Germination of *Liatris spicata* (L.) Willd. Seed Is Enhanced by Stratification, Benzyladenine, or Thiourea but not Gibberellic Acid

Constance A. Parks¹ and Thomas H. Boyle²

Department of Plant and Soil Sciences, French Hall, University of Massachusetts, Amherst, MA 01003

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Abstract. The effects of stratification, BA, thiourea, and GA₃ were examined on germination of *Liatris spicata* (L.) Willd. seed. Seeds were germinated at 20 °C and numbers of germinated seed were counted daily for 21 days. The final germination percentage (G) for seeds stored dry at 4 °C for 0 to 10 weeks ranged from 52% to 64%, while stratification at 4 °C for 10 weeks increased G to 98% and decreased the days to 50% of final germination (T₅₀) and the days between 10% and 90% germination (T₉₀–T₁₀). Aqueous solutions of BA at 10 or 100 mg·L⁻¹ applied to blotter paper increased G and decreased T₅₀ but did not affect T₉₀–T₁₀. In a separate experiment, dry seeds were treated for 3 minutes in BA at 0 to 1126 mg·L⁻¹ dissolved in acetone. G values increased quadratically, whereas T₅₀ and T₉₀–T₁₀ values decreased quadratically in response to BA concentration. A 3-minute preplant acetone permeation of seeds with BA at 225 or 1127 mg·L⁻¹ yielded G and T₅₀ values similar to those obtained with 10 weeks of stratification. Seeds immersed in thiourea at 0.76 or 7.61 mg·L⁻¹ for 24 hours prior to sowing had higher G and lower T₅₀ values than controls (0 mg·L⁻¹ thiourea), but T₉₀–T₁₀ values were similar for all treatments. Seeds treated with GA₃ at 1, 10, or 100 mg·L⁻¹ in H₂O did not differ from controls (0 mg·L⁻¹ GA₃) in G, T₅₀, or T₉₀–T₁₀. Infusion of BA via acetone may be a practical means of breaking seed dormancy and accelerating germination in *L. spicata*. Chemical names used: *N*-(phenylmethyl)-1*H*-purine-6-amine [benzyladenine (BA)]; gibberellic acid (GA₃); thiocarbamide (thiourea).

Liatris spicata (Asteraceae) is a herbaceous perennial native to eastern North America (Dress, 1959). It is cultivated widely as a cut flower (fresh or dried) and flowering potted plant (Nau, 1996). Plants usually are propagated by seeds or division. Despite its horticultural importance, little is known about germination requirements of *L. spicata* seed. Literature from seed companies (Ernst Benary Samen-zucht, 1987; Jelitto Staudensamen, 1998) states that *L. spicata* seeds will germinate within 20 to 30 d at ≈20 °C.

Seed dormancy occurs in several *Liatris* species (Baskin and Baskin, 1989; Blake, 1935; Greene and Curtis, 1950; Nichols, 1934; Salac and Hesse, 1975) and can be overcome by stratification. The effects of thiourea or growth regulators such as gibberellins or cytokinins on germination of *Liatris* seed are not known. Khan and co-workers (Khan et al., 1973; Tao et al., 1974) demonstrated the feasibility of using organic solvents to apply growth regulators to dry seeds. The present study was conducted to determine the effects of stratification, BA, GA₃, and thiourea on seed germination in *L. spicata*, and to determine if the

application method (aqueous solutions or acetone permeation of dry seed) affects the responsiveness of *L. spicata* seed to BA.

Materials and Methods

Plant material. Seeds (achenes) were harvested in Oct. 1995 from plants growing in a field plot at the Univ. of Massachusetts, Amherst (lat. 42°22.5'N). To minimize genetic variation, half-sib seed from the cross 'Callilepis' × 'Kobold' (asexually propagated, self-incompatible maternal parent and seed-propagated, open-pollinated pollen parent) was used for study. Seeds were cleaned by Ball Seed Co. (West Chicago, Ill.) and returned to the Univ. of Massachusetts in Dec. 1995. Seeds were dusted with 3α,4,7,7α-tetrahydro-2-[(trichloromethyl)thio]-1*H*-isoindole-1,3(2*H*)-dione (captan) and stored at 4 °C and 40% relative humidity until experiments commenced. Experiments were performed between Jan. and July 1996.

Germination methods. Seeds were sown in 15-cm glass petri plates on top of a single layer of blue blotter paper. Germination tests were performed in incubators (model I-35LVL; Percival Scientific, Boone, Iowa) kept at 20 ± 1 °C with cool-white fluorescent lamps providing 50 ± 8 μmol·m⁻²·s⁻¹ photosynthetic photon flux for 12 h daily. Blotter paper was moistened initially with deionized water (dH₂O) or a growth regulator solution. After the first watering, dH₂O was used exclusively

for moistening blotter paper and was applied as needed.

Data collection. Numbers of germinated seeds (radicle ≥1 mm) were counted daily for 21 d. Germinated seeds were discarded daily. Four 100-seed replications (plates) were used for each treatment. The final germination percentage (G) at 21 d, number of days to 50% final germination (T₅₀), and number of days between 10% and 90% germination (T₉₀–T₁₀) were calculated for each treatment. Five experiments were conducted.

Expt. 1. Effects of stratification. Seeds were sown in plates containing dry blotter paper (dry storage) or paper moistened with dH₂O (stratification). Plates were placed in 34 × 24 × 7-cm covered plastic boxes that were then sealed in a polyethylene bag. Seeds were stored for 2, 4, 6, 8, or 10 weeks in a dark incubator (model I-35LVL; Percival Scientific) kept at 4 ± 1 °C. After storage, plates were transferred to a 20 °C incubator for germination.

Expt. 2. Effects of BA in aqueous solutions. Blotter paper was moistened with BA at 0 (control), 10, or 100 mg·L⁻¹ in dH₂O. Crystalline BA (Sigma, St. Louis) was dissolved in a small volume of 0.1 M HCl and diluted with dH₂O. Solutions were adjusted to pH 6.5 with 0.1 M HCl (BA at 0 mg·L⁻¹) or 0.25 M NaOH (BA at 10 or 100 mg·L⁻¹) prior to application.

Expt. 3. Effects of permeating seeds with BA via acetone. BA was dissolved in ≈5 mL of 95% ethanol and diluted with acetone for a final volume of 25 mL. Treatments included BA at 0 (control), 13, 23, 113, 225, and 1127 mg·L⁻¹. About 500 seeds were immersed for 3 min in 20 mL of a BA/acetone solution. Seeds were dried 1 h in a vacuum desiccator (0.07 MPa) and stored overnight in open containers at 20 °C.

Expt. 4. Effects of thiourea. Seeds were immersed for 24 h in thiourea at 0 (control), 0.08, 0.76, or 7.61 mg·L⁻¹ in dH₂O. After treatment, seeds were rinsed briefly in dH₂O and transferred to petri plates containing blotter paper moistened with dH₂O for germination testing.

Expt. 5. Effects of GA₃. Blotter paper was moistened with GA₃ at 0 (control), 1, 10, or 100 mg·L⁻¹ in dH₂O. Crystalline GA₃ (grade III; Sigma) was dissolved in a small volume of ethanol (95%) and diluted with dH₂O.

Data analysis. Data were transformed (arcsin for G and square root for T₅₀ and T₉₀–T₁₀) prior to analysis. Data were analyzed using the general linear models (GLM) or analysis of variance (ANOVA) procedure of SAS (SAS Institute, Cary, N.C.). Pooled *t* tests were used to compare G values for dry- and moist-stored seeds in Expt. 1. Coefficients for linear and quadratic terms in Expts. 2, 3, and 4 were calculated using the procedure of Gomez and Gomez (1984) for treatments with unequal intervals.

Results and Discussion

Expt. 1. Treatment and duration interacted significantly (*P* ≤ 0.001) to affect G, T₅₀, and T₉₀–T₁₀. At week 0, the G, T₅₀, and T₉₀–T₁₀ values were 37%, 12.8 d, and 13.3 d, respec-

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¹Graduate Research Assistant.

²Professor. E-mail address: tboyle@pssci.umass.edu.

tively (Fig. 1). For dry-stored seeds, G increased between weeks 0 and 4 then remained nearly constant after week 4. T_{50} and $T_{90-T_{10}}$ values for dry-stored seeds varied during the 10-week storage period but did not change markedly except for a decrease in T_{50} between weeks 8 and 10 (Fig. 1 B and C). The G value for stratified seeds increased from 29% to 98% between weeks 4 and 10 (Fig. 1A). G values were greater for dry-stored seeds at weeks 2, 4, and 6, were similar for dry-stored and stratified seeds at week 8, and were significantly higher for stratified seed at week 10.

G values never exceeded 64% for dry-stored seed, whereas seeds stratified for 10 weeks germinated 98% (Fig. 1A). This situation is indicative of physiological dormancy [or endodormancy, according to Lang et al. (1987)] in which G was low despite high seed viability. Our results concur with previous studies (Baskin and Baskin, 1989; Blake, 1935; Greene and Curtis, 1950; Nichols, 1934; Salac and Hesse, 1975), which have shown that seed dormancy in *Liatris* can be overcome with stratification. In the current study, stratification for 10 weeks markedly increased G and decreased T_{50} and $T_{90-T_{10}}$ (Fig. 1). Seeds stratified for 8 weeks or less time exhibited lower G values and delayed germination compared to those stratified for 10 weeks (Fig. 1).

During the first 4 weeks of storage at 4 °C, G values declined slightly for stratified seeds but increased from 38% to 61% for dry seeds (Fig. 1A). This increase in G during dry storage can be attributed to after-ripening. After-ripening occurs in recently harvested seeds of many herbaceous species (Baskin and Baskin, 1998) and is characteristic of seeds with non-deep physiological dormancy (Nikolaeva, 1977). Seeds of *L. squarrosa* (L.) Michx. after-ripened during 12 weeks of dry storage (Baskin and Baskin, 1989) but to a lesser extent than we obtained with *L. spicata*.

Expt. 2. Aqueous solutions of BA applied to blotter paper significantly affected G and T_{50} values ($P < 0.0001$; Fig. 2) but did not influence $T_{90-T_{10}}$ values ($P = 0.95$; data not shown). G increased quadratically and T_{50} decreased quadratically as BA concentration increased (Fig. 2).

Expt. 3. BA concentration in acetone had a significant ($P < 0.01$) effect on G, T_{50} , and $T_{90-T_{10}}$ values. G increased quadratically, whereas T_{50} and $T_{90-T_{10}}$ values decreased quadratically in response to BA concentration. G increased from 61% to 95% as BA concentration increased from 0 to 1127 mg·L⁻¹ (Fig. 3A). Increasing the BA concentration from 0 to 225 mg·L⁻¹ decreased T_{50} values from 11.5 to 6.3 d (Fig. 3B). $T_{90-T_{10}}$ values remained constant as BA concentration increased from 0 to 113 mg·L⁻¹ but decreased when BA concentration increased from 113 to 1127 mg·L⁻¹ (Fig. 3C).

Exogenously applied cytokinins can overcome seed dormancy in a few species such as *Rosa arvensis* Huds. (Jackson and Blundell, 1963) and *Xanthium pensylvanicum* Wallr. (Khan, 1966) but are only moderately or marginally effective in releasing seed dormancy in many other species (see review by Black, 1980). In *L. spicata*, germination was en-

Fig. 1. Influence of storage conditions at 4 °C [dry (●, solid line) or moist (■, dashed line)] and treatment duration on (A) germination percentage, (B) days to 50% of final germination (T_{50}), and (C) days between 10% and 90% germination ($T_{90-T_{10}}$) for *Liatris spicata* seed (Expt. 1). Data are means of four 100-seed replications ± 1 SD.

Fig. 2. Effects of BA concentration on germination percentage (●, solid line) and days to 50% of final germination (T_{50} ; □, dashed line) for *Liatris spicata* seed (Expt. 2). Aqueous solutions of BA were applied on the day of sowing. Data are means of four 100-seed replications ± 1 SD.

hanced when cytokinin was applied as an aqueous solution (Fig. 2) or infused into seeds via acetone (Fig. 3). G and T_{50} values obtained with acetone permeation of *L. spicata* seeds with BA at 225 or 1127 $\text{mg}\cdot\text{L}^{-1}$ (Fig. 3) were similar to those values attained with 10 weeks of stratification (Fig. 1). Seeds that were stored dry in Expt. 1 and those treated with acetone alone (Expt. 3) yielded similar G, T_{50} , and $T_{90}-T_{10}$ values (Figs. 1 and 3), indicating that acetone had little or no effect on germination responses. It is noteworthy that the 3-min infusion treatment that we used in Expt. 3 is much shorter than the 1- to 24-h infusion treatments reported elsewhere (Khan et al., 1973; Persson, 1993; Tao and Khan, 1974; Tao et al., 1974).

Water-soluble compounds such as BA are retained in the aqueous solution when seeds are germinated in a closed system (petriplates), but these compounds may leach readily or become bound by substrate particles when seeds are sown in open systems like those used commercially, e.g., trays containing germination medium. From a horticultural perspective, it would be useful to know if BA is as efficacious in open systems as it is in closed systems. We therefore applied an aqueous solution of 100 $\text{mg}\cdot\text{L}^{-1}$ BA to three plug trays (91 seeds per tray, 1 seed per plug cell) 1 d after sowing and another three plug trays received only dH_2O . Germination (radicle emergence) was assessed daily for 21 d. $T_{90}-T_{10}$ values were similar for BA-treated seeds and controls, but BA had a significant ($P \leq 0.008$) effect on G and T_{50} . G and T_{50} values were 56.3% and 14.3 d, respectively, for controls and 86.3% and 11.0 d, respectively, for BA-treated seeds. These results show that BA improves germination of *L. spicata* seed in open and closed systems.

Expt. 4. Thiourea had a significant effect on G ($P < 0.0001$) and T_{50} ($P < 0.004$) but $T_{90}-T_{10}$ values were similar ($P = 0.43$) for all treatments. G increased quadratically and T_{50} decreased linearly with increasing thiourea concentration (Fig. 4). The highest G and lowest T_{50} values were obtained when seeds were immersed in 7.61 $\text{mg}\cdot\text{L}^{-1}$ thiourea for 24 h. The response of *L. spicata* to thiourea (Fig. 4) is similar to that of numerous other species (Mayer and Poljakoff-Mayber, 1982).

Erez (1978) studied the effect of thiourea on growth of cytokinin-requiring callus tissues of *Glycine max* (L.) Merrill, *Malus pumila* Mill., and *Nicotiana tabacum* L. and found that thiourea compensated for lack of cytokinins in all three species. These data suggest that tissues exhibiting a stimulatory effect from thiourea may also be responsive to cytokinin. Cytokinin and thiourea have been found to increase seed germination in two other Asteraceae species: *Lactuca sativa* L. (Reynolds and Thompson, 1973; Thompson and Kosar, 1939) and *X. pensylvanicum* (Esashi et al., 1975; Khan, 1966).

Expt. 5. Germination responses of *L. spicata* seeds were not influenced by GA_3 concentration ($P > 0.05$). G, T_{50} , and $T_{90}-T_{10}$ values were 38%, 13.4 d, and 12.9 d, respectively, when averaged over the four GA_3 treatments.

Fig. 3. Influence of BA concentration on (A) germination percentage, (B) days to 50% of final germination (T_{50}), and (C) days between 10% and 90% germination ($T_{90}-T_{10}$) for *Liatris spicata* seed (Expt. 3). Data are means of four 100-seed replications ± 1 sd. Dry seeds were permeated with BA in acetone for 3 min.

Fig. 4. Effects of thiourea concentration on germination percentage (●, solid line) and days to 50% of final germination (T_{50} ; □, dashed line) for *Liatris spicata* seed (Expt. 4). Seeds were immersed in thiourea solutions for 24 h prior to sowing. Data are means of four 100-seed replications ± 1 sd.

Applied gibberellins overcome physiological seed dormancy in many species (Baskin and Baskin, 1998) but failed to stimulate germination in *L. spicata*. This may have been due to suboptimal concentrations or lack of sensitivity of *L. spicata* seed to applied gibberellins or GA₃ per se. In some species, GA₄ and GA₇ are more effective than GA₃ in promoting seed germination (Thompson, 1969; Watkins and Cantliffe, 1983).

Conclusions

Stratification, BA, or thiourea overcame seed dormancy in *L. spicata*. The most effective treatment for enhancing and accelerating germination was stratification at 4 °C for 10 weeks. However, infusion of BA via acetone appears to be a practical means of breaking seed dormancy and accelerating germination in *L. spicata*. Seeds can be permeated with BA and vacuum-dried within a 2-h period. Additional studies are needed to 1) determine the effects of seed treatments (stratification and BA infusion via acetone) on growth and development of *L. spicata* seedlings, and 2) ascertain if the promotive effect of BA is retained when *L. spicata* seeds are stored for extended durations after acetone infusion.

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