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Optimization of Seed Germination and Seedling Emergence of *Medicago arborea* L.

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Abstract: Laboratory and greenhouse experiments were conducted in Athens, in order to investigate the germination behaviour of untreated seeds and seeds subjected to several pretreatments and subsequent emergence of the seedlings. In general, the speed and percentage of seed germination and seedling emergence were greatly increased by some treatments, including hot water immersion (especially for 4 min) immersion in sulphuric acid for 2 min and in water for 20 h, clearly confirming that there is a physical (seed coat) dormancy in this species, while untreated seeds (i.e., control) had relatively moderate germination and emergence percentages. On the contrary, very high temperatures and prolonged immersion in sulphuric acid were rather harmful for the germination and further emergence of *M. arborea* and therefore they have certainly to be avoided.

Key words: *Medicago arborea*, seed germination, hot water, dry heat, emergence

INTRODUCTION

Cultivated fodder shrubs have received considerable attention in recent years because they can provide available biomass during periods of zero or low production of grassland resources. Especially in marginal areas, these shrubs might lead to greater exploitation of land in danger of progressive abandonment and degradation. Moreover, compared with herbaceous species, cultivated fodder shrubs ensure soil cover throughout the year, contributing to a reduction in soil erosion (Andreu *et al*., 1994) an increase in the accumulation of organic matter and on the whole the stability of agroecosystems. Forage legumes are widely used in many grassland farming areas of the world; their importance having arisen principally because of their ability to fix atmospheric N biologically and secondly because of their high nutritional value and other advantages (Rochon *et al*., 2004).

Medicago arborea (Tree Medic) is an important perennial woody leguminous shrub which can grow between 2-4 m high under favourable conditions. This species originates from the Mediterranean regions, where it is found growing from the Canary Islands along southern Europe to Asia Minor (Lesins and Lesins, 1979). It is very drought and cold tolerant and it can reduce soil erosion (Andreu *et al.* 1994; ICARDA, 1998). Besides, the good forage quality of the species was recognised by ancient Greeks and Romans (Lesins and Lesins, 1979) while its production is located in the late summer and early autumn period, i.e., when a significant feed gap occurs in Mediterranean climates typified by cool wet winters and hot dry summers.

It is well recognized that *M. arborea* may act as a strategic forage species supporting conventional resources in forage systems for sheep farming in semiarid Mediterranean environments (Stringi *et al*., 1996; Papanastasis *et al*., 1998) particularly during periods of shortage (Amato *et al*., 2004). However, in order to fully exploit this potential, we have primarily to pay attention to the establishment and dispersal of the species, starting from seed germination and seedling emergence. Until now little information is available on the influence of specific pretreatments on seed germination and seedling emergence of tree medic. Within this scope, the objective of this study was to improve this knowledge, by assessing the response of this species seed germination and further seedling emergence to the effects of several pretreatments. The highly desirable features of *M. arborea* make imperative the need of such a study, in order to optimize its seed germination and first growth.

MATERIALS AND METHODS

Experimental details: The *M. arborea* seeds were collected directly from the wild from several locations of Mt Hymettus, Athens, Greece (latitude 37°58′ N;

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longitude 23°48′ E; altitude 350-420 m) in 2003. After collection, immature seeds and those attacked by insects were removed and the healthy seeds were stored at 5°C and 50% RH (relative humidity) until their use.

Three germination experiments were conducted in the Laboratory of Agronomy of the Agricultural University of Athens (AUA) during the summer and autumn of 2003. Both tests were carried out in a completely randomized design under laboratory conditions, in incubators (Conviron T 38/Lb/AP) at constant temperature (30°C) and total darkness.

In the first experiment the effects of the following treatments on seed germination were evaluated: (1) immersion in hot water (100°C) for 1 min; (2) immersion in hot water (100°C) for 2 min; (3) immersion in hot water (100°C) for 4 min and (4) immersion in hot water (100°C) for 8 min.

In the second germination assay, seeds were placed in an aluminum dish and exposed to the desired temperatures in a preheated oven for 5 min: (1) at 50°C; (2) at 100°C; (3) at 150°C and (4) at 200°C. These temperatures were selected because they are likely to be reached at the soil surface or the first few centimetres below ground in fires in arid and semi arid regions (DeBano *et al*., 1998) while a preliminary experiment showed that prolonged exposure (>5-10 min) at higher temperatures is probably lethal for the *M. arborea* seeds.

In the third germination experiment the following pretreatments were tested: (1) immersion in water at room temperature (25-30°C) for 20 h; (2) immersion in concentrated (95%) sulphuric acid for 2 min followed by thorough rinsing with running water; (3) immersion in concentrated sulphuric (95%) acid for 6 min and thorough rinsing with running water and (4) immersion in a gibberellic acid (GA_3) solution (100 mg $GA_3.L^{-1}$ water) for 30 min.

There were used five replicates (Petri dishes) for each treatment, while untreated seeds were used as control for each experiment. Twenty two seeds were placed between two Whatman No. 1 paper filter disks (Whatman Ltd., Maidstone, England) in each 9 cm Petri dish and 5 mL of distilled water was added. Additionally, distilled water was added whenever there was a need to keep filter papers moist.

Seed germination was recorded every other day and expressed as a % percentage of the total number of tested seeds (Germination percentage, GP). Seeds were considered germinated at the emergence of the radicle (Bewley and Black, 1994). The germination rate index (GRI) was also calculated for each treatment using the following equation:

$$(GRI) = (G_1/1)+(G_2/2)+\ldots+(G_x/x)$$

where G is the germination on each day after placement and 1, 2,...x represents the corresponding day of germination (Esechie, 1994). Corrected germination rate index (CGRI) was obtained by dividing GRI by the Final Germination Percentage or FGP (GP at 20 days after the beginning of seed incubation for our experiments) and multiplying by 100. The number of days lapsed to reach 50% of the final germination percentage (GT_{50}) another widely used index in order to compare relative rate of germination in perennial forage plants, was also calculated (Hsu *et al*., 1985).

Subsequently, three pot experiments were conducted in a glasshouse of the Agricultural University of Athens (AUA) in 2003. Minimum/maximum air temperature and relative humidity were: 17/40°C and 35/60%, respectively and the plants were subjected to a natural day length ranging between 12-15 h during the experiments.

Twenty five pregerminated seeds of each treatment reaching a radicle of 1-3 cm length were sown at 1 cm depth. Five seeds were planted in each plastic pot (15 cm in diameter) filled with 2.4 l of a slightly calcareous sandy clay loam (SCL) soil. Table 1 shows the physical and chemical properties of this soil. Irrigation was carried out with 200 mL of distilled water in each pot every two days, in order to promote plant emergence. The number of days from sowing to emergence was recorded for all the seedlings.

Statistical analysis: The percentages of germination and emergence (after arcsine transformation) and the rest raw data were subjected to one-way analysis of variance (ANOVA) using the Statgraphics statistical software package (v.5.0, Statistical Graphics Corporation, Englewood Cliffs, NJ, USA). Mean comparison was performed using Fisher's least significant difference (LSD) method ($p<0.05$).

Table 1: The physical and chemical properties of the soil used for seedling emergence of *Medicago arborea*

Parameter	SCL
Sand (%)	50.2
Clay (%)	15.8
Silt (%)	34
pH(in H_2O)	7.4
Total $CaCO_3$ (%)	20.4
Organic matter (%)	3.96
Nitrogen (g kg^{-1})	0.32
Phosphorus (g kg^{-1})	101.34
Potassium (g kg^{-1})	1.5
Sodium (g kg^{-1})	0.19
CEC (meq 100 g^{-1})	8.7

SCL = Sandy Clay Loam

RESULTS

In Table 2 it is shown that immersion in hot water for 4 min increased significantly the FGP of *M. arborea* seeds from 45 (control) to 71% and it was consistently the most effective pretreatment. Furthermore, seed immersion in hot water for 1, 2 and 8 min also resulted to significantly highest germination percentages than untreated seeds (Table 2).

The second germination experiment revealed that dry heat can either promote or hinder seed germination. The temperatures of 100 and 150°C were the significantly most stimulative temperature for the treatment of *M. arborea* seeds, but still less effective than hot water treatments. In contrast, dry heating of tree medic seeds at 200°C resulted to slightly lower germination percentage even than the untreated seeds (Table 3).

The enhancing or preventing influence of sulphuric acid on seed germination of *M. arborea* (due to the time of immersion) is shown in the Table 4. Moreover, immersion in water for 20 h has also improved seed germination, while immersion in gibberellic acid for 30 min was rather ineffective on *M. arborea* seed germination. The germination rate of seeds after an immersion in hot water for 4 min (and secondly for 2 and 8 min) was greatly enhanced, as long as the CGRI values calculated in this method was the highest among all treatments and the 50% of the final germination percentage was obtained after 11 (Table 5). Concerning the dry heat treatments, none of them was really effective on the germination rate, while the temperature of 200°C had clearly negative effects on germination percentage, although it was a quite rapid method. Concerning the germination rate of *M. arborea*seeds, immersion in sulphuric acid for 2 min and immersion in water for 20 h were also among the most satisfactory pretreatments. Besides, in Table 5 it is shown that although the emergence percentage and rate of untreated tree medic seeds (control) were relatively satisfactory, some pretreatments, such as immersion in

Table 2: Time course for the germination of *Medicago arborea* seeds in response to different periods of immersion in hot water

Days after seed placement	Pretreatment Control	Hot water for 1 min	Hot wate for 2 min	Hot water for 4 min	Hot water for 8 min
2	0c	0c	2b	4a	0c
4	0d	0d	5b	8a	2c
6	5c	5c	12b	18a	8bc
8	7d	10cd	16b	24a	14bc
10	12c	18b	23b	32a	21b
12	18c	30b	36b	45a	31b
14	25c	44b	46b	54a	42b
16	33c	49b	52b	60a	50b
18	39c	55b	58b	66a	55b
20	45c	63b	64b	71a	62b

Means (germination percentages, %) followed by the same letter(s) within a row are not significantly different at p = 0.05 Fisher's least significant difference test

Table 3: Time course for the germination of *Medicago arborea* seeds in response to several dry heating pretreatments

Days after seed placement	Pretreatment Control	50°C	100°C	150°C	200°C
2	0a	0a	0a	0a	0a
4	0b	0b	2a	2a	0b
6	2b	0c	6a	6a	2b
8	6b	4b	12a	10a	6b
10	10b	8b	16a	15a	10b
12	17b	15b	24a	22a	16b
14	24b	23b	32a	31a	16c
16	31b	32b	40a	40a	20c
18	37b	40b	48a	50a	25c
20	42b	46b	56a	54a	25c

Means (germination percentages, %) followed by the same letter(s) within a row are not significantly different at p = 0.05 Fisher's least significant difference test

Table 4: Time course for the germination of *Medicago arborea* seeds in response to several pretreatments

Days after seed placement	Pretreatment Control	Water for 20 h	Sulphuric acid for 2 min	Sulphuric acid for 6 min	Gibberellic acid for 30 min
2	0b	0b	5a	0b	0b
4	0c	2b	8a	2b	0c
6	2c	5bc	16a	8b	2c
8	8cd	10bc	22a	12b	6d
10	12b	16b	30a	14b	11b
12	18c	24b	40a	16c	18c
14	22cd	32b	52a	18d	24c
16	30cd	38b	58a	24d	32bc
18	37c	46b	62a	28d	38c
20	44c	56b	68a	30d	44c

Means (germination percentages, %) followed by the same letter(s) within a row are not significantly different at p = 0.05 Fisher's least significant difference test

Table 5: Seed germination rate and seedling emergence of *Medicago arborea* in response to different pretreatments

	Pretreatments	CGRI (% day^{-1})	GT_{50} (days)	Mean emergence (%)	Average days of emergence
A	Control	12.7d	13a	68b	5a
	Hot water for 1 min	18.8cd	13a	72b	5a
	Hot water for 2 min	24.5ab	11c8	0a	4b
	Hot water for 4 min	31.8a	11c	84a	4b
	Hot water for 8 min	20.5bc	12b	72b	5a
B	Control	11.3b	13.5ab	72a	6b
	50°C	10.7b	14a	76a	6b
	100°C	16.9a	13b	68a	5c
	150°C	16.3a	13b	72a	5c
	200°C	8.4b	11.5c	48b	7a
C	control	11.7c	14a	68c	6a
	Water for 20 h	16.2b	12.5b	76ab	5b
	Sulphuric acid for 2 min	30.4a	11c	80a	4c
	Sulphuric acid for 6 min	11.7c	11c	52d	5b
	Gibberellic acid for 30 min	11.7c	13b	72bc	5b

Means followed by the same letter(s) within a column are not significantly different at p = 0.05 Fisher's least significant difference test within each experiment. A: First, B: Second and C: Third germination and emergence assay hot water for 2 and 4 min, in sulphuric acid for 2 min and in water for 20 h contributed to the further optimization of seedling emergence. On the contrary, it has to be noted the negative effect of very high temperatures (e.g., 200°C) and prolonged immersion in sulphuric acid, not only on seed germination, but on seedling emergence, too.

DISCUSSION

Our results indicated that all of the hot water treatments increased significantly the germination and emergence percentage and rate of *M. arborea*. The beneficial effect of hot water bath on seed germination is common among perennial legumes widespread in arid and semi-arid zones (Clemens *et al*., 1977; Muhammad and Amusa, 2003). Furthermore, it is well documented that the immersion of the seeds in hot water may lead to the rapture of the coat wall, allowing water to permeate the seed tissues and causing seed germination and further rapid emergence of several other species of arid regions (Agboola and Etejere, 1991; Emongor *et al*., 2004).

Some of the tested dry heat treatments were also very effective in terms of germination percentage (100 and 150°C for 5 min) likewise in other woody species (Tarrega *et al*., 1992; Vilela and Ravetta, 2001) while only the temperature of 200°C showed a marked inhibition of germination and emergence, similarly to other woody species (Reyes and Casal, 2006). This positive (or damaging at high temperatures or prolonged exposure) effect of dry heat is well documented in many species, while it underlines the potential effect of fire on seed germination and seedling emergence of *Medicago arborea* in Mediterranean-type ecosystems (Arianoutsou and Margaris, 1981; Thanos and Goerghiou, 1988; Qaderi and Cavers, 2003).

The beneficial effect of sulphuric acid on seed germination of several *Medicago* species has already mentioned (Ruiz and Devesa, 1998). Furthermore, concerning the germination promotion of the seeds treated with sulphuric acid and their possible damages caused by prolonged immersion (6 min in our case) or low integumental resistance, there are many reports in several arid-adapted legumes (Sy *et al*., 2001; Vilela and Ravetta, 2001).

In a contrary way to some-and in accordance to-others hard-seeded legumes native to arid and semi-arid environments, water immersion was also effective on tree medic seed germination and emergence (Sy *et al*., 2001). Our results confirm previous reports on the positive effect of water immersion on *M. arborea* seed germination (Huxley, 1992). However, gibberellic acid treatment (immersion for 30 min) did not have significant effects on this species germination and emergence, implying that probably the primary control of germination in *M. arborea* seeds resides in the seed coats, i.e., the type of seed dormancy is not physiological but mainly physical dormancy, likewise many leguminous plants (Travlos *et al*., 2006).

In addition, there was a significantly positive correlation between germination percentage (FGP) and germination rate (CGRI and GT_{50}) (r^2 = 0.88, $p<0.05$ and r^2 = -0.6, $p<0.05$, respectively, suggesting that in our experiments on tree medic, the rapid germination was closely associated with the high germination percentage. Additionally, it seems that *M. arborea* germination was highly significantly correlated with the emergence of the seedlings (r^2 = 0.86, $p<0.05$) and the rate of seedling emergence (r^2 = -0.79, $p<0.05$).

Seed dormancy is usually associated with the factors of the protective covering, the seeds coat or the enclosed embryo. Our results indicated that immersion in hot water, in sulphuric acid for 2 min and in water for 20 h can induce significantly seed germination. These results are in agreement with previous reports, providing evidence that *Medicago arborea* seeds certainly involve physical dormancy (Bass *et al*., 1998) while there are differences between the several species, ecotypes and specific pretreatments (Uzun and Aydin, 2004). This physical (coat-imposed) dormancy favors the accumulation of persistent seed banks in the soil, spreads germination over time and increases the chance that some seeds will germinate, establish and complete the life cycle successfully (Gutterman, 1993) but is exceptionable for the cultivation of the species.

Medicago arborea is not only included to the national strategies for rangeland rehabilitation in many countries, but it is often recommended as a model legume for revegetation, regeneration and biological reactivation of degraded lands in semi-arid mediterranean areas (González-Andrés *et al*., 1999; Valdenegro *et al*., 2001; Amato *et al*., 2004). Unfortunately, in many cases the cost of the establishment by means of transplanting is relatively high (Bouzid and Papanastasis, 1996) while direct seeding has been shown to be successful in establishing native trees and shrubs in low rainfall environments (Knight *et al*., 1998). Consequently, there is a need of optimization of seed germination, seedling emergence and first growth of tree medic, in order to exploit the inarguable potential of this species. Therefore, such information can be of considerable practical importance and specific field experiments, monitoring and further laboratory studies must be continued in order to optimize rapid and uniform seed germination and seedling emergence of *M. arborea*.

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