

## Effect of different treatments on dormancy-breaking and germination of perennial pepperweed (*Lepidium latifolium*) (Brassicaceae)

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### Abstract

The purpose of our study was to better understand the seed-germination ecology of the invasive plant *Lepidium latifolium*, which grows in orchards and among cool-season crops in Iran. The seed dormancy of this weed was studied using soil burial, scarification, cold stratification, constant temperatures, flooding, and potassium nitrate (KNO<sub>3</sub>) and gibberellic acid (GA<sub>3</sub>) treatments. The results showed that the seeds' dormancy can be broken most effectively by scarification, KNO<sub>3</sub>, flooding and after-ripening. The highest seed-germination percentage was achieved from physical scarification (66%), while chemical scarification had a comparatively low effect (35%). KNO<sub>3</sub> could induce the seed germination (61%) in 0.02 M concentration. waterlogging resulted in 60% germination, and the seeds survived in the flooding conditions for more than 90 days. Constant temperature increased the seeds' germination and promoted its percentage (37%). Soil burial had a positive effect on the germination of *L. latifolium* during the first 30 days of warm conditions, with germination rates of 34.5% for 1cm soil-burial depth, and 26% for 10 cm. Increasing GA<sub>3</sub> concentration to 20 ppm resulted in 29% germination; greater GA<sub>3</sub> concentrations did not increase the germination percentage significantly.

**Keywords:** dormancy-breaking, chemicals, afterripening, flooding, *Lepidium latifolium*

### Introduction

Perennial pepperweed (*Lepidium latifolium*) is known or thought to be an invasive plant (Reynolds & Boyer, 2010). It is native to western Asia and southeastern Europe, and has moved through Europe to Norway and east to the western Himalayas. It has been introduced to Australia, Mexico and much of the U.S (Reynolds & Boyer, 2010).

In general *L. latifolium* is the most problematic in riparian areas and wetlands in the U.S. However, in Iran it is a common winter weed in orchards, and also is a problem in wheat and other field crops (Shimi & Termeh, 2004). *L. latifolium* is a highly competitive weed, and infestations can cause severe reductions in crop yield (Young et al., 1995). *L. latifolium* reproduces from seeds, creeping roots and semi-woody crowns. Each mature *L. latifolium* plant has the capacity to produce thousands of seeds each year (Howald, 2000).

Weed control is necessary to achieve higher yields, as weeds compete with crops for water and nutrients, causing significant yield reduction (Olorunmaiye & Olorunmaiye, 2009; Bijanzadeh et al., 2010). Seed germination is a key event in determining the success of a weed in an agricultural ecosystem (Cousens & Mortimer, 1995). Therefore, knowledge about the influence of climatic and edaphic factors on germination is essential to implement a rational strategy of weed management (Baskin et al., 2003). Dormancy is a condition within the seed that prevents germination in a viable seed, even though the environmental conditions are suitable (Baskin & Baskin, 1998). Seed dormancy and germination are complex developmental processes that are regulated by a variety of endogenous and environmental signals. For instance, dormancy in many

species has a seasonal variation (Milberg & Andersson, 1997), which is related to seasonal temperature changes (Hilhorst & Karssen, 1992).

For many weed species, particularly those that produce persistent seed banks, the seasonal pattern of emergence under field conditions is mainly controlled by seasonal changes in the dormancy status of buried seeds (Batlla & Benech-Arnold, 2007). In summer annual species, cold stratification during winter weakens dormancy, thus enabling seeds to germinate in spring. In contrast, high temperatures during summer induce dormancy, and seeds do not germinate in late summer or early autumn (Baskin & Baskin, 1998). In the case of winter annuals, high summer temperatures promote the full loss of dormancy, while low winter temperatures may wholly or partially, depending on the species, prevent loss of dormancy (Baskin & Baskin, 1998). Understanding the temporal patterns in seed dormancy is important for predicting the time of weed germination and emergence in crop fields. To extend the weeds' seedling emergence, modelled data is needed on the changing dormancy status of seeds that had been stored both in a laboratory and natural seed-bank condition (Cardina & Sparrow, 1997).

The seed-germination stage and temporal changes in dormancy of *L. latifolium* in relation to environmental and chemical regulation factors is still not well understood. Thus, this study has aimed to determine the effects of storage environment, storage duration and chemicals on changing seed-dormancy status and germination of this species.

**Table 1.** Average minimum air temperature, maximum air temperature and precipitation (mm) during the experiments

Month	Average minimum air temperature(°C)	Average maximum air temperature(°C)	Precipitation (mm)
August 01-September 01, 2009	20	37.6	0
September 01-October 02, 2009	15.53	31.94	0.9
October 02-November 01, 2009	9.71	24.58	0.12
November 01-December 01, 2009	4.52	16.48	30.6
December 01-December 31, 2009	0.03	10.32	43.00
December 31, 2009-January 30, 2010	0.58	14.19	14.3

## Materials and methods

### Plant materials

#### Seed collection

Samples of the mature seeds were collected from orchards in Isfahan province, Iran, in mid-June 2009. After cleaning, all seeds were stored in paper bags in a refrigerator ( $-20 \pm 1^\circ\text{C}$ ) until used in the experiments. The number of seeds used in the each experiment was determined by the surface area of the study unit (Petri dishes); adequate numbers (50 seeds in every Petri dish) were used to evaluate the percentage of germination.

#### General procedure

In all experiments, the seeds were surface sterilized in 1% sodium hypochlorite solution (household-bleach solution) for five seconds, rinsed three times in sterile distilled water and placed on a layer of filter paper in a Petri dish. The filter paper was moistened with distilled water or test solution, according to the specifications for each experiment. All Petri dishes were covered with plastic film to reduce loss of water, placed in germination incubators (model Arvin Tajhiz Espadana, Iran) for 14 days, and adjusted to  $22^\circ\text{C}$ . A cycle of 12 hours of light and 12 of darkness was maintained in these incubators throughout the experiments. Although germination was usually complete after a week to 10 days of incubation, the seeds were stored for 14 days in the incubator. The seeds were considered to have germinated when radicals emerged from the seed coat. Germination tests were performed using four Petri dishes for each solution (each containing 50 seeds). Mean germination percentage and SE were calculated for each germination test.

#### Effect of after-ripening

After-ripening patterns were investigated using variations in three germination conditions:

#### Effect of burial depth

Freshly matured seeds were placed in polyethylene mesh bags and buried at depths of 1 and 10 cm in sandy soil between August 1, 2009 and January 30, 2010. Meteorological conditions during the experiment were characterized by average maximum and minimum air temperatures and total monthly precipitation during the experiment period (Table1). Each bag was exhumed every 30 days and the seeds were placed in Petri dishes to germinate, as described above. The other procedures in the experiment were conducted as described above.

#### Effect of dry storage

To evaluate the effect of dry storage over time on dormancy, freshly matured seeds were placed in transparent, sealed Petri dishes and dry-stored at a constant temperature ( $20^\circ\text{C}$ ) for six months. Every 30 days the lid was removed and seeds were allowed to germinate in accordance with the general procedure.

#### Effect of cold stratification

Freshly matured seeds were wet-stored at ( $5^\circ\text{C}$ ) for six months and allowed to stratify in refrigerator. As with the dry storage, every 30 days seeds were allowed to germinate in accordance with the general procedure.

#### Effect of flooding

Irrigation and flooding are common practices in agricultural systems; seeds in orchards and fields must be able to germinate at low oxygen concentrations. To evaluate this effect, freshly matured seeds were placed in polyethylene mesh bags and buried in plastic pots (30 cm diameter  $\times$  30 cm height) filled with sandy soil. The pots were maintained under saturated or flooded conditions by applying water as required. This experiment was conducted in a greenhouse. During the first fifteen days of the experiment, seeds were placed in Petri dishes to germinate every three days. Subsequently, seeds were placed in Petri dishes to germinate every fifteen days. The other procedures were in accordance with the general procedure.

#### Effect of chemical solutions

##### Influence of $\text{GA}_3$

The effect of  $\text{GA}_3$  on embryo growth of freshly matured seeds was investigated. A range of  $\text{GA}_3$  concentrations (10, 20, 50, 100, 150 and 200 ppm) were prepared, and 3 ml of solution was added to the filter paper in each Petri dish. The effects of combining either after-ripening or cold stratification with  $\text{GA}_3$  were also investigated. Seeds were stored at  $5^\circ\text{C}$  or  $20^\circ\text{C}$  for six months, or buried in soil at depths of 1 or 10 cm for four months, then placed into Petri dishes on filter papers soaked with 3 ml of different concentrations of  $\text{GA}_3$  solution (10, 20, 50, 100, 150 and 200 ppm). The other procedures were followed in accordance with the general procedure. Solution was added to the Petri dishes as necessary during germination to keep the filter paper moist.

##### Influence of $\text{KNO}_3$

To determine the effect of potassium nitrate on seed germination, freshly matured seeds were placed into Petri

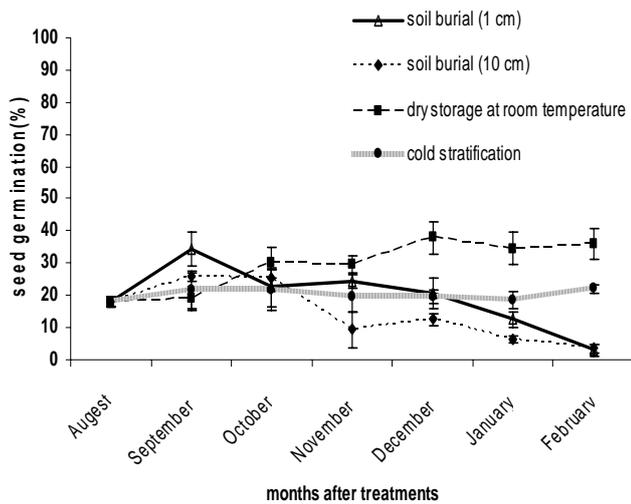


Fig.1 effects of different incubation treatments and time on *L. latifolium* seed germination

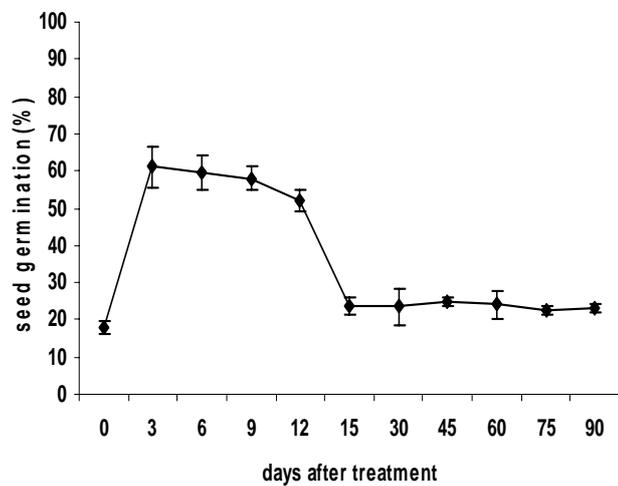


Fig.2 effects of flooding time on *L. latifolium* seed germination

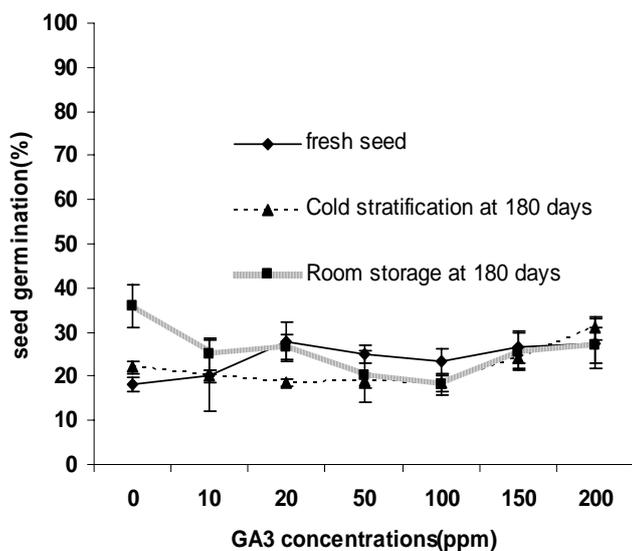


Fig 3. Effects of different GA3 concentrations on *L. latifolium* seed germination

dishes on filter papers soaked with 3 ml of different concentrations of  $KNO_3$  solutions (0.2, 0.02, 0.002 and 0.0002 M). Solution was added to the Petri dishes as necessary during germination to keep the filter paper moist. The other procedures were followed in accordance with the general procedure.

### Effect of scarification

To evaluate the effect of scarification on the germination of freshly matured seeds, two experiments were conducted. The first experiment was carried out to investigate the effect of physical scarification on germination. Seeds were rubbed with sandpaper. In the second experiment, the seeds were chemically scarified with highly concentrated (97%)  $H_2SO_4$  at different time intervals (5, 10, 15, 30 and 60 seconds). The seeds were washed in running tap water for five minutes before placing them in the dishes. The other procedures were similar to the general procedure.

## Results

### Effect of after-ripening

#### Effect of burial depth on dormancy-breaking

Table 1 shows the air temperatures and rainfall during seed burial. Fresh seeds of *L. latifolium* exhibited negligible germination. Burial had a positive effect on the germination of *L. latifolium* at both depths only in the first 30 days. The 1 cm burial resulted in a higher germination rate (34.5%) than the 10 cm burial (26%) (Figure 1). In general, between September and February the germination rate of buried seeds declined rapidly as temperature dropped in autumn and winter (Figure 1 and Table 1). For example, the germination rate declined from 34.5% and 26% (in September) to 3% and 3.5% (in February) for seeds buried at 1cm and 10 cm respectively.

#### Effect of dry storage on dormancy-breaking

Constant temperature increased the germination percentage of the seeds to 37% (Figure 1).

#### Effect of cold stratification on dormancy-breaking

Cold stratification had no significant effect on dormancy, and the maximum germination rate was 22% over the 30 days of cold stratification (Figure 1).

#### Effect of flooding on dormancy-breaking

Flooding for three days gave a high germination percentage (60%), and a considerable number of the seeds survived in flooding conditions for more than 90 days (Figure 2).

#### Effect of chemical solutions on dormancy-breaking

##### Influence of $GA_3$ on embryo growth

Increasing  $GA_3$  concentration up to 20 ppm promoted the germination rate (29%). Increasing the concentration of  $GA_3$  resulted in no significant increase in germination rate compared with 20 ppm (Figure 3). Wet storage at (5°C) for 180 days at different concentrations of  $GA_3$  improved germination rates. The maximum germination occurred at a 200 ppm concentration of  $GA_3$  (31%). Germination was

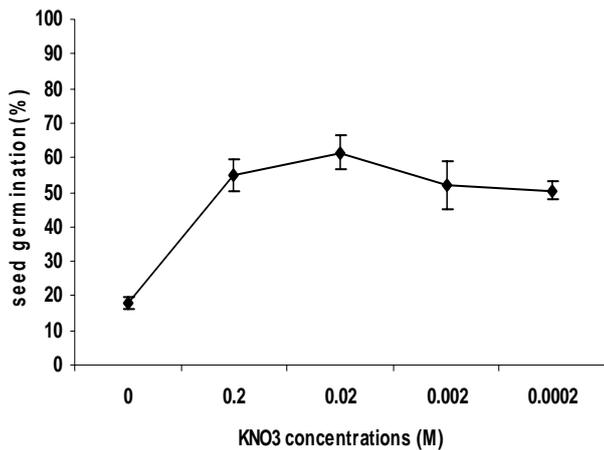


Fig.4 effects of different KNO<sub>3</sub> concentrations on *L. latifolium* seed germination

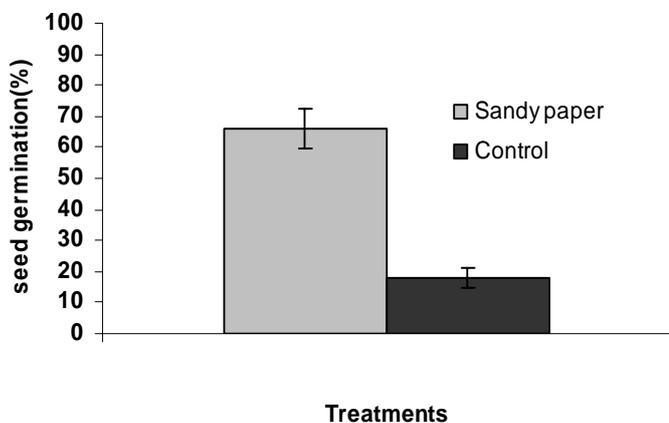


Fig 5. Effects of physical scarification on seed dormancy breaking of *L. Latifoli*

minimally affected by changes in GA<sub>3</sub> concentration at a constant temperature (20°C) in dry conditions. Maximum germination (25%) occurred at a 100 ppm concentration of GA<sub>3</sub> (Figure 3).

#### Effect of KNO<sub>3</sub> on germination

Potassium nitrate induced germination. The highest germination rate (61%) was obtained using a 0.02 M concentration of KNO<sub>3</sub>; at higher concentrations, the germination rate decreased (Figure 4).

#### Effect of scarification on dormancy-breaking

Physical scarification greatly influenced the germination rate (66%) (Figure 5). Chemical scarification with H<sub>2</sub>SO<sub>4</sub> gave a high germination percentage, which increased with increasing duration of scarification with H<sub>2</sub>SO<sub>4</sub>. The highest germination rate (35%) was observed after a 60-second exposure (Figure 6).

#### Discussion

Treatments that included burial in 1 and 10 cm and dry storage at 20 °C enabled an increasing proportion of the weed

seeds to germinate. However, after six months, 60% to 70% of weed seeds still had not germinated (Figure 1).

Cold stratification for 180 days at 5°C had a minimal effect on germination. This result agrees with other research into the germination ecology of crucifer weeds, which showed that the germination of these weeds is temperature-dependent on warm stratification (Baskin et al., 2004).

Outdoor burial, especially for seeds at 1 cm depth exhumed after 30 days, resulted in more germination than cold stratification. These results suggest that dormancy was easily broken following the warmer, drier conditions (see Table 1). This is consistent with Baskin et al.'s studies (2004) on seed-dormancy breaking for *Capsella bursa-pastoris* and *Descurainia Sophia*, species that originate in high northern latitudes. The more rapid decline in dormancy of weed seeds buried 1 cm deep might be due to greater temperature fluctuations close to the soil surface compared to those at 10 cm (Stoller & Wax, 1973), and helps explain the long-term survival of *L. latifolium* seeds that are buried in soil by tillage (Batlla & Benesch-Arnold, 2007). These differences indicate that burial depth is an important source of variation that must be considered to accurately predict changes in dormancy and potential emergence of *L. latifolium*. The low germination rates for *L. latifolium* seeds buried in soil for the last two months of fall and the entire winter are attributed to decreasing temperature, which prevents the breaking of dormancy. Baskin et al.'s (2004) study of the germination ecology of *Descurainia Sophia* found similar results. The current study of *L. latifolium* suggests that the annual minimum temperature observed in the middle of January caused secondary dormancy. Low germination in fall and winter is a natural mechanism in *L. latifolium* that preserves the plant in harsh weather and prepares the seeds for germination in late winter and early spring.

The relatively small effect of different GA<sub>3</sub> concentrations on the fresh mature seeds, as well as the inconclusive results of combining different GA<sub>3</sub> concentrations with after-ripening and cold stratification, indicated that *L. latifolium* dormancy may not be related to seed maturity, and that coat-imposed dormancy may inhibit germination. The *L. latifolium* seeds were released from dormancy by both physical and chemical scarification, demonstrating that this species has hard-seed/physical dormancy. Similarly, physical scarification in *Mimosa pudica* stimulates the germination and releases the seeds from dormancy (90.3%) (Chauhan & Johnson, 2008).

The seeds of *L. latifolium* germinated successfully after being placed in saturated or flooding conditions. Our result showed that flooding conditions eliminated the seeds' white coats, thus removing one of the main reasons for failure to germinate. Another possible reason for dormancy-breaking in flood conditions is the low concentration of oxygen surrounding the seeds, which may terminate dormancy. Similarly, *Crassocephalum* and *Ischaemum rugosum* seeds showed 89% and 98% germination respectively in flooding conditions. (Bakar & Nabi, 2003; Nakamura & Hossain, 2009). According to these results we suggest the conclusion that *L. latifolium* seeds possess coat-imposed dormancy.

*L. latifolium* seeds were released from physical dormancy in 0.2 and 0.02 M concentration of KNO<sub>3</sub>; this could be because of the effect of KNO<sub>3</sub> on the seed membrane. Other research has shown that nitrogenous compounds play a regulatory role in breaking seed dormancy in some plant species (Bethke et al., 2004; Li et al., 2005; Rouhi et al. 2010).

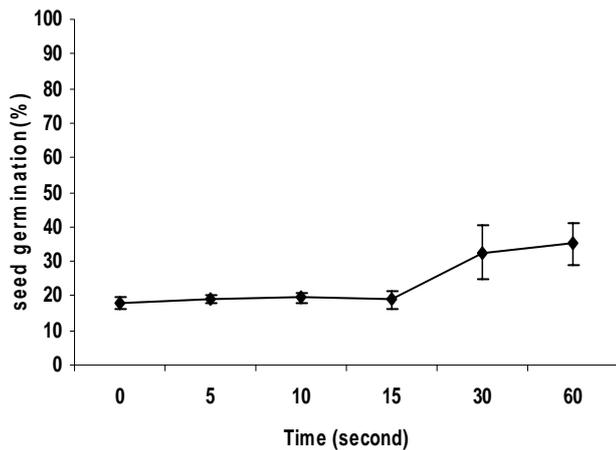


Fig.6 effects of diffents time of chemical scarification with H<sub>2</sub>SO<sub>4</sub> on *L. latifolium* seed germination

### Implications for weed management

The results of many studies highlight the significant efficacy of winter flooding in reducing weed infestations in fields and orchards (Fogliatto et al., 2010). Results of this and other studies (Blank et al., 2002) showed that *L. latifolium* seeds can survive and germinate under flooding conditions, and that the low oxygen concentration may even terminate dormancy in these seeds; therefore it should be noted that this cultural practice is not very effective in reducing *L. latifolium* seed density.

Manipulation of fertilization is a cultural practice to reduce weed competition in crops (Kirkland & Beckie, 1998). Kirkland and Beckie's research (1998) demonstrated that nitrogen fertilizer breaks dormancy in *L. latifolium*, and thus may directly affect weed-infestation densities. Therefore, manipulation of nitrogen has the potential to protect crop yield, and also may contribute to long-term reductions in *L. latifolium* populations.

These results would suggest that *L. latifolium* seeds are stimulated by hot temperatures to release their dormancy in late summer and beginning of the fall and to be ready for germination amongst cool-season crops such as wheat and barley. Only a few seeds of *L. latifolium* become non-dormant during mid- to late fall and winter, while most remain dormant. These results could help farmers manage *L. latifolium* in cool-season crops by shifting their planting date from early fall to mid- or late fall if possible.

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