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EFFECTS OF GIBBERELLIC ACID AND COLD STRATIFICATION ON SEED GERMINATION OF TWO *SORBUS* SPECIES

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Abstract

The present study aims at investigating the effects of gibberellic acid (GA_3) application and cold stratification on the germination of *Sorbus domestica* L. and *Sorbus torminalis* (L.) Crantz seeds. In particular, seeds of both species were treated with 500, 1000 and 2000 ppm GA_3 for 30 hours and were, subsequently, cold stratified at 3–5 ºC for 0, 1, 2 and 3 months. In addition, seeds from each species were only cold stratified for 0, 1, 2 and 3 months (control). In both species, non-stratified and 1-month cold stratified seeds, despite having been treated with GA_3 solutions, exhibited very low germination percentages (0.8–4.2 % for *S. domestica* and 0.0–1.7 % for *S. torminalis*). However, in control seeds and seeds treated with GA_3 solutions an increase in the cold stratification period from 2 to 3 months increased germination percentages significantly in both species. In *S. domestica*, after a 2-month period of cold stratification, the germination percentage of the seeds treated with 2000 ppm GA_3 (30.8 %) was significantly higher than that of control seeds (18.3 %). In 3 months of cold stratification, there were no significant differences in the germination percentages between control seeds (87.5 %) and seeds treated with GA_3 solutions (89.2–91.7 %). In *S. torminalis*, the GA_3 application significantly improved the germination of 2 and 3-month cold stratified seeds. After a 2-month period of cold stratification, the germination percentage of seeds, which had been treated with 2000 ppm GA_3 (30.0 %), was higher than the germination percentages of the seeds which had been treated with 500 and 1000 ppm (15.8 and 14.2 % respectively). After a 3-month period of cold stratification there were no significant differences in the germination percentages between seeds treated with GA_3 solutions (88.3–91.7 %). The results demonstrated that the optimum germination percentages of *S. domestica* and *S. torminalis* were obtained after a 3-month cold stratification period. In both species, the application of GA_3 did not replace or shorten the required cold stratification period.

Key words: seed dormancy, service tree, *Sorbus domestica*, *Sorbus torminalis*, wild service tree.

Introduction

The genus *Sorbus* L. (Rosaceae) includes about 100 species of deciduous trees and shrubs which are widely distributed in the Northern Hemisphere (Krussmann 1984). Two of the most widespread European species of the genus *Sorbus* are *Sorbus*

domestica L. (Service tree) and *Sorbus torminalis* (L.) Crantz (Wild service tree) (Tutin et al. 1968–1992). In Greece, where *S. domestica* is rather rare, scattered individuals were mainly found in the continental part of the country, whereas *S. torminalis* is mainly found in the northern part of the country, mostly in sub-mountain and mountain regions (Boratynski et al. 1992). Both of them grow in deciduous forests (Browicz 1982, Boratynski et al. 1992). In a forest ecosystem, the ecological significance of both species is great due to the forage (fruits) which they offer to wildlife species. Furthermore, their timber is valuable due to its excellent aesthetic and technical characteristics (Coello et al. 2013). As a result, the specific species have to be propagated in nurseries and, subsequently, introduced in reforestation programmes.

Sorbus species are usually propagated by seeds (Stein 2008). However, seeds of most *Sorbus* species, similarly to many other species of temperate deciduous forests, have only a dormant embryo (Dirr and Heuser 1987), which complicates germination. Therefore, overcoming seed dormancy is one of the major steps to ensure a rapid, uniform and high germination. Cold moist stratification is a technique, which is widely used for breaking embryo dormancy and enhancing the germination of seeds in numerous species (Macdonald 2006). According to Stein (2008), *Sorbus* seeds do not germinate readily and require a period of cold moist stratification. Despite the fact that the seeds of *Sorbus* species have a fully developed embryo (Martin 1946, cited in Baskin and Baskin 1998) warm followed by cold stratification is recommended for seeds of some *Sorbus* species (Dirr and Heuser 1987, Piotto et al. 2003). The length of the cold stratification period, which is necessary for dormancy removal, varies among the species of the genus. In general terms, the seeds of *Sorbus* species require at least a 2-month stratification period for good germination (Hartmann et al. 1997, Young and Young 1992). According to ISTA (1999), a 4-month stratification period at 3–5 ºC is required for the seeds of *Sorbus* species to release from dormancy. In *S. domestica* and *S. torminalis,* a 45-day period of cold stratification is sufficient to break dormancy in fresh seeds (Gultekin et al. 2007). Var et al. (2010), studying the seed germination of four *S. torminalis* provenances, found that the maximum germination percentage for all provenances was achieved after 4 months of stratification at 2 ºC. Apart from cold stratification, various chemical solutions are used to stimulate seed germination (Macdonald 2006). Gibberellic acid (GA) is one of the growth regulators that can be used to partially or fully replace the necessary period of cold moist stratification in a number of plant species (Baskin and Baskin 1998). In the relevant literature, however, the effects of GA_3 treatment on seed germination of *S. domestica* and *S. torminalis* have not been reported.

The objectives of the present study are to: i) examine the effectiveness of gibberellic acid and cold stratification on germination, ii) describe the effects of gibberellic acid and cold stratification treatment combinations on germination, and iii) propose effective treatments to maximize germination of *S. domestica* and *S. torminalis* seeds.

Materials and Methods

In autumn 2010, mature fruits of *S. domestica* and of *S. torminalis* were collected from trees growing in their natural habitat in Northern Greece (41º12´40´´N,

25º15´31´´E, 500 m elevation and 40º28´06´´N, 22º15´45´´E, 360 m elevation, respectively). In particular, fruits of *S. domestica* were collected in the middle of September and fruits of *S. torminalis* – in late October. After collection, the fruits of both species were pulped manually and the seeds were separated from the pulp using sieves and running water. It is worth noting that with flotation, apart from light debris, empty seeds were also removed. Subsequently, the cleaned seeds of both species were spread out on filter paper in laboratory conditions and left to dry. After drying, the seeds were stored in glass containers in refrigerator (3–5 ºC) until they were used in the experiments.

Seed treatment

Germination experiments were carried out in the following December in the laboratory of Silviculture, Department of Forestry and Natural Environment, Aristotle University of Thessaloniki. For each species, an experiment was carried out to determine the effects of gibberellic acid (GA_3), cold stratification (CS) and combination of GA_3 with CS on seed germination. Seeds of each species were soaked in solutions of GA_3 for 30 hours. The concentrations of GA_3 solutions were 500, 1000 and 2000 ppm. Subsequently, the treated seeds were placed in plastic containers and were mixed with moist sterilized river sand and left to stratify at 3–5 ºC for 0, 1, 2, and 3 months. For each species, three plastic containers, which corresponded to the concentrations of GA_3, were used and 12 treatments (combinations of GA_3 solutions with CS periods) were applied. In addition, seeds (control) from each species were soaked in distilled water for 30 hours and then were subjected to CS for 0, 1, 2, or 3 months. For each species there was a plastic container. During stratification, sand moisture was checked periodically and water was added, when necessary, to keep it moist.

Germination test

For each species, at the end of each CS period, a random sample of 120 seeds was taken out from each plastic container and randomly placed in 4 plastic Petri dishes (30 seeds per Petri dish). For each treatment, there were 4 replications of 30 seeds. The seeds were placed on sterilized river sand moistened with distilled water in 9-cm plastic Petri dishes. Prior to the arrangement of seeds in Petri dishes, the seeds were dusted with fungicide (Captan) to avoid fungi development. The Petri dishes were randomly arranged on the shelves of the growth chamber and were watered with distilled water, as necessary. The temperature in the growth chamber was set at 20 ºC for a 16-hour dark period and 25 ºC for an 8-hour light period. The germinated seeds were counted once a week for a period of 7 weeks. A seed with at least 2 mm long radicle was considered to be germinated (ISTA 1999). Finally, for each treatment of each species, the germination percentage (GP) was calculated as the average of the 4 replications.

Statistical analysis

For each species, a completely randomised experimental design was used. In both species, treatments, in which none of the seeds germinated or the germination percentage was lower than 5.0 %, were not included in the statistical analysis. The germination percentage

data were arc-sine square root transformed before analysis (Snedecor and Cochran 1980). The transformed data were checked for normality and homogeneity of variances and then analysed by one-way ANOVA. Comparisons of the means were made using the Duncan test (Klockars and Sax 1986). All statistical analyses were carried out using SPSS 20.0 (SPSS, Inc., USA).

Results

There were significant differences in GPs (α = 0.05) among the treatments applied in *S. domestica* and *S. torminalis* seeds [$F_{(7,24)}$ = 77.69, p = 0.00 for the *S. domestica* and $F_{(7,24)}$ = 149.26, p = 0.00 for the *S. torminalis*].

In both species, regardless of GA_3 treatment, the seeds, which were not stratified or stratified at 3–5 ºC for 1 month exhibited very low GPs (0.8–4.2 % for *S. domestica* and 0.0–1.7 % for *S. torminalis*) (Table 1). In control seeds, as well as in the seeds treated with GA_3 solutions, an increase in CS period from 2 to 3 months demonstrated a significant increase in GPs in both species.

In *S. domestica*, after a 2-month period of CS, the germination percentage of seeds treated with 2000 ppm GA_3 (30.8 %) was higher (p < 0.05) than that of control seeds (18.3 %). However, after a 3-month period of CS there were no significant differences in GPs between control seeds (87.5 %) and seeds treated with GA_3 solutions (89.2–91.7 %). Furthermore, in seeds stratified for 2 as well as 3 months, no significant differences in GPs among the treatments with the GA_3 solutions were observed.

Table 1. Effects of GA_3 and CS on GP of *S. domestica* and *S. torminalis* seeds.

GA_3, ppm	CS, months	*S. domestica* GP, % ±S.D.	*S. torminalis* GP, % ±S.D.
Control	0	2.5 ±1.67	0.0
	1	1.7 ±1.92	0.0
	2	18.3 c ±4.30	7.5 e ±3.19
	3	87.5 a ±3.19	79.2 b ±5.69
500	0	0.8 ±1.67	0.8 ±1.67
	1	2.5 ±3.19	0.8 ±1.67
	2	20.8 bc ±5.00	15.8 d ±5.00
	3	89.2 a ±5.00	90.8 a ±5.00
1000	0	0.8 ±1.67	0.0
	1	4.2 ±1.67	1.7 ±1.92
	2	28.3 bc ±5.78	14.2 d ±4.19
	3	90.0 a ±6.09	88.3 a ±4.30
2000	0	2.5 ±1.67	0.8 ±1.67
	1	3.3 ±2.72	0.0
	2	30.8 b ±5.69	30.0 c ±6.09
	3	91.7 a ±6.38	91.7 a ±4.30

Note: Means are statistically different at p < 0.05, when they share no common letter. The comparisons were made using the Duncan test.

In *S. torminalis*, GA_3 application significantly improved the germination of 2 and 3-month cold stratified seeds. After a 2-month period of CS, the germination percentage of seeds which had been treated with 2000 ppm GA_3 (30.0 %) was higher than the GPs of the seeds, which had been treated with 500 and 1000 ppm (15.8 and 14.2 %, respectively). However, after a 3-month period of CS there were no significant differences in GPs between seeds treated with GA_3 solutions (88.3–91.7 %).

Discussion

Non-stratified and 1-month stratified seeds of *S. domestica* and *S. torminalis*, regardless of GA_3 treatment, exhibited very low germination or failed to germinate, which confirms that the seeds of both species are dormant. As mentioned in the introduction, *Sorbus* seeds are characterized by having a fully developed but dormant embryo (Martin 1946, cited in Baskin and Baskin 1998, Dirr and Heuser 1987) and require a period of cold moist stratification to germinate (Stein 2008). According to Hartmann et al. (1997), hormonal changes occur during the CS of seeds with dormant embryo. A number of studies have shown that the ABA (a hormone, which is responsible for maintenance of dormancy) levels in embryo or seed decrease during the warm or cold stratification (Pinfield et al. 1987, Chien et al. 1998, Chen et al. 2007) and the gibberellin (GAs) (hormones that promote seed germination) levels in embryo increase during CS (Powell 1987, Chen et al. 2007). Furthermore, Oster et al. (1987) reported that germination inhibitors are present both in the seeds and fruits of *S. aucuparia*. The seeds of both species, receiving only a 2-month period of CS exhibited low GPs (18.3 and 7.5 %, Table 1), which demonstrates that a 2-month period of CS was insufficient to release dormancy of seeds. Possibly, a 2-month period of CS is not sufficient to drastically decrease the ABA levels and increase the level of GAs in seeds of both species. In contrast, Gultekin et al. (2007) state that a 45-day period of cold stratification is sufficient to break dormancy in fresh seeds of *S. domestica* and *S. torminalis*. In their experiment, *S. domestica* and *S. torminalis* seeds stratified at 6 ±1 ºC for 45 days exhibited GPs equal to 92.25 and 91.75 %, respectively. In the present study, the seeds of both species, which were only stratified at 3–5 ºC for 3 months, exhibited high GPs (87.5 and 79.2 %, respectively). According to Orsanic et al. (2009), stratification for 105 days at 3 ºC is sufficient to break the dormancy of *S. torminalis* seeds. However, Var et al. (2010), studying the seed germination of four *S. torminalis* provenances, found that the maximum GP for all provenances was achieved after a 4-month stratification period at 2 ºC. In addition, Taylor and Gerrie (1987) reported that CS of *S. glabrescensis* seeds at 1 ºC for 12 weeks resulted in the highest germination and they also demonstrated that the germination of *S. glabrescensis* seeds was optimum at temperatures of 10 and 15 ºC; in addition, they argued that high temperature (25 ºC) inhibited germination and induced secondary dormancy. However, ISTA (1999), for seed germination of *Sorbus* species, recommends a 4-month stratification period at 3–5 ºC and then alternating temperature regimes of 20/30 ºC. Furthermore, at about the end of the 3-month CS pe-

riod germinated seeds appeared in both species and therefore, a longer than a 3-month period of CS of seeds could not be applied in the present study.

The results of the present study demonstrate that the application of GA_3 in the seeds of both species does not replace or shorten the required CS period. According to Pipinis et al. (2012a), the application of GA_3 or kinetin in *Pyrus pyraster* (L) Burgsd. and *Malus dasyphylla* Borh. seeds does not replace or shorten the required CS period. However, exogenous GA_3 application has been reported to be effective in breaking dormancy and substituting for the CS requirement in the seeds of many species (Karam and Al-Salem 2001, Pipinis et al. 2011, Pipinis et al. 2012b, Pipinis et al. 2014). In addition, the application of GA_3 improved seed germination of the two studied *Sorbus* species. In particular, GA_3 application in *S. domestica* significantly improved the germination of 2-month only cold stratified seeds, whereas in *S. torminalis* significantly improved the germination of 2 and 3-month cold stratified seeds (Table 1).

Conclusions

The results of the present study demonstrate that the *S. domestica* and *S. torminalis* seeds exhibit physiological dormancy. A 3-month period of stratification at 3–5 ºC is essential for breaking dormancy in seeds of both species. It is also worth emphasizing that the application of GA_3 in seeds of both species does not replace or shorten the required CS period. However, GA_3 application prior to a 3-month period of CS maximizes germination in *S. torminalis* seeds.

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