# Berseem-annual ryegrass intercropping: effect of plant arrangement and seeding ratio on $N_2$ fixation and yield

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## Abstract

Various agronomic factors can affect the productivity and the efficiency of legume-grass intercropping systems. This research was carried out in a Mediterranean semi-arid environment (37°30'N; 13°31'E; 178 m a.s.l.) with the aim to study on berseem clover- annual ryegrass mixture (*Trifolium alexandrinum* L. – *Lolium multiflorum* Lam subsp. *wersterwoldicum*) the effects of different plant arrangement (sowing of the two components in alternate rows or in the same row) and seeding ratios (100:0; 75:25; 50:50; 25:75; 0:100) on forage yield, nitrogen content and nitrogen fixation. The experimental design was a split-plot with four replications. The $^{15}N$ isotope dilution technique was used to estimate nitrogen fixation by berseem clover. All plots were cut four times (first cut 85 DAS; rest period of four weeks). The DM yield of mixtures and pure stand clover were similar; annual ryegrass in pure stand produced the lowest yields. No significant differences for DM yield were observed due to the plant arrangement and seeding ratio in the mixture. Intercropping berseem had always a significant higher percentage of Ndfa than monocropped berseem. The %Ndfa of mixed berseem was not influenced by plant arrangement but gradually decreased when proportion of berseem in the mixture increased.

Keywords: berseem clover, annual ryegrass, mixture, plant arrangement, seeding ratio, nitrogen fixation

## Introduction

Intercropping is the growing of two (or more) crops together on the same area of ground and often produces an advantage in terms of more yield and less variation in yield than comparable areas of sole crops (Willey, 1979; Bulson *et al.*, 1997). The most commonly accepted reason explaining why it is possible to obtain better yields with crop mixtures is that the component crops can use more efficiently the limiting resources differing in their growth requirements. Systems which combine grass and legume crops are highly regarded because of their potential for replacing fertilizer N with symbiotically fixed atmospheric $N_2$ and the economic and environmental benefits which may result from this (Stern, 1993). However, in the literature there are few data available on biological nitrogen fixation by annual forage legumes in Mediterranean environments. As well as few data is available on the contribution of biological nitrogen fixed by annual legume to the nitrogen nutrition of a non-legume grown in mixed stand and on the effects of intercropping with grass on the quantity of $N_2$ fixed by forage legume component. Moreover the productivity and efficiency of grass-legume intercrop system are affected by various agronomic variables such as component crop density, plant spacing and arrangement, time of sowing of component crop, N fertilization (Ofori and Stern, 1987).
The aim of this research was to evaluate on berseem clover – annual ryegrass mixture the effects of different plant arrangement and seeding ratios on forage yield, nitrogen content and nitrogen fixation in a typical rain-fed Mediterranean environment.

## Materials and Methods

The research was carried out during 2002/03 in a hilly area of Sicily (37°30'N; 13°31'E; 178 m a.s.l.) on a *Vertic haploxerepts* soil and with wheat as previous crop. The topsoil (0–40 cm) had the following characteristics: 38% clay, 25% silt, 36% sand, 8.4 pH (in water), 1.27% organic matter, 0.85‰ total nitrogen.

The experiment was set up in a complete randomized block design with four replications. Treatments were: 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 with different plant arrangement [sowing of the two components in alternate rows ($BR_{AR}$) or in the same row ($BR_{SR}$)] and seeding ratios ($B_{25}$-$R_{75}$; $B_{50}$-$R_{50}$; $B_{75}$-$R_{25}$). Pure stands were seeded at 50 kg seeds $ha^{-1}$. Mixed and pure stands were sown by hand on 16 December 2002. No herbicide treatments were used; all plots were kept free of weeds by hand-weeding.

The $^{15}N$ isotope dilution technique was used to estimate nitrogen fixation by clover. $^{15}N$ fertilizer [$(NH_4)_2SO_4$ with an isotopic composition of 10 atom% $^{15}N$] was uniformly applied in a liquid form, following the application procedure described by Hogh-Jensen and Schjoerring (1994). $^{15}N$ fertilizer (8 kg N $ha^{-1}$) was applied to a 2.88 $m^2$ microplot in the middle of each plot at the emergence of the crops and again two days after the 1st and 2nd utilizations. The rest of the plot outside the $^{15}N$-labelled area received a top-dressing of ammonium sulphate in an amount equivalent to the microplots. After each application of labelled fertilizer an irrigation of all plots was used in order to avoid the retention of $^{15}N$ fertilizer on the plant leaves.

All plots were cut simultaneously at 85d after sowing and defoliation frequency of 28d was used (4 times). All plots were harvested by hand at 5 cm stubble height and total fresh weight was determined. At each harvest, a sample of plant material, taken in the centre of microplots, was hand-disaggregated into its botanical components (B, R), dried at 60 °C for 36 h, weighed and ground to a fine powder (sieved using a 0.1 mm mesh size) in a fast running mill 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 were used to calculate the percentage of clover nitrogen derived from symbiotic $N_2$ fixation (%Ndfa) according to Fried and Middleboe (1977).

The GLM procedure of the Statistical Analysis System (SAS) was used to analyze the variance. Least significant difference (LSD at $P \leq 0.05$) was used to make comparisons among treatments.

## Results and Discussion

During growing season total rainfall was close to the long-term mean (532 mm vs 550 mm) and distribution was generally favourable for crop growth.

Dry matter yield (kg $ha^{-1}$) for annual ryegrass and berseem clover in pure and mixed stands in relation to the different plant arrangement and seeding ratio are reported in Table 1.

The yield of sole-cropped berseem was significantly higher than ryegrass in pure stand (9449 *vs* 4826; $P<0.0001$). The DM yield of mixture and pure stand clover were similar. No significant differences for DM yield were observed due to the arrangement of plants and to the different seeding ratio in the mixture.

The legumes competed very well in the mixed stands, producing on average 80% of the total yield. Both seeding ratio and plant arrangement had small effects on %clover on DM basis.

Annual ryegrass intercropped with berseem consistently had a higher nitrogen concentration in the dry matter than ryegrass grown in pure stand ($P<0.001$). This is in agreement with the reports of Jorgessen *et al*. (1999) and Malhi *et al*. (2002). Moreover, mixing the components within the row, compared to sowing in alternate rows, significantly increased the N content in annual ryegrass.

On the contrary, the N concentration of berseem was much greater in pure stand than in mixture; similar results have been found by Scarpello (2003) for berseem clover intercropped with annul ryegrass in a Mediterranean semi-arid environment.

In the mixture the N concentration (g $kg^{-1}$ DM) of berseem and ryegrass forage increased significantly with increase in berseem portion of stand.

Table 1. Dry matter yield (kg $ha^{-1}$), N concentration (g $kg^{-1}$ DM) and percent of N uptake derived from atmospheric nitrogen fixation (%Ndfa) for annual ryegrass and berseem clover in pure and mixed stands as affected by plant arrangement (alternate rows – $BR_{AR}$; same row – $BR_{SR}$) and seeding ratio

| | Dry matter yield (kg $ha^{-1}$) | | | N concentration (g $kg^{-1}$ DM) | | Ndfa (%) |
|---|---|---|---|---|---|---|
| | Berseem | Ryegrass | Total | Berseem | Ryegrass | Berseem |
| Berseem (B) | 9449 | – | 9449 | 35.0 | – | 73.3 |
| Ryegrass (R) | – | 4826 | 4826 | – | 20.6 | – |
| $BR_{AR}$ ($B_{25}$-$R_{75}$) | 6746 | 2000 | 8746 | 31.6 | 29.9 | 81.1 |
| $BR_{AR}$ ($B_{50}$-$R_{50}$) | 7083 | 1707 | 8790 | 32.6 | 29.2 | 80.7 |
| $BR_{AR}$ ($B_{75}$-$R_{25}$) | 6948 | 1718 | 8666 | 33.5 | 31.3 | 78.8 |
| $BR_{SR}$ ($B_{25}$-$R_{75}$) | 6824 | 2458 | 9282 | 31.4 | 28.4 | 84.1 |
| $BR_{SR}$ ($B_{50}$-$R_{50}$) | 7980 | 1266 | 9246 | 33.7 | 33.3 | 80.2 |
| $BR_{SR}$ ($B_{75}$-$R_{25}$) | 8258 | 1438 | 9696 | 33.9 | 33.6 | 78.8 |
| *$LSD_{0.05}$* | *970* | *852* | *811* | *1.3* | *2.5* | *3.0* |
| *Mean effect of plant arrangement* | | | | | | |
| Alternate rows | 6925 | 1809 | 8734 | 32.6 | 30.1 b | 80.2 |
| Same row | 7688 | 1721 | 9409 | 33.0 | 31.8 a | 81.0 |
| *Mean effect of seeding ratio* | | | | | | |
| BR ($B_{25}$-$R_{75}$) | 6785 b | 2228 a | 9013 | 31.5 b | 29.1 b | 82.6 a |
| BR ($B_{50}$-$R_{50}$) | 7531 a | 1487 b | 9018 | 33.1 a | 31.2 a | 80.4 ab |
| BR($B_{75}$-$R_{25}$) | 7603 a | 1579 b | 9182 | 33.7 a | 32.5 a | 78.8 b |

Different letters within the columns indicate significant different at the $P \leq 0.05$ level

On average, *Trifolium alexandrinum* achieved a higher proportion of its N from nitrogen fixation when grown in mixture with ryegrass than when it was grown in monoculture ($P<0.0001$). In mixture ryegrass has been found to be stronger competitor for soil nitrogen (data not shown), and berseem relied more on $N_2$ fixation as N source. Similar observations were made by Papastylianou (1988), Loiseau *et al.* (2001), Carlsson and Huss-Danell (2003) and Stringi *et al.* (2004). This suggests that nitrogen competition plays a key role in intercropping legume-nonlegume systems and its knowledge can enable to manage better N inputs in order to make the intercropping system more efficient.

No significant differences for %Ndfa were observed due to the arrangement of plants in the mixture. The percentage of nitrogen derived from $N_2$-fixation decreased with increase in berseem portion of stand. Such result seems related to the increase of soil N uptake by

ryegrass as its density increased in the mixture; this, on turn, encouraged the legume to obtain a greater proportion of its nitrogen through biological fixation.
The apparent transfer of fixed nitrogen from clover to grass was negligible in all treatment. In fact, no significant lower values for % atom $^{15}N$ of intercropped annual ryegrass compared to monocropped were observed, indicating that transfer of the nitrogen from legume to grass was marginal or nonexistent (Cowell *et al*., 1989). This is in agreement with the reports of Peoples and Herridge (1990), Danso *et al*. (1993) and Stringi *et al.* (2004) that highlighted how in annual grass-legume intercropping N transfer may not be common phenomena.

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