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Germination trials of annual autochthonous leguminous species of interest for planting as herbaceous cover in olive groves

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ABSTRACT

The germination of five species of annual native leguminous plants (*Astragalus hamosus* L., *Medicago minima* (L.) L., *Medicago orbicularis* (L.) Bartal, *Medicago polymorpha* L., and *Scorpiurus muricatus* L.), present spontaneously in the ground cover of olive groves, was tested under experimental garden conditions using different scarification and planting depth treatments. Surface and deep planting were carried out after submitting seeds to different treatments: mechanical scarification (MS) with sandpaper, chemical scarification consisting of immersion in sulphuric acid for five (S_5) or ten (S_10) minutes and non-scarified seeds (Con). Germination success was calculated by analysing (i) the final germination and (ii) the pattern of germination that occurred during the 27 months that the study lasted. The most effective method for interrupting the dormancy of all the species (except *M. polymorpha*) was surface seeding with MS. Unlike the other treatments, in which there were germination peaks in each successive autumn, manually scarified seeds only germinated in the first autumn after sowing, and only within 10 days in the case of surface planting and within 20 days in deep-sown seeds. The speed and abundant germination obtained after manual scarification indicates that the dormancy of the tested species is a product of the hardseededness of the seed coat. Chemical scarification only managed to break down the tough seed cover in the case of deep-sown *M. orbicularis*.

1. Introduction

Most of the world's olive groves are found in the Mediterranean region. In total, there are 2.5 million ha of olive groves in Spain, of which 60% are in Andalusia (S Spain). About 60% of Spanish olive cultivation is carried out in adverse locations (Repullo-Ruibérriz, 2014). Factors such as the low percentage of this woody crop in open plantations, the situation of many olive groves on steep hillsides and the use of inappropriate practices in the control of spontaneous herbaceous plants all favour high rates of soil erosion (Gómez et al., 2009; Schoorl and Veldkamp, 2001) that are currently way above sustainable thresholds (Montgomery, 2007). Furthermore, the continuous use of herbicides for more than 50 years has provoked a loss of seed banks in spontaneous pastures and soil compaction, which in turn has led to lower resilience and an increase in the amount and speed of water run-off (Gómez et al., 1999; Lindstrom and Onstad, 1984).

In integrated production and ecological olive groves plant cover is encouraged and conserved via mechanical cutting and/or grazing (see Torres et al., 2013a for a revision of the techniques used to manage plant cover in ecological olive groves). Recent studies have shown that the use of plant cover (1) reduces the loss of soil compared to other techniques that opt for bare soil all-year round (Bruggeman et al., 2005; Francia et al., 2006; Gómez et al., 2004, 2009), (2) increases the soil's organic matter content and lessens the loss of organic carbon and nitrogen, available K and the phosphorous associated with water-born sediments (García-Ruiz et al., 2012; Gómez et al., 2009; Repullo-Ruibérriz, 2014), (3) improves notably the capacity for water infiltration (Simoes et al., 2014) and (4) halts the contamination of the surface water by synthetic chemical herbicides and fertilizers (Rodríguez-Lizana et al., 2007).

Numerous trials have been conducted involving the planting of ground cover with species of interest as forage crops such as grasses (Castro, 1994) and legumes (Hall et al., 1993; Repullo-Ruibérriz, 2014), or with species such as crucifers that help control *Verticillium dahliae* (Alcántara et al., 2009, 2011). Nevertheless, few farmers opt to plant herbaceous ground cover. In part, this reticence is due to the inherent problems with the planting and tending of ground cover, i.e. seed selection, the choice of method and timing of controls, and the need to reseed in many of species that are used. Recent studies with other types of crops have shown

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that the use of fodder grass species as monospecific ground cover improves soils but also implies an increase in the use of nitrogen-based fertilizers if crop yields are to be maintained (Alvarez and Steinbach, 2009).

The maintenance and conservation of spontaneous plant cover in olive groves is a potentially good alternative to the use of fodder species. Spontaneous cover has great specific diversity. Numerous adventitious species belonging mainly to the botanical families *Fabaceae*,*Poaceae*, *Asteraceae* and *Brassicaceae* (Foráster, 2010; García-Fuentes and Cano, 1998) have been described as growing in olive groves. Recent studies have demonstrated that the use of plants of these families reduces both erosion and the loss of organic carbon in soils in olive groves (Repullo-Ruibérriz et al., 2014). Most spontaneous leguminous species in olive groves have high seed production (Conway et al., 2001; Zoghlami and Zouaghi, 2003), are prostrate (Meloni et al., 2000), are adaptable to a wide range of environmental conditions (Chebouti and Abdelguerfi, 1999; Ehrman and Cocks, 1990), are able to improve soil quality by fixing atmospheric nitrogen (Faria et al., 1989), have high protein contents, and are high-quality forage for cattle (Derkaoui et al., 1993; Evers, 2011; Licitra et al., 1997; Zhu et al., 1996). Leguminous species are thus ideal for restoring plant cover (Meloni et al., 2000), for conserving and improving degraded soils in olive groves, and for diversifying exploitations by permitting the introduction of cattle (Torres et al., 2013b). The seeds of leguminous plants are characterised by having impermeable coats (Lodge, 1996; Souza and Marcos-Filho, 2001), which play an important role in germination patterns under natural conditions since they ensure that seed germination only occurs at optimum moments for seedling growth (Lodge and Whalley, 2002). This mechanism, which guarantees the perpetuation and regeneration of natural pastures, is determinant when high germination success rates over a short period of time are sought (Argel and Paton, 1999).

In all plant restoration programmes it is important to examine closely all components of the production chain, that is, the potential and efficiency of seed production, the obtaining, management and storage of seeds, germination and plant production capacity, and the establishment of plants in the field (Prieto and López, 2006). Numerous studies exist that have attempted to identify pre-germination techniques or treatments that break dormancy and stimulate seed germination in annual leguminous plants. Scarification is one of the most commonly used pre-germination treatments (Can et al., 2009; Martin and De la cuadra, 2004; Patanè and Gresta, 2006; Uzun and Aydin, 2004; Wang and Grusak, 2005) but its effectiveness depends on the genus, species and type of treatment employed (Demir and Ermis, 2004; Kimura and Islam, 2012). Most of these studies have been carried out under laboratory conditions and use the germination capacity achieved by the end of the study as an index for evaluating the success of germination under different pre-germination treatments. Nevertheless, if the conclusions drawn from germination trials with leguminous species are to be put into practice in restoration plans or used to improve soil cover in olive groves, it is necessary to undertake account field studies that analyze germination success in terms of both germination capacity and the time needed for germination to occur (McNair et al., 2012).

This study describes the germination under experimental garden conditions of five species of annual leguminous species that commonly occur in olive groves. The objectives of the study were: (1) to analyse the accumulated germination percentage, (2) identify the germination curves throughout the study and (3) evaluate the differences in germination patterns under different pre-germination treatments.

2. Material and methods

In 2012 mature pods of five annual leguminous species that act as ground cover in olive groves were collected: *Astragalus hamosus*L. (*A. hamosus*), *Medicago minima* (L.) L. (*M. minima*), *Medicago orbicularis* (L.) Bartal (*M. orbicularis*), *Medicago polymorpha* L. (*M. polymorpha*) and *Scorpiurus muricatus* L. (*S. muricatus*). Pods were gathered in ecological olive groves in the county of Sierra Mágina (Jaén), which has average annual precipitation of 400 mm and a precipitation regime characterized by severe summer drought and a concentration of precipitation in autumn (Estación Agroclimática de Mancha Real, X:447571; Y:4196710). Pods were separated by species and conserved under laboratory conditions until the beginning of autumn, when their seeds were manually separated from the pods and planted in experimental garden conditions under different pre-germination treatments and at different depths.

Four pre-germination treatments (Treat) and two planting depths (Depth) were tested. The treatments were as follows: mechanical scarification using a medium-grain sandpaper (MS), two chemical scarifications consisting of immersion in 95% sulphuric acid for five (S_5) and ten (S_10) minutes and a control in which seeds were not treated in any way (Con). There were two planting depths: surface (S) and buried (B). burial depth was three times the average seed size, varying between 0.6 and 1 cm (depending on species). The burial depth (<1 cm) was optimal for

Table 1
Results of the generalized mixed linear models testing all fixed effects (treatments (Treat), Planting depth (Depth) and the interaction Treat × Depth) and the decomposition of the interaction effect between treatments and planting depth on the final germination for five species of annual native plants. F statistics are used for fixed effects. Significance of effects is indicated as *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. The treatments were as follows: mechanical scarification (MS), five minutes in sulphuric acid (S-5), ten minutes in sulphuric acid (S-10) and control (Con). The seed planting depths were: on the surface (S) and burial (B).

	A. hamosus			*M. minima*			*M. orbicularis*			*M. polymorpha*			*S. muricatus*		
	DF	F	p	DF	F	p	DF	F	p	DF	F	p	DF	F	p
Treatment	3;792	51.66	***	3;792	26.67	***	3;800	48.68	***	7;856	1.42		7;792	12.677	***
Depth	1;792	9.31	***	1;792	0.02		1;800	13.26	***	7;856	16.01	***	7;792	3.31	
Treat × Depth	3;792	7.68	***	3;792	5.14	**	3;800	11.84	***	7;856	0.69		7;792	2.89	*
Variation in final germination percentage of different planting depths for each treatment															
MS	1;792	7.80	**	1;792	107.49	***	1;800	12.89	***	1;856	7.8	**	1;792	1.91	
S_5	1;792	11.11	**	1;792	0.77		1;800	5.17	*	1;856	6.71	*	1;792	6.41	*
S_10	1;792	14.49	***	1;792	7.79	**	1;800	10.09	**	1;856	3.99	*	1;792	0.568	
Con	1;792	9.72	**	1;792	4.06	*	1;800	35.42	***	1;856	0.74		1;792	1.81	
Variation in final germination percentage of different treatments for each planting depth															
B	3;792	42.565	***	3;792	11.46	***	3;800	64.21	***	3;856	0.74		3;792	3.08	*
S	3;792	468.5	***	3;792	48.2	***	3;800	234.89	***	3;856	1.46		3;792	12.66	***

weed germination and emergence (Gardarin et al., 2010). The same number of seeds per treatment and planting depth was used for each species (*A. hamosus*: 100; *M. minima*: 100; *M. orbicularis*: 101; *M. polymorpha*: 108 and *S. muricatus*: 100). In 2012 after the first autumn rains the seeds were planted in seedling trays filled with blonde peat enriched with coconut fibre. Four seeds of the same species subject to the same treatments and planted at the same depth were placed in each container (3.5×3 cm). 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. Seed generation was monitored over the following 27 months. Germination was defined as the development of a functional radicles and the presence of cotyledons. Germination success was analyzed in terms of (i) the accumulated germination and (ii) the germination patterns recorded during the study period. Acceptable germination for an introduction programme or project aimed at improving natural herbaceous cover was defined as a germination success rate of over 75%. Statistical analyses were carried out using the software SPSS 22.0 (IBM Corp., Armonk, NY, USA). Separate analyses were conducted for each species.

The final germination were modelled with Generalized Linear Mixed Models, GLMM (Lee et al., 2006) using a binomial error distribution and logit ling function. The final germination was modelled, with ‘container’ as a random block and ‘pre-treatment’, ‘seed depth’ and the interaction ‘pre-treatment $\times$ seed depth’ as fixed factors. Multiple comparisons were made with Sidak sequential.

The Kaplan–Meier method (Kaplan and Meier, 1958) was used to calculate cumulative germination curves for each pre-treatment and seed depth. Differences in the shape of the curves between pre-treatments for each seed depth and between seed depths for each pre-treatment were tested using the log-rank test (Pyke and Thompson 1986).

3. Results

3.1. Final germination

The analysis of the final germination showed an effect for the treatment and for the interaction Treat $\times$ Depth in four of the five tested species (Table 1), which indicates that the responses to the treatment depended on the depth of planting. Only one significant effect for planting depth was found in *M. polymorpha* ($F_{7856} = 16.01$, $p < 0.001$), which suggests that the germination percentage increases in this species if it is planted on the surface, regardless of the treatment that is applied to its seeds. The final germination probability for each species by treatment and planting depth are given in Table 2.

3.2. Analysis of the variation between planting depths for each treatment type

For scarified seeds, our results show for all species greater final germination on the surface (significant differences between depths in *A. hamosus*, *M. minima*, *M. orbicularis* and *M. polimorpha*), with final germination percentages of around 90% in *A. hamosus*, *M. minima* and *M. orbicularis*. Likewise, for these three species non-scarified seeds showed significant differences between depths of planting, with the greatest final germination obtained in deep-planted *A. hamosus* and *M. minima* seeds (Table 2 and Fig. 1).

In general, chemical scarification treatments gave very low final germination (Table 2), with significant differences between depth of planting in *A. hamosus*, *S. muricatus*, *M. orbicularis* and *M.*

Table 2
Final germination probability (FG) and germination time (day) required to reach the 25% (GT-25), 50% (GT-50) and 75% (GT-75) germination success under different treatments and planting depths. Values $\pm$ SE are given. The treatments were as follows: mechanical scarification (MS), five minutes in sulphuric acid (S_5), ten minutes in sulphuric acid (S_10) and control (Con). Letters indicate statistical differences between treatments for each planting depth and species; * indicates a significant effect for the planting depth for each treatment at $p < 0.05$ (Sidak-sequential).

	Surface				Burial			
	FG	GT-25	GT-50	GT-75	FG	GT-25	GT-50	GT-75
A. hamosus								
MS	0.96 $\pm$ 0.02 a*	4 $\pm$ 0.2	6 $\pm$ 0.1	6 $\pm$ 0.2	0.83 $\pm$ 0.04 a	7 $\pm$ 0.40	8 $\pm$ 0.3	10 $\pm$ 0.5
S_5	0.07 $\pm$ 0.03 bc*				0.26 $\pm$ 0.05 b	683 $\pm$ 0.0		
S_10	0.03 $\pm$ 0.02 c*				0.22 $\pm$ 0.05 b			
Con	0.17 $\pm$ 0.04 b*				0.39 $\pm$ 0.06 b	350 $\pm$ 11.8		
M. minima								
MS	0.89 $\pm$ 0.03 a*	4 $\pm$ 0.1	5 $\pm$ 0.1	6 $\pm$ 0.2	0.70 $\pm$ 0.06 a	5 $\pm$ 0.0	8 $\pm$ 0.9	
S_5	0.23 $\pm$ 0.05 c				0.33 $\pm$ 0.05 b	683 $\pm$ 66.2		
S_10	0.43 $\pm$ 0.06 b	18 $\pm$ 13.2			0.43 $\pm$ 0.06 b	350 $\pm$ 13.8		
Con	0.47 $\pm$ 0.06 b*	331 $\pm$ 61.5			0.68 $\pm$ 0.05 a	303 $\pm$ 54.1	674 $\pm$ 131.5	
M. orbicularis								
MS	0.96 $\pm$ 0.03 a*	7 $\pm$ 0.2	8 $\pm$ 0.17	9 $\pm$ 0.19	0.80 $\pm$ 0.04 a	8 $\pm$ 0.3	10 $\pm$ 0.5	26 $\pm$ 41.9
S_5	0.28 $\pm$ 0.04 c*	683 $\pm$ 0.0			0.15 $\pm$ 0.04 b			
S_10	0.09 $\pm$ 0.02 d*				0.26 $\pm$ 0.04 c	350 $\pm$ 0.0		
Con	0.52 $\pm$ 0.05 b*	674 $\pm$ 53.4	683 $\pm$ 0.0		0.16 $\pm$ 0.04 b			
M. polymorpha								
MS	0.58 $\pm$ 0.07 a*	6 $\pm$ 0.3	26 $\pm$ 292.2		0.31 $\pm$ 0.06 a	303 $\pm$ 147.1		
S_5	0.48 $\pm$ 0.07 a*	350 $\pm$ 3.5			0.25 $\pm$ 0.06 a	683 $\pm$ 0.0		
S_10	0.57 $\pm$ 0.07 a*	350 $\pm$ 6.8	350 $\pm$ 52.2		0.38 $\pm$ 0.07 a	303 $\pm$ 64.7		
Con	0.40 $\pm$ 0.07 a	350 $\pm$ 7.1			0.32 $\pm$ 0.06 a	674 $\pm$ 68.9		
S. muricatus								
MS	0.59 $\pm$ 0.08 a	9 $\pm$ 0.8	18 $\pm$ 1.5		0.44 $\pm$ 0.08 a	18 $\pm$ 1.25		
S_5	0.08 $\pm$ 0.03 b*				0.26 $\pm$ 0.06 ab	350 $\pm$ 0.0		
S_10	0.12 $\pm$ 0.04 b				0.16 $\pm$ 0.05 b			
Con	0.20 $\pm$ 0.06 b				0.32 $\pm$ 0.07 ab	350 $\pm$ 8.03		

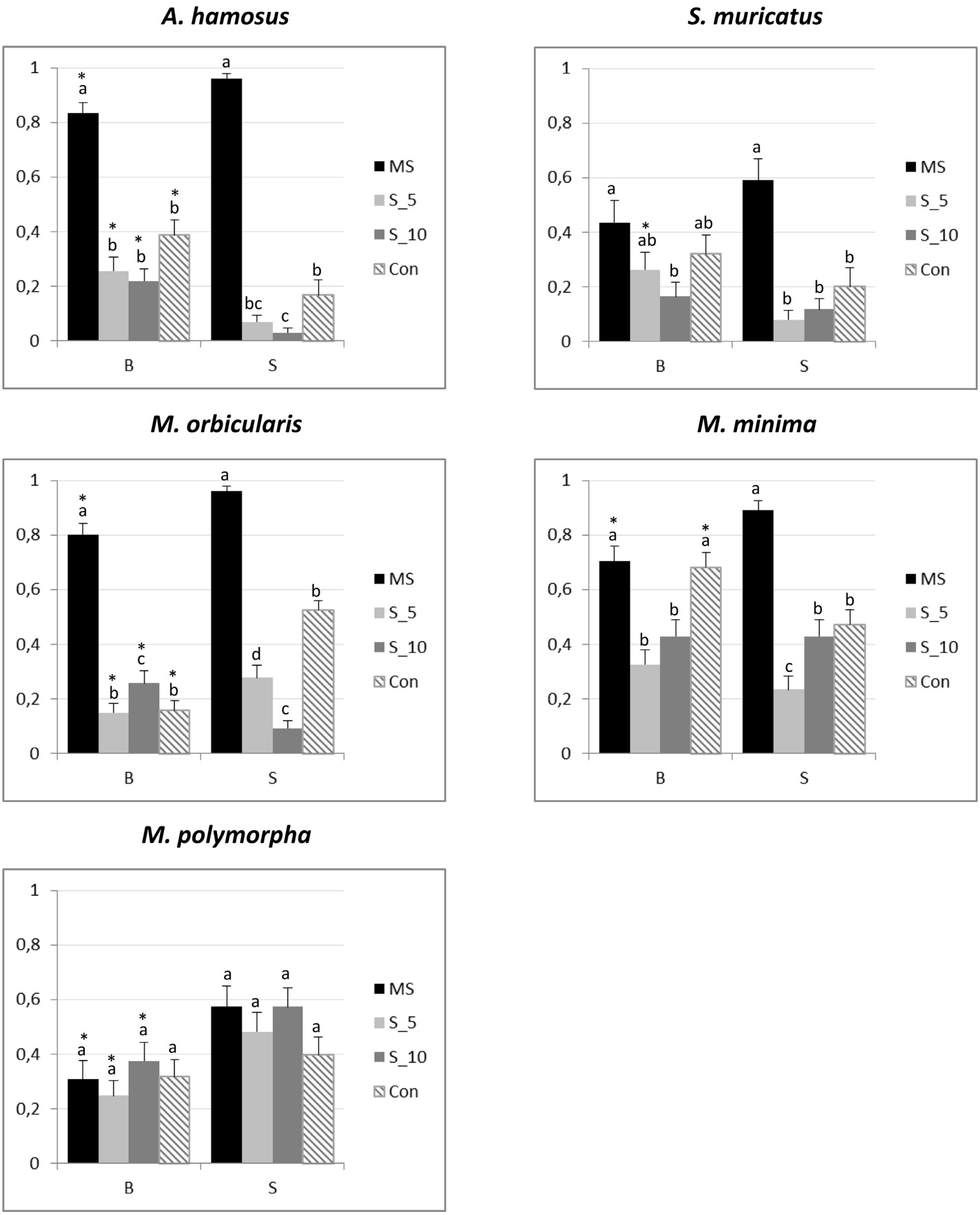

Fig. 1. Variation in germination (germination probability, *y*-axis, means ± ES) under different treatments and planting depths. The treatments were as follows: mechanical scarification (MS), five minutes in sulphuric acid (S_5), ten minutes in sulphuric acid (S_10) and control (Con). The seed planting depths were as follows: on the surface (S) and burial (B). Letters indicate statistical differences between treatments within planting depths and the asterisk indicates significant effects for planting depths for each treatment at $p < 0.05$ (Sidak-sequential).

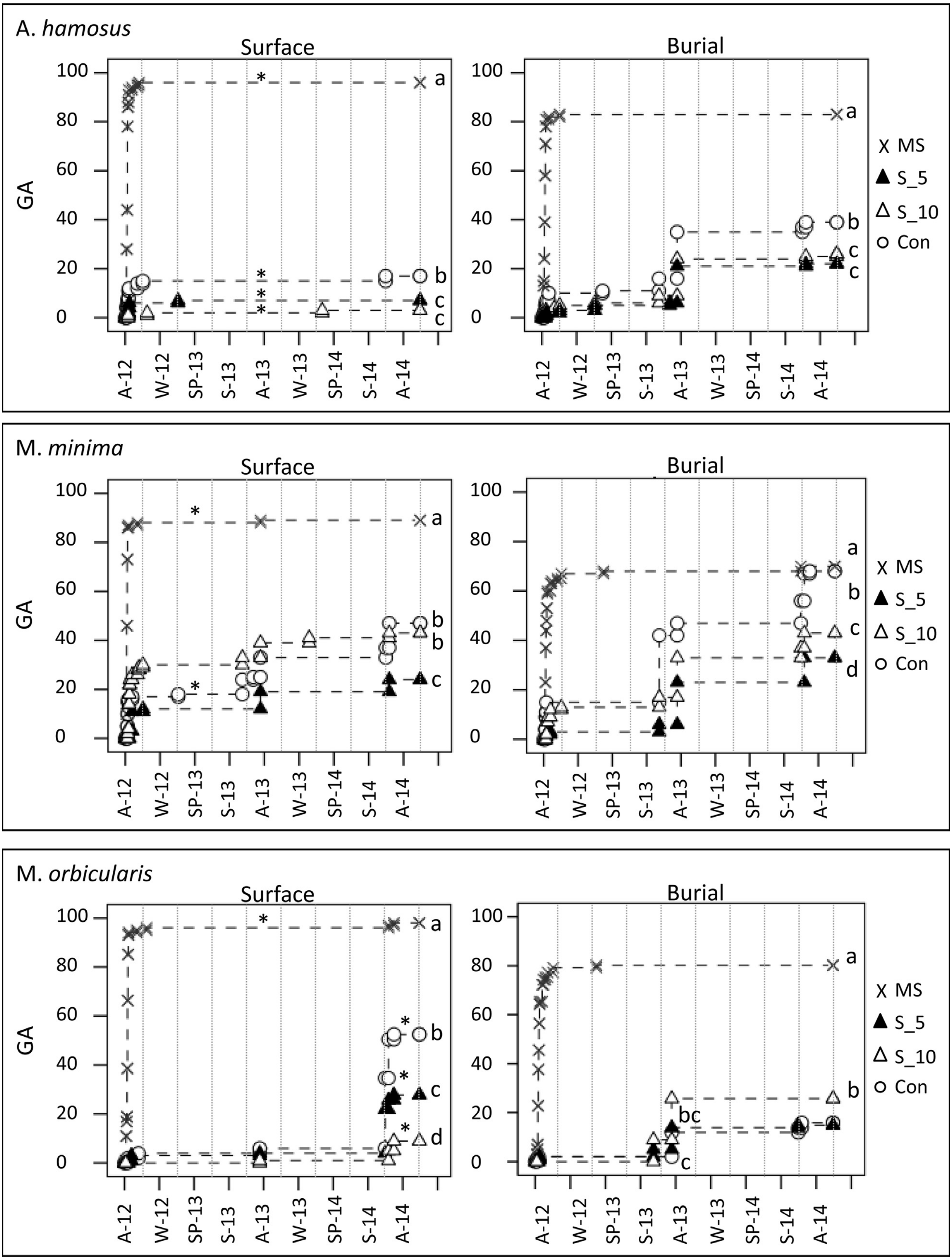

Fig. 2. Curves of accumulated germination (AG) during the study period (autumn (A), winter (W), spring (SP) and summer (S); *x*-axis). The treatments were as follows: mechanical scarification (MS), five minutes in sulphuric acid (S_5), ten minutes in sulphuric acid (S_10) and control (Con). Different letters indicates significant differences between different treatments for each planting depth; the asterisk indicates significant differences between planting depths for each treatment (Kaplan Meier analysis; Log-rank test, $p < 0.05$).

polymorpha (Table 1). There was a positive effect for surface sowing in *M. polymorpha* and *M. orbicularis* (S_5) and for deep sowing in *A. hamosus*, *M. orbicularis* (S_10) and *S. muricatus* (S_5) (Fig. 1).

3.2.1. Analysis of the variation between different treatments for each planting depth

Our results showed significant differences between treatment types for both depths in all studied species except *M. polymorpha* (Table 1).

In surface planting the treatment that gave the greatest final germination was mechanical scarification (Table 2), with significant differences compared to other treatments in *A. hamosus*, *M. orbicularis*, *M. minima* and *S. muricatus* (Fig. 1). As well, we found significant differences between the chemical scarification treatments and the control in both *A. hamosus* and *M. orbicularis*, and between S_5 and Con in *M. minima* (in S_5), with, in general, less final germination when seeds were immersed in sulphuric acid (see Fig. 1).

In deep planting the treatment that gave the greatest final germination – up to 80% in *A. hamosus* and *M. orbicularis*– was mechanical scarification (Table 2), and our results show significant differences between this treatment and the other treatments in *A. hamosus* and *M. orbicularis.* Mechanical scarification in both *S. muricatus* and *M. minima* gave no benefits over non-scarified seeds but did give benefits over the immersion in sulphuric acid (see Fig. 1).

3.3. Germination patterns

The accumulated germination curves during the study period estimated by the Kaplan Meier method are shown in Fig. 2. Germination peaks occur at the end of summer and beginning of autumn, and there was almost no germination during the spring. The results of the comparative analysis of the different germination patterns are given in Table 3. The mechanical scarification method was the only treatment that showed significant differences between planting depths for all species. In addition, we found significant differences between the germination patterns of the tested treatments at each planting depth in all species except for the buried seeds of *M. polymorpha* (Table 3).

3.3.1. Analysis of the variation between different depths of planting for each treatment

In all species scarification was the only treatment that produced significant differences between the different depths of planting (Table 3). Surface-sown seeds required less time to germinate (Fig. 2) and reached acceptable seed germination levels in less than 10 days: *A. hamosus* (6 ± 0.20), *M. orbicularis* (9 ± 0.19) and *M. minima* (6 ± 0.17) (see Table 2).

Non-scarified seeds showed significant differences in germination patterns between depth of planting in *A. hamosus*, *M. minima* and *M. orbicularis* ($p < 0.01$), with a positive effect for surface planting only in *M. orbicularis* (Fig. 2).

The treatment consisting of the immersion in acid for five minutes (S_5) showed significant differences for depth of planting in *A. hamosus*, *M. polymorpha*, *M. orbicularis* and *S. muricatus*. There was a positive effect for surface planting in *M. polymorpha* and *M. orbicularis*, which was reflected in the shorter time needed to reach 25% germination, although both species still needed a number of autumns to reach this percentage (Table 2 and Fig. 2).

The immersion treatment in S_10 showed significant differences between different depths of planting in *A. hamosus*, *M. orbicularis* and *M. polymorpha*, with a positive effect for surface planting in *M. polymorpha*. This species achieved 50% germination in the second autumn of the study period, while deep planting had a germination percentage during the same period of only around 25%.

3.3.2. Analysis of the variation between different treatments for each planting depth

In surface planting the comparison between the germination patterns in mechanically scarified seeds and other treatments showed significant differences in all species (Table 3 and Fig. 2). Under mechanical scarification in all species seeds required fewer than 10 days to reach 25% germination, in contrast to the control and chemical treatments that required more than one autumn to reach a similar percentage by the end of the experiment (Table 2). Mechanical scarification was the only treatment that achieved acceptable germination, which was reached very rapidly in three of the five species studied (Table 3).

Chemical scarification treatments showed significant differences compared to non-scarified seeds in *A. hamosus*, *M. orbicularis* and *S. muricatus* (in both S_5 and S_10), *M. minima* (in S_5) and *M. polymorpha* (in S_10). Other than in *M. polymorpha,*chemical scarification requires more time for germination and gives poorer germination rates.

In deep-sown seeds the comparison between germination patterns for mechanical scarification and for the other treatments showed significant differences for all species except *M. polymorpha* (see Fig. 2). Mechanical scarification needed in four out of five species fewer than 20 days to reach 25% of germination, in contrast to the other treatments that generally needed more than three months during autumn to obtain this figure (Table 2). Mechanical scarification also managed to reduce the time needed to obtain acceptable seed germination in two of the five studied species, *A. hamosus* (10 ± 0.54) and *M. minima* (26 ± 41.92).

There were significant differences between the chemical scarification treatments and non-scarified seeds in *A. hamosus*

Table 3
Results of the decomposition of the interaction effect between treatments and planting depth on germination patterns in each native legume species. The treatments were as follows: mechanical scarification (MS), five minutes in sulphuric acid (S_5), ten minutes in sulphuric acid (S_10) and control (Con). The seed planting depths were as follows: on the surface (S) and burial (B). The significance of the effects is indicated as $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

	A. hamosus			*M. minima*			*M. orbicularis*			*M. polymorpha*			*S. muricatus*		
	DF	χ^2	*p*	DF	χ^2	*p*	DF	χ^2	*p*	DF	χ^2	*p*	DF	χ^2	*p*
Variation in germination patterns of different planting depths for each treatment															
MS	1792	35.53	***	1792	25.32	***	1800	33.89	***	1856	16.79	***	1792	6.75	**
S_5	1792	11.73	**	1792	1.35		1800	3.66		1856	12.25	***	1792	10.69	**
S_10	1792	16.31	***	1792	0.33		1800	10.91	**	1856	4.79	*	1792	0.96	
Con	1792	9.27	**	1792	7.87	**	1800	25.17	***	1856	1.71		1792	1.69	
Variation in germination patterns of different treatments for each planting depth															
B	3792	442.4	***	3792	261.3	***	3800	269.8	***	3856	5.3		3792	23.02	***
S	3792	242.7	***	3792	75.91	***	3800	511.3	***	3856	2.32	***	3792	98.99	***

and *M. minima*(in both S_5 and S_10), and in *S. muricatus* (in S_10), with a negative effect in all for immersion in sulphuric acid (Fig. 2).

4. Discussion

Dormancy is a common characteristic in leguminous plants. This group of species has hard seed coats and low germination rates when subject to unfavorable environmental conditions (Aydin and Uzun, 2001). These factors play an important role in the germination patterns of this family (Lodge and Whalley, 2002) and ensure that germination will be prolonged; this is a good survival strategy (Bewley and Black, 1982) for guaranteeing the preservation of well-established natural pastures. Nevertheless, this temporal distribution of seed germination complicates the use of these species in projects to improve degraded pastures since one of the key aims of this type of management strategy is the quick, uniform germination of seeds and emergence of seedlings. Laboratory experiments have demonstrated that scarification is a good way of breaking the dormancy of leguminous species (Hardegree and Emmerich, 1991; Ibanez and Passera, 1997; Uzun and Aydin, 2004). A number of authors have reported that the effectiveness of scarification treatments depends on the genus, species and type of treatment applied (Demir and Ermis, 2004; Kimura and Islam, 2012). Some of the most widely used treatments include heating or cooling, humidification, and mechanical or chemical seed scarification.

Our results show that the seeds of the native annual leguminous species used in this study have a physical dormancy that is the product of the hardness of their seed coats. This is confirmed by the quick and abundant germination that occurred in seeds whose coats were mechanically scarified by removing part of the seed coat with sandpaper. Our results confirm those of Patanè and Gresta (2006) in *A. hamosus* and *M. orbicularis*, Gresta et al. (2007) in *S. muricatus*, and Sadeghi and Khaef (2012) in *M. polymorpha*.

Our results also indicate that mechanical scarification is the best way of stimulating seed germination. Of all the treatments tested in this study, the surface planting of mechanically scarified seeds gave the greatest germination success. This method had benefits over other treatments, both in the obtained germination percentage (except in *M. polymorpha*, for which no difference was found between the treatments) and in the less time needed for seeds to germinate. These results corroborate laboratory trials using the same species and show the greater efficiency of mechanical scarification as opposed to chemical scarification and non-scarified seeds in *A. hamosus* and *M. orbicularis* (Patanè and Gresta, 2006) and in *S. muricatus* (Gresta et al., 2007). We found no positive effect on the final germination percentage for mechanical scarification in *M. polymorpha*, as also reported by Can et al. (2009). Nevertheless, an analysis of this species' germination patterns showed a considerable decrease in the time needed to germinate, which was reduced from more than one autumn to less than a month.

Some studies have reported a positive effect for chemical scarification in leguminous species, although the effectiveness of the immersion in acid depends on the species involved, the concentration of the acid and the length of the immersion period (Balouchi et al., 2006). Our results did not demonstrate any improvement in the germination capacity of seeds scarified by sulphuric acid. The exception was in the deep planting of *M. orbicularis*, where we found an increase in the final germination percentage in comparison to non-treated seeds after immersing seeds in sulphuric acid for 10 min. Patanè and Gresta (2006) report a significant improvement in chemical scarification when increasing exposure time to acid in both *A. hamosus* and *M. orbicularis*. In *M. polymorpha* our results differed from those of Can et al. (2009), who found that immersion in sulphuric acid for five minutes produces a positive effect on the germination capacity when compared to non-scarified seeds or mechanical scarification. Nevertheless, we perceived a tendency towards greater germination capacity in S_10 and, in addition, also found a positive effect on the time needed to germinate in chemically scarified seeds as compared to control seeds. Patanè (1998) found a positive effect for chemical scarification over non-scarified seeds in *S. muricatus*. These discrepancies in our results, which showed no such positive effect, could be due to the greater exposure to acid – over 35 min – in this author's experiment.

The analysis of the germination patterns shows that mechanically scarified seeds only germinated in the first autumn and, unlike the other treatments, in which further germination peaks occur even in the third autumn after sowing, no subsequent germination takes place. These results indicate that the success of any plan for enhancing or improving plant cover using mechanical scarification will depend on the presence of environmental conditions favourable for the germination of leguminous species (temperatures in the range 20–25 °C and regular rainfall; Gresta et al., 2007). Thus, if rainfall is not regular enough, emergency irrigation – whenever available – for at least the first ten days might represent a good alternative method for dampening and reducing seed loss due to adverse environmental conditions.

The final germination percentage and the germination patterns observed in some of the tested species (e.g. *M. minima* and *M. orbicularis*, two of the best-represented species in spontaneous pastures) suggest that, if irrigation is not possible, another option is the mixed sowing of scarified and non-treated seeds. In this way, germination is staggered over more than one year and its success will not be so dependent on the environmental conditions of the year in which sowing takes place. As discussed above, the germination of these species occurs in late summer and early autumn when the soil is completely bare due to the extreme environmental conditions of the Mediterranean summer, and when other adventitious species have not yet established themselves, even if they are already present in the seed bank.

Several studies have shown the positive effect of seed burial on the breaking of dormancy. Our results show that the effects of seed burial vary between species: in two of the five species studied (*A. hamosus* and *M. minima*) burial improved germination rates in untreated seeds, although in *M. orbicularis* burial decreased this rate. The effects of burial in soil may be determined by soil moisture and temperature and therefore will vary depending on the depth of planting. Zeng et al. (2005) found that the breaking of physical dormancy decreased with depth of burial below 2 cm in seeds of *M. polymorpha* and several species of *Trifolium*. On the other hand, several studies have shown that grazing by sheep is a fundamental tool for enhancing the persistence of Mediterranean annual legumes and productivity (Rochon et al., 2004), possibly because the presence of sheep favours dispersion and pod burial. Further advantages of seed burial include reduced seed loss, which may reach serious proportions due to consumption by birds and other living organisms, and less germination failure as a consequence of unfavorable environmental conditions such as drought immediately following seed germination.

Thus, in the case of programmes involving the planting of non-treated seeds of leguminous species, we recommend direct planting followed either by light surface ploughing or the presence of sheep flocks that will trample the seeds a few centimetres into the soil. Nevertheless, the burial depth determined by present tillage operations – even superficial tillage – can cause different germination patterns to those found in this study. Thus, field studies are still required to both search for tillage techniques that allow for the shallow burial of seeds and to corroborate these findings, which should be complemented by more detailed establishment and competiveness studies. Moreover, more detailed studies are still needed regarding (i) the floristic composition of green natural cover in these olive groves, (ii) the problems of predation suffered by these species at seed and seedling stages (severe in some cases due to the abundance of rabbits in these crops, as is becoming apparent in light of certain studies), and (iii) the planting and control systems required for these species, which are very dependent on the time that they wilt.

5. Conclusions

The time required for germination and the germination percentages achieved in the surface sowing of mechanically scarified seeds reveal that this combination of techniques is a good option for restoring or establishing natural herbaceous ground cover in olive groves. We recommend that sowing takes place at the end of summer or beginning of autumn (to reduce seed losses due to predation) and, if rainfall is not sufficient, that emergence irrigation is employed.

If the mechanical scarification of seeds is not possible, the shallow burial of seed by light ploughing or grazing by sheep is a good option for increasing the germination of leguminous plants. This technique is compatible with any type of olive grove and is particularly favorable for improving cover in organic crops.

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