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A second generation of pasture legumes: An opportunity for improving the biodiversity in farming systems of Mediterranean basin?

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Abstract. A screening of second generation pasture legumes was carried out through three experiments in different pedoclimatic regions of Sardinia (Italy). The main objective was to evaluate the adaptation and persistence of most new species and varieties selected in Australia. The response was quite different in relation to the site specific environmental characteristics. Some varieties shown difficulties of establishment and persistence and lower or similar forage productions compared to subterranean clovers, especially in the sites with higher edaphic and climatic limitations. For some accessions this was confirmed even with a satisfactory self-reseeding capability. The results give some indications about the potentiality of some second generation legumes for Sardinian farming systems and indicate that it may be possible to rely just on a restricted number of new varieties, accurately chosen site by site in regard of specific pedo-climatic conditions, if the main aim is the pasture improvement.

Keywords. Annual pasture legumes – Establishment – Persistence – Biodiversity.

Une seconde génération de légumineuses de pâturage : Une opportunité pour l'amélioration de la biodiversité dans les systèmes agricoles du bassin méditerranéen ?

Résumé. Un triage des légumineuses de parcours de deuxième génération a été effectué à travers trois expériences conduites dans différentes régions pédoclimatiques de la Sardaigne (Italie). L'objectif principal a été d'évaluer l'adaptation et la persistance de la plupart des nouvelles espèces et variétés sélectionnées en Australie. La réponse était très différente par rapport aux caractéristiques environnementales spécifiques des sites. Quelques variétés ont montré des difficultés d'établissement et de persistance et une production de fourrage égale ou inférieure comparée aux trèfles souterrains, particulièrement dans les sites présentant des limitations édaphiques et climatiques élevées. Pour quelques accessions, ce fait a été confirmé même avec des capacités individuelles de réensemencement satisfaisantes. Les résultats donnent quelques indications concernant la potentialité de quelques légumineuses de deuxième génération pour les systèmes d'exploitation agricoles sardes et montrent qu'il n'est possible de compter que sur un nombre restreint de nouvelles variétés, soigneusement choisies site par site en fonction des conditions pédoclimatiques, si le but principal est l'amélioration des parcours.

Mots-clés. Légumineuses annuelles de parcours – Établissement – Persistance – Biodiversité.

I – Introduction

Annual self-regenerating legumes have the important potential role to be utilised in the widest range of environmental conditions and relevant farming systems of the Mediterranean areas, because of the large number of species and the relevant diversity of their adaptive characteristics. These species were introduced to Mediterranean-type areas, particularly to Australia where annual legumes have effectively contributed to sustain and increase cereal and animal productions ("ley farming" and "phase farming" systems). But several limitations led to a serious re-examination of the traditional pasture legume components in the last decade, as subterranean clovers and annual medics, required for contemporary ley farming, which has

resulted in the recognition that alternative legume species with different traits were required (Nichols *et al.*, 2007). Many Australian cultivars of a second generation of annual pasture legumes are now commercially available, being the Mediterranean pasture seed market heavily reliant on the Australian selections often originating from the Mediterranean basin (Sulas, 2005). Such availability of a wider range of species for Mediterranean agricultural systems may be of benefit to local farmers, who could be in the position to choose from a wider range of species with different characteristics (Porqueddu and González, 2006). The question posed by pasture specialists is if these alternative pasture legumes are really of interest for European farmers. Some of the traits considered important for ley farming systems seem not so relevant for permanent pasture establishment in southern Europe (e.g. high hardseededness, insect tolerance, etc.) and preliminary results on the evaluation of these alternative pasture species in Central Italy are contrasting (Campiglia *et al.*, 2005). The aim of the research was to evaluate the potential contribution of the introduction of most new species and varieties of annual pasture legumes into the Sardinian farming systems. In this paper data on the establishment, soil covering and persistence of the introduced legumes are presented.

II – Materials and methods

Three experimental trials under rainfed conditions were carried out in Sardinia along the N-S axis and the main pedo-climatic characteristics are reported in Table 1. The climate is typical of central Mediterranean area with long-term average annual rainfall of 635, 600 and 550 mm in Chilivani, Bolotana and Oristano, respectively. A total of 27 annual self-reseeding legumes, between *Biserrula*, *Medicago*, *Lotus*, *Ornithopus* and *Trifolium* genera, were observed (Table 2). Annual medics and subterranean clovers were utilized as tests; within them, four accessions (Anglona, Campeda, Funtana Bona and Pabarile) were native. Plots were laid out in a completely randomised block design with 3 replicates; each plot was 4.5 m² in size at Oristano, and 10 m² at Chilivani and Bolotana. The plots were established in autumn, after soil ploughing and seed bed preparation, at the ordinary sowing time at each site and according to the sowing rates recommended for these varieties (ranging from 8 to 30 kg ha⁻¹ of uninoculated seed).

Table 1. Main pedo-climatic characteristics of the three experimental sites during the trial period

	Chilivani	Bolotana	Oristano
Trial duration	Sept 04-Mar 08	Sept 06-June 09	Sept 02-Jun 04
GPS position	40° 36' N, 8° 56' E	40° 16' N, 8° 58' E	40° 45' N, 8° 29' E
Altitude (m a.s.l.)	226	200	80
Climate			
Average temperature (°C)	15.2	16.3	16.6
Average rainfall (mm)	560	495	590
Average frost days (no.)	35	16	7
Maximum temperature (°C)	42.8	44.3	42.8
Soil			
Gravel (%)	15	7	–
Sand (%)	53	68	72
Silt (%)	29	12	10
Clay (%)	18	20	18
pH	5.5	6.3	6.7
Total N (g kg ⁻¹)	1.4	0.7	0.6
Organic C (g kg ⁻¹)	18	10	10
P (mg kg ⁻¹) (Olsen)	14	6	40

Table 2. Coverage (%) of the 21 varieties with soil covering rates greater than 40% at least in one out of the three experimental sites along the trial period

	Variety	Chilivani				Bolotana			Oristano	
		2004	2005	2006	2008	2007	2008	2009	2003	2004
<i>B. pelecinus</i>	Casbah					61 ^b	10 ^a	0	49 ^{abcde}	30 ^{abc}
<i>B. pelecinus</i>	Mauro	57 ^{de}	23 ^a	40 ^{bc}	26 ^{abc}				62 ^{cdefg}	79 ^{de}
<i>M. polymorpha</i>	Anglona	43 ^{bc}	35 ^{bcd}	23 ^a	40 ^c					
<i>O. compressus</i>	Avila								34 ^{ab}	59 ^{bcd}
<i>O. compressus</i>	Pabarile					96 ^c	77 ^b	60 ^b		
<i>O. compressus</i>	Santorini					66 ^b	27 ^a	38 ^{ab}	48 ^{abcde}	58 ^{bcd}
<i>O. sativus</i>	Cadiz					96 ^c	67 ^b	15 ^a	50 ^{abcdef}	20 ^a
<i>O. sativus</i>	Marguerita	48 ^{cd}	21 ^a	20 ^a	17 ^a					
<i>T. brachycalycinum</i>	Funtana Bona	57 ^{de}	90 ^f	42 ^c	40 ^c	84 ^{bc}	67 ^b	48 ^b		
<i>T. glanduliferum</i>	Prima	48 ^{cd}	43 ^d	19 ^a	26 ^{abc}	68 ^{bc}	65 ^b	50 ^b	78 ^g	77 ^{de}
<i>T. hirtum</i>	Hykon	26 ^a	23 ^a	19 ^a	23 ^{ab}				67 ^{defg}	90 ^{de}
<i>T. incarnatum</i>	Caprera								65 ^{defg}	61 ^{cd}
<i>T. michelianum</i>	Bolta	71 ^e	41 ^{cd}	23 ^a	26 ^{abc}				77 ^g	68 ^{de}
<i>T. michelianum</i>	Paradana	67 ^e	57 ^e	26 ^a	19 ^a	7 ^a	8 ^a	0	73 ^{fg}	78 ^{de}
<i>T. resupinatum</i>	Laser								51 ^{abcdef}	11 ^a
<i>T. resupinatum</i>	Nitro Plus								55 ^{bcddefg}	63 ^{cd}
<i>T. resupinatum</i>	Prolific								71 ^{efg}	85 ^{de}
<i>T. spumosum</i>	WCT36	67 ^e	23 ^a	19 ^a	38 ^{bc}				74 ^g	87 ^{de}
<i>T. subterraneum</i>	Campeda	66 ^e	90 ^f	38 ^{bc}	23 ^{ab}					
<i>T. subterraneum</i>	York								47 ^{abcd}	83 ^{de}
<i>T. vesiculosum</i>	Cefalu	50 ^{cd}	31 ^{abc}	31 ^{ab}	28 ^{abc}	59 ^b	13 ^a	13 ^a	74 ^g	94 ^e

Values among columns not followed by the same superscript (a, b, c, d, e, f, g) differ significantly ($p < 0.05$).

Before sowing, all plots were fertilized with 100 kg ha⁻¹ of P₂O₅ using triple superphosphate. No fertilizer or herbicide was applied after sowing. In the sowing year the spring sward management was oriented to maximize self-reseeding capability of introduced legumes avoiding utilization before seed ripening, except for Funtana Bona in the 2nd year, when most of the seed was harvested for starting field multiplication of such promising native accession. Legume adaptation was estimated combining observations on soil covering rate, determined by seasonal visual rating (%), with the potential contribution to the seed bank. Seeds were harvested within two sample areas of 0.125 m² for each plot in July. Both characters were subjected to multifactor analysis of variance and differences between means were compared by least significant differences for $P \leq 0.05$ (Statgraphics Centurion XV). *L. ornithopodioides* ITA, *M. murex* Zodiac, *M. sphaerocarpa* Orion, *O. sativus* Erica, *T. formosum* Portolu, *Trigonella balanse*, were not included in the analysis because they never reached soil covering rates greater than 40% at least in one of the sites. *T. resupinatum* Laser produced a negligible no. of seeds and was not included in the respective analysis.

III – Results and discussion

The response was quite different in relation to the site specific environmental characteristics. Some new Australian varieties have shown difficulties of adaptation and lower or similar forage productions compared to subterranean clovers (data not showed), especially in Chilivani and Bolotana. Both sites were characterized by higher edaphic (e.g. lower P content, shallow soils) and climatic (e.g. higher number of frost days) limitations. At Chilivani, only half of the accessions presented a satisfactory soil covering in the year of establishment; this was probably due a severe winter temperatures (Table 2). In the 2nd year just subterranean clovers assured

high coverage. Native accessions *M. polymorpha* Anglona and *T. brachycalycinum* Funtana Bona showed higher persistence. The establishment and spring growth was generally high at Bolotana, except for Paradana. Subsequently, soil covering decreased along years and only the native *O. compressus* Pabarile reached 60% during the spring of the 3rd year. Moreover, a progressive contribution of the unsown legumes was observed where the introduced accessions decreased in term of plant density. In the first year the establishment, the soil seasonal covering for most of the accessions was also satisfactory at Oristano where 11 out of 18 accessions have shown coverage rates over 60% in the 2nd year. This influenced positively the self-reseeding and most of the accessions reached high or satisfactory level of produced seeds both at 1st and 2nd year of the trial (Table 3). In Bolotana, and with a major extent in Chilivani, a strong decrease of seed production after the establishing year was recorded. A remarkable number of seeds m⁻² for *T. glanduliferum* Prima and *T. vesiculosum* Cefalù, within the smaller seed size group of accessions, were observed both in Bolotana and Oristano. *T. spumosum* WCT36, within the group of accessions with larger seed size, showed the highest self-reseeding ability both in Chilivani and Oristano. *T. hirtum* Hykon showed high self-reseeding capability in the 2nd year but only at Oristano, producing up to 148,000 seeds m⁻². For some accessions, the low covering rate and persistence was recorded even with a satisfactory seed production of the previous spring season, suggesting that probably high rates of hard seeds negatively affected seedlings re-establishment. A higher hard seed level was one of the traits sought in the new legume species (Nichols *et al.*, 2007). Plot establishment of some new introduced legume species was successful even in absence of seed or soil inoculation. This suggests that effective native rhizobia may be present in the soil and, consequently, the need of inoculation should be carefully considered.

Table 3. Number of seeds m⁻² for the varieties grown in each site. Varieties were grouped on the basis of the seed size

	Variety	Chilivani		Bolotana			Oristano	
		2004	2005	2007	2008	2009	2003	2004
1000 seed weight < 2 g								
	<i>B. pelecinus</i> Casbah			35743 ^{ab}	11761 ^a		96640 ^b	35851 ^{ab}
	<i>B. pelecinus</i> Mauro	89726 ^b					86772 ^b	112103 ^{bc}
	<i>T. glanduliferum</i> Prima	24004 ^a		44132 ^{ab}	20280 ^a	35740 ^a	198063 ^c	47108 ^{ab}
	<i>T. michelianum</i> Bolta	18879 ^a					15715 ^a	59713 ^{ab}
	<i>T. michelianum</i> Paradana	62482 ^b		6126 ^a			89654 ^b	20804 ^a
	<i>T. resupinatum</i> Nitro Plus						9357 ^a	78136 ^{ab}
	<i>T. resupinatum</i> Prolific						109678 ^b	89733 ^{abc}
	<i>T. vesiculosum</i> Cefalu			59522 ^b	49463 ^a	6728 ^a	193527 ^c	169830 ^c
1000 seed weight > 2 g								
	<i>M. polymorpha</i> Anglona	17209 ^b						
	<i>O. compressus</i> Avila						13994 ^{ab}	14492 ^a
	<i>O. compressus</i> Pabarile			32135 ^b	29280 ^b	13809 ^b		
	<i>O. compressus</i> Santorini			25255 ^{ab}	7347 ^a	7334 ^a	20152 ^{abc}	13757 ^a
	<i>O. sativus</i> Cadiz			17792 ^{ab}	7965 ^a	2200 ^a	14746 ^{ab}	
	<i>O. sativus</i> Marguerita	2512 ^a						
	<i>T. brachycalyc.</i> Funt. Bona	5972 ^a	2162 ^a	1877 ^a	7387 ^a	1843 ^a		
	<i>T. hirtum</i> Hykon	4149 ^a					93164 ^d	147868 ^c
	<i>T. incarnatum</i> Caprera						46083 ^c	24714 ^{ab}
	<i>T. spumosum</i> WCT36	60615 ^c					138831 ^e	62869 ^b
	<i>T. subterraneum</i> Campeda	3262 ^a	3245 ^a					
	<i>T. subterraneum</i> York						30330 ^{bc}	16462 ^{ab}

Values among columns not followed by the same superscript (a, b, c) differ significantly (p < 0.05).

Moreover, some of the promising accessions (*T. vesiculosum*, *T. glanduliferum*, *T. hirtum*) are not present in the native flora of Sardinia. For such reason, the introduction of exotic material should be evaluated considering the possible positive or negative consequences in term of biodiversity.

These results do not partially agree with the findings from experiments carried out in Western Australia, whose climate and soils are similar but not really so widespread in Mediterranean basin. Since most of such new pasture legumes are already marketed in South Europe as complex mixtures, it is strongly recommended to extend their evaluation in the environments of introduction (Franca *et al.*, 2007).

IV – Conclusions

The agronomic performances of the new Australian varieties under study were sometime satisfactory the first year but, subsequently, the presence of sown varieties often decreased as native species became more competitive. Consequently, it seems possible rely on a restricted number of new species and varieties, accurately chosen site by site in regard of high pedo-climatic variability of Mediterranean basin, if the main aim is the establishment of long-term pasture. More options are available for their inclusion in short rotation with cereals and annual forage crops. The results indicate also that local ecotypes and/or varieties of traditional pasture species are more suitable than most of the new commercial varieties for Sardinian farming systems. Some accessions may be included into simple legumes-based pasture mixtures taking into account the high spatial variability for environmental characteristics present in Sardinia.

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