

Postprint on the Overwintering Performance of Seeds of Invasive Alien Plants *Xanthium italicum* and *Xanthium spinosum* in Xinjiang

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Abstract

Fruits of the invasive alien plants *Xanthium italicum* and *Xanthium spinosum* in Xinjiang begin to abscise successively after maturing in late autumn. Detached fruits may fall onto dry ground, drop into water bodies, or remain on branches without abscising until the following spring. Since differences in overwintering habitats directly affect seed germination activity in the subsequent spring, we conducted this study to evaluate the invasive capacity of these alien plants, predict their potential distribution areas, and provide scientific guidance for effective prevention and control. We simulated winter habitat types that seeds might encounter under natural conditions in Xinjiang, designing four experimental treatments: low-temperature water immersion with water changes (simulating habitats in flowing water or large water bodies before freezing), low-temperature water immersion without water changes (simulating habitats in small static water bodies before freezing), freezing (simulating habitats in water bodies after freezing), and dry freezing treatment (simulating habitats on dry ground or remaining on plants), with room-temperature dry storage as the control. After 90 days of treatment, seed germination experiments were conducted. The results showed that for *Xanthium italicum*, germination rates after dry freezing, freezing, and low-temperature water immersion with water changes showed no significant difference from the control group, whereas water immersion without water changes reduced seed viability and significantly decreased germination capacity. Dry freezing treatment had no effect on seed germination of *Xanthium spinosum*, while cold water immersion (both with and without water changes) significantly reduced its germination rate, decreasing by 37% and 65% compared with the control group, respectively. No germination occurred in *Xanthium spinosum* after freezing treatment. These findings indicate that *Xanthium italicum* seeds possess good tolerance to various overwintering habitats in Xinjiang, whereas *Xanthium spinosum* seeds exhibit poor tolerance to low-temperature and waterlogging environments. Given the distinct seed germina-

tion characteristics of the two species, we recommend comprehensive monitoring for *Xanthium italicum* across mesic, hygric, and seasonal waterlogged habitats throughout Xinjiang, as farmland, forest understories, roadsides, canal edges, and areas around lakes and reservoirs could all become potential invasion sites. For *Xanthium spinosum*, monitoring efforts should focus on low-latitude, low-altitude mesic and meso-xeric habitats, with particular emphasis on strengthening quarantine inspections of imported goods at international ports in Southern Xinjiang.

Full Text

Seed Overwintering Performance of Two Invasive Plants, *Xanthium italicum* and *Xanthium spinosum*, in Xinjiang

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Abstract

The fruits of Italy cocklebur (*Xanthium italicum* Moretti) and spiny cocklebur (*Xanthium spinosum* Linn.), two invasive plant species in Xinjiang, begin to fall after maturing in late autumn. Detached fruits may land on dry ground, fall into water bodies, or remain on branches until the following spring. Since overwintering habitat conditions directly affect seed germination viability the next spring, we simulated four natural winter habitat types that seeds might encounter in Xinjiang to evaluate the invasive potential of these species and predict their potential distribution areas, thereby providing scientific guidance for effective prevention and control. The experimental treatments included: (1) low-temperature water immersion with water changes, simulating habitats in flowing water or large water bodies before freezing; (2) low-temperature water immersion without water changes, simulating habitats in small stagnant water bodies before freezing; (3) freezing after water immersion, simulating habitats in frozen water bodies; and (4) dry freezing treatment, simulating habitats on dry ground or seeds remaining on plants. Room-temperature dry storage served as the control.

The results showed that after dry freezing, freezing, and low-temperature water immersion with water changes, the germination rate of *X. italicum* showed no significant difference from the control group, though the treatment without water changes significantly reduced seed vigor and germination capacity. Dry freezing had no effect on *X. spinosum* seed germination, while both water immersion treatments (with and without water changes) significantly reduced its germination rate by 37% and 65% compared to the control, respectively. No seeds germinated after freezing treatment. These findings indicate that *X. italicum* seeds exhibit good tolerance to various overwintering habitats in Xinjiang, whereas *X. spinosum* seeds have poor tolerance to low temperature and waterlogging environments. We recommend comprehensive monitoring of *X.*

italicum across mesic, hygric, and seasonally flooded habitats throughout Xinjiang, as areas around farmland, understory, roadsides, riversides, lakes, and reservoirs could become potential invasion sites. For *X. spinosum*, monitoring efforts should focus on low-latitude, low-altitude mesic and semi-arid habitats, with particular emphasis on strengthening quarantine inspections of imported goods at international ports in southern Xinjiang.

Introduction

As China's western opening strategy deepens and freight trade along the New Silk Road increases, numerous alien species have quietly entered Xinjiang. Particularly, plants from the Asteraceae family, genus *Xanthium*—including *Xanthium italicum* Moretti and *Xanthium spinosum* Linn.—are spreading rapidly across Xinjiang, causing serious damage to local agriculture and animal husbandry and further stressing the region's fragile oasis ecosystems. Effective prevention and control require prior evaluation of their invasive potential and prediction of their potential distribution ranges.

Current research on these two invasive *Xanthium* species has focused primarily on morphological descriptions, distribution ranges, and chemical composition and allelopathic effects. However, studies on their invasion mechanisms and population expansion trends remain limited. Seed germination represents a critical stage in plant life cycles, significantly influencing species propagation, range expansion, and environmental adaptation. As reproductive units, seeds' adaptive capacity to environmental conditions directly affects individual survival in new habitats. Extreme low temperatures and annual accumulated temperature are important factors influencing the geographic distribution of alien plants. For many alien species native to warm regions, surviving winter low temperatures in introduced areas poses a severe challenge and often constitutes a bottleneck for successful invasion. Studies on seed overwintering performance are not only key to elucidating invasion mechanisms but also provide important evidence for evaluating invasive capacity and potential distribution ranges.

Xinjiang's unique landscape—characterized by alternating mountains and basins, and coexisting deserts and wetlands—means seeds must endure not only severe winter cold on land but also potential immersion in ice-water environments. Therefore, this study employed experimental ecology methods to simulate various natural overwintering habitats, investigating the germination performance of *X. italicum* and *X. spinosum* seeds after experiencing different winter conditions. By exploring the relationship between habitat types and seed overwintering performance, we aim to provide scientific evidence for elucidating invasion mechanisms, evaluating invasive capacity, and predicting potential distribution areas.

Materials and Methods

Seed Collection Fruits of both species were collected in October 2015 from the vicinity of Yining City, Xinjiang (81°0 -83°59 E, 42°50 -44°03 N) and transported to the laboratory for air-drying. Healthy, mature fruits of uniform size were selected for experiments. In this study, “fruits” actually refer to infructescences with involucre.

Experimental Treatments Based on four potential overwintering habitat types that seeds might encounter in Xinjiang, we designed corresponding experimental treatments with room-temperature dry storage as the control:

1. **Room-temperature dry storage:** Fruits stored in dark, dry conditions at 20°C.
2. **Low-temperature water immersion with water changes:** To avoid microbial contamination during long-term water immersion, experimental containers and distilled water were autoclaved, and fruits were surface-sterilized. Fruits were rinsed with distilled water to remove dust and debris, soaked in 75% ethanol for 30 seconds, rinsed with sterile water, then soaked in 0.1% mercuric chloride for 10 minutes, and finally transferred to sterile water. They were sealed with sterile breathable film and stored in a dark refrigerator at 5°C, with water changed every 3 days to simulate fruits falling into flowing water that had not frozen.
3. **Low-temperature water immersion without water changes:** Seed sterilization and sealing procedures were identical to treatment 2, but stored in dark, stagnant water at 5°C to simulate fruits falling into small stagnant water bodies.
4. **Dry freezing treatment:** Fruits stored in dark, dry, frozen conditions at -20°C to simulate fruits on dry ground or remaining on plants.
5. **Freezing after water immersion:** Fruits were rinsed, soaked in water at 5°C for 24 hours, then frozen at -20°C to simulate fruits falling into water bodies that subsequently froze.

All treatments lasted 90 days.

Germination Experiments After treatment completion, fruits were removed for germination experiments. Fruits from room-temperature dry and dry freezing treatments were soaked in distilled water at room temperature for 24 hours to soften the involucre. Frozen fruits were thawed at room temperature. Because the two seeds within each fruit exhibit different germination characteristics, the involucre was carefully removed without damaging the internal seeds, and the larger seeds were selected and placed uniformly in plastic germination boxes (20 cm × 12 cm × 5 cm) filled with river sand (1 cm depth, 45% relative humidity). Each treatment had 3 replicates with 45 seeds per replicate. Germination boxes were placed in a growth chamber with a 25°C/20°C day/night temperature cycle, 12-hour photoperiod, and light intensity of 450 mol · m² · s⁻¹. Soil relative humidity was maintained at 45% throughout the experiment.

Germination was recorded when cotyledons emerged above the sand surface, with counts performed daily. Germination initiation time (days until first seed germinated), germination potential (percentage of seeds germinated by day 7), germination rate (percentage of seeds germinated by day 15), and germination index were calculated. The germination index was calculated as: $GI = \Sigma(Gt/Dt)$, where Gt is the number of germinated seeds on day t , and Dt is the corresponding germination day.

Seed Viability Testing After the germination experiment, 30 ungerminated seeds of each species were randomly selected from each treatment group. Embryos were sliced transversely with a blade, placed on clean slides, and stained with 1% TTC solution to test viability.

Data Analysis Experimental data were analyzed using SPSS 19.0 software. One-way ANOVA was used to analyze seed germination rates and germination indices among different treatments, with Duncan's multiple range test used to detect significant differences between treatments ($P < 0.05$).

Results

Seed Germination Potential *Xanthium italicum* seeds in the room-temperature dry treatment began germinating on day 3, while seeds in other treatments started on day 4. Germination potential was significantly affected by treatment ($P < 0.01$). Low-temperature water immersion without water changes significantly reduced germination potential. Room-temperature dry treatment showed the highest germination potential (95.56%), but this was not significantly different from water immersion with water changes, dry freezing, or freezing treatments.

Different treatments strongly affected *X. spinosum* seed germination. Seeds in room-temperature dry treatment began germinating on day 4, while no seeds germinated in the freezing treatment throughout the experiment. In dry freezing treatment and water immersion with water changes, seeds began germinating on day 5. Germination potential in room-temperature dry and dry freezing treatments was 42.22%. Low-temperature water immersion significantly reduced *X. spinosum* germination potential ($P < 0.01$).

Seed Germination Rate Germination rate results were consistent with germination potential findings. For *X. italicum*, germination rate under low-temperature water immersion without water changes was significantly lower than other treatments ($P < 0.01$), while no significant differences were observed among dry freezing, freezing, and water immersion with water changes. For *X. spinosum*, no seeds germinated after freezing treatment. Germination rates under room-temperature dry and dry freezing treatments were both 42.22%, representing decreases of 37% and 65% compared to the control ($P < 0.01$).

Germination rate under water immersion with water changes was significantly higher than without water changes.

Germination Index For *X. italicum*, the germination index under room-temperature dry storage was highest, but not significantly different from water immersion with water changes, freezing, or dry freezing treatments ($P > 0.05$). Water immersion without water changes resulted in a significantly lower germination index compared to other treatments ($P < 0.01$). For *X. spinosum*, room-temperature dry treatment produced the highest germination index, though not significantly different from dry freezing ($P > 0.05$). Both water immersion treatments significantly reduced the germination index ($P < 0.01$). Under identical treatments, *X. italicum* consistently showed higher germination indices than *X. spinosum*.

Seed Viability Testing TTC staining revealed that ungerminated *X. italicum* seeds from various treatments remained viable, though with reduced vigor. In contrast, ungerminated *X. spinosum* seeds had completely lost viability, with some seeds already rotted. Among 30 ungerminated seeds, only 3 showed activity while the rest had completely lost viability. In this study, germination rate served as the primary indicator of seed vigor.

Discussion

Dry freezing simulates the overwintering habitat of fruits on dry ground or remaining on plants. This treatment significantly reduced the germination index of *X. italicum* seeds but did not affect germination potential or rate. For *X. spinosum*, dry freezing had no significant effect on germination. Germination potential and rate reflect seed uniformity and quality, while germination index indicates germination speed. Xinjiang's winter low temperatures delay the germination process of *X. italicum* seeds on dry ground or plants but do not significantly affect final germination rates, whereas *X. spinosum* seeds remain unaffected. Thus, both species can safely overwinter in this habitat.

In hygric environments, mature seeds often become immersed in water after detachment, making waterlogging a major environmental stressor affecting germination. Previous studies have shown that long-term water immersion reduces cocklebur seed germination and viability, with germination rates decreasing as waterlogging duration extends. Waterlogging affects seed germination through two primary mechanisms: (1) dissolved oxygen pressure in water is far lower than in air, inhibiting normal respiratory metabolism; and (2) leaching of inorganic salts and starch from the embryo damages the embryo itself, reducing seed vigor.

For *X. spinosum*, both water immersion treatments significantly reduced germination potential and rate compared to the control, indicating embryo damage and reduced vigor after prolonged immersion. The absence of water changes further decreased oxygen pressure, inhibiting normal respiration, which explains

why germination rate was higher with water changes than without. For *X. italicum*, water immersion with water changes maintained high germination potential (93.33%) and rate (97.78%) with no significant difference from the control, indicating that prolonged water immersion does not damage the embryo. However, germination index was significantly lower without water changes, likely due to hypoxia-induced vigor loss. While *X. italicum* seeds at high density (45 seeds/400 mL) in stagnant shallow water showed lower overwintering safety, such densities are unlikely in natural conditions, suggesting that *X. italicum* seeds falling into cold water generally have high overwintering safety.

Freezing is a common winter event in Xinjiang. Freezing treatment only reduced *X. italicum* germination index without significantly affecting germination potential or rate. However, freezing after water immersion severely impacted *X. spinosum* seed viability, with no germination observed, indicating that *X. spinosum* seeds cannot overwinter in frozen wetlands.

Xanthium italicum and *X. spinosum* were first recorded in Xinjiang in 2009. *Xanthium italicum* is now widely distributed in Bortala, Changji, and Urumqi regions, while *X. spinosum* is mainly found in Urumqi and Yili areas. Their distinct distribution patterns can be explained by differences in seed germination characteristics. *Xanthium italicum* seeds show good tolerance to Xinjiang's winter hydrothermal conditions, allowing distribution across a wide latitudinal range, including high-latitude, low-temperature areas like Tacheng, Emin, and Altay, and can grow in mesic, hygric, and seasonally flooded environments. In contrast, *X. spinosum* seeds have poor tolerance to low temperature and waterlogging, restricting its distribution to lower-latitude areas along the northern Tianshan slopes and favoring mesic habitats like farmland or drier desert steppe.

Although both species are currently limited to northern Xinjiang, southern Xinjiang's relatively warmer winter temperatures would be more favorable for seed overwintering. Their absence in southern Xinjiang may be due to the Tianshan Mountains' barrier effect. However, as Xinjiang's role as a bridgehead for China's western opening strategy strengthens and trade between northern and southern Xinjiang and neighboring countries increases, this geographical barrier may be breached. Therefore, comprehensive prevention measures should be considered across the entire Xinjiang region.

Successful plant invasion depends on both species invasiveness and habitat invasibility. Invasiveness depends on biological characteristics, while invasibility depends on abiotic and biotic resistance to invasion. In addition to enemy release and vacant ecological niches, broad environmental adaptability is crucial for successful invasion. Based on the germination characteristics of these two species, we recommend comprehensive monitoring of *X. italicum* across mesic, hygric, and seasonally flooded habitats throughout Xinjiang, as areas around farmland, understory, roadsides, riversides, lakes, and reservoirs could become potential invasion sites. For *X. spinosum*, monitoring should focus on low-latitude, low-altitude mesic and semi-arid habitats, with particular emphasis on strengthening quarantine inspections of imported goods at international ports

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