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EFFECTS OF ECOLOGICAL FACTORS AND REPRODUCTIVE STRATEGIES ON GERMINATION CAPABILITY OF *AILANTHUS ALTISSIMA* (MILL.) SWINGLE

Ecological factors and reproductive strategies in *Ailanthus altissima* germination

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Abstract – The tree of heaven *Ailanthus altissima* is one of the most invasive plant species in Europe and Italy and poses a threat especially in urban environments. A more profound comprehension of how germination traits respond to environmental factors could help elucidate the invasion process. Germination tests on *A. altissima* seeds collected at different altitudes were carried out at different light conditions (12-hr light/12-hr darkness and continuous darkness) and temperature regimes (15-6 °C, 20-10 °C and 25-15 °C). A further test was also performed to assess the germinative response of seeds retained on the plant during winter. Seeds haven't shown clear preferences for temperature and light, nor an effect of the altitude at which they develop on their germination capacity. However, a coast-inland gradient emerged in relation to seeds' sensitivity to cold stratification. Moreover, the continuous release of seeds throughout the year may maximize the chances of a successful germination, offsetting low germinability against a significant number of offsprings. These characteristics endow *A. altissima* with substantial adaptability and highlight the pioneer nature of the species during its reproduction and germination, both in its tolerance to different environmental conditions and in adopting a reproduction strategy oriented towards the extreme of r-strategist species, contributing to the species' invasiveness.

Keywords: altitudinal differences, ecological adaptation, germination, invasive alien species, reproductive strategies.

Introduction

Biological invasions are considered one of the primary drivers of global change (Crutzen, 2002), posing significant threats to the environment, economy and human health (IUCN, 2000; Rai & Singh, 2020). Human activities play a crucial role in introducing numerous species beyond their native ranges, enabling them to overcome biogeographical barriers (Richardson et al., 2000; Nentwig et al., 2018). When a species establishes a self-sustaining population that rapidly spreads from the introduction site, it is classified as an invasive alien species (IAS) (Pyšek et al., 2020). Alien species are the second global cause of extinctions (Bellard et al., 2016), leading to a reduction in biodiversity and to biotic homogenization (Sax & Gaines, 2003; Olden et al., 2016). Often, plant species are introduced for ornamental and horticultural purposes (Heywood, 1989; Pergl et al., 2016), and many of them have become permanent additions to local flora (Seebens et al., 2018). Globalization and intensified trades have contributed to a significant increase in the introduction of exotic species over the last century (Meyerson & Mooney, 2007). In Europe, the number of plant IAS has increased 4-fold (Hulme, 2009), and invasions are particularly prominent in urban areas, where

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28% of the local flora comprises alien species (Aronson et al., 2014; Godefroid & Ricotta, 2018). According to the checklist of the vascular flora alien to Italy by Galasso et al. (2024), 1782 exotic species are present on the Italian territory, and 250 of them are invasive.

A common question in the study of alien species is which attributes contribute to a species' invasiveness (Rejmanek & Richardson, 1996). Factors that are considered crucial for the success of an IAS include dispersal ability, tolerance thresholds, adaptability to different ecological factors and phenotypic plasticity (Cain et al., 2000; Funk, 2008; van Kleunen et al., 2016). The ability to adapt to various environments allows species to display adaptive characteristics, which can influence their ecological distribution and, as a result, their likelihood of invading new territories (Hulme, 2008; Molina-Montenegro et al., 2018). Traits associated with reproduction and growth are also commonly linked to invasion success. Many invasive plant species exhibit high fecundity or efficient mechanisms of vegetative propagation (Pyšek & Richardson, 2008). Seed production, early and rapid germination, and the capacity to germinate under a broad range of environmental conditions are crucial processes that determine the outcome of invasion in new regions (Moravcová et al., 2006; Pyšek & Richardson, 2008). Furthermore, the prolonged viability of seeds and the ability to form persistent seed banks can significantly contribute to determining the invasion potential of alien plants in their new distribution ranges (Gioria et al., 2019).

Many lists of the most harmful alien species have been compiled to raise awareness amongst the general public, politicians and stakeholders (Nentwig et al., 2018). European Parliament has adopted the Regulation No 1143/2014 to prevent, minimize, and mitigate the adverse impact on biodiversity of the introduction and spread of invasive alien species within the Union (EU 2014). In 2019 the plant species *Ailanthus altissima* was included within the species of Union concern. *Ailanthus altissima* (Mill.) Swingle 1916 (Simaroubaceae), also called the "tree of heaven", is a deciduous medium-sized tree native to China and northern Vietnam. Introduced in Europe in 1740 by the French missionary Pierre d'Incarville, it is currently invasive on all continents except Antarctica (Kowarik & Säumel, 2007). Due to its rapid growth and efficient photosynthesis in open, bright areas, it is considered a pioneer ruderal species occupying highly disturbed sites (Knapp & Canham, 2000; Call & Nilsen, 2003; Kowarik & Säumel, 2007). Disturbances such as frost, fires and cutting induce the production of shoots from the roots and the stem (Bory et al., 1991), forming dense monoclonal stands and thus allowing the rapid colonization of the invaded sites. *A. altissima* is tolerant to many ecological factors, such as drought, high temperatures, different soil compositions, salinity and pollution (Kovacs et al., 1982; Kowarik & Säumel, 2007; Sladonja et al., 2015). Nevertheless, it is a shade-intolerant species and it is sensible to severe frost (Knapp & Canham, 2000; Kowarik & Säumel, 2007; Knüsel et al., 2019). *A. altissima* can be found at <1000 m in temperate Europe, but in the Mediterranean climate most of *A. altissima* observations occur at <300 m of altitude (Fotiadis et al., 2011). *A. altissima* is one of the most frequent non-native species in Italy, particularly in urban areas where it thrives on walls, sidewalks, embankments of roads and railways and abandoned lots (Kowarik & Säumel, 2007). Its growth in cities poses a threat as its roots can cause significant damage, particularly to archaeological remains in historically rich Mediterranean cities like Rome (Celesti-Grapow & Blasi, 2004; Casella & Vurro, 2013; Trotta et al., 2020). In the rare cases that the species occupies natural areas, competition with native species and the production of allelopathic substances causes a reduction of local biodiversity (Constán-Nava et al., 2015). It is a dioecious species: female individuals produce a conspicuous number of winged fruits (samaras). A single tree can produce up to 325.000 seeds (Clair-Maczulajtys, 1984), but according to Martin & Canham (2010) a mature individual can produce more than a million seeds per year. Seeds can remain on the tree for an extended period, be dispersed after the subsequent winter or retained until the following autumn.

Due to the high ability of IAS to adapt to different environmental conditions, it is crucial to acquire as much information about their germination strategies as possible. A deeper insight into how germination traits react to environmental conditions can help clarify the mechanisms behind the invasion process. This, in turn, may enhance our ability to predict and anticipate future invasions. As for all plants, seed germination of invasive species is a crucial process in their life cycle and is highly vulnerable to environmental change (Wu et al., 2019). Since biological invasions act in synergy with other global change drivers, climate change can impact each stage of exotic species' invasion pathways depending on the species' ecology (Hellmann et al., 2008; Sage, 2020). Changes in temperature and precipitation regime may indeed strongly impact vulnerable life-history stages such as germination (Vesela et al., 2020). Furthermore, climate change may enable lower altitude species to expand and adapt to higher altitudes (Walther, 2007). Differences in the germination pattern among populations of the same species could provide information about its adaptability to different environments and help predict how the species would behave under the ongoing climate changes. In this study, germination tests were conducted on seeds collected from various environments at different altitudes. The aim was to gain new insights into the adaptability of the germination process of *A. altissima*. Additionally, tests were performed to assess the invasive tree's ability to employ reproductive strategies associated with seed dispersal and seed retention on the parent plant.

MATERIALS AND METHODS

Sampling

Seeds of *A. altissima* were collected from three sites in Rome Province: Castel Fusano (FUS) (41° 43' 12.6"N, 12° 21' 32.8"E; 5 m s.l.m.), Appia Antica Regional Park (Caffarella Valley, CAF) (41° 51' 58.4"N, 12° 31' 29.5"E; 40 m s.l.m.) and Grottaferrata (GRO) (41° 46' 56.7"N, 12° 40' 46.5"E; 365 m s.l.m.) (Fig. 1).

The three sites have a Mediterranean climate. The average total yearly rainfall, mostly distributed in autumn and winter, is 1079,0 mm in Castel Fusano, 700,5 mm in Rome and 723,3 mm in Grottaferrata. The average mean air temperature of the hottest month (August) is 23,13 ± 0,38 °C in Castel Fusano, 26,68 ± 0,35 °C in the city of Rome and 24,90 ± 0,38 °C in Grottaferrata. The average mean air temperature of the coldest month (January) is 6,02 ± 0,49 °C in Castel Fusano, 8,34 ± 0,35 °C in the city of Rome and 7,87 ± 0,40 °C in Grottaferrata (data provided by SIARL, Integrated Agrometeorological Service of Lazio Region; Meteorological Stations of Fiumicino-Maccarese, Roma-Via Lanciani and Grottaferrata-Valle Marciana, for the period 2010 to 2020). Seeds were collected in December 2020. For the site of Caffarella, a second harvest was performed in March 2021 to evaluate the germinative response of seeds retained on the plant during winter. Samaras were sampled from individuals placed at a distance of more than 10 m from each other to prevent the collection of seeds from closely related individuals and thus to ensure the genetic variability of the sample. To avoid the prevalence of certain genotypes, an equal number of seeds was collected for each individual (Bacchetta et al., 2006). Samaras were collected from different points of the tree canopy to avoid biases

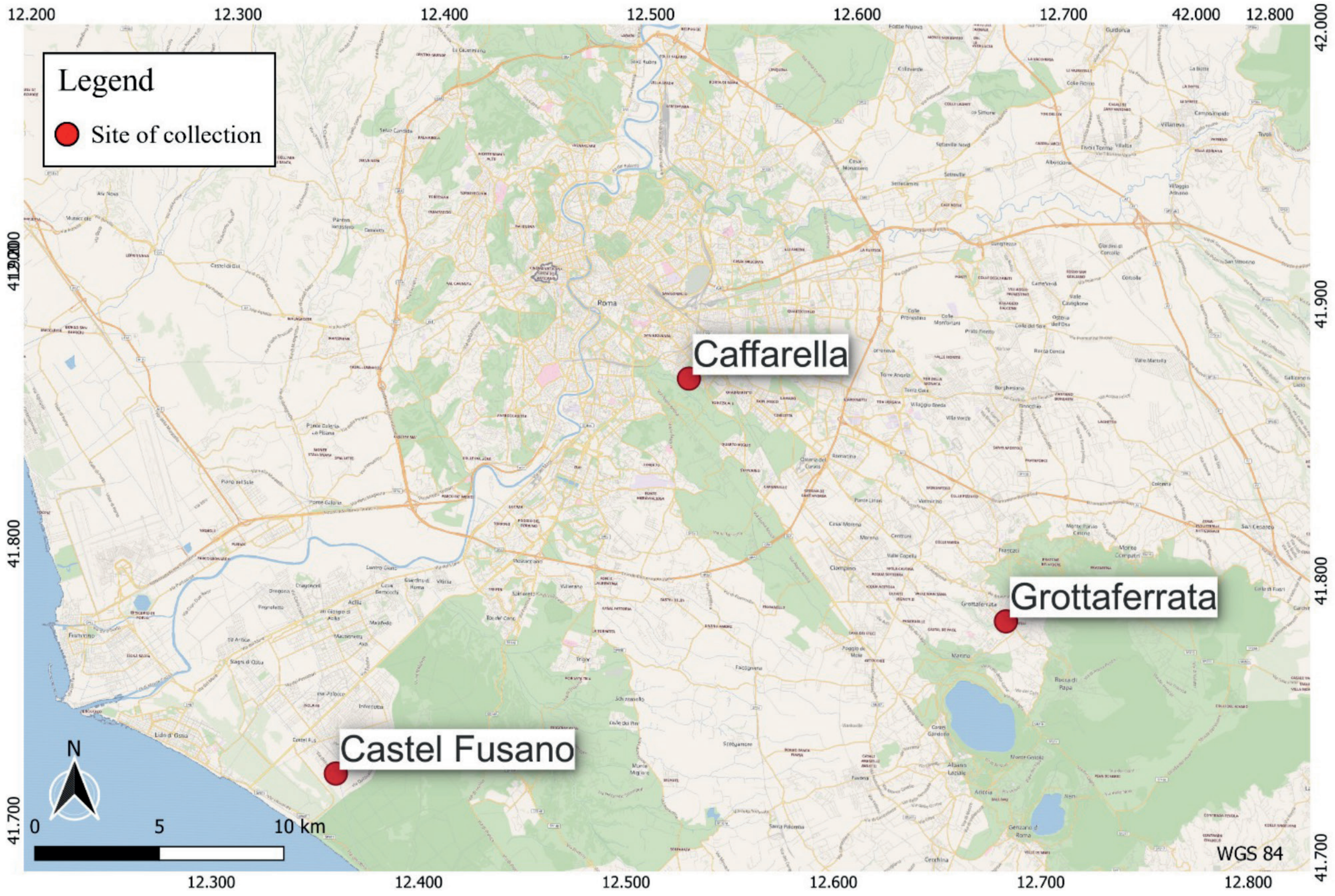

Figure 1. Sites of collection of *A. altissima* seeds. Castel Fusano (FUS) (41° 43' 12.6"N, 12° 21' 32.8"E; 5 m s.l.m.), Appia Antica Regional Park (Caffarella Valley, CAF) (41° 51' 58.4"N, 12° 31' 29.5"E; 40 m s.l.m.) and Grottaferrata (GRO) (41° 46' 56.7"N, 12° 40' 46.5"E; 365 m s.l.

resulting from the operator's ease of collection. The fruits were placed in paper bags during harvesting and immediately transported to the Germplasm Bank of the Botanical Garden, where seeds were manually separated from the fruits and post-ripened in open containers at room temperature of 20 °C and with a relative humidity of 40-60% for 15 days.

Morphometric traits

The following morphometric traits were measured from 100 seeds for each collection site: seed fresh mass (SFM, g), seed dry mass (SDM, g), moisture content (mc, %), major axis (A, mm) and minor axis (a, mm).

SFM and SDM were obtained by weighing 5 samples, each containing 20 seeds, with a Gilbertini precision balance (precision of 0.1 mg). SFM was measured immediately after seed collection, while SDM was determined after drying at 103 °C for 17h the same seeds used to measure SFM. Mc was calculated as a weight loss according to ISTA (2014) ($mc_{\%} = 100 \times [(SFM-SDM)/SFM]$) and expressed as a percentage. A and a were measured individually for 100 seeds per collection site using NIS-Element Br. 2.10 with images from a digital camera Nikon Digital Sight DS-120 U1, mounted on a stereo microscope Carl Zeiss.

Effect of ecological factors on seed germination

For each site of collection, seed germination was tested at three alternating temperature regimes (15-6°C, 20-10°C and 25-15°C), according to Bacchetta et al. (2006). The chosen temperatures simulate the thermal conditions of the different seasons in the region of interest, and they are recommended in literature (Martínez-Díaz et al., 2018; Wu et al., 2019). For each temperature, seeds were exposed to an alternating light/darkness regime (L/D: 12h light – 12h dark) and continuous darkness (D: 24h dark). Since dormancy in *A. altissima* seeds is reported in the literature (Redwood et al., 2019), three trials were carried out to evaluate seed germination. In trial 1, seeds were germinated without undergoing any pre-treatment to overcome their dormant state. Trial 2 was carried out after cold stratifying seeds for three months at 4°C in river sand, and trial 3 after six months of cold stratification under the same conditions. For each trial and at each temperature regime, germination was analyzed for eight experimental units (four in the light and four in the darkness) of 25 seeds. Seeds were placed in Petri dishes on a layer of sterile filter paper. Sterile distilled water was periodically added to each Petri dish to keep the substrate moistened. The Petri dishes destined for light treatments were incubated for 12h light and 12h darkness (L/D), with coolwhite fluorescent tubes providing a photon flux density of 80 μmol (photon) m^{-2} s^{-1}. The 12h light period corresponded to the daily high temperature, thus simulating natural conditions. Petri dishes destined to complete darkness treatments (D) were covered with a double aluminum foil wrapping to shield seeds from light.

For seeds collected in March 2021 (after winter) in Caffarella site (CAFw), a further test (trial 4) was set up in which seeds were subjected to the same combinations of temperature (15-6°C, 20-10°C and 25-15°C) and light (L/D and D) of the previously described germination tests.

All seeds were incubated for 60 days, and germinated seeds were counted every day. A seed was considered germinated when the length of the emerging radicle was >1 mm (ISTA, 1985). Seeds incubated in darkness were examined daily in a darkroom with a green safety light (Joly & Felippe, 1979).

Germination traits

For each treatment, two germination traits were calculated:

Germination percentage (grp, %): the percentage of seeds germinated at the end of germination period. Corresponds to the ratio between the number of germinated seeds and the total number of seeds, multiplied by one hundred. It is calculated as:

$$grp = \left(\frac{\sum_{i=1}^{k} n_i}{N}\right) \times 100 \qquad (1.1)$$

where n_i is the number of seeds germinated on day i, k is the last day of the germination test and N is the total number of seeds present in an experimental unit (Lozano-Isla et al., 2019) ($0 \leq grp \leq 100$).

Mean germination time (mgt, days): the average time a seed takes to germinate. It corresponds to a weighted average of the germination time, where the weight is represented by the number of seeds germinated in the time interval established for the count (in this case one day) (Czabator, 1962). It is calculated as:

$$mgt = \left(\frac{\sum_{i=1}^{k} n_i t_i}{\sum_{i=1}^{k} n_i}\right) \qquad (1.2)$$

where n_i is the number of seeds germinated on day i, t_i is the number of days passed since the beginning of the experiment and k is the last day of the germination test (Lozano-Isla et al., 2019) ($0 \leq mgt \leq k$).

Germination traits were calculated using R package version 2.1.3 GerminaR: indices and graphs to evaluate the seed germination process (Lozano-Isla et al., 2019).

Data analysis

To compare values of SFM and SDM of the seeds collected in the three sites (CAF, FUS and GRO), a one-way ANOVA was carried out, followed by a post-hoc Tukey test for pairwise multiple comparisons between sites. A Kruskal-Wallis test was used to compare A, a, and mc, followed by a post-hoc Dunn test for multiple pairwise comparisons.

To evaluate the effect of site of collection (three levels: CAF, FUS and GRO), temperature (three levels: 15-6°C, 20-10°C and 25-15°C) and light (two levels: L/D and D) a three-way ANOVA was carried out separately for trials 1, 2 and 3.

A four-way ANOVA was performed to evaluate the effect of cold stratification on grp and mgt. Factors were pre-treatment (three levels: no pre-treatment, three months of cold stratification and six months of cold stratification), site of collection (three levels: CAF, FUS and GRO), temperature (three levels: 15-6°C, 20-10°C and 25-15°C) and light (two levels: L/D and D).

A three-way ANOVA was carried out to compare, for CAF alone, grp and mgt of seeds harvested in December and not pre-treated (CAF0) with those of seeds harvested in March after the winter season (CAFw). The effects of the period of collection (two levels: CAF0 and CAFw), temperature (three levels: 15-6°C, 20-10°C and 25-15°C) and light (two levels: L/D and D) were evaluated.

Data were log-transformed if tests for the ANOVA assumptions of normality and equal variance failed. Following ANOVA, a post-hoc Tukey test was run for pairwise multiple comparisons.

For all the tests the level of significance chosen was $\alpha \leq 0.05$. Data are shown as mean value ± SE.

RESULTS

Morphometric traits

Analyses highlighted differences between seeds collected in the three sites (Tab. 1). GRO showed the highest values of both SFM and SDM, while those traits didn't differ significantly for CAF and FUS. Mc significantly differed between CAF (6,56 ± 0,09 %) and FUS (7,21 ± 0,07 %). CAF seeds were also characterized by the lowest size, since they showed a significantly inferior value for both A and a.

Trial 1: germinability with no cold stratification

Unstratified seeds showed very low germination rates, in all cases less than or equal to 5%, confirming the presence of dormancy in *A. altissima* seeds. The results of the three-way ANOVA (Tab. 2) showed that neither temperature nor site of collection nor light influenced germination percentage of non-stratified seeds.

Table 1. Morphometric traits of seeds collected in Caffarella (CAF), Castel Fusano (FUS) and Grottaferrata (GRO). Seed fresh mass (SFM), seed dry mass (SDM), moisture content (mc), major axis (A) and minor axis (a) are shown. Different letters indicate significant differences ($p < 0.05$; for SFM and SDM: one-way ANOVA followed by post-hoc Tukey tests; for mc, A and a: Kruskal-Wallis test followed by post-hoc Dunn test).

	SFM (g)	SDM (g)	mc (%)	A (mm)	a (mm)
CAF	0.19 ± 0.00 a	0.18 ± 0.00 a	6.56 ± 0.09 a	4.80 ± 0.05 a	3.78 ± 0.04 a
FUS	0.19 ± 0.00 a	0.18 ± 0.00 a	7.21 ± 0.07 b	5.15 ± 0.03 b	4.09 ± 0.04 b
GRO	0.23 ± 0.01 b	0.21 ± 0.01 b	6.91 ± 2.04 ab	5.05 ± 0.03 b	3.94 ± 0.02 b

Table 2. Germination percentage (grp, %) and mean germination time (mgt, days) of seeds collected in Caffarella (CAF), Castel Fusano (FUS) and Grottaferrata (GRO) from trial 1 (not stratified seeds). Seeds were tested at three temperatures (15-6 °C, 20-10 °C, 25-15 °C) and two light conditions (alternating light/dark: L/D, dark: D). The absence of mgt values is caused by a corresponding grp = 0 %. In cases where "NA" (Not Available) standard error occurs, at least one seed has germinated in only one of the four replicates, so it was not possible to calculate the standard error. Different letters indicate significant differences ($p < 0.05$; three-way ANOVA followed by post-hoc Tukey tests).

Trial 1

	grp (%)		mgt (days)	
15-6 °C	**L/D**	**D**	**L/D**	**D**
CAF	3 ± 1.00 a	0 a	42.33 ± 2.33 ab	-
FUS	4 ± 1.63 a	0 a	41.17 ± 2.92 ab	-
GRO	4 ± 0.00 a	2 ± 1.16 a	44.75 ± 3.97 ab	48.00 ± 12.00 b
20-10 °C				
CAF	3 ± 1.92 a	0 a	18.00 ± 3.00 ab	-
FUS	0 a	1 ± 1.00 a	-	14.00 ± NA a
GRO	4 ± 1.63 a	1 ± 1.00 a	21.00 ± 3.51 ab	18.00 ± NA ab
25-15 °C				
CAF	4 ± 2.31 a	2 ± 1.16 a	16.00 ± 9.50 a	28.00 ± 6.00 ab
FUS	5 ± 3.79 a	1 ± 1.00 a	15.25 ± 2.25 a	47.00 ± NA ab
GRO	0 a	0 a	-	-

Table 3. Germination percentage (grp, %) and mean germination time (mgt, days) of seeds collected in Caffarella (CAF), Castel Fusano (FUS) and Grottaferrata (GRO) from trial 2 (seeds cold stratified for three months). Seeds were tested at three temperatures (15-6 °C, 20-10 °C, 25-15 °C) and two light conditions (alternating light/dark: L/D, dark: D). The absence of mgt values is caused by a corresponding grp = 0 %. Different letters indicate significant differences (p < 0.05; three-way ANOVA followed by post-hoc Tukey tests).

Trial 2

	grp (%)		**mgt (days)**	
15-6 °C	**L/D**	**D**	**L/D**	**D**
CAF	0 a	0 a	-	-
FUS	0 a	0 a	-	-
GRO	0 a	0 a	-	-
20-10 °C				
CAF	3 ± 1.00 ab	18 ± 4.16 c	34.33 ± 2.67 a	37.93 ± 5.47 a
FUS	4 ± 1.63 ab	4 ± 1.63 ab	44.00 ± 8.02 a	27.33 ± 6.44 a
GRO	14 ± 3.83 bc	16 ± 3.27 c	42.60 ± 1.85 a	36.65 ± 2.69 a
25-15 °C				
CAF	10 ± 4.76 abc	18 ± 2.58 c	31.83 ± 7.37 a	30.83 ± 3.70 a
FUS	4 ± 2.83 ab	9 ± 2.52 abc	26.83 ± 3.83 a	27.00 ± 3.05 a
GRO	3 ± 1.00 ab	10 ± 1.16 abc	29.00 ± 1.73 a	28.38 ± 2.73 a

Table 4. Germination percentage (grp, %) and mean germination time (mgt, days) of seeds collected in Caffarella (CAF), Castel Fusano (FUS) and Grottaferrata (GRO) from trial 3 (seeds cold stratified for six months). Seeds were tested at three temperatures (15-6 °C, 20-10 °C, 25-15 °C) and two light conditions (alternating light/dark: L/D, dark: D). Different letters indicate significant differences (p < 0.05; three-way ANOVA followed by post-hoc Tukey tests).

Trial 3

	grp (%)		**mgt (days)**	
15-6 °C	**L/D**	**D**	**L/D**	**D**
CAF	57 ± 5.26 c	55 ± 3.00 c	38.75 ± 1.11 cdef	36.28 ± 0.80 cde
FUS	15 ± 3.42 ab	19 ± 5.26 ab	40.52 ± 1.81 def	35.89 ± 1.75 bcde
GRO	19 ± 4.44 ab	31 ± 5.75 b	44.58 ± 1.60 ef	43.57 ± 3.59 ef
20-10 °C				
CAF	6 ± 1.16 a	18 ± 2.58 ab	39.13 ± 3.62 cdef	31.48 ± 2.57 abcde
FUS	4 ± 2.82 a	8 ± 1.63 a	53.67 ± 0.33 f	43.88 ± 5.04 ef
GRO	9 ± 5.26 a	14 ± 3.46 ab	39.33 ± 4.17 cdef	33.25 ± 3.51 abcde
25-15 °C				
CAF	15 ± 4.12 ab	14 ± 3.83 ab	22.02 ± 2.23 ab	21.53 ± 1.82 a
FUS	4 ± 2.83 a	5 ± 3.79 a	25.50 ± 3.50 abcd	23.13 ± 1.13 abc
GRO	7 ± 4.73 a	15 ± 4.44 ab	29.05 ± 0.55 abcde	36.60 ± 2.14 cdef

Mean germination time values showed a greater variability. However, the analysis didn't recognize a strong significance in the differences between mgt values. The model highlighted the effect of temperature on the average germination time, suggesting the presence of longer germination times for the lowest temperature (15-6°C) and lower for the higher temperatures (20-10°C and 25-15°C): high temperatures, therefore, seem to accelerate the germination times of *A. altissima* seeds; however, the differences were not significant after pairwise comparisons (Tab. 2). At 15-6°C mgt was more than 40 days for all sites, while at 20-10°C and 25-15°C mgt almost never exceeded 30 days. Furthermore, even if not significant, a notable difference can be observed at 25-15°C between L/D and D for CAF (L/D: 16.00 ± 9.50 days; D: 28.00 ± 6.00 days) and FUS (L/D: 15.25 ± 2.25 days; D: 47.00 ± NA days).

Trial 2: germinability after three months of stratification

For grp, the three-way ANOVA highlighted a greater distinction between the temperature of 15-6°C and those of 20-10°C and 25-15°C: at the lowest temperature no germination occurred (Tab. 3). A significant difference in D occurred between the percentage observed for CAF at 15-6°C (0 ± 0.00 %) and those observed at 20-10°C (18 ± 4.16 %) and 25- 15°C (18 ± 2.58%). For GRO there was a significant difference both in L/D and D between seeds incubated at 15-6°C (L/D: 0 ± 0.00%; D: 0 ± 0.00 %) and those incubated at 20-10°C (L/D: 14 ± 3.83 %; D: 16 ± 3.27 %). FUS, on the other hand, didn't show significant variations in grp between different temperatures. The only significant difference between sites was observed at 20-10°C in D: GRO and CAF had a similar behavior (GRO:

16 ± 3.27%; CAF: 18 ± 4.16%), while FUS maintained a lower grp (4 ± 1.63%). The only statistically significant difference between L/D and D occurred for CAF at 20-10°C where in D (18 ± 4.16 %) seeds germinated 6 times more than in L/D (3 ± 1.00 %). Mgt values were all similar and not significantly different to each other: neither temperature nor site of collection nor light conditions affect the mean germination time (Tab. 3).

Trial 3: germinability after six months of stratification

Table 5. Germination percentage (grp, %) for every combination of temperature (15-6 °C, 20-10 °C, 25-15 °C), light (alternating light/dark: L/D, dark: D), site of collection (Caffarella: CAF, Castel Fusano: FUS, Grottaferrata: GRO) and pre-treatment (trial 1: not pre-treated seeds, trial 2: three months of cold stratification, trial 3: six months of cold stratification). Different letters indicate significant differences ($p < 0.05$; four-way ANOVA followed by post-hoc Tukey tests).

grp (%)

	15-6 °C		**20-10 °C**		**25-15 °C**	
	L/D	**D**	**L/D**	**D**	**L/D**	**D**
Trial 1						
CAF	3 ± 1.00 cde	0 e	3 ± 1.92 cde	0 e	4 ± 2.31 cde	2 ± 1.16 cde
FUS	4 ± 1.63 bcde	0 e	0 e	1 ± 1.00 de	5 ± 3.79 cde	1 ± 1.00 de
GRO	4 ± 0.00 bcde	2 ± 1.16 cde	4 ± 1.63 bcde	1 ± 1.00 de	0 e	0 e
Trial 2						
CAF	0 e	0 e	3 ± 1.00 cde	18 ± 4.16 abc	10± 4.76 abcd	18 ± 2.58 abc
FUS	0 e	0 e	4 ± 1.63 bcde	4 ± 1.63 bcde	4 ± 2.83 cde	9 ± 2.52 abcd
GRO	0 e	0 e	14 ± 3.83 abc	16 ± 3.27 abc	3 ± 1.00 cde	10± 1.16 abcd
Trial 3						
CAF	57 ± 5.26 a	55 ± 3.00 a	6 ± 1.16 bcde	18 ± 2.58 abc	15 ± 4.12 abc	14 ± 3.83 abc
FUS	15 ± 3.42 abc	19 ± 5.26 abc	4 ± 2.82 cde	8 ± 1.63 abcd	4 ± 2.83 cde	5 ± 3.79 cde
GRO	19 ± 4.44 abc	31 ± 5.75 ab	9 ± 5.26 bcde	14 ± 3.46 abc	7 ± 4.73 bcde	15 ± 4.44 abc

The main difference which clearly emerges compared to the previous trials concerns the temperature of 15-6°C, at which seeds achieved the highest germination rates among all the tests performed, for all sites of collection. However, only for CAF (L/D: 57 ± 5.26%; D: 55 ± 3.00%) grp values were significantly higher than those measured at higher temperatures, in both light conditions (Tab. 4). CAF grp values were also significantly greater than GRO and FUS ones. For temperatures of 20-10°C and 25-15°C the three-way ANOVA didn't recognize differences between stations, temperatures, and light conditions. Mgt was significantly lower for seeds incubated at higher temperatures (Tab. 4): in L/D, CAF had a smaller mgt at 25- 15°C (22.02 ± 2.23 days) compared to the other temperature ranges (20-10°C: 39.13 ± 3.62 days; 15-6°C: 38.75 ± 1.11 days). Instead, in D at 25-15°C (21.53 ± 1.82 days) mgt was significantly different only with respect to 15-6°C (36.28 ± 0.80 days). For FUS, at 25-15°C (L/D: 25.50 ± 3.50 days; D: 23.13 ± 1.13 days) mgt values were only significantly lower than those observed at 20-10°C (L/D: 53.67 ± 0.33 days; D: 43.88 ± 5.04 days), for both light conditions. No significant difference between temperatures was observed for GRO. In addition, at 25-15°C and in D, CAF had a lower mgt than GRO (36.60 ± 2.14 days).

Effect of cold stratification on germinability

The four-way ANOVA on all germination tests highlighted a mild effect of stratification on the interruption of seed dormancy. Not stratified seeds showed the lowest grp, while seeds stratified for six months showed the highest grp (Tab. 5). Only in D, three months of stratification caused a significant increase in grp, compared to non-stratified seeds, at incubation temperatures of 20-10 °C and 25-15 °C (Tab. 5). The same stratification did not cause an increase in grp at low temperatures (15-6 °C), making a longer pre-treatment duration necessary. At the lowest temperature, six months of stratification resulted in a significant and particularly large increase of grp, especially in D, while the increase of grp wasn't significant at 20-10 °C and 25-15 °C compared to three months of stratification (Tab. 5).

Thus, the effect of cold stratification on grp was more evident when seeds were incubated at the lowest temperature range, and in complete darkness. Cold stratification didn't influence the timing of germination: mgt values were nearly always not significantly different (Tab. 6).

Trial 4: overwintering germinability of seeds retained on the plant

Statistical analyses highlighted the non-significance of the period of seed collection (Tab. 7); therefore,

Table 6. Mean germination time (mgt, days) for every combination of temperature (15-6 °C, 20-10 °C, 25-15 °C), light (alternating light/dark: L/D, dark: D), site of collection (Caffarella: CAF, Castel Fusano: FUS, Grottaferrata: GRO) and pre-treatment (trial 1: not pre-treated seeds, trial 2: three months of cold stratification, trial 3: six months of cold stratification). The absence of values is caused by a corresponding grp = 0 %. In case "NA" (Not Available) standard error occurs, seeds germinated in only one of the four replicates, so it was not possible to calculate the standard error. Different letters indicate significant differences ($p < 0.05$; four-way ANOVA followed by post-hoc Tukey tests).

mgt (days)

	15-6°C		**20-10°C**		**25-15°C**	
	L/D	**D**	**L/D**	**D**	**L/D**	**D**
Trial 1						
CAF	42.33 ± 2.33 abc	-	18.00 ± 3.00 a	-	16.00 ± 9.50 a	28.00 ± 6.00 ab
FUS	41.17 ± 2.92 abc	-	-	14.00 ± NA a	15.25 ± 2.25 a	47.00 ± NA abc
GRO	44.75 ± 3.97 abc	48.00 ± 12.00 bc	21.00 ± 3.51 a	18.00 ± NA a	-	-
Trial 2						
CAF	-	-	34.33 ± 2.67 abc	37.93 ± 5.47 abc	31.83 ± 7.37 abc	30.83 ± 3.70 abc
FUS	-	-	44.00 ± 8.02 abc	27.33 ± 6.44 ab	26.83 ± 3.83 ab	27.00 ± 3.05 ab
GRO	-	-	42.60 ± 1.85 abc	36.65 ± 2.69 abc	29.00 ± 1.73 abc	28.38 ± 2.73 ab
Trial 3						
CAF	38.75 ± 1.11 abc	36.28 ± 0.80 abc	39.13 ± 3.62 abc	31.48 ± 2.57 abc	22.02 ± 2.23 a	21.53 ± 1.82 a
FUS	40.52 ± 1.81 abc	35.89 ± 1.75 abc	53.67 ± 0.33 c	43.88 ± 5.04 abc	25.50 ± 3.50 ab	23.13 ± 1.13 ab
GRO	44.58 ± 1.60 abc	43.57 ± 3.59 abc	39.33 ± 4.17 abc	33.25 ± 3.51 abc	29.05 ± 0.55 abc	36.60 ± 2.14 abc

Table 7. Germination percentage (grp, %) and mean germination time (mgt, days) of seeds collected in Caffarella in December (CAF0) and March (CAFw) from trial 4 (seeds retained on the plant overwinter). Seeds were tested at three temperatures (15-6 °C, 20-10 °C, 25-15 °C) and two light conditions (alternating light/dark: L/D, dark: D). The absence of mgt values is caused by a corresponding grp = 0 %. In cases where "NA" (Not Available) standard error occurs, at least one seed has germinated in only one of the four replicates, so it was not possible to calculate the standard error. Different letters indicate significant differences ($p < 0.05$; three-way ANOVA followed by post-hoc Tukey tests).

Trial 4

	grp (%)		**mgt (days)**	
15-6 °C	**L/D**	**D**	**L/D**	**D**
CAF0	3 ± 1,00 ab	0 a	42,33 ± 2,33 bc	-
CAFw	1 ± 1,00 a	0 a	53,00 ± NA c	-
20-10 °C				
CAF0	3 ± 1,92 ab	0 a	18,00 ± 3,00 ab	-
CAFw	15 ± 4,73 b	2 ± 2,00 a	40,33 ± 3,89 abc	41,00 ± NA abc
25-15 °C				
CAF0	4 ± 2,31 ab	2 ± 1,16 ab	16,00 ± 9,50 a	28,00 ± 6,00 abc
CAFw	15 ± 4,12 b	13 ± 1,92 b	44,81 ± 4,90 c	29,52 ± 1,04 abc

overwinter permanence of seeds on the tree didn't affect germination rates. However, at higher temperatures an increase of grp was observed between CAF0 and CAFw seeds. The model highlighted the effect of temperature and light on germination rates. At 20-10°C, a significant difference of grp emerged for CAFw between D (2 ± 2.00 %) and L/D (15 ± 4.73 %). For CAFw, in L/D there were significantly higher percentages at 20-10 °C and 25-15 °C compared to those obtained at 15-6 °C. In D instead, 25-15 °C generated significantly higher percentages than those obtained both at 20-10 °C and 15-6 °C. The effect of the collection period on mgt could be observed at 25-15°C in L/D, conditions under which CAF0 seeds (16.00 ± 9.50 days) germinated more rapidly than CAFw ones (44.81 ± 4.90 days) (Tab. 7). In the other conditions no significant differences between CAF0 and CAFw were observed. For CAFw seeds no effect of temperature and light emerged on mgt values.

Discussion

Germination can be considered as part of the invasiveness mechanism (Richardson et al., 2000). Reproductive traits such as abundant seed production, rapid germination, and the ability to germinate under a wide range of environmental conditions characterize many invasive species (Pyšek & Richardson, 2008). Since germination is a critical phase in the life cycle of a plant and plays an important role in seedling establishment, seed characteristics and the adaptation of their germination to the environment are fundamental determinants for the invasion success of an alien plant (Wen, 2015). In order to evaluate the germinative response of many species, it is necessary to break the dormancy of their seeds (Finch-Savage & Leubner-Metzger, 2006). Nevertheless, cold stratification had only a mild effect on seed germinability. Soler & Izquierdo (2024) pointed out that several intrinsic factors could play a crucial role in *A. altissima* seed dormancy, since environmental requirements for its seeds to germinate have been shown to be greatly variable. In the present study the effect of stratification could be observed only under complete darkness. An interaction between stratification and light was also found in the study by Milberg & Andersson (1998) in which, following stratification of seeds of 33 annual species, the response to light changed for 21 of them. They also highlighted the possibility of an interaction between population and stratification for some species. The present study seems to reveal a population-stratification-temperature interaction, as the effect of stratification was different at different temperatures for each site of collection: for FUS stratification had a significant effect on increasing germination percentages only at 15-6 °C, for CAF the effect was significant at 15-6 °C and 20-10 °C, and for GRO it was significant at all temperatures. Seeds collected at higher altitude therefore appear to be more susceptible to the effect of cold stratification, while those collected at the lowest altitude respond to the same pre-treatment only at the lowest germination temperature. This pattern reveals the existence of a gradient from the coast to the inland, possibly indicating an ecotype differentiation. Although *A. altissima* tolerates a wide range of climatic conditions, it is in fact most common and abundant in temperate climatic zones (Knapp & Canham, 2000; Kowarik & Säumel 2007; Clark et al., 2014). In a more typical Mediterranean climate, as that of Italian Tyrrhenian coasts, it thrives less well, thus resulting in a lower germinative response to higher temperatures after cold stratification.

For nearly all conditions, instead, no significant differences were observed in the germination capability among seeds collected from different sites. The average germination time was also not found to be influenced by the site of collection. The germinative characteristics of *A. altissima* seeds seem to be independent of the altitude at which they are produced. In other words, the environmental characteristics in which the mother plant is found may not have an influence on the germinative characteristics of the seeds it produces. This demonstrates that the morphological variability of *A. altissima* seeds observed in the measurements of A, a and SFM may not have an adaptive value to the different environmental conditions in which the seeds are produced, but would rather be the result of inter- and intra-individual variability, as well as of several ecological and genetic factors (Vaughton and Ramsey, 1997; Alonso-Blanco et al. 1999; Halpern, 2005). The absence of an effect of the site of seed production on their germination dynamics may contribute to the high invasive potential of the tree of heaven, as it demonstrates how the species can easily adapt to a variety of environmental conditions without consequences on the early stages of its development. The lower abundance of invasive species at higher elevations (Medvecká et al., 2014; Beniak et al., 2015) may not necessarily reside in their lower adaptability to such environments: it could also be due to a time-lag effect, related to invasion dynamics or to less intense human activities present at higher elevations (McDougall et al., 2011). This could be true for *A. altissima*, which is a typically urban species (Miller, 2003). Urban environments are also characterized by the phenomenon of the heat island, which produces higher temperatures in cities than in surrounding areas (Santamouris, 2007; Godefroid & Ricotta, 2018). However, although *A. altissima* is considered a thermophilic species (Sudnik-Wójcikowska, 1998), no clear preference for higher temperatures has been found during the germination process in the present study.

In fact, the incubation of seeds at different temperatures has highlighted contrasting relationships between temperature and germination capacity, depending on the type of pre-treatment undergone by seeds (Soler & Izquierdo, 2024). In the absence of stratification, temperature had no effect on germination. Seeds stratified for three months showed, for GRO and CAF, an increase in germination percentages at a temperature of 20-10°C, indicating a partial contribution of intermediate temperatures in determining higher germination rates. CAFw seeds showed a significant increase in germination percentages at higher temperatures (at 20-10°C in L/D and at 25-15°C in both L/D and D): for these seeds, germination seems to be favored by higher temperatures (Little, 1974; Kheloufi et al., 2020). Since overwintering seeds are located above the tree canopy and thus unable to germinate during the cold season, displaying higher germination percentages at higher temperatures could be an adaptation to the period when they are dispersed. For seeds stratified for six months, however, lower temperatures generated the highest germination percentages, although this increase was significant only for CAF. This last observation agrees with that of Constán-Nava & Bonet (2012) who found, for seeds incubated in growth chambers, higher germination percentages at 15°C and lower at 30°C. Higher temperatures can in fact determine a greater demand for oxygen by the embryo, and a lack of oxygen could induce secondary or induced dormancy (Hilhorst, 2007). These results demonstrate that temperature is a secondary control factor of the germination of *A. altissima* seeds. The main limiting factor could indeed be water: in the experiments, seeds were not restricted in water availability. The field experiment by Constán-Nava & Bonet (2012) showed that seedling emergence is related to rainfall rather than temperature, while Sevik & Cetin (2015) demonstrated in a laboratory study that *A. altissima* seeds are particularly sensitive to water stress, which affects their germination rates. The absence of a clear trend in germinations in relation to temperature is an indication of the high invasiveness of this species, which is not limited in the initial stages of its development by the temperature factor and thus germinates independently of the environment in which it is found. Nevertheless, as stated above, pre-treatment could highlight a coast-inland gradient: after undergoing cold stratification, seeds from inlands (CAF and GRO) enhance their germination in a wider range of temperatures, while seeds from coastal areas (FUS) are less susceptible to this kind of pre-treatment, increasing their germination rates only at the lowest temperature and resulting in being less sensitive to cold stratification. Germination timing was almost never affected by temperature, in agreement with Kheloufi et al. (2020). A significant effect was observed when the higher temperature of 25-15 °C accelerated the germination of seeds from CAF and FUS stratified for six months, both in L/D and D. Following longer cold stratification, higher temperatures seem to favor faster mgt.

As for temperature, light didn't affect seeds germination, resulting in no differences between L/D and D conditions. Kota et al. (2007) obtained higher germination rates in laboratory conditions with lower light conditions, while in the field germination increased with increasing light. These contrasting trends indicate that the amount of light is not the main regulator of *A. altissima* germination. The heliophilous nature of this species would not depend on its germination, but on subsequent phases: *A. altissima* is in fact a ruderal pioneer species characterized by efficient photosynthesis in open sites (Kowarik and Säumel, 2007). Given that light does not appear to be a limiting factor for germination, the seed's depth in the soil would not influence the species' germination capacity.

Regardless of the different conditions seeds were undergone, the germination tests carried out in the present study produced low germination percentages, almost always less than 20%. The overall low germination rates obtained, in agreement with other studies (Kota et al., 2007; Constán-Nava & Bonet, 2012; Cabra-Rivas & Castro-Diez, 2016), contrast with the high invasiveness of *A. altissima* (Kowarik & Säumel, 2007). Indeed, numerous studies emphasize the importance of vegetative propagation in the invasion mechanism of *A. altissima* (Bory et al., 1991; Vilà et al., 2006; Kowarik & Säumel, 2007). Seeds are the main mechanism by which this species reaches new habitats, but then the root system contributes to the rapid increase in the tree density and the subsequent expansion of the population nearby, forming populations of scattered clones (Soler & Izquierdo, 2024). However, this species has a high fecundity: a single individual can in fact produce up to a million samaras every year (Wickert et al., 2017). *A. altissima*, in addition to spreading vegetatively, would rely on the large quantity of seeds produced rather than their quality (viability and germinability) in colonizing new areas for its reproductive success. Thus, low germination rates would be offset by a high seed production. According to Rejmànek & Richardson (1996), invasive species are often characterized by a reproductive r-strategy. These organisms invest many resources into reproduction but provide a minimal amount of resources to each offspring, thereby obtaining the highest possible number of progenies (Pianka, 1970) and ensuring the reproductive success of the species. Among plant species, r-strategists are often pioneer or early successional species (Pianka, 1970; Rejmànek & Richardson, 1996), and since most invasions occur in disturbed habitats it is not surprising that invasive species are r-strategists (Whitmore, 1991; Rejmànek & Richardson, 1996). *A. altissima* would therefore adopt such strategy, relying on an extremely high number of seeds rather than their vitality for its reproductive success.

The efficiency of *A. altissima* reproduction through seeds is not only due to the large number of seeds produced, though. In fact, this species seems to implement an additional reproductive strategy aimed at maximizing the probability of success of young seedlings. *A. altissima* exhibits a large variability in samaras' dispersal period. Some trees completely lose their fruits by mid-winter, while others keep samaras on their branches until spring and in some cases even until the following autumn. Germination tests have shown that permanence on tree during winter doesn't determine an increase in seeds germination rates. However, grp obtained are still equal to or greater than those produced by non-stratified seeds, demonstrating that these seeds maintain their viability. The enormous amount of seeds produced allows this species to keep a portion of them on the tree: a "reservoir effect" that results in a delayed release of samaras over time. Seeds that remain on the plant constitute a genetic reservoir of potentially reproductive material, which is gradually released throughout the year. Worldwide, over 1200 species employ the strategy of storing seeds on their mother plants, forming what is known as an aerial seed bank (Silvestro et al., 2020). This phenomenon, referred to as serotiny, has usually been associated with extreme environments as a strategy to reduce the risk of offspring mortality and await better conditions for seedling establishment (Lamont & Enright, 2000; Bastida et al., 2010; Saracino et al., 2017; Silvestro et al., 2020). Furthermore, the prolonged emergence of seedlings resulting from gradual seed release can avoid intense competition among seedlings and act as a mechanism for plant population maintenance in the face of unpredictable environments (Günster, 1994; Gao et al., 2014). For *A. altissima*, the gradual and continuous dispersal of samaras may contribute to its success as an invasive species: the continuous seed release allows the generation of new individuals in different conditions of temperature and water availability, maximizing the chances of success for a greater number of seedlings throughout the year.

In addition to an aerial seed bank, *A. altissima* may also constitute a soil seed bank, a deposit of viable seeds present on or in the soil, which acts as a reserve of propagules and genetic diversity for many species (Gioria et al., 2019). For every site of collection, seeds' internal humidity was less than 15 %, allowing to classify *Ailanthus* seeds as orthodox and possibly favoring their ability to constitute a soil seed bank. The formation of soil seed banks is a common strategy in plants that grow in environments subject to disturbance, such as invasive ones (Miller, 2000; Bacchetta et al., 2006). Since different studies report the ability of *A. altissima* seeds to germinate even in the years following their production (Kota et al., 2007; Cabra-Rivas & Castro-Dìez, 2016; Wickert et al., 2017; Rebbeck & Jolliff, 2018), the formation of a soil seed bank may thus contribute to the invasive potential of this species.

CONCLUSIONS AND FUTURE PERSPECTIVES

This study provides evidence that seeds of the invasive species *A. altissima* are capable of germinating under a wide range of conditions, showing no preferences for temperature, light or altitude at which they develop. These findings align with previous studies by Kota et al. (2007) and Moore & Lacey (2009), who found that the germination rate of *A. altissima* is not influenced by the habitat type in which its seeds germinate. This characteristic makes *A. altissima* highly adaptable to different environments without being constrained by specific conditions. It suggests that the anticipated temperature increase associated with climate change may not affect the germination capability of this species. The presence of a coast-inland gradient in response to cold stratification may indicate, however, that seeds produced in coastal areas wouldn't perform as effectively at high temperatures as those produced in more temperate-like areas, leading to a possible ecotype differentiation. Adaptability is typical of many invasive species, which are often capable of germinating in a wide range of environmental conditions (Pyšek & Richardson, 2008). It is therefore possible that the nature of pioneer species of *A. altissima* emerges already during its reproduction and germination, a crucial phase in a plant's life cycle, both in its tolerance to different environmental conditions and in the adoption of a reproduction strategy oriented towards the extreme of r-strategist species. The continuous release of numerous seeds throughout the year may maximize the chances of a successful germination. Unlike many pioneer species, however, germination of *A. altissima* is not limited by light, contributing even more significantly to its adaptability and therefore to its invasiveness. Nevertheless, the potential impact of climate change on water availability should be more deeply investigated, as it appears to be a limiting factor for the germination process of this species. Understanding the germination capabilities and environmental requirements of invasive species like *A. altissima* is crucial for developing effective and targeted management strategies, in order to mitigate their spread and impacts.

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