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Response to N-fertilizer of Italian ryegrass grown alone and in mixture with berseem clover under continental irrigated mediterranean conditions

Rendement du raygrass Italien seul ou en association avec le trefle berseem dans les conditions méditerranéennes continentales irriguées

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Abstract

In a field experiment over three growing seasons, the potential benefits of planting berseem clover (*Trifolium alexandrinum L*) with Westerwold Italian ryegrass (*Lolium multiflorum* Lam.) were examined under irrigated continental Mediterranean conditions.Similar N rates (0, 30, 60, 90 and 120 kg N ha^{-1} cut^{-1}) were applied to both pure Italian ryegrass stands and mixtures, each given three successive cuts. One previously unfertilized cut was performed in late winter. Species in the mixture were established at 50:50 seed ratio but the mean proportion of berseem clover was 14%. Mean winter survival of berseem was 87% but 88% of the plants had leaves damaged by the frost. Forage production varied with both N rate and cutting sequence in both the pure stand and the mixture but differences between the two types of swards were significant only at low levels of fertilizer N. Total DM production over the four cuts in plots with N applications of 0,90, 180, 270 and 360 kg N ha^{-1} a^{-1} were 7.14, 9.51, 11.66, 13.91 and 14.36 t DM ha^{-1} a^{-1} in pure stand, respectively. Corresponding values for the mixture were 8.80, 10.94, 12.90, 14.05 and 13.64 t DM ha^{-1} a^{-1}. The mean response of Italian ryegrass in the range of 0-360 kg N ha^{-1} a^{-1} was 20 kg DM per kg N applied. The corresponding value for the mixture was 13 kg DM per kg N applied. At the berseem clover proportions reached in this work, N equivalence showed values of about 80 kg N ha^{-1} a^{-1}. As rates of N increased from 0 to 120 kg N ha^{-1} cut^{-1}, nitrogen concentration increased by 78%. In the applied range of N fertilizers, $N0_3$-N was not affected.

Résumé

Dans un essai réalisé au cours de trois saisons agricoles, on a étudié le potentiel de l'association du bersim avec le raygrass italien. Les cultures ont été emenées et irrigué dans des conditions continentales méditerranéennes. On a appliqué, aussi bien pour la culture pure du raygrass que pour l'association, une fertilisation azotée avec les doses suivantes (0, 30, 60, 90 et 120 kg N/ha/coupe) après chacune des trois coupes successives. Une autre coupe avant fertilisation a été faite à la fin de l'hiver. Les proportions du bersim et du raygrass dans le mélange de graines étaient de 50:50. Cependant, dans la culture en association, les plantes du bersim n'étaient préentés qu'avec un 14 pourcent. 87% des plantes du bersim ont pu survivre en hiver, dont 88% avaient des feuilles endommagées par les gelées. La production d'herbe a été proportionnelle aux doses de fertilisation pour la culture pure et l'association. Néanmoins, différence entre les rendements de chacune de ces dernières était d'autant plus nette que les doses d'azote incorporées dans le sol étaient faibles. La production de la MS pour les quatre coupes dans les parcelles avec les applications de 0, 90, 180, 270 et 360 kg N ha^{-1} a^{-1} étaient de 7.14, 9.51, 11.66, 13.51, 14.36 tMS ha^{-1} a^{-1}. Le rendement moyen du raygrass italien dans un intervalle de 0-360 kg N ha^{-1} a^{-1} a été de 20 kg MS par kg de N de fertilisation. Concernant le bersim, les valeurs équivalentes de N étaient de l'ordre de 80 kg N ha^{-1} a^{-1}. Au fur et à mesure que les doses de fertilisation azotée augmente de 0 à 120 kg ha^{-1} coupe, la concentration en azote augmente de 78%. Dans l'intervalle de la fertilisation azotée appliqué NO_3 -N n'a pas été affectée.

Introduction

Year-long feeding programmes for ruminant livestock in semiarid areas of the Mediterranean basin cannot rely only on seasonally distributed and low quality resources. Alternatives must be found to fill in the forage deficit. Italian ryegrass (*Lolim multiflorum* Lam.) has been promoted as a winter-spring fodder crop in irrigated conditions as one of the possible alternatives. Earlier results suggested that in irrigated Mediterranean conditions, good forage potential cannot be achieved without considerable amounts of fertilizer nitrogen. Reyne and Garambois (1977) in France, Rosello and Hidalgo (1977) in Spain, Ahmin *et al.*, (1975) in Algeria, and Ameziane (1979) in Morocco have observed up to 18 t DM ha^{-1} with applications of up to 300 kg ha^{-1}. Our own results in Mediterranean conditions, but with continental influence, showed yields of up to 13.5 t DM ha^{-1} in four cuts with 360 kg N ha^{-1}. DM yields were reduced by half without application of fertilizer nitrogen (Caballero and López-Goicoechea, 1980). Economic analysis of the fitted quadratic curves indicated that optimum rates were in the range of 70-100 kg N ha^{-1} cut^{-1} for the three fertilized cuts. In recent years, low-input agricultural practices have been promoted with emphasis on forage legumes (BGS, 1989). Reliance on high levels of fertilizer N has reduced the efficiency of use of renewable resources such as biologically fixed nitrogen (Camlin *et al.*, 1983).

A mixture of Italian ryegrass and berseem clover (*Trifolium alexandrinum* L.) has been promoted in some Mediterranean countries as a way of improving forage yields compared with pure berseem stands or enhancing silage or haymaking suitability (Ameziane, 1979; Badawy *et al.*, 1976). Nevertheless in frost-free growing conditions such as in Egypt (Radwan *et al.*, 1977) pure berseem clover produced higher seasonal yields than its mixtures with Italian ryegrass.

In the experiment reported in this paper, berseem clover was mixed with Italian ryegrass with the aim of assessing the ability of berseem to withstand competition and winterhardiness and to evaluate the response of mixtures to fertilizer N in comparison with that of pure ryegrass stand. The ultimate aim of our experiment was to test the possibility of obtaining good dry matter yields of quality forage with a lower amount of fertilizer N by mixing berseem clover with Italian ryegrass.

Material and methods

Field and crops conditions

The experiment was conducted during three growing seasons (1985-86; 1986-87 and 1987-88) at La Poveda Field Station near Madrid. The main climatic conditions during the growing seasons are described in Table 1. The main characteristics of the alluvial soils are shown in Table 2. The study was conducted under irrigation by sprinkling at variable rates, in relation to spring rainfall, up to the field capacity of the soils.

Barley stubble was ploughed under and after common seedbed preparation, 650 kg ha^{-1} of an 8:15:15 (N-P_2O_5 -K_2O) compound were applied. Seeds of tetraploid Italian ryegrass cv. Tewera and commercial berseem clover of Egyptian origin were broadcast between September 18 and September 29 at the rate of 900 seeds m^{-2}. Mixtures were established at 50:50 seed ratio computed from seed number.

Experimental design

A completely randomized experimental design with three replications was laid out each year with species (pure Italian ryegrass stand or Italian ryegrass plus berseem clover) and N rates (0, 30, 60, 90 and 120 kg N ha^{-1} cut^{-1}) acting as main factors of analysis in the statistical model. Mean date of the first cut was February 13, with both Italian ryegrass and berseem clover at the vegetative stage. Before this date, plots of both crops did not receive differential N rates as unfavourable climatic conditions limited crop growth and N application could have enhanced N leaching to groundwater. The plots measured 25 m × 3 m with strips of 2 m between them. Fertilizer N was applied at the stated rates as ammonium nitrate (33% N).

Crop controls

Plant establishment in both crops was assessed on October 31 and just prior to the first cut (February 13), allowing for calculation of winter survival rate. Winterhardiness was assessed by controlling the plants with frost-damaged leaves. All controls were made in two 0.5 m × 0.5 m quadrats per plot. By the first cutting date, mean of 52 frost-days were registered. Inside the plots, yields were measured in a central strip of 21 m × 1 m by harvesting above ground biomass with a scythe at 4 cm stubble.Two 0.5 m × 0.5 m quadrats were harvested to assess the botanical composition by separating total biomass into its species components. Dry matter (DM) content of Italian ryegrass, berseem

Table 1. Mean meteorological data during the crop growth seasons. Station 182 E. Madrid

Month	1985-86			1986-1987			1987-88		
	R[a]	D	M	R	D	M	R	D	M
September	0.0	0	23.4	54.7	0	20.7	8.0	0	22.6
October	0.0	0	16.4	122.0	0	15.1	75.2	0	13.8
November	46.0	9	9.3	15.0	13	10.7	32.0	12	9.2
December	106.0	15	6.0	19.2	23	5.3	77.8	6	9.0
January	19.5	21	5.4	87.0	20	4.8	50.2	5	8.0
February	101.0	10	7.1	50.5	11	7.2	17.0	11	7.6
March	36.5	10	9.4	4.0	7	10.9	0.0	9	10.5
April	68.2	6	8.8	56.0	0	13.5	79.0	1	12.5
May	21.0	0	17.8	18.0	1	15.8	50.0	0	15.5
June	0.0	0	21.3	4.5	0	18.5	99.5	0	18.2

[a]R = Rainfall (mm); D = Days with frost; M = Mean air temperatures(ºC).

clover and adventitious species was evaluated following drying in an air-forced oven at 80°C for 24 h.

After the first cut, differential N rates were applied to plots and the same procedure was followed after the 2nd and 3rd cuts. Thus we had the first cut without and three subsequent cuts with influence of fertilizer N. Mean dates of the three last cuts were April 18, May 25 and June 20 respectively, with a maximum range, of 14 d for the 3rd cut between the first and 2nd growing seasons. Harvesting stages of both species were vegetative (2nd cut) to emerged inflorescences (3rd and 4th cuts) in Italian ryegrass, and vegetative (2nd cut), early flowering (3rd cut) and full flowering (4th cut) in berseem clover.

Table 2. Mean properties of experimental soils

Texture	Sandy-loam
pH (H_2O)	7.75
C/N ratio	7.92
Organic matter (%)	1.65
N (%)	0.121
$CaCO_3$ (%)	4.15
Available P (ppm)	113.7
Available K (ppm)	127.3

Analytical techniques

Samples from the three last cuts in 1985-86 were randomly selected for chemical analysis of individual species. Samples were dried at 60°C for 22h. and subsequently at 80°C for 4 h. N content was analyzed by a colorimetric method (Law *et al.*, 1971) after Kjeldahl digestion. Italian ryegrass samples of the 2nd harvest in 1987-88 were analyzed for total N, non-protein N (NPP) (Goswami and Willcox, 1969) and NO_3-N (Humphries, 1956).

Statistical analysis

Analysis of variance was carried out on a per-plot basis. Cuts were combined within a year and yearly totals over the three years were computed (Little and Hills, 1978). Quadratic vs linear regression analysis was performed yearly within seeding components using non linear statistics (Draper and Smith, 1966). A F-statistic was calculated to test the fit of higher degree polynomial vs linear regression. As no difference between years was found, pooled data on species × cuts were used in the regression of the herbage production upon the fertilizer nitrogen.

Results

Plant establishment and winterhardiness

By the time of the first cut, there were no significant differences over the three years in Italian ryegrass establishment, either in pure stand (33% of planted seeds) or combination with berseem clover (36%). Total plant establishment in the mixture (31%) showed no significant difference with that of Italian ryegrass. On the average, 88% of surviving berseem clover plants had

Table 3. Mean effect of N levels (kg N ha^{-1} cut^{-1}) on herbage production (t DM ha^{-1} cut^{-1}) over three years

	0	30	60	90	120	SE(±)
Italian ryegrass						
Total DM	1.61	2.41	3.12	3.87	4.01	0.16**
Italian ryegrass plus berseem clover						
Total DM	2.25	2.98	3.52	4.00	3.87	0.12**
Berseem DM	0.61	0.46	0.45	0.31	0.29	0.05**
Clover (%)	27.1	15.4	12.8	7.8	7.5	2.62**

leaves damaged by the frost, but winter survival was 86%.

Plant proportions

Mean relative biomass proportions of Italian ryegrass were 89.0, 90.1, 83.9 and 78.1% at the four successive cuts. Adventitious species which showed a mean proportion of 5.6% in the first cut almost disappeared in the 3rd and 4th cuts. Significant differences were found in the proportion of both species as related to the effect of fertilizer N. Mean proportions of Italian ryegrass increased steadily at increasing rates of N from 71.7 at 0 to 91.5% at 120 kg N ha^{-1} cut^{-1} (Table 3)

Forage production

The year or year/species effects showed no significant difference in DM yields. Forage production was unevenly distributed among consecutive cuts. Differences were found between mid-season cuts (2nd and 3rd-cuts) and both 1st and 4th-cuts. Both types of sward showed a similar trend as related to the cutting sequence. Significant differences were found in the DM yields of both types of sward at different rates of N (Table 3).

The total mean forage production in the three N fertilized cuts amounted to 11.32 and 11.94 t DM ha^{-1} in Italian ryegrass and Italian ryegrass plus berseem clover respectively. Significant differences between the pure stand and the mixture were observed up to 0, 30 and 60 kg N ha^{-1} cut. Within sward types differences between 60 and 90 kg N ha^{-1} cut^{-1} were significant in Italian ryegrass but not in the mixture. Between sward types, the difference between Italian ryegrass at 90 kg N ha^{-1} cut^{-1} (3.87 DM ha^{-1} cut^{-1}) and Italian ryegrass plus berseem clover at 60 kg N ha^{-1} cut^{-1} (3.52 t DM ha^{-1} cut^{-1}) was significant only at the 0.05 probability level (Table 3).

The total DM production over the four cuts in plots with total applications of 0,90, 180, 270 and 360 kg N ha^{-1} a^{-1} were 7.14, 9.51, 11.6, 13.91 and 14.36 t DM ha^{-1} a^{-1} in Italian ryegrass pure stands. Corresponding values from the mixture of Italian ryegrass plus berseem clover were 8.80, 10.94, 12.60, 14.05 and 13.64 t DM ha^{-1} a^{-1}. The differences in DM yield between the 90 kg N ha^{-1} cut^{-1} Italian ryegrass and the 60 kg N ha^{-1} cut^{-1} mixture were slightly increased due to the difference in DM yield at the 1st non-fertilized cut, between the grass alone (2.30 t DM ha^{-1}) and the mixture (2.03 t DM ha^{-1}).

Fertilizer N equivalence and N effect

Fertilizer N effect showed decreasing values in the 2nd and 4th-cuts as rate of N increased. In the 3rd-cut, the sward response fell significantly only at the level of 120 kg N ha^{-1} cut^{-1} in both Italian ryegrass and the mixture. For any given range of increase in N fertilizer rates, the effect was higher in Italian ryegrass than in Italian ryegrass plus berseem clover (Table 4).

Plotting the total DM production in the three N-fertilized cuts, showed that herbage production in Italian ryegrass at 77.5 kg ha^{-1} a^{-1} was equivalent to DM production of Italian ryegrass plus berseem clover without added nitrogen (Fig. 1).

Regression analysis

When DM yields were regressed on N levels, quadratic curves almost always lowered the residual mean square as related to the corresponding linear regression. Nevertheless, differences were found between sward types and successive cuts within sward types. N levels explained the variations in DM yield better in Italian ryegrass (mean $R^2 = 0.77$) than in the mixture (mean $R^2 = 0.63$). In the mixture the goodness of the fit of the equations became less as cuts progressed.

Table 4. Yield response (kg DM kg N^{-1}) as related to range of N rates (kg N ha^{-1} cut^{-1}) and cutting sequence

	0-30			30-60			60-90			90-120		
	Apr18	May25	Jun20	Apr18	May25	Jun20	Apr18	May25	Jun20	Apr18	May25	Jun 20
Italian ryegrass	24.3	30.0	24.7	20.0	29.7	22.0	13.7	43.7	17.7	3.67	7.7	3.7
Italian ryegrass plus berseem clover	33.0	16.0	22.3	17.7	23.0	6.0	19.7	22.7	14.7	-2.7	-4.0	-7.0
					SE (±) 2.53							

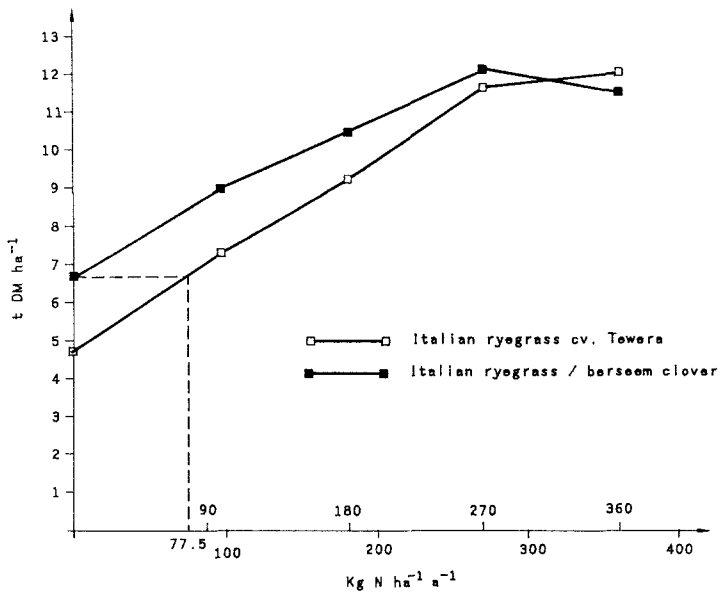

Fig. 1. Total herbage production of three accumulated cuts from Italian ryegrass and Italian ryegrass/berseem clover (mean of three years).

Table 5. Regression ($Y = a + bX + cX^2$) of the herbage production (kg DM ha^{-1}) upon the fertilizer nitrogen rates (kg N ha^{-1} cut^{-1})[a]

	n	a	b	c	R^2	Sy.x	Significance
Italian ryegrass							
Apr 18	9	2303	29.35	-0.11	0.71	505	**
May 25	9	1584	38.40	-0.0	0.86	579	**
Jun 20	9	827	30.65	-0.10	0.73	500	**
Italian ryegrass plus berseem clover							
Apr 18	9	2215	35.78	-0.15	0.77	439	**
May 25	9	2821	21.80	-0.052	0.61	619	**
Jun 20	9	1607	23.61	-0.12	0.51	481	**

[a]N rates: 0, 30, 60, 90 and 120 kg N ha^{-1} cut^{-1}

Mean R^2 showed values of 0.77, 0.61 and 0.51 for the 2nd, 3rd and 4th-cuts, respectively (Table 5). This is probably related to increasing proportions of berseem clover in the mixture as cuts progressed.

Chemical analysis

The mean nitrogen concentration in Italian ryegrass was related to both the cutting sequence and to the effect of nitrogen levels (Table 6). The N concentration of pure berseem clover was not related to the rate of fertilizer N but differences were found among successive cuts. The mean N concentration of the 2nd, 3rd and 4th-cuts were 23.3, 19.4 and 27.2 g N kg DM^{-1} for Italian ryegrass, and 39.7, 29.1 and 33.1 g N kg DM^{-1} for berseem clover The mean N concentration in Italian ryegrass increased from 16.7 to 28.7 g N kg DM^{-1} as the fertilizer N rate was increased from 0 to 120 kg N ha^{-1} cut^{-1}.

Nitrogen fractions of the 2nd-cut were analyzed in 1987-88 (Table 7). Both total nitrogen (TN) and non-protein nitrogen (NPN) increased significantly as the fertilizer N rate increased, NO_3-N was unaffected. In the 3rd cut^{-1} in 1987-88, only herbage from plots receiving 0 and 120 kg N ha^{-1} cut^{-1} was analyzed. In this case, TN varied from 13.9 to 18.6 g N kg DM^{-1}; NPN from 4.8 to 7.6 g N kg DM^{-1} and NO_3-N showed value of 0.018 g kg DM^{-1} at both N rates.

Discussion

Forage composition and production

The contribution of legumes to the herbage biomass either in pasture or fodder crops has always been a subject of concern (Frame, 1973; Wolton and Brockman, 1970), with interest in reducing fertilizer N rates and improving the quality of herbage (Frame, 1989; Frame and Newbould, 1984; Thomson, 1984). The growth of grass/white clover swards in temperate countries is impaired in early spring since temperatures are suboptimal for clover. N fertilization is thus recommended outside the clover's main growing season and at minimal strategic rates (Frame, 1989). In Mediterranean countries, mixed stands of annual forage legunes such as common vetch (*Vicia sativa* L.) and small grain cereals showed the competitive advantage of cereals (Caballero and López-Goicoechea, 1984). Italian ryegrass almost completely depressed growth of common vetch at 50:50 seed ratio (Caballero and Treviño, 1984).

In the first year of this study (1985-86) red clover (*Trifolium pratense* L.) was mixed with Italian ryegrass in similar conditions of those of berseem. Although red clover showed good plant establishment and better winterhardiness than berseem clover (37.3 vs 70.8 of frost-damaged plants) its DM proportion in the forage mixture showed mean values of only 1.5, 1.8, 2.2 and 7.7% for the four successive cuts. Red clover was thus discarded in the following years of the study as companion crop for Italian ryegrass.

The effect of fertilizer N on the DM proportion of berseem clover in the mixture showed contrasting results in successive cuts. The highest was reached at 0 N rate in the fourth cut (32%) and the lowest at 120 N in the second cut (2%). As the growing season progressed, increasing soil temperature allowed better growth conditions for berseem clover.

The depressing effect of fertilizer N on clover proportions has been observed elsewhere in grass/white clover swards (Frame, 1973; Mc Kenzie and Daly, 1982; Reid, 1983).

Some authors have shown that the production of white clover is increased as the defoliation interval is lengthened within N levels of 0-400 kg N ha^{-1} a^{-1} (Frame and Newbould, 1984; Wilman and Asiegbu, 1982). In our experiments the successive cuts represented decreasing length of cutting interval. Nevertheless the proportion of berseem increased significantly because it coincided with increasing soil temperatures.

Nitrogen effect

At the proportions of berseem attained in this work, N equivalence showed values of about 80 kg N ha^{-1} a^{-1} (Fig. 1). Frame and Newbould (1984) summarized data in the range of 125 to 280 kg N ha^{-1} a^{-1} in perennial grass vs perennial grass/white clover swards.

The mean responses of Italian ryegrass in the range of 0-360 kg N ha^{-1} a^{-1} were 15.4, 27.8 and 17.0 kg DM per kg N applied in the three successive fertilized cuts. Corresponding results for Italian ryegrass plus berseem clover showed values of 16.9, 14.4 and 9.0 kg DM per kg N applied, respectively. It is evident that N efficiency is related to the clover percentage in the mixture which showed an increasing trend as the cutting sequence progressed.

Nitrogen concentration and recovery

Increasing N concentration as fertilizer N increased has been shown by Willman and Ojuederie (1978) and Treviño *et al.*, (1980) in Italian ryegrass and

Table 6. Mean N concentrations (g N kg^{-1} DM) of three successive cuts as related to fertilizer N rate (kg N ha^{-1} cut^{-1}) 1986

Species	0	30	60	90	120	SE (±)
Italian ryegrass	16.75	21.14	23.57	26.27	28.70	0.32**
Berseem clover	34.37	34.08	33.57	33.44	34.61	
SE (±)			0.95*			

Table 7. N fraction (%) DM) of 2nd cut italian ryegrass as related to N fertilizer rate (kg N ha^{-1} cut $^{-1}$) 1988

N fraction	0	30	60	90	120	SE(±)
Total N	1.34	1.74	2.06	2.55	2.53	0.18**
Non-protein N	0.47	0.61	0.75	1.04	1.00	0.08**
NO_3-N	0.18	0.21	0.22	0.19	0.22	0.03NS

Frame (1991) in other grass species. Our results on the effect of fertilizer N on N fractions of Italian ryegrass, confirmed earlier extensive research in the subject (Treviño *et al.*, 1980). In their experiment, protein N increased by 50% and non-protein N two fold as N rate increased from 0 to 120 kg N ha^{-1} cut^{-1}. Nevertheless NO_3-N was not affected within this range. In our experiments, N concentration increased by 71% within the same range of N fertilizer rates. In contrast increasing rates of fertilizer N did not affect the N concentration of berseem clover.

The apparent recovery of the fertilizer N was calculated in 1986-87 in Italian ryegrass by subtracting the N uptake into herbage on plots receiving no fertilizer from that of those receiving fertilizer and expressing this as a percentage of the fertilizer N applied (Long *et al.*, 1991). The mean apparent recovery of the three fertilized cuts was 77.5, 81.0 and 52.2% for the N_{0-30} N_{60-90} and N_{90-120} kg N ha^{-1} cut^{-1}intervals respectively. These values showed higher percentage of recovery and no significant effect up to the rate of 90 kg N ha^{-1} cut^{-1}. Nevertheless, even at lower rates of fertilizer N there always remains an amount of unrecovered nitrogen. Apparent recovery in the mixture was not calculated as clover N content of N-fixing origin was not estimated.

In conclusion, this work showed that it is possible to reduce the use of fertilizer nitrogen on Italian ryegrass by sowing it in a mixture with berseem clover in continental Mediterranean climate. The benefit is impaired by frost damage to berseem clover and the aggressiveness of Italian ryegrass. Nevertheless, even with relatively low proportion of clover, N equivalence in the order of 80 kg N ha^{-1} a^{-1} has been obtained. Improving berseem clover winterhardiness or changing seed ratios could lead to more promising results.

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