International Journal of Botany

ISSN: 1811-9700

science alert

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International Journal of Botany 5 (2): 181-185, 2009
ISSN 1811-9700

The Effect of Stratification on Seed Germination of *Jasminus fruticans* L. (Oleaceae): A Contribution to a Better Insight on the Species Germination Ecology

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Abstract: A germination experiment was carried out in the laboratory to evaluate the effect of stratification on seed germination of *Jasminus fruticans*. Nine different treatments were used to break the embryo imposed dormancy. Seeds were subjected to warm stratification (20/25°C) for 1 or 2 months, cold stratification (2-4°C) for 1, 2 or 3 months and to the following warm plus cold stratification combinations: 1 month warm plus 1 month cold stratification (1W+1C), 1 month warm plus 2 months cold stratification (1W+2C), 2 months warm plus 1 month cold stratification (2W+1C) or 2 months warm plus 2 months cold stratification (2W+2C). Maximum germination (86.00%) and minimum mean germination time (11.26 days) were attained after 3 months of cold stratification without warm stratification. Seeds that were subjected to 2 months cold stratification exhibited 70.50% germination, whereas those stratified for 1W+2C or 2W+2C exhibited 69.00 or 67.50%, respectively. One month of cold stratification resulted in a germination percentage equal to 21%, whereas seeds that were subjected to warm stratification for 1 or 2 months prior to 1 month cold stratification gave germination percentages equal to 18.50 and 20.00%, respectively. None of the control seeds or those that were warm stratified for 1 or 2 months germinated. Results revealed that several months of cold stratification (3 months) were required to overcome physiological dormancy and to enhance *Jasminus fruticans* seed germination.

Key words: *Jasminus fruticans*, seed germination, seed dormancy, stratification

INTRODUCTION

Jasminus fruticans L. is a native shrub of South Europe. It is an evergreen or half-evergreen shrub up to 3 m, with slender, erect or patent, 4 angled branches (Amaral Franco and Rocha Afonso, 1972). The flowers are yellow and flowering starts in May. The fruits ripen in September and have a black color. *Jusminus fruticans* fruit is a two-seeded berry consisting of a fleshy exocarp and mesocarp and a soft endocarp. It is found in the *Quercetalia ilicis* and *Quercetalia pubescentis* floristic zone. This shrub has potential use as a landscape plant due to its ornamental flower and decorative fruit. However, in order to introduce this plant into the landscape industry or forestry use, a feasible propagation method should be available. Seeds are important for propagating woody plants. According to Macdonald (1993), propagation from seeds is the most frequent and cheapest method. *Jasminus fruticans* seeds are dormant, yet the type of dormancy is not identified. There is no study on dormancy breaking of *J. fruticans* seeds and on the germination of *Jasminus* species in general.

There are three fundamentally different types of seed dormancy: morphological, physiological and physical (Baskin and Baskin, 1998, 2004). Morphological dormancy is conspicuous in seeds with embryos that are underdeveloped and simply need a period of growth before germination. According to Nikolaeva (1977), this type of dormancy is usually broken under conditions of warm stratification. Physiological dormancy is the most prevalent dormancy form in seeds of species that are distributed in the temperate climate. The causes for this type of dormancy are basically hormonal and the phenomenon is mainly due to the disorder of the balance between the substances that cause inhibition (ABA) and promotion of growth (GAs) of the embryo (Bewley, 1997; Finch-Savage and Leubner-Metzger, 2006; Seeley, 1990). According to Nikolaeva (1977), the most important condition for breaking physiological dormancy of seeds of many plants is cold stratification. Seeds with physical dormancy have a seed coat impermeable to water or gases. Various methods of scarification are used to break physical dormancy (chemical, mechanical, hot water) (Bonner *et al*., 1994). Furthermore, dormancy types may

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be combined in the same seed. The combination of morphological and physiological (morphophysiological) dormancy is evident in seeds, having an underdeveloped embryo and in addition have a physiological component to their dormancy (Baskin and Baskin, 2004). These seeds therefore require a defined combination of warm and cold stratification (Nikolaeva, 1977). Physical and physiological dormancy is another combination in seeds with water impermeable coats and physiological embryo dormancy. The combination of physical and morphological dormancy is impossible according to Fenner and Thompson (2005).

The aim of the present study is to evaluate the effect of various stratification treatments (warm, cold and their combinations, for various (different) periods) on dormancy breaking of seeds of *J. fruticans* and to understand the nature of seed dormancy exhibited by the species.

MATERIALS AND METHODS

Mature fruit (berry) of *Jasminus fruticans* were collected in September 2006 from plants growing in its natural habitat, approx. 20 km West of Komotini (41°08′70′′N-25°18′23′′E), in North Greece. Fruits were collected from more than 10 individual plants. The fruit were soaked in water for 24 h before they were pulped by hand. Sieving and flotation were used to clean the seeds. Flotation removed trash (parts of fruit) and also empty or insect - infested seeds. The clean seeds were spread on filter paper and left to dry. After drying, the seeds were stored in glass containers in the refrigerator (2-4°C) until the beginning of the experiment (Bonner *et al*., 1994).

Seed treatment: The germination experiment was conducted in the Laboratory of Silviculture, School of Forestry and Natural Environment, Aristotle University of Thessaloniki during the winter and spring of 2007. Due to lack of hard endocarp the seeds were not subjected to scarification treatments. Several treatments were conducted to determine the effect of (a) various stratification periods and (b) temperature during stratification on seed dormancy breaking and germination. In total, 9 treatments were conducted and there were four replicates of 50 seeds for each treatment. Seeds were placed with wet sterilized river sand in poly bags and underwent (1) warm stratification for 1 or 2 months, (2) cold stratification for 1, 2 or 3 months and (3) to the following warm+cold stratification combinations: 1 month of warm plus 1 month of cold stratification (1W+1C), 1 month of warm plus 2 months of cold stratification (1W+2C), 2 months of warm plus 1 month of cold stratification (2W+1C) and 2 months of warm plus 2 months of cold stratification (2W+2C). In total, 9 poly bags were used (each bag had 200 seeds and corresponded to a different treatment). Moisture was checked twice a week during warm stratification and once a week during cold stratification. Warm stratification was accomplished with daily alternating temperatures of 20°C (16 h dark) and 25°C (8 h light). Cold stratification was performed at 2-4°C in the dark (Nikolaeva, 1977; Baskin and Baskin, 1998; Falleri, 2004). Before the treatments the seeds were dusted with fungicide (Captan) to avoid fungi development. At the end of each stratification period the seeds were taken out from poly bag and were subjected to a germination test. Moreover, untreated seeds were used as means of control.

Germination test: The seeds that were subjected to the germination test were placed on filter paper moistened with distilled water in 9 cm plastic Petri dishes. Seeds of each replication were randomly placed in 2 Petri dishes (25 seeds per Petri dish). The Petri dishes were randomly arranged on the shelves of the growth chamber. The temperature in the growth chamber was set at 20°C for 16 h dark period and 25°C for 8 h light period (Young and Young, 1992). Seed germination was defined as the appearance of a radicle, at least 2 mm long, according to the rules of the International Seed Testing Association (1985). Germinated seeds were being counted every week for a period of 7 weeks. Each germinated seed was removed in order to avoid confusion in the measurement. Petri dishes were moistened (as needed) with distilled water to secure adequate moisture for seed germination. The germination percentage was determined for each replication per treatment. Finally, the germination percentage for each treatment was calculated from the average of germination percentages of the 4 replications.

In addition, mean germination time (MGT) for each treatment was calculated to assess the rate of germination. The MGT was calculated for each replication per treatment according to the following equation:

$$MGT = \Sigma (Dn)/\Sigma n$$

where, n is the number of seeds which germinate on day D and D is the number of days counted from the beginning of the test (Ellis and Roberts, 1981). The MGT of each treatment was calculated using the 4 replications (per treatment).

Statistical analysis: The effect of the different stratification treatments on germination (%) and on mean germination time (MGT) was tested using the analysis of variance (ANOVA) and the Duncan test (Matis, 1991). In

order to increase normality and to achieve homogeneity of variances, the germination percentage data was transformed to arc-sine square root values, before the analysis (Snedecor and Cochran, 1988). The germination data of warm stratification for 1 or 2 months and control treatment were not analyzed, since not a single seed germinated. The statistical analysis were performed using SPSS ver. 11.5.

RESULTS

No germination occurred without pre-treatment (control seeds) indicating that the seeds were dormant (Table 1). Similarly, seeds that were stratified only at 20/25°C (warm stratification treatments) did not germinate. On the contrary, germination was achieved with seeds that were subjected to cold stratification without preceding warm stratification. Furthermore, germination took place after the combination of warm and cold stratification. The germination percentage significantly increased with increasing duration of cold stratification. Maximum germination (86.00%) was attained after 3 months of cold stratification without warm stratification. Seeds that were subjected only to cold stratification for 1 or 2 months gave a germination percentage of 21.00 or 70.50%, respectively. The germination percentage of seeds that were subjected to 1 or 2 months warm stratification followed by 1 month cold stratification was 18.50% and 20.00% respectively. There were no statistically significant differences in the germination percentages among the treatments of 1 month cold stratification and the combinations 1 or 2 months warm stratification with 1 month cold stratification. The combinations 1 or 2 months warm stratification with 2 months cold stratification gave satisfactory germination percentages that were equal to 69.00 and 67.50%.

Table 1: Mean germination and mean germination time of *J. fruticans* seeds in different stratification treatments

Warm stratification at 20/25°C (months)	Cold stratification at 2-4°C (months)	Germination percentage [A] (% ±SD)	Mean germination time[B] (days ±SD)
0 (control)	0 (control)	0	-
1	0	0	-
2	0	0	-
0	1	21.00±2.58c	29.62±1.63c
0	2	70.50±4.43b	13.33±1.13b
0	3	86.00±3.65a	11.26±0.73a
1	1	18.50±2.52c	30.20±1.73c
1	2	69.00±3.46b	13.84±0.73b
2	1	20.00±2.31c	28.74±1.68c
2	2	67.50±4.12b	13.75±1.23b

Mean values in a column are statistically different at $p<0.05$, when they share no common letter(s). The comparisons were made using the Duncan test A: Average of the 4 germination percentages (4 replications per treatment) in each treatment, B: Average of the 4 mean germination times (4 replications per treatment) in each treatment

Germination speed reached the maximum value (minimum MGT: 11.26 days) after 3 months of cold stratification. The MGT in seeds that were subjected to the combinations 0 or 1 or 2 months warm plus 1 month cold stratification ranged from 28.74 to 30.20 days. The MGT in seeds that were subjected to the combinations 0 or 1 or 2 months warm followed by 2 months cold stratification ranged from 13.33 to 13.84 days.

DISCUSSION

Stratification has been applied to many species in order to satisfy embryo needs for the purpose of stimulating germination (Karam and Al-Salem, 2001; Falleri, 2004; Gebre and Karam, 2004; Smiris *et al*., 2006). As a result of stratification, changes occur in the levels of the substances that cause inhibition and promotion of growth of the embryo. Physiological dormancy in seeds of some plants is dependent on abscisic acid (ABA)/gibberellic acids (GAs) ratio (Finch-Savage and Leubner-Metzger, 2006). ABA is an important hormone involved in inducing dormancy and in maintaining the dormant state (Bewley, 1997; Kucera *et al*., 2005). On the other hand, GAs appears to be important in dormancy release and in the promotion of germination. According to Nicolas *et al*. (1996), in some species with cold stratification requirements, ABA and GAs are thought to play antagonistic roles in the maintenance and breaking of dormancy. The seeds remain dormant if the level of ABA is higher than that of GAs, whereas they germinate if the GAs level is higher than that of ABA (Macdonald, 1993; Ali-Rachedi *et al*., 2004; Cadman *et al*., 2006). Frankland and Wareing (1966) and Rudnicki (1969) as well as Copeland and Macdonald (1985) reported considerable changes in the levels of ABA and GAs during stratification and suggested that low temperature during stratification enhanced the production of GA and consequently promoted seed germination. Ali-Rachedi *et al*. (2004) and Le Page-Degivry *et al*. (1997) demonstrated a strong reduction in ABA when chilled seeds were transferred to germination conditions. Furthermore, Powell (1987) referred that cold stratification is believed to activate the gibberellin-synthesizing mechanism.

In the present study, applying cold stratification led to an increased germination percentage and decreased the time of germination. Increasing the duration of stratification (from 0 to 3 months) resulted in a significant increase in germination percentage. A period of cold stratification relieves dormancy of many northern hemisphere species according to Baskin and Baskin (1998). Warm stratification alone did not promote

germination. Furthermore, the germination percentages were not improved when warm stratification was applied prior to 1 or 2 months of cold stratification. According to Ellis and Hong (1985) the warm stratification prior to cold stratification is beneficial because it may enable underdeveloped embryos to develop. It therefore seems that the *J. fruticans*' embryo is fully elongated at seed maturity and is not morphologically dormant. Similarly, seeds without pre-treatment (control seeds) did not germinate. Consequently, the seed germination might have been inhibited by a high ABA/ GAs ratio. The cold stratification reversed this ratio and led to germination of seeds (Table 1).

In the natural environment, after fruit maturity in September and seed dispersal (which occurs in Autumn), the seeds are exposed to low temperatures (cold stratification) during Winter for adequate period and finally the seedlings emerge the following spring. Thus, *J. fruticans* have developed a dormancy mechanism that prevent germination during the cold winter period until spring, when conditions are favourable for germination, as well as, for seedling growth and survival.

From the results presented in the current study, it can be concluded that *J. fruticans* seeds are in dormancy. Seeds failed to germinate probably because of a physiological inhibitory mechanism of germination. Future studies are needed to assess if the use of exogenous GA_3 can reduce the period of cold stratification and enhance germination.

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