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Germination success under different treatments and pod sowing depths in six legume species present in olive groves

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Abstract

This study analysed the germination success of pods of six annual native legumes species: *Astragalus hamosus, Medicago minima, Medicago orbicularis, Medicago polymorpha, Medicago rigidula* and *Scorpiurus muricatus*. The use of these species has been proposed as a means of generating and improving herbaceous cover in olive groves. Germination success was studied in terms of the variability in the number of seeds germinated per pod after 18 months at two different sowing depths, on the surface (S) and buried 10 mm (B). Pods were subject to five different pre-germination treatments: chemical scarification, consisting of immersion in sulphuric acid for 15 min (S_15) and 20 min (S_20), immersion in water for 48 h (W_48), pod precooled to -18ºC for one month (P_18º) and untreated pods (Con). The results showed that the effectiveness of the different treatments and sowing depths depended on the species, and that there were no problems of 'sibling-competition' in any of the treatments or at any of the sowing depths. Species with larger, non-spiralled pods, such as *A. hamosus* or *S. muricatus*, or with very loosely spiralled pods such as *M. orbicularis*, had greater germination rates when buried, mainly in the case of untreated pods and pods that were immersed in sulphuric acid for 20 minutes.

Additional keywords: burial depth; spontaneous cover; scarification

Abbreviations used: B (buried); Con (untreated pods); Dmax (maximum diameter); GEE (generalized estimating equations); GS/P (germinated seeds per pod); P_-18° (precooled to -18 °C for one month); S (surface sowing); S_15 (soaking pods in 95% concentrated sulphuric acid solution for 15 min); S_20 (soaking pods in 95% concentrated sulphuric acid solution for 20 min); SPE (seed production efficiency); SPP (seed production potential); W_48 (soaking pods in distilled water for 48 h).

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Introduction

Pressure on water resources and biodiversity, spreading contamination caused by the use of pesticides, and the degradation of soils by water erosion are just three of the problems related to the expansion and intensification of olive production that have become patent in recent years in Spain (Scheidel & Krausmann, 2011). A possible strategy for avoiding soil degradation – as well as a way of increasing biodiversity and fixing the organic carbon content in soils – is the use of herbaceous plant cover (Gómez *et al*., 2009; Fernández-Escobar *et al*., 2013). Annual legume forage plants have been proposed as species that can be used to restore pastures and conserve degraded soils since they improve soil quality by fixing atmospheric nitrogen (Faria *et al.,* 1989); likewise, they adapt well to a wide range of environmental variables (Beale *et al*., 1991), have prostrate growth habitats (Meloni *et al*., 2000) and have a high forage value for cattle (Abdelguerfi & Abdelguerfi-Berrekia, 1989; Derkaoui *et al*., 1993; Zhu *et al.*, 1996). Recently, the pure sowing of annual legumes or in combination with grasses has also been proposed as a means of restoring herbaceous ground cover in olive groves and vineyards (Porqueddu et al., 2000; Rodrigues *et al*., 2015; Siles *et al*., 2016). Nevertheless, certain factors such as the dormancy of these species' pods – which may hinder quick homogenous germination (Argel & Paton, 1999) – and the cost of such techniques may complicate the use of these species in restoration and management projects designed to improve herbaceous cover.

Currently, the most commonly proposed technique in the generation of herbaceous cover is the sowing of pre-treated commercial seeds. Numerous experiments under laboratory conditions (Hardegree & Emmerich, 1991; Ibáñez & Passera, 1997; Uzun & Aydin, 2004) or experimental garden conditions (Siles *et al.*, 2016) have shown that scarification is a good way of breaking the dormancy of the seeds of certain legumes species. The effectiveness of this type of treatment varies according to the scarification method employed, i.e. physical or chemical, the genus and the species of the plants used, and even the different accessions that exist between plants (Can *et al*., 2009; Martin & De la Cuadra, 2004; Uzun & Aydin, 2004; Kimura & Islam, 2012; Siles *et al*., 2016). Success rates were also found to be highly dependent on environmental conditions during the year of sowing. Some studies have shown that the shallow burial of untreated annual legume seeds increases germination success and that this sowing technique is less dependent on the rainfall regime in the first autumn after sowing. Nevertheless, the effectiveness of this method varies between species and the sowing depth (Loi *et al.*, 1999; Taylor & Revell, 2002; Zeng *et al.*, 2005; Siles *et al*., 2016).

The sowing of commercial seeds represents an additional expense for farmers, who have to resort to purchasing allochthonous varieties and ecotypes, which in many cases require re-sowing. It is thus vital to continue investigating other techniques that may help minimise the costs of establishing herbaceous plant cover and foment the use of local species and ecotypes. This will also help create and maintain a seed bank that can guarantee the permanence of natural legume plant cover, thereby avoiding re-sowing costs and increasing the capacity of agricultural systems to thrive even in face of the rainfall variability that is so characteristic of Mediterranean ecosystems.

One aspect that increases the cost of using commercial seeds is the need to extract seeds from pods, since most annual legumes exhibit synaptospermy, a reproductive strategy in which diaspores containing more than one seed form and then disperse as a single unit (Cheplick, 1992); moreover, many pods of annual legumes are indehiscent.

The advantages of synaptospermy include the protection of seeds during long dormancy periods and a better retention of moisture during germination (Pijl, 1982), two factors that influence the establishment and persistence of the seed bank. In addition, studies of some legumes have shown that the loss of viability is faster and greater when sowing seeds than when sowing a pod with seeds (Kim *et al.*, 2010). Other studies are currently experimenting with the sowing of non-scarified legume seeds alongside herbaceous crops (Loi & Nutt, 2010). Despite the above-mentioned advantages, if all the seeds from a single pod germinate simultaneously or if the seeds are not dispersed efficiently, individual competition between genetically related individuals, *i.e.* 'sibling competition', may occur (Cheplick, 1992). Nevertheless, information on the use of pods to establish, improve and maintain spontaneous annual plant cover in woody crops is still very scarce. It is thus necessary to investigate the success of generating spontaneous herbaceous cover using pods as the unit of seeding, and to analyse techniques that can break the dormancy of seeds in pods without engendering problems of sibling competition.

This study analysed germination per pods under experimental garden conditions in six species of annual spontaneous legumes that are frequently found in olive groves. The aims were to (1) characterise the morphological traits of pods and seeds of the assayed species; (2) evaluate the potential and efficiency of seed production per pod of the most frequent annual legume species in the study area; (3) test for variability in the germination of pods subject to different treatments and sowing depths; and (4) evaluate whether problems of sibling-competition occur in any of the tested treatments and/or sowing depths.

Material and methods

Mature pods of *Astragalus hamosus* L., *Medicago minima* (L.) L., *Medicago orbicularis* (L.) Bartal, *Medicago polymorpha* L., *Medicago rigidula* (L.) All. and *Scorpiurus muricatus* L. were collected in summer 2013 from a spontaneous pasture in a biologically run olive (*Olea europaea* L.) grove in Sierra Mágina (Jaén province, Southern Spain). The climate is Mediterranean in type, with warm winters and hot dry summers. Pods were stored in paper bags at room temperature (25°C) under laboratory conditions until sowing was performed in the experimental garden at the University of Jaén. In autumn, 420 apparently well-formed and vigorous pods from each species were selected.

Pods were classified by shape, the compactness of their spirals and the hardness of their spines (Blanca *et al.*, 2009). In total, 100 pods per species were selected to calculate the seed production potential (SPP) and seed production efficiency (SPE). Seeds were separated from pods by hand and classified according to their size and surface roughness (Bojňansky & Fargašová, 2007) to establish whether or not they were developed.

We calculated the SPP as the sum of the developed and undeveloped seeds present in each pod, and the SPE as the ratio per pod between developed seeds and the SPP (Rodríguez *et al.*, 2001). We also calculated the maximum diameter (Dmax) of 100 seeds chosen at random for each species. Descriptive statistics including the mean and standard deviation were calculated for each species. Data were analysed using a one-way ANOVA. Multiple comparisons of the means were calculated based on T2 of Tamhane (1979).

To test the variability in the number of germinated seeds per pod under different treatments and sowing depths, 320 pods of each species were selected, which were then submitted to either physical or chemical treatment. The first physical treatment consisted of soaking pods in distilled water for 48 h (W_48), while in the second treatment the pods were placed in sterile containers and precooled to -18 °C for one month (P_-18°). The two chemical treatments consisted of soaking pods in 95% concentrated sulphuric acid solution for 15 min (S_15) or 20 min (S_20). After these treatments, pods were thoroughly rinsed, first with tap water and then distilled water. The last treatment was the control (Con, untreated pods).

In early autumn pods were sown in different forest trays under natural conditions of temperature and sunlight, with a substrate of blonde peat (60%), sand (30%) and perlite (10%), at sowing depths of 0 mm (surface, S) or 10 mm (buried, B), to simulate the stratification of pods in field crops after tilling or pod-burial caused by summer grazing. For each species, 32 pods were used for each treatment and sowing depth. In addition to the natural precipitation, at the beginning of autumn and spring seedlings were watered to ensure that soil humidity was not a limiting factor for seed germination. Germination per pod was monitored over the following 18 months, with weekly revisions in spring and autumn. The presence of cotyledons was taken as the end-point of germination. At the end of the experiments, the number of germinated seeds per pod was calculated (GS/P).

Separate analyses were conducted for each species. Generalized Estimating Equations (GEE) using a Poisson distribution and logit-link function were applied to fit the GS/P data. GEE is an appropriate statistical tool for analysing repeated measures and takes into account the correlation of responses in pods in trays through the use of an exchangeable working correlation matrix, which was adjusted to the model. This technique is equivalent to a Generalised Linear Mixed Model with only random intercepts (Twisk, 2003; Gardiner *et al*., 2009). We selected 'treatment', 'sowing depth' and the interaction 'treatment*sowing depth' as factors. Multiple comparisons of the marginal means were conducted using the Sidak sequential adjustment.

All statistical analyses were carried out with the software SPSS (IBM, 2013).

Results

The classification of the shape and size of the pods and seeds of the tested species is given in Table 1. We found great differences between genera in the shapes of the pods. In addition, the *Medicago* species have several types of spines and spirals. Of the six tested species, four have spiralled-shaped pods, although only one (M. *rigidula*) has very compact spirals. Neither *A. hamosu*s nor *M. orbicularis* have spines on their pods, unlike *M. rigidula* and *S. muricatus*, which have thick rigid spines, or *M. minima* and *M. polymorpha*, which have thin flexible spines. The roughness of the surface of the seeds also varied

Table 1. Classification of *Astragalus hamosus, Medicago minima, Medicago orbicularis, Medicago polymorpha, Medicago rigidula* and *Scorpiurus muricatus* in terms of pod shape (S_pod), compactness of the pod spiral (Spiral), hardness of the spine (Spine) and roughness of the seed surface (R_seed). Also shown are the summary descriptive statistics including mean and standard deviation (SD) of the seed production efficiency (SPE, %), the seed production potential (SPP, seed), and the average size of the seed (Dmax, cm).

Legume	*S_pod*	*Spiral*	*Spine*	*R_seed*	SPE ± SD	SPP ± SD		Dmax ± SD	
A. hamosus	falcate	-	-	tuberculate	83.18±17.89	19.62±3.46	a	0.26±0.04	c
M. minima	spiralled	loose	soft	smooth	92.05±21.38	4.87±1.23	b	0.21±0.02	a
M. orbicularis	spiralled	loose	-	tuberculate	91.95±15.38	12.59±2.51	c	0.24±0.03	b
M. polymorpha	spiralled	loose	soft	smooth	87.34±22.49	3.68±0.93	d	0.27±0.04	c
M. rigidula	spiralled	compact	hard	smooth	86.02±26.83	5.95±1.15	e	0.35±0.04	d
S. muricatus	scorpioid	-	hard	smooth	96.48±8.33	7.93±1.26	f	0.36±0.05	d

Values with different letters in the columns differ significantly at a significance level of $p<0.05$.

and two of the studied species, *A. hamosus* and *M. orbicularis*, have seeds with tubercles, *i.e.* small protuberances on the surface; the other species, however, all have seeds with smooth surfaces. Table 1 shows the values of SPP, SPE and Dmax for each species. The species that had most seeds per pod was *A. hamosus*, although it also showed the greatest variation in the 100 pods; the species with the fewest seeds per pod was *M. polymorpha*. The species with the smallest seeds was M. minima, which were significantly smaller than those of the other species; *S. muricatus* and *M. rigidula* had the largest seeds. All species had an SPE of over 80%, the most efficient being *S. muricatus* (>95%).

Figure 1 shows the accumulated absolute values of germinated seeds per pod for each species during the study period. Seed germination per pod in the six species was concentrated in autumn of both experimental years following sowing. There was a seed germination peak in the second autumn after sowing, *i.e.* there were generally very low germination rates in the first autumn-winter. The only exception was the surface sowing of *S. muricatus*, in which hardly any germination was observed in any of the different treatments in the second autumn. *Astragalus hamosus* and *S. muricatus* had the lowest germination rates per pod at both depths in the first autumn; the highest germination rates during this period were in frozen *M. polymorpha* and *M. rigidula* seeds in surface sowing.

The analysis of the accumulated germination after two years shows a significant effect for pod treatment, sowing depth and the interaction treatment*sowing depth for all species except *M. rigidula*, in which the only effect observed was due to the interaction (Table 2). The average number of seeds germinated per pod for each species, treatment and sowing depth is given in Figure 2. Although burial sowing gave a larger number of germinated seeds per pod (except for M. *minima* and *M. rigidula*), the most effective treatment varied between species. For *M. minima* and *M. rigidula*, the best germination rates were obtained from surface sowing with seeds immersed in water.

Variability in germination at different sowing depths for each treatment

The effect of the sowing depth varied between treatments (Fig. 2). Deep sowing significantly improved the number of seeds germinated per pod under all treatments in *A. hamosus, S. muricatus* and *M. orbicularis* (except for S_20 in this latter species, for which no significant differences were found). By contrast, a negative effect for burial in the pods of both M. minima (in Con, S_15 and W_48) and *M. rigidula* (in W_48 and P_-18) was obtained.

The greatest values of germination after pod burial were obtained when sowing untreated pods and in pods that had been immersed in acid. The sowing at depth of untreated pods resulted in higher germination values in two of the six species, *A. hamosus* (S: 0.10 *vs*. B: 2.08) and *M. orbicularis* (S: 0.74 vs. B: 2.14). Sowing pods that had been immersed in sulphuric acid at depth increased germination success in all species except *M. minima* (in S_15) and especially in *S. muricatus* (S: 0.28 vs. B: 2.4 in S_20) (Fig. 2).

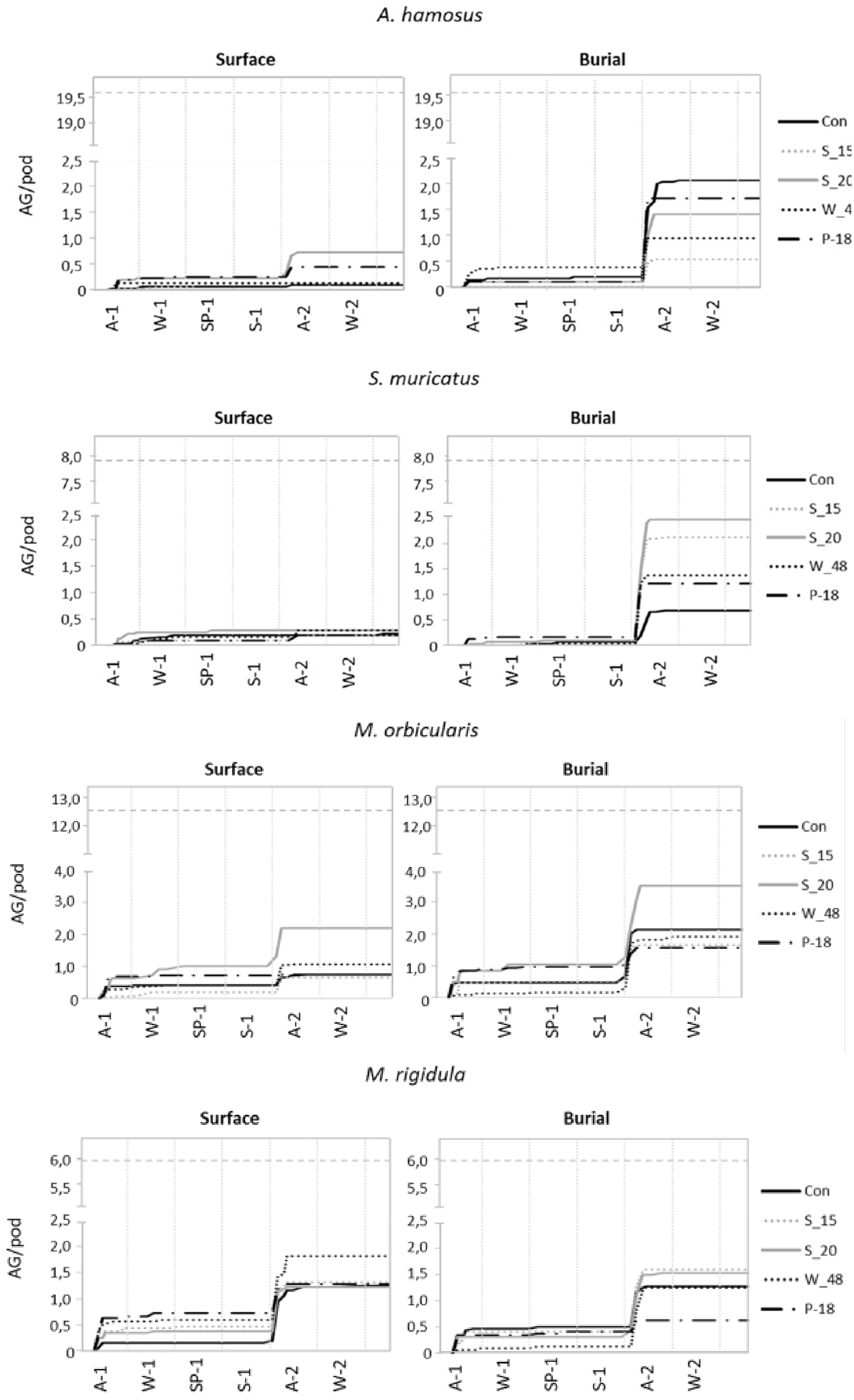
Variability in germination between different treatments at each sowing depth

In surface sowing, the species with the poorest germination success were *A. hamosus*, with between 0.10 (Con) and 0.73 (S_20) germinated seeds, and *S. muricatus*, with between 0.19 (Con) and 0.28 (S_20) germinated seeds. Immersion in sulphuric acid for 20 min significantly increased the number of seeds that germinated per pod compared to untreated pods

Table 2. Results of the Generalized Estimating Equations (GEE) testing the effects of Treatments (Treat), sowing depth (Depth) and the interaction Treat*Depth on the final number of germinated seed in pods for six species of annual native pods.

Legume	Treat			Depth			Treat*Depth		
	DF	χ^2	*p*	DF	χ^2	*p*	DF	χ^2	*p*
A. hamosus	4	1470.82	***	1	49.44	***	4	47.80	***
M. minima	4	191.054	***	1	13.370	***	4	170.556	***
M. orbicularis	4	110.79	***	1	158.65	***	4	20.27	***
M. polymorpha	4	274.4	***	1	66.443	***	4	139.88	***
M. rigidula	4	70.58	***	1	2.84		4	49.01	***
S. muricatus	4	202.08	***	1	142.11	***	4	43.38	***

The significance of effects is indicated as * $p<0.05$; ** $p<0.01$; *** $p<0.001$.



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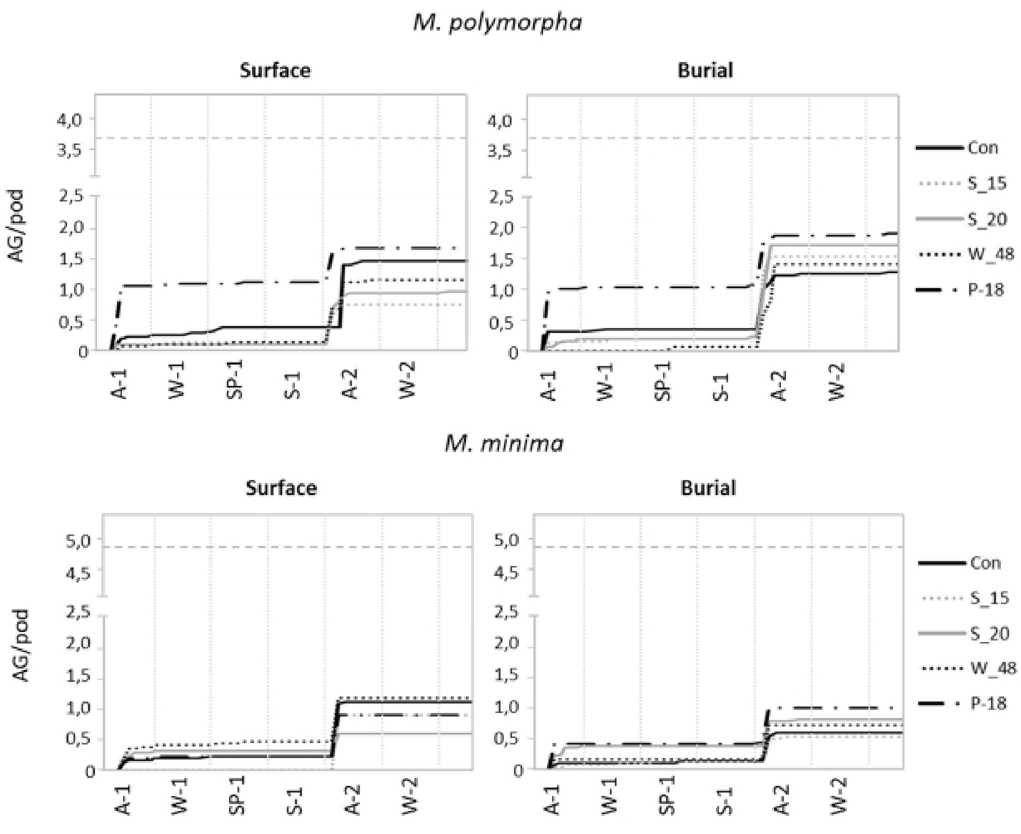

Figure 1. Accumulated absolute values of germinated seeds per pod (AG) during: autumn (A), winter (W), spring (SP) and summer (S) in the first (1) and the second year (2) of study. The treatments were: untreated pods (Con), 15 min in sulphuric acid (S_15), 20 min in sulphuric acid (S_20), forty-eight hours in water (W_48), and precooled to -18ºC (P_-18). The horizontal discontinuous line for each graph indicates the average number of seeds per pod for each species.

in *A. hamosus*, *M. orbicularis* and *S. muricatus* (Fig. 2). In both M. rigidula and *M. minima*, the highest germination rates were obtained from pods immersed in water, although significant differences were only found with respect to the untreated pods of M. rigidula. Freezing gave rise to a significant increase in germination compared to untreated pods in *A. hamosus* and *M. rigidula*.

In burial sowing, the species with least germination success was *M. minima* (Fig. 2), while the species with the greatest germination success was M. *orbicularis* (S_20 and Con); nevertheless, there were no significant differences between these two treatments (Fig. 2).

In both *M. rigidula* and S. *muricatus* the greatest germination success was obtained in pods immersed in sulphuric acid (S_20), which tripled in S. *muricatus* the number of seeds germinated in comparison to untreated pods. In *M. minima* and M. *polymorpha* there was a significant increase in germination in frozen pods. Immersion in water only led to an increase in germination in comparison with untreated pods in *S. muricatus.*

Discussion

Throughout the twentieth century a number of studies have demonstrated the important role played by spontaneous annual legumes in agricultural systems (Piano & Talamucci, 1996; Roggero & Porqueddu, 1999) and have highlighted the advantages of using spontaneous plants rather than commercial varieties. The annual legume species tested in this study are common in herbaceous plant cover in Mediterranean environments (Beale *et al*., 1991; Chebouti & Abdelguerfi, 1999; Sokoloff & Lock, 2005; Abbate *et al*., 2010) and grow abundantly in ecologically farmed olive groves (own data). All the species use the reproductive strategy known as synaptospermy. Nevertheless, great variability exists between different species in terms of the degree of dehiscence, shape, structure and productive potential of their pods. All the tested species have small hard seeds and only a small percentage of the seeds in the untreated pods germinated in the two years after sowing. Piano & Francis (1992) have shown how in arid zones annual legumes with high productive potential and small hard seeds predominate.

Our study shows that the annual legumes present in olive groves in dry climates also have small hard seeds.

The results show that high values of SPP per pod are not related to high values of germination rate per pod. In the surface sowing of untreated pods, which simulates the natural conditions that pods encounter when they drop from their mother plants, the species with the largest number of seeds per pod had the lowest germination rates.

This study also reveals how the different treatments applied to the pods increase seed germination rates, although the effectiveness of each treatment depends on the species, the treatment used and the sowing depth. The immersion of pods in sulphuric acid increased the germination rate per pod in the surface sowing of *A. hamosus, M. orbicularis* and *S. muricatus* and, when sowing at depth, in S. muricatus and *M. rigidula*. We found that the positive effect of sulphuric acid on final germination rates in *A. hamosus* and *M. orbicularis* was similar to that reported by Patanè & Gresta (2006) for seeds exposed to concentrations of sulphuric acid for long periods of time. Our study did not reveal any effect on germination in *M. rigidula* for surface sowing after immersion in sulphuric acid, although this effect did occur when pods were sown at depth. Laboratory studies of this species involving the immersion of seeds in sulphuric acid (Balouchi & Sanavy, 2006; Can *et al.*, 2009) have led to an increase in germination rates after chemical scarification. We found a negative effect for chemical scarification in *M. minima* and *M. polymorpha* after surface sowing. Other authors have reported similar effects for the seeds of *M. minima* (Siles *et al.*, 2016). Nevertheless, our germination data for *M. polymorpha* differ from the results derived from other seed germination studies (Can *et al.*, 2009, Siles *et al.,* 2016).

Our results show that the effect of sowing depth depends on the species and treatment: in four of the six species tested, the best germination rates were for seeds sown at depth. For the other two species, *M. minima* and *M. rigidula*, the best germination rates were recorded for pods immersed in water and sown on the surface. Zeng *et al.* (2005) found that the effect of seed burial varied according to the species and the sowing depth, the breaking of dormancy decreasing with depths of burial below 2 cm for seeds of *M. polymorpha* and other clover species. Other authors have demonstrated that sowing depth not only influences seed dormancy loss but also affects their viability. The persistence and viability of 'loose' *Astragalus sinicus* seeds sown at different depths – as opposed to seeds still inside their pods – were studied by Kim et al. (2010). These authors report that the loss of viability was greater and quicker in the 'loose' seeds than in those still inside the pods, and was also greater and quicker in surface-sown seeds than in shallow-planted seeds. The effect of burial on the viability of seeds decreased as the sowing depth increased. Differences found in germination success between species, treatments and sowing depths may be related to the morphological traits of pods and seeds. The degree of spiralization of the pod may obstruct the arrival of sulphuric acid and water to the seed, thereby affecting the chemical scarification of the seed and its imbibition of water. This may be one of the causes of the differences in how species are affected by immersion in sulphuric acid detected in this study and in other studies conducted directly on seeds.

The pods of both *A. hamosus* and *S. muricatus* are unspiralled; by contrast, the pods of *M. orbicularis* are very open and loosely spiralled, have soft walls and a degree of dehiscence that is regarded as exceptional in spiralled pods. All these characteristics favour greater exposure of the seed to moisture under the different treatments, which may increase their germination rates, above all in burial sowing where the humidity of the pods is maintained for longer. Some authors have shown that a number of seeds from the same pod in *Medicago* species with soft-walled pods are likely to germinate simultaneously (Small *et al.*, 1991). Our data for sowing at depth confirm this tendency and, of the four species of *Medicago* tested, *M. orbicularis* had the greatest germination success per pod, despite the fact that this species had the fewest germinated seeds per pod in surface sowing.

Puri (1979) hypothesized that the protuberances on the surface of seeds could act as a way of increasing their surface area, thereby augmenting their ability to absorb water and contributing to better germination rates. Our data are consistent with this hypothesis since *A. hamosus* and *M. orbicularis* were the only two species that significantly benefitted from the burial of untreated seeds and both are species whose seeds have tubercles (the other four species all have smooth seeds) (Bojňansky & Fargašová, 2007). Siles *et al.* (2016) have shown that the effect of burial is species-specific. Blumenthal & Ison (1996) found that marginal humidity conditions in the shallow sowing of pods of *Medicago murex* could be beneficial for the germination and emergence of seedlings, and that this effect may vary in terms of the environmental stress to which seeds are subjected. Our study shows that the effect of burial varies in terms of the species and pod traits (*i.e.* spiralled *vs*. unspiralled pods) but also in relation to the degree of seed roughness (*i.e.* smooth *vs*. tuberculate seeds). Sowing retains the moisture in the pod, less compaction of the spires facilitates the arrival of water to the seeds, and the roughness of the seeds increases the surface area in contact with water, thereby facilitating the imbibition of the seeds and germination success. However, more work still needs to be carried out on the effects on germination of (1) the type and degree of roughness of seeds, (2) soil humidity, (3) different techniques of seed burial, and (4) pod morphology.

In recent years, two novel sowing techniques known as 'twin-sowing' and 'summer sowing' have been used to introduce legume species into cereal fields and olive groves as a means of increasing soil fertility (Loi & Nutt, 2010). These techniques employ untreated annual pods or seeds with high degrees of dormancy in combination with the target cultivated plant. The aim is to break legume seeds' dormancy in the first year in which the target crop is planted, thereby facilitating for the following season better germination of the annual legumes. Abbate *et al*. (2010) have proposed *S. muricatus* as an ideal species for this type of sowing. The germination patterns registered in this species support its suitability for use in twin-sowing. Data for *A. hamosus* and *M. orbicularis* show that these two species are also good candidates for use in this type of sowing. Twin-sowing techniques could be used for establishing mixed natural ground cover in olive groves given that

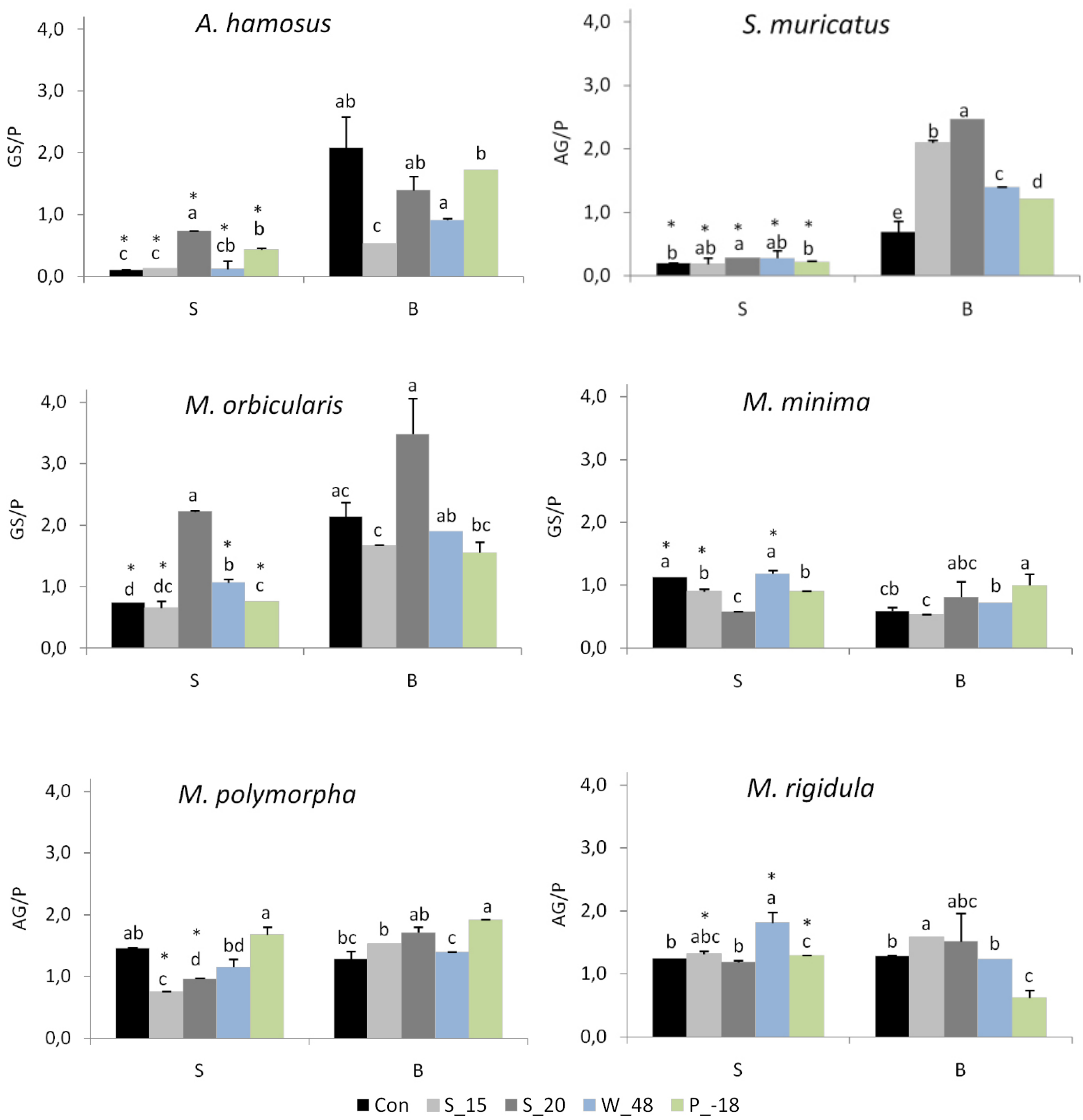

Figure 2. Average number of germinated seeds per pod (GS/P) for each species, treatment and sowing depth (S, surface; B, buried). Letters indicate statical differences between treatmens within each sowing depth and species; * indicates differences between sowing depths in each treatment at p<0.05 (Sidak-sequential),

such methods favour both temporal and spatial diversity and biodiversity within the established herbaceous plant cover.

Our study shows how species with large and unspiralled or loosely spiralled pods respond best to burial. Some authors have also associated pod size and shape with the degree and type of predation they suffer. Roggero & Porqueddu (1999) found that large pods are more grazed than small pods, and are more at risk to predation by ants. Small (2011) showed that a greater openness in pods implies a greater likelihood of being trampled and buried by cattle. Thus, the morphological characteristics of pods that facilitate burial are an important evolutionary advantage in environments with high pressure from livestock and irregular rainfall. These morphological traits reduce grazing pressure and increase the exposure of seeds in pods to the humidity, which is greater in the first few centimetres of the soil than on the surface.

The differences between the mean number of seeds per pod and the mean number of germinated seeds per pod (Fig. 2) show that none of the tested treatments or sowing depths stimulated the germination of the seeds to the extent of causing problems of sibling competition. Thus, when the aim of sowing is to improve the establishment and permanence of a seed bank, the sowing of legumes pods should be seen as a viable alternative to the sowing of commercially purchased seeds. The shallow burial of pods represents an easy-to-use management tool that can guarantee the longevity of the seed bank, improve germination rates and provide protection against herbivores.

The effects of the treatments is specific-species and so, in monospecific sowing aimed at generating herbaceous ground cover, we would recommend the use of the treatment that best suits the target species. By contrast, several options exist for establishing mixed herbaceous ground cover. The first is to carry out specific individual treatment for each species and then mix pre-treated species before sowing. Another option is to use the shallow burial of pods that have not been treated or have only been immersed in sulphuric acid − the exceptions are M. minima and M. rigidula, whose pods should be sowed on the surface having previously been submerged in water to increase their germination rate.

The presence of structures that aid dispersion by adhesion to animals in four of the six tested species, and the benefits of the shallow burial of pods demonstrated during this study, suggest that sheep flocks could be a key element in the dispersal and increase in recruitment rates of new legume populations in olive groves, especially in traditional crops and marginal olive groves in mountainous areas. Other authors have also shown that endozoochoric seed dispersal by sheep flocks is a promising way of improving pastures in Mediterranean areas (Russi *et al.*, 1992; Malo & Suárez, 1995; Ramos *et al.,* 2010).

Aware of the limitations of our experiments under garden conditions, we have undertaken further experiments involving the sowing of legume seeds and of other potentially important families in olive groves. The monitoring of their establishment over the years under different climatic and edaphic conditions will allow us to detect the most promising techniques for farmers wanting to implant and maintain these species as cover crops.

The effects of the different treatments depend on the species and vary in terms of the depth of burial. No problems of sibling competition were encountered and so the sowing of seeds in pods should be regarded as a useful technique for improving the establishment of spontaneous ground cover in olive groves, above all in the mid-term given that maximum germination takes place in the second autumn after sowing. The shallow burial of untreated pods and pods that have been immersed in acid for 20 minutes were the two most effective treatments for large, unspiralled or loosely spiralled pods. By contrast, the germination rate in pods with compact spirals increased when sown on the surface.

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