





# Mucilage affects seed water imbibition and germination time of subtropical monsoonal forbs

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## Abstract

The role of seed mucilage in the moist environments of monsoonal subtropics is poorly understood. We studied germination of six forb species from subtropical monsoonal China. Mucilage presence had little to no effect on germination percentage (G%), except for a significant decline for the large-seeded *Prunella vulgaris*. Dark treatments reduced G% for all species, while the combination of mucilage removal and high temperatures delayed the mean germination time (MGT). Seed fresh mass was negatively correlated with G%, but only for intact seeds incubated at 12/12 hours of 25/35 °C. The MGT of de-mucilaged seeds varied with seed shape index, also under the warmer temperature regime. Temperature and light are fundamental to drive germination processes, and the presence of mucilage influences MGT of subtropical monsoonal species. Presence or absence of mucilage had little to no difference in germination percentage, but can be important to drive germination timing, also promoting water uptake and seed adhesion to soil.

**Key words:** mucilage, germination, photoperiod, seed traits, temperature, forbs

## Résumé

Le rôle du mucilage des graines dans les environnements humides des régions subtropicales de mousson est mal compris. Les auteurs ont étudié la germination de six espèces d'herbes non graminéennes de la Chine subtropicale de mousson. La présence de mucilage n'avait que peu ou pas d'effet sur le pourcentage de germination (G%), à l'exception d'une baisse significative chez *Prunella vulgaris* à grosses graines. Les traitements à l'obscurité réduisaient le G% chez toutes les espèces, tandis que la combinaison de l'élimination du mucilage et des températures élevées retardait le temps moyen de germination (TMG). La masse fraîche des graines était négativement corrélée avec le G%, mais seulement chez les graines intactes incubées 12/12 heures à 25/35 °C. Le TMG des graines démucilaginées variait en fonction de l'indice de forme des graines, également sous un régime de températures plus chaudes. La température et la lumière sont fondamentales pour mener les processus de germination, et la présence de mucilage influence le TMG des espèces subtropicales de mousson. Il n'y avait que peu ou pas de différences dans le pourcentage de germination en fonction de la présence ou de l'absence de mucilage, mais celui-ci peut être important pour déterminer le moment de la germination, favorisant également l'absorption d'eau et l'adhésion des graines au sol. [Traduit par la Rédaction]

**Mots-clés :** mucilage, germination, photopériode, caractéristiques des graines, température, herbes non graminéennes

## Introduction

The production of mucilage on a seed/fruit dispersal unit during hydration (myxodiaspory) can serve one of several functions. It may protect the seed from predators and pathogens (Kreitschitz 2012; Western 2012), aid in seed dispersal via endozoochory or epizoochory (Yang et al. 2012), or maintain seed hydration in water-stressed surroundings (Western 2012). Mucilage is primarily composed of polysaccharides, such as pectins, hemicelluloses, and cellulose (Naran et al. 2008; Phan et al. 2016; Kreitschitz et al. 2021). Mucilage has a mixed effect on germination, which it may

initiate and enhance (Huang and Gutterman 1999; Yang et al. 2010; Sun et al. 2012), or inhibit by acting as a physical barrier to water and oxygen diffusion to the inner seed tissues (Huang and Gutterman 1999). Alternatively, it may have no role in germination (Bhatt et al. 2019). Hence, the use of mucilage varies among species and environments (Yang et al. 2012; Bhatt et al. 2016; Chen et al. 2018).

Seed mucilage is ecologically important in species of arid habitats where germination and seedling establishment benefit from the attraction and retention of moisture (Gutterman and Shem-Tov 1996; Gorai et al. 2014; Zhang

**Table 1.** Seed collection details of the studied species.

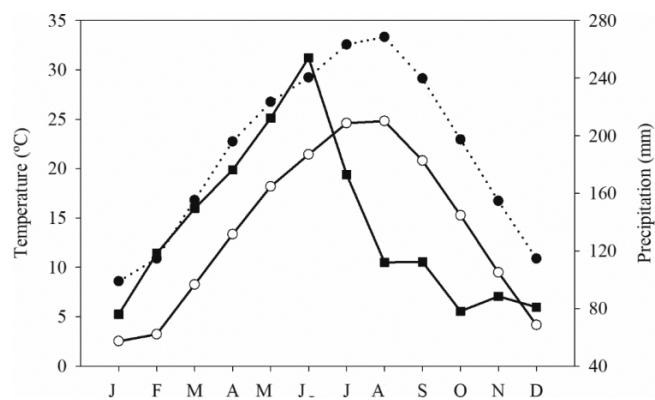
Species	Family	Collection month (2020)	Locality	Lat (N)	Long (E)	Altitude (m a.s.l.)	Habit	Collection habitat
<i>Lepidium virginicum</i>	Brassicaceae	June	Saiyang	29°32'	115°53'	107	Annual forb	Stream side
<i>Plantago asiatica</i>	Plantaginaceae	June	Saiyang	29°32'	115°53'	130	Perennial forb	Forests
<i>Plantago virginica</i>	Plantaginaceae	May	Gutang	29°38'	116°8'	7	Annual forb	Stream side
<i>Prunella vulgaris</i>	Lamiaceae	June	Wentang	27°39'	114°17'	443	Perennial forb	Open area
<i>Salvia plebeia</i>	Lamiaceae	May	Wucheng	29°8'	115°58'	21	Annual forb	Open area
<i>Solanum americanum</i>	Solanaceae	October	Lunshan North Road	29°36'	116°0'	920	Annual forb	Open area

et al. 2014; Huang et al. 2015; Bhatt et al. 2016). In wetter environments, the role of seed mucilage is usually thought to be for dispersal or defense (Wu et al. 2003; Thapliyal et al. 2008; Sun et al. 2012), and therefore its influence on germination has received little attention. Nevertheless, precise regulation of water and air concentrations around the embryo may still influence germination, even in moist habitats.

Temperature and light are the main environmental factors that regulate seed germination (Bewley et al. 2013) when availability of moisture is not a constraint (Chauhan and Johnson 2008). Temperature regulates timing of germination (Baskin and Baskin 2014) and influences hormone synthesis and enzyme activities during germination (Nonogaki et al. 2010). Light requirement determines whether germination is improved by seed burial (Fenner and Thompson 2005). In addition to abiotic factors, seed traits such as seed mass, life form, and mode of dispersal also influence germination patterns worldwide (Guo et al. 2000; Willson and Traveset 2000; Figueroa 2003; Jurado and Flores 2005; Moles et al. 2007; Rubio de Casas et al. 2017; Bhatt et al. 2020). Therefore, observation of these biotic factors can also improve our understanding of germination biology of a species.

The aim of this study was to evaluate the role of seed mucilage, temperature, and light on germination of six small-seeded species (annual and perennial forbs) from a Chinese subtropical monsoonal region. Water availability is unconstrained in this Southeast Chinese habitat (Kang et al. 2017). Specifically, we asked how germination of the species was influenced by (i) seed mucilage presence, (ii) temperature and photoperiod regime, and (iii) seed fresh mass and seed shape index of intact and de-mucilaged seeds. We hypothesized that seed mucilage would have little to no effect on germination percentage since water is mostly available. However, the presence/absence of mucilage might affect seed imbibition patterns and mean germination time (MGT) by influencing enzyme activities that drive the embryo growth. Small-seeded species are often photoblastic, requiring light exposure for germination (Milberg et al. 2000; Zhang et al. 2014). Morphological traits and seed size may help to explain ecological behavior and germination timing of intact and de-mucilaged seeds.

**Fig. 1.** Five-year averages (2015–2019) of minimum (white) and maximum temperature (black circles), and precipitation (squares) for Jiujiang, Jiangxi, China.



## Materials and methods

### Seed collection and seed trait measurements

Seeds of *Lepidium virginicum*, *Plantago asiatica*, *Plantago virginica*, *Prunella vulgaris*, *Salvia plebeia*, and *Solanum americanum* were collected from Jiujiang, China, from May to October 2020, when ripening fruits showed their phenological maturity (Table 1). These species were selected based on the following criteria: (i) production of copious mucilage by diaspores after hydration and (ii) easy accessibility from wild populations. In wetter environments, the role of seed mucilage on germination has received little attention. Therefore, our results would be helpful for understanding the ecological roles of seed mucilage in a monsoonal ecosystem.

The Jiujiang climate is subtropical monsoonal, in which winter (December–February) is cold with a min/max temperature of 3–11 °C, while in mid-summer (July–August) the maximum temperature may exceed 39 °C. Rainfall events occur throughout the year, but precipitation is greatest during the monsoon (May–July; Fig. 1).

Seeds were collected from 35 to 50 maternal plants per species, spaced > 2 m apart, to ensure the genetic diversity

of the population. Seeds were extracted from the collected diaspores in the laboratory, and seeds that visually appeared non-viable were removed. Seeds were dried to 5%–8% moisture content (optimal conditions for most of orthodox seeds) using 15 °C and 20% relative humidity, then stored at –18 °C at the Lushan Botanical Garden, China, until the experiment started in December 2020. Seeds retrieved from –18 °C storage were allowed to equilibrate to room temperature for 24 h before starting the germination trials.

Fresh seed mass of each species was determined by weighing three 100-seed replicates of two treatments (intact and de-mucilaged) using an analytical balance (Sartorius Analytical Balance; accurate to 0.1 mg). Seed length, breadth, and width were measured on 15 seeds per species using a Stereo Microscope (Nikon SMZ800 N; Nikon Instruments Inc. Melville, NY, USA) fitted with a microscope camera IMG-SC600 C (iMG Biotechnology Co., Ltd, Suzhou, Jiangsu, China). Seed shape index was calculated by dividing each seed dimension by the individual seed's length and then calculating the variance among them. Index values vary from 0 (spherical) to 1 (elongated/flattened; Thompson et al. 1993; Perez-Harguindeguy et al. 2013). Seed color, morphology, and dispersal mode were also observed (Table 1).

### Mucilage removal

Mucilage was removed by soaking seeds in distilled water for 10 minutes, then gently blotting with filter paper until mucilage production ceased (Gorai et al. 2014; Bhatt et al. 2019). To determine the effect of mucilage presence on water uptake (water gain, %), three 100-seed replicates of each treatment (intact and de-mucilaged seeds) were placed on filter paper in 9 cm plastic Petri dishes; 10 mL distilled water was added and seeds were incubated for 19 hours at 20 °C in darkness (Gorai et al. 2014; Bhatt et al. 2019). Water gain was calculated as the percentage change in weight from before and after incubation.

### Germination trials

Effect of temperature (alternating day/night temperatures of 20/30 °C and 25/35 °C) and photoperiod of dark and light treatments (0 and 12 hours of light per day, respectively) were studied under laboratory conditions. Temperature regimes were chosen from a preliminary study (data not shown) that indicated these temperatures were suitable for germination of these species. Dark and light treatments were used to simulate exposed and buried seeds, respectively. The hottest temperature period of each alternating temperature regime coincided with the light cycle in the 12 hour light photoperiod.

A total of 48 treatments (six species, two temperature regimes, two photoperiods, and two mucilage conditions) were tested, using four replicates of 25 seeds each. Germination trials were conducted in 9 cm tight-fitting Petri dishes containing one disk of Whatman No. 1 filter paper and 10 mL of distilled water. Constant darkness was simulated by wrapping Petri dishes in two layers of aluminium foil. Germinated seeds were counted and removed daily from the 12 hour light treatments, while seeds under the dark treatments were evaluated only at the end of experiment (after 30 days). Germination

was defined as the presence of a radicle ( $\geq 2$  mm). At the end of the germination trials, all the ungerminated seeds (both intact and de-mucilaged) from the 12 hour light treatments were dissected to evaluate the embryo status and viability of the ungerminated seeds (living and therefore white; turgid and brown and therefore dead) under a stereoscope.

### Data analysis

The influence on germination percentage of all first-order treatments within species and their interactions was evaluated using generalized linear models (GLMs) with a binomial distribution. The analysis was repeated for MGT but without the photoperiod treatment as MGT could not be calculated for seeds in the dark treatment. Values for MGT were log-transformed. All analyses were conducted using “lme4” and “nlme” packages (Bates et al. 2015; Pinheiro et al. 2021) in R software (R Core Team 2020). Among treatments, multiple comparisons were performed using post-hoc HSD (Tukey) tests in the “agricolae” package (De Mendiburu 2021).

To evaluate the relationship of seed traits (seed fresh mass and seed shape index) with germination patterns, we conducted mixed models (GLMMs) using species as a random effect (Zuur et al. 2009). Germination percentage (G%) was evaluated as a function of seed fresh mass + seed shape index, separately under the temperature regimes of 20/30 and 25/35 °C, with a binomial distribution. Values of log-transformed MGT were evaluated as a function of the same seed traits, under the same temperature regimes, using linear mixed models (LMMs).

## Results

### Seed characterization

All studied species had relatively small seeds, with seed fresh mass varying from 0.012 (*S. plebeia*) to 0.043 g (*P. vulgaris*; Table 2). Water imbibition in fresh seeds ranged from an increase of 56% to 182%, and in de-mucilaged seeds ranged from 44% to 119%. Removal of mucilage reduced water imbibition in all species except for *P. asiatica*. The most dramatic change was in seeds of *P. vulgaris*, which had the largest seed fresh mass, the largest imbibition of intact seeds, and the largest decline of imbibition with mucilage removal (Table 2).

Seed length ranged from 0.9 mm in *S. plebeia* to 1.8 mm in *P. asiatica* and *P. vulgaris* (Table 2). Seeds of *S. plebeian* were the most rounded (seed shape index 0.029), but all other species exhibited a rounded shape with a seed shape index around 0.1 (Table 2). Hence, seed morphology was classified from ovoid to fusiform/discoid in all study species. Dispersal mode is mostly related to animals (birds and small mammals), except for *L. virginicum* and *S. plebeia*, which can be dispersed by wind and (or) water (Table 2).

### Mucilage, temperature, and light

Seed mucilage presence had little to no effect on germination patterns (Table 3), except for *P. vulgaris*, where G% decreased from 83%  $\pm$  5% in intact to 47%  $\pm$  6% in de-mucilaged seeds under 12 hours of light and 20/30 °C (Table 3; Fig. 2). Maximum G% values were observed under the 12 hour light

**Table 2.** Seed characteristics and water imbibition (mean  $\pm$  SD).

Species	Fresh mass (g)	Intact seed imbibition (% w/w)	De-mucilaged imbibition (% w/w)	Seed length (mm)	Seed shape index	Seed color	Seed morphology	Dispersal mode	References
<i>Lepidium virginicum</i>	0.026 $\pm$ 0.006	113 $\pm$ 1	77 $\pm$ 25	1.685 $\pm$ 0.085	0.149 $\pm$ 0.020	Reddish	Ovate-oblong	Wind	Zhang et al. 2020
<i>Plantago asiatica</i>	0.027 $\pm$ 0.005	92 $\pm$ 20	119 $\pm$ 20	1.844 $\pm$ 0.201	0.154 $\pm$ 0.018	Blackish-brown	Ovoid-ellipsoid	Animals	Yu et al. 2012
<i>Plantago virginica</i>	0.036 $\pm$ 0.006	111 $\pm$ 20	85 $\pm$ 17	1.652 $\pm$ 0.087	0.122 $\pm$ 0.019	Light-brown	Ovoid	Animals	Xu et al. 2019
<i>Prunella vulgaris</i>	0.043 $\pm$ 0.009	182 $\pm$ 16	64 $\pm$ 13	1.806 $\pm$ 0.115	0.097 $\pm$ 0.009	Brown	Fusiform	Animals	Picard et al. 2015
<i>Salvia plebeia</i>	0.012 $\pm$ 0.004	56 $\pm$ 10	44 $\pm$ 10	0.917 $\pm$ 0.091	0.029 $\pm$ 0.013	Dark-brown	Ovoid	Wind/water	Zona 2017
<i>Solanum americanum</i>	0.020 $\pm$ 0.003	63 $\pm$ 15	45 $\pm$ 28	1.325 $\pm$ 0.157	0.118 $\pm$ 0.014	Dark	Discoïd	Animals	Carlo 2005

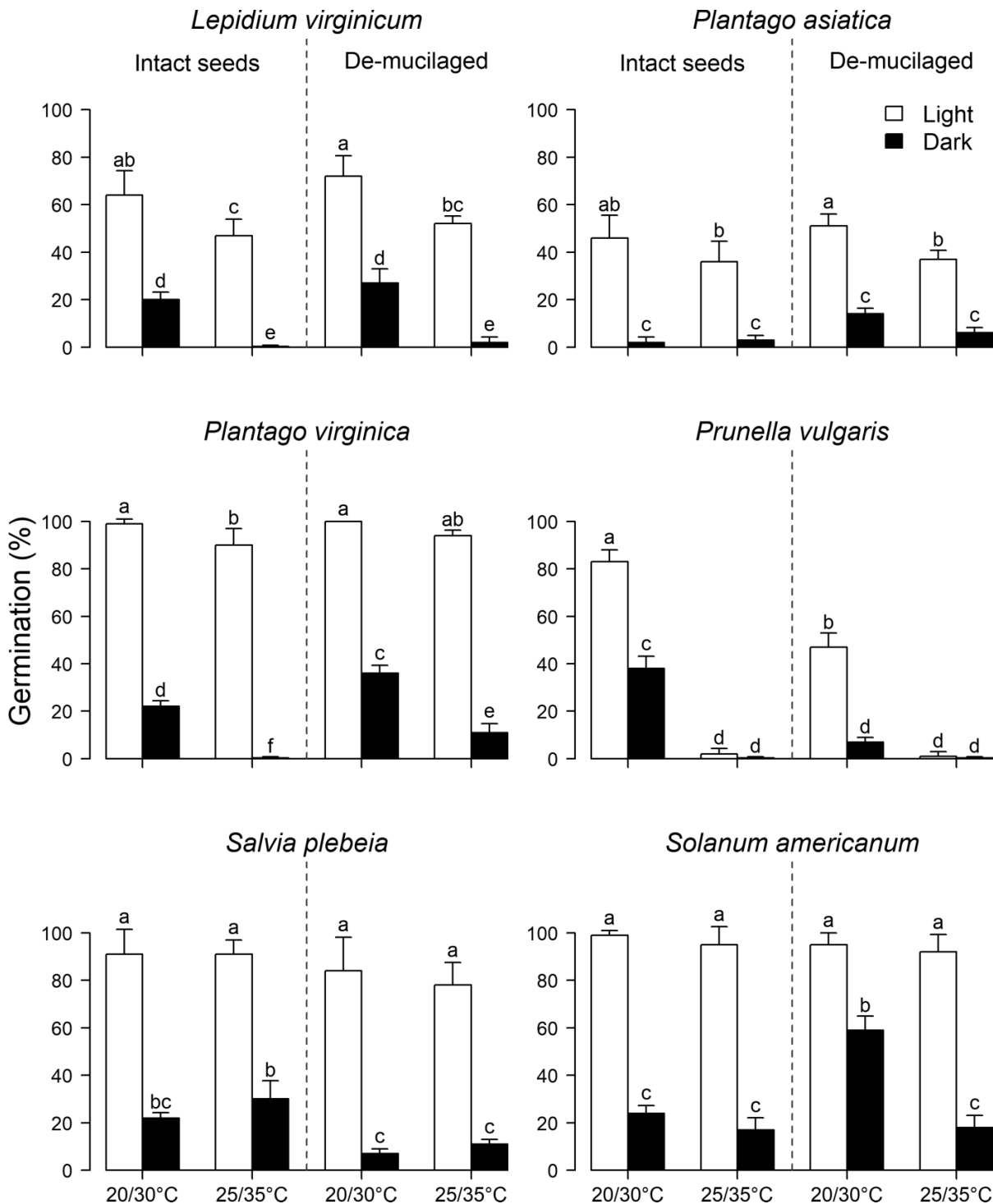
**Note:** Water gain (% w/w) refers to the gravimetric increase of seed mass after 19 hours of soaking.

**Table 3.** Generalized linear models with binomial distribution of seed germination.

Treatments	<i>Lepidium virginicum</i>			<i>Plantago asiatica</i>			<i>Plantago virginica</i>			<i>Prunella vulgaris</i>			<i>Salvia plebeia</i>			<i>Solanum americanum</i>		
	Est.	SE	P value	Est.	SE	P value	Est.	SE	P value	Est.	SE	P value	Est.	SE	P value	Est.	SE	P value
Intercept	0.58	0.21	–	–0.16	0.20	–	3.89	0.71	–	1.59	0.27	–	2.20	0.33	–	4.60	1.01	–
Mucilage (M)	0.37	0.31	0.23	0.20	0.28	0.48	0.70	1.23	0.57	<b>–1.71</b>	<b>0.33</b>	<b>&lt;0.001</b>	–0.54	0.43	0.21	–1.65	1.11	0.14
Temperature (T)	<b>–0.70</b>	<b>0.29</b>	<b>0.02</b>	–0.42	0.29	0.15	<b>–1.70</b>	<b>0.79</b>	<b>0.03</b>	<b>–5.48</b>	<b>0.76</b>	<b>&lt;0.001</b>	0.12	0.48	0.81	–1.65	1.11	0.14
Photoperiod (P)	<b>–1.96</b>	<b>0.33</b>	<b>&lt;0.001</b>	<b>–3.73</b>	<b>0.74</b>	<b>&lt;0.001</b>	<b>–5.16</b>	<b>0.75</b>	<b>&lt;0.001</b>	<b>–2.08</b>	<b>0.34</b>	<b>&lt;0.001</b>	<b>–3.46</b>	<b>0.41</b>	<b>&lt;0.001</b>	<b>–5.75</b>	<b>1.03</b>	<b>&lt;0.001</b>
M $\times$ T	–0.17	0.42	0.69	–0.16	0.41	0.70	–0.15	1.35	0.91	1.00	1.28	0.43	–0.51	0.61	0.40	1.15	1.25	0.36
M $\times$ P	0.02	0.45	0.96	<b>1.88</b>	<b>0.82</b>	<b>0.02</b>	–0.01	1.27	0.99	–0.39	0.55	0.48	–0.78	0.63	0.22	<b>3.17</b>	<b>1.15</b>	<b>0.01</b>
T $\times$ P	<b>–2.51</b>	<b>1.08</b>	<b>0.02</b>	0.83	0.97	0.39	–1.64	1.30	0.21	1.37	1.28	0.28	0.30	0.58	0.60	1.22	1.16	0.29
M $\times$ T $\times$ P	0.48	1.34	0.72	–1.20	1.13	0.29	1.96	1.74	0.26	1.10	1.96	0.58	0.59	0.85	0.49	–2.60	1.34	0.05

**Note:** Significant values ( $P < 0.05$ ) are highlighted in bold. Est., estimate; SE, standard error.

**Fig. 2.** Germination percentage (G%) for the six study species from Chinese subtropical monsoon regions in the tested treatments (presence of mucilage, temperature regime, light/dark photoperiod). Different letters refer to significant differences among treatments, separately for each species. For statistical tests and interactions, see **Table 3**.



photoperiod, reaching ~80%–100% for all the study species, except *L. virginicum* (64% ± 10%) and *P. asiatica* (46% ± 9%; **Fig. 2**). Darkness reduced G% of all species (**Table 3**), except for *S. americanum* seeds that reached ~60% of germination of de-mucilaged seeds in dark conditions under 20/30 °C (**Fig. 2**). Hence, G% of *S. americanum* seeds responded positively to

mucilage removal and germination under darkness (**Table 3**). *Plantago asiatica* also showed an interaction between mucilage and photoperiod, but to a lesser extent (**Table 3**; **Fig. 2**). Photoperiod and temperature had a negative interaction on *L. virginicum* G%, which dropped to almost zero in the warmer conditions (25/35 °C) in the dark (**Table 3**; **Fig. 1**). Irrespective

**Table 4.** Mean germination times (MGT) in days for intact and de-mucilaged seeds under two temperature regimes and 12 hours of light per day.

Species	Intact seeds		De-mucilaged	
	20/30 °C	25/35 °C	20/30 °C	25/35 °C
<i>Lepidium virginicum</i>	6.8 ± 0.5bc	6.5 ± 0.7c	8.7 ± 1.3b	12.3 ± 1.4a
<i>Plantago asiatica</i>	6.9 ± 1.3b	6.6 ± 1.2b	8.4 ± 0.6ab	10.5 ± 1.2a
<i>Plantago virginica</i>	3.3 ± 0.2c	4.8 ± 0.4b	4.5 ± 0.4b	9.5 ± 0.4a
<i>Prunella vulgaris</i>	15.3 ± 1.0 <sup>n.s.</sup>	–	13.9 ± 2.2 <sup>n.s.</sup>	–
<i>Salvia plebeia</i>	4.9 ± 0.1b	5.5 ± 0.1ab	6.4 ± 1.0a	6.2 ± 0.6a
<i>Solanum americanum</i>	6.2 ± 0.5c	8.0 ± 0.4b	6.6 ± 0.4c	10.1 ± 1.0a

Note: Letters indicate significant differences among treatments within each species. n.s., indicates nonsignificant; en-dashes (–) indicate null germination values.

of photoperiod or mucilage presence, temperature regime reduced *P. vulgaris* G% to almost zero at 25/35 °C (Fig. 2).

Mean germination times of intact seeds mostly ranged from three to eight days, with a significant delay (two to six days longer) in at least one of the tested temperature regimes when compared to de-mucilaged ones (Table 4). The exception was *P. vulgaris*, for which MGT lasted 14–15 days for both intact and de-mucilaged seeds under the temperature regime of 20/30 °C. Null germination values did not allow the calculations of MGT for *P. vulgaris* under the warmer condition. The temperature of 25/35 °C also caused longer MGT values (two to five days longer) for all study species, mostly in de-mucilaged seeds, except for *S. plebeia*, which kept germination of de-mucilaged seeds lasting around 6 days at both temperature regimes (Table 4).

### Role of seed traits

Seed fresh mass and shape index had little to no influence on G% or MGT (Supplementary Material Table S1; Fig. 3). Nevertheless, G% was negatively correlated with seed fresh mass ( $P = 0.03$ ), but the relationship was only found for intact seeds at 25/35 °C (Supplementary Material Table S1; Fig. 3). A significant relation was found between MGT and the seed shape index for de-mucilaged seeds at 25/35 °C (Supplementary Material Table S1; Fig. 3). All other conditions (mucilage presence and temperature regimes) had no relationship from either of the seed traits or germination parameters.

## Discussion

Water gain of intact seeds ranged from 12% to 118% higher than the de-mucilaged ones, except for *P. asiatica* (Table 2). Mucilage presence therefore plays a role in facilitating water uptake and retention, consistent with other species bearing mucilaginous seeds (e.g., Gu et al. 2008; Wu et al. 2009; Lu et al. 2010; Sun et al. 2012; Bhatt et al. 2016, 2019). Timing of seed dispersal can be important for optimizing moisture availability for germination, as reported in tropical mesic savannas (Salazar et al. 2011; Escobar et al. 2018). In our study site, most species disperse mature seeds in the rainy season (from May to July), thus seed mucilage had little influence on G% per se, as expected.

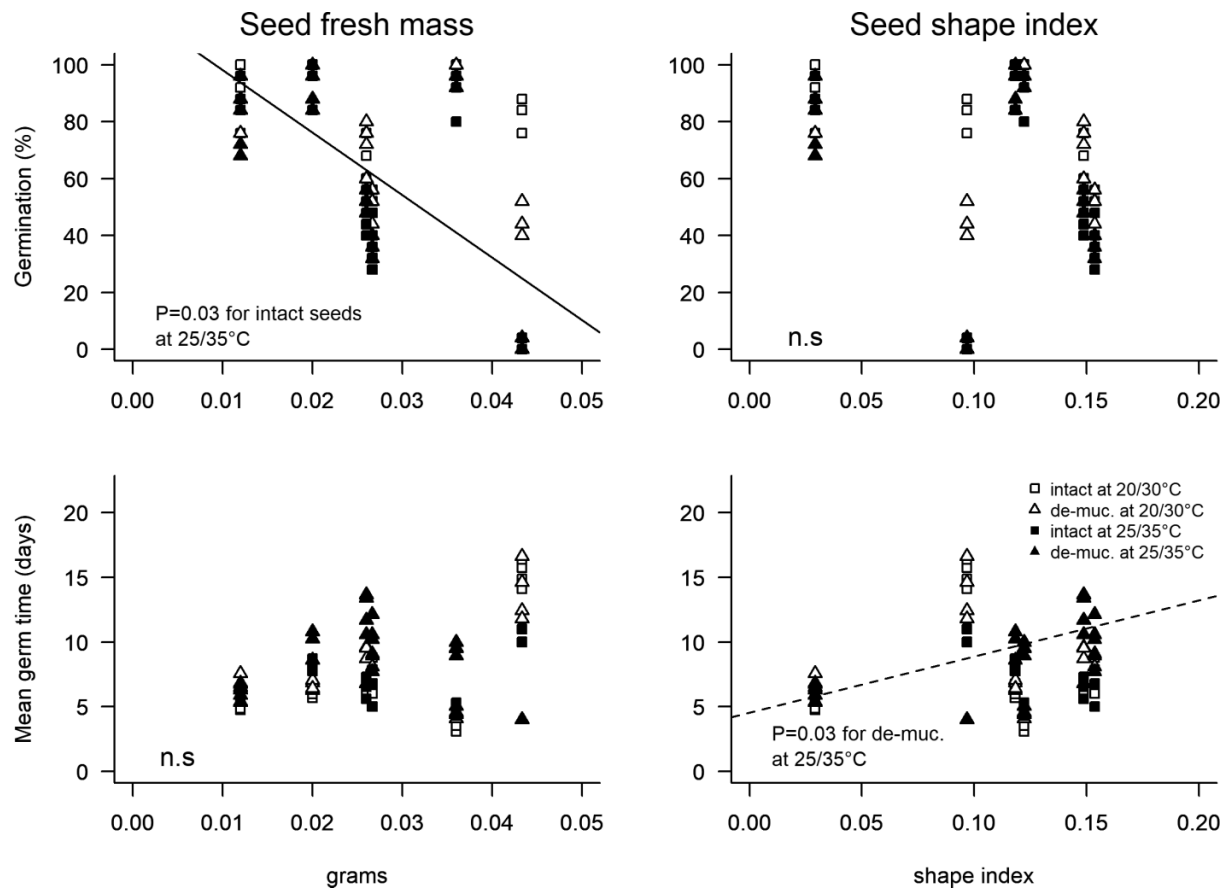
The most affected species was *P. vulgaris*, which bears the largest seeds among the studied species, also showing the

greatest reduction in water imbibition from mucilage removal and a significant decrease of G% (Table 3; Fig. 2). The larger mass of *P. vulgaris* seeds may increase the probability of granivory (Fenner et al. 2002). Water absorption by mucilaginous seeds of different species could be linked to the variation in the quantity of mucilage deposited in the mucilage-secreting cells (Kreitschitz and Gorb 2018). Previous studies reported that the increase of seed fresh mass of mucilaginous seeds after hydration can be species-specific. For example, in *Linum usitatissimum* seeds, fresh mass can increase around 3 times the initial weight, reaching up to 167 times in *Alyssum minus* and up to 589 times in *Artemisia sphaerocephala* (Huang and Gutterman 1999; Sun et al. 2012; Kreitschitz et al. 2015).

Despite the minimal effect of mucilage presence on G%, the warmer temperature regime decreased germination of *L. virginicum*, *P. asiatica*, and *P. vulgaris* seeds, while both intact and de-mucilaged seeds of *P. virginica*, *S. plebeia*, and *S. americanum* germinated equally well in both temperature regimes. On the other hand, MGT was delayed in de-mucilaged seeds by two to six days, with a greater delay associated with warmer temperature (Table 4). A combination of higher G% and shorter germination time indicates optimal conditions for germination. This G%/MGT combination could be important to ensure germination and seedling success during short windows of suitable conditions (Kadereit et al. 2017), or to minimize risk of seed predation (Vallejo-Marín et al. 2006; Norden et al. 2009). Variation in temperature requirement is common, with asynchronous germination timing being desirable in natural conditions for population persistence (Alvarado and Bradford 2002; Bewley et al. 2013). Study of germination sensitivity to warmer temperatures has become increasingly common as researchers seek to understand the impacts of climate change (Fernández-Pascual et al. 2019). Altitudinal gradients might as well be a source of temperature variation affecting plant populations, but seeds of *S. americanum* (from >900 m in elevation) were unaffected by our tested temperature regimes.

Light, on the other hand, was necessary for reaching high G% in all studied species regardless of mucilage presence. Positive photoblastism is a strong driver of both G% and MGT, acting as a limiting factor for germination of small-seeded species (Milberg et al. 2000; Zhang et al. 2014) in tropical rainforests where water availability is not a constraint (Vázquez-Yanes and Orozco-Segovia 1993; Válio and Scarpa 2001). Light requirement of the studied species indicates a preference for

**Fig. 3.** Relationship of germination percentage (G%) and mean germination time (MGT) in days with seed fresh mass (g; shown in the left panels) and seed shape index (shown in right panels) for intact and de-mucilaged seeds under two temperature regimes. n.s., non-significant. For statistical tests, see Supplementary Material Table S1.



non-burial (Milberg et al. 2000). This is common in small-seeded species, where there are fewer resources for establishing an above-ground photosynthetic surface (Pons 2000).

Seed traits, life form, and environmental factors play important roles in determining seed dormancy and germination patterns (Bu et al. 2007; Moles et al. 2007; Baskin and Baskin 2014; Wang et al. 2016; Saatkamp et al. 2019). Usually, larger seeds are expected to exhibit less dormancy and reach higher germination rates than smaller seeds (Norden et al. 2009). Conversely, most of our small-seeded forbs displayed non-dormant seeds, with a high G% (>80%) in at least one of the tested temperature regimes in the presence of light. *P. asiatica* was the only species with a G% < 50%, but most seeds remained viable to the end of the trials (data not shown), indicating the presence of physiological dormancy (Hu et al. 2013). In previous studies, *P. virginica* also displayed dormancy (Hicks et al. 2019), but not in the present study. *P. vulgaris* seeds are non-dormant at the time of maturation (Russell 2011), while *L. virginicum* seeds will germinate in adverse light and temperature conditions if treated with gibberellins (Toole and Cathey 1961). The presence of variability in seed dormancy and germination could be linked to the geographical variation in temperature and precipitation, and different forms of dormancy among species (Baskin and Baskin 2014; Xu et al. 2019; Seguí et al. 2021).

Small seeds are often more susceptible to drought stress and thus more likely to use mucilage to prevent desiccation during germination (Thapliyal et al. 2008). However, the highest seed mass and lowest G% were observed in *P. vulgaris* seeds at 25/35 °C, indicating greater sensitivity to temperature increase. Therefore, seed fresh mass had a negative influence on G% of intact seeds in the warmer temperatures (Fig. 2). Likewise, MGT was slower in more ovate seeds (seed shape index approx. 0.15) such as *L. virginicum* and *P. asiatica*, lasting 10–12 days under 25/35 °C. In contrast, the rounder seeds of *S. plebeia* (shape index = 0.03) germinated with an MGT of around 6 days. However, seed size is only loosely connected to G% (Baskin and Baskin 2014) and needle- or disc-shaped seeds with high shape index values tend to be short-lived in soil seed banks (Thompson et al. 1993).

Inclusion of more species from subtropical monsoonal regions would improve our understanding of recruitment strategies in these conditions. Growth form (e.g., forbs, shrubs, and trees) may influence seed traits such as seed size/mass (Moles et al. 2005). Phylogenetic relationships could then be investigated to better understand the evolutionary bias of plant lineages in expressing dormancy and seed traits (Bu et al. 2008; Wang et al. 2009; Dayrell et al. 2017), including germination responses to temperature in annual vs. perennial plants (Arène et al. 2017).

In conclusion, subtropical monsoonal forbs have evolved to recruit during the monsoon season. The presence of seed mucilage influences germination times and may also play other ecological roles, such as seed adhesion to soil and anchorage, detention of predators by avoiding seed ingestion by animals, or facilitating seed dispersal by wind or by attachment to animals. Environmental factors, such as temperature and light, are fundamental to drive germination process of the study species, showing small-seeded photoblastic seeds, while the warmer temperature regime usually delayed MGT values. Seed fresh mass and shape index may help to explain germination parameters under 25/35 °C, but further investigation is required to fully understand the role of seed traits on germination of de-mucilaged seeds in the context of a changing climate.

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A.B., X.C., D.Y., and Y.N. collected the seeds. A.B. conceived and designed the experiments; both A.B. and X.C. performed the experiments. A.B., L.F.D., and D.J.G. analyzed the data. A.B., L.F.D., and D.J.G. wrote, revised, and edited the manuscript.

### Competing interests

The authors declare there are no competing interests.

## Supplementary material

Supplementary data are available with the article at <https://doi.org/10.1139/cjb-2022-0034>.

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