Effect of temperature and salinity on germination and seedling establishment of *Ailanthus altissima* (Mill.) Swingle (*Simaroubaceae*)

Abdenour Kheloufi✉, Lahouaria Mounia Mansouri, Rawnak Zerrouni, Okba Abdelhamid

Faculty of Natural and Life Sciences, Department of Ecology and Environment, University of Batna2, Batna 05000, Algeria

✉ abdenour.kheloufi@yahoo.fr

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Abstract

The tree of heaven *Ailanthus altissima* (Mill.) Swingle is a multipurpose tree in forestry. However, it is considered an invasive and dangerous plant for native species, and in particular for national parks, where many studies have recorded their involvement in the disturbance of the already developed floral diversity. Assessing the impact of certain abiotic conditions on this species may identify the expected areas to be colonized by its seed propagation. Germination of tree of heaven were tested for germination at constant temperatures of 25, 30, 40°C, and at room temperature varying from 25-30°C coupled with total darkness. Seeds were sown in Petri dishes (0.8% agar water) for 6 days of incubation. The kinetic of germination was determined according to five closely related parameters *viz.* final germination percentage (FGP), mean germination time (MGT), coefficient of velocity of germination (CVG), time to 50% germination (T50) and seedling length (SL). For the saline condition, the seeds underwent various NaCl concentrations from 0, 50, 100 or 200 mM. For each treatment, there were four replicates with 50 seeds incubated in a plastic container between two layers of moist sand at 15% of the appropriate treatment and then placed in a culture chamber at 27°C (± 2°C) for 30-day period. The effect of temperature was not significant on the MGT, CVG and T50. However, it was significant ($p < 0.0001$) on FGP and SL. The maximum germination of 94% was obtained at a temperature of 30°C and the lowest FGP of 40% was obtained at 25°C. For the salinity effect, the FGP of 75% in the control was much higher compared to the seeds treated at 50 mM NaCl with only 17.2% of FGP. Germination was completely inhibited from 100 mM NaCl. *A. altissima* can be classified as sensitive to salt stress during seed germination and seedling emergence. The salinity effect then joined the temperature to monitor nature's *A. altissima* seed propagation.

Keywords

Forestry; Invasive; NaCl; Tree of heaven; *Simaroubaceae*; Seed behaviour

Contents

1 Introduction

Seed germination is of great importance for plant regeneration, whether natural or artificial. However, several environmental factors, such as temperature, salinity, light, soil moisture, oxygen concentration and pH, often limit the germination stage (Kigel 2017). It is also affected by the temperature regimes that directly influence the rate of water absorption and the biochemical reactions that play a decisive role in germination processes (Vertucci and Farrant 2017). At the other side, salinity contributes to a plant water deficit due to osmotic stress, potentially associated with biochemical disruptions induced by sodium ion influx (Zivcak et al. 2016). The degree of salinity response depends on the salt concentration, the species itself, its variety and the stage of the plant's development (van Zelm et al. 2020). However, plant tolerance to salinity is not a fixed trait, and often varies with plant growth (Kheloufi et al. 2019).

Ailanthus altissima (Miller) Swingle, commonly known as the tree of heaven, is a deciduous tree of the family of Simarubaceae, up to 30 m height (Mouillefert 1898). This species is native to southern China (Hu 1979). It is one of the most distributed hardwood species that prefers disturbed urban and rural areas such as agricultural land and transportation corridors, especially abandoned parcels, meadows, vineyards or cracked town sidewalks (Filipou et al. 2014; Sladonja et Poljuha 2018). *A. altissima* rises fast and reaches 18-21 m in 10 years and it is slightly thermophilic (Rebbeck and Jolliff 2018). Tree of heaven grows rapidly in environments where other trees do not survive. The first years, this tree is sensitive to cold. It was noted, however, that 6-year-old plants could withstand severe cold, down to -33°C (Hoshovsky 1988). Stevens et al. (2018) showed that tree of heaven seedlings from North America are uniformly and negatively affected by drought stress.

The development of stands in *A. altissima* is based on a double strategy of reproduction: sowing and sucking. This tree is capable of producing viable seeds from 3 to 5 years old and from 14 000 to 325 000 per tree in very huge quantities (Clair-Maczulajtys 1985). Besides its fast growth, *A. altissima* has many features that are often associated with highly invasive weed species (Landenberger et al. 2007). *A. altissima's* aggressive behaviour can be due to physiological features and allelopathic compounds in the roots, leaves and wood (Filippou et al. 2014). This species can however be used as a source of active compounds such as aileranthone (quassinoids) which is an important phytotoxic component that can potentially be used as a broad-spectrum herbicide (Heisey and Heisey 2003). Indeed, recent studies have shown that quassinoids can be used for the production of firewood, paper, environmental remediation, bee pasture and slope erosion control (Sladonja et al. 2015). Graves (1990) reported that tree of heaven seeds germinate without stratification. However, seeds have dormant embryos and require a 30 to 60-day cold stratification before germination (Little 1973; Dirr et al. 1987).

This research aimed to evaluate the germination of *Ailanthus altissima* seeds under various temperature and saline conditions to contribute nursery practices, and seed science and technology, and also future studies in the species.

2 Material and methods

2.1 Collection and seed characteristics

The seeds of *A. altissima* used in the present experiment were obtained from freshly mature samaras (fruits) harvested on November 2019 from 10 different trees (approximately the same size of height: 2.5 m) growing at 1025 meters of altitude in an area located in the semi-arid region of Batna (Algeria) (Latitude: 35°34'13.39"N; Longitude: 6°10'56.47"E). In this study, we have retained the average annual temperature (15.8°C), average annual maximum temperature (23.2°C), average annual minimum temperature (8.0°C), total annual precipitation and total rainy days of 67 days during the year 2019 (WCD Tutiempo 2020).

The seed sample was obtained by fruit mixing to reduce intergenetic variability (Kheloufi et al. 2018). Samples were properly selected, identified, photographed and then stored in a glass bottle at 4°C for a month (Graves 1990) (Figure 1A). After manual decortication, seeds were reserved for germination test. Fruit and seed morphological characteristics are shown on Table 1.

Table 1. Morphological characteristics of fruits and seeds of *Ailanthus altissima* (n=100).

	Samara (Fruit)	Seed
Length (cm)	4.17 ± 0.17	0.43 ± 0.03
Width (cm)	0.97 ± 0.04	0.35 ± 0.02
1000-sample weight (g)	33.7	13.2

2.2 Temperature effect on seed germination in Petri dishes

The research experiment was carried out at the Laboratory of the Department of Ecology and Environment, University of Batna 2, Algeria. The experiment was performed in January 2020 at three constant temperatures of 25, 30, 40°C, and at room temperature varying from 25-30°C. To determine the effect of temperature during seed germination of *A. altissima*, a total of four replicates of 25 seeds were disinfected with 1% sodium hypochlorite for one minute, rinsed with distilled water and immediately sown on 0.8% (water agar) in 9 cm Petri dishes under aseptic conditions (Figure 1B). Seeds were incubated simultaneously for 6 days in the dark under three continuous temperature regimes (25, 30 and 40°C) and room temperature (25-30°C). Seed germination was assessed under dark conditions because we were only interested in studying the impact of temperature on seed germination, ignoring the influence of other factors, such as light. Regular germination counts were performed to determine kinetics of germination. Seeds were considered germinating only when 2 mm radicles emerged.

Figure 1. Overview of fruits (samaras) and seeds of *Ailanthus altissima* (Mill.) Swingle (A) and experimental design in Petri dishes (B) and plastic containers (C).

The parameters evaluated were as follows:

$$\mathrm{FGP}\ (\%) = \frac{\sum ni}{N} \times 100,$$

where *FGP* is the final germination percentage, *ni* is the number of seeds germinated on the last day of testing, and *N* is the total number of seeds incubated per test (Côme 1970). However, FGP only reflects the final percentage of germination attained and provides no picture of the speed or uniformity of germination.

$$\mathrm{MGT}\ (\mathrm{days}) = \frac{\sum(ti.ni)}{\sum ni},$$

where *MGT* is the mean germination time, *ti* is the number of days since the start of the test, *ni* is the number of germinated seeds recorded at time *ti*, and *Σni* is the total number of germinated seeds (Orchard 1977).

$$\mathrm{CVG}\ (\%) = \frac{\sum \mathrm{Ni}}{\sum NiTi}\ \mathrm{x}\ 100,$$

where *CVG* is the coefficient of velocity of germination, *Ni* is the number of seeds germinated each day, *Ti* is the number of days from sowing corresponding to *N* (Jones and Sanders 1987). The coefficient of velocity of germination gives an indication of the rapidity of germination. It increases when the number of germinated seeds increases and the time required for germination decreases. Theoretically, the highest CVG possible is 100, which is the case if all seeds germinated on the first day (Jones and Sanders 1987).

$$\mathrm{T50}\ (\mathrm{days}) = t1 + \left(\frac{0.5 - \mathrm{n1}}{\sum NiTi}\right) \mathrm{x}\ 100$$

where *T50* (days) is the time up to 50% germination, *n1* is the cumulative percentage of germinated seeds which value is closest to 50% by a lower value, *n2* is the cumulative percentage of germinated seeds which value is the closer to 50% by higher value, *t1* is the time necessary for the germination of *n1* of seeds, *t2* is the time necessary for the germination of *n2* of seeds (Côme 1970).

At the end of the experimental period, the lengths of eight randomly selected seedlings per thermal condition were also reported using a digital caliper.

2.3 Effect of salinity on seed germination

For the effect of saline condition, the samaras underwent various concentration of NaCl from 0 (Control), 50, 100 or 200 mM. For each treatment, there were four replicates with 50 seeds incubated in a plastic container hermetically sealed (16 cm Length × 10 cm Height × 12 cm Width) between two layers (1 cm each) of moist sand at 15% for the appropriate NaCl solution, and then placed in a culture chamber at 27°C (± 2°C) (Figure 1C). In each saline treatment, the final germination rate (FGP) was expressed after 30 days as a percentage of the total number of seeds. Seeds were counted as germinated when the radicle growth reached 2 mm. The lengths of 10 seedlings per treatment have also been recorded using a digital caliper.

2.4 Statistical analysis

The results were expressed as mean ± standard deviation (SD). All the data were subjected to one-way analysis of variance (ANOVA) and Duncan's multiple range test ($P < 0.05$) using SAS Version 9.0 (Statistical Analysis System) (2002) software.

3 Results and discussion

3.1 Effect of temperature on seed germination

Based on the results of the analysis of variance, the effect of time, temperature and their correlation (time × temperature) was significant ($p < 0.0001$) on the kinetic of germination of *A. altissima*. Figure 2 indicates the differences in germination percentages under various temperature treatments according to time. *A. altissima* seeds were able to germinate in all temperature treatments; but the percentage of germination varied from treatment to another. The results shown in Figure 2 indicate that germination kinetics expressed three phases under all treatments: a first latency phase, expressed by seed imbibition; a second exponential phase, where germination is accelerated; and finally, a third phase, characterized by a stationary period, indicating germination interruption. For all temperatures, the first emergence of seedlings was observed on the second day of incubation except for the alternating temperature 25-30°C (room temperature) where germination began on the third day. The seeds under a 25°C treatment expressed a low percentage of germination with a very slow exponential process and that took longer to stabilize on the 5th day of the experiment (Figure 2).

The optimum temperature for *A. altissima* seed germination was observed at 30°C, reaching maximum germination on the 5th day (Figure 2). The seeds germinated at 40°C are reduced in germination and stabilized from the fourth day. Hilhorst (2018) stated that as the germination temperature increases, the embryo needs more oxygen, lack of oxygen can cause the embryo to go into secondary dormancy (induced dormancy). Respiration, reserves hydrolysis, and enzymatic activities remain temperature-dependent. Indeed, any variation in the incubation temperature can affect, in addition to the activity of certain enzymes, certain processes important for germination control, such as membrane permeability and cell wall extensibility (Bewley et al. 2012; Allen et al. 2018).

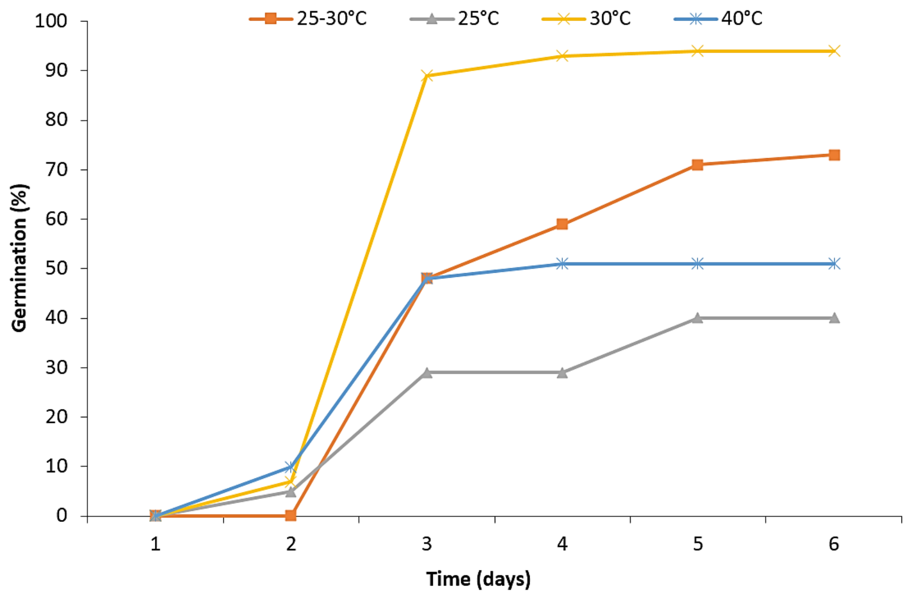

Figure 2. Temperature effect on seed germination of *Ailanthus altissima* for 6 days of incubation in Petri dishes (0.8% Agar water).

Seed germination is a physiological process in which the seed goes from slow to active state (Delouche 1980; Rao et al. 2017). It is characterized as the sum of the events leading to the germination of the dry seed, it begins with the crucial stage of seed absorption of water and ends with the elongation of the embryonic axis and the emergence of the radicle through the structures surrounding the embryo (Kheloufi 2017). Temperature is one of the variables that has a significant direct or indirect impact on germination rate, average germination period, coefficient of germination speed and length of seedlings. The temperature effect on the MGT (P=0.0925), CVG (P=0.0819), and T50 (P=0.0855) was not significant, showing only small differences (Table 2). The evolution of the seedlings' emergence over 6 days is shown in Figure 3.

Table 2. Final germination percentage (FGP), mean germination time (MGT), coefficient of velocity of germination (CVG), time to 50% germination (T50) (n=4) and seedling length (SL) (n=8) for *Ailanthus altissima* exposed to different temperature.

Temperature	FGP (%)	MGT (days)	CVG (%)	T_{50} (days)	SL (cm)
25-30 °C	73 ± 8.25^{b}	4.68 ± 0.03^{a}	21.4 ± 0.14^{b}	4.26 ± 0.03^{a}	2.89 ± 0.53^{c}
25 °C	40 ± 9.80^{c}	4.56 ± 0.28^{ab}	21.9 ± 1.30^{ab}	4.16 ± 0.35^{ab}	3.64 ± 0.88^{b}
30 °C	94 ± 4.00^{a}	4.48 ± 0.03^{ab}	22.3 ± 0.13^{ab}	3.99 ± 0.04^{ab}	7.71 ± 0.66^{a}
40 °C	51 ± 8.25^{c}	4.40 ± 0.06^{b}	22.7 ± 0.31^{a}	3.93 ± 0.06^{b}	0.27 ± 0.02^{d}
F of Fisher	37.10	2.70	2.85	2.80	506.49
P	<0.0001	0.0925	0.0819	0.0855	<0.0001

The same alphabet along the column indicates no significance difference (Duncan Multiple Range Test).

Figure 3. Overview of *Ailanthus altissima* seedling emergence during 6-day incubation period in Petri dishes at 30°C.

The analysis of the temperature effect on the percentage of germination showed, according to Table 2, that the maximum germination was achieved at a temperature of 30°C (94%) and the lowest FGP was obtained at 25°C (40%). Under the various temperature, seed germination rate was according to this order 30°C > 20-25°C > 40°C > 25°C (Figure 2, Table 2). (Figure 2, Table 2). The optimum temperature for most seeds to germinate is between 5°C and 30°C (Bellairs and Bell 1990).

The effect of temperature was significant on the SL parameter (P< 0.0001) (Table 2). The increase in temperature to 30°C was favourable for the seedlings emergence by recording an average value of 7.71 cm. This condition is important for the acquisition of good water hydration from the first hours of imbibition to trigger protease activity, which implies a degradation of the protein reserves accumulated in the cotyledons and ensure the root breakthrough growth (Bewley et al. 2013). Table 2 shows that the temperature of 40 °C resulted in seedlings not exceeding 0.3 cm despite a good percentage of germination that has been obtained from the first days. According to our observations at this temperature, the emergence of the seedlings underwent a very pronounced inhibition and delay after germination induction. The coefficients of germination velocities showed that the maximum velocity was found at 40°C (22.7%) which was not significantly different from that observed at 30°C (22.3%) where the seeds were almost fully germinated (Table 2).

3.2 Effect of salinity on seed germination

The results presented in Table 3 correspond to the average values of the final germination percentages and the seedlings lengths of *A. altissima* exposed to different NaCl concentrations (0, 50, 100 and 200 mM) after 30 days of incubation. These results showed that the impact of saline treatments on both FGP and SL (Table 3) was significant (P< 0.0001). The FGP in the control was much higher (75%) compared to the seeds treated at 50 mM NaCl with only 17.2% of FGP, which is actually a very low concentration. Ibrahim (2016) reported that salinity affects germination, facilitating the absorption of toxic ions, which can cause changes in seed enzymatic or hormonal activity.

Table 3. Final germination percentage (FGP) and seedling length (SL) of *Ailanthus altissima* exposed to various levels of salinity after 30 days of incubation.

Salinity treatments		FGP (%) (n=4)	SL (mm) (n=10)
0 mM (Control)	0 g.L^{-1} NaCl (Control)	75.0 ± 4.16^{a}	8.77 ± 1.23^{a}
50 mM	2.99 g.L^{-1} NaCl	17.2 ± 2.58^{b}	1.08 ± 0.79^{b}
100 mM	5.84 g.L^{-1} NaCl	0	0
200 mM	11.7 g.L^{-1} NaCl	0	0
Standard Error of the Mean (SEM)		31.9	3.75
P value		< 0.0001	< 0.0001

The same alphabet along the column indicates no significance difference (Duncan Multiple Range Test).

At very high concentrations, the effect of salt on germination can be an inhibitor or toxic (Kheloufi et al. 2016; Liang et al. 2018). Concentrations of 100 mM and 200 mM NaCl have completely inhibited seed germination (Table 3). These results can be explained by a high absorption of Na^+ and Cl^- ions during seed germination, resulting in an increase in osmotic potential which induces a cellular toxicity that inhibits or reduces germination rates (Carillo et al. 2011; Kheloufi et al. 2017). Other studies showed that high concentrations of salt cause complete germination inhibition and a depressive effect on germination rate, biological growth and seed development (Aslam et al. 2011).

The impact of salinity on the length of the seedling was also very harmful. Indeed, at a concentration of 50 mM, SL was considerably reduced with a length of 1.08 cm compared to 8.77 cm at 0 mM NaCl. This reduction is explained by the inhibition of water absorption induced by excessive Na^+ and Cl^- ion accumulation (Tavakkoli et al. 2010). According to Zhang et al. (2012), high salt concentrations, especially sodium chloride (NaCl), can inhibit seed enzymatic activity and delay the radicle's development and evolution. Seeds of halophytes are more salt tolerant than seeds of non-halophytes by remaining dormant until the substrate salt levels are reduced by rain water flushing the salt from the soil, improving germination conditions.

4 Conclusion

Like many species, seed germination of *Ailanthus altissima* are also affected by changes in temperature and salinity. The constant temperature of 30°C was the optimal temperature for the germination of *Ailanthus altissima* seeds with a percentage of 94% obtained in just 5 days of incubation. The effect of salinity on the germination of *A. altissima* has been very remarkable. Indeed, seeds were sensitive to 50 mM NaCl and germination was inhibited from 100 mM NaCl. A reversibility test of the salt or viability activity with tetrazolium chloride would be conclusive to confirm sodium chloride's toxic/osmotic effect on seeds which could not germinate. The salinity then joins the temperature to monitor the species' seed propagation. More work will be needed to determine the salt tolerance of *A. altissima* at other stages of growth. Furthermore, the use of *A. altissima* for reforestation in degraded lands as a fast-growing species could be very interesting but other researches must be carried out to control and avoid its invasion.

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