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Seasonal changes in germinability, dormancy and viability of field bindweed (*Convolvulus arvensis*) seeds as affected by storage and duration

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Abstract: Background: Field bindweed (*Convolvulus arvensis* L.) is a hardy perennial weed currently threatening many agroecosystems and orchard ecosystems in China. It can be spread farther by seed with a long dormancy.

Objective: This study aimed to evaluate the seasonal changes in germinability, dormancy and viability of field bindweed seeds in the unique arid environment in northwest China.

Methods: Seeds were subject to burial at various depths in the field, outdoor and indoor storage, or dry- and wet-cold stratification (5 C) treatments for 12 months. Seed germinability was tested at monthly intervals.

Results: No seed at 0- or 1-cm burial depths germinated *in situ*. Seeds buried at 3-20 cm started to germinate after 4 months, with steady increase in germination *in situ* over the next 7-month burial period. Some ungerminated seeds under field conditions released dormancy and could germinate *ex situ* under optimal laboratory conditions. The rapid dormancy release occurred under outdoor storage conditions and 13%–30% seed germinated *ex situ*. However, seeds from the indoor storage maintained a high level of dormancy with germination less than 12%. The dry/cold stratification was more effective in breaking seed dormancy than the wet/cold treatment. Most of the seeds (> 87%) retained their viability during the 12-month period.

Conclusions: Low temperature could partially break seed dormancy of field bindweed. The optimal emergence depth was 3–5 cm and the best time to control this weed species was from April to October. In view of the high seed viability, late season weed control is necessary to stop seedset.

Keywords: Buried seed; Cold stratification; *Convolvulus arvensis*; Seed persistence; Weed management

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1. Introduction

Field bindweed (*Convolvulus arvensis* L.), a member of the *Convolvulaceae* family, is one of the ten worst weeds in the world and common in the temperate zone (Holm et al., 1977; Gianoli, 2001). It has incredible ability to adapt to extreme barren and drought environments, making this plant a widespread weed (Mitich, 1991; Weaver, Riley, 1982). The presence of field bindweed can induce major yield and product quality reductions of several crops, such as winter wheat (*Triticum aestivum* L.) (Bayat, Zargar, 2020), maize (*Zea mays* L.) (Pfirter et al., 1997) and tomato (*Lycopersicum esculentum* L.) (Sosnoskie, Hanson, 2016), through competition and by interfering with harvesting operations. In recent decades, field bindweed has been noted as a problematic weed in northwest China, often forming a single dominant community and seriously infesting cotton (*Gossypium hirsutum* L.) fields and orchards (Zhang et al., 2016).

The ability of field bindweed to invade and persist in a variety of habitats can be explained by its specific reproductive traits. As a perennial weed species, field bindweed is capacity of vegetative reproduction through rhizomes on its extensive root system and sexual reproduction by its long-lived seeds (Gaskin et al., 2023). Under cultivation, both rhizome fragments and seeds may be spread and establish new plants (Degennaro, Weller, 1984; Sosnoskie et al., 2020). Field bindweed can rely on vegetative growth via roots and rhizomes to spread locally, forming large single individuals (Davis et al., 2018), while bindweed seeds can allow for long distance dispersal and establish population in new environments (Karaman, Tursun, 2021).

Herbicides, soil disturbance (tillage, cultivation, hoeing, or harrowing), mulch, grazing, fertilizer, and biological control have been suggested as effective approaches to managing field bindweed (Davis et al., 2018; Moretti, Peachey, 2022). However, this plant continues to invade and persist in temperate regions of the world and remains difficult to control partly due to its long-lived seeds. Field bindweed seed had a long dormancy and could last for up to 60 years in the soil. When plants of field bindweed were completely controlled for two consecutive years, there were still 753 viable seeds per square meter in the top 0–15 cm soil layer, and 430 seeds retained a considerable degree of viability after six years (Timmons, 1949). The dormancy of field bindweed seed is due to the physical barrier of a hard seed coat (Xiong et al., 2018). Brown and Porter (1942) reported that 87%–99% of the freshly harvested seeds were viable, but only 5%–25% of them were non-dormant and could germinate immediately. The hard seed could withstand silage, soaking, digestion by birds, heat, and fumigation with methyl bromide (Proctor, 1968; Phillips, 1978). Fifty five percent of the seeds could germinate after 54 months of soaking in water (Zimdahl, 1993).

Seed dormancy of field bindweed could be alleviated under laboratory conditions by using mechanical scarification, sulfuric acid, hot water scarification and cold stratification (Xiong et al., 2018). It is not clear how the seed dormancy of field bindweed is released under natural conditions to ensure its survival and spread? At maturity, some seeds fell on the soil surface and some remained on the stem. The seeds on the ground can be buried into different soil layers by tillage implements. Previous studies have demonstrated that the depth and duration of soil burial has significant effect on weed seed dormancy, viability and longevity (*Amaranthus retroflexus*, Omami et al., 1999; *Veronica hederifolia*, Mennan, Zandstra, 2006; *Solanum elaeagnifolium*, Stanton et al., 2012). Moreover, seed dormancy was affected by dry storage or cold stratification (Bourgeois et al., 2019; Kundu et al., 2019). There is little biological and ecological information for field bindweed in the unique temperate continental arid environment in northwest China. Therefore, the series of experiments were conducted to clarify the impact of depth and duration of burial in the soil, dry storage, and cold stratification on germinability, dormancy and viability of field bindweed seeds during a 12-month period. The generated information will aid better evaluating which strategy of weed control may be most effective at stopping the establishment and spread of this noxious weed in northwest China.

2. Materials and Methods

2.1 Seed collection

Field bindweed seeds were collected from naturally senescing plants in November 2016 at the Research Farm of Tarim University (40.55° N, 81.30° E, 1100 m a.s.l.), Xinjiang Province, China. The collection site had an extreme dry climate, with a mean annual temperature of 10.7 °C, annual rainfall of 50 mm, and annual sunshine hours of 2,900 h. Immediately after collection, initial germination and viability were determined at 7 days after incubation under optimal temperature conditions (30/20 °C, 14 h light/10 h dark photoperiod, Brown, Porter, 1942). Non-germinated seeds were subsequently treated with concentrated (98%) sulfuric acid for 30 min (Xiong et al., 2018) and incubated for a further 7 days at 30/20 °C in light (14 h d^{-1}) to determine seed viability.

2.2 Seed burial in the field

In this experiment, total 288 nylon mesh bags (15 cm × 10 cm) were used and samples of 50 seeds were placed in each bag. These bags with seeds were then placed at the soil depths of 0, 1, 3, 5, 10 or 20 cm, respectively, in a cultivated field at the Research Farm of Tarim University on November 17, 2016. A split-plot design with four replications (bags) was used, with exhumation time as the main plot and burial depth as the subplot. Four bags (50 seeds × 4) each from the six burial depths were exhumed from the field at monthly intervals, washed under running tap water in the laboratory and blotted dry with the filter paper before opening to determine the total number of seeds, including germinated (treated as *germination in situ*) and non-germinated seeds. This experiment was repeated on November 20, 2016 to ensure the accuracy of the experimental results.

2.3 Outdoor and indoor storage

To evaluate the effect of the seasonal change of environmental conditions on seed dormancy and viability, total 48 nylon mesh bags were used and 50 seeds were placed in each nylon mesh bag, and then all bags were hung on an outdoor fence in the field on November 17, 2016 to imitate the seeds remained on the stem under natural conditions. This outdoor storage treatment was compared with an indoor storage treatment, where 48 Petri dishes were used and 50 seeds were stored in each Petri dish sealed with Parafilm at room temperature (approximately 20 °C). The seed germination was continuously tested on monthly basis up to 12 months from four randomly selected nylon mesh bags or Petri dishes from both outdoor and indoor storage treatments. This experiment was also repeated.

2.4 Cold stratification

Cold stratification was compared between dry and wet treatments at 5 °C . Under dry conditions, 50 seeds were placed in each Petri dish (total 48 dishes) and sealed with Parafilm, while under wet conditions, 50 seeds were spread between two sheets of filter paper (Whatman No. 1) in each Petri dish (total 48 dishes) moistened with 4 ml of deionized water and sealed with Parafilm. All 96 Petri dishes were then stratified at 5 °C in a refrigerator. The seed germination was continuously tested on monthly basis up to 12 months by four randomly selected Petri dishes from each of the dry and wet treatment groups. The number of germinated seeds during the wet and cold stratification treatments was determined as *germination in situ* and non-germinated seeds were subject to the following germination test. And this experiment was also repeated.

2.5 General germination procedure

Intact, non-geminated seeds from either nylon mesh bags or Petri dishes were transferred to new Petri dishes (90-mm-diam) lined with two pieces of filter paper moistened with 4 ml of deionized water. Dishes were sealed with Parafilm to minimize water losses from evaporation. The dishes were then randomly placed in an incubator set at fluctuating temperatures of 30/20 °C with a photoperiod of 14 h light/10 h dark and a photosynthetic photon-flux density of 150 μmol m^{-2} s^{-1} provided by cool white fluorescent tubes. Seeds with visible radicle protrusion from the seed coat were considered germinated and the number of germinated seeds (treated as *germination ex situ*) was counted after incubating for 7 days. Non-germinated seeds were subsequently treated with concentrated (98%) sulfuric acid for 30 min to break the physical dormancy and incubated for a further 7 days (Xiong et al., 2018). The number of germinated seeds (treated as *germination operando*) was again recorded. Seeds that did not germinate after treatment with sulfuric acid were considered to be non-viable.

2.6 Statistical analyses

Percent germination data were square root arcsine transformed to fulfill the homogeneity of variance condition. All experiments were repeated and data were combined as there were no significant differences over time. Data were analyzed with ANOVA and Fisher's Least Significant Difference test at $P < 0.05$ was used to determine the statistically different means. Separate analyses were performed for: (a) germination in nylon bags or in petri dishes (i.e. *in situ*) during the treatment periods in the field or under laboratory conditions, respectively; (b) germination in the laboratory under favourable incubation conditions (i.e. *ex situ*); (c) germination after treatment with sulfuric acid (i.e. *operando*); (d) total germination (i.e. a + b + c), which was considered to represent total viability. Additionally, regression analysis was used to evaluate the effect of burial duration (months) on germination percentage. Coefficients of determination (R^2) were used to determine the goodness of fit of different models. Analysis was performed with the statistical software SPSS 13.0. Local climate data during the experimental periods was obtained from the meteorological station at the University (Figure 1).

3. Results and Discussion

Fresh matured seed of field bindweed had low germination rate (2.0% ± 0.82%), and germination percentage could reach to 91.5%–100% after the treatment of concentrated (98.0%) sulfuric acid. These results indicated that newly harvested field bindweed seeds were viable but with strong physical dormancy, which was in agreement with the study of Brown and Porter (1942).

3.1 Seed burial in the field

The seasonal change in germination of field bindweed seeds in the field was affected by burial depth ($P < 0.001$). Seeds on the soil surface or buried at 1 cm did not emerge *in situ* during the 12-month experimental period, which may have been resulted from the drought conditions with high evaporation and very little rainfall (Figure 1). When seeds were buried at 3–20 cm, there was an apparent positive linear correlation between seed germination *in situ* and burial duration ($R^2 > 0.81$, $P < 0.001$; Figure 2). There was no seed germination in the field (*in situ*) in the first four months from December 2016 to March 2017. The proportion of seeds buried at 3–20 cm germinated *in situ* increased gradually from April until October, which coincided with the crop growing season in northwest China and was the best time to control this weed species. However, the monthly germination rate *in situ* in the 20-cm burial depth was lower than that of seed buried at 3–10 cm depths, which indicated that it was not conducive to the germination of field bindweed seeds with the deeper burial depths. The previous studies shown that the optimum soil burial for seed emergence of field bindweed was 0–1 cm and the emergence rate decreased gradually with the increase of soil depth. When the soil depth reached 8 cm, the seeds could not emerge normally (Tanveer et al., 2013; Kumari et al., 2010). Moreover, the seeds of field bindweed could enter the secondary dormancy with the increase of soil depth (Asgharipour, 2011). These published protocols showed that the optimal emergence depth of field bindweed is 1–5 cm and the depth of 8 cm is the maximum depth to emerge. In this study, although the seeds of field bindweed buried at 10–20 cm in the field could germinate

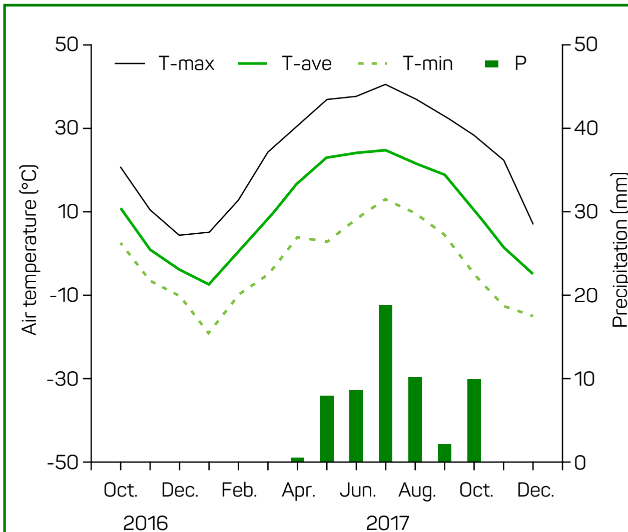

Figure 1 - Maximum (T-max), minimum (T-min), average (T-ave) air temperatures (°C) and precipitation (P, mm) at the Research Farm of Tarim University during the experimental periods between October 2016 and December 2017

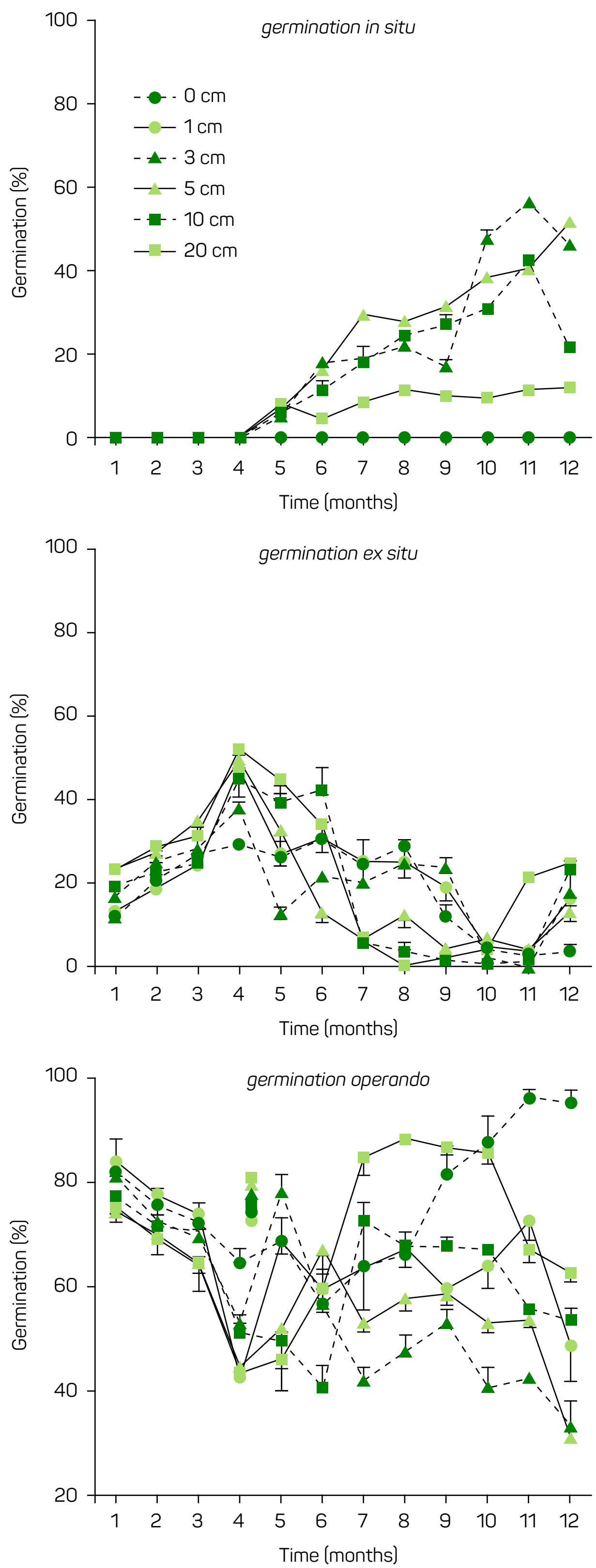

Figure 2 - Germination of field bindweed seeds buried in the field, consisting of the seeds that germinated in the field (*in situ*) and the seeds germinated in the laboratory before (*ex situ*) and after (*operando*) treatment with sulfuric acid

in situ, those germinated seeds obviously had no chance of reaching the soil surface. Therefore, an effective method to control field bindweed involves the use of mechanical soil-turning tools, e.g., moldboard plowing, to place field bindweed seeds at least 10 cm in the soil conducive to fatal germination (Sousa-Ortega et al., 2023).

Although field bindweed seeds had a high degree of dormancy after harvest, the level of dormancy gradually decreased during the time the seeds were buried in the field. After exposing to about 0 °C low temperature for one month, 12.1%–23.6% seeds buried in the field could germinate in the laboratory (*ex situ*) under suitable conditions (30/20 °C , 14 h light/10 h dark photoperiod), which indicated that low temperature in winter could release seed dormancy of field bindweed to some extent. As the seed exposure time in the field prolonged, the percentage of seeds released from dormancy (*ex situ*) increased gradually and reached to 29.6%–52.3% after 4 months. In the subsequent months, germination *ex situ* declined and only less than 4.9% seeds could germinate under suitable conditions among September and October 2017, except for the seeds retrieval from the 20 cm soil in October. The relationship between seed germination *ex situ* and burial time (months) was fitted with the cubic regression model ($R^2 > 0.72$, $P < 0.01$), except for the seeds buried at 3 cm. These findings indicated that after exposing the low winter temperature freshly matured seeds of field bindweed could partially release dormancy and germinate under favorable conditions, but some seed released dormancy did not germinate in the field, allowing it to persist the soil seedbank (Ngo et al., 2017b).

Non-germinated seeds *ex situ* were subsequently treated with concentrated (98%) sulfuric acid for 30 min and then incubated for a further 7 days. Only 3.5% seeds were still in dormant with hard seed coat and most seeds could germinate *operando*. This result indicated that seeds of field bindweed was highly viable (i.e. *in situ*, *ex situ* and *operando* germinated seeds combined), which was significantly affected by burial duration and burial depth ($P < 0.01$; Figure 3). Viability of field bindweed seeds in the first five months was between 90.2% and 99.5% and burial depth had no effect on seed viability. After 8 months of seed burial in the field, seed buried at 1 cm depth had significantly lower viability than the other burial depths, due to significant seed decay (20.2%–34.9%) at this depth. Dormancy is considered to be an effective mechanism for seeds to persist in the soil, and persistence of the soil seedbank is an important adaptive feature of weed species (Kleemann, Gill, 2018).

3.2 Outdoor and indoor storage

After one month of exposure to low temperature in the field, the germination *ex situ* increased from the initial 2.0% to about 15.7%, indicating the rapid dormancy release under natural conditions. The germination *ex situ*

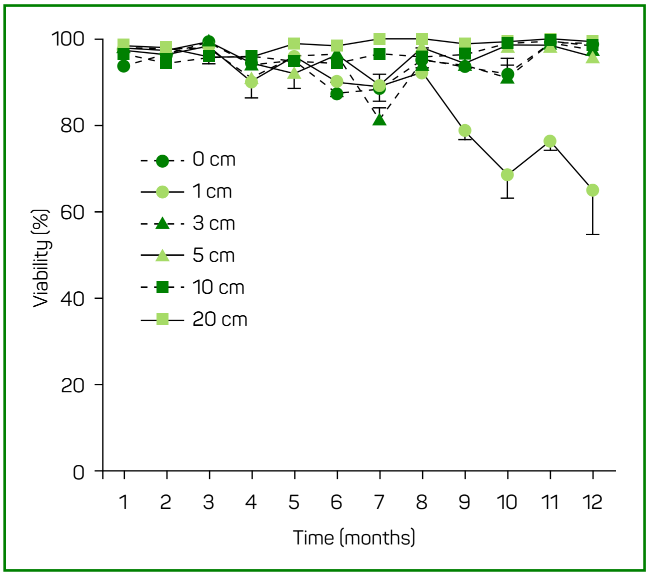

Figure 3 - Seed viability of field bindweed, consisting of the sum of seeds that germinated *in situ* and those that germinated in the laboratory before (*ex situ*) and after (*operando*) treatment with sulfuric acid

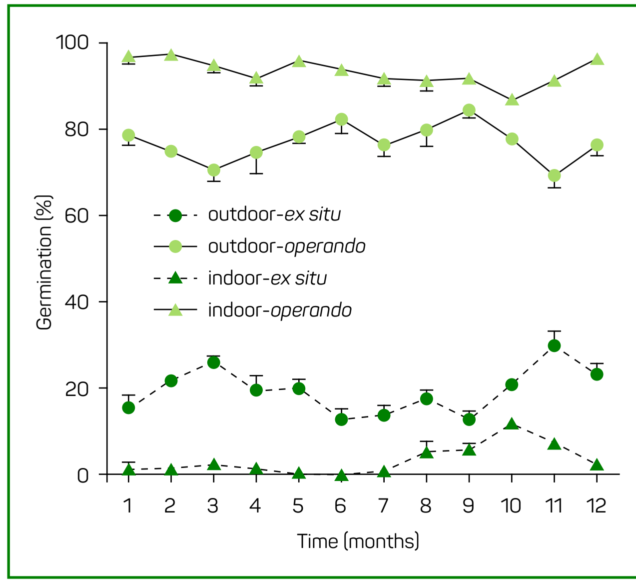

Figure 4 - Effect of outdoor- and indoor-storage on seed germination of field bindweed, consisting of the seeds that germinated in the laboratory before (*ex situ*) and after (*operando*) treatment with sulfuric acid

increased steadily and peaked (26.3%) in February after 3-month outdoor treatment in the field, followed by a slight declining trend. The germination *ex situ* then increased again with a second peak 30.0% after 11 months in October 2017 (Figure 4). The results of seed burial and seed storage in the field showed that low temperature in winter was an effective method to partially release dormancy of field bindweed seeds. In northwest China, seeds of field bindweed mature around October and November and are exposed to a cold winter period (about 5 months), which can help them to break dormancy and improve germination. This phenomenon was also reported by Meulebrouck et al. (2008), who found that low autumn and winter temperatures tended to partially remove seed physical dormancy in the holoparasite dodder [*Cuscuta epithymum* (L.) L.]. According to field observation, seeds of field bindweed had poor seed-shedding ability. After seed maturation, most of the seeds stayed on the climbing vines during winter, even until autumn and winter of the next year. Once seeds shed onto the ground under cultivation, they could germinate under suitable environmental conditions and cause problems in agricultural or horticultural systems.

The dormancy of field bindweed seeds could not be effectively released under room temperature and dry storage condition, and seed germination (indoor *ex situ*) was only 0%–12.0% during the experiment period (Figure 4). Although freshly harvested seeds of feather fingergrass (*Chloris virgata* Sw., Ngo et al., 2017a), Japanese foxtail (*Alopecurus japonicas*, Wang et al., 2017), were also nil, seed germination of these weed species could gradually increased after an after-ripening period under room storage conditions. The dormancy of field bindweed seed resulted from a hard seed coat barrier (Xiong et al., 2018) and room storage was not an efficient setting for breaking seed coat-mediated dormancy of weed species (Adams et al., 2017; Lujan Rocha et al., 2022). However, after treatment with concentrated sulfuric acid to break dormancy, the germination *operando* of seeds in all months could reach more than 87.0%. After one year of dry-storage indoor, the viability of field bindweed seeds remained above 99.0%. Moreover, the total viability was the similar between the indoor (97.0%) and outdoor (96.8%) treatments (independent sample t-test, $t = -0.32$, $P = 0.75$). These results further showed that seed germination of field bindweed should be prevented by the seed coat, which might involve permeability (preventing water uptake or gaseous exchange) and mechanical (preventing embryo expansion) barrier to germination.

3.3 Cold stratification

Low temperature treatment could effectively release the seed dormancy of field bindweed, but the ambient humidity also had a significant effect on the degree and seasonal variation of seed dormancy. Under dry storage conditions, seed germination *ex situ* could reach to 8.5% after one month treatment at 5 °C, and increase to 35.0% after two months. As the storage time progressed, the seed germination rate increased gradually. The seed germination reached to a maximum 87.5% after 7-month dry storage and maintained at a high level within the next five months. The relationship between treatment time and germination *ex situ* was a typical cubic curve model ($R^2 = 0.97$, $P < 0.001$; Figure 5). The seeds under dry storage at 5 °C maintained high seed vitality, and the total germination including *ex situ* and *operando* was more than 89.0%.

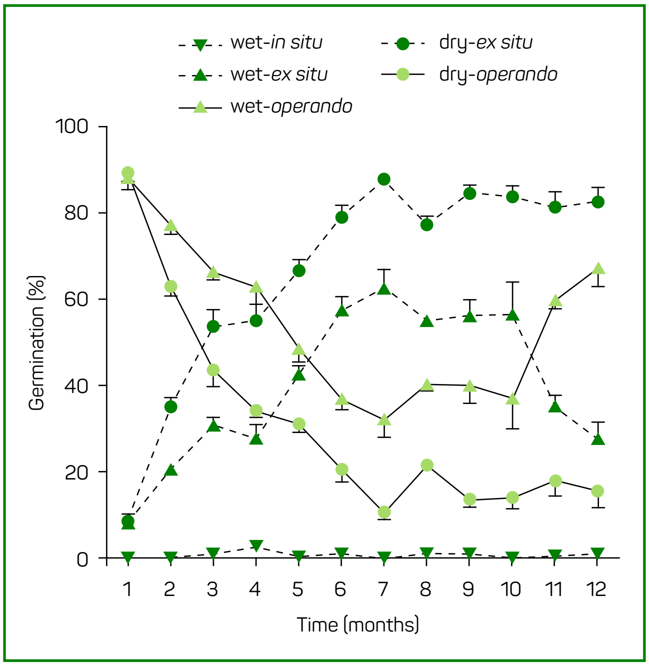

Figure 5 - Effect of dry- and wet-cold stratification on seed germination of field bindweed, consisting of the seeds that germinated in the stored dish (*in situ*) and the seeds germinated in the laboratory before (*ex situ*) and after (*operando*) treatment with sulfuric acid

The wet and cold stratification were less effective in breaking dormancy as compared to the dry/cold treatment. Germination *in situ* was very low (0.5%–2.6%) during the wet and cold stratification. Seed germination *ex situ* increased from 8.1% at one month to 62.5% at 7-month after treatments, and the germination then decreased gradually to less than 35.0% at 12 months after treatments. Moreover, seed dormancy release rate (i.e. germination *ex situ*) showed a cubic model with the increasing storage time under wet and cold conditions (R^2 = 0.98, $P < 0.001$; Figure 5). Correspondingly, germination *operando* showed a concave trend with the storage duration (cubic model, R^2 = 0.95, $P < 0.001$). The percentage of viable seeds, including the seeds germinated *in situ*, *ex situ* and *operando*, were more than 91.5%. Cold stratification was typically required to break dormancy of seeds with physical dormancy (Page, Nurse, 2015; Rouhi et al., 2015) due to allowing water to penetrate the seed coat (López-Granados, García-Torres, 1996). However, several studies have shown that cold stratification had no effect on dormancy release of seeds with physical dormancy (Adams et al., 2017; Susko et al., 2001).

The information on the level of dormancy and viability in weed seed is useful for developing predictive models and for determining optimal timing for the control of weed in a crop. Previous studies showed that total seed viability of weed species decreased with time of burial in the soil or laboratory storage (Burnside et al., 1996; Mennan, Zandstra, 2006; Stanton et al., 2012). However, the seasonal decline in seed viability did not occur in our study and field bindweed seeds always maintained a high level (65.1%–100%) of viability during the 12-month experimental period. Therefore, effective control is required to prevent field bindweed plants from producing seed and enriching the soil seed bank. Seedlings of field bindweed need to be controlled with cultivation or herbicides before 3 to 4 weeks after germination (Vasilakoglou et al., 2013; Wright et al., 2011). After this period, regenerative root buds formed, and its control became much more difficult (Jurado-Expósito et al., 2004). Moreover, late season weed control is necessary to avoid weeds from setting seed and this can be done by hand pulling or shallow hoe weeding (e.g., scraping). If the field bindweed plants are mature, harvest weed seed control (Lee, Thierfelder, 2017; Winans et al., 2023) is advisable to remove the mature seeds from the field and destroy weed seed, thus reducing the amount of weed seed returning to the soil.

Authors' contributions

XM, HW, and XR: investigation, methodology, formal analysis, writing. HH, experiment, investigation. LW and YM: funding acquisition.

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