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Study of dormancy-breaking and optimum temperature for germination of Russian knapweed (*Acroptilon repens* L.)

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ABSTRACT

This work aimed to examine dormancy breaking and optimum temperature for germination of Russian knapweed seed (*Acroptilon repens* L.). To examine germination characteristic of this weed, several experiments were conducted. First experiment was a factorial with 3 replications to break seed dormancy of this weed. First factor was KNO_3 and Second factor was duration of storage in concentrated sulfuric acid with 6 periods of scarification. Second experiment was conducted as a completely randomized design with 26 treatments (constant temperature including 5, 10, 15, 20, 25, 30 and 35°C in both light or darkness situations and fluctuate temperatures including 2/10, 7/15, 12/20, 17/25, 22/30, 27/35°C and fluctuate temperatures including 0/10, 5/15, 10/20, 15/25, 20/30 and 25/35°C to determine optimum temperature of germination. In order to break of seed dormancy, 20 minutes treatment with concentrated sulfuric acid was required and KNO_3 (1 and 3% v/v) treatments did not have significant effect on seed germination. The Optimum temperature for germination was 20/30˚C (8 hours dark/16 hours light) with 42% germination. Seed germination was strongly influenced by temperature. Light did not play a crucial role on seed germination of this weed. Therefore Russian knapweed seeds were not photoblastic and fluctuating temperature increased seed germination. The above characteristics are very important in making Russian knapweed an invasive weed.

Keywords: Constant temperature, Fluctuating temperature, Russian knapweed, Seed germination, Seed dormancy

INTRODUCTION

Russian knapweed (*Acroptilon repens* L.) is a perennial weed in the family Asteraceae. It has been reported that this perennial weed can decrease the yield of dryland farming up to 80%. It also can be reduce the quality of crop plant and reduce the quality of farm land. Some characteristics of germination in this plant were evaluated by researchers (Selleck 1964). Seed germination is a key event in determining the success of a weed in an agroecosystem because of it is the first stage of weed competition in an ecological niche (Cousnse, Mortimer 1995). Seed dormancy is common in wild plants (especially in weeds), where it may ensure the ability of a species to survive natural catastrophes, decrease competition between individuals of the same species, or prevent germination out of season (Finkelstein et al. 2008). Dormant seed does not have the capacity to germinate in a specified period of time under any combination of normal physical environmental factors that are otherwise favourable for its germination, i.e. after the seed becomes nondormant (Finch-Savage, Leubner 2006). All types of dormancy impose a delay between seed shedding and germination, but the underlying causes may vary. This variety has been classified in terms of whether germination is inhibited owing to embryonic immaturity or physical or physiological constraints, and whether the controlling structure or substances are embryonic or in the surrounding tissues of the seed, i.e., coat imposed (Finch-Savage, Leubner 2006). Seed dormancy has been further negatively categorized in terms of the requirements for release from this block, such as disruption of the seed coat (scarification), a period of dry storage (after-ripening) or moist chilling (stratification), or exposure to light (Rouhi et al. 2010). Appling sulfuric acid is one of the scarification methods for stimulating the seed germination such as *Centaurea repens* (Selleck 1964); *Capparis spinosa* (Sozzi, Chiesa 1995) and *Cyclocarya paliurus* (Fang et al. 2006).

Sixtus et al. (2003) found that sulfuric acid and sand paper treatment increased germination of *Ulex europaeus* seeds, while hot water treatment did not affect seed germination. Aliero (2004) reported that use of hot water, sulfuric acid and sand paper scarification affected *Parkia biglobosa* seed dormancy-breaking. Seeds of Russian knapweed have a dormancy and breaking dormancy for germination is essential (Selleck 1964).

Various environmental components, such as temperature, light, pH, and soil moisture, have been known to influence weed seed germination (Chachalis and Reddy 2000; Koger et al. 2004). Temperature is the primary environmental factor regulating both seed dormancy and germination (Wang et al. 2004).

Little information exists about the effect of environmental factors on germination of Russian knapweed. Knowledge about Russian knapweed germination would help in estimating the potential for its spread to new croplands. Therefore, the objective of this study was to find out the best treatment for dormancy breaking and to determine the effect of temperature (constant and fluctuating) on Russian knapweed seed germination.

MATERIALS AND METHODS

First experiment

This study was carried out at the Weed Research Department, Plant Pest and Diseases Research Institute of Tehran, Islamic Republic of Iran. Seeds were received from Firuz-kuh, Tehran province, Islamic Republic of Iran. Germination percentage of the Russian knapweed was studied using following treatments: KNO_3 levels including: 1 and 3% (v/v). Sulfuric acid durations for 5, 10, 15, 20, 25 and 30 minutes were done. Seeds were incubated in 10°C for 18 month before treating with KNO_3 or Sulfuric acid.

Seed treatments

Chemical scarification: Seeds of Russian knapweed (*Acroptilon repens* L.) were soaked in sulfuric acid (98% v/v) for 5, 10, 15, 20, 25, and 30 min respectively, and then washed thoroughly by distilled water three times before transfer to the germination test process. Likewise, similar to previous treatment, the incubated seeds were treated by 1, and 3% (v/v) KNO_3 for 7 days.

Germination tests

Three replicates of fifty seeds were placed evenly in a 9-cm-diam Petri dish containing two pieces of No. 2 filter paper. The filter paper was moistened with 7 ml deionized water. The Petri dishes were sealed with parafilm to avoid moisture loss. Seeds were allowed to germinate at 20-30±1◦C (8 hours dark/16 hours light) for 7 days. Germination was considered to have occurred when the radicals were 2mm long. Germination percentage was recorded every 24 h for 7 days.

Statistical analysis

The statistical design was a completely randomized design in a factorial arrangement with two factors. Data for abnormal germination percentage were subjected to arcsine transformation before analysis of variance. Statistical analysis was carried out using MSTAT-C program (Michigan State University). Mean comparison was performed with Duncan's Multiple Range Test at the 1% level of significance and graph drawing was performed by means of Excel 2003 software.

Second experiment

This experiment was done for comparing the effects of constant and fluctuate temperature as well as light and dark treatment on the germination percentage of Russian knapweed seeds. This experiment had four sub experiments which were arranged in a randomized completely block design with 8 replication and 50 seeds per replicate with following treatments:

a) Seven treatments include complete light and constant temperature of 5, 10, 15, 20, 25, 30 and 35°C

b) Seven treatments include complete darkness and constant temperature of 5, 10, 15, 20, 25, 30 and 35°C

c) Six treatments include 16 hours light and 8 hours darkness in fluctuate temperature of 2/10, 7/15, 12/20, 17/25, 22/30 and 27/35°C

d) Six treatments include 16 hours light and 8 hours darkness in fluctuate temperature of 0/10, 5/15, 10/20, 15/25, 20/30 and 25/35°C

in all of the experiments, seeds were first treated in sulfuric acid (98% v/v) for 20 minutes. The seeds were surface sterilized by soaking in 10% sodium hypochlorite (NaOCl) for 1 min and subsequently rinsed thoroughly with sterilized water prior to applying any treatment.

Data for abnormal germination percentage were subjected to arcsine transformation before analysis of variance. Statistical analysis was carried out using MSTAT-C program (Michigan State University). Mean comparison was performed with Duncan's Multiple Range Test at the 1% level of significance and graph drawing was performed by means of Excel 2003 software.

RESULTS AND DISCUSSION

First experiment

Analysis of variance showed that the effect of sulfuric acid durations after stratification in 10°C for 18 month (data for preliminary experiment from stratification treatment not shown) was significant (Table. 1). Regarding to results, the highest germination percentage was obtained for the use of sulfuric acid for 20 min. However it was not different significantly to use of sulfuric acid for 15 min (Fig1.). In contrast, the lowest germination was detected for treatment with sulfuric acid for duration of 30 min. this treatment was not different significantly to use of sulfuric acid for duration of 5 min (Fig1.). In longest duration (30 min with H_2SO_4) germination was zero. Presumably, acid penetrated into the seed structure and tissues were destroyed by the sulfuric acid. Rahman et al. (1999) noted a significant reduction of germination after treatment with 98% sulfuric acid for 30 minutes compared to little effect after 10 minutes. Farhoudi et al. (2007) reported that the highest percentage of germination was observed in the case of 90% sulfuric acid applied for 15 minutes, but a 20 minute treatment resulted in abnormal seedlings with reduced growth. KNO_3 treatment in both levels failed to stimulate the germination but interaction between sulfuric acid and KNO_3 treatment was significant (Table. 1). Potassium nitrate has been used for many years, with positive studies beginning in the 1980's but it often increased the germination of photo-dormant seeds (Shanmugavalli et al. 2007). Similar results were reported in *Tulipa kaufmanniana* by Rouhi et al. (2010). It seems that Russian knapweed seeds have a physiological dormancy because of PD is the abundant dormancy class 'in the field' and it is the major form of dormancy in most seed model species 'in the laboratory', including *Helianthus annuus* and *Lactuca sativa* in Asteracea family (Finch-Savage and Leubner, 2006). The great majority of seeds have nondeep PD (Baskin *and* Baskin, 2004). Embryos excised from these seeds produce normal seedlings; dormancy can be broken by scarification, after-ripening in dry storage, and cold or warm stratification (Finch-Savage and Leubner, 2006). In our experiment, application of sulfuric acid (98% v/v) for 20 min stimulated the germination of this species but after stratification (data for stratification not shown), it can be report that Russian knapweed seeds have a nondeep physiological dormancy.

Table 1. Analysis of evaluation variance of germination percentage of studied via dormancy breaking treatment in Russian knapweed

Mean of squares		
SOV	df	final germination percentage
KNO_3 (A)	1	10.028 ^{ns}
Duration of sulfuric acid (B)	5	49.294**
KNO3 * Duration of sulfuric acid (AB)	5	0.628 ^{ns}
Error	24	5.889

ns,**, Respectively non significant and significant of 1 of probability

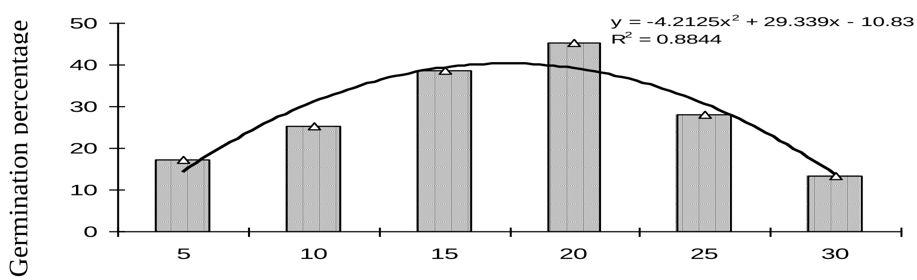

Fig 1. Role of sulfuric acid durations on the response of dormant perennial Russian knapweed seeds

Second experiment

Seed germination was affected by changing in temperature (Fig2.). In complete light situation, seed germination was better than complete darkness situation in all of temperature except of 5 and 10°C. In complete darkness and complete light situations seeds were no germinated in 5 and 10°C. Germination variation curves following complete darkness and complete light were rather similar, but maximum amount of germination from complete darkness was observed in 30°C whereas maximum amount of germination from complete light was observed in 25°C (Fig2.). Germination of Russian knapweed seeds in both situations (light and darkness) indicates that light is not a requirement for germination. Indifferent behavior to presence or absence of light is a positive factor which spreads this species rapidly in any region. Similar results were also reported in *Cirsium arvense* (Wilson, 1979) and *Bidens pilosa* (Reddy et al. 1992). In 15 and 20°C germination improved by complete light rather than complete darkness (Fig2.). Some researchers believed that in low temperatures, light can have a role of the temperature (Benvenuti and Macchia, 1995). While germination was clearly better when fluctuating temperatures were provided (Fig3.), constant temperatures per se during the incubation of seeds in germinator did not increase germination (Fig2.). Germination progress curves following the application of sulfuric acid for in both fluctuating temperatures (with an 8°C interval and 10°C interval) were similar, but fluctuating temperatures with a 10°C interval were more effective (Fig3.B). In temperature fluctuations with an 8°C interval, the maximum germination percentage (36%) occurred at 22/30 °C (Fig2.A). At 2/10°C, no seed germination occurred (Fig2.A). Simultaneously, in temperature fluctuations with a 10°C interval, the maximum germination percentage (42%) detected at 20/30 °C (Fig3.B) but it was better than from maximum germination at 22/30 °C (Fig3.). At 0/10°C, no seed germination occurred (Fig3.B). In many weed species, seed germination improved by fluctuating temperatures (Jain and Singh, 1989; Esno et al. 1996; Webster et al. 1999). For example seed germination of *Amaranthus quitensis* and *Setaria viridis* increased from 30% in constant temperatures to 90% in fluctuating temperatures (Vitta and Faccini, 2005).

4. Conclusion

The dormancy of Russian knapweed seed was released by using of sulfuric acid treatment after stratification, but KNO3 levels had no effect. Light did not affect germination. Seeds germinated equally in darkness and light, hence it could be concluded that Russian knapweed seeds were not photoblastic. In second experiment for finding the optimum temperature, maximum germination for this species (42%) was reached at 20/30 °C, so fluctuating temperatures was better than constant temperatures.

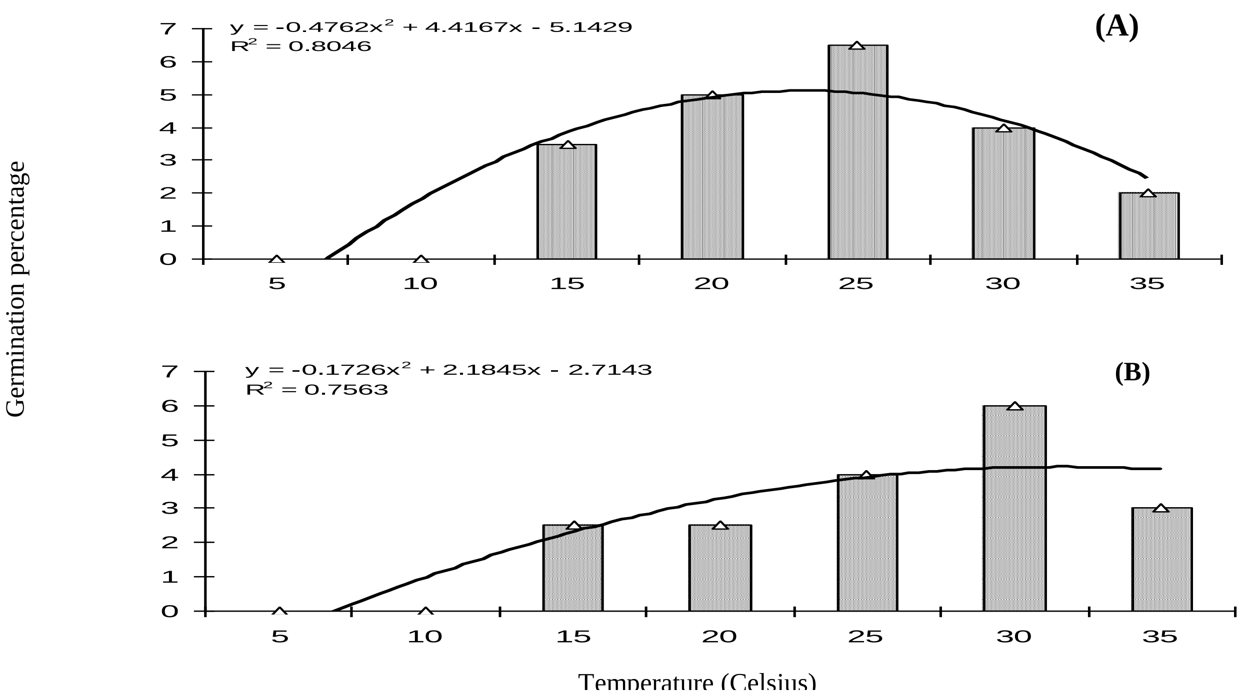

Fig2. Role of constant temperatures on the response of Russian knapweed seed germination, A) complete light, B) complete darkness

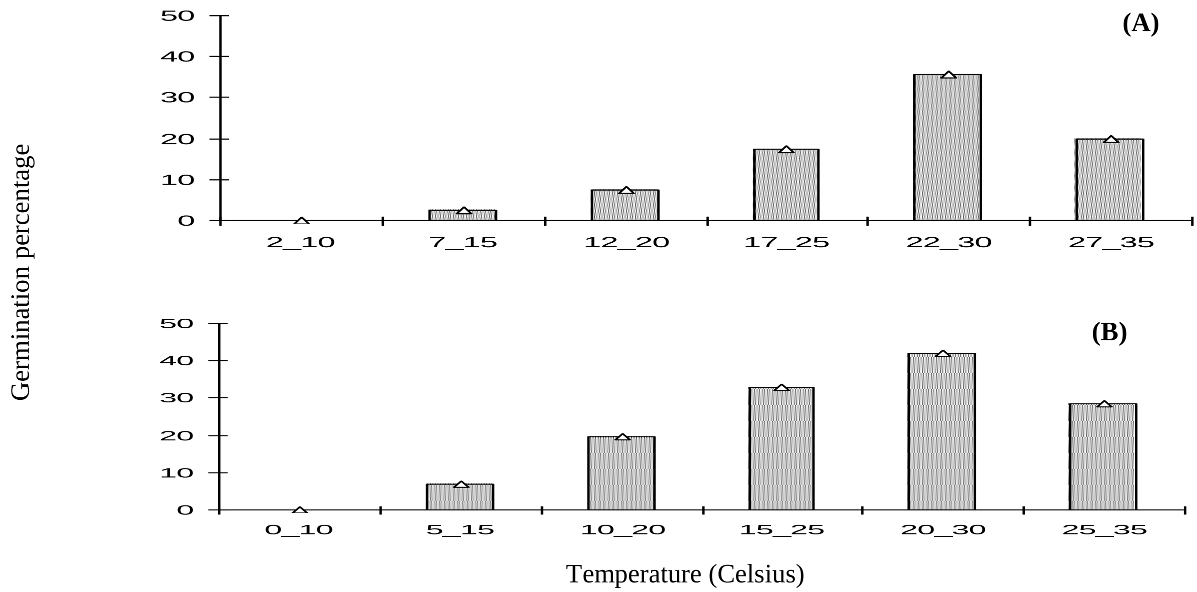

Fig3. Role of fluctuating temperatures on the response of Russian knapweed seed germination, A) fluctuation with an 8°C interval, B) fluctuation with a 10°C interval

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