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RESEARCH ARTICLE

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Vicia narbonensis-Avena strigosa mixture, a viable alternative in rainfed cropping systems under Mediterranean conditions

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Abstract

The demand of vegetable protein for animal feed and the need to diversify the crop rotation in rainfed Mediterranean climates has led to study the viability as new forage crop of the *Vicia narbonensis-Avena strigosa* mixture. Therefore, a 3-year field trial was conducted at two different and representative locations of the area to evaluate the capacity of both species to form a balanced mixture and to define its potential for high yield and forage quality. Different seeding ratios (65:35, 50:50 and 35:65) were compared with their pure stands and the standard mixture *Avena sativa-Vicia sativa* (65:35). Forage mixtures establishment and growth varied according to the environmental conditions with a negative influence of a dry year 1 for legumes (<300 mm) and a wet year 2 for oats (>630 mm). However, competition ratio indicated that there were not significant differences between mixed treatments, displaying intermediate ground coverage values, dry matter yield and crude protein regarding pure stands. Higher dry matter yield than control mixture in addition to similar crude protein, acid-detergent fibre, neutral-detergent fibre and digestible dry matter values, were produced at 35:65 in dry years and 65:35 and 50:50 in rainy years with loamy and clay soils, respectively. The appropriate development of both species in the mixture at different soil and rainfall conditions, as well as a good yield often higher than control mixture and a great forage quality, confirm to the narbon bean-black oat mixture as a viable and profitable crop alternative in rainfed cropping systems under Mediterranean conditions.

Additional keywords: black oat; narbon bean; mixture rates; intercropping; dry matter yield; forage quality.

Abbreviations used: ADF (Acid-Detergent Fibre); CAP (Common Agricultural Policy); CP (Crude Protein); CR (Competition Ratio); DAS (Days After Sowing); DDM (Digestible Dry Matter); DM (Dry Matter); GDD (Growing Degree Days); NDF (Neutral-Detergent Fibre).

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Introduction

Rainfed arable areas occupy over 28 million hectares in southern Europe, which are mainly devoted to cereal-industrial crop rotation (Eurostat, 2015). Nowadays, this traditional practise is facing the urge to modify the current crop management and to diversify the crop production. They are especially influenced by the stagnating products price, the need to improve and achieve more balanced agro-ecosystem through the crop rotation (Flower *et al.*, 2012), as well as the accomplishment of the new Common Agricultural Policy (CAP) greening measures. The most noteworthy measure included is the 'crop diversification', where the farm holdings with an arable land of 10-30 ha have to cultivate at least two crops, and farms exceeding 30 ha must have at least three crops, with no more than 75% of the cultivated area assigned to one crop. Moreover, Southern Europe is also a great livestock region with an important number of heads of cattle (over 14 million) and sheep (over 24 million) next to the rainfed arable crops areas. The major constraint of the livestock development in these regions is the dependence of imported powerful food sources due to the limited on-farm production of forage, especially in the dry years of Mediterranean countries (Sadeghpour *et al.*, 2014). Therefore, it is necessary to satisfy the increasing demands for livestock (Alizadeh & da Silva, 2013) and there is the possibility of obtaining supplementary forage sown with arable crops in Southern Europe. Intercropping of winter grasses with annual legumes is extensively used in Mediterranean areas for forage production under rainfed conditions (Lithourgidis *et al.*, 2006). This system increases fodder yield concerning dry weight thanks to the grasses (Hashemi *et al.*, 2013) and improves feed quality owing to the higher crude protein concentration of legumes (Umunna *et al.*, 1995).

The environmental conditions (Gierus *et al.*, 2012) in addition to the seeding ratios of the selected species (Caballero *et al.*, 1995) are crucial factors affecting yield and quality of intercropping systems. The vetch (*Vicia sativa* L.)-oat (*Avena sativa* L.) mixture is the most traditionally used in Southern Europe and many studies have reported that these species are the most appropriate for mixture in Mediterranean regions (Lithourgidis *et al.*, 2006). Nevertheless, common vetch is described as susceptible to broomrape (*Orobanche crenata* Forsk.) (Gil *et al.*, 1987), one of the most serious legume pests in Mediterranean areas. However, findings of Nadal & Moreno (2007) revealed that narbon bean (*Vicia narbonensis* L.) is a high-yielding annual legume with an inbreed line resistant to broomrape. It would be a good alternative for cropping in broomrape-infested areas under rainfed Mediterranean conditions. In these areas it is also known for being a promising crop in rotation with wheat (Durutan *et al.*, 1990) and a good source of protein fodder for cattle (van der Veen, 1960) and sheep (Jacques *et al.*, 1994). On the other hand, Flower *et al.* (2012) showed that black oat (*Avena strigosa* Schreb.) is a winter cereal crop with a more rapid growth and higher biomass production than other oats, widely used in Australia (Flower *et al.*, 2012) and USA (Reeves & Price 2005). It has been used not only for grain production and as a green cover crop due to its weed control potential (Santos *et al.*, 2010), but also to obtain forage for dairy cows feed (Salgado *et al.*, 2013).

Given the capacity for high dry matter and protein yield documented in rainfed conditions of each species separately, mixtures of narbon bean with black oat could be a promising alternative for increased forage production in these areas. However, despite the fact that there is much published information on the forage yield and quality of legume-grass intercropping system worldwide, no information exists on the adaptation of intercropping black oat-narbon bean, seeding ratios, forage yield and quality both in Mediterranean regions and overseas.

In order to provide a new viable rotation crop alternative based on the black oat-narbon bean mixture, two main objectives were considered in this study: (i) determine if a stable forage mixture for balanced growth of the two species can be formed by narbon bean and black oat at different seeding ratios (65:35, 50:50 and 35:65) compared with the pure stands and the common vetch-common oat mixture (65:35), and (ii) define if the studied mixture can achieve a high forage yield and quality production under rainfed Mediterranean conditions.

Material and methods

Experimental design and field procedures

Field studies were conducted during the 2011/2012, 2012/2013 and 2013/2014 growing seasons (hereafter year 1, 2 and 3, respectively) at two different locations representing the typical soils occupied by rainfed arable crops in Southern Spain. The experiments were established on a Vertisol clay soil (*Chromic Haploxerert*) at the 'Las Torres-Tomejil' experimental farm (hereafter Tomejil) in Carmona (Sevilla) (37º24'07''N, 05º35'10''W) and an Entisol loam soil (*Typical Xerufluvent*) at the 'Alameda del Obispo' experimental farm (hereafter Alameda) located in Córdoba (37º51'42''N, 04º48'00''W). Soil samples were collected at 20 cm depth and analysed showing that Tomejil soils have higher pH (7.9 *vs.* 7.61), organic matter content (1.67 *vs.* 1.15 %), P (Olsen) (24 *vs.* 12.2 ppm) and K (634 *vs*. 289 ppm) than those at Alameda. Mean monthly temperature and rainfall were monitored by the Weather Station located *in situ* at each location (Fig. 1), being obtained the mean daily temperature by averaging minimum and maximum air temperatures. Cumulative rainfall during the 3-year study in addition to the cumulative 10-year average rainfall from October to June was included. The highest rainfall amounts were recorded in year 2 (784 mm and 636 mm in Alameda and Tomejil, respectively) and the lowest values in year 1 (283 mm and 220 mm, respectively). The year 3 showed intermediate values (443 mm and 374 mm) without significant differences with the 10-year average rainfall (613 mm and 519 mm).

Species studied were narbon bean (*Vicia narbonensis* L., an inbreed line from the IFAPA Germplasm Collection) and black oat (*Avena strigosa* cv. *Saia*) besides the traditional common vetch (*Vicia sativa* cv. *Vaguada*)-common oat (*Avena sativa* cv. *Chimene*) used as control mixture. Narbon bean: black oat seeding

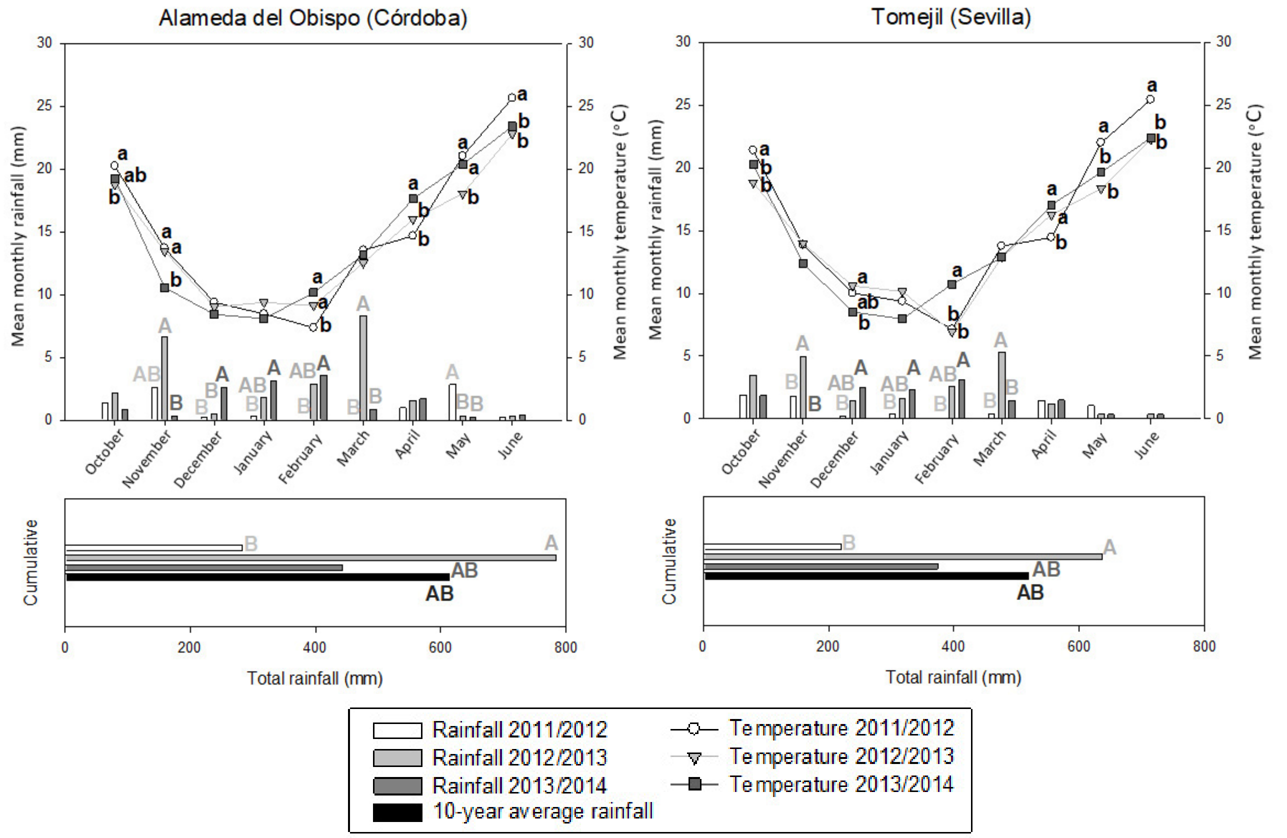

Figure 1. Mean monthly temperature and rainfall in addition to total rainfall during all the growing seasons and the cumulative 10-year average rainfall from October to June. Only significant differences between years are shown as different small letters within each monthly temperature per column and different capital letters within each monthly and cumulative rainfall per column (Tukey's, $p \leq 0.05$).

ratios were 100:0 (D1), 65:35 (D2), 50:50 (D3), 35:65 (D4) and 0:100 (D5). They were established creating a proportional mixture according to the most common seeding rates of the pure stands of each species in the area and increasing and/or decreasing respectably in 35, 50 and 65 %. Seeding rates for each treatment are given in Table 1. Common vetch: common oat seeding ratio was 65:35 (C) based on the traditional sowing rate in the study area. The experimental layout consisted of a randomized complete block design with six treatments and four replicates. The total number of elementary plots was 24 with a size of 24 m^2 (2 m × 12 m).

The previous crop was durum wheat harvested in mid-June each growing season except the year 1 when

Table 1. Seeding rates and 1000 seed weight for pure stands and mixtures in each legume-grass seeding ratio treatment.

Species	Seeding rates (kg/ha)						
	D1 100:0	**D2 65:35**	**D3 50:50**	**D4 35:65**	**D5 0:100**	**Species**	**C 65:35**
V. narbonensis	140	91	70	49	0	*V. sativa*	91
A. strigosa	0	24.5	35	45.5	70	*A. sativa*	49
			1000 seed weight (g)				
V. narbonensis			226.5			*V. sativa*	72.70
A. strigosa			19.43			*A. sativa*	25.25
			Seeding rates (seeds/m^2)				
V. narbonensis	61	40	30	21	0	*V. sativa*	125
A. strigosa	0	126	180	234	360	*A. sativa*	194

a fallow land preceded the trial in Alameda. Before sowing, the land was ploughed and harrowed, and the seedbed was prepared with a vibro-cultivator pass. All plots were hand sowed in 10 lines spacing 200 mm and the seed uniformly distributed by qualified staff. Soil was not fertilized and weeds were controlled by hoeing. Sowings were performed after the first autumn rains on mid-November in 2011 and 2012 and early November in 2013 in both locations.

Assessments

Plant density and ground cover were evaluated for each component of the mixtures separately. Plant density was estimated counting the established plants in each seeding rate treatment 22 and 32 days after sowing (DAS) in 12 randomly selected 0.1 m^2 area of each plot during the three years. Plants with the first pair of true leaves were considered established plants. Plant ground coverage was determined photographically based on the methodology described by Laflen *et al.* (1981). Five random photographs per plot were taken with a camera at the height of 1.5 m above the frame during 5 dates from January to April over the cover (from 60-65, 85-90, 105-115, 130-135 to 145-160 DAS). Total percentage ground cover was determined by counting the different species coverage in each treatment using a digital grid with 100 crossing points. The template points coinciding with green parts of each species came to their percent coverage in each photo.

Phenological events for species were determined based on the maximum and minimum daily temperatures to calculate growing degree days (GDD) with a 0ºC baseline temperature for narbon bean (Mwanamwenge *et al.*, 1999) and black oat (Bauer *et al.*, 1984) and based on the BBCH scale (Lancashire *et al.*, 1991) of faba bean and cereals.

Dry matter (DM) yield for the different mixed treatments was also assessed at the time of harvesting. All plants were harvested on the same day in mid-April each year, on the closest stage to the optimum phenological development in both species according to Muslera & Ratera (1991), at the flat pods stage for legumes (71-78 stage in the BBCH-scale) and early milk stage for grasses (71-73 stage). Two biomass samples were randomly collected from a 0.5 m^2 area of each plot by cutting at 5-7 cm above ground level with a sickle. Species were separated by hand, and the weight after drying for 48 h in a forced air oven at 70 ºC was determined.

The effect of competition between the two species used in the mixture was calculated using the competition ratio (CR) which indicates the degree to which one species competes with the other in an intercrop. The CR is calculated according to the following formula (Lithourgidis *et al.*, 2011):

$$CR_{nbean} = \left(\frac{Y_{nbeani}/Y_{nbean}}{Y_{boati}/Y_{boat}}\right)\left(\frac{Z_{boati}}{Z_{nbeani}}\right)$$

$$CR_{boat} = \left(\frac{Y_{boati}/Y_{boat}}{Y_{nbeani}/Y_{nbean}}\right)\left(\frac{Z_{nbeani}}{Z_{boati}}\right)$$

where Y_{nbean} and Y_{boat} are the yields of narbon bean and black oat, respectively, as monocrops and Y_{nbeani} and Y_{boati} are the yields of the species as intercrops. Z_{nbeani} and Z_{boati} are the sown proportion of intercropped narbon bean and oat respectively.

For determining the forage quality, 50 g from the oven-dried samples were used for the control and mixed treatments. Crude protein (CP) content and CP yield according to AOAC (1990) were determined. Besides, the study included acid-detergent fibre (ADF) and neutral-detergent fibre (NDF) values according to Goering & Van Soest (1970) and digestible dry matter (DDM) content according to the Tilley & Terry method modified by Alexander & McGowan (1966).

Statistical analysis was done by combined analysis of variance (McIntosh, 1983). The comparison of mean values was estimated using a randomized complete block experimental design for each dependent measured variable influenced by the treatments (fixed factor) at the two field locations and the three years (random factors). The fixed factor 'treatment' represented the different crop mixtures (D1, D2, D3, D4, D5 and C). The random factors 'location' and 'year' represented the effect of the environmental variables 'geographical location and soil features' (Alameda and Tomejil) and 'rainfall and temperature each year' (year 1, 2 and 3). Differences between treatments were submitted to contrast analysis. The overall variance was partitioned by orthogonal contrasts to assess differences between pure stands-intercrops treatments and between control-mixed treatments. Contrast coefficients are shown in each row of Table 2. Differences between means were compared using the Tukey test at the 0.05 probability test including in the STATISTIX 9 program.

Results

Effect of environmental conditions

Statistical analysis showed an interaction between the factors year and location in all variables evaluated, except for *Avena* plant density and the DDM that all

Table 2. Application of orthogonal constrasts to the forage mixtures experiment.

Contrast	Treatment					
	D1 100:0	D2 65:35	D3 50:50	D4 35:65	D5 0:100	C 65:35
Pure *A. strigosa vs.* Narbon bean-black oat mixtures	0	-1	-1	-1	3	0
Pure *V. narbonensis vs.* Narbon bean-black oat mixtures	3	-1	-1	-1	0	0
Control *vs.* Narbon bean-black oat mixtures	0	-1	-1	-1	0	3

Table 3. Summary of ANOVAs for testing the effect of treatments, year, location and interactions between factors on the different variables evaluated.

Variables evaluated	Treatments[1] (TR)	Year[2] (Y)	Location[3] (L)	TR × Y	TR × L	Y × L
	F					
Plant density						
Vicia	20.20***[4]	3.79*	13.87**	0.10 n.s.	0.66 n.s.	12.81***
Avena	35.53***	364.35***	52.40***	8.28**	0.64 n.s.	2.20 n.s.
Ground cover						
Vicia	70.62***	367.84***	102.78**	1.76 n.s.	1.24 n.s.	119.44***
Avena	10.59***	155.81***	122.01***	0.23 n.s.	0.16 n.s.	60.44***
CR						
Vicia	1.14 n.s.	10.79***	11.69**	0.74 n.s.	1.14 n.s.	37.86***
Avena	0.47 n.s.	10.79***	1.75 n.s.	0.44 n.s.	0.85 n.s.	4.39*
DM yield	0.69 n.s.	141.61***	185.90***	2.68 n.s.	0.32 n.s.	155.76***
CP content	59.91***	20.64***	0.27 n.s.	8.08**	1.22 n.s.	18.56***
CP yield	3.14 n.s.	35.26***	125.54***	1.52 n.s.	0.50 n.s.	141.85***
ADF	1.69 n.s.	427.69***	43.10***	0.70 n.s.	0.02 n.s.	29.64***
NDF	5.14*	175.84***	23.95***	1.23 n.s.	0.11 n.s.	13.89***
DDM	7.39*	248.57***	20.88***	0.73 n.s.	0.05 n.s.	1.49 n.s.

[1]Treatments: D1, D2, D3, D4, D5, C. [2] Year: year 1 (2011/2012), year 2 (2012/2013), year 3 (2013/2014). [3] Location: Tomejil, Alameda. [4] p <0.05*, p <0.01**, p <0.001***, n.s.: not significant

years showed the highest values in Alameda and Tomejil, respectively (Table 3). However, there was no interaction between treatments-year or treatments-location for any variable (except for *Avena* plant density and CP content that showed treatments-year interaction). The results have been represented by each factor (year, location and treatment) separately in the tables and figures.

Study of species interaction in the mixture

The success of the mixture installation depended on the success of each species separately, which varied in relation to the narbon bean or black oat proportion in the mixture and the weather conditions in each location (Fig. 2). *Vicia* plant density results indicated the highest values in Alameda year 2 (ranged from 17 to 25 % higher) and more similar results every year in Tomejil. However, *Avena* plant density showed the highest values the year 1 (about 56-82 and 79-89 % higher in Alameda and Tomejil, respectively). *Vicia* plant density values of C were greater than those in the proposed mixture treatments all years and locations. However, *Avena* plant densities for D4 and D3 were higher or similar to C in most of the cases.

The temporal evolution of the ground coverage sole crops registered the highest values compared to the mixed treatments, with significant differences on most of the sampling dates (Fig. 3). Results for year 1 showed that ground coverage of narbon bean-mixtures decreased (6 % in Alameda and 3 % in Tomejil) from the 50 stage in Alameda and an early 30 stage (after 796 GDD accumulated) in Tomejil. Meanwhile, black oat increased (38-57 % in Alameda and 21 % in Tomejil) coinciding with an extended late winter-early spring drought (5.8 and 12.8 mm accumulated from February to March, respectively). By contrast, the narbon bean ground cover increased as black oat mixes scarcely evolved from the 35-39 stage (995-1076 GDD) during the year 2 in both locations. It was probably due to the high rainfall recorded from February to March (354 and 239 mm in Alameda and

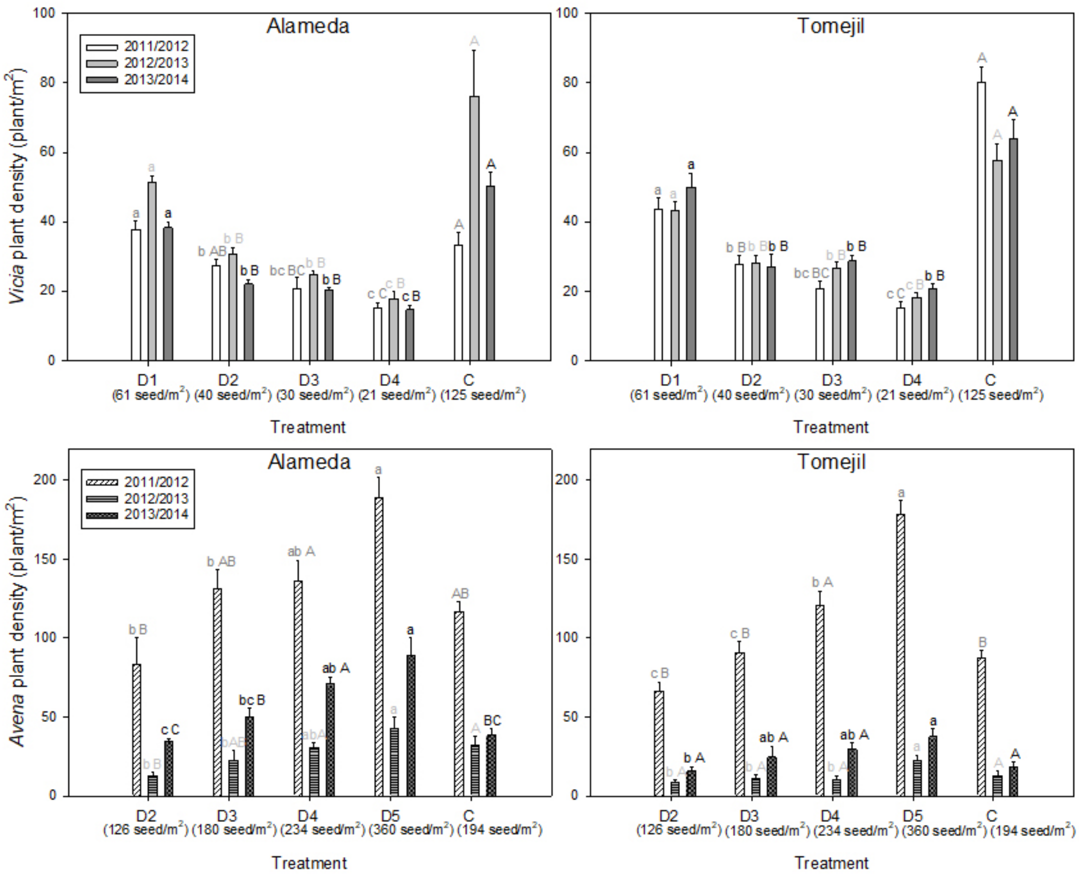

Figure 2. Plant density (plant/m^2) of the component species of the mixture (D2, D3, D4) compared to pure stands (D1 and D5) and the control mixture (C) in each treatment and location during all the growing seasons. Significant differences between species in mixture are shown as different small letters compared to pure stands and different capital letters compared to control within each treatment per column (Tukey's, $p \leq 0.05$).

Tomejil, respectively). A similar trend occurred the year 3 in Alameda, with 139 mm recorded from February to March. During year 3 in Tomejil (374 mm accumulated), both species registered the highest growth stages of the study period at the time of harvest (73 black oat stage and 75 narbon bean stage with 1700 GDD). The lowest growth stage at both locations occurred the year 1 (219-283 mm) for narbon bean (71 stage with 1479-1555 GDD) and the year 2 (636-784 mm) for black oat (69-70 stage with 1529-1633 GDD). Narbon bean species grown at D2 and D3 and black oat species at D4 showed the lower decrease compared to their pure stands in all case studies.

Forage yield and quality

All narbon bean-black oat intercrops resulted in intermediate DM yields between pure stands each year and location except for the year 3 when D2 and D3 in Alameda and D4 in Tomejil produced the highest DM yield (Table 4). Moreover, intercropping treatments were not affected by seeding ratio. Differences with C were only shown in Alameda the year 1 and 3, with the highest DM yields produced by D4 the first year and D2-D3 the third one.

Competition ratio results of both narbon bean and black oat species showed no significant differences between the seeding ratio treatments neither location nor year (Table 5). The CR was lower than unity in all the intercropped narbon bean cases except for year 2 in Alameda and year 3 in Tomejil. Conversely, CR for the intercropped black oat was significantly greater than unity in all cases except for Alameda the year 2 and Tomejil the year 3.

Crude protein content results showed that all mixed forages were enhanced by increasing the proportion of legume seeding ratio (Table 4). Mixed treatments were not affected by seeding ratio the year 1 and year 2, and this second year there were no significant differences with sole legume (D1) (from 142 to 167 g/kg of DM in Alameda and 110-139 g/kg of DM in

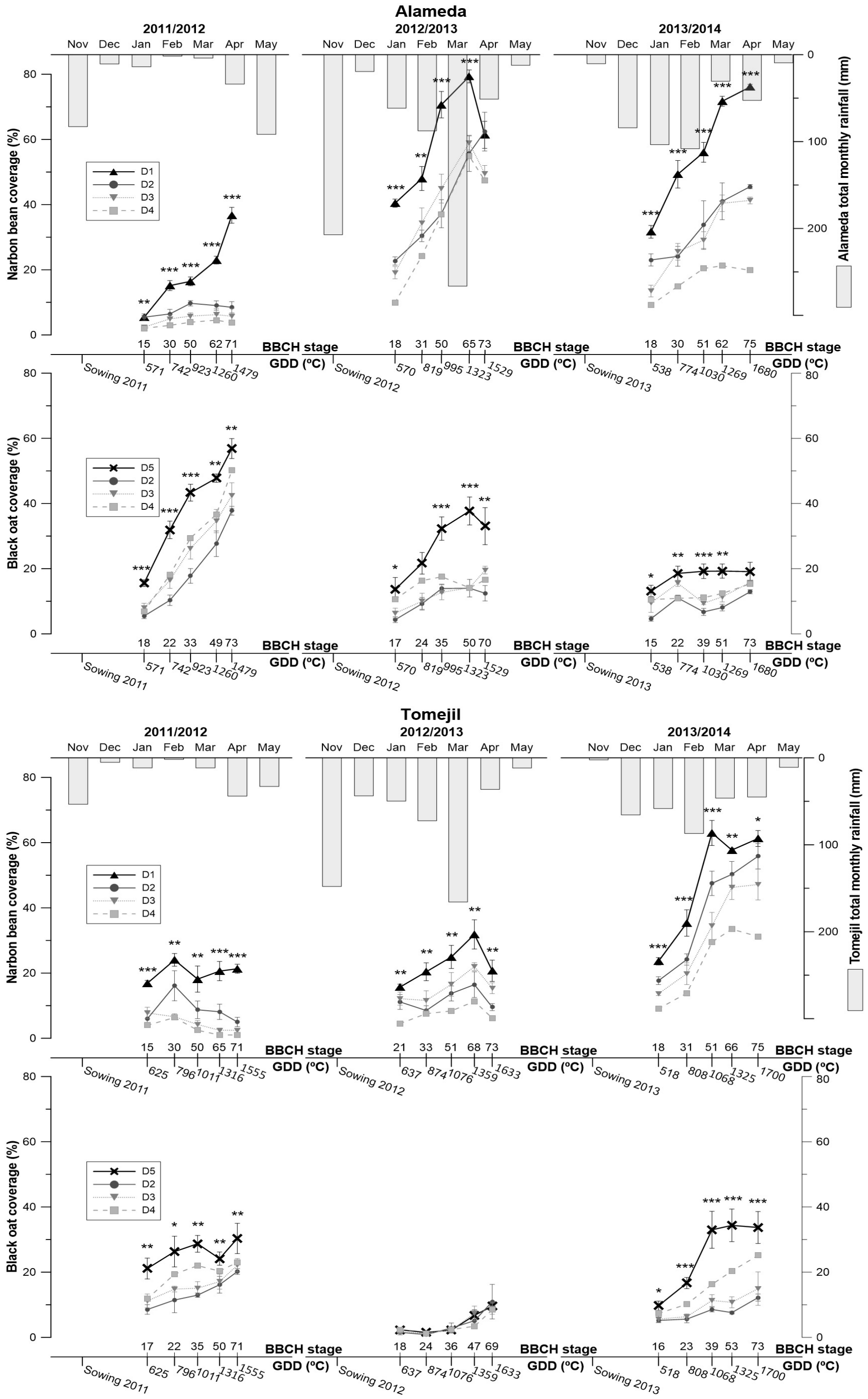

Figure 3. Temporal evolution of the monthly cumulative rainfall, phenological growth stage and ground cover mean values of the component species in mixture (D2, D3, D4) compared with pure stands (D1 and D5) in both locations all the growing seasons. Temporal scale was expressed as the growing degree days (GDD) accumulated in five different dates (60-65, 85-90, 105-115, 130-135 and 145-160 DAS) with the last interval corresponding to the harvest time. The phenological growth stage was based on the BBCH-scale for faba bean and cereals (1, leaf development; 2, side shoots/tillering; 3, stem elongation; 5, inflorescence emergence; 6, flowering; 7, development of fruit). Differences between treatments were determined by contrasts. Only significant differences are shown: * p <0.05, ** p <0.01 and *** p <0.001.

Table 4. Dry matter (DM) yield (kg/ha), crude protein (CP) content (g/kg of DM) and CP yield (kg/ha) of the different treatments during 2011/2012, 2012/2013 and 2013/2014 in Alameda and Tomejil.

Legume-oat seeding ratio	2011/2012			2012/2013			2013/2014		
	DM yield (kg/ha)	CP		DM yield (kg/ha)	CP		DM yield (kg/ha)	CP	
		g/kg of DM	kg/ha		g/kg of DM	kg/ha		g/kg of DM	kg/ha
Alameda									
100:0 (D1)	5003 b[1]	158 a	793 a	5870 a	167 a	981 a	2849a	108 a	313 a
65:35 (D2)	8668 aA[2]	89 bAB	766 aA	4549 aA	158 aA	716 aA	3020aA	88 abA	261 abA
50:50 (D3)	9452 aA	74 bcB	700 aA	4430 aA	148 aA	667 aA	2941aA	82 abcA	247 abA
35:65 (D4)	10385 aA	77 bcB	807 aA	4374 aA	142 aA	614 aA	2461aAB	79 bcA	187 abA
0:100 (D5)	11279 a	68 c	766 a	1639 b	55 b	90 b	1693a	61 cA	99 b
65:35 (C)	5724 B	119 A	686 A	5354 A	157 A	838 A	1296B	105 A	145 A
Tomejil									
100:0 (D1)	1807 b	149 a	278 a	1892 a	139 a	347 a	4153 a	133 a	572 a
65:35 (D2)	2928 abA	95 bB	282 aAB	929 abA	121 aA	113 abA	4696 aA	122abA	573 aA
50:50 (D3)	3343 abA	99 bAB	320 aAB	1407abA	122 aA	179 aA	4046 aA	124 aA	499 aAB
35:65 (D4)	3476 abA	79 bB	272 aB	556 abA	110 aA	61 abA	4753 aA	108 abA	496 aAB
0:100 (D5)	4436 a	75 b	328 a	193 b	50 b	10 b	3742 a	81 b	293 a
65:35 (C)	3884 A	128 A	496 A	707 A	125 A	89 A	3419 A	109 A	363 B

[1] Different small letters within each location per column indicate that the differences between narbon bean:black oat mixtures-pure stands were statistically significant (Tukey's, $p \leq 0.05$). [2] Different capital letters within each location per column indicate that the differences between narbon bean:black oat mixtures-control were statistically significant (Tukey's, $p \leq 0.05$).

Tomejil). During the year 3, the highest CP contents were produced by D2 in Alameda (88 g/kg of DM) and D3 and D1 in Tomejil (124 and 133 g/kg of DM, respectively), without significant differences between them. Differences between C and intercrops were only shown during the year 1. C registered the highest CP contents (119 g/kg of DM in Alameda and 128 g/kg of DM in Tomejil), but with a lower difference with D2 in Alameda (89 g/kg of DM) and D3 in Tomejil (99 g/kg of DM). Following the same previous trend, the CP yield improved as narbon bean seeding proportion increased (Table 4). All mixed treatments showed similar values to pure narbon bean. Significant differences were only found in Tomejil the year 2 (D1 got 347 kg/ha *vs.* 61 and 179 kg/ha of D4 and D3, respectively) and in Alameda the year 3 (D1 produced 313 kg/ha against 187-261 kg/ha of the mixtures). Significant differences between intercrops and C were only noted in Tomejil the years 1 (C achieved 496 kg/ha *vs.* 282 and 320 kg/ha of D2 and D3) and year 3 (when intercrops reached 496-573 kg/ha and C showed 363 kg/ha).

Table 5. Competition ratio (CR) index for narbon bean: black oat mixtures in three seeding ratios (65:35, 50:50 and 35:65) during 2011/2012, 2012/2013 and 2013/2014 in Alameda and Tomejil.

Seeding ratio	2011/2012		2012/2013		2013/2014	
	CR_{nbean}	CR_{boat}	CR_{nbean}	CR_{boat}	CR_{nbean}	CR_{boat}
Alameda						
65:35 (D2)	0.20 a[1]	5.77 a	2.28 a	0.51 a	0.38 a	1.64 a
50:50 (D3)	0.17 a	8.96 a	1.80 a	0.61 a	0.34 a	2.87 a
35:65 (D4)	0.16 a	7.69 a	2.38 a	0.43 a	0.69 a	1.48 a
Tomejil						
65:35 (D2)	0.26 a	6.93 a	0.36 a	2.93 a	1.15 a	0.71 a
50:50 (D3)	0.22 a	6.17 a	0.57 a	2.31 a	1.66 a	0.69 a
35:65 (D4)	0.09 a	8.95 a	0.88 a	1.59 a	1.60 a	0.56 a

[1]Different small letters within each location per column indicate that the differences between mixtures were statistically significant (Tukey's, $p \leq 0.05$)

Table 6. Acid-detergent fibre (ADF) (g/kg of DM), neutral-detergent fibre (NDF) (g/kg of DM) and digestible dry matter (DDM) (g/kg of DM) of mixed stands and control treatments during 2011/2012, 2012/2013 and 2013/2014 in Alameda and Tomejil.

Seeding ratio	2011/2012			2012/2013			2013/2014		
	ADF	NDF	DDM	ADF	NDF	DDM	ADF	NDF	DDM
Alameda									
65:35 (D2)	298 a	485 a	746 ab	400 ab	498 a	602 a	397 a	604 a	559 b
50:50 (D3)	297 a	507 a	743 ab	361 b	491 a	640 a	401 a	610 a	546 b
35:65 (D4)	319 a	534 a	720 b	377 ab	478 a	634 a	385 a	593 ab	577 b
65:35 (C)	228 b	384 b	831 a	412 a	514 a	639 a	368 a	529 b	668a
Tomejil									
65:35 (D2)	241 a	440 a	772 b	353 a	526 a	645 ab	415 a	552 ab	573 b
50:50 (D3)	228 ab	438 a	812 a	346 a	510 a	653 ab	420 a	566 a	578 b
35:65 (D4)	220 b	434 a	821 a	372 a	508 a	627 b	411 a	569 a	586 b
65:35 (C)	217 b	393 b	819 a	333 a	480 a	708 a	368 b	508 b	700 a

Different small letters within each location per column indicate that the differences between mixtures-control were statistically significant (Tukey's, $p \leq 0.05$)

The ADF values (Table 6) displayed significant differences between mixing ratios in Alameda during year 2 and Tomejil the year 1, with the lowest ADF contents produced by D3 and D4 (361 and 220 g/kg of DM, respectively). However, C showed lower ADF values in absolute terms all locations and years, although significant differences were only noticed in Alameda the year 1 and Tomejil the years 1 and 3. NDF contents for intercrops were not affected by seeding ratio, except for year 3 when D4 in Alameda (593 g/kg of DM) and D2 in Tomejil (552 g/kg of DM) showed the lowest values. Nevertheless, C showed lower NDF values than intercropping treatments the years 1 and 3. The highest DDM contents were shown by C all locations and years, except for D4 in Tomejil the year 1 (821 g/kg of DM) and D2 in Alameda the year 2 (640 g/kg of DM), which registered the highest values.

Discussion

The study of the species and their interaction in the mixture disclosed that it is possible to get a balanced forage mixture with narbon bean and black oat in rainfed Mediterranean conditions. However, the variables studied in order to characterize the intercropped narbon bean and black oat species (plant density and ground cover evolution) displayed the importance of the 'year' effect on the establishment and development of the species in mixture, such as was previously reported by Gierus *et al.* (2012). Although variation due to year was expected, differences of rainfall recorded were so different over the three experimental years that a negative influence of the drier year 1 for legumes and the wet year 2 for grasses was verified. Narbon bean plants were affected by drought conditions (<300 mm) and winter frost period registered from the stem elongation/inflorescence emergence stage, as also occurred in white clover-mixtures studied by Annicchiarico & Tomasoni (2010). Conversely, black oat showed lower development and growth during year 2, when total rainfall values were significantly higher than the 10-year average (784 mm and 636 mm in Alameda and Tomejil, respectively). The amount of rainfall accumulated, especially in November, February and March led to waterlogging during critical growth stage period as emergence, heading and flowering which reduced root growth and penetration and therefore production of tillers and fertile heads as was also reported by Watson *et al.* (1976). Taken together to the highest water retention capacity of the Vertisol clay soil, the decrease of oats plant density in Tomejil was high due to the adverse effects of excess moisture, as was previously reported by Urban *et al.* (2015). However, total rainfall amounts the year 3 (443 mm and 374 mm in Alameda and Tomejil, respectively) were statistically similar to the 10-year average rainfall. Both species showed a positive ground cover evolution accompanied by the highest phenological growth stage at the time of harvest and an accumulation of 1700 GDD, which was similar to the requirements showed by Mwanamwenge *et al.* (1999) in other Mediterranean climate zones.

Besides the 'year' effect, our results also showed the 'seeding ratio' important effect on the final mixture due to the individual species growth and the interaction between them (Caballero *et al.*, 1995; Lithourgidis *et al.*, 2006). In spite of the highest plant density and ground coverage reached by sole cropping treatments, the 65:35 and 50:50 seeding ratios showed the most similar trend to pure stand for the intercropped narbon bean. Moreover, the 50:50 and 35:65 displayed the greatest establishment and growth for black oat in mixture, without significant differences with the control during year 2 (even higher) and year 3. Our results also highlighted that a lower seeding rate and plant density of the proposed mixture compared to the control produced similar forage yield. This fact implies lower installation costs for farmers and an additional advantage of this mixture to include in the cropping systems since the economic feasibility of intercropping systems is ultimately determined by their monetary benefits, as was pointed out by Lithourgidis *et al.* (2011).

The absence of significant differences in CR in both components in the mixed treatments also evidenced their suitability to grass-legume mixture binary forage. The CR_{boat} was significantly higher than unity, showing the greatest competitive ability of black oat to exploit resources in association with narbon bean in all the case studies (Lithourgidis *et al.*, 2011) except year 2 in Alameda and year 3 in Tomejil, when CR_{nbean} was higher than unity in all intercrops. In all other cases, CR results for both species were positive but lower than unity, indicating that there was a positive benefit and these species can be grown in an intercrop (Dhima *et al.*, 2013). Indeed, all different narbon bean-black oat mixtures (D2, D3 and D4) showed good DM yields with similar or higher values than control mixture.

However, the fact that the species in the mixture displayed a different behaviour influenced by the weather conditions each study year, affected not only their growth and development, but also to subsequent forage yield and quality production (Caballero *et al.*, 1995). The different mixtures reached DM yields between black oat and narbon bean pure stands although similar to black oat in monoculture during year 1 when the weather conditions favoured the oats growth (Vandermeer, 1984). In contrast, during the year 2 with the highest rainfall occurring from the emergence to the flowering black oat stage, the intercrops DM yields were similar to narbon bean sole crop, especially noteworthy in Alameda. However, the intercropping system improved black oat yields the year 3 and year 2, increasing the DM yields from 20-44 % to 62-79 %, respectively, and this latter range displayed significant differences with the sole grass, as was reported by Dhima *et al.* (2013). The greatest DM yields for the intercropping narbon bean-black oat were provided by 35:65 in both locations during the drier year 1, by 65:35 the other study years in Alameda and by 50:50 and 35:65 in Tomejil. These average DM yields produced by the new mixture proposed were the highest in absolute terms. This fact could provide greater monetary advantages if farmers decide to cultivate intercropping systems, as were previously reported by Lithourgidis *et al.* (2011).

Regarding forage quality, increasing on-farm protein production it is also desirable to achieve more economically feasible farming systems (Sadeghpour *et al.*, 2014). The CP content is often considered as the most important component of forage quality (Lithourgidis *et al.*, 2007; Cazzato *et al.*, 2012), and CP yield (based on forage CP content and total DM produced) is a valuable measure of the total protein that can be harvested for livestock enterprises (Caballero *et al.*, 1995; Cazzato *et al.*, 2013). Our results showed that CP values enhanced with the seeding proportion of narbon bean in the intercrops (Sadeghpour *et al.*, 2014). The greatest CP contents were reached by the narbon bean sole crops but existing mixed treatments with similar CP values the year 2 (all mixed treatments in both locations) and year 3 (50:50 seeding ratio in Tomejil). In addition, CP contents achieved by 65:35 in Alameda the year 2 and by 50:50 in Tomejil the year 3 were higher than control mixture in absolute terms. Previous studies reported the highest CP content recorded when the legume was grown as a sole crop or formed a high proportion in intercrops with grasses (Lithourgidis *et al.*, 2007; Dhima *et al.*, 2013). Moreover, our findings showed that 65:35 and 50:50 achieved CP contents ranged from 80 to 160 g/kg of DM all years and locations, which comply with the CP content requirement for good quality forage content (Leng, 1990). The total CP yields observed in narbon bean-black oat intercrops resulted in intermediate values between monocrops, without significant differences between intercrops, except for CP yield produced the year 2 in Tomejil and year 3 in Alameda due to their lower DM forage yield which is based for measurement (Dhima *et al.*, 2013). Similar results without significant differences in CP yield were reported by Lithourgidis *et al.* (2011) for faba bean-oat intercrops in Greece. Nonetheless, 65:35 in Alameda and 50:50 in Tomejil gave the more analogous results all the years, that is, the mixing ratios with higher legume proportion in the mixture, as was reported in many other studies and constitutes the reason for including vetches in an intercropping system (Lithourgidis *et al.*, 2006; Sadeghpour *et al.*, 2014).

Important criteria for evaluating forage quality are also ADF and NDF, being ADF a better efficient predictor of forage digestibility (Caballero *et al.*, 1995; Lithourgidis *et al.*, 2007; Corleto *et al.*, 2009; Sadeghpour *et al.*, 2014). In fact, as the ADF percentage decrease, quality and digestibility of forage usually increase. Our results showed all the ADF values (ranging from 220 to 419 g/kg of DM) were in agreement with the high quality forage levels (Van Saun, 2015). In addition, ADF contents at 50:50 in Alameda and 35:65 in Tomejil were lower or similar, respectively, in some cases to those obtained by the control, all of them values consistent with those presented in previous studies for Southern Europe (Caballero *et al.*, 1995; Lithourgidis *et al.*, 2007). On the other hand, the NDF content is a good measure of voluntary intake of the feed and it is inversely related to its digestibility or energy density (Sayar *et al.*, 2014). All NDF values obtained ranged between 485-600 g/kg of DM, forming reasonably good quality forage. The lowest NDF levels were found at 35:65 in almost all the cases, without significant differences with the control treatment the year 2. Lithourgidis *et al.* (2006) reported similar results, decreasing % NDF ratio while vetch ratio declined in a mixture. Finally, high DDM contents are required by ruminants to digest the greatest portion of the dry matter in a feed intake (Van Saun, 2015). The highest DDM contents were found at 50:50 during the rainiest year 2 (639-652 g/kg of DM). Meanwhile, the control mixture showed the highest values during the years 1 and year 3, quite similar to the digestibility levels described by Caballero *et al.* (1995) but closely followed by 35:65 (from 577 to 821 g/kg of DM) in both locations.

In summary, the study of narbon bean and black oat species at different seeding ratios showed good ground cover evolution and adequate phenological growth according to the GDD accumulation despite the influence of environmental fluctuations. Plant density for the intercropped narbon bean and black oat species was lower than sole cropping and control mixture, but the CR of all intercrops did not show differences between mixed treatments and both species in monoculture. It confirms that both species despite becoming dominant under different environmental conditions can be grown successfully as an intercrop. Furthermore, narbon bean-black oat mixtures reached higher DM yields and CP yield than common vetch / common oat traditional mixture and all quality traits (CP content, ADF, NDF and DDM) complied with the high-quality forage requirements. Higher dry matter yields and similar quality values than control mixture were displayed at 35:65 with the scarcity of rain and 65:35 and 50:50 with increased rainfall recorded in loamy and clay soils, respectively. The fact that it is possible to form a balanced mixture between narbon bean-black oat and to get a forage of high quality, jointly to their adaptation capacity at different soil and rainfall conditions, makes the mixture *V. narbonensis- A.strigosa* an economically and environmentally promising option to be considered as a forage mixture for livestock diets in Mediterranean environments. Moreover, it is a viable rotation crop alternative to be implemented by farmers in the CAP greening framework for all Southern Europe countries. In order to improve the emergence and subsequent development of both species, especially in difficult environmental conditions (poor and waterlogging soils, frosty periods or drought), further research should be focused on studying the water balance and N role in the intercropping system. Under rainfed Mediterranean conditions, it would be useful to define the water use efficiency for the studied mixture as well as to determine the seeding ratio that maximizes the N_2 atmospheric fixation for improving forage production and the residual N resource for subsequent crops in low-inputs cropping systems.

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