). This is not unusual and is probably due to dissimilar N uptake patterns by plants in pure or mixed systems (Danso *et al.*, 1993). On the whole, our data suggests that N transfer from berseem to ryegrass did not occur. Also Peoples and Herridge (1990), in their review, commented that N transfer in annual legume to non-legume may not be a common phenomenon.

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