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Influence of Heat on Seed Germination and Seedling Emergence of Chaste Tree (*Vitex agnus castus* L.)

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Abstract: Chaste tree (*Vitex agnus castus* L.) is one of the most important native shrubs in arid and semi arid Mediterranean and western Asia regions, widely used from ancient years for medicinal and other purposes. 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. In general, the speed and percentage of seed germination was greatly increased by the most treatments including hot water immersion and dry heating (germination increased up to 83%), clearly indicating that there is a physical (seed coat) dormancy in this species, while untreated seeds (i.e., control) had relatively moderate germination and emergence percentages. This positive (or damaging at high temperatures) effect of dry heat on seed germination and emergence rate and percentage, implies the potential effect of fire on promotion of *V. agnus castus* seed germination and in some cases it could be taken into account, as long as fire is a frequent ecological factor in Mediterranean-type ecosystems.

Key words: *Vitex agnus castus*, seed germination, hot water, dry heat, emergence

INTRODUCTION

Vitex agnus castus (chaste tree) is a deciduous, very ornamental, aromatic shrub native in arid and semi arid Mediterranean and western Asian regions and widely cultivated elsewhere in warm temperate and subtropical regions (Schopmeyer, 1974). Chaste tree (likewise other *Vitex* species) is also very desirable in several rehabilitation programs in degraded lands, as long as it can grow in nutritionally poor soils and dry regions and it has many edible (condiment), medicinal and other uses (Hirobe *et al*., 1997; Watanabe *et al*., 1995). This species is probably one of the most important herbs used by medical herbalists for centuries (mentioned by Hippocrates from the fourth century BC) and it is also known as the women's herb, because of its great ability to treat several female hormonal disorders (Bartram, 1995).

It is well documented that seed germination is a critical stage in the establishment and growth of many plants of arid and semi arid regions (Radosevich *et al*., 1997). However, it has been mainly studied in annual species (Gutterman, 1993), while their germination patterns differ widely from those of perennial species. Furthermore, there is considerable interest in increasing and accelerating the emergence ability of the plants, in order to optimize their growth and establishment. Concerning the seed germination of *V. agnus castus*, there have been carried out some studies, supporting either the rapid and easy seed germination of the species, or the existence of dormancy and the need of several pretreatments (Dirr and Heuser, 1987; Belhadj *et al*., 1998).

Understanding of the seed germination is crucial for its development and life cycle, but until now little information is available on the influence of several abiotic factors (such as fire) on seed germination and seedling emergence of chaste tree. Within this scope, the objective of this study was to improve this knowledge, by assessing the response of seed germination and further seedling emergence of this species to the effects of several pretreatments and especially heat, by means of dry heating and immersion in hot water. The beneficial qualities of *V. agnus castus* make imperative the need of such a study, in order to optimize its seed germination and first growth.

MATERIALS AND METHODS

Experimental details: The *V. agnus castus* seeds were collected directly from the wild from several locations of Vonitsa, Greece (latitude 38°40′ N; longitude 20°43′ E) in 2005. 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.

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Two germination experiments were conducted in the Laboratory of Agronomy of the Agricultural University of Athens (AUA) during the summer of 2005. 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 90°C; (3) at 130°C and (4) at 170°C. These temperatures were selected because they are likely to be reached at the soil surface or the first few centimeters 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 high temperatures is probably lethal for the *V. agnus castus* seeds.

Five replicates (Petri dishes) were used for each treatment, while untreated seeds were used as control for each experiment. Twenty five 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 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 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, was also calculated (Hsu *et al*., 1985).

Subsequently, two pot experiments were conducted in a glasshouse of the Agricultural University of Athens (AUA) in 2005. 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 1-3 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 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) 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 4 min increased significantly the FGP of *V. agnus castus* seeds from 53 (control) to 80% 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 1).

The second germination experiment revealed that dry heat can either promote or hinder seed germination. The temperature of 130°C (followed by 90 and 50°C) was the significantly most stimulative temperature for the

Table 1: Time course for the germination of *Vitex agnus castus* seeds in response to different periods of immersion in hot water

Days after seed placement	Control	Pretreatment: Hot water for 1 min	Hot water for 2 min	Hot water for 4 min	Hot water for 8 min
2	0a	0a	0a	0a	0a
4	0a	0a	0a	0a	0a
6	4c	8b	11ab	14a	12ab
8	7c	12b	16ab	19a	20a
10	14c	21b	27a	29a	30a
12	24c	30b	37a	42a	39a
14	33c	41b	50a	56a	55a
16	40d	49c	61b	68a	64ab
18	45d	57c	67b	74a	69ab
20	53d	63c	73b	80a	72b

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 2: Time course for the germination of *Vitex agnus castus* seeds in response to different dry heat pretreatments

Days after seed placement	Pretreatment Control	50°C	90°C	130°C	170°C
2	0a	0a	0a	0a	0a
4	0c	0c	2b	8a	4b
6	3d	8c	10bc	16a	12ab
8	8c	14b	18b	28a	26a
10	16d	24c	27bc	42a	30b
12	27c	30c	38b	48a	36b
14	35c	41bc	54a	56a	43b
16	42b	49b	61a	67a	48b
18	48c	58b	65b	74a	50c
20	56c	67b	70b	83a	53c

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: Seed germination rate and seedling emergence of *Vitex agnus castus* in response to different pretreatments

Pretreatments	CGRI (%.day^{-1})	GT_{50} (days)	Mean emergence (%)	Average days of emergence
A				
Control	14.9c	13a	68c	6a
Hot water for 1 min	16.6c	13a	74.2b	5ab
Hot water for 2 min	25.4ab	12ab	81a	4b
Hot water for 4 min	27.5a	11.5b	85.3a	4b
Hot water for 8 min	22.9b	11b	83c	5ab
B				
Control	15.9c	12ab	69bc	6a
50°C	21.1b	12.5a	74b	5ab
90°C	25.1b	11bc	75ab	4b
130°C	32.8a	10c	82a	4b
170°C	23.7b	9d	66.3c	5ab

Means followed by the same letter(s) within a column are not significantly different at P = 0.05 Fisher's least significant difference test. A: first and B: second germination and emergence assay

treatment of *V. agnus castus* seeds. In contrast, dry heating of *V. agnus castus* seeds at 170°C resulted to slightly lower germination percentage even than the untreated seeds (Table 2).

The germination rate of seeds after an immersion in hot water for 2, 4 and 8 min was greatly enhanced, as long as the CGRI values calculated in this method were the highest among all treatments and the 50% of the final germination percentage was obtained after 11-12 days (Table 3). Concerning the dry heat treatments, the 5 min treatment at 130°C yielded one of the fastest germination rates, while the temperature of 170°C had clearly negative effects on germination percentage, although it was a quite rapid method. Besides, in Table 3 it is shown that although the emergence percentage and rate of untreated Chaste tree seeds (control) were relatively satisfactory, some pretreatments (immersion in hot water and most dry heating treatments) contributed to the optimization of seedling emergence.

DISCUSSION

In the present study, hot water treatments increased significantly the germination and emergence percentage and rate of *V. agnus castus*. 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). 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).

The most of the tested dry heat treatments were also very effective in terms of germination and emergence percentage or germination rate, likewise in other woody species (Tarrega *et al.*, 1992; Vilela and Ravetta, 2001). 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 *Vitex agnus castus* (Thanos and Goerghiou, 1988; Qaderi and Cavers, 2003).

In addition, there was a significantly positive correlation between germination percentage (FGP) and germination rate (CGRI) ($r^2 = 0.83$, $p<0.05$), suggesting that in our experiments on Chaste tree, the rapid germination was closely associated with the high germination percentage. Besides, the CGRI was significantly correlated with GT_{50} ($r^2 = -0.67$, $p<0.05$) indicating that CGRI was a relatively good index in order to express relative speed of germination. Moreover, it seems that *V. agnus castus* germination was highly significantly correlated with the emergence of the seedlings ($r^2 = 0.94$, $p<0.05$) and the rate of seedling emergence ($r^2 = -0.83$, $p<0.05$).

Seed dormancy is usually associated with the factors of the protective covering, the seeds coat or the enclosed embryo. Our results indicated that hot water immersion and dry heat induce significantly seed germination. These results are in agreement with previous reports, providing evidence that *Vitex agnus castus* seeds certainly involve physical dormancy (Belhadz *et al.*, 1998). This physical 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). It has been already mentioned that the chaste tree seeds can germinate with no pretreatment at all (Dirr and Heuser, 1987), while in other studies seeds incubated at 20 or 30°C without any pretreatment failed to germinate massively (Belhadz *et al.*, 1998). Our results clearly indicate that hot water treatment and dry heating can greatly increase the germination percentage of this species, equally or more than some previously mentioned methods, such as scarification with sulphuric acid, gibberellic acid and cold stratification (Schopmeyer, 1974; Belhadz *et al.*, 1998).

Conclusively, the authors recommend the use of dry heat or hot water, as some farmers of the arid and poor regions may not have access to other chemicals or do not know how to handle them. Furthermore, fire is a frequent ecological factor in many Mediterranean regions and other Mediterranean-type ecosystems and consequently in some cases it could be taken into account for seed germination and seedling emergence of *V. agnus castus*. Therefore, specific field experiments, monitoring and further laboratory studies must be continued in order to optimize rapid and uniform seed germination and seedling emergence of chaste tree.

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