Factors Affecting Germination and Emergence of Dame's Rocket (*Hesperis matronalis*)

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Factors Affecting Germination and Emergence of Dame's Rocket (*Hesperis matronalis*)

David J. Susko and Yara Hussein*

Laboratory experiments were conducted to determine the effects of temperature, light, cold stratification, dry storage, solution pH, solution osmotic potential, and planting depth on germination and emergence of dame's rocket. Maximal germination (> 80%) of fresh seeds occurred at alternating temperatures ≥ 25/15 C in both alternating light/dark and continuous darkness. However, < 10% of seeds germinated at or below 20/10 C, with lower germination in the presence of light than in darkness. Cold stratification at 4 C for 4 to 16 wk enhanced germination at low alternating temperatures (≤ 20/10 C), but depressed germination at warm temperature regimes (≥ 25/15 C). After 1 yr of dry storage (after-ripening), germination exceeded 94% and did not differ significantly among temperature regimes. Germination exceeded 60% in solutions with pH 3 to 10. Germination was reduced below 50% in solutions with osmotic potentials below -0.6 MPa. Percent emergence was greater than 56% at burial depths in soil of 0 to 5 cm, with maximal emergence (93 to 99%) at 0 to 2 cm. Dame's rocket seeds possess non-deep physiological dormancy at maturity, but when dormancy is alleviated, the seeds are capable of germinating in a variety of climatic and edaphic conditions.

Nomenclature: Dame's rocket, *Hesperis matronalis* L. HEVMA.

Key words: Light, temperature, cold stratification, pH, osmotic stress, planting depth.

Dame's rocket is a herbaceous perennial belonging to the mustard family (Brassicaceae). The species is native to Eurasia and was introduced into North America in the 1600s (Gleason and Cronquist 1991). Its range extends throughout the continental United States except for eight extreme southern states, including Arizona, Texas, Oklahoma, Louisiana, Mississippi, Alabama, Florida, and South Carolina (USDA, NRCS 2007). It is currently listed as a noxious, prohibited, or banned weed in Colorado, Massachusetts, and Connecticut (USDA, NRCS 2007). In Canada, dame's rocket is common in southern Ontario, where it is typically found in old fields, ditches, disturbed sites, and along roadsides (D. Susko, personal observation). Dame's rocket can form monotypic stands in meadows, orchards, forest edges, and bottomland woods where it can crowd out native wildflower species. In addition, dame's rocket is an alternate host for a number of plant viruses including turnip, cucumber, and ribgrass mosaic viruses (Ford et al. 1988).

In order to understand the expansion of the geographic range of this species in North America, we need to learn how its seeds respond to varied climatic and edaphic conditions. Several environmental variables, such as temperature, light, pH, salinity, and nutrient and moisture content regulate the timing and probability of seed germination (Koger et al. 2004; Susko et al. 1999; for a review see Baskin and Baskin 1998), yet no specific seed research has been reported to date for dame's rocket. The objectives of this research were to determine: (1) the effect of temperature regime and light exposure on the germination of dame's rocket seeds, (2) the dormancy status of seeds at maturity, (3) the effect of cold stratification on seed germination, (4) the effect of dry storage on seed germination, (5) the effect of pH on seed germination, (6) the effect of osmotic potential on seed germination, and (7) the effect of depth of burial within the soil on seedling emergence.

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Materials and Methods

On September 6, 2006, a bulk seed collection was made from 60 dame's rocket plants growing in a 4-ha old field adjacent to Black Oak Heritage Park in Windsor, Ontario, Canada. Seeds were stored at room temperature (22 to 25 C) until experiment initiation. All laboratory germination experiments described below were initiated within 3 wk of seed collection and were repeated once for a total of two trials carried out concurrently in two separate growth chambers.

Unless stated otherwise, each experiment consisted of three replications of 50 seeds placed on two sheets of fitted Whatman #4 filter paper in 9-cm petri dishes. Initially, the filter paper was moistened with about 5 ml of distilled water or test solution. 1 to 5 ml of water or test solution was added as necessary to maintain adequate moisture during each experiment. All dishes were placed in polyurethane bags to slow desiccation. Germination studies were conducted in temperature- and light-controlled environmental growth chambers maintained with a daily alternating thermoperiod and photoperiod (12 h light/12 h dark). Relative humidity was maintained at 50%. Dishes were placed in a completely random format within each chamber and their positions were rearranged daily. Fluorescent lamps produced a photosynthetic photon flux of 100 μmol m^{-2} s^{-1}. Dishes designated for dark treatments were wrapped separately in two layers of aluminum foil and were opened for examination in a dark room equipped with a green safety light. Germination was monitored daily for a period of 21 d. A seed was considered germinated when the radicle protruded 1 mm from the seed coat.

Effects of Temperature and Light. This experiment had a split-plot design with temperature as the whole-plot factor and light treatment as the subplot factor. The whole-plot design was a randomized complete block with growth chamber as the blocking factor. Fifty seeds were placed on each of 24 moistened Petri dishes. Six replications were placed in a growth chamber set for alternating temperature regimes of 15/5, 20/10, 25/15, or 30/20 C, with half of the dishes exposed to alternating light/dark (as described above) and the other half treated to continuous darkness.

Effect of Moist Chilling. Fifty seeds were placed on each of 60 moistened Petri dishes. Twelve Petri dishes were not chilled (control), whereas the rest were exposed to either 4, 8, 12, or 16 wk of chilling in darkness in a refrigerator at 4 C. Following each respective chilling period, three replications were incubated in alternating light/dark at 15/5, 20/10, 25/15, or 30/20 C. Viability of all nongerminated seeds was tested using a 1% tetrazolium chloride method (ISTA 1985).

Effect of Dry Storage. Fifty seeds were placed in each of 36 paper envelopes. After 0 (control), 180, or 360 d of storage at room temperature (22 to 25 C), three replications of 50 seeds were transferred to moistened Petri dishes and placed in an incubator in alternating light/dark at 15/5, 20/10, 25/15, or 30/20 C. Viability of all nongerminated seeds was tested using a 1% tetrazolium chloride method (ISTA 1985).

Effect of pH. Fifty seeds were placed on each of 27 Petri dishes moistened with test solution. Solutions with pH levels 3, 4, 5, and 6 were prepared using 0.1 M potassium hydrogen phthalate, and solutions with pH 7, 8, 9, and 10 were prepared with 25 mM borax (Shaw et al. 1991); distilled water (pH 6.5) was used as a control. Buffer solutions were adjusted to the appropriate pH using 0.5 M NaOH or 1 N HCl. Three replications of 50 seeds were assigned to each pH solution and placed in alternating light/dark at 25/15 C.

Effect of Osmotic Stress. Fifty seeds were placed on each of 18 Petri dishes moistened with test solution. Solutions with osmotic potentials of 0 (control), −0.3, −0.4, −0.6, −0.9, and −1.3 MPa were prepared by dissolving 0, 154, 191, 230, 297, and 350 g of polyethylene glycol (PEG 8000[1]) in 1 L of deionized water. The equations of Michel (1983) were used to calculate the required quantities of PEG 8000. Three replications of 50 seeds were assigned to each solution and placed in alternating light/dark at 25/15 C.

Effect of Planting Depth. Fifty seeds were buried at depths of 0 (control), 0.5, 1, 2, 5, or 10 cm below the soil surface in a pot (9.5 cm diam by 11 cm depth) for each of three replications. Pots were filled with Shoals silt loam. All pots were placed in a randomized design on a bench in a growth chamber with alternating light/dark at 25/15 C. On a daily basis, pots were rearranged and surface irrigated to field capacity. Seedling emergence was recorded daily for 28 d.

Statistical Analyses. Prior to analysis, germination percentages were arcsin square root transformed because such a transformation improved homogeneity of variance. The GLM procedure of SYSTAT (SYSTAT 2004) was used to assess significant differences among trials and treatments. Because analysis of variance revealed no significant trial by treatment interactions ($P > 0.05$), data for each trial were pooled for subsequent analyses and cumulative percent germination or emergence data (mean ± SE) presented herein represent the average of the two trials. Factorial analysis of variance was used to assess the effects of incubation temperature and light regime, incubation temperature and duration of cold stratification, and incubation temperature and duration of dry storage on percent germination. Significant differences among treatments were identified using Fisher's LSD test ($P < 0.05$). Nonlinear regression analysis was used to determine how pH and osmotic stress affected percentage germination (SYSTAT 2004). The relationship between depth of burial and percent emergence was determined via logistic regression analysis (SYSTAT 2004).

Results and Discussion

Effects of Temperature and Light. Germination percentages differed among incubation temperature regimes ($F_{3, 40} = 1787.8$, $P < 0.001$) and light regimes ($F_{1, 40} = 41.3$ $P < 0.001$; Figure 1). There was also a significant temperature by light regime interaction ($F_{3, 40} = 5.6$, $P < 0.001$). In general, the effect of temperature on germination depended on the light regime; seeds germinated readily (> 80%) when placed in warm alternating temperatures (≥ 25/15 C) in both light and darkness. At cooler temperature regimes (≤ 20/10 C), however, percent germination was extremely low in the presence of light (< 1%), but was greater (8 to 10%) when seeds were placed in complete darkness. Based on these germination responses to temperature, we conclude that dame's rocket seeds possess nondeep physiological dormancy. Such seeds are conditionally dormant in autumn and only germinate at high temperatures. Similar changes in germination responses to temperature were reported for the seeds of several other summer annual species, including bearded flatsedge (*Cyperus inflexus* Muhl.; Baskin and Baskin 1978), redroot flatsedge (*Cyperus erythrorhizos* Muhl.), slender fimbry [*Fimbristylis autumnalis* (L.) Roemer & J. A. Schultes], and Vahl's fimbry [*Fimbristylis vahlii* (Lam.) Link] (Baskin et al. 1993). The seeds of all four species possessed nondeep physiological dormancy at maturity; they were conditionally dormant in autumn and germinated in high percentages only at high temperatures. Following after-ripening in soil outdoors over winter at lower temperatures, the seeds of all four species came out of conditional dormancy and were able to germinate over a broader range of temperatures the following spring.

Dame's rocket seeds do not have a light requirement for germination, and might be somewhat negatively photoblastic at low temperatures. However, the seeds should germinate readily when shaded by litter or a leaf canopy, or following burial in soil. Although the physiological mechanism for negative photoblasticity at low temperatures in dame's rocket was not determined, we note that negative photoblasticity of seeds has been documented only rarely (Batanouny 1974; Pirovano et al. 1999). Pirovano et al. (1999) found that germination of lacy phacelia (*Phacelia tanacetifolia* Benth.) had optimal germination in darkness at 16 C, but was inhibited in light at 16 C. Their results showed that the presence of light was a factor that inhibited the activation of transport and protein synthetic activities in the embryo, blocking the embryo from operating as a sink for the absorption of nutrients from the endosperm, and thereby preventing the embryo's growth and metabolism.

Effect of Moist Chilling. Germination percentages differed significantly among moist chilling treatments for different periods of cold stratification ($F_{4, 100} = 7.4$, $P < 0.001$) and incubation temperature regimes ($F_{3, 100} = 77.7$, $P < 0.001$; Figure 2). Also, there was a significant cold stratification period by incubation temperature interaction ($F_{12, 100} =$

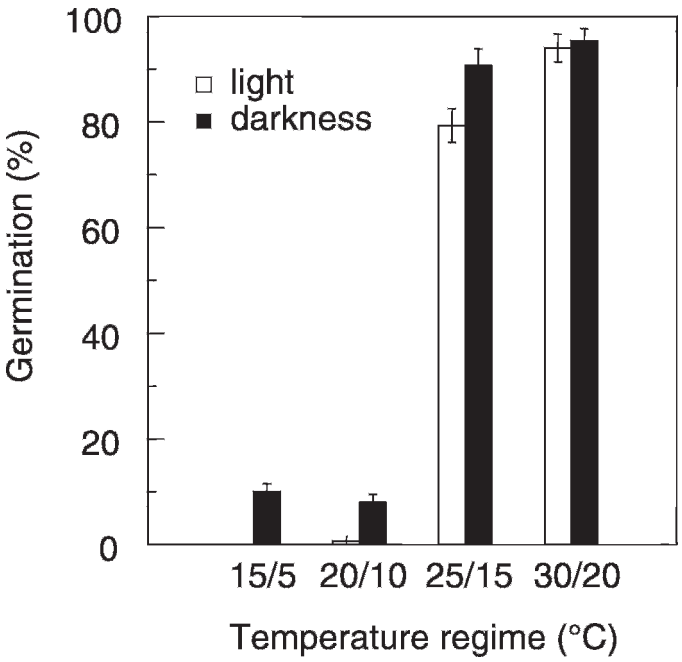

Figure 1. Germination percentages (mean ± SE) of dame's rocket seeds at various temperature regimes in an alternating photoperiod (12 h light/12 h dark) or continuous darkness after 21 d.

12.4, $P < 0.001$); at cooler thermoperiods (15/5 and 25/15 C), percentage germination increased with 4 to 8 wk of exposure to chilling while extended periods had no additional effect. Dame's rocket seeds chilled for 16 wk had 2- to 3-fold greater germination than did nonchilled seeds. Baskin and Baskin (1987) also noted that the minimum temperature at which seeds could germinate declined and percentage germination increased following 1 to 5 mo of cold-stratification at 5 C for seeds of four summer annuals: annual ragweed (*Ambrosia artemisiifolia* L.), Pennsylvania smartweed (*Polygonum pensylvanicum* L.), slim amaranth (*Amaranthus hybridus* L.) and common lambsquarters (*Chenopodium album* L.). At warmer thermoperiods (25/15 and 30/20 C), percentage germination of dame's rocket seeds was greatest for nonchilled seeds, whereas seeds that were chilled had depressed germination relative to controls. The failure of cold-stratified seeds to germinate at warmer thermoperiods cannot be attributed to low viability, because tetrazolium tests determined that > 96% of these nongerminated seeds were still viable. Rather, chilling seems to induce secondary dormancy that warm temperatures cannot overcome in these nongerminated seeds. As stated earlier, dame's rocket seeds possess nondeep physiological dormancy at maturity. Such seeds become nondormant when exposed to (a) warm temperatures or (b) following prolonged periods of cold exposure, allowing them to germinate at lower minimum temperatures (Baskin and Baskin 1998). This means that germination of dame's rocket in the field can occur in both spring and summer.

Effect of Dry Storage. Seed viability was ≥ 95% in all dry storage treatments. Germination percentages for seeds stored dry at room temperature (after-ripened) differed among temperature regimes ($F_{3,120} = 1497.9$; $P < 0.001$), light regimes ($F_{1,120} = 8.8$; $P < 0.001$), and storage periods ($F_{2,120} = 1402.3$; $P < 0.001$; Figure 3). Two interaction terms, temperature regime by storage period ($F_{6,120} = 413.0$; $P < 0.001$) and light regime by storage period ($F_{2,120} = 8.6$; $P < 0.001$), differed as well, but all others did not. Freshly collected seeds germinated poorly at low temperature regimes (≤ 20/10 C), but high germination percentages were observed at high temperature regimes (≥ 25/15 C). After 180 d of dry storage, percent germination at low temperature regimes (≤ 20/10 C) increased to about 24 to 26% and did not differ among light regimes. Following one year of dry storage, percent germination was high (≥ 94%) in all temperature regimes and physiological dormancy was completely alleviated via after-ripening. Similarly, Chauhan et al. (2006a) noted that germination of seeds of Oriental mustard (*Sisymbrium orientale* L.) required after-ripening and that percent germination increased with increased durations of after-ripening in dry storage over 1 yr. In comparison to the loss of dormancy due to cold stratification, the period of after-ripening for dry seeds of dame's rocket at room temperature was much longer. Baskin and Baskin (1998) also noted similar temporal differences in after-ripening for cold-stratified seeds vs. those stored dry at room temperature.

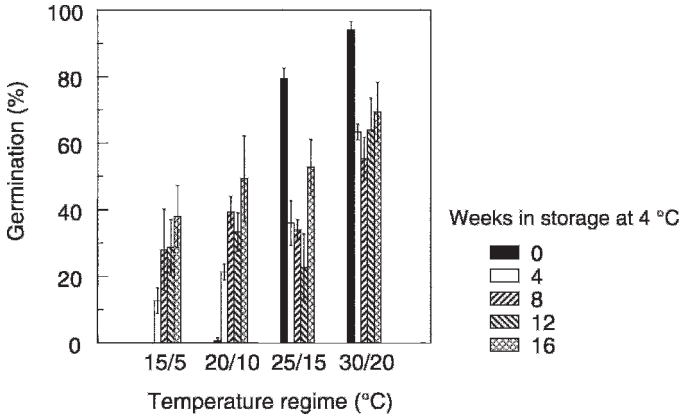

Figure 2. Effect of moist, cold stratification (4 C) treatments (0, 4, 8, 12, or 16 wk) and subsequent germination thermoperiods on percentage germination (mean ± SE) of seeds of dame's rocket after 21 d.

Effect of pH. A quadratic regression ($y = 48.1 + 19.4x - 1.8x^2$; $R^2 = 0.86$) best described how germination of dame's rocket seeds changed with regard to solution pH. Dame's rocket seeds germinated well (> 60%) over a wide range of

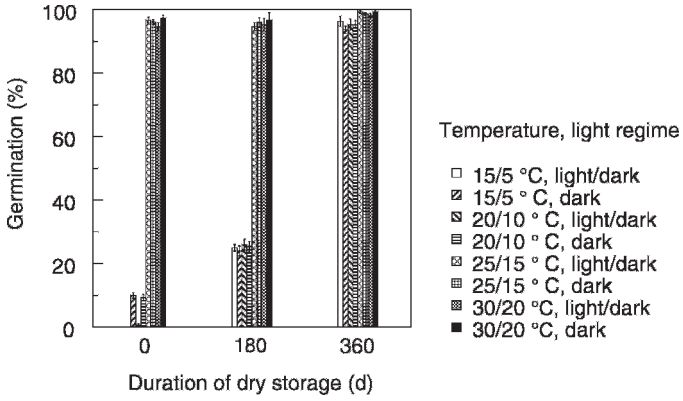

Figure 3. Effect of dry storage periods (0, 180, or 360 d) on percentage germination (mean ± SE) of seeds of dame's rocket. Seeds were incubated at 15/5, 20/10, 25/15, or 30/20 C (12 h light/12 h dark) for 21 d.

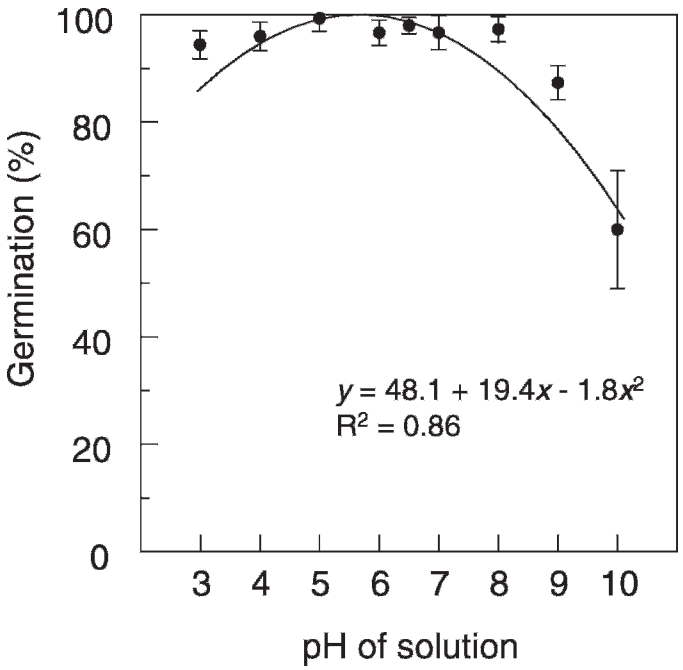

Figure 4. The relationship between pH and percent seed germination (mean ± SE) of dame's rocket. Seeds were incubated at 25/15 C (12 h light/12 h dark) for 21 d.

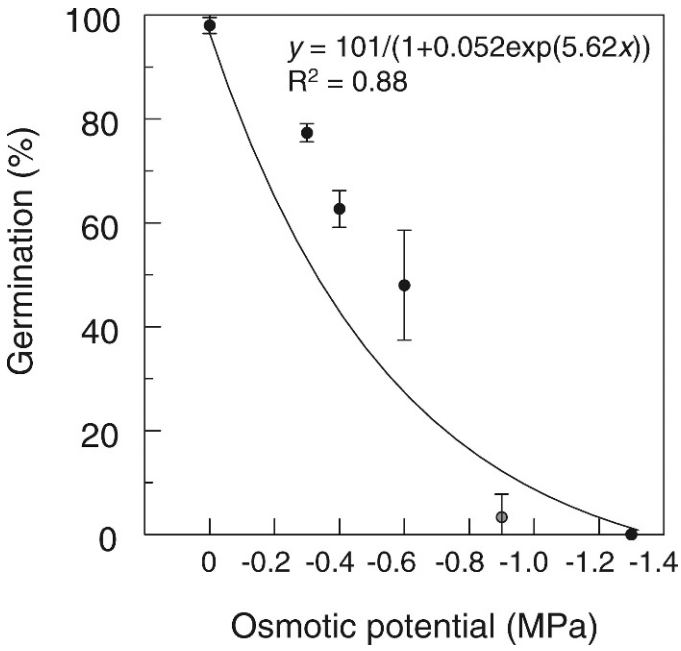

Figure 5. The relationship between osmotic potential and percent seed germination (mean ± SE) of dame's rocket. Seeds were incubated at 25/15 C (12 h light/12 h dark) for 21 d.

pH (3 to 10; Figure 4). High seed germination of dame's rocket over a broad range of pH levels (3 to 10) indicates that pH should not limit germination in most soil types. Germination over a broad range of pH has been reported in other mustard species (pH 4 to 10 in *Sisymbrium orientale* Torn., Chauhan et al. 2006a; and pH 4 to 10 in *Brassica tournefortii* Gouan, Chauhan et al. 2006b), as well as numerous other weedy species (Chauhan et al. 2006c,d; Horak and Wax, 1991; Norsworthy and Oliveira, 2006; Oliveira and Norsworthy 2006; Zhou et al. 2005), but in general, the optimum pH range for seed germination falls within about 5 to 8; germination typically decreased in more acidic ($\leq$ 4) or alkaline ($\geq$ 9) pH conditions. In contrast, seed germination of dame's rocket was high ($>$ 94%) and did not differ significantly over a pH range of 3 to 8, indicating that seeds should germinate well, even in extremely acidic soils. However, Clapham et al. (1990) reported that the species tends to avoid acidic soils, and grows best in very alkaline soils.

Effect of Osmotic Stress. A nonlinear regression ($y = 101/[1 + 0.052 \exp\{5.62x\}]$; $R^2 = 0.88$) best described how germination of dame's rocket seeds decreased as osmotic stress increased from 0 to −1.3 MPa (Figure 5). Percent germination was greatest ($>$ 97%) in distilled water at 0 MPa and dropped with decreased osmotic potential to 48% at −0.6 MPa. Germination was completely inhibited at an osmotic potential of −1.3 MPa, whereas about 3% seed germination occurred at −0.9 MPa. Some weedy species, such as mayweed chamomile (*Anthemis cotula* L.; Gealy et al. 1985) and tropical soda apple (*Solanum viarum* Dunal; Akanda et al. 1996) can germinate relatively well at osmotic potentials as low as −1.0 MPa, whereas others such as redvine [*Brunnichia ovata* (Walt.) Shinners] (Shaw et al. 1991) and trumpetcreeper [*Campsis radicans* (L.) Seem. ex Bureau] (Chachalis and Reddy 2000) are highly sensitive to osmotic stress and fail to germinate at an osmotic potential of −0.2 MPa. Although optimal germination of dame's rocket seeds should occur on moist soils given that the seeds germinated best when osmotic stress was low, dame's rocket seeds are tolerant of marginal water stress conditions and, hence should germinate even on moderately dry soils.

Effect of Planting Depth. Dame's rocket seedlings emerged on the surface and from all burial depths to 10 cm; a logistic regression best described how percentage emergence decreased with increased depth of burial ($y = 98.7/[1 + \{x/5.15\}^{4.0}]$, $R^2 = 0.96$; Figure 6). Seed germination was highest (93 to 99%) within the first 0 to 2 cm of the soil surface. Similarly, maximum emergence at shallow planting depths was noted for two other mustard species: 0 cm for field pennycress (*Thlaspi arvense* L.) and 1 to 2 cm for wild mustard (*Sinapis arvensis* L.) (Boyd and Van Acker 2003) For dame's rocket, seed germination decreased to 56% and 5% at the greatest burial depths of 5 and 10 cm, respectively. The mean predicted depth for 50% emergence from a fitted response was 5.2 cm. Such a sigmoidal decline in emergence with increasing planting depth has only been reported in a few other weedy species (Norsworthy and Oliveira 2005, 2006; Oliveira and Norsworthy 2006). Because dame's rocket seeds do not have a light requirement for germination and are moderately tolerant of osmotic stress, their seeds can germinate readily on the surface (where hydraulic conductivity can be low) and when buried; this could explain why individuals are frequently associated with disturbed areas, such as roadsides, trails, and forest edges.

In summary, germination of fresh dame's rocket seeds occurred primarily at warm alternating temperatures, indicating that the seeds possess nondeep physiological dormancy at the time of dispersal. Dormancy was alleviated by cold stratification and by after-ripening via dry storage at room

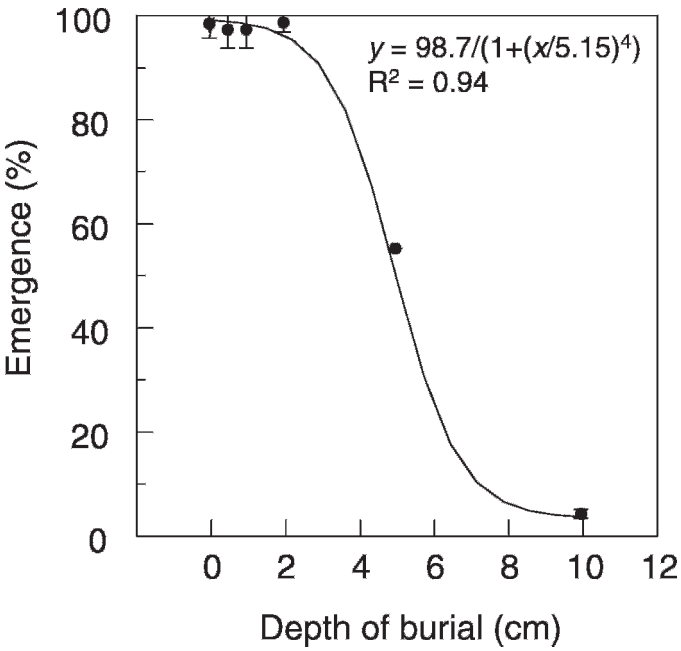

Figure 6. The relationship between planting depth and percent seedling emergence (mean ± SE) of dame's rocket after 28 d.

temperature for 1 yr. Germination occurred over a broad range of pH, osmotic stress, and depth of burial in soil, although apparent optima were observed. Thus, seeds of this species can be expected to germinate in a variety of soils with different levels of acidity and moisture.

Source of Materials

[1] PEG 8000. Sigma-Aldrich Co., 3050 Spruce St., St. Louis, MO 63103.

Acknowledgment

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