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Seed Germination and Seedling Emergence of *Spartium junceum* L. In Response to Heat and Other Pre-Sowing Treatments

I.S. Travlos, G. Economou and A.J. Karamanos
Department of Crop Production, Laboratory of Agronomy,
Agricultural University of Athens, 75, Iera Odos st., 11855 Athens, Greece

Abstract: Laboratory and greenhouse experiments were conducted in Greece, in order to investigate the germination behavior of untreated seeds and seeds subjected to several pretreatments and subsequent emergence of the seedlings. Germinability of untreated (control) seeds was relatively high (ca. 70%), indicating the presence of primary (coat-imposed) dormancy in a fraction of the seeds studied. Hot water immersion, dry heat, sulphuric acid treatment and water soak effectively relieved dormancy of the hardcoated portion of the seed population and consequently increased rate and final percentage of germination (92, 84, 80 and 78%, respectively). This positive effect of heat on seed germination followed by a massive and rapid seedling emergence might be used as a tool for the control of invasive *Spartium* populations.

Key words: *Spartium junceum*, seed germination, hot water, dry heat, emergence

INTRODUCTION

Several authors have reported the dramatic increase of introduced leguminous woody plants on many arid regions, with Spanish broom (*Spartium junceum* L.) being one of the major invasive woody plants found (Bush and Van Auken, 1991; Hickman, 1993). This species is a perennial, leguminous, ornamental shrub native to the Mediterranean region in southern Europe, southwest Asia and northwest Africa. In contrast to its important advantages (e.g., it can grow in nutritionally poor soil, prevent soil erosion, it is drought tolerant and nitrogen fixating), it is generally regarded as undesirable in some regions (likewise south Africa) and it will no longer be tolerated, as long as it displaces the native species and threatens the ecosystem structure and function of natural habitats (Chiej, 1984; Henderson, 2001).

Most research approaches of Spanish broom to date, have concentrated on finding ways to control this species because of its invasive nature. However, a truly effective method of eradication has not been found, as long as only a few experiments have been conducted to discover the basic ecology of the species (Nilsen, 1992; Nilsen and Semones, 1997). Besides, seed germination in arid and semi-arid regions has been studied mainly in annual species (Gutterman, 1993), but their germination patterns differ widely from those of perennial species. Therefore, we have primarily to pay attention to the seed germination and seedling emergence of the species, which spreads by abundantly producing seeds of high viability (one plant can easily produce more than 7000 seed in one season) (Nilsen, 2000).

Understanding of the germination of seeds is crucial for its effective control, but until now little information is available on the influence of several abiotic factors on seed germination and seedling emergence of Spanish broom. Within this scope, the present study is an attempt towards assessing the response of this species seed germination and further seedling emergence to the effects of several pretreatments and especially heat and demonstrating the potential use of fire as a management tool for the population control of *S. junceum*. The aggressive nature of this plant makes imperative the need of such a study, in order to control its disturbance effectively.

MATERIALS AND METHODS

Experimental details: The *S. junceum* seeds (readily dehiscent) were collected directly from the wild of Vonitsa region in Greece (latitude ranging from 38°40′ N to 38°60′ N; longitude ranging from 20°40′ E to 20°60′ E) in the summer of 2003. After collection, immature seeds and those attacked by insects were removed and the healthy seeds were stored at 5°C and 50% RH until their use.

Two germination experiments were conducted in the Laboratory of Agronomy of the Agricultural University of

Corresponding Author: I.S. Travlos, Department of Crop Production, Agricultural University of Athens, 75, Iera Odos st., 11855 Athens, Greece Tel: +3 210 5294482 Fax: +3 210 5294482

Athens (AUA) during the summer of 2004. 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 water at room temperature (25-30 °C) for 24 h; (2) immersion in hot water (100°C) for 5 min; (3) immersion in concentrated (95%) sulphuric acid for 5 min followed by thorough rinsing with running water and (4) cold stratification (pre-chilling) at 5°C for 5 days.

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 80°C; (3) at 110°C (4) at 140°C and (5) at 170°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 Africa (DeBano *et al*., 1998), while a preliminary experiment showed that prolonged exposure (>5-10 min) at high temperatures is probably lethal for the *S. junceum* seeds.

The above mentioned treatments of each experiment were planned in such a way that the incubation period was begun at the same time for all the treated and untreated (control) seeds. Six replicates (Petri dishes) were used for each treatment. Twenty seeds were placed between two Whatman No. 1 paper filter disks (Whatman Ltd., Maidstone, England) in each 9 cm petri dishand 5 mL of distilled water was added. Additionally, distilled water was also 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 placementand 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 18 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, was also calculated (Hsu *et al*., 1985).

Subsequently, two pot experiments were conducted in a glasshouse of the Agricultural University of Athens (AUA) in 2004. Minimum/maximum air temperature and relative humidity were: 20/40°C and 40/60%, respectively and the plants were subjected to a natural day length ranging between 13-15 h during the experiments.

Thirty pregerminated seeds of each treatment reaching a radicle of 1-2 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 mixture of peat and perlite (2:1, v/v). Irrigation was carried out with 200 mL of distilled water in each pot every three 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 according to Sokal and Rohlf. (1995) 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 ($p<0.05$).

RESULTS

Immersion in hot water for 5 min increased significantly the FGP of *S. junceum* seeds from 67 (control) to 92% and was consistently the most effective pretreatment. Furthermore, seed immersion in water for 24 h and in sulphuric acid for 5 min also resulted to significantly highest germination percentages than control and cold stratification (Table 1).

The second germination experiment revealed that dry heat can either promote or hinder seed germination. The temperature of 110°C (followed by 80 and 50°C) was the significantly most stimulative temperature for the treatment of *S. junceum* seeds. In contrast, dry heating of *S. junceum* seeds at 170°C (and secondly 140°C) resulted to significantly lower germination percentage than the untreated seeds (Table 2).

The germination rate of seeds after an immersion in hot water was greatly enhanced, as long as the CGRI value calculated in this method was the highest among all treatments and the 50% of the final germination percentage was obtained after only nine days (Table 3). Additionally, immersion in sulphuric acid for 5 min was also a very effective method for seed germination, while brief cold stratification of the seeds clearly resulted to a low rate of germination (the CGRI value was significantly lower than the corresponding value for the control batch).

Table 1: Time course for the germination of *Spartium junceum* seeds in response to different pretreatments

		Pretreatment			
Days after seed placement	Control	Water for 24 h	Hot water for 5 min	Sulphuric acid for 5 min	Cold stratification for 5 days
2	0±0.0a	0±0.0a	0±0.0a	0±0.0a	0±0.0a
4	5±0.1c	8±0.1bc	15±0.2a	11±0.3ab	5±0.2c
6	12±0.1c	16±0.2bc	27±0.2a	21±0.2ab	13±0.1c
8	20±0.2c	23±0.2c	38±0.3a	30±0.2ab	20±0.3c
10	29±0.3c	34±0.3bc	50±0.2a	41±0.2b	31±0.3c
12	35±0.3c	47±0.4b	65±0.4a	57±0.3a	39±0.2bc
14	47±0.6c	60±0.3b	81±1.1a	68±0.7b	44±0.4c
16	58±0.3c	69±0.4b	85±0.7a	73±0.6b	57±0.5c
18	67±0.4c	78±0.6b	92±0.6a	80±0.8b	68±0.8c

Results (germination percentages, %) are represented as means of six replications±standard error (duration of the germination experiment = 18 days). Means within the same row followed by the same letter(s) do not differ significantly (p>0.05). All percent germination data were arcsine transformed for analysis

Table 2: Time course for the germination of *Spartium junceum* seeds in response to different dry heat pretreatments

		Pretreatment				
Days after seed placement	Control	50°C	80°C	110°C	140°C	170°C
2	0±0.0a	0±0.0a	0±0.0a	0±0.0a	0±0.0a	0±0.0a
4	4±0.1c	5±0.2c	9±0.2b	14±0.3a	12±0.4ab	2±0.1c
6	14±0.2b	15±0.2b	14±0.3b	26±0.3a	20±0.4ab	8±0.3c
8	20±0.4b	22±0.3b	28±0.4b	42±0.5a	24±0.3b	12±0.3c
10	31±0.4b	32±0.4b	40±0.4ab	48±0.3a	31±0.5b	16±0.3c
12	38±0.3b	40±0.3b	47±0.5ab	56±0.6a	39±0.4b	20±0.3c
14	49±0.5bc	52±0.4b	60±0.3ab	69±0.4a	48±0.3bc	24±0.4d
16	61±0.6b	66±0.7b	70±0.6ab	79±0.8a	50±0.7c	26±0.5d
18	69±0.4b	72±0.8b	74±0.6b	84±0.9a	54±0.8c	27±0.4d

Results (germination percentages, %) are represented as means of six replications±standard error (duration of each dry heat pretreatment = 5 min and duration of the germination experiment = 18 days). Means within the same row followed by the same letter(s) do not differ significantly (p>0.05). All percent germination data were arcsine transformed for analysis

Table 3: Seed germination rate and seedling emergence of *Spartium junceum* in response to different pretreatments

Pretreatments	CGRI (% day^{-1})	GT_{50} (days)	Mean emergence (%)	Average days of emergence
A				
Control	38.8±0.2bc	11.5±0.2	70±0.7c	7±0.2b
Water for 24 h	35.6±0.4cd	11±0.2a	80±0.6b	6±0.1c
Hot water	43.1±0.5a	9±0.3c	86.7±1.2a	5±0.2d
Sulphuric acid	40.9±0.6ab	10±0.4b	83.3±0.7ab	5±0.4d
Cold stratification for 5 days	33.5±0.4d	11±0.3a	73.3±0.7c	8±0.4a
B				
Control	33.6±0.5d	11±0.5a	73.3±0.8b	7±0.5c
50°C	34.5±0.4d	11±0.6a	70±0.6b	7±0.4c
80°C	38.9±0.3c	9.5±0.4b	80±0.9a	6±0.4d
110°C	45.1±0.6a	8±0.2c	80±0.7a	6±0.3d
140°C	46.7±0.2a	9±0.4bc	63.3±0.6c	8±0.7b
170°C	42.4±0.5b	9.5±0.7b	53.3±1.3d	11±0.6a

All data are represented as means of all replications±standard error (duration of each dry heat pretreatment = 5 min and duration of the germination experiment = 18 days). A: first and B: second germination and emergence assay. Means within the same column followed by the same letter(s) do not differ significantly (p>0.05). All percent germination data were arcsine transformed for analysis

Concerning the dry heat treatments, the 5 min treatment at 110°C yielded the highest emergence and the fastest germination and emergence rate, while the temperature of 170°C has clearly negative effects not only on germination percentage, but on seedling emergence, too.

Besides, in Table 3 it is shown that although the emergence percentage and rate of untreated Spanish broom seeds (control) were relatively satisfactory, some pretreatments (immersion in hot water and sulphuric acid, or dry heating at 80°C or 110°C) contributed to the improvement of *S. junceum* emergence.

DISCUSSION

In the present study, hot water treatment increased significantly the germination and emergence percentage and was the most rapidly effective seed pretreatment. 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, immersion of seeds in boiling water may lead to the rapture of the coat wall allowing water to permeate the seed tissues causing seed germination and further rapid emergence of other Africa-known (Agboola and Etejere, 1991) or relative to Spanish broom species, such as *Cytisus scoparius* (Abdullah *et al*., 1989).

Concerning the germination promotion of the seeds treated with sulphuric acid, it is common among arid-adapted legumes (Sy *et al*., 2001; Vilela and Ravetta, 2001). In accordance to some legumes native to arid and semi-arid environments, water immersion was not very effective on Spanish broom seed germination and emergence (Sy *et al*., 2001). Although cold stratification has been reported to break dormancy and enhance germination in many species (Kullkarni *et al*., 2006), it has also been reported to cause lethal or no effect at all on seeds (Ren and Tao, 2004; Kambizi *et al*., 2006). The latter might have been the case with *S. junceum* seeds when they were subjected to pre-chilling treatment in our experiment, probably due to the low duration of cold stratification.

Some dry heat treatments were very effective in terms of germination and emergence percentage or germination rate, likewise similar invasive woody species (Tarrega *et al*., 1992; Vilela and Ravetta, 2001), while only 140 and 170°C showed a marked inhibition of germination, similar to other woody species (Reyes and Casal, 2006). This positive (or damaging at high temperatures) effect of dry heat suggests that seedlings of *Spartium junceum* (likewise other plants) may emerge sooner and in greater numbers following a fire event (Qaderi and Cavers, 2003), while a part of their seed bank can be destroyed.

In addition, there was not a significant positive correlation between germination percentage (FGP) and germination rate (CGRI) ($r = -0.11$, $p>0.05$), suggesting that the rapid germination was not always associated with the high germination percentage. However, the CGRI was significantly correlated with GT_{50} ($r = -0.85$, $p<0.05$) indicating that CGRI was a good index in order to express relative speed of germination. Besides, it seems that *S. junceum* germination was closely associated with the emergence of the seedlings ($r = 0.96$, $p<0.05$).

The results from the present study are in agreement with previous reports, providing evidence that *Spartium junceum* seeds have a seed-coat dormancy (physical) and do not involve physiological dormancy (Pipinis *et al*., 2005), as long as they do not require a dormancy-breaking pretreatment *per se* in order to germinate (Baskin and Baskin, 2004). This physical dormancy favours 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). It is already known that the Spanish broom seeds can germinate readily with no pretreatment at all (Hellmers and Ashby, 1958; Nilsen and Semones, 1997), but our results clearly indicate that hot water treatment, acid treatment or dry heating (some of them previously mentioned by Hoshovsky, 1986) can greatly increase the germination rate and percentage of this species.

According to Nilsen (2000), a relatively effective control combination of Spanish broom may be cutting followed by an application of a herbicide (e.g., glyphosate) to cut stems. However, even if the plants are killed and root sprouting is prevented, seeds within the substantial soil seed bank can stay dormant for at least five years and germinate discontinuously over long periods of time (Nilsen, 2000). Alternatively, a severe (not low-temperature) fire that kills all aboveground stems and burns hot and close to the ground could completely kill standing individuals and most likely remove some of the seed bank (of the upper 2-3 cm of the soil) (Bossard, 1993; Nilsen, 2000; Newton *et al*., 2006) and make some of the rest to germinate massively and rapidly, as our results indicate. Fire is a frequent ecological factor in many Mediterranean-type ecosystems (likewise South Africa) and consequently in some cases it could be taken into account and locally applied for an effective control of *S. junceum* seed bank and population. Therefore, specific field experiments, monitoring and further laboratory studies must be continued in order to optimize rapid and uniform seed germination and seedling emergence, as long as they are certainly preadequate for the understanding of the distribution and the further effective control of the large seed bank in Spanish broom stands (Mack, 1996; Nilsen, 2000).

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