Substrate Moisture Level Governs the Germination of Verbena Seed

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Abstract. *Verbena* (*Verbena x hybrida* Voss) seed germination varied with the water content of the substrate. Total germination percentages (G) were highest when substrates were 75% to 100% saturated and progressively declined with increased free distilled water (FDW) on the blotter paper substrate. Natural differences in G among cultivars at favorable substrate moisture levels increased when free water was present. Removing the seed hilums did not increase G of 'Romance Scarlet' on a substrate with FDW, but significantly increased the G of 'Showtime Blaze' and 'Red A'. An inverse relationship was found between seed moisture contents and G at high substrate moisture levels. 'Romance Scarlet', 'Showtime Blaze', and 'Red A' had 64%, 73%, and 84% seed moisture contents and G of 72%, 18%, and 10%, respectively. The period of sensitivity to excessive water during germination was day 2 for 'Red A' and days 2 and 3 for 'Showtime Blaze'. The G of 'Romance Scarlet' seed was not reduced when placed on a substrate containing FDW for 1 day. Removal of seed hilums from 'Red A' significantly increased G during day 2 for seeds on a substrate containing FDW.

Germination of the garden verbena is erratic, with total germination for most cultivars between 40% and 60%. Total germination was found to vary from 19% to 64% when nine seed laboratories, using the identical cultivar seed lot, germinated seeds at 20C (Heit, 1963). Heit attributed these results to verbena seed sensitivity to water and the variable substrate moisture levels used among the laboratories. High medium moisture content has been reported to reduce the

seed germination of numerous plant species, including bean (*Phaseolus vulgaris* L.), spinach (*Spinacea oleracea* L.) (Orphanos and Heydecker, 1968), and beets (*Beta vulgaris* L.) (Heydecker and Chetram, 1971). Ely (1979) reported 'Blaze' verbena water sensitivity was caused by the formation of a water film over the seeds' surface, which was a barrier to O₂ diffusion. Maekawa and Carpenter (1991) showed that the testa of verbena seed hilum is hydrophilic and expands during water imbibition, thus restricting the hilum aperture. Free water in the substrate was believed to block the reduced hilum opening of many imbibed verbena seeds, thus limiting O₂ diffusion through the micropyle and reducing total germination. The objectives of these experiments were to determine the effect of substrate moisture level, hilum removal, day of application of free distilled water (FDW), and seed moisture content on the rate, uniformity, and total germination of verbena seed.

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Fig. 1. Comparison of verbena seed germination on saturated blotter paper (5 ml) and with free water (10 ml) using logistic regression models of the form percent $G = G_{50} / \{1 + \exp[-RC(\text{days} - G_{50})]\}$ as in Eq. [1] for expressing percent germination as a function of days after sowing, where G is an estimate of the final germination, G_{50} represents days to 50% of final germination, and RC is the rate constant, with lower numbers indicating a slower rate of germination. Cultivar treatment values were compared using the asymptotic 95% confidence intervals.

Cultivar	ml DW	G_{50}	RC
Romance Scarlet	5	6.2 a	1.5 a
	10	7.8 b	0.6 b
Showtime Blaze	5	8.0 a	0.8 a
	10	9.3 b	0.6 a
Red A	5	5.9 a	1.8 a
	10	11.6 b	0.3 b

Substrate water content during germination. Seeds of 'Romance Scarlet' (Sluis and Groot, Quality Seeds, Enkhuizen, Holland) and 'Showtime Blaze' and seedling 'Red A' (Goldsmith Seeds, Gilroy, Calif.) were received in Dec. 1988 and immediately germinated in 9-cm petri dishes on Anchor blue blotter paper 100 (Anchor Paper Co., Charlotte, N.C.) moistened with 5 or 10 ml of distilled water (DW). Tests using blotter paper from several lots indicated that saturation occurred after adding 5.1 ± 0.1 ml DW. In preliminary studies, total germination varied among these cultivars when germinated on mediums containing FDW, with 74%, 26%, and 10% for 'Romance Scarlet', 'Showtime Blaze', and 'Red A', respectively. 'Red A' is an outstanding cultivar, but has low total and highly irregular germination that prevents introduction by Goldsmith Seeds. Treatments of four 100-seed replications were germinated in darkness at 25°C. Daily germination counts were made when seeds had visible radicle protrusion through the testa. Final germination percent (G), days to 50% of final germination (G_{50}), and rate constant of germination (RC) were measured using logistic regression models of the form:

$$\%G = \frac{G}{1 + e^{-RC(\text{Days} - G_{50})}} \quad [1]$$

The RC for the percentage of increase during germination was calculated for all treatments. The design was a randomized block, with data analyzed by nonlinear regression procedures using SAS (1982). Treatment values were compared using the asymptotic 95% confidence intervals.

Hilum removal. In a second study, the hila were removed from 250 seeds each of 'Romance Scarlet', 'Showtime Blaze', and 'Red A' by use of a stainless steel scalpel with a #11 (5×20 mm) blade. Intact seeds and those having the hilum removed were germinated as above with 10 ml of DW added to each petri dish, which resulted in FDW on the blotter paper. Treatments contained five 50-seed replications and seeds were germinated in darkness at 25°C. Seed counts and statistical analyses were conducted as in the previous experiment.

Seed water content and germination. Seeds of the three cultivars were germinated as above with 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 9, or 10 ml DW added to the blotter paper in each petri dish. Treatments consisted of six 50-

Table 1. Comparison of final germination percentage and days to 50% of final germination for verbena seed germinated on saturated blotter paper (5 ml DW), in free water (10 ml DW), or on saturated blotter paper with free water on day 1, 2, 3, 4, or 5.

Cultivar	Treatment	Germination (%)	(days)
Red A	Constant 5 ml	62	8.4
	10 ml, day 1	56	8.7
	10 ml, day 2	31	11.3
	10 ml, day 3	53	9.8
	10 ml, day 4	59	8.8
	10 ml, day 5	64	8.5
	Constant 10 ml	8	16.7
Showtime Blaze	Constant 5 ml	73	8.9
	10 ml, day 1	73	9.0
	10 ml, day 2	52	10.8
	10 ml, day 3	57	10.5
	10 ml, day 4	70	9.3
	10 ml, day 5	71	9.2
	Constant 10 ml	28	14.4
Romance Scarlet	Constant 5 ml	89	6.0
	10 ml, day 1	90	5.8
	10 ml, day 2	89	6.2
	10 ml, day 3	87	6.1
	10 ml, day 4	86	6.1
	10 ml, day 5	89	5.9
	Constant 10 ml	66	7.8
Significance			
Cultivar (C)		**	**
Treatment (T)		**	**
C $\times$ T		**	**

**Significant at $P = 0.01$.

seed replicates, and germination was in darkness at 25°C. Germination counts were made daily when seeds had visible radicle protrusion. The data were analyzed by establishing regression equations for each quantity of water added for each cultivar. This experiment was repeated to determine percent moisture contents of seeds following 3 days of imbibition in petri dishes with each of the above levels of DW added to the blotter paper. Seeds were blotted dry and the remaining surface moisture was removed by 10 min of vacuum aspiration before reweighing. Seeds were placed in open petri dishes, dehydrated in forced-draft ovens at 40°C for 48 h, and weighed again after the seeds cooled. The data for G were analyzed by establishing regression equations for seed moisture contents for each cultivar.

Water-sensitive period during germination. Seeds of 'Romance Scarlet', 'Showtime Blaze', and 'Red A' were germinated continuously, as described above, in 5 or 10 ml DW, or in 5 ml with 24 h in 10 ml DW on day 1, 2, 3, 4, or 5. The addition of 10 ml DW on these days permitted 5 ml of FDW to remain on the blotter paper. Treatments consisted of six replications of 50 seeds, and germination counts were made daily. The design was a randomized complete block with analysis of variance (ANOVA) at $P = 0.01$.

In a subsequent study, comparisons of G were made between seeds of 'Red A' intact or with the hilums removed that were then placed on blotter paper containing FDW (10 ml) only during day 2 of germination. Dur-

Fig. 2. Germination of verbena seeds with and without hilum on blotter paper with free water (10 ml) using logistic regression models of the form percent $G = G/[1 + \exp[-RC(\text{days} - G_{50})]]$ as in Eq. [1] for expressing percent germination as a function of days after sowing, where G is an estimate of the final germination, G_{50} represents days to 50% of final germination, and RC is the rate constant, a measure of the rate of germination. Cultivar treatment values were compared using the asymptotic 95% confidence intervals.

Cultivar	Treatment	G
Romance Scarlet	With hilum	61 a
	Without hilum	65 a
Showtime Blaze	With hilum	19 a
	Without hilum	44 b
Red A	With hilum	10 a
	Without hilum	39 b

ing the other 13 days of the germination period all seeds were on blotter paper saturated with 5 ml DW. Treatments contained six replications of 50 seeds and germination was as described previously. Germination counts were made daily with the estimate of the final germination (G), days to 50% of final germination (G_{50}), and rate constant of germination (RC) measured using logistic regression models as in Eq. [1]. Treatment values were compared using the asymptotic 95% confidence intervals.

Substrate moisture content significantly influenced the G of each cultivar (Fig. 1). Larger G accompanied saturating blotter papers with 5 ml DW. Applying 10 ml DW, which permitted 5 ml of FDW in petri dishes, significantly reduced the G for all cultivars, but 'Romance Scarlet' had a much smaller reduction than 'Showtime Blaze' or 'Red A' (Fig. 1). The days required for G_{50} were increased for each cultivar when the blotter paper contained free water, with the largest delay for 'Red A' (Fig. 1). Lower RC readings, indicating slower germination rates, occurred when seeds were germinated with FDW for 'Romance Scarlet' and 'Red A', but no differences were found for 'Showtime Blaze' (Fig. 1). These results support the

conclusions of Heit (1963), who found that verbena seed germination is a function of substrate moisture level, and excessive moisture causes variability in G .

'Showtime Blaze' and 'Red A' seeds with hilums removed had higher G than intact seeds when germinated with FDW on the blotter papers. In contrast, 'Romance Scarlet' seeds with or without the hilum had similar G (Fig. 2). 'Showtime Blaze' and 'Red A' seeds with and without hilums had similar G at favorable (5 ml DW) substrate moisture levels (data not presented). Comparisons by Maekawa and Carpenter (1991) of the morphology of dry and imbibed 'Romance Scarlet' seeds showed that wetting caused only limited thickening of the hilum walls and reduction of the hilum apertures, while wetted 'Showtime Blaze' and 'Red A' seeds had more wall thickening and hilum aperture closure. Removing the hilum had no significant effect on the days required for G_{50} (7.6 to 11.6 days) or RC (0.3 to 0.6) at which seeds germinated on blotter paper in FDW.

Highest G occurred when the blotter paper substrate was 75% to 100% saturated with ≈ 4 or 5 ml DW (Fig. 3A). The G for all cultivars progressively declined as blotter paper moisture contents declined below 75%

of saturation or when the amount of free water increased. 'Romance Scarlet' had maximum G when the blotter paper was saturated (5 ml), the other cultivars when at 4 ml. Maximum G occurred at seed moisture contents of 53% for 'Showtime Blaze', 60% for 'Romance Scarlet', and 63% for 'Red A' (Fig. 3B). 'Romance Scarlet' had lower seed moisture percentages at each blotter paper water level than the other cultivars. Percentages of germination for 'Romance Scarlet' declined from a maximum of 83% to 78% as FDW increased, G of 'Showtime Blaze' declined from 65% to 26%, while G of 'Red A' declined from 70% to 22% (Fig. 3B). Blacklow (1972) reported 75% water content was optimum for germinating dent corn (*Zea mays* L.) seed, which was accompanied by a 261% water content, on a dry-weight basis, of the embryo. He associated very high seed water contents with excessive embryo hydration and reduced G .

The period of sensitivity to excessive water during germination of verbena seed encompassed the 2nd or 3rd day. Placing seeds of 'Red A' on blotter paper containing FDW (10 ml) during only the second day greatly reduced G , while similar treatment on days 1, 3, 4, or 5 did not reduce G (Table 1). Reduced G of 'Showtime Blaze' resulted when seeds were placed in FDW during days 2 or 3 of germination, with no reductions in G from similar treatment on days 1, 4, or 5. 'Romance Scarlet' G was reduced when seeds were kept continually in FDW on the substrate during germination, but 1 day in FDW did not reduce G (Table 1).

The germination of 'Red A' seed was significantly reduced by placing imbibed seeds on substrates with FDW for 24 h during the second day of germination (Table 1). 'Red A' seeds without hilums during this treatment had significantly higher G than seeds with hilums (Fig. 4). Seeds with hilums removed had 84% and intact seeds 51% the G of seeds on saturated blotter paper without FDW. Seeds with hilums removed required fewer days to G_{50} , and had faster rates of germination, indicating a lower level of retardation during germination (Fig. 4). The improved germination of seeds without hilums possibly resulted from the more rapid re-establishment of O_2 exchange through micropyles following removal from a substrate containing FDW. The extensive closure of hilum apertures for imbibed 'Red A' seeds with hilums intact reported by Maekawa and Carpenter (1991) could extend temporary anaerobiosis and further lower G .

The verbena is an excellent bedding plant with a wide color range, exceptional climatic adaptability, and high consumer demand. Hausbeck et al. (1982) reported that verbena are not commonly available to retail garden centers or consumers because growers have problems with the germination of its seed. Providing adequate but not excessive substrate moisture during germination is the major problem encountered. Maekawa and Carpenter (1991) found seed hilum morphology was responsible for reduced G on

Fig. 3. (A) The percentages of total germination for 'Romance Scarlet', 'Showtime Blaze', and 'Red A' verbena seeds on blotter paper wet by 1 to 10 ml DW. Blotter paper was saturated at 5 ml. Regression equations with water (ml) = x: Romance Scarlet, $R^2 = 0.94$, percent G = $-37.3 + 64.6x - 10.2x^2 + 0.48x^3$; Showtime Blaze, $R^2 = 0.90$, percent G = $-24.4 + 65.0x - 12.0x^2 + 0.59x^3$; Red A, $R^2 = 0.91$, percent G = $-12.3 + 56.2x - 10.5x^2 + 0.51x^3$. (B) The percentage of total germination for each cultivar in relation to percent seed moisture content. Regression equations with percent seed moisture = x: Romance Scarlet, $R^2 = 0.74$, percent G = $-131.5 - 51.8x + 1.2x^2 - 0.01x^3$; Showtime Blaze, $R^2 = 0.65$, percent G = $1256.6 + 61.7x - 0.9x^2 + 0.01x^3$; Red A, $R^2 = 0.62$, percent G = $404.1 + 14.5x - 0.1x^2 + 0.01x^3$.

Fig. 4. Comparison of cumulative germination curves for 'Red A' verbena seeds with and without hilum that were placed on blotter paper containing free water (10 ml DW) only during day 2 of germination. Logistic regression models of the form percent G = $G/[1 + \exp\{-RC(\text{days} - G_{50})\}]$ as in Eq. [1] were used for expressing percent germination as a function of days after sowing, where G is an estimate of the final percent germination, G_{50} represents days to 50% of final germination, and RC is the rate constant, a measure of the rate of germination. Treatment values were compared using the asymptotic 95% confidence intervals.

Treatment	G_{50}	RC
Without hilum	5.4 a	1.3 a
With hilum	7.1 b	0.6 b

wet substrates. Our current research showed that differences in G among cultivars was small at favorable substrate moisture levels, but increased with greater FDW. 'Romance Scarlet', which is more tolerant of FDW on the substrate, had similar G for seeds with or without hilum in the presence of free water, while removal of seed hilums significantly increased G in the presence of free water for the sensitive 'Showtime Blaze' or 'Red A'.

No attempt was made in this study to determine the changes in seed physiology resulting from excessive FDW on the substrate. Instead, the studies were conducted to determine the possible causes for the irregular total and delayed germination experienced by commercial propagators. Our results indicate verbena cultivars have a narrow percent seed moisture tolerance range for acceptable germination. The ranges were: 'Romance Scarlet' 56% to 63%, 'Showtime Blaze' 49% to 58%, and 'Red A' 58% to 70%. Germination rapidly declines above or below these seed moisture ranges. Increasing FDW on the blotter paper from 0 to 5 ml per petri dish progressively increased the seed moisture content of 'Romance Scarlet' from 57% to 65% and reduced G from 83% to 78% (Fig. 3B). 'Showtime Blaze' G declined from 65% to 26% as seed moisture contents progressively increased from 57% to 73%, while 'Red A' G declined from 70% to 22% as seed moisture levels increased from 66% to 84% (Fig. 3B). These results indicate that reductions in verbena germination in the presence of free water on the substrate could be associated with high seed water contents, causing a deleterious degree of embryo hydration, as reported for corn by Blacklow (1972).

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