Effects of soil type and competition on *Bituminaria bituminosa var. albomarginata* cv. LANZA® biomass production: preliminary results

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Abstract

The novel perennial legume *Bituminaria bituminosa* var. *albomarginata* is a promising forage for Mediterranean climates. It has shown outstanding drought resistance and good forage quality. Recently, an improved variety named LANZA® has been developed as a result of a collaborative breeding programme led by Australian and Spanish researchers. Further research is needed to study its adaptation to the varied environmental conditions of the Mediterranean regions. Previous research suggests that soil water saturation could limit the initial development of *B. bituminosa* var. *albomargina*. Slow initial development might compromise its successful establishment in natural grasslands, where grass species might outcompete *B. bituminosa*. This study aims to investigate the effect on the development of LANZA® in two contrasting soil textures (sandy and clay), the competition with *Lolium rigidum* and the interaction between both factors in a pot experiment with six replicates per treatment. Plants were grown in 6-litre pots for five months maintaining high soil water content. The results showed significant reduction in dry mass production of LANZA® due to *L. rigidium* competition and lower dry mass of thin rootsin clay soils.

Keywords: soil texture, *Lolium rigidum*, development, adaptation

Introduction

The use of perennial legumes in Mediterranean extensive livestock systems is recognized as one of the most promising measures to improve the sustainability of farms. Research efforts have been focused on the search for species that could meet the requirement of Mediterranean livestock systems (Rognli *et al.*, 2021). One of the most promising species is *Bituminaria bituminosa*, especially var. *albomarginata* from the Canary Islands. Previous research has demonstrated that this species is productive under very low rainfall (<200 mm) and it is suitable for feeding livestock (Mendez *et al.*, 1991; Oldham *et al.*, 2015). An improved variety named LANZA® has been developed as a result of a collaborative breeding programme lead by Australian and Spanish researchers (Real *et al.,* 2014).

There is still research to be done in order to clarify the potential adaptation of this species to the wide range of environmental conditions of Mediterranean environments. Previous research suggested that soil water saturation could be unfavourable for *B. bituminosa* var. *albomargina* (Fernández-Habas *et al.*, 2021). Also, the slow initial development of this plant might compromise successful establishment in temporary or permanent grasslands, where annual grass species might outcompete *B. bituminosa*. This study aimed to investigate the effect on cv. LANZA® in two contrasting categories of soil texture (sandy and clay), the competition with *Lolium rigidum* and the interaction between both factors.

Materials and methods

A complete block design with 6-litre pots was established in a shade house. The experiment consisted of six replicates per treatment and the treatments were: Soil type (sandy soil, denoted by S, or clay soil denoted by C) and Competition (pure culture of Lanza® and pure culture of *L. rigidum*, or Mixed culture of LANZA® and *L. rigidum*). In pure cultivation of LANZA®, one plant with three folioles was planted per pot. In pure cultivation of *L. rigidum*, two plants of *L. rigidum* with three leaves were planted per pot. In mixed cultivation one plant of LANZA® and two of *L. rigidum* were planted per pot. Sandy soil texture was: 73% sand, 13% silt and 14% clay. Clay soil texture was: 29% sand, 25% silt and 46% clay. Soil pH, cation exchange capacity and organic matter content was: 7.10, 11 (meq 100 g^{-1}) and 1.97% for sandy soil and 8.12 (1 2.5^{-1}), 31 (meq 100 g^{-1}) and 3.21% for clay soil. Both soils were mixed at 1/5 (v:v) with soil collected from natural populations of *B. bituminosa* to promote rhizobium inoculation (soil data correspond to the soil already mixed). Soil water content was maintained at a high level during the experiment (between 90-100% of soil water content at dripping point) and was controlled by weighing the pots and manually irrigating to the desired soil water content. The experiment was conducted from March to July 2021. At the end of the experiment, plants were cut to ground level. In pure cultivation, roots were also separated from soil and divided in thin (<2 mm) and thick roots (2> mm). Biomass was oven-dried at 60 °C for 72 h and then weighed. Firstly, the differences in dry mass production of pure culture (LANZA® or *L. rigidum* only) and mixed culture (summed production of LANZA® and *L. rigidum*) and by soil type were tested by two-way ANOVA. Then, we tested the shoot dry mass production of each species when cultivated without competition (Pure) and with competition (Mixed) by two-way ANOVAs independently for each species. Finally, thick and thin roots were compared between soil types in pure cultivation for each species by one-way ANOVA since separation by species in mixed cultivation was not possible. When differences were significant (P<0.05), post-hoc Tukey's test at the 0.05 level was carried out to separate homogeneous groups.

Results and discussion

Differences in dry mass production of pure and mixed cultures by soil type were not significant (Table 1). The total dry mass production of pure LANZA® culture was significantly higher than the total dry mass production of the mixture of LANZA® and *L. rigidum* and *L. rigidum* pure culture alone, whose dry mass production was significantly lower than the production of the mixture. These differences remain when shoot and root dry mass were compared separately (Table 1).

When the shoot dry mass production of each species in mixture culture was compared to their dry mass production in pure culture, LANZA® showed a significant reduction of 86% (Figure 1). Conversely, *L. rigidum* showed a significant increase of 43% of shoot dry mass production in mixed culture. A slower development of LANZA® in clay soils was observed during the initial stages, although the shoot dry mass at the end of the experiment showed no significant differences by soil type in either pure or mixed culture (df=1; F=3.97; P=0.06) (Figure 1).

Table 1. Pure and mixed dry mass of LANZA® and *Lolium rigidum* in sandy (S) and clay (C) soil types

Culture	Soil type	Total dry mass (g)	Shoot dry mass(g)	Root dry mass(g)
LANZA®	S	48.1±3.8 a	32.0±2.1 a	16.1±2.0 a
	C	38.1±5.1 a	26.3±3.3 a	11.8±1.8 a
LANZA® and *L. rigidum*[1]	S	26.1±5.0 b	17.0±2.6 b	9.1±2.6 b
	C	26.9±4.3 b	19.7±2.4 b	7.2±2.0 b
L. rigidum	S	11.4±0.8 c	9.4±0.5 c	2.0 ±0.3 c
	C	12.9±1.5 c	10.7±1.0 c	2.2±0.5 c

[1] Summed dry mass of LANZA® and *L. rigidum*.

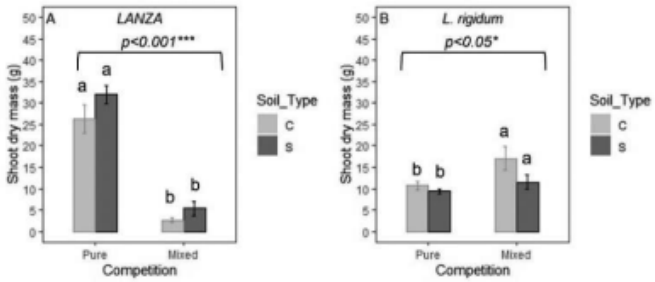

Figure 1. Effect of soil type and competition on shoot dry mass of LANZA® and *Lolium rigidum*; *P*-values indicate the main effect of competition. Level of significance: *P*<0.001***; *P*<0.01**; *P*<0.05*. *Pure* refers to cultivation without competition and *Mixed* refers to cultivation with competition.

LANZA® showed no significant difference between both soil types in thick roots dry mass. However, the dry mass of thin roots of LANZA® was 44% significantly lower when cultivated in clay soils while the soil type did not affect the dry mass of thin roots of *L. rigidum*. *L. rigidum* did not produce thick roots (2> mm).

These results suggest that LANZA® in pure cultivation could be more productive than when mixed with annual grasses. The establishment and production of LANZA® can be compromised when competing with annual grasses. This might be a major limitation for this species to be introduced into Mediterranean permanent grasslands where annual grasses are abundant (Olea and San Miguel, 2006). It also highlights the importance of avoiding competition in the initial phases of development to achieve a proper establishment in temporary grasslands.

Although shoot dry mass and total root dry mass production were unaffected by soil type, the lower dry mass of thin roots of LANZA® in clay soils indicates that sandy soils could be more suitable for its development. Given the possible effects of the culture in pots on the natural development of the plants, further field-scale trials are needed to verify these results.

Conclusions

Further research is needed to investigate measures aimed at reducing early competition of grasses and forbs to successfully establish LANZA®. The higher dry mass of thin roots of LANZA® in sandy soils shows a better adaptation of this species to poor sandy soils.

Acknowledgements

This study was funded by the Horizon 2020 project SUPER-G (Sustainable Permanent Grassland, grant agreement 774124).

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