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Germination of Spotted Spurge (*Chamaesyce maculata*) Seeds in Response to Different Environmental Factors

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Spotted spurge, a C_4 species, is a summer annual weed, introduced to the Golestan province of Iran in 2006. A series of laboratory experiments were conducted at the Faculty of Agriculture, Ferdowsi University of Mashhad, Iran, to evaluate the influence of environmental factors on seed germination of spotted spurge. Seeds germinated over a constant temperature range of 25 to 45 C with a 14-h photoperiod and at 30 to 50 C in continuous darkness. Seeds germinated at alternating day/night temperature regimes of 25/15 to 45/35 C, with maximum germination ($>$ 80%) at alternating day/night temperatures of 35/25, 40/30, and 45/35C. Germination increased from 23.5 to 98% as water potential increased from -0.6 to 0 MPa (control). Germination was $>$ 85% at sodium chloride (NaCl) concentrations up to 80 mM, with no germination at 160 mM NaCl. Germination was not affected by pH, and it was $>$ 95% at pH ranging from 4 to 9. The germination of seeds submerged in water for a period less than 3 wk was $>$ 60%, whereas no germination was observed after 9 wk of submergence in water. The results of our study could help to develop effective management strategies for this species. The results also suggest that spotted spurge could invade most tropical regions of Iran.
Nomenclature: Spotted spurge, *Chamaesyce maculata* (L.) Small EPHMA; soybean, *Glycine max* (L.) Merr.
Key words: Light, longevity in water, salinity stress, temperature, water potential.

Spotted spurge is a C_4 summer annual herb from the Euphorbiaceae family, and it is native to the eastern United States (Pahlevani and Akhani 2011). This weed contains a milky, sticky sap that is toxic to some animals, and it can cause contact dermatitis in human and animals (Molinar et al. 2009; Young 2012). Spotted spurge seeds are dispersed by an explosive mechanism (autochory) in summer, and those produced in autumn are dispersed by ants (myrmecochory) (Ohnishi and Suzuki 2008). Wet seeds are adhesive, hence, they easily stick to animal feet, bird feathers, human feet, and vehicles and, thus, are able to disperse widely (Anonymous 2012).

Spotted spurge was collected and reported in the north of Iran in 2006 for the first time (Nasseh et al. 2006). Soybean fields in Golestan province were infested by this weed, where it emerged simultaneously with soybean. Spotted spurge generally has procumbent stems, but stems can grow erect when competing for light with other plants. It reduces crop yield and interferes with mechanical harvesting. Humidity from the spotted spurge canopies is transferred to soybean grains, and therefore, delays their drying process. The reasons for the spread and survival of the weed in Golestan province could be its adaptability to local climate conditions and a lack of efficient herbicides for its control (Savari-Nejad 2009).

Hope (1982) reported that spotted spurge contains many of the characteristics required for the longevity of a weedy plant to survive in monoculture cropping systems. These characteristics included (1) broad geographic range, (2) growth adaptability to different temperatures faced within this range, (3)superiority for mesophytic habitats, (4) many seeds produced per plant, (5) sporadic germination throughout the normal growing season, and (6) dormancy to ensure viable seed maintenance in unfavorable environmental conditions. Bararpour et al. (1994) reported that spotted spurge densities of 5, 10, and 50 plants m^{-1} in rows reduced cotton (*Gossypium hirsutum* L.) seed yield by 47, 57, and 85%, respectively.

When a species is introduced to a new area, seed germination is one of the most critical phases in plant development because it is when the weed can compete for an ecological niche and influence the success of an annual plant (Forcella et al. 2000). Each plant species requires a specific range of

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environmental conditions for germination (Lu et al. 2006; Singh et al. 2012). The environmental factors governing seed germination in arable soils are temperature, light, water potential, and pH (Chauhan and Johnson 2009, 2010; Chauhan et al. 2006a). However, there is very limited information available regarding the germination characteristics of spotted spurge. For instance, Baskin and Baskin (1979) reported that spotted spurge requires high temperatures of 30 to 35 C, whereas Hope (1982) stated the optimum germination percentage at 25 to 30 C.

Spotted spurge has been problematic in current years in the north of Iran, and no information, to our knowledge, is available on the biology of this weed in the climatic conditions of this region. The information on seed ecology is crucial because it could help predict the probability of its dispersal in new areas in Iran, as well as adopt appropriate management methods for its control. The objectives of the present study were to evaluate the effects of constant and alternating temperatures, light, drought and salinity stresses, pH, and submergence on germination of this invasive species. This information provides a biological basis for understanding and estimating its invasive potential under future climate changes.

Materials and Methods

Seed Source. Spotted spurge plants were harvested from soybean fields of Kordkuy (36°79′N, 54°10′E) in Golestan province, Iran, with loam soil during the growing season of soybean in the summer of 2011. The plants were dried at room temperature (25 ± 2 C) for 7 to 10 d before collecting seeds, and seeds were cleaned to remove any extraneous plant or floral material using sieves. Seed viability and dormancy were tested immediately after seed collection using 100 seeds in four replicates. Germination percentage was > 90%, and there was no dormancy in the collected seeds. Seeds were stored in paper bags and placed in a black plastic and kept in the cold room at 4 C until initiation of the experiment. Experiments were started in the fall (November).

General Information. Four replicates of 25 seeds were placed in 70-mm-diam petri dishes on a layer of Whatman No. 1 filter paper moistened with 5 ml of distilled water or experimental solutions. Dishes were placed in sealed plastic bags to minimize water losses from evaporation. After that, dishes were placed in an incubator at a constant temperature of 35 C, until otherwise specified. The photoperiod was set at 14 h. The number of germinated seeds was counted 14 d after the start of the experiment. Seeds with a radicle of ≥ 2 mm length were considered germinated seeds. The following equation (Maguire 1962) was used to calculate the rate of germination:

$$Rs = \sum_{i=1}^{n} (Si/Di) \quad [1]$$

where *Rs* is the germination rate (germinated seeds d^{-1}), *Si* is the number of germinated seeds on day *i*, and *Di* is the number of days after the start of the experiment.

Effect of Constant Temperature and Light on Germination. Germination tests were conducted in incubators set at constant temperatures of 15, 20, 25, 30, 35, 40, and 45 C. Photoperiod was set at 14 h. For germination in complete darkness, the dishes were wrapped in two layers of aluminum foil, and germination was counted daily in a dark room with a safe green light. Fluorescent lamps were used to produce a photosynthetic photon-flux density of 120 μmol m^{-2} s^{-1}.

Effect of Alternating Temperature on Germination. Seed germination was evaluated at fluctuating day/night temperatures of 20/10, 25/15, 30/20, 35/25, 40/30, and 45/35 C. Difference between minimum temperature and maximum temperature in April to September during recent years in the region was about 10 C. Photoperiod was set at 14 h, and it coincided with the higher temperature.

Effects of Drought and Salinity Stresses on Germination. Germination, as affected by drought stress, was determined at osmotic potentials of 0, −0.2, −0.4, −0.6, −0.8, and −1.0 MPa, prepared by dissolving 0, 137.0, 199.0, 246.7, 286.9, and 323.3 g, respectively, of polyethylene glycol (PEG) 6000 in 1 L of distilled water (Michel and Kaufmann 1973). The effect of salt stress on spotted spurge germination was determined by incubating seeds in dishes containing sodium chloride (NaCl) solutions of 0, 10, 20, 40, 80, 120, and 160 mM.

Effect of pH on Germination. The effect of pH on spotted spurge germination was studied using buffer solutions with pH of 4 to 9. Solutions with pH

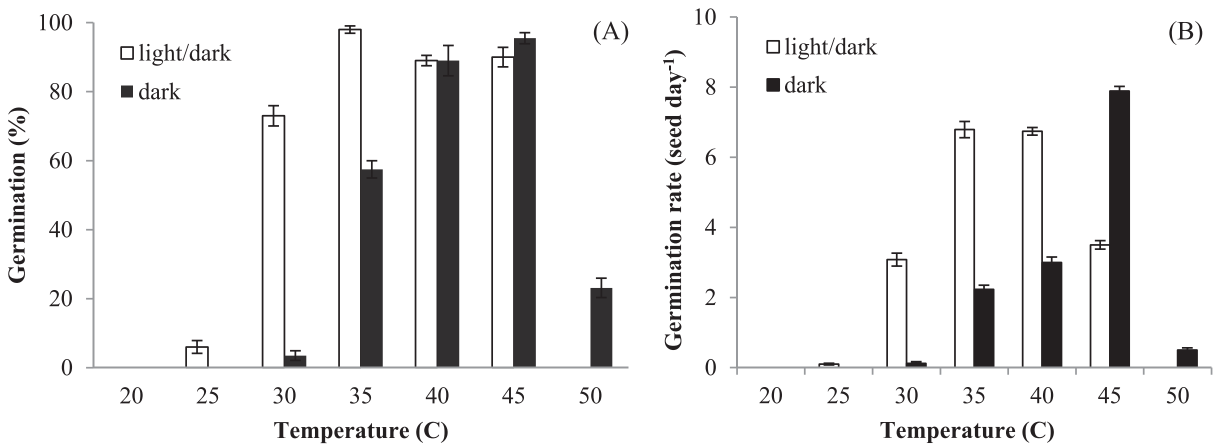

Figure 1. Effect of constant temperatures and light (light/dark and dark) on spotted spurge seed (A) germination percentage and (B) germination rate. Vertical bars represent standard errors of the means.

levels 4, 5, and 6 were prepared using 0.1 M potassium hydrogen phthalate, and solutions with pH 7 and 9 were prepared with 25 mM borax. Distilled water (pH 8) was used as a control. Buffer solutions were adjusted to the appropriate pH using 1 M NaOH or 1 M HCl.

Seed Longevity in Water. To investigate the seed longevity in water, the seeds were kept in 500-ml beakers containing tap water at ambient conditions of 25 ± 2 C. The water was changed daily. One hundred seeds were removed from the water every 7 d and placed in petri dishes for the germination test to study the viability of seeds in water. Viability of nongerminated seeds was tested with a 1% tetrazolium chloride solution. Seeds showing a pink to reddish color were considered viable.

Statistical Analyses. All laboratory experiments were conducted in a completely randomized design with four replicates. All experiments were repeated twice. The experimental runs were not statistically significant; therefore, the data from the two runs were pooled before further analysis. Data on the percentage of germination were transformed before statistical analysis to ensure homogeneity of variance. Transformation of data did not improve homogeneity; thus, ANOVA and regression analysis were performed on the nontransformed percentage of germination. The GLM procedure of Minitab (version 16) was used to assess significant differences among trials and treatments. Significant differences among treatment means were identified by the Fisher's protected LSD test at the 0.05 level of significance.

Regression analysis was used to evaluate the effect of drought and salinity stresses and seed submergence time on germination. Data were fitted to a functional three-parameter sigmoidal model using SigmaPlot 2008 (version 11.0). The applied model was

$$G = G_{\max}/\{1 + \exp[-(x - x_{50})]/G_{rate}\} \qquad [2]$$

where G is the total germination (%) at different concentrations of salt and osmotic potential or storage time in water, $G_{\max}$ is the maximum germination (%), x_{50} is the concentration or storage time in water required for 50% inhibition of the maximum germination, and G_{rate} indicates the slope.

Results and Discussion

Effect of Constant Temperature and Light on Germination. Temperature and light both significantly affected spotted spurge germination ($P < 0.001$). Seeds germinated over a temperature range of 25 to 45 C with a 14-h photoperiod and at 30 to 50 C in complete darkness. As temperature increased, both the percentages and the rate of germination increased and reached maximum at 35 C in light/dark and 45 C in complete darkness (Figure 1).

Hope (1982) reported that the optimum temperatures for spotted spurge germination were 25 to 30 C, and germination began at 15 C. In our study, seeds started to germinate at 25 C and maximum germination occurred at 35 C. Temperature is a good indicator of the time of year, and it is, therefore, implicated strongly in determining the timing of germination (Fenner and Thompson 2005). Seed germination of this weed coincides with the time of soybean seedbed preparation in the Gorgan region (June and July). In recent years, the average minimum temperatures in June and July in

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Table 1. Average maximum (max) and minimum (min) temperatures (C) in the observed region of Gorgan, Golestan Province, Iran, during 2006–2011.

	2006		2007		2008		2009		2010		2011	
Month	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min
Apr	21.4	11.9	19.0	10.6	24.4	12.0	18.6	8.9	19.7	11.2	21.8	11.3
May	26.9	16.0	28.4	15.3	27.4	15.4	26.5	15.9	28.6	16.7	26.5	17.1
Jun	33.5	22.2	31.5	21.4	30.5	20.2	32.6	20.8	35.4	23.1	31.7	21.9
Jul	32.4	24.1	33.0	22.6	33.3	23.7	33.7	24.2	35.9	25.6	36.4	25.1
Aug	36.3	24.0	34.8	24.0	35.1	23.8	30.2	22.0	35.2	24.1	31.7	23.6
Sep	30.6	20.2	31.6	19.8	31.0	20.8	28.7	19.3	31.8	20.0	28.8	19.6
Oct	23.4	12.7	26.5	13.8	24.0	14.6	26.3	14.4	25.1	16.1	22.4	12.9
Nov	18.3	8.0	18.9	8.5	18.1	7.8	18.0	8.6	22.7	8.6	17.9	7.7
Dec	12.1	4.0	13.2	3.1	12.7	3.7	15.6	6.2	18.5	5.6	12.4	2.9
Jan	15.0	3.1	8.1	−2.7	12.0	1.8	14.9	4.9	13.3	4.2	12.1	2.6
Feb	13.7	3.4	12.2	2.4	15.0	6.0	12.6	6.0	11.4	3.7	10.1	1.3
Mar	14.8	6.2	22.9	9.1	18.7	7.4	15.7	7.5	15.3	6.0	14.7	4.3

this region have been 21.6 and 24.2 C, respectively (Table 1). Consequently, the weed adapted itself to the new climatic conditions in this region.

In previous studies, maximum germination of other tropical species from the Euphorbiaceae family, such as wild poinsettia (*Euphorbia heterophylla* L.), nodding spurge [*Chamaesyce nutans* (Lag.) Small], cassava (*Manihot esculenta* Crantz), and texasweed [*Caperonia palustris* (L.) St. Hil.], also occurred at 35 C (Brecke 1995; Koger et al. 2004; Pujol et al. 2002; Serviss 1998). We also observed that the germination temperature range of spotted spurge is almost similar to *Amaranthus* species (Ghorbani et al. 1999; Guo and Al-Khatib 2003; Thomas and Wilcut 2006).

The interaction of light and constant temperatures had significant effects on the percentage (Figure 1A) and rate (Figure 1B) of spotted spurge germination ($P < 0.001$). In general, the effect of temperature on germination was dependent on the light regime. At the temperature regimes below 40 C, germination was greater (6 to 70%) in light than it was in complete darkness (Figure 1A). There was no significant difference in germination between light regimes at 40 and 45 C. No germination was observed at 50 C in the light/dark condition, but 23% germination occurred in dark. A seed may require light for germination at one temperature but may not require it at another one. For example, European white birch (*Betula pendula* Roth) seeds require light at low temperatures but not at high temperatures (Fenner 2000). In contrast, damesrocket (*Hesperis matronalis* L.) germination at cooler temperatures was extremely low in the light conditions (Susko and Hussein 2008). As spotted spurge seeds require light at low temperatures, crop residue on the soil surface can reduce its germination by preventing light penetration (Ramakrishna et al. 2006), decreasing soil temperature in the hot season (Jodaugiene et al. 2006) and moderating diurnal fluctuations in soil temperature (Ghosh et al. 2006).

Varied germination responses to light have been reported among different weed species. Molinar et al. (2009) expressed that the spotted spurge seeds need light for maximum germination. Wild poinsettia did not require light for germination (Bannon et al. 1978). Prostrate spurge (*Euphorbia supina* Raf. ex Boiss.) germination in darkness was much lower than it was in the light (Krueger and Shaner, 1982). Texasweed seeds germinated in light and darkness, although germination was higher in the light (Koger et al. 2004).

Effect of Alternating Temperature on Germination. Spotted spurge seeds did not germinate at an alternating day/night temperature of 20/10 C, but a germination of 19.5% was recorded at a day/night temperature of 25/15 C (Figure 2A). Maximum germination (> 90%) occurred at alternating day/night temperature ranges of 35/25, 40/30, and 45/35 C. The germination rate increased with the increase in the day/night temperature regimes from 25/15 to 40/30 C, and then declined at day/night temperature of 45/35C (Figure 2B). Hence, the rate of emergence is closely correlated with soil temperatures (Chauhan et al. 2006b).

Spotted spurge germination was greater at high and alternating temperatures. Because temperature fluctuations are higher on the soil surface (Fenner and Thompson 2005), and soil temperature and its fluctuations reduce with increasing soil depth, spotted spurge germination is expected to occur when seeds are on or near the soil surface and

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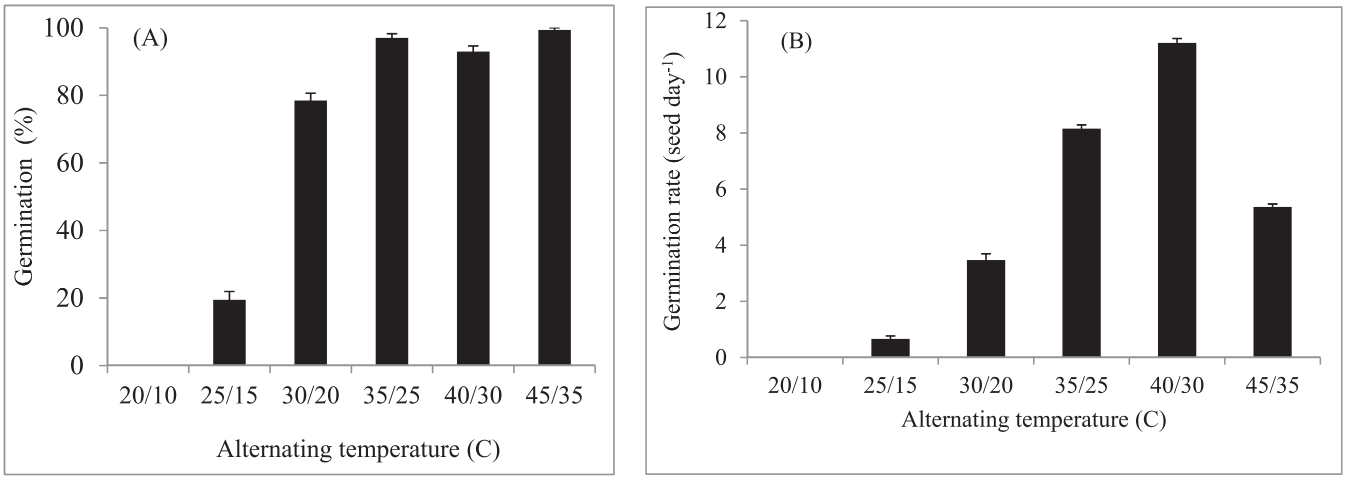

Figure 2. Effect of alternating day/night temperatures on spotted spurge seed (A) germination percentage and (B) germination rate. Vertical bars represent standard errors of the means.

emerged on bare ground. In addition, temperatures below a constant 25 C and alternating day/night temperatures of 20/10 C were not desirable for germination. Therefore, the distribution of this weed may be restricted to tropical and subtropical regions.

Effect of Drought Stress on Germination. Spotted spurge germination decreased from 98 to 23.5% as water potentials decreased from 0 to −0.6 MPa, and no germination was observed at −0.8 MPa. Germination exceeded 80% at water potentials of 0 to −0.4 MPa (Figure 3). These results suggest that the spread of spotted spurge is possibly restricted to moist soils because of its inability to germinate under low soil moisture conditions.

Water stress delayed the onset of germination and the time to 50% germination increased from 4.3 to 11.8 d when water potential decreased from 0 to −0.6 MPa (Figure 4). One of the factors that control seed imbibition is the differences in water potential between the soil and the seeds. The seed imbibition rate decreases with reducing water potential in the soil and, subsequently, the germination rate decreases.

Similar to spotted spurge, sicklepod [*Senna obtusifolia* (L.) H.S. Irwin & Barneby] (Norsworthy and Oliveira 2006), garden huckleberry (*Solanum melanocerasum* All.) (Zhou et al. 2006), and annual sowthistle (*Sonchus oleraceus* L.) (Chauhan et al. 2006a) germination was completely inhibited at a water potential of −0.8 MPa. Evans and Etherington (1990) found that none of the wetland

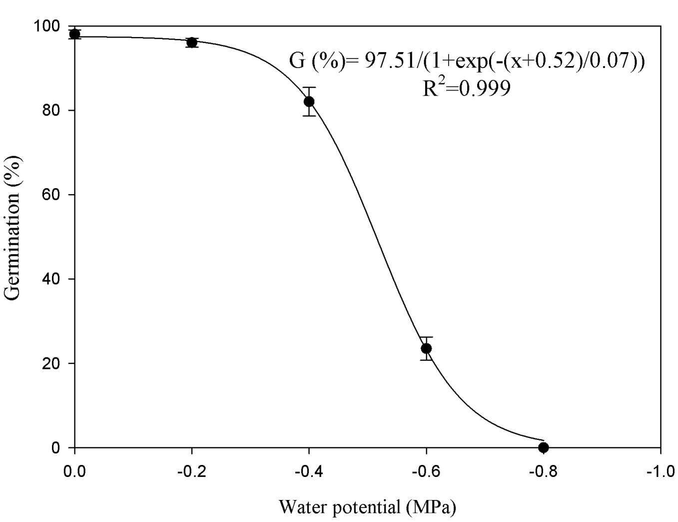

Figure 3. The relationship between water potential and seed germination percentage (mean ± SE) of spotted spurge. Seeds placed in an incubator at 35 C with a 14-h photoperiod for 14 d.

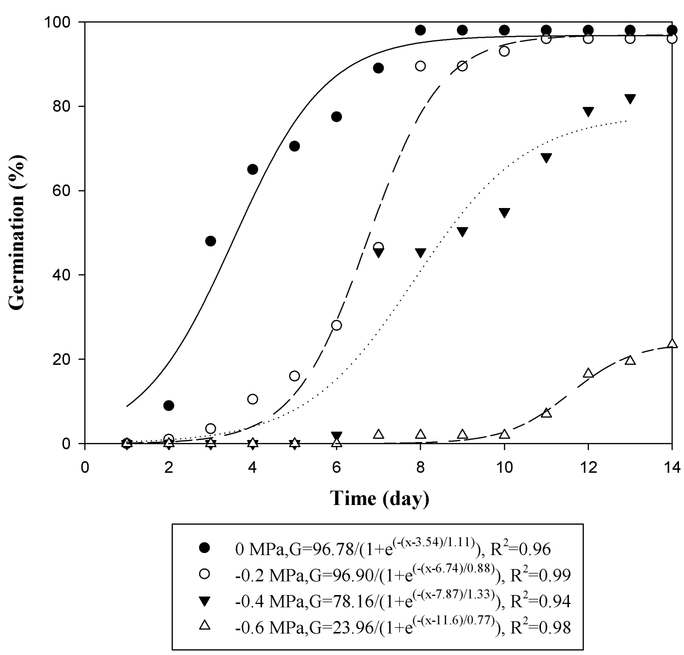

Figure 4. Effect of water potential on cumulative germination of spotted spurge seeds.

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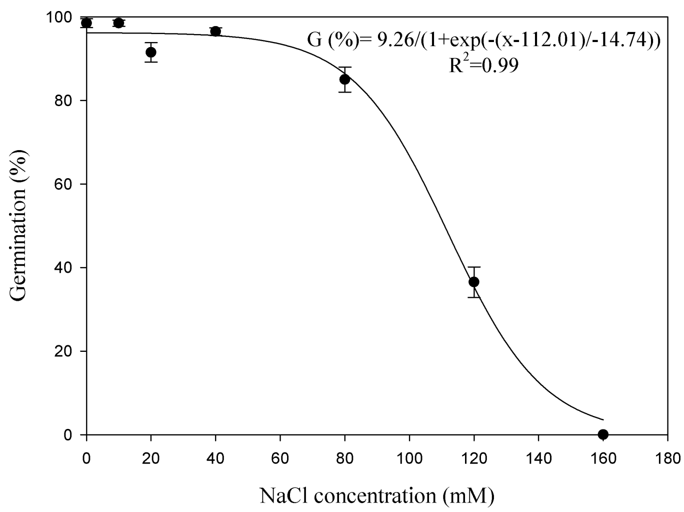

Figure 5. The relationship between sodium chloride (NaCl) concentration and seed germination percentage (mean ± SE) of spotted spurge. Seeds placed in an incubator with 35 C at 14-h photoperiod for 14 d.

species tested could germinate effectively at low water potentials.

Effect of Salinity Stress. Different levels of salinity had significant effects on spotted spurge germination ($P < 0.001$). Germination at < 80 mM NaCl was > 85%, and it declined to 36.5% at 120 mM NaCl and was completely inhibited at 160 mM NaCl (Figure 5). Seedling growth reduced severely at salt concentrations > 40 mM NaCl (data not shown). These results indicate that, at high soil salinity, a proportion of spotted spurge seeds may germinate, but they may not grow vigorously. The germination rate reduced with increasing salinity; for example, the time for 50% germination (T_{50}) increased from 4.3 d at 0 mM NaCl (control) to 11.8 d at 120 mM NaCl (Figure 6).

Soils with electrical conductivity of 4 dS m^{-1} (about 40 mM NaCl) are classified as saline soils (USDA 1954). Salinity affects seed imbibition, germination, and root elongation. The influence of NaCl is a combination of osmotic and specific ion effects (Katembe et al. 1998). NaCl reduces the rate of seed imbibition by reducing water potential, and thereby, the germination rate declines. The salinity tolerance of spotted spurge was similar to garden huckleberry, with germination of 20% at 120 mM NaCl and no germination at 160 mM NaCl (Zhou et al. 2006). However, its germination was more sensitive than texasweed (Koger et al. 2004), trumpetcreeper [*Campsis radicans* (L.) Seem. ex Bureau] (Chachalis and Reddy 2000), annual sowthistle (Chauhan et al. 2006a), and silverleaf

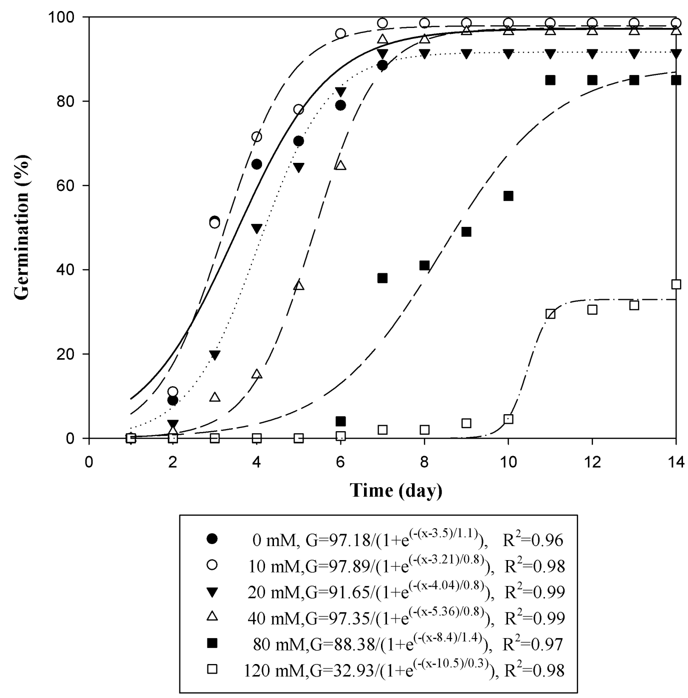

Figure 6. Effect of sodium chloride (NaCl) concentrations on cumulative germination of spotted spurge seeds.

nightshade (*Solanum elaeagnifolium* Cav.) (Stanton et al. 2012), which had germination percentages of 27, 20, 7.5, and 5%, respectively, at 160 mM NaCl. Salt stress delayed the onset of germination of Chinese sprangletop [*Leptochloa chinensis* (L.) Nees] (Chauhan and Johnson 2008). The time to 50% germination increased with increasing NaCl concentrations from 0 to 100 mM. The germination rate of *Salsola affinis* C.A. Mey. also decreased with increasing salinity (Wei et al. 2008).

Effect of pH. Spotted spurge germination was not affected by the tested levels of pH (data not shown). Germination was > 95% over a broad pH range from 4 to 10. This characteristic has been observed in several weed species, such as wild poinsettia (Brecke 1995), buffalobur (*Solanum rostratum* Dunal) (Wei et al. 2009), cadillo (*Urena lobata* L.) (Wang et al. 2009), garden huckleberry (Zhou et al. 2006), and texasweed (Koger et al. 2004). A broad pH range for germination indicates that pH should not be a limiting factor for spotted spurge germination and such a trait would help this weed species to colonize various habitats.

Longevity of Seeds in Water. Germination of seeds soaked in water for a period shorter than 3 wk was > 60%, whereas no germination was observed after a soaking period of 9 wk (Figure 7). The

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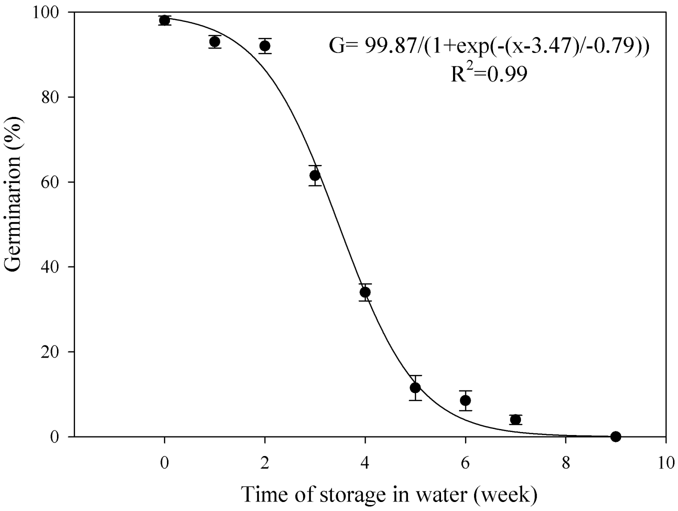

Figure 7. Longevity of spotted spurge seeds in water. Vertical bars represent standard errors of the means.

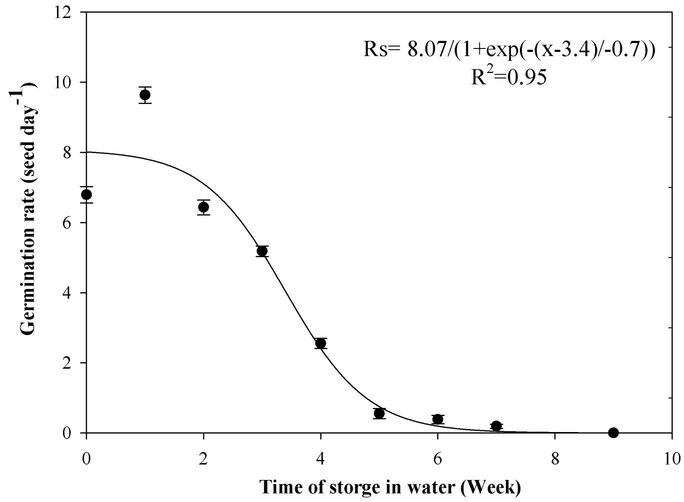

Figure 8. Germination rate of spotted spurge seeds stored in water. Vertical bars represent standard errors of the means.

nongerminated seeds were not viable. The germination rate increased after the seeds were submerged in water for 1 wk. Thereafter, the germination rate reduced with increasing time the seeds were in water (Figure 8).

These results indicate that the seed longevity of spotted spurge is relatively low in water. However, seeds can be transferred by water and infest other farms. Irrigation water is an important agent for spreading of weed seeds. Water dispersal is frequently blamed for the very rapid spreading of some invasive aliens (Thebaud and Debussche 1991; Pysek and Prach 1993). Wilson (1980) found seeds of 77 species in irrigation water, which disseminated 48,400 seeds ha^{-1} in the sampled field. Comes et al. (1978) investigated seed viability of 82 species of weeds and crops after being stored in freshwater. They reported that 24 species did not germinate after being stored for 12 mo in water, whereas 27 species germinated after storage in water for 60 mo. Leafy spurge (*Euphorbia esula* L.) and prostrate spurge germinated 1% after being kept in water for 60 mo. Creeping bentgrass (*Agrostis stolonifera* L.) seeds did not lose their viability after 17 wk of being in water at 20 C, although their germination percentage was reduced to 46% after seeds were kept in water for 17 wk at 4 C (Zapiolaand Mallory-Smith 2010).

In summary, the germination of spotted spurge seeds occurred at warm temperatures. Determination of germination time can help to improve the efficacy of herbicide application, tillage, and other weed management strategies. It helps to coordinate seedbed preparation with the cultivation time and method. Because spotted spurge seeds require light at lower temperatures, crop residue can reduce spotted spurge germination. Also, fluctuating temperatures promoted spotted spurge germination. So, a deep-tillage operation that buries the seeds might inhibit germination. Seeds geminated at water potentials less than -0.8 MPa, indicating that it the weed was sensitive to water stress. Thus, spotted spurge seeds are expected to germinate in moist soils. Germination occurred in salinity below 160 mM NaCl concentration. However, seedling growth was reduced severely at NaCl concentrations $>$ 40 mM. Therefore, it may be difficult for spotted spurge to establish in saline soils. Germination was not affected by pH. Seeds of this species can be expected to germinate in soils with different levels of acidity. Because seeds of this weed maintain their viability for several weeks in water, they can remain viable in flooded conditions and be dispersed by water safely. The results of our study indicate that this invasive weed species can spread by several methods, and it will be able to invade most of the warm regions of Iran that have moist soils.

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