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Effects of after-ripening, stratification and GA_3 on dormancy release and on germination of wild asparagus (*Asparagus acutifolius* L.) seeds

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ABSTRACT

Seeds of wild asparagus (*Asparagus acutifolius* L.) were treated and compared in this research to investigate seed dormancy class and level involved in this species. Four seed lots were compared: (i) freshly harvested seeds in 2007 (07Fr); (ii) freshly harvested seeds in 2008 (08Fr); (iii) after-ripened (AR) 2007 seeds dry stored in glass jars (AR*g*); (iv) AR 2007 seeds dry stored in paper bags (AR*p*). The 07Fr seeds were exposed to (1) chemical scarification combined with gibberellic acid (GA_3) levels (0, 200, 400, and 600 mg L^{-1}) and to (2) 28-day moist stratification at 5 and 23 °C, and two sequences of 5/23 °C combined with 0 and 400 GA_3 mg L^{-1} levels, and (3) together to the 08Fr and AR seeds were exposed to 56-day moist stratification at 5, 23, or 5/23 °C. With the 08Fr and AR seed lots this last stratification treatment was combined with 0 or 800 GA_3 mg L^{-1} levels. The dormancy depth of 08Fr (32% germination) was less than 07Fr seeds (2%). The latter after-ripened during dry storage and when stored in glass germinated more (47.5%) than in paper (12%). Stratification for 4 weeks was ineffective in improving germination of 07Fr seeds; when chemically scarified they did not germinate at all. The highest (nearly 70%) and the most rapid and uniform germination were observed for all the lots when they were warm stratified for 56 days. Warm stratification improved germination more than alternate temperature stratification, while cold stratification inhibited germination especially for the 08Fr and ARg lots, thus seeds seem not to have a morphological component to their dormancy. GA_3 only improved germination of 07Fr seeds, at a low rate. *A. acutifolius* seeds fit the characteristics of a non-deep physiological dormancy.

1. Introduction

Wild asparagus (*Asparagus acutifolius* L.), formerly classified in the *Liliaceae* family, has been recently included in the *Asparagaceae* family (Angiosperm Phylogeny Group II, 2003). The centre of origin and its geographic distribution is South Europe as far east as southeast Bulgaria (Štajner et al., 2002). Currently, wild asparagus is a common species in all Mediterranean areas (Sica et al., 2005). In Italy, this evergreen species is found mainly in its southern regions, where it grows spontaneously in uncultivated areas, on dry stonewalls, fences and especially in the Mediterranean macchia ecosystem. The spears are highly valued and consumed in a vast number of regional dishes (Ghirardini et al., 2007). Wild asparagus could become a new crop with high income potential, especially for marginal areas where its cultivation will fit perfectly within a sustainable agriculture framework of both biodiversity and environmental conservation. Currently, some limitations exist in the cultivation of this vegetable, the most important is related to its low and erratic seed germination that limits seedling or crown production (Rosati, 2001). Rosati and Falavigna (2000) found that dry storage of *A. acutifolius* seeds for more than one year did not completely break dormancy, obtaining a satisfactory germination (~90%) of freshly harvested seeds only after moist stratification in sand for at least 8 months. They also observed that irrespective of dry storage or stratification treatments, germination responses were strongly influenced by ecotype. Other authors, working with different ecotypes, reported 60–70% germination with seeds maintained in open field conditions of a Mediterranean environment under 70% shading for almost 5 months (January–May: winter–spring period), independently of whether stratified in sand or directly sown in peat (Fiori et al., 2001).

A more recent study with an *A. acutifolius* ecotype from an hilly area (Conversa and Elia, 2009) has reported that a period of 13 months of dry storage in paper bags at room temperature (21 ± 2 °C; R.H. 50 ± 10%) is effective in after-ripening seeds by enhancing seed sensitivity to subsequent seed treatments (soaking in warm water at 35 °C and moist stratification): the highest germination (76%) was obtained when after-ripened seeds were stratified and soaked,

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without any significant difference between cold or warm stratification. When singularly applied, soaking or warm stratification were found to be less effective (47% germination) while cold stratification did not improve germination. Seeds appeared fully imbibed at the end of the pre-germination, which points to the absence of physical dormancy, as already noted by other authors (Rosati and Falavigna, 2000). Following these findings a non-deep physiological dormancy (according to seed dormancy classification proposed by Baskin and Baskin, 2004) has been hypothesized for the seeds of this species, nevertheless more information is necessary to confirm this and to exclude a morphological component.

Scarification is reported to be effective to break non-deep physiological dormancy, by weakening the embryo cover layer, as well as gibberellic acid application. Moreover, to overcome most morpho-physiological dormancy types, a sequence of warm and cold moist stratification is required (Baskin and Baskin, 2004).

To investigate seed dormancy class and level involved for this species, in this paper we report the results of chemical scarification, gibberellic acid levels, temperature regime and sequence during the 28- and 56-day periods of moist stratification applied to non-after-ripened and after-ripened seeds of *A. acutifolius* collected in Southern Italy.

2. Materials and methods

2.1. Seed collection

In November 2007 and 2008, plant stems with mature green-brown berries were collected from wild *A. acutifolius* L. plants selected in Manfredonia territory (41°37′36″84 N, 15°54′36″72 E, Foggia province, Apulia Region). The site is the wetland of Lake Salso Oasis in the Gargano National Park. It is about 1 km far from the coastline and 2 m above sea level and is dominated by a Mediterranean climate with mild winter and dry-and-warm summer (Macchia et al., 2000); mean minimum and maximum temperatures are 10.8 ± 1.7 and 19.9 ± 2.2 °C, respectively, and the mean temperature of the coldest (January) and hottest (August) months are 7.1 and 24.5, respectively. Mean annual rainfall is 537 mm (Caliandro et al., 2005). The stems were air-dried for 2 weeks under 90% shading before removing berries. Once the berries were picked off from stems, the pericarps were manually removed, thus releasing the seeds. In both years the weight of 1000 seeds and moisture content of seeds were on average 39 ± 1.5 and 6.8 ± 1.0 g 100 g^{-1} dw, respectively.

2.2. Preparation of seed lots

In total four seed lots were prepared. Seeds harvested in the first year (2007) were randomly divided into three lots: the first was used for the tests carried out 2 months after harvest, hereinafter, referred to as the 2007 fresh seed lot (07Fr), the other two were dry-stored for 14 months (after-ripening—AR) in glass jars (AR*g*) or in paper bags (AR*p*), at room temperature (21 ± 2 °C; R.H. 50 ± 10%). The last lot was represented by the seeds harvested in 2008 that were used in germination tests 2 months after harvest and hereinafter referred to as the 2008 fresh seed lot (08Fr).

At the end of the after-ripening period the AR*g*, and AR*p* seed lots had 7.1 ± 0.6 and 8.1 ± 0.4 g 100 g^{-1} dw of water content, respectively.

2.3. Seeds were sterilized according to the Reid et al. (2002) procedure

Soaking, which in a previous work (Conversa and Elia, 2009) proved effective in improving *A. acutifolius* germination, was always applied. Seed soaking was performed by putting seeds in 100 ml vials (50 seeds/vial—average weight 3 g) filled with 50 ml of distilled water. The vials were maintained in a thermostatic bath at 35 °C for 12 h in the dark.

2.4. Seed treatments

Seed treatments were arranged (Table 1) in order to evaluate the following effects.

2.4.1. Experiment 1—chemical scarification and gibberellic acid level on 2007 fresh seed germination

The test was aimed to evaluate the effect of chemical scarification combined with different level of GA_3 treatment on 2007 freshly harvested seeds (07Fr). The trial started in January 2008, seeds were submerged in a sulphuric acid solution (96%) for 2 min and then rinsed with running water. Both scarified (*SC*) and control seeds (no*SC*) were soaked, sterilized and then treated with GA_3 at 0, 200, 400, and 600 mg L^{-1} by maintaining seeds constantly for 24 h under submerged conditions (12 g/20 ml) in the GA_3 solution at room temperature.

2.4.2. Experiment 2—GA_3 and temperature regimes/sequences during 28 days moist stratification on 2007 fresh seed germination

In January 2008 a separate test was carried out to evaluate the effect of 28 days of moist stratification on 07Fr seeds, performed with different temperature regimes and sequences and combined with the GA_3 treatment.

Sterilized seeds were first treated with GA_3 at 0 and 400 mg L^{-1} ($GA_3$0 and $GA_3$400) (as above described), and then they were subjected to moist stratification for 28 days (*ST28*) with the following temperature regimes and sequence: 28 days at 23 °C ($ST28_{23}$); 28 days at 5 °C ($ST28_5$); 14 days at 5 °C followed by 14 days at 23 °C ($ST28_{5/23}$); a repetition of two cycles of 7 days at 5 °C plus 7 days at 23 °C ($ST28_{5/23/5/23}$) (these latter two to simulate seasonal fluctuations). After the stratification period the seeds were soaked. For each GA_3 level non-stratified controls ($GA_3$0-no*ST* and $GA_3$400-no*ST*) were also carried out; they were soaked, sterilized and finally treated with gibberellic acid.

Stratification was carried out in flat aluminium containers (6 cm × 10 cm × 20 cm) filled with a 5 cm layer of washed river-sand saturated with distilled water. The containers were placed in plastic bags to avoid dehydration of the substrate and were stored in the dark in a growth chamber.

2.4.3. Experiment 3—seed lot and temperature regimes/sequences during 56 days of moist stratification on seed germination

The seed lots used in this test were: 07Fr, 08Fr, AR*p* and AR*g*. The tests on 07Fr seeds were carried out in January 2008, while those on the other seed lots were carried out in January 2009.

The effect of a longer stratification period was investigated, performed with the same temperature regimes (23 and 5 °C), but with different time sequences, than those applied in the previous "Experiment 2". Sterilized seeds were subjected to moist stratification (in the conditions described above) for 56 days (*ST56*) with the following temperature regimes: 56 days at 23 °C ($ST56_{23}$), 56 days at 5 °C ($ST56_5$), 28 days at 5 °C followed by 28 days at 23 °C ($ST56_{5/23}$). After the stratification period, seeds were soaked before placing them in Petri dishes. For each seed lot, non-stratified (no*ST*) controls were carried out which were only soaked and sterilized before starting incubation in Petri dishes.

2.4.4. Experiment 4—GA_3 and temperature regimes during 56 days moist stratification on 2008 fresh and after-ripened seed germination

A separate test was performed in January 2009 to evaluate the effect of 800 mg L^{-1} GA_3 on the 08Fr, AR*g* and AR*p* seed lots, higher

Table 1
Treatments applied in the different experiments.

Experiment	Seed lot	Scarification	GA_3 (mg L^{-1})	Moist stratification			
				Temperature		Total duration (d)	Treatment code
				Regime (°C)	Sequence (from day to day)		
1	07Fr		0	–	–		–
		Yes	200				
		No	400				
			600				
2	07Fr	No		Not stratified		–	no*ST*
				23	1–28	28	$ST28_{23}$
			0	5	1–28	28	$ST28_{5}$
			400	5	1–14	28	$ST28_{5/23}$
				23	15–28		
				5	1–7	28	$ST28_{5/23/5/23}$
				23	8–14		
				5	15–21		
				23	21–28		
3		No	0	Not stratified		–	no*ST*
	07Fr			23	1–56	56	$ST56_{23}$
	AR*g*			5	1–56	56	$ST56_{5}$
	AR*p*			5	1–28	56	$ST56_{5/23}$
	08Fr			23	29–56		
4		No		Not stratified		–	no*ST*
	AR*g*			23	1–56	56	$ST56_{23}$
	AR*p*		0	5	1–56	56	$ST56_{5}$
	08Fr		800	5	1–28	56	$ST56_{5/23}$
				23	29–56		

than the most effective GA_3 level (600 mg L^{-1}) tested on 07Fr seeds ("Experiment 1"); this GA_3 treatment ($GA_3$800) was carried out as described in "Experiment 1" evaluation. It was combined with 56-day long stratification treatments as described in "Experiment 3".

2.5. Germination test

Germination tests were carried out by placing 50 seeds on moist Whatman No. 1 filter paper inserted in 9 cm diameter Petri dishes (experimental unit). Four replicates were used for each treatment. Petri dishes were arranged in a completely randomized design within a growth chamber at 15/23 °C (12 h/12 h) in the dark. From the beginning of germination of each single treatment and for a period of ninety days, the germinated seeds (young radicles over 2 mm in length) were counted and removed every 2 days from every single Petri dish. No germination occurred for any of the treatments beyond the 51st day after the start of germination, so germination occurred by the 50th day is presented. The germination percentage was calculated as the number of germinated seeds/viable seeds × 100.

2.6. Seed viability evaluation

Seeds resulting non-germinating after the conclusion of the tests were analysed using the tetrazole test (ISTA, 2003) to evaluate seed viability. Seeds were bisected along the longitudinal axes to evaluate the reaction of seed tissue to 2,3,5-triphenyltetrazolium chloride (Carlo Erba) after incubation at 30 °C, in the dark for 24 h. Sections were observed on a stereoscope (Nikon SMZ800) under incandescent light and viable seeds with embryo and endosperm blue stained, were counted.

2.7. Statistical analysis

Cumulative proportion of germinated seeds curves for each seed lot and no*ST*, ST56 and $GA_3$800 treatment were constructed using Weibull's function with three parameters (Damato et al., 1994):

$$Y = a[1 - 100^{-(X/q)^b}]$$

where Y is the cumulative proportion of germinated seeds at time X, a is the maximum germination that may be reached, b is a shape parameter and q is the time required to achieve 99% of a from the beginning of the germination period.

The three parameters of Weibull's function for each treatment were estimated using PROC NLIN (SAS Institute, 1999). Time to 50% cumulative germination (T_{50}) was calculated using the following formula derived from Weibull's function:

$$T_{50} = \left\{ \frac{\mathrm{Log}_{10}[-a/(Y-a)]}{2} \right\}^{(1/b)} q$$

Final germination percentage and T_{50} data were subjected to ANOVA and mean separation was carried out using the $LSD_{0.05}$ test. The values expressed as percentages were transformed by arcsin $\sqrt{x}$ before data analysis.

To evaluate the effect of after-ripening compared to freshly harvested seeds of both years, data from the January 2008 test of the 07Fr lot were merged in the ANOVA and in the graphs with those of 08Fr, AR*g* and AR*p* treated and tested in January 2009, the germination tests being carried out in the same experimental conditions.

3. Results

3.1. Experiment 1—scarification and gibberellic acid levels (0, 200, 400, and 600 mg L^{-1}) on 2007 fresh seed germination

In the seeds collected in 2007 (07Fr), not scarified (no*SC*) and treated with 600 mg L^{-1} of GA_3, 38% germination was recorded. It was 21 percentage points higher than that observed in no*SC*-$GA_3$400 seeds and 36 percentage points higher than that obtained, on average, with 200 mg L^{-1} GA_3 or 0 mg L^{-1} GA_3. Germination of no*SC* seeds started 88 days after placing them in Petri dishes, with 94 days as T_{50}, on average. Seeds subjected to scarification did not

Table 2
Effect (significance of *F*-test) of temperature regimes/sequences over the 56-day moist stratification period on the germination and on the time required to achieve 50% cumulative germination (T_{50}) in fresh seeds of *A. acutifolius* collected in 2007 and 2008 and in seeds (only harvested in 2007) after-ripened under glass or in paper.

	Germination (%)	T_{50} (days)
Seed lot (SL) (07Fr–08Fr–ARg–ARp)	***	***
Temperature regime (ST)	***	***
SL*ST	***	***

*** Significant at $P \leq 0.001$.

exhibit germination at all after an incubation period of 88 + 90 days.

3.2. Experiment 2—GA_3 (0 and 400 mg L^{-1}) and temperature regime/sequence during 28 days moist stratification on 2007 fresh seed germination

In the non-stratified and non-GA_3 treated 07Fr lot (no*ST*-$GA_3$0) germination was 2%, and it increased to 17% in no*ST*-$GA_3$400 treatment; germination started after 85 days of incubation and T_{50} was about 89 days, on average. The 28th day stratification period at 23 (*ST28*$_{23}$) and 5 °C (*ST28*$_5$) or with the shift of these temperatures every 14 (*ST28*$_{5/23}$) or 7 (*ST28*$_{5/23/5/23}$) days, did not allow germination after 85 + 90 days of seed incubation.

3.3. Experiment 3—seed lot and temperature regime/sequence over the 56 days period of moist stratification on seed germination

3.3.1. Germination percentage (Fig. 1)

The interaction between the seed lot and the temperature regime/sequence over the 56 days of moist stratification was significant (Table 2). The highest percentage of germination (73.5%, on average) was observed both in fresh seeds from 2007 (07Fr) or those after-ripened (ARg and ARp) when warm stratified, while germination was slightly lower in the 08Fr seeds (65%). The germination was not statistically different from the latter when stratification was given with alternate cold/warm temperatures (28 days at 5 °C followed by 28 days at 23 °C) (*ST56*$_{5/23}$), but only if seeds were after-ripened (ARg or ARp) (61%), while with 08Fr and 07Fr seeds germination was lower (45% and 19%, respectively). The germination percentage of 08Fr-*ST56*$_{5/23}$ seeds was similar to that of 08Fr-no*ST* ones (32%). Among no*ST* treatments, the seeds after-ripened in glass jars (ARg-no*ST*) had the highest germination percentage (47.5%), that was 5-fold higher than that of ARp-no*ST*, while the 07Fr seed lot had the lowest (2%). Almost null germination was observed in fresh seeds harvested in 2007 and 2008 when they were subjected to cold stratification (*ST56*$_5$); only seed lots after-ripened under glass or in paper germinated when submitted to cold stratification (19% and 10%, respectively) (Fig. 1).

3.3.2. T_{50} (Fig. 2)

The time required to achieve 50% cumulative germination (T_{50}) for each lot was almost always lower in *ST56* treatments than in corresponding no*ST* controls; the highest T_{50} decrease was observed between 07Fr non-stratified (90 days) and 07Fr seeds warm or cold/warm stratified (*ST56*$_{5/23}$) (44 days, on average). The T_{50} of ARg and 08Fr non-stratified lots (31 days, on average) decreased by nearly 18 days when they gave both warm (*ST56*$_{23}$) and cold stratification (*ST56*$_5$); this was also true for ARg-*ST56*$_{5/23}$ seeds, while 08Fr cold/warm stratified seeds showed a lower decrease (11 days). The seed lot stored in paper bags had the lowest T_{50} (19 days) among no*ST* seeds; it further decreased, by 6 and 3 days when this lot was warm and cold/warm stratified, respectively, on the contrary it increased by 6 days when cold stratified (Fig. 2).

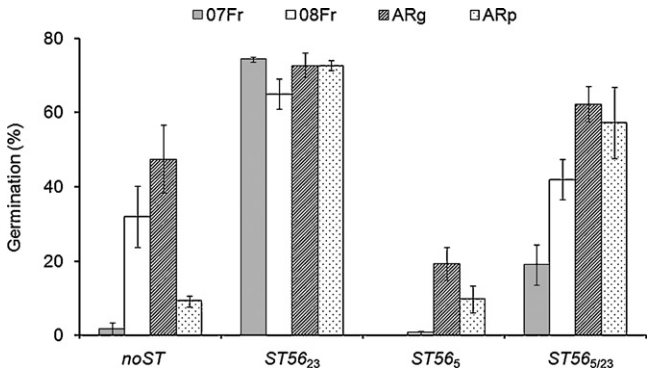

Fig. 1. Effect of temperature regimes during 56 days moist stratification on the germination percentage of fresh seeds collected in 2007 and 2008, and in seeds (only in those harvested in 2007) after-ripened under glass or in paper (seed lots) of *A. acutifolius* (vertcal bars indicate ±SE).

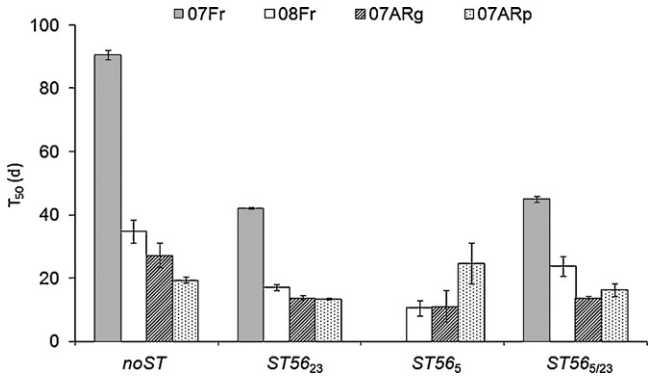

Fig. 2. Effect of temperature regimes during 56 days moist stratification on the time required to achieve 50% cumulative germination (T_{50}) of fresh seeds of *A. acutifolius* collected in 2007 and 2008 and in seeds (only in those harvested in 2007) after-ripened under glass or in paper (vertcal bars indicate ±SE).

3.3.3. Germination pattern

Among non-stratified seeds (Fig. 4a), in those fresh harvested in 2008 germination started 25 days after incubation in Petri dishes, achieving its maximum after about 1 month. On the contrary in 07Fr lot germination started after 84 days of incubation, showing a very low initial increase that remained unchanged during the following period of observation. The after-ripened seed lots started germination after 13 days of incubation. The germination of those stored under glass increased throughout the period, while in those stored in paper germination ended 30 days after the beginning.

When seed lots were warm stratified for 8 weeks (Fig. 4b) germination started 6 days after placing seeds in Petri dishes, except for 07Fr. Germination rapidly increased in the ARp lot, reaching a maximum 15 days later, while it evolved more slowly for the ARg and 08Fr seed lots (maximum at 36th and 40th day, respectively). For 07Fr-*ST56*$_{23}$ germination started 35 days after placing the seeds in Petri dishes and ended 35 days later.

With cold stratification for 8 weeks (Fig. 4c) the incubation period before the start of germination was 10 days long in 08Fr and ARp seeds. For the latter, germination increased slowly for the following 26 days, while for the 08Fr seed lot germination occurred within a few days. The ARg seeds soon started germination reaching a maximum at the end of the 50 day period.

The *ST56*$_{5/23}$ seeds (Fig. 4d) started germination after 7 days of incubation, except for 07Fr that delayed the beginning of germination to the 41st day. The seeds stored under glass completed germination 24 days after the beginning, while those stored in paper about 20 days later. The fresh seeds from 2008 reached the maximum value of germination at the end of the observation

Table 3
Significance of *F*-test for temperature regimes/sequences after 56 days of moist stratification and of gibberellic acid on the germination percentage and on the time required to achieve 50% cumulative germination (T_{50}) in fresh seeds of *A. acutifolius* collected in 2008 and in seeds (harvested in 2007) after-ripened in glass or in paper.

	Germination (%)	T_{50} (days)
Seed lots (SL) (08Fr-ARg-ARp)	***	***
Gibberellic acid (GA_3)	ns	ns
Temperature regime (ST)	**	*
SL × GA_3	ns	ns
SL × ST	***	*
GA_3 × ST	ns	ns
SL × GA_3 × ST	ns	*

ns, not significant.
* Significant at $P \leq 0.05$.
** Significant at $P \leq 0.01$.
*** Significant at $P \leq 0.001$.

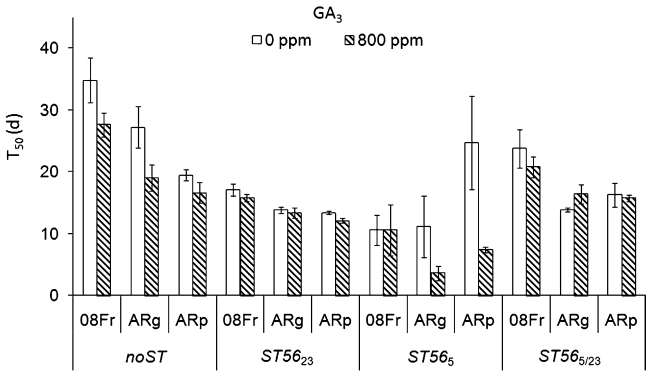

Fig. 3. Effect of GA_3 treatment and of temperature regimes during 56 days moist stratification on the time required to achieve 50% cumulative germination (T_{50}) of fresh seeds of *A. acutifolius* collected in 2007 and 2008 and in seeds (only in those harvested in 2007) after-ripened in glass or in paper (seed lots) (vertcal bars indicate ±SE).

period, while germination of those from 2007 occurred within the first 2 weeks of observation.

3.4. Experiment 4—GA_3 (0 and 800 mg L^{-1}) and temperature regime/sequence during 56 days of moist stratification on 2008 fresh and 2007 after-ripened seeds

3.4.1. Germination percentage

The germination percentage of 08Fr and after-ripened seeds was not influenced by gibberellic acid treatment or by the interaction of GA_3 with any of the other treatments (Table 3). The interaction between seed lot and stratification treatments was significant, independently of GA_3 level; therefore the results confirm the behavior observed when GA_3 was not used according to Experiment 3 results (Fig. 1).

3.4.2. T_{50}

The time required to achieve 50% cumulative germination was influenced by the interaction of the three factors (Table 3). In non-stratified seeds an 800 mg L^{-1} GA_3 dose decreased T_{50} by 7 days for the 08Fr and ARg lots and by 2 days for AR*p*. In the stratified seeds, with warm or alternate temperatures, gibberellic acid did not influence T_{50}, while with cold stratification and only in after-ripened lots, GA_3 treatment decreased T_{50} by 7 and 18 days in ARg and AR*p*, respectively (Fig. 3).

3.4.3. Germination pattern

Among non-stratified seeds gibberellic acid produced an effect only on fresh seeds (08Fr) bringing forward the beginning of germination from 25 to approximately 12 days (Fig. 4a); in ARg, GA_3 also brought forward the point of maximum germination by about 20 days.

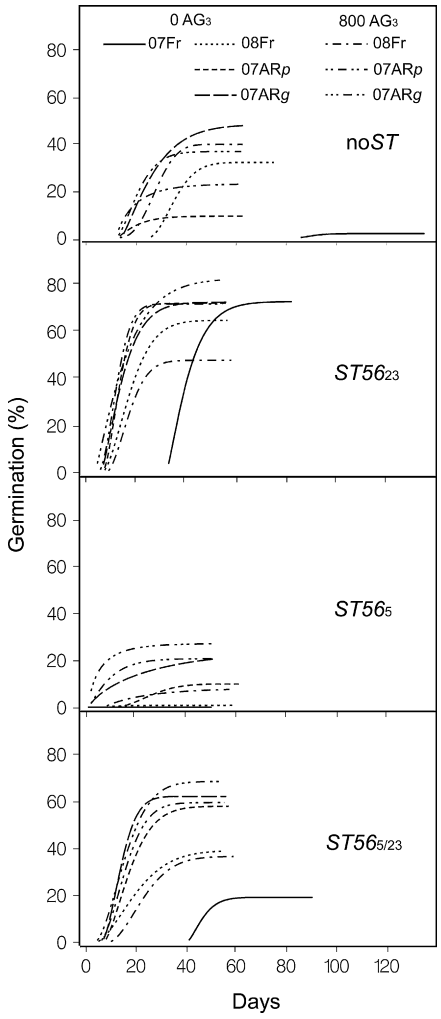

Fig. 4. Effect of seed lot (2007 and 2008 fresh and after-ripened under glass or in paper) and of GA_3 treatment on cumulative germination of *A. acutifolius* seeds when not stratified (a), stratified for 56 days at 23 °C (b), at 5 °C (c) and (days) at 23 °C for the first 28 days and at 5 °C for the following 28 days.

No effect of GA_3 treatment on germination speed was observed in warm stratified seeds (Fig. 4b).

The only effect of GA_3 in cold stratified seeds was to bring forward the beginning of germination by 10–1 day in the AR*p* lot (Fig. 4c). The germination pattern following the GA_3 treatment was almost unchanged, when seeds were stratified with alternate warm/cold temperatures (Fig. 4d).

4. Discussion

When non-scarified, non-stratified and non-GA_3 treated, the fresh seeds harvested in 2007 germinated at a very low rate (2%), after about 6 months of incubation, thus confirming the presence of seed dormancy in the species. However, the fresh seeds harvested in 2008 showed 32% germination within 36 days, therefore the 07Fr seeds were more dormant than the 08Fr ones.

The primary dormancy depth is strongly related to the nutritional status of the mother plant (Geneve, 2003; El-Keblawy and Al-Rawai, 2006) and to the environmental conditions occurring during seed development (Finch-Savage and Leubener-Metzger, 2006). These conditions strongly influence the abscissic/gibberellic acid ratio in the late phase of maturation (Gutierrez et al., 2007)

on which depends induction of dormancy. It is likely that the conditions experienced by parent plants during the growing season in 2008 compared to 2007 were more suitable for a reduction of primary dormancy depth of seeds.

Physiological dormancy can have an embryo and/or coat (testa, endosperm, pericarp) component; and their sum and interaction determine the depth of seed physiological dormancy (deep, intermediate, non-deep level). While embryo dormancy is characterized by the inhibition of extension growth, coat dormancy is manifested as mechanical resistance from testa and endosperm to embryo growth (Finch-Savage and Leubener-Metzger, 2006), or as chemical dormancy due to the presence of inhibitor compounds in the covering layers of the seeds (Baskin and Baskin, 2004).

Although scarification is reported to be effective to break non-deep physiological dormancy, the fresh seeds harvested in 2007 did not germinate at all when chemically scarified. On the contrary, when not scarified, 07Fr seeds were sensitive to the application of gibberellic acid with 38% and 17% germinating with 600 and 400 mg L^{-1}, respectively. Therefore, it is likely that seeds submersion in sulphuric acid for few minutes impaired the embryos that were found not viable in the viable test (data not shown).

Seed after-ripening by dry storage can contribute to relieve primary dormancy and particularly non-deep physiological dormancy (Baskin and Baskin, 2004). Seed changes that occur in low-hydration conditions depend on species and ecotypes and they modify responses to environmental signals (Müller et al., 2009).

Compared to the 07Fr lot, AR*g* and AR*p* dry-stored seeds were less dormant. Dry storage under glass for 14 months improved germination (+50%) compared to fresh seeds (Fig. 1), while by dry-storing seeds in paper they germinated with a higher speed (Fig. 4a) and earlier than 07Fr ones, but at a very similar percentage (9% vs. 2%). Thus seeds of this *A. acutifolius* ecotype are after-ripened during dry storage, but with some differences relying on the type of containers.

The different release from dormancy observed as a function of container type used for storing the seeds could be related to the low humidity fluctuation throughout the storage period assured by the glass compared to the paper, the after-ripening rate being affected by seed water content (Finch-Savage and Leubener-Metzger, 2006).

In a previous work after-ripening was found effective in *A. acutifolius* ecotype seeds collected in hilly areas. Storing these seeds for 13 months in paper bags brought forward (but did not improve) germination compared to freshly harvested seed, and after-ripening was also effective in improving seed sensitivity to pre-germination treatments (moist stratification and soaking) (Conversa and Elia, 2009).

After-ripening and moist (warm or cold) stratification affect metabolic and physiological changes in seeds that involve both the embryo and its covering layers (Leubner-Metzger, 2005; Bair et al., 2005) linked to a rapid decline in the abscissic acid (ABA) content and in ABA sensitivity, and to increase in gibberellic acid (GA) sensitivity of imbibed dormant seeds (Gubler et al., 2005).

In this ecotype of *A. acutifolius* the stratification for 4 weeks (*ST28*), irrespective of temperature regimes and sequence or of GA_3 treatment was ineffective in improving germination of freshly harvested seeds in 2007. Moreover, *ST28* reduced sensitivity to 400 mg L^{-1} GA_3 treatment compared to non-stratified 07Fr seeds.

The ecotype of *A. acutifolius* collected from a hilly area (687 m a.s.l.) showed 56% germination when soaked seeds were stratified for 1 month (at 23 or 5 °C) (Conversa and Elia, 2009). This confirms that the different habitat from which the ecotype comes from can influence the response to the same seed treatment (Nikolaeva, 2004).

In this research a longer stratification period was necessary to break dormancy and the temperature regimes within this period were crucial in influencing break in dormancy, depending on the depth of dormancy of seed lots. It was confirmed that the efficacy of stratification is temperature and duration dependent.

Even with large differences in initial dormancy degree between the different seed lots, the most copious (Fig. 1), rapid and uniform (Figs. 2 and 4b) germination was observed when seeds were warm stratified for 56 days. Warm stratification was able to relieve dormancy in about 70% of freshly harvested seeds and AR seeds. It was much more effective in 07Fr and AR*p* seeds (Fig. 1), though the latter germinated more slowly and later than the other seed lots (Figs. 2 and 4b).

Physiological dormancy relief is under genetic control; the mechanisms are different and they can partially overlap (Cadman et al., 2006; Finch-Savage et al., 2007). In this research warm stratification and after-ripening seem to act on different mechanisms of dormancy relief, but some of them could be in common, particularly in glass after-ripened seeds.

Cold stratification inhibited germination, it reduced the number of germinated seeds especially when it was applied on 08Fr and AR*g* lots (Fig. 1), but also AR*p* seeds germinated more slowly when cold stratified (Fig. 4c). The inhibiting effect of cold stratification was also detected in the previous work (Conversa and Elia, 2009) using *A. acutifolius* seed collected from a hilly area, but only when this seed was AR and not soaked after stratification. In this research although seeds were soaked after stratification, low temperature showed an inhibiting effect in particular with the lots that were presumably at a lower degree of dormancy (08Fr and AR*g*). From an ecological point of view, cold stratification acts as an inhibiting factor (secondary dormancy) on the germination of those seeds that are ready to germinate to avoid unfavorable weather conditions (Baskin and Baskin, 1998).

In addition, in seeds with morpho-physiological dormancy the embryo is underdeveloped, so temperature sequence could be useful to break dormancy. In the present research, the stratification of the 07Fr lot for 4 weeks with alternate and repeated temperature sequences (cold/warm/cold/warm) did not improve germination. The alternate temperature during the 8-week stratification period ($ST56_{5/23}$) never improved germination in any of the seed lots compared to warm temperature; $ST56_{5/23}$ seed germination was at least equal to that of warm stratification, but only if seeds were after-ripened (Figs. 1 and 4d). Only 07Fr cold/warm stratified seeds germinated more and earlier compared to non-stratified ones (Fig. 4a and d). Even when temperature sequences were applied to the 07Fr lot with more frequent changes (2 series of 14 days at 5 and 23 °C; 4 series of 7 days at 5 and 23 °C) germination results were similar to $ST56_{5/23}$ treatment (data not shown), thus indicating that seeds do not have a morphological component of dormancy.

Increasing the level of exogenous gibberellic acid (from 200 to 600 mg L^{-1}) caused a slight increase germination in freshly harvested in 2007 seeds; the same was not observed in 08Fr seeds, or in those after-ripened whether stratified or not, even if they were treated with a higher GA_3 level (800 mg L^{-1}). Gibberellic acid proved effective in speeding up germination only in fresh non-stratified or cold stratified seeds from 2008 (Fig. 4a and c) as reported by others authors (Yang et al., 2007).

These findings, except for the scarce response to GA_3, firmly support the hypothesis that *A. acutifolius* seeds fit the characteristics a non-deep physiological dormancy according to the dormancy classification of Baskin and Baskin (2004). Moreover environmental cues able to break dormancy appear to act differently in different ecotypes. It seems that as mechanisms to prevent germination in unfavorable conditions, the seeds of the ecotypes collected in the areas characterized by dry–warm summer and rainy–mild winter need after-ripening and/or warm moist stratification to release primary dormancy; when these seeds are releasing from dormancy moist conditions at low temperatures induce secondary dormancy.

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References

Angiosperm Phylogeny Group, 2003. An update of Angiosperm Phylogeny Group classification for orders and families of flowering plants: APG II. Bot. J. Linn. Soc. 141, 399–436.

Bair, N.B., Meyer, S.E., Allen, P.S., 2005. A hydrothermal after-ripening time model for seeds dormancy loss in *Bromus tectorum* L. Seed Sci. Res. 16, 17–28.

Baskin, C.C., Baskin, J.M., 1998. Seeds; Ecology, Biogeography, and Evolution of Dormancy and Germination. Academic Press, San Diego.

Baskin, J.M., Baskin, C.C., 2004. A classification system for seed dormancy. Seed Sci. Res. 14, 1–16.

Cadman, C.S.C., Toorop, P.E., Hilhorst, H.W.M., Finch-Savage, W.E., 2006. Gene expression profiles of *Arabidopsis* Cvi seeds during dormancy cycling indicate a common underlying dormancy control mechanism. Plant J. 46, 805–822.

Caliandro, A., Lamaddalena, N., Stelluti, M., Steduto, P., 2005. Caratterizzazione agro-ecologia della Regione Puglia in funzione della potenzialità produttiva. Progetto ACLA 2, Bari, Italy (Ideaprint).

Conversa, G., Elia, A., 2009. Effect of seed age, stratification, and soaking on germination of wild asparagus (*Asparagus acutifolius* L.). Sci. Hort. 119, 241–245.

Damato, G., Vannella, S., Downs, R.J., 1994. Temperatures, incubation periods, osmotic potentials and rate of germination of Florence fennel "seeds" at optimal and critical temperatures. Acta Hort. 362, 167–172.

El-Keblawy, A., Al-Rawai, A., 2006. Effects of seed maturation time and dry storage on light and temperature requirements during germination in invasive *Prosopis juliflora*. Flora 201, 135–143.

Finch-Savage, W.E., Leubener-Metzger, G., 2006. Seed dormancy and the control of germination. New Phytol. 171, 501–523.

Finch-Savage, W.E., Cadman, C.S.C., Toorop, P.E., Lynn, J.R., Hilhorst, H.W.M., 2007. Seed dormancy release in *Arabidopsis* Cvi by dry after-ripening, low temperature, nitrate, and lights shows common quantitative patterns of gene expression directed by environmentally specific sensing. Plant J. 51, 60–78.

Fiori, P.P., Giola, M., Ledda, M.G., Tedde, M., 2001. Valorizzazione dell'asparago selvatico. Inftore agrario 50 (57), 47.

Geneve, R., 2003. Impact of temperature on seed dormancy. HortScience 3 (38), 336–341.

Ghirardini, M.P., Carli, M., del Vecchio, N., Rovati, A., Cova, O., Valigi, F., Agnetti, G., Macconi, M., Adamo, D., Traina, M., Laudini, F., Marcheselli, I., Caruso, N., Gedda, T., Donati, F., Marzadro, A., Russi, P., Spaggiari, C., Bianco, M., Binda, R., Barattieri, E., Tognacci, A., Girardo, M., Vaschetti, L., Caprino, P., Sesti, E., Andreozzi, G., Coletto, E., Belzer, G., Pieroni, A., 2007. The importance of a taste. A comparative study on wild food plant consumption in twenty-one local communities in Italy. J. Ethnobiol. Ethnomed. 3, 22, doi:10.1186/1746-4269-3-22.

Gubler, F., Millar, A.A., Jacobsen, J.V., 2005. Dormancy release. ABA and pre-harvest sprouting. Curr. Opin. Plant Biol. 8, 183–187.

Gutierrez, L., Van Wuytswinkel, O., Castelain, M., 2007. Combined networks regulating seed maturation. Trends Plant Sci. 7 (12), 294–299.

ISTA, 2003. Working Sheets on Tetrazolium Testing, 1st ed., vol. II—Tree and Shrub Species. International Seed Testing Association, Bassersdorf, Switzerland.

Leubner-Metzger, G., 2005. β-1,3 Glucanase gene expression low-hydrated seeds as a mechanism for dormancy release during tobaco after-ripening. Plant J. 41, 133–145.

Macchia, F., Cavallaro, V., Forte, L., Terzi, M., 2000. Vegetazione e clima della Puglia. In: Marchiori, S., De Castro, F., Myrta, A. (Eds.), La cooperazione italo-albanese per la valorizzazione della biodiversità. CIHEAM-IAMB, Bari, pp. 33–49.

Müller, K., Carste, A.C., linkies, A., Torres, M.A., Leubner-Metzger, G., 2009. The NADPH-oxidase *AtrbohB* plays a role in Arabidopsis seed after-ripening. New Phytol. 184, 885–897.

Nikolaeva, M.G., 2004. On criteria to use in studies of seed evolution. Seed Sci. Res. 14, 315–320.

Reid, T.C., Hausbeck, M.K., Kizilkaya, K., 2002. Use of fungicides and biological controls in the suppression of *Fusarium* crown and root rot of asparagus under greenhouse and growth chamber conditions. Plant Dis. 86, 493–498.

Rosati, A., Falavigna, A., 2000. Germinazione dei semi di asparago selvatico. Inftore agrario 46 (56), 53–55.

Rosati, A., 2001. Un possibile futuro per l'asparago selvatico. Inftore agrario 7 (57), 89–92.

Institute, S.A.S., 1999. SAS/STAT User's Guide, Ver. 7-1, vol. 3. SAS Institute Inc., Cary, NC.

Štajner, N., Bohanec, B., Jarornik, B., 2002. Genetic variability of economically important Asparagus species as revealed by genome size analysis and rDNA ITS polymorphisms. Plant Sci. 162, 931–937.

Sica, M., Gamba, G., Montieri, S., Gaudio, L., Aceto, S., 2005. ISSR markers show differentiation among Italian populations of *Asparagus acutifolius* L. BMC Genet. 6, 17–23.

Yang, Q.H., Ye, W.H., Yin, X.J., 2007. Dormancy and germination of Areca triandra seeds. Sci. Hort. 113, 107–111.