PART OF A HIGHLIGHT SECTION ON SEEDS

Fruit and seed heteromorphism in the cold desert annual ephemeral *Diptychocarpus strictus* (Brassicaceae) and possible adaptive significance

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INTRODUCTION

Heterocarpy or fruit (seed) heteromorphism is the production of seeds of different morphologies and/or behaviour on different parts of the same plant (Imbert, 2002) and is an adaptation of species to the spatio-temporal variability of habitats (Venable and Lawlor, 1980; Venable et al., 1998). It may be viewed as a bet-hedging strategy in which organisms evolve traits that reduce short-term reproductive success in favour of long-term risk reduction (Venable, 1985, 2007) or that allow escape from the negative effects of density (Levin et al., 1984; Sadeh et al., 2009) or sib competition (Lloyd, 1984; Cheplick, 1992). Heterocarpy appears to be confined to a limited number of families of the phylogenetically advanced angiosperms (i.e., 18 families and >200 species), in particular the Asteraceae, Brassicaceae, Chenopodiaceae and Poaceae (Imbert, 2002). Heteromorphic fruits or seeds may vary in size, colour and morphology/anatomy, as well as in dispersal, dormancy and germination (Baskin and Baskin, 1998).

In Brassicaceae, fruit and seed heteromorphism has been studied most extensively in the genus *Cakile*. Fruit heteromorphism of *Cakile* may be associated with differences in dispersal (Payne and Maun, 1981; Donohue, 1998; Cordazzo, 2006), germination behaviour (Barbour, 1970a; Maun and Payne, 1989; Zhang, 1993) and seedling emergence and survival rates (Barbour, 1970b; Maun and Payne, 1989; Zhang and Maun, 1992; Zhang, 1993, 1995). However, other than for the genus *Cakile*, little is known about the role of dimorphic fruits and seeds in the dispersal and germination stages of the life cycle of Brassicaceae species.

*Diptychocarpus strictus* is an annual ephemeral Brassicaceae species that occurs in Middle Asia, Iran, Turkey, Caucasia and China (An and Zhou, 1995). This is the only species in the genus, and in China it grows only in the southern part of the Junggar Basin, Xinjiang province. The species has two flower colour morphs (purple and white), and two morphologically distinct types of fruits (upper and lower) are produced by each of them. Additionally, seeds from upper and lower siliques of each flower colour morph vary in width of the wing and in amount of mucilage (Fig. 1). *Diptychocarpus strictus* is one of the very common ephemeral (annual) species that germinates in autumn and early spring (mostly) in the Junggar Desert. Flowering of this species occurs from mid-April to...
early May, and seeds mature in late May to early June. Siliques and/or seeds are dispersed within a few days after maturity by wind or rain; those at the bottom of the infructescence are shed first. Pericarps of upper siliques first begin to dehisce at the end of the fruit nearest to the peduncle, and the pericarp remains attached to the mother plant via connection of the peduncle to the membranous fruit partition. The seeds with a wide wing from the upper siliques are units of dispersal and of germination. However, lower siliques with highly lignified pericarps do not dehisce, and usually whole (but sometimes transversally broken segments) siliques are dispersed near the mother plant. Thus, seeds are the dispersal units of upper siliques, and both intact and transversally broken siliques are the dispersal units of lower siliques.

We hypothesized that fruit and seed heteromorphism in *D. strictus* aids the distribution of this species in time and space in its cold desert habitat. To test this hypothesis, for both flower colour morphs we (a) compared the morphology and anatomy of upper and lower siliques and their seeds; (b) compared dispersal of seeds after release from dehiscent upper siliques with that of seeds retained inside indehiscent lower siliques; (c) determined the role of mucilage on seeds from upper and lower siliques that vary in amount of mucilage; and (d) compared dormancy and germination characteristics of seeds freed from the two silique types with each other and with seeds retained in lower siliques. Since the results of the two flower colour morphs were essentially identical, only those for the purple flower morph will be presented herein.

The importance of fruit/seed heteromorphism in the life cycle of *D. strictus* is not due to differences in the germination ecophysiology between the two seed morphs *per se*, as has been reported for many species, but to the release at maturity of seeds from the upper dehiscent fruits and the long-term retention and imposed dormancy by the lower indehiscent fruits of the seeds within them.

**MATERIALS AND METHODS**

**Field site description and silique collection**

Freshly matured siliques, i.e. with khaki coloured pericarps and dispersing naturally, were collected from natural *Diptychocarpus strictus* (Fisch. ex M. Bieb.) Trautv. populations growing in the gravel desert in the vicinity of Ürümqi on the southern edge of the Junggar Basin of Xinjiang (43°48′N, 87°32′E, 850 m a.s.l.) on 1 June 2008. Siliques from the infructescences were bulked into one collection and separated into upper and lower fruits. In the laboratory, siliques were separated from other plant material and stored in paper bags under ambient conditions [18–25 °C, 30 % relative humidity (RH)] until used.

This area of the Junggar Basin has typical desert vegetation dominated by Chenopodiaceae and Asteraceae, gravelly grey desert soil and a continental climate. Mean annual temperature is 6.8 °C, and the extreme temperatures of the coldest (January) and hottest (July) months are −32.8 and 40.5 °C, respectively. Average annual precipitation (including rain and snow) is 234 mm, 62.7 % of which occurs in spring and summer, and the snow that falls in winter begins to melt in March or April. The annual potential evaporation is >2000 mm (Wei et al., 2003).
Characteristics and dispersal of siliques and seeds

Colour, shape, size and mass. Colour, shape, size and mass were determined for siliques of each type collected in June 2008 and stored dry under laboratory conditions for 2 months. The length and width of the siliques were measured individually using a Sartorius BS210S electronic balance (0.0001 g), and then the mass of the pericarp and seeds inside these siliques was determined. Ten replications of 1000 seeds of each of the two morphs (10 groups of 1000 seeds in each replication) were weighed to the nearest 0.0001 g. Bulk colour, shape, size and mass were determined for 50 dry siliques of each of the two morphs. A total of 1000 seeds of each of the two morphs (upper and lower) was weighed before and after imbibition. Ten groups of 100 seeds were used for mass determination of each morph. The increase in mucilage mass (mm) (\( W_m \)) of imbibed seeds was calculated as \( W_m = (W_i - W_o)/2 \), where \( W_i \) and \( W_o \) are the widths of imbibed seeds with and without mucilage, respectively; the mucilage width ratio (\( W_m/2 \)) of imbibed seeds as \( W_m/2 = (W_i - W_o)/2W \); the number of times the mass (\( M_i \)) increased after imbibition as \( M_i = M_i/M_a \), where \( M_i \) and \( M_a \) are the mass of imbibed and dry seeds, respectively; the mucilage mass of dry seeds (\( M_{md} \)) as \( M_{md} = M_{wd} - M_{wod} \), where \( M_{wd} \) and \( M_{wod} \) are the mass of dry seeds with and without mucilage, respectively; the mucilage mass of imbibed seeds (\( M_{mi} \)) as \( M_{mi} = M_{wi} - M_{wot} \), where \( M_{wi} \) and \( M_{wot} \) are the mass of imbibed seeds with and without mucilage, respectively; and the proportion of mucilage to seed mass before (\( M_{mb} \)) and after (\( M_{ma} \)) imbibition as \( M_{mb} = M_{md}/M_{wd} \) and \( M_{ma} = M_{mi}/M_{wi} \), respectively.

Water absorption and dehydration. Water absorption (water uptake) was monitored in the same groups of seeds under laboratory conditions (22–25°C, 30% RH). The mass of each group of seeds was recorded at time 0 and every 10 min until final constant mass.

Dehydration (water loss) was monitored in the same groups of seeds under laboratory conditions (22–25°C, 30% RH). The mass of each group of seeds was recorded at time 0 and every 10 min until final constant mass.

Mass of seeds with and without mucilage plus adhered soil particles.

(a) Adherence to wet soil particles by dry seeds with and without mucilage. Four replicates of 100 dry seeds of each of the two morphs, with and without mucilage, were weighed. Seeds were distributed on water-saturated desert soil in 9 cm diameter Petri dishes under room conditions (22–25°C, 30% RH). After swelling to full water capacity, the seeds were dried at room temperature. The mass of the dry seeds including attached soil particles was compared with the mass of dry seeds before adherence to soil particles. During incubation, soil particles adhered to the mucilage surrounding the seeds (= mass of imbibed seeds with mucilage plus soil particles).

(b) Adherence to dry soil particles by imbibed seeds with and without mucilage. Four replicates of 100 seeds of each of the two morphs with and without mucilage were weighed.
and the seeds submerged in distilled water until they were fully imbibed. Then, the seeds were placed on fine-particle dry soil in 9 cm diameter Petri dishes and rolled until they would not adhere to any more particles, after which the seed–soil particle units were allowed to dry under room conditions. The mass of the dry seeds with fully developed mucilage including the soil particles that had adhered to them was compared with the mass of dried seeds before adherence to soil particles. The number of times seed mass increased ($M'_s$) after adherence of sand particles was calculated as $M'_s = M_s / M'_s$, where $M'_s$ and $M_s$ are the mass of imbibed seeds with and without mucilage plus sand particles and dry seeds, respectively.

Germination ecophysiology

Effect of dry storage (after-ripening) on germination. To investigate the germination responses of seeds during dry storage, seeds stored in laboratory conditions for 0 (fresh), 1, 2, 3 and 12 months were tested for germination. For each test, three replicates of 50 seeds of each of the two morphs for each of the five storage periods were incubated on two layers of Whatman No.1 filter paper moistened with 2.5 mL of distilled water in 9 cm diameter Petri dishes. Seeds were incubated at daily (12/12 h) temperature regimes of 5/2, 15/10, 20/10, 25/15 and 30/15 °C in light (12 h of ~100 μmol m$^{-2}$ s$^{-1}$, 400–700 nm, cool white fluorescent light each day) or in constant darkness (Petri dishes with seeds in them placed in light-proof black bags) for 14 d. The four higher temperature regimes represent the mean daily maximum and minimum monthly temperatures in the vicinity of Ürümqi during the growing season: 15/2 °C (March, early April and November), 20/10 °C (late April and October), 25/15 °C (May and September) and 30/15 °C (June, July and August). The 5/2 °C temperature regime was used to test the effect of a cold-stratifying temperature regime on seed germination (Baskin and Baskin, 1998). A seed was considered to be germinated when the radicle had emerged. Germination in light was examined daily for 14 d; germinated seeds were removed at each counting. Seeds incubated in darkness were checked only after 14 d; therefore, they were not exposed to any light during the incubation period.

After the germination trials were complete, the non-germinated seeds were tested for viability. Seeds were cut open and the embryo observed. Seeds with white and firm embryos were considered non-viable and excluded from the calculations of germination percentages. Only a very few seeds were non-viable. The tests of fresh seeds (0 months old) were initiated on 3 June 2008, using seeds collected on 1 June 2008.

Effect of storage in soil in the field on germination. After collection on 2 June 2008, approx. 1000 freshly mature seeds from upper siliques and 300 whole intact lower siliques were enclosed in 12 fine-mesh nylon bags. Each bag was buried at a depth of 5 cm in plastic pots (18 cm deep, 21 cm in diameter) with drainage holes at the bottom and filled with a mixture of 75% habitat soil and 25% sand. The pots were buried with the top of the pot level with the soil surface in the experimental garden on the campus of Xinjiang Agricultural University. Seeds from upper siliques and the whole intact lower siliques were subjected to natural temperature and soil moisture conditions; temperature and rainfall data were recorded at a weather station about 30 m from the buried seeds and siliques.

Germination tests of fresh seeds from upper siliques, seeds mechanically removed from lower siliques (by carefully cutting them open with a knife) and whole intact lower siliques with seeds inside (0 months old) were tested on 3 June 2008. Except for months with a snow cover on the ground (December 2008 to February 2009), one buried pot containing seeds from upper siliques and intact lower siliques was randomly selected and retrieved at monthly intervals, starting on 2 July 2008 (seeds buried for 1 month) and ending on 2 June 2009 (12 months). After pots were retrieved, seeds from upper siliques, seeds mechanically removed from lower siliques and those inside intact whole lower siliques were tested for germination. However, only data for fresh seeds and for those buried for 4 months (October) and 10 months (April) are presented. These three sets of data are sufficient to tell our ‘story’. Seeds and whole intact lower siliques were incubated in 9 cm diameter plastic Petri dishes, and three replicates of 50 seeds from the upper and lower siliques, and five replicates of five whole intact lower siliques were placed in light at each of the five temperature regimes, as previously described, for 2 weeks. Germination was examined daily for 2 weeks, and germinated seeds were removed at each counting. Seeds and whole, intact lower siliques incubated in darkness were checked only after 2 weeks, at the end of the experiment; therefore, they were not exposed to any light during the incubation period. After the germination trials were complete, the non-germinated seeds were tested for viability. Seeds were cut open and the embryo observed. Seeds with white and firm embryos were counted as viable, and those with tan, soft embryos were considered non-viable and excluded from the calculations of germination percentages. More than 98% of the seeds were viable.

Germination phenology. The purpose of this experiment was to compare the germination phenology of seeds of D. strictus in the field under supplemented and non-supplemented (natural) soil moisture conditions. Seeds from upper and lower siliques and intact lower siliques collected in June 2008 were sown on bare soil in plots (1.5 × 0.8 m) on 23 August 2008. There were six treatments, each consisting of three replications of 200 seeds from upper and lower siliques or 200 lower siliques, for a total of 18 plots. Seeds from upper siliques, seeds from lower siliques and whole, intact lower siliques were watered and not watered.

The experiment was carried out in the experimental garden on the campus of Xinjiang Agricultural University, Ürümqi, China. In the watered treatment, the soil was watered to field capacity every 3 d throughout the experiment, except during the winter when the soil was frozen, while in the non-watered treatment the soil received water only via precipitation or snowmelt. At 7 d intervals, from August 2008 to May 2009, germinated seeds (seedlings) were counted and marked. Survivorship of marked seedlings was monitored until plants died, either before or after reproduction. Information on
temperature and precipitation at the study site was obtained from data collected at the Xinjiang Agricultural University weather station near the study plots.

To compare the spring germination phenology of *D. strictus* seeds further, three replications of 50 seeds of each of the two morphs collected in June 2008 and stored in the laboratory were sown on 20 March 2009 in plastic pots 18 cm deep and 21 cm in diameter (with drainage holes in the bottom) filled with soil from the natural habitat of *D. strictus*. Sown seeds were exposed to near-natural temperatures in a non-temperature-controlled metal framehouse (top covered, with plastic, only when it rained) in the experimental garden of Xinjiang Agricultural University and monitored for germination. Soil was watered daily, and therefore it remained at or near field capacity throughout the experiment. Seeds were monitored for germination (emerged radicle) and seedlings removed from the pots daily until 29 April 2009, when no germinants had appeared for 2 weeks.

**Effect of pericarp and mucilage on germination.**

(a) Seeds from upper siliques.

(1) Control: seeds were left intact.

(2) Seeds without mucilage were used to determine whether the mucilage inhibited germination, either mechanically or chemically.

(3) Fifty seeds with mucilage removed, along with the mucilage removed from the 50 seeds, were placed in each of three Petri dishes to test whether soluble chemicals that would inhibit germination might be leached from the mucilage.

(b) Seeds from lower siliques.

(1) Control: unmanipulated dispersal units (seeds with pericarps) were placed in Petri dishes.

(2) Segments of dispersal units were placed in Petri dishes.

(3) Mucilaginous seeds without pericarps were placed in Petri dishes to determine whether the pericarps inhibited germination, either mechanically or chemically.

(4) Mucilaginous seeds and pericarps were placed together in each Petri dish to test whether soluble chemicals that would inhibit germination might be leached from the pericarps.

(5) Seeds without mucilage were placed in Petri dishes to determine whether the mucilage inhibited germination, either mechanically or chemically.

(6) Seeds without mucilage and mucilage from the 50 seeds were placed together in Petri dishes to test whether soluble chemicals that would inhibit germination might be leached from the mucilage.

Experiments with seeds from both upper and lower siliques were conducted at 15/2 °C in light using fresh seeds (0 months old) and seeds that had been stored dry in the laboratory for 1, 2 and 3 months after they were collected in June 2008. Three replicates of 50 seeds for each morph were used for each treatment. Germinated seeds were counted daily for 14 d, and germination percentages were calculated from the data.
differences among dimorphic seeds and treatments. Statistical tests were conducted at $P = 0.05$. Regression analyses were used to determine the relationship between dispersal unit and fall time and between dispersal unit and fall rate. All data analyses were performed with the software SPSS 13.0 (SPSS Inc, Chicago, IL, USA). Values are means ± 1 s.e. (Sokal and Rohlf, 1995).

RESULTS

The results of this study generally provide support for our hypothesis that fruit and seed heteromorphism in _D. strictus_ aids in the distribution of this species in space and time in its cold desert habitat. Thus, we have shown that there is a high degree of difference in morphology and anatomy of both seeds and siliques of this species that is correlated with differences in timing of germination and in capacity for dispersal between seeds in upper and lower siliques. Namely, dehiscence of the thin-walled upper siliques and their winged seeds allows for dispersal in space, whereas the thick-walled indehiscent lower siliques prevent the nearly wingless non-mucilaginous seeds inside them from germinating for $>1$ year, thus forming a persistent seed bank of unknown length. In spite of the dimorphism in shape, size and amount of mucilage between seeds in upper and lower siliques, they do not differ physiologically, i.e. they are not physiologically dimorphic. The thick mucilage layer on the winged seeds in the upper siliques aids in lodgment on the soil surface and increases the time for which they are hydrated, thereby increasing the chances for germination once the seeds have been dispersed from the mother plant.

**Characteristics and dispersal of siliques and seeds**

**Colour, shape, size and mass.** The upper siliques are elongated (i.e. 46-4 mm in length and 3-5 mm in width) and compressed (Fig. 1; Table 1), while the lower siliques are relatively shorter, about 33.0 mm in length and 3.0 mm in width, approximately columnar and highly lignified (Fig. 1; Table 1). Moreover, the mass of upper siliques, seeds and pericarps was significantly greater than that of lower siliques, seeds and pericarps (Table 1). Both upper and lower siliques are khaki coloured and have a peduncle and a beak.

The colour and shape of seeds from the upper and lower siliques are similar, i.e. dark brown and oval to ovate and slightly compressed. The mean size of seeds from the upper and lower siliques is 2.7 × 2.0 mm and 2.7 × 1.8 mm, respectively. The hilum is obvious at one end of the seed, and there is a transparent wing around the edge of the seed (Fig. 1). However, the wing of seeds from upper siliques is obviously wider than that of seeds from lower siliques (Fig. 1; Table 1). When fully hydrated, a transparent, gelatinous coating of mucilage develops within a few minutes (Fig. 1), but mucilage on seeds from upper siliques is wider than that of mucilage on seeds from lower siliques. Also, the mean mass of 1000 seeds from upper siliques (about 1.8 g) is greater than that of 1000 seeds from lower siliques (1.4 g) (Table 1).

**Anatomy.** The general structure of the pericarp in the two types of siliques is similar and mainly consists of three layers (i.e. epiderc, mesocarp and endocarp), which are fused together (Fig. 2A–D). The epiderc consists of only a single layer of small rectangular epidermal cells, which are closely arranged. The outer pericarpal wall of epidermal cells is thickened by a covering of cutin. The mesocarp has two distinctive cell layers, i.e. palisade cells and large parenchyma cells. Cells in the endocarp are much smaller than those in the epiderc, and they usually are irregularly polygonal in shape and closely arranged.

The anatomical structure of the pericarp, however, differs greatly between the two types of siliques. In cross-section, the upper siliques are radially elongated to oval, and its length is several times greater than its width (Fig. 2A), while the lower siliques are approximately round in cross-section (Fig. 2C). The pericarp of lower siliques is nearly 1.5 times thicker than that of upper siliques. In addition, orientation of small rectangular epidermal cells, which are closely arranged. The cell wall of palisade tissue in the mesocarp of lower siliques is thicker than that of upper siliques, but the cell wall of palisade tissue in the mesocarp of lower siliques is thinner than that of upper siliques. In addition, orientation of parenchyma cells has a tangential arrangement in mesocarp of upper siliques, while it is radial in lower siliques (Fig. 2B, D).

The episperm and endopleura are the outermost cell layers of the seed coat in both siliques morphs (Fig. 2E–H). The episperm is surrounded by a mucilaginous layer, which adheres tightly to the surface of the seed, and the endopleura, which ruptures, has two layers of cells, the inner of which presumably contains tannin and the outer contains mucilage. The thickness of the seed coat is much greater for seeds from lower siliques than seeds from upper siliques (Fig. 2F, H). Conversely, the mucilaginous layer on the episperm is much thicker and better defined on seeds from upper siliques than on those from lower siliques.

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**Table 1. Comparison of the morphology and mass of dimorphic siliques and seeds of Diptychocarpus strictus (mean ± s.e.)**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Upper Silique</th>
<th>Lower Silique</th>
</tr>
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<tbody>
<tr>
<td>Silique Morphology (mm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length of peduncle</td>
<td>3.414 ± 0.012</td>
<td>2.908 ± 0.006</td>
</tr>
<tr>
<td>Length of beak</td>
<td>4.990 ± 0.019</td>
<td>6.143 ± 0.022</td>
</tr>
<tr>
<td>Length of siliques</td>
<td>46.400 ± 0.132</td>
<td>32.977 ± 0.147</td>
</tr>
<tr>
<td>Width of siliques</td>
<td>3.543 ± 0.008</td>
<td>3.402 ± 0.013</td>
</tr>
<tr>
<td>Mass (g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Per siliques</td>
<td>0.142 ± 0.004</td>
<td>0.064 ± 0.002</td>
</tr>
<tr>
<td>Pericarp</td>
<td>0.087 ± 0.003</td>
<td>0.051 ± 0.002</td>
</tr>
<tr>
<td>Seeds per siliques</td>
<td>0.055 ± 0.001</td>
<td>0.013 ± 0.001</td>
</tr>
<tr>
<td>No. of seeds per siliques</td>
<td>31.700 ± 0.666</td>
<td>11.267 ± 0.783</td>
</tr>
<tr>
<td>Seed Morphology (mm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length of seed</td>
<td>2.674 ± 0.044</td>
<td>2.658 ± 0.042</td>
</tr>
<tr>
<td>Width of seed</td>
<td>2.047 ± 0.042</td>
<td>1.763 ± 0.046</td>
</tr>
<tr>
<td>Width of wing</td>
<td>0.085 ± 0.032</td>
<td>0.025 ± 0.005</td>
</tr>
<tr>
<td>Mass (g) of 1000 seeds</td>
<td>1.844 ± 0.024</td>
<td>1.370 ± 0.018</td>
</tr>
</tbody>
</table>

Different letters within a row indicate significant differences (Tukey’s HSD, $P = 0.05$).
more than 2-fold, whereas there was no obvious increase in the width of seeds with mucilage from upper siliques increased lower siliques at wind speeds of both 1 and 4 m s\(^{-2}\). Air were usually considerably greater than those of whole distances of seeds from upper siliques in a parallel stream of P, (Table 2; ques differed significantly in landing time and fall rate (\(r = 0.83, P < 0.01\)). Dispersal distance (cm, mean ± s.e.) and effect of wind speed on dispersal units of Diptychocarpus strictus

<table>
<thead>
<tr>
<th>Dispersal units</th>
<th>Seeds from upper siliques</th>
<th>Whole, intact lower siliques</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fall time (s)</td>
<td>0.523 ± 0.006(^b)</td>
<td>0.377 ± 0.013(^a)</td>
</tr>
<tr>
<td>Fall rate (cm s(^{-1}))</td>
<td>230.376 ± 4.327(^a)</td>
<td>334.720 ± 19.793(^b)</td>
</tr>
<tr>
<td>Dispersal distance (cm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wind speed 1 m s(^{-1})</td>
<td>25.228 ± 1.244(^b)</td>
<td>6.018 ± 0.534(^a)</td>
</tr>
<tr>
<td>Wind speed 4 m s(^{-1})</td>
<td>57.772 ± 2.486(^b)</td>
<td>16.842 ± 1.367(^a)</td>
</tr>
</tbody>
</table>

Different letters within a row indicate significant differences (Tukey’s HSD, \(P = 0.05\)).

Dispersal

In still air, seeds from upper siliques and whole lower siliques differed significantly in landing time and fall rate (Table 2; \(P < 0.01\)), the seeds from upper siliques taking longer to fall a specific distance. This suggests that the dispersal ability of the seeds from upper siliques will be much greater than that of the whole lower siliques. The dispersal unit was correlated significantly with fall time (\(r = 0.92, P < 0.01\)) and fall rate (\(r = 0.83, P < 0.01\)). Dispersal distances of seeds from upper siliques in a parallel stream of air were usually considerably greater than those of whole lower siliques at wind speeds of both 1 and 4 m s\(^{-1}\) (Table 2).

Seed mucilage

Change in width and mass of imbibed seeds. After imbibition, the width of seeds with mucilage from upper siliques increased more than 2-fold, whereas there was no obvious increase in width of seeds without mucilage (mucilage removed) from upper siliques. In imbibed seeds from lower siliques, there was no difference in seeds with mucilage vs. those without mucilage vs. dry seeds (Table 3). After they were fully imbibed, seeds with mucilage from upper siliques increased in mass by 30-118 times (3011.8\%) in comparison with the mass of the dry seeds before wetting. On the other hand, the increase in mass of seeds without mucilage from upper siliques was only 1.921 times (192.1\%). However, the imbibed seeds with and without mucilage from lower siliques increased in mass by only 1.9–2.2 times in comparison with the original mass of dry seeds. In imbibed seeds from upper siliques, mucilage accounted for >50% of the width and >90% of the total seed mass. However, for seeds in the lower siliques mucilage accounted for only 5% of the width and about 20% of the total mass of the seeds (Table 3).

Water absorption and dehydration. The process of water absorption by the two seed morphs with and without mucilage was similar and can be divided into three stages: (1) the rate of water absorption was rapid; (2) the rate of water absorption decreased; and (3) water absorption stopped. Seeds were fully imbibed after 240–300 min (Fig. 3). Intact seeds from upper siliques had a high capacity to take up water, and their mass increased 1800\% (from 0.2 to 3.6 g) within only 30 min from the start of imbibition, which differed significantly from seeds without mucilage (\(P < 0.01\)). Thus, water absorbed by seeds alone contributed little to the total amount of water absorbed by intact seeds from upper siliques. Since seeds with mucilage in the lower fruits had little mucilage, they absorbed only 0.14 g of water in 30 min.

The dehydration process was almost the reverse of the water absorption process and can also be divided into three stages: (1) the loss of water was rapid; (2) the rate of dehydration decreased; and (3) seeds had reached their original mass.
TABLE 3. Width (mm, mean ± s.e.) of Diptychocarpus strictus seeds with and without mucilage and mass (g, mean ± s.e.) of dry and of imbibed seeds with and without mucilage and of only mucilage

<table>
<thead>
<tr>
<th></th>
<th>Upper silique</th>
<th>Lower silique</th>
</tr>
</thead>
<tbody>
<tr>
<td>Width (mm) Seeds W&lt;sub&gt;d&lt;/sub&gt;</td>
<td>2.047 ± 0.042&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.763 ± 0.046&lt;sup&gt;aa&lt;/sup&gt;</td>
</tr>
<tr>
<td>W&lt;sub&gt;i&lt;/sub&gt;</td>
<td>4.420 ± 0.056&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.890 ± 0.034&lt;sup&gt;aa&lt;/sup&gt;</td>
</tr>
<tr>
<td>W&lt;sub&gt;m&lt;/sub&gt;</td>
<td>2.055 ± 0.023&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.785 ± 0.031&lt;sup&gt;aa&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mucilage W&lt;sub</td>
<td>m&lt;/sub&gt;</td>
<td>1.183 ± 0.035&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>W&lt;sub&gt;m&lt;/sub&gt;</td>
<td>0.532 ± 0.010&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.045 ± 0.009&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mass (g) of 100 seeds Seeds with mucilage M&lt;sub&gt;N&lt;/sub&gt;</td>
<td>0.175 ± 0.001&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.124 ± 0.011&lt;sup&gt;aa&lt;/sup&gt;</td>
</tr>
<tr>
<td>M&lt;sub&gt;i&lt;/sub&gt;</td>
<td>5.278 ± 0.084&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.272 ± 0.005&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>M&lt;sub&gt;e&lt;/sub&gt;</td>
<td>3.0118 ± 0.469&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.193 ± 0.042&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Seeds without mucilage M&lt;sub&gt;d&lt;/sub&gt;</td>
<td>0.125 ± 0.001&lt;sup&gt;aa&lt;/sup&gt;</td>
<td>0.128 ± 0.002&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>M&lt;sub&gt;i&lt;/sub&gt;</td>
<td>0.240 ± 0.008&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.239 ± 0.012&lt;sup&gt;aa&lt;/sup&gt;</td>
</tr>
<tr>
<td>M&lt;sub&gt;e&lt;/sub&gt;</td>
<td>1.921 ± 0.070&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.869 ± 0.080&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mucilage M&lt;sub&gt;d&lt;/sub&gt;</td>
<td>0.046 ± 0.002&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.006 ± 0.001&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>M&lt;sub&gt;i&lt;/sub&gt;</td>
<td>4.057 ± 0.054&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.055 ± 0.010&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>M&lt;sub&gt;e&lt;/sub&gt;</td>
<td>9.654 ± 6.735&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.446 ± 3.193&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mucilage/seed M&lt;sub&gt;mb&lt;/sub&gt;</td>
<td>0.248 ± 0.016&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.045 ± 0.010&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>M&lt;sub&gt;mn&lt;/sub&gt;</td>
<td>0.944 ± 0.002&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.185 ± 0.030&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Different letters indicate significant differences. Lower case letters compare the two morphs of seeds and upper case letters different treatments within an index (Tukey’s HSD, P = 0.05). W<sub>d</sub>, width of dry seeds; W<sub>i</sub> and W<sub>m</sub>, widths of imbibed seeds with and without mucilage, respectively; W<sub>m</sub>, mucilage width; W<sub>m</sub>, mucilage width ratio of imbibed seeds; M<sub>d</sub>, dry mass of seeds and mucilage; M<sub>i</sub>, mass of imbibed seeds with and without mucilage and mucilage; M<sub>e</sub>, number of times mass increased after imbibition; proportion of mucilage to seed in mass before (M<sub>mb</sub>) and after (M<sub>mn</sub>) imbibition.

(Fig. 3). Seeds with mucilage from upper siliques of both morphs lost their absorbed water more slowly than those without mucilage. Seeds with mucilage from upper siliques of the two morphs retained >50% of their water for 360 min and did not return to their original masses until 1200 min, whereas the seeds without mucilage became fully dehydrated (i.e. had reached the same moisture level as that of seeds at the beginning of absorption) after 240 min. After 240 min, however, seeds with and without mucilage from lower siliques had almost reached their original masses.

**Mass of seeds with and without mucilage and with adhered soil particles.**

(a) Adherence of wet soil particles to dry seeds with and without mucilage. The original mean mass of seeds with and without mucilage from upper siliques was 0.18 and 0.13 g, and that of seeds with and without mucilage from lower siliques was 0.14 and 0.13 g, respectively. When dry seeds with mucilage from upper and lower siliques were placed on wet soil particles, a mucilaginous layer formed after a few minutes at the contact area between the seed and the wet soil surface on the lower side of the seed. However, mucilage had not yet covered the upper part of the seed; thus, the upper side of the seed was still dry. The mass of seeds from upper siliques with mucilage and adhered soil particles was significantly greater than that of seeds from upper siliques without mucilage (and soil particles) and of those from lower siliques (P < 0.01). Compared with its original mass, however, the mass of seeds of the lower morph increased very little (Table 4).

(b) Adherence of dry soil particles to imbibed seeds with and without mucilage. Fully hydrated seeds with mucilage from upper siliques adhered to many soil particles, resulting in an increase of more than seven times the total mass of dry seeds. On the other hand, soil particles did not adhere to imbibed seeds without mucilage from upper siliques or to those from lower siliques with or without mucilage (Table 4).

**Germination ecophysiology**

**Effect of dry storage (after-ripening) on germination.** A four-way ANOVA showed that germination was significantly affected by light condition (P < 0.01), storage time (P < 0.01), temperature (P < 0.01) and seed type (P < 0.01) (Table 5). No significant interactions were observed in germination percentage.
between light and seed type ($P = 0.30$), between light, storage time and temperature ($P = 0.10$), between light, storage time and seed type ($P = 0.72$) or between light, temperature and seed type ($P = 0.19$), or among the interaction of the four factors ($P = 0.23$) (Table 5). All freshly harvested seeds were dormant, and thus no seeds germinated at any of the five temperature regimes in either light or constant darkness. Dormant seeds from the two silique types gradually after-ripened during storage in the field (Fig. 5). After 4 months of storage, i.e. October 2008, germination was about $47-87\%$ in light and $43-85\%$ in darkness at 15/2 and 5/2°C. Seeds did not go back into dormancy during winter, and 10-month-old seeds (October 2008) germinated to about $47-85\%$ in light and $43-87\%$ in darkness at 15/2 and 5/2°C in spring (April 2009) (Fig. 5). The germination percentage of whole intact lower siliques was 0% in all test conditions at time 0 and at the two retrieval times. They also did not germinate when retrieved after 12 months of burial (J. J. Lu, unpubl. res.).

**Effect of storage in soil in the field on germination.** A four-way ANOVA showed that germination was significantly affected by light condition ($P < 0.01$), retrieval time ($P < 0.01$), temperature ($P < 0.01$) and seed type ($P < 0.01$) (Table 6). Also, significant interactions were observed in germination percentage between retrieval time and temperature ($P < 0.01$), between retrieval time and seed type ($P < 0.01$), between temperature and seed type ($P < 0.01$) and between retrieval time, temperature and seed type ($P < 0.01$) (Table 6). In June 2008, all freshly harvested seeds were dormant, and thus no seeds germinated at any of the five temperature regimes in either light or constant darkness. Dormant seeds from the two silique types gradually after-ripened during storage in the field (Fig. 5). After 4 months of storage, i.e. October 2008, germination was about $47-87\%$ in light and $43-85\%$ in darkness at 15/2 and 5/2°C. Seeds did not go back into dormancy during winter, and 10-month-old seeds (October 2008) germinated to about $47-85\%$ in light and $43-87\%$ in darkness at 15/2 and 5/2°C in spring (April 2009) (Fig. 5). The germination percentage of whole intact lower siliques was 0% in all test conditions at time 0 and at the two retrieval times. They also did not germinate when retrieved after 12 months of burial (J. J. Lu, unpubl. res.).

**Germination phenology.** Germination of seeds from upper and lower siliques on wet soil began between 6 and 13 September, when mean daily maximum and minimum air temperatures were 24-3 and 11-7°C, respectively (Fig. 6A, B). Essentially all seeds that germinated in autumn did so in September. We did not observe germination of any seeds from upper or lower siliques on dry soil or in intact lower siliques on either wet or dry soil in autumn. In spring 2009, the period in which the

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**Table 4. Comparison of mass (mean ± s.e.) of Diptychocarpus strictus seeds with and without mucilage and with adhered soil particles after maximum water absorption and of dry seeds adhered to wet soil**

<table>
<thead>
<tr>
<th>Seed mass (g) with adhered soil particles after maximum water absorption</th>
<th>With mucilage</th>
<th>Without mucilage</th>
</tr>
</thead>
<tbody>
<tr>
<td>M$_d$</td>
<td>0.182 ± 0.011$^{a,b}$</td>
<td>0.131 ± 0.001$^{a,b}$</td>
</tr>
<tr>
<td>M'$_d$</td>
<td>1.069 ± 0.05$^{a,b}$</td>
<td>0.333 ± 0.003$^{a,b}$</td>
</tr>
<tr>
<td>M$_t$</td>
<td>7.106 ± 0.085$^{a,b}$</td>
<td>1.023 ± 0.036$^{a,b}$</td>
</tr>
</tbody>
</table>

**Table 5. Four-way ANOVA of effects of seed type, temperature, light condition, storage time and their interactions on germination of Diptychocarpus strictus seeds stored dry under laboratory conditions**

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>SS</th>
<th>MS</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light (L)</td>
<td>1</td>
<td>367,413</td>
<td>367,413</td>
<td>32.496</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Storage time (T)</td>
<td>4</td>
<td>296,308,453</td>
<td>74,077,113</td>
<td>6551,631</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Temperature (T)</td>
<td>4</td>
<td>422,049,87</td>
<td>105,521,477</td>
<td>933,188</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Seed type (S)</td>
<td>1</td>
<td>573,781</td>
<td>573,781</td>
<td>507,472</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>L × T</td>
<td>4</td>
<td>255,653</td>
<td>63,913</td>
<td>5,653</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>L × T'</td>
<td>4</td>
<td>196,453</td>
<td>49,113</td>
<td>4,344</td>
<td>0.002</td>
</tr>
<tr>
<td>L × S</td>
<td>4</td>
<td>12,000</td>
<td>12,000</td>
<td>1,061</td>
<td>0.034</td>
</tr>
<tr>
<td>T × T'</td>
<td>16</td>
<td>301,515,477</td>
<td>188,447,2</td>
<td>166,669</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>T × S</td>
<td>4</td>
<td>206,520</td>
<td>51,530</td>
<td>45,582</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>T × T' × S</td>
<td>4</td>
<td>168,253</td>
<td>42,231</td>
<td>37,351</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>L × T × T'</td>
<td>16</td>
<td>270,480</td>
<td>16,905</td>
<td>1,495</td>
<td>0.104</td>
</tr>
<tr>
<td>L × T × S</td>
<td>4</td>
<td>23,867</td>
<td>5,967</td>
<td>0.528</td>
<td>0.715</td>
</tr>
<tr>
<td>L × T' × S</td>
<td>4</td>
<td>70,267</td>
<td>17,567</td>
<td>1,554</td>
<td>0.188</td>
</tr>
<tr>
<td>T × T' × S</td>
<td>16</td>
<td>259,473</td>
<td>162,338</td>
<td>14,358</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>L × T × T' × S</td>
<td>16</td>
<td>226,533</td>
<td>14,158</td>
<td>1,252</td>
<td>0.232</td>
</tr>
</tbody>
</table>
The greatest number of seeds from upper and lower siliques on wet and dry soil germinated was 3–28 March, when mean daily maximum and minimum air temperatures were 7.3 and 2.5 °C, respectively. The germination percentage from upper and lower siliques on dry soil was lower than it was on wet soil. For example, of a total of 600 seeds sown on wet and dry soil, 210 and 117 of them from the upper silique germinated on wet and dry soil, respectively, and 101 and 36 from lower siliques germinated on wet and dry soil, respectively. There was no further germination on either wet or dry soil after 25 April 2009. On the other hand, no seeds inside the intact lower siliques germinated on either wet or dry soil. Plants from 100% of the 41 seeds that germinated in the six treatments in autumn 2008 reproduced in spring 2009, and

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**Fig. 4.** Final germination percentages (mean ± s.e.) of the two types of seeds in *Diptychocarpus strictus* (A) incubated in the light/darkness regime (left panels) and (B) in constant darkness (right panels) at five temperature regimes following 1, 2, 3 and 12 months of dry storage under laboratory conditions. Germination percentages of fresh (0-months-old) seeds from upper and lower siliques were 0% in both light/darkness and constant darkness at all temperature regimes. Bars with different letters are significantly different in multiple range comparison within the upper and within lower silique (Tukey’s HSD, *P* = 0.05).
97–100% (depending on treatment) of the plants from the 464 seeds that germinated in spring 2009 reproduced in spring 2009. When 9-month-old laboratory-stored seeds were sown on soil at natural temperatures in March 2009, very little or no germination occurred during the first week after sowing, even on soil kept at or near field capacity, when the mean maximum and minimum daily temperatures averaged 9.1 °C (range 3.2–15.5 °C) and −0.6 °C (range −5.8 to 2.9 °C), respectively (Fig. 6A, C). Most of the germination of seeds from upper siliques occurred from day 9, when mean maximum and minimum daily temperatures in the metal framehouse averaged 18.9 and 5.8 °C, respectively. The highest germination of seeds from lower siliques occurred from day 12, when the mean maximum and minimum daily temperatures in the metal framehouse averaged 15.3 and 6.1 °C, respectively. There was no further germination of seeds from upper siliques after 9 April (i.e. 20 d after sowing) and from lower siliques after 14 April (i.e. 25 d after sowing). Seeds from upper siliques germinated faster than those from lower siliques, reaching 50% germination after 10 and 15 d, respectively. Also, total germination was higher (88%) for seeds from upper siliques than for those from lower siliques (77%).

Effect of pericarp and mucilage on germination. None of the untreated freshly matured seeds (with mucilage) from upper siliques germinated. Furthermore, seeds without mucilage and those without mucilage with added mucilage germinated to <10% (Fig. 7). After storage for 2 months, seeds without mucilage from upper siliques germinated to >95%. When seeds without mucilage were placed in close contact with mucilage, their germination was not inhibited (Fig. 7). Germination percentages of treated seeds from upper siliques were significantly higher than that of those of complete dispersal units, i.e. seeds with mucilage (P < 0.01). However, there was no difference between the two seed treatments. After storage for 3 months, there was no significant difference among the intact dispersal units and treated seeds (P = 0.39).

None of the untreated freshly matured seeds from the lower siliques germinated. Removing the fruit coat and mucilage did not increase the germination percentage of seeds from lower siliques, which was still 0%. Storage influenced germination of seeds from lower siliques in a similar way to seeds from upper siliques. The germination of the dispersal unit (i.e. intact silique) was still 0% in 3-month-old intact dispersal units. However, removing the fruit coat was very effective in promoting germination, and there were no significant differences in germination among seeds with mucilage, seeds with mucilage plus pericarp, seeds without mucilage or seeds

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**Table 6. Four-way ANOVA of effects of seed type, temperature, light condition, retrieval time and their interactions on seed germination of Diptychocarpus strictus stored in soil in the field**

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>SS</th>
<th>MS</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light (L)</td>
<td>1</td>
<td>115-282</td>
<td>115-282</td>
<td>5-289</td>
<td>0-022</td>
</tr>
<tr>
<td>Retrieval time (R)</td>
<td>9</td>
<td>150107-535</td>
<td>16678-615</td>
<td>765-250</td>
<td>&lt;0-01</td>
</tr>
<tr>
<td>Temperature (T)</td>
<td>4</td>
<td>129186-993</td>
<td>32296-748</td>
<td>1481-842</td>
<td>&lt;0-01</td>
</tr>
<tr>
<td>Seed type (S)</td>
<td>1</td>
<td>5871-882</td>
<td>5871-882</td>
<td>269-414</td>
<td>&lt;0-01</td>
</tr>
<tr>
<td>L x S</td>
<td>4</td>
<td>6-727</td>
<td>1-682</td>
<td>0-077</td>
<td>0-989</td>
</tr>
<tr>
<td>R x T</td>
<td>36</td>
<td>72929-540</td>
<td>2025-821</td>
<td>92-949</td>
<td>&lt;0-01</td>
</tr>
<tr>
<td>T x S</td>
<td>9</td>
<td>4880-402</td>
<td>542-267</td>
<td>24-880</td>
<td>&lt;0-01</td>
</tr>
<tr>
<td>T x R x T</td>
<td>36</td>
<td>8-415-060</td>
<td>2103-765</td>
<td>96-525</td>
<td>&lt;0-01</td>
</tr>
<tr>
<td>L x R x T</td>
<td>9</td>
<td>119-175</td>
<td>13-242</td>
<td>0-680</td>
<td>0-791</td>
</tr>
<tr>
<td>L x T x S</td>
<td>4</td>
<td>15-167</td>
<td>3-792</td>
<td>0-174</td>
<td>0-952</td>
</tr>
<tr>
<td>R x T x S</td>
<td>36</td>
<td>7995-073</td>
<td>222-085</td>
<td>10-190</td>
<td>&lt;0-01</td>
</tr>
<tr>
<td>S x T x L x R</td>
<td>36</td>
<td>2-1367</td>
<td>5-927</td>
<td>0-272</td>
<td>1-000</td>
</tr>
</tbody>
</table>

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**Fig. 5.** Final germination percentages (mean ± s.e.) of the two types of seeds in *Diptychocarpus strictus* (A) incubated in the light/darkness regime (left panels) and (B) in constant darkness (right panels) at five temperature regimes following 4 and 10 months of storage in soil in field conditions. Germination percentages of fresh (0-months-old) seeds from upper and lower siliques were 0% in both light/darkness and constant darkness at all temperature regimes. No seeds inside fresh or buried whole intact lower siliques germinated in any of the tests. Bars with different letters are significantly different in multiple range comparison within upper and within lower siliques (Tukey’s HSD, P = 0-05).
Imbibition by indehiscent lower siliques and of seeds within them. Imbibition of water by intact siliques, by silique segments of fresh and 1-year-old siliques and by the seeds within them did not differ in water uptake. Siliques in all treatments imbibed water readily and followed a typical pattern of rapid initial water uptake, with whole silique and segment mass increasing by 136% and 145%, respectively, after 1 d; after 5 d their mass had increased by 180–190% (data not shown). Seeds within fresh and 1-year-old siliques and silique segments also became fully hydrated. There was no significant difference in dry mass of non-imbibed seeds from fresh and 1-year-old siliques or silique segments. In addition, after 5 d, when whole siliques and segments of siliques were fully imbibed, there was no significant difference in mass of seeds removed from imbibed siliques, increase in the mass...
types of dispersal units in
by mucilage may increase differences in dispersal of the two
and the soil is moist, seeds from upper siliques rapidly
mental conditions, for example rainfall. When rain occurs
(Meyer and Carlson, 2001). Our results showed that, in
the better dispersal ability of the lighter dispersal units
part of the dispersal ability, an observation that reinforces
wing, the mass of dispersal units can explain a significant
primarily to be dispersed near the mother plants, which serve
usually are dispersed near the mother plants, which serve
lower siliques remain within the highly lignified pericarp and
plant by wind currents. However, seeds in the indehiscent
silique morphs is their seed dispersal ability. The wide wing
of seeds from dehiscent upper siliques decreases their terminal
range of temperature regimes in either light or darkness, prob-
D. strictus confers differences in the capability for dispersal
and germination of seeds of this species in time and space,
which may be viewed as adaptive bet-hedging in an unpredict-
able environment.

The passive mode and local dispersal by wind are the two
principal mechanisms of dispersal in D. strictus. After plants
D. strictus complete their life cycle in late May to early
June, one of the most obvious differences between the two
silique morphs is their seed dispersal ability. The wide wing
of seeds from dehiscent upper siliques decreases their terminal
velocity and thus prolongs their fall time, which increases the
chance of these seeds being carried away from the mother
plant by wind currents. However, seeds in the indehiscent
lower siliques remain within the highly lignified pericarp
and usually are dispersed near the mother plants, which serve
only to leave descendants in safe sites previously occupied
by their parents (primary dispersal). Apart from the seed
wing, the mass of dispersal units can explain a significant
part of the dispersal ability, an observation that reinforces the
better dispersal ability of the lighter dispersal units
(Meyer and Carlson, 2001). Our results showed that, in
D. strictus, the fall rate of whole lower siliques is faster than
that of seeds from upper siliques. Moreover, seeds from
upper siliques were dispersed a greater distance than whole
lower siliques at wind speeds of both 1 and 4 m s⁻¹.

During summer and autumn, secondary dispersal as affected
by mucilage may increase differences in dispersal of the two
types of dispersal units in D. strictus under some environment-
al conditions, for example rainfall. When rain occurs
and the soil is moist, seeds from upper siliques rapidly
absorb water and develop a thick layer of mucilage, which
aids adherence of the seeds to the soil surface and in settling
in local sites. Although lower siliques are indehiscent, a thin
mucilaginous layer forms on the seeds; the role of this muci-
lagence on the seeds is not clear. Mucilage on seeds from upper
siliques significantly increased dehydration time, but it did
not affect dehydration time of seeds from lower siliques.
Gutterman and Shem-Tov (1997) found that the dehydration
time of mucilaginous seeds was much longer than that of non-
mucilaginous seeds. Thus, on the soil surface mucilaginous
seeds from upper siliques should have an advantage over mucil-
aginous seeds from lower siliques (if they were able to escape
from the silique). Low or non-mucilaginous seeds would take
up less water from wet soil because of poor contact with the
soil particles (Huang et al., 2001).

Moreover, mucilaginous seeds could adhere to the soil
surface, and mucilage could hold soil particles around the
seed, thereby increasing seed mass (Gutterman and
Shem-Tov, 1996; Huang et al., 2001). The more mucilage
the seed has, the more sand particles that can adhere to it.
Since soil particles tenaciously adhere to mucilaginous seeds
from upper siliques, the great increase in mass of these seeds
may prevent them from being further dispersed by wind
from favourable microhabitats (Gutterman, 1993; Huang
and Gutterman, 2004), delay or prevent seed collection by
insects, thereby avoiding seed loss (Gutterman, 1993; Huang
and Gutterman, 2004), or promote settlement of seeds onto
the soil (Huang et al., 2001).

Freshly matured seeds from both silique morphs of
D. strictus are dormant and thus do not germinate over a
range of temperature regimes in either light or darkness, prob-
ably because of the low growth potential of the embryo
(Baskin and Baskin, 1998). After 3–4 months of after-ripening
under laboratory (Fig. 4) and field (Fig. 5) conditions during
summer, the seeds germinate to high percentages at cool but
not at warm temperatures. After 12 months of after-ripening
under laboratory conditions, seeds had high germination in
both light and darkness over the range of cool to warm tem-
perature regimes at which they were tested. Seeds stored in
the field germinated to 46.7–87.3% in light and 43.3–85.3% in
darkness at 5/2 and 15/2°C, but to <14.0% in light and darkness
at 25/15 and 30/15°C when tested in October. The
optimum temperatures for germination in light and darkness
of seeds stored in the field until April of the following year
were still 15/2 and 5/2°C. Dormancy break occurs in
summer, but, unlike seeds of temperate zone obligate winter

### Table 7. Imbibition of water (mg, mean ± 1 s.e.) by seeds of Diptychocarpus strictus within whole lower siliques and by segments of lower siliques

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Seed from fresh siliques</th>
<th>Seed from 1-year-old siliques collected from the field</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unmanipulated</td>
<td>Segments</td>
</tr>
<tr>
<td>Mass of seed removed from imbibed silique</td>
<td>2.218 ± 0.020³A</td>
<td>2.237 ± 0.041³A</td>
</tr>
<tr>
<td>Mass of redried seeds</td>
<td>1.301 ± 0.014³A</td>
<td>1.273 ± 0.039³A</td>
</tr>
<tr>
<td>Dry mass of non-imbibed seed</td>
<td>1.333 ± 0.015³A</td>
<td>1.267 ± 0.039³A</td>
</tr>
<tr>
<td>Increase in mass per seed (%)</td>
<td>70-674 ± 2-100³</td>
<td>77-264 ± 4-318⁸</td>
</tr>
</tbody>
</table>

Different lower case letters within a row and upper case letters within a column indicate significant differences (Tukey’s HSD, P = 0.05).
annuals, those of *D. strictus* are not induced back into dormancy (secondary dormancy) during winter. Although the seeds can germinate to high percentages at low temperatures in autumn, in most years they are prevented from doing so by the low amount of rainfall in autumn (Fig. 6A, B). Thus, germination of most seeds is delayed until spring when soil moisture is high due to rainfall and snow melt. Seeds of *D. strictus* have non-deep physiological dormancy (Baskin and Baskin, 2004).

After-ripened seeds of *D. strictus* can germinate to high percentages in both light/darkness and darkness. Thus, it is likely that should seeds become covered with soil or litter, they would not be prevented from germinating. In fact, after a spring rain soil below the surface or under litter would remain moist for longer periods of time than that on the soil surface, and thus burial of seeds in soil or litter would favour germination. Based on these results, it seems unlikely that *D. strictus* could form a large persistent seed bank from seeds of the upper siliques, which are dispersed soon after these dehiscent siliques mature.

The pericarp of dehiscent upper siliques of *D. strictus* is relatively thin, whereas the indehiscent lower siliques have a thick pericarp. Thus, the pericarp plays little or no role in controlling germination of seeds from upper siliques, but it physically restricts/prevents germination of seeds within the lower siliques. However, even when fresh seeds are removed from the lower siliques the presence of some embryo dormancy (or non-deep physiological dormancy) prevents germination. Germination of the excised seeds from the upper and lower siliques of *D. strictus* was not inhibited by mucilage, indicating that water-soluble germination inhibitors were not leached from it. However, the presence of a thick mucilaginous layer might play a regulating role in diffusion of water and oxygen to the embryo and thus influence germination (Witztum *et al.*, 1969; Liu and Tan, 2007; Thapliyal *et al.*, 2008).

After dry storage at room temperature for several months, most of the seeds from upper siliques and those removed from lower siliques, but not those held within intact lower siliques, could germinate. In the field, after-ripening also occurs in summer, and seeds are non-dormant in autumn. However, before the first snowfall in Ürümqi in November 2008, we searched for seedlings of *D. strictus* in the field but found none. This was probably due to soil being wet for a period of only 1–2 d at a time, which did not allow the seeds enough time to germinate even though temperatures were favourable. However, many seedlings were found in the field in spring (mid-March to early April), when soil moisture (precipitation plus snow melt) and temperature were suitable for germination. Presumably, these seedlings were from upper siliques seeds, or perhaps from lower siliques that had been produced several years earlier (seed bank). In the plots of the experimental garden, only seeds from upper siliques and those removed from lower siliques germinated. After 1 year on the soil surface, pericarps of the lower siliques were still hard, and seeds inside them were viable. The dormancy breaking requirements of this morph have not been identified, but it is not due to lack of water uptake by seeds within the intact siliques or silique segments (Table 7; Fig. 9). We suggest that seeds within these indehiscent siliques and silique segments are prevented from germinating during the first spring germination season (and perhaps also in the second and later germination seasons) by the mechanical restraint of the thick, rigid pericarp. The time required for pericarps of lower siliques to release their seeds or to become soft enough for the seeds inside to germinate in the field is not known.

Thus, the lower siliques can form a persistent seed bank (*sensu* Thompson and Grime, 1979), which is an advantage in arid zones with unpredictable rainfall, such as the Junggar Desert. Buffering variation in reproductive success by delayed germination in desert annuals is a classic example of bet-hedging (Philippi, 1993; Venable, 2007). While the best thing a non-germinating seed can do is to survive, a germinating seed may either die or produce many new offspring. Thus, producing a fraction of dormant seeds is the hedge against the risk associated with germination. The germination fraction favoured by natural selection is lower when there is a high risk of seedling death than when there is a low risk (Ellner, 1985; Evans *et al.*, 2007).

Seeds from the upper siliques of *D. strictus* after-ripen during summer and can germinate in autumn or the following spring. Seedlings produced in autumn and in spring can survive and produce seeds in late May to early June and, in contrast to obligate winter annuals, the non-dormant seeds do not re-enter dormancy (secondary dormancy) during winter (Baskin and Baskin, 1998). Thus, the species has the potential to behave both as a winter annual and as a spring-ephemeral annual, i.e. facultative winter annuals. In the field, *D. strictus* behaves mostly as a spring-ephemeral annual, however, apparently because most seeds, which are non-dormant in autumn, are prevented from germinating at that time by low soil moisture content. The probable reason why only a low percentage of seeds sown on the soil surface in summer and watered at 3 d intervals during autumn germinated in autumn is that the soil did not remain moist for a long enough period of time for the seeds to germinate (Fig. 6B). It also seems likely that seeds in the lower siliques would have the same germination phenotype as that described above for seeds from upper siliques, once they are released from the siliques or the pericarp becomes soft enough for seeds to germinate within it. The dormancy breaking and germination characteristics of seeds extracted from these indehiscent lower siliques are, in fact, the same as those released from the dehiscent upper siliques (see Figs 4 and 5).

In contrast to extreme hot desert habitats, such as those in the Mojave Desert of North America (*MacMahon and Wagner*, 1985) and the Negev Desert of Israel (*Gutterman*, 1993), the Junggar Desert is a cold desert characterized by snow and very low temperatures in winter and by aridity in summer (*Wei et al.*, 2003). Differences in morphology of siliques and seeds, dispersal and time delay between dispersal and germination enable *D. strictus* to spread its offspring in space and time, which is one of the ecological adaptive strategies to the cold desert of the Junggar Basin. Like the majority of species with heteromorphic seeds (*Imbert*, 2002), our results suggest that high dispersal ability and reduced dormancy of seeds (from upper siliques) provide *D. strictus* with the chance of rapidly colonizing new sites. Furthermore, lack of seed dispersal and delay of germination of seeds from lower
siliques give the species a better chance of retaining the mother site and of colonizing suitable local sites. This constitutes a very safe means of reproduction and an available seed reserve on/in soil of the desert habitat, thus increasing the probability of persistence of D. strictus populations.

These studies have revealed differences in the behaviour of the heteromorphic fruits and seeds of D. strictus compared with those of Cakile, another member of the Brassicaceae. Cakile is a genus of sandy freshwater lakeshores and of sandy seashores (Rodman, 1974; Maun et al., 1990) and its fruit and seed biology have been studied extensively (see references in Introduction). Whereas heteromorphism in D. strictus is between upper and lower fruits and their seeds, in Cakile spp. it is between proximal and distal segments of a fruit and their seeds. In Cakile, the distal segment of the fruit abscises and is dispersed by water, whereas the proximal segment remains attached to the mother plant. Further, neither segment is dehiscent, and thus seeds (usually one per segment) germinate within the segment. In both Diptychocarpus and Cakile, fresh seeds have non-deep physiological dormancy, and non-dormant seeds germinate in both light and darkness. However, seeds of D. strictus come out of dormancy (after-ripen) in summer, and those of Cakile generally need cold stratification (winter) to do so. Thus, at least in the northern parts of its range (in the Northern Hemisphere) seeds of Cakile germinate in spring and fruits mature in autumn. This summer annual life cycle is in contrast to the winter or spring annual ephemeral life cycle of D. strictus.

In summary, then, in Cakile the distal segment of the heteromorphic fruit distributes the species in space and the proximal segment remains near the mother plant. In D. strictus, on the other hand, the winged and mucilageous seed of the upper distal segment remains near the mother plant. In Cakile, the distal segment of the heteromorphic fruit distributes the species through time via imposed dormancy of the seeds inside it.

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LITERATURE CITED


