



## Mucilage secretion: an adaptive mechanism to reduce seed removal by soil erosion?

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Diaspores of many plant species inhabiting open vegetation in semi-arid environments secrete mucilage after wetting (myxospermy) that glues the diaspores to the ground and prevents movement when the mucilage dries. In the present study, we test whether mucilage secretion can be considered as a selective response to soil erosion in plant species inhabiting semi-arid environments. We relate the amount and type of mucilage secretion by seeds of *Helianthemum violaceum* and *Fumana ericifolia* (Cistaceae) to the number of raindrop impacts needed to remove these seeds after gluing them with their own mucilage to the ground and also the time that these seeds resist water run-off without detaching. We also compare the amount of seed mucilage production by plants growing in habitats without erosion and plants affected by severe erosion by fitting mixed effect models. Our results show an important phenotypic variation in the amount of mucilage secretion in both species, although it is suggested that the effect of mucilage secretion in the rate of seed removal by erosion is species- and mechanism-dependent. For *F. ericifolia*, the amount of mucilage secreted by the seeds is directly proportional to their resistance to raindrop impacts and is positively related to the intensity of the erosive processes that the plants experience. Nevertheless, all the seeds resist the force of run-off during 60 min, irrespective of the amount of mucilage they produce. In *H. violaceum*, mucilage secretion *per se*, and not the amount of mucilage produced by the seeds, has an effect on the rate of seed removal by erosive processes. Furthermore, cellulosic fibrils were found only in the mucilage of *F. ericifolia* but not in *H. violaceum*. Overall, our results only partially support the hypothesis that a selective response to soil erosion exists. © 2013 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2014, **111**, 241–251.

ADDITIONAL KEYWORDS: drop impact – *Fumana* – *Helianthemum* – mucilage – myxospermy – pectin – run-off.

### INTRODUCTION

There is consensus that the cause of natural selection is any environmental or biotic agent that results in differential fitness among phenotypes (MacColl, 2012). However, current understanding of the causes of natural selection is still poor and it is necessary to deepen our knowledge of selective agents by focussing on the structure and consequences of ecological variation on the traits that we suspect are under selection (Siepielski, DiBattista & Carlson, 2009). We can tackle this by discovering how selection changes the phenotypic distribution of traits in nature and why selection operates in this way (Wade & Kalisz, 1990).

The light environment, water conditions, temperature, mineral nutrition, competition, herbivory, plant pathogen interactions, pollinator and dispersal services, and perturbations have been frequently cited as selection agents for plants (Niklas, 1997; Gurevitch, Scheiner & Fox, 2002; Herrera & Pellmyr, 2002; Pausas & Keeley, 2009). However, soil erosion has not yet been considered as an agent of selection for plants and is usually only viewed as an environmental problem (Boardman, 2006). Arid and semi-arid regions of the world are characterized by sparse vegetation cover and, in these regions, most of the annual precipitation is concentrated into a small number of high-intensity events, which have a high potential for soil erosion (Whitford, 2002; Rodriguez-Iturbe & Porporato, 2004; Thompson, 2005). Seeds resting on the soil surface of hillslopes after seed dispersal are at

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risk of losing opportunities for plant recruitment as a result of erosive processes that move them downhill, where they can become buried (García-Fayos *et al.*, 1995), or suffer strong competition with more competitive seedlings or from pre-established vegetation (Cantón *et al.*, 2004). Under these circumstances, anchoring to the ground surface can help seeds from stress tolerant and less competitive plant species to resist removal by erosive processes and therefore increase their fitness.

There are two main mechanisms by which soil erosion proceeds: soil particle splash detachment by raindrops and detachment and transport of soil particles by run-off or surface wash along hillslopes (Lal, 2001). The severity of these soil erosive processes depends on the characteristics of the rainfall (duration, intensity, amount, drop size), on the physical and chemical properties of the soil (aggregation, infiltration, and hydraulic conductivity), and on the topography of hillslopes (angle and length of the slope) (Thornes, 1985). Similar to what happens with the mineral particles of soil, the susceptibility of seeds to be removed from the ground by erosion mainly depends not only on seed size (García-Fayos & Cerdà, 1997), but also on characteristics such as shape, presence of appendages, and the ability to secrete mucilage from the seed coating or the pericarp (myxospermy) (García-Fayos, Bochet & Cerdà, 2010; Wang *et al.*, 2013).

Once the myxospermic diaspores reach the soil surface, light rain, dew or even ground humidity is sufficient to induce mucilage secretion in seconds or minutes (M. Engelbrecht, personal observation; Huang *et al.*, 2008) and, after drying, it anchors the seed to the surface that it is resting on (Van Rheede van Oudtshoorn & Van Rooyen, 1999). Two main types of mucilage has been recognized: 'true' mucilage, consisting of almost exclusively of pectin, which occurs, for example, in the genus *Linum*; and 'cellulosic' mucilage, consisting of pectin and cellulose fibrils, which occurs in the genus *Salvia* and *Artemisia*. Pectin increases the amount of water and viscosity around the seed coat and, once it dries, results in adherence of the seed coat to the surface that it rests on. Cellulose fibrils increase the amount and strength of the contact between the seed and the surface, thus enhancing the anchoring power of the pectin (Gutterman, Witzum & Evenari, 1967; Witzum, Gutterman & Evenari, 1969; Grubert, 1974; Werker, 1997; Kreitschitz, 2009).

Mucilage secretion by diaspores was initially related to seed dispersal functions in plants inhabiting semi-arid environments (Zohary, 1962; Friedman & Stein, 1980). However, other functions, such as protecting seeds from predation, flooding, and the regulation of germination, have been demonstrated (Witzum *et al.*, 1969; Gutterman & Shem-Tov, 1996,

1997a). Several studies then confirmed that mucilage secretion significantly hinders seeds from predation by granivorous ants (Fuller & Hay, 1983; Gutterman & Shem-Tov, 1997a; Engelbrecht & García-Fayos, 2012) and reduces the probability of seed removal by soil erosion, especially small seeds (Ellner & Shmida, 1981; García-Fayos & Cerdà, 1997; García-Fayos *et al.*, 2010). Other studies have found that the proportion of plant species whose diaspores are myxospermic varies among plant communities that differ in the severity of soil erosion and also that this proportion correlates with those soil properties linked to run-off generation (García-Fayos, Engelbrecht & Bochet, 2013). Similarly, other studies have shown that mucilage secretion plays a role in the germination of seeds in semi-arid environments by increasing the water retention of seeds and increasing the seed-soil particle contact (Gutterman & Shem-Tov, 1997b; Huang *et al.*, 2008; Lu *et al.*, 2010).

Recent reviews have examined the functions proposed for mucilage secretion in diaspores (Kreitschitz, 2009; Western, 2012; Yang *et al.*, 2012) and the emerging view is that the adaptive value of mucilage may vary between plant lineages and may respond to several not mutually exclusive forces, thus hindering the establishment of general patterns. A way to disentangle the role of mucilage secretion of a plant species or a plant lineage is to analyze the variation in the amount of mucilage secreted by diaspores along gradients of selected environmental factors that presumably affect this amount (i.e. gradients of increasing climatic drought crossed with gradients of increasingly severe soil erosion). A complementary or alternative approach would be to analyze the variation in mucilage secretion in plants whose seeds have traits that simultaneously preclude some of the possible functions of mucilage. For example, in the case of plants whose seeds show physical dormancy or deep physiological dormancy (*sensu* Baskin & Baskin, 1998) and then require long periods of cold or warm stratification before germinating, it is unlikely that the role of mucilage secretion is related to enhance the rate or the speed of germination. Similarly, under sunny conditions in temperate and tropical climates, the water captured by the mucilage is lost less than 1 h after rainfall as a result of evaporation (Grubert, 1981; Ryding, Harley & Reynolds, 1992), thus making improbable the use of this water for germination.

To test the hypothesis that myxospermy is an adaptive response of plants to soil erosion, it is necessary to show that: (1) the amount of mucilage produced per seed is positively related to the reduction in the rate or in the distance of seed removal by soil erosion (mechanism selection) and (2) increasing the intensity of soil erosion in the environment where plants develop produces a positive phenotypic selection at

the population level with respect to the amount of mucilage secretion (character selection) with other potential selective forces being equal.

In the present study, we analyze the relationship between mucilage secretion by seeds and the intensity of soil erosion processes in several populations of *Fumana ericifolia* and *Helianthemum violaceum* (Cistaceae) and then discuss the results in the light of the type of mucilage secreted by these species. Cistaceae species are characteristic of open plant communities in dry and sunny temperate habitats, and their seeds have hard seed coats that impede germination until it is removed or scarified (Thanos *et al.*, 1992; Baskin & Baskin, 1998; Ferrandis, Herranz & Martínez-Sánchez, 1999; Herranz, Ferrandis & Martínez-Sánchez, 1999; Thompson, 2005; Guzmán & Vargas, 2009; Moreira *et al.*, 2010). In the family of the Cistaceae, pectin was assumed to be the main component of mucilage but, for *Fumana laevipes*, cellulose components have been described as well (Grubert, 1981).

## MATERIAL AND METHODS

### TYPE OF SEED MUCILAGE

To detect differences in the type of seed mucilage of *F. ericifolia* and *H. violaceum* we observed wetted seeds of both species under the binocular. Chemical staining was used to distinguish pectin mucilage from cellulose mucilage. We therefore used methylene blue (0.1% solution) to detect the cellulose content, (Kreitschitz & Vallès, 2007; Kreitschitz, 2009) and ruthenium red was used to stain the pectin part of the mucilage red (Hanke & Northcote, 1975; Western, Skinner & Haughn, 2000; Western *et al.*, 2001).

### SEED RESISTANCE TO DROP IMPACT

To measure the resistance to direct drop impacts of mucilaginous seeds adhered to the ground, we applied the water drop test method to fully-developed seeds of *H. violaceum* and *F. ericifolia*. This method simulates the rain drop impact that seeds undergo in the field during storms and is usually used to test the stability of soil aggregates to erosion forces (Imeson & Vis, 1984). Seeds of both species were equally collected from the different study areas and then mixed. We measured mucilage production by submerging 83 fully-developed seeds of *F. ericifolia* and 104 fully-developed seeds of *H. violaceum* in distilled water until the maximum mucilage production of each seed was reached (in less than 45 min). Although the technique may not reflect the exact natural conditions that seeds experience, this method gives us an idea about the amount of mucilage produced by each seed and permits us to homogenize the experimental con-

ditions. High resolution photographs were taken with an incorporated camera in a binocular (Leica LED 2500;  $\times 80$ ) at an augmentation of  $1 \times 80$ . The projected area of the seed surface and the mucilaginous layer were measured with SIGMASCAN PRO, version 5 (1999). Then, seed surface was used as a proxy of seed size and relative mucilage production per seed was calculated as the quotient of mucilage area by seed area.

After taking the photograph, each seed was immediately placed on a wet filter paper (2 mm thick) and completely air-dried. The filter paper with the seed was placed on an inclined plastic surface ( $20^\circ$  angle) and the number of drop impacts required to detach each seed from the filter paper was counted. The inclination angle was selected because it is in the range of the slope angles where plant populations of highly eroded habitats develop. Drops were produced by a pipette with a water supply system with a constant head and fell on each seed with a frequency of approximately one drop per second from a height of 1 m. Mean drop size diameter was 5 mm, which is in the range of rainfall drops during medium intensity storms with a return period of approximately 5 years in eastern Spain (Cerdà, Garrigós & García-Fayos, 2002; Arnaez *et al.*, 2007). The number of direct drop impacts needed to detach the seeds was evaluated separately for each species (*F. ericifolia* and *H. violaceum*) because they varied significantly in seed size and absolute and relative mucilage production (Table 1). Linear and quadratic regression models were used to fit the relationship between the relative mucilage production, as well as seed size, with the number of drop impacts needed to detach the seeds. Statistical analyses were performed using SPSS, version 19.0 (SPSS Inc.) and residuals were checked for normality.

### SEED RESISTANCE TO RUN-OFF

To simulate run-off, water flux experiments were conducted under laboratory conditions with a methacrylate flume (length 250 cm, width 30 cm) modified from Poesen *et al.* (1999). The slope of the flume was set to an inclination of  $25^\circ$  and tap water was used to generate run-off. The strength of the overland flow caused by run-off was estimated by means of the flow shear stress, which was calculated in accordance with the equation described in De Baets *et al.* (2007). In the experiments, a flow shear stress of 5 Pa was applied because it was the minimum flow strength under which seed removal was observed and, at the same time, it is in the range of values that produces detachment and transport of particles on hillslopes, although is still below the critical flow strength that forms rills and gullies (1.8 to 10.6 Pa; Poesen *et al.*, 2003). The return period of rainfall events with this intensity in

**Table 1.** Overview of the range (minimum – maximum) of seed mass, seed area, mucilage area, relative mucilage area, minimum time to secrete mucilage, and time until maximum mucilage is released from seeds of *Fumana ericifolia* and *Helianthemum violaceum* for both the flat geomorphologic site with no erosion and the associated hillslopes with severe erosion, separately

| Species                       | Seed weight (mg) | Seed area (mm <sup>2</sup> ) | Mucilage area (mm <sup>2</sup> ) | Relative mucilage area (mucilage area/seed area) | Minimum time to mucilage segregation (min) | Time until maximum mucilage is segregated (min) | N   |
|-------------------------------|------------------|------------------------------|----------------------------------|--|--|---|-----|
| No erosion                    |                  |                              |                                  |  |  |   |     |
| <i>Fumana ericifolia</i>      | 0.57–2.58        | 1.41–4.18                    | 2.24–12.93                       | 1.22–5.11  | 1.0  | 5   | 480 |
| <i>Helianthemum violaceum</i> | 0.40–1.06        | 0.69–1.69                    | 1.31–2.84                        | 1.40–2.23  | 1.5  | 45  | 451 |
| Severe erosion                |                  |                              |                                  |  |  |   |     |
| <i>Fumana ericifolia</i>      | 0.90–3.17        | 1.73–4.44                    | 6.08–15.8                        | 2.03–6.80  | 1.0  | 5   | 464 |
| <i>Helianthemum violaceum</i> | 0.53–1.35        | 0.69–1.97                    | 1.38–3.27                        | 1.42–2.45  | 1.5  | 45  | 461 |

Seeds measured (N) were recollected in the different study sites from year 2007 to 2009.

western Mediterranean semi-arid environments is 10 years but, for some coastal regions, this period is shorter (Grove & Rackman, 2001). A thin layer of sand particles between 0.02 and 1 mm, with the major proportion between 0.25 and 0.5 mm, was previously glued to the surface of the flume channel to provide roughness that approximated natural conditions.

The quantity of mucilage in seeds was measured to establish a relationship between the strength of seed adherence and the resistance to run-off. Fully-developed seeds of *H. violaceum* and *F. ericifolia* collected in the study areas were moistened to provoke the secretion of mucilage and then photographed to measure the relative mucilage production and seed size. Ten seeds were then placed on plastic sheets (50 × 30 × 0.5 cm) over a thin layer of the same sand material glued to the bottom of the flume. Seeds were arranged along a horizontal line, at intervals of 1.5 cm, and at 8 cm from the left and right margin and 10 cm from the upper margin of the sheet to prevent edge effects. For each species, ten plastic sheets with ten or 11 seeds each (total of 104 seeds) were used. Once the seeds were dried, and subsequently glued to the sand layer, the sheet was placed in the flume, 85 cm from the water flux entry, in a cavity equalling the size of the sheet so that the sample surface was at the same level as the flume surface. Once the water flow discharge began, the time to detachment from the plastic sheet was measured for each seed with a maximum run time of 60 min per experiment. We designed a complementary test with seeds of the two species in which the mucilage layer was eliminated and their resistance to being removed by run-off was compared with that of intact seeds using the same method as in the previous experiment. In each experiment, we used seeds of similar weight to enable the comparison of results (García-Fayos *et al.*, 2010). To eliminate the mucilage layer, seeds were first wetted for 15 min and then rubbed on filter paper until the mucilage was removed.

Linear and quadratic regression models were used to fit the relationship between the relative mucilage production, as well as the seed size, with the time (min) needed to detach the seeds. Statistical analyses were performed using SPSS, version 19.0, and residuals were checked for normality.

#### DIFFERENCES IN AMOUNT OF SEED MUCILAGE FROM PLANTS GROWING UNDER CONTRASTING EROSION REGIMES

We looked for variations in the amount of mucilage produced by seeds of *F. ericifolia* and *H. violaceum* collected from plants growing in several populations that experienced contrasting soil erosion pressures. We also analyzed the variation in seed weight (mg) and seed area (mm<sup>2</sup>) of these seeds to control for the



indirect effects that erosion might have on the amount of mucilage through changes in seed size.

The study area was located in the Alfambra River basin (province of Teruel, Spain), which is an area of 4000 m<sup>2</sup> that lies at an altitude of 900 to 1100 m a.s.l. The study site has a yearly mean temperature of 11.9 °C and precipitation of 368 mm. A more detailed description of the study area is provided in García-Fayos & Bochet (2009).

We selected two geomorphological conditions, almost flat highlands (< 5° slope angle) and their associated hillslopes (25–30°), as surrogates for ‘no erosion’ and ‘severe erosion’ treatments, respectively. For ‘no erosion’ sites, we used forest clearings larger than 0.05 km<sup>2</sup> and, for ‘severe erosion’ sites, we used sectors of hillslopes longer than 100 m in length. All sites were southerly (García-Fayos & Bochet, 2009). In spring and summer 2009, we identified three populations per treatment of the selected species, each at least 500 m from any other sampled slope and all containing more than 100 individuals. In each population, we collected mature seeds from 15 individuals, each at least 5 m distant from other sampled plants. Under laboratory conditions, we weighed the seeds and measured the size (seed area in mm<sup>2</sup>) and the relative mucilage amount (surface of secreted mucilage/seed surface) of ten fully-developed seeds from each individual plant.

Because the seeds of the studied species were food items for granivorous ants (Engelbrecht & García-Fayos, 2012), ants may also potentially exert a selective pressure on the amount of mucilage secreted by the seeds. However, the mean ± SE nest density of granivorous ants (as a surrogate of seed harvesting pressure) in the ‘no erosion’ and ‘severe erosion’ hillslopes was 97.6 ± 36.4 and 81.8 ± 15.8 nests ha<sup>-1</sup>, respectively (García-Fayos *et al.*, 2013) and did not significantly differ between them ( $t = 0.1512$ , d.f. = 13.977,  $P = 0.882$ ).

#### STATISTICAL ANALYSIS

We fitted linear mixed models to analyze the relative amount of mucilage from the seeds. All these models included an erosion regime where plants grow as a fixed effect (‘no erosion’ versus ‘severe erosion’) and various alternatives for random effects: (1) the individual plant from which the seeds were collected; (2) the population where these plants develop; (3) the individual plant nested within the population; and (4) no random effects. Akaike’s information criterion was used to choose among competing statistical models, as suggested by Zuur *et al.* (2009) and Crawley (2007). We performed linear mixed-effect models using the R ‘lmer’ function from ‘lme4’ (Bates, Maechler & Bolker, 2011). We used the ‘lmer’ function because it fits a greater range of models and is more reliable than other

similar functions (Bates, 2005). We constructed the models using the restricted maximum likelihood estimation procedure (Zuur *et al.*, 2009). The significance of the fixed effects was evaluated by means of likelihood ratio tests by calling the function ‘anova’ to compare models with and without the factor being tested, which was previously fitted using maximum likelihood estimates (Crawley, 2007; Zuur *et al.*, 2009). In addition, to assess the uncertainty of parameter estimates from the linear mixed models, we ran 10000 Markov chain Monte Carlo samples using the function `mcmc` in `lme4`, estimating the posterior probability density of model parameters. Parameter estimates for which the 95% confidence interval (CI) (defined by the 2.5% and 97.5% quantiles from the resampled distributions) did not overlap with zero were considered significant. The confidence interval was calculated using the `HPDinterval` function in `lme4`.

Differences in seed weight and size under contrasting soil erosion pressures were evaluated for the two species with mixed effect models using the same steps as described above. All statistical analyses were carried out in R, version 2.12.2 (R Development Core Team, 2011).

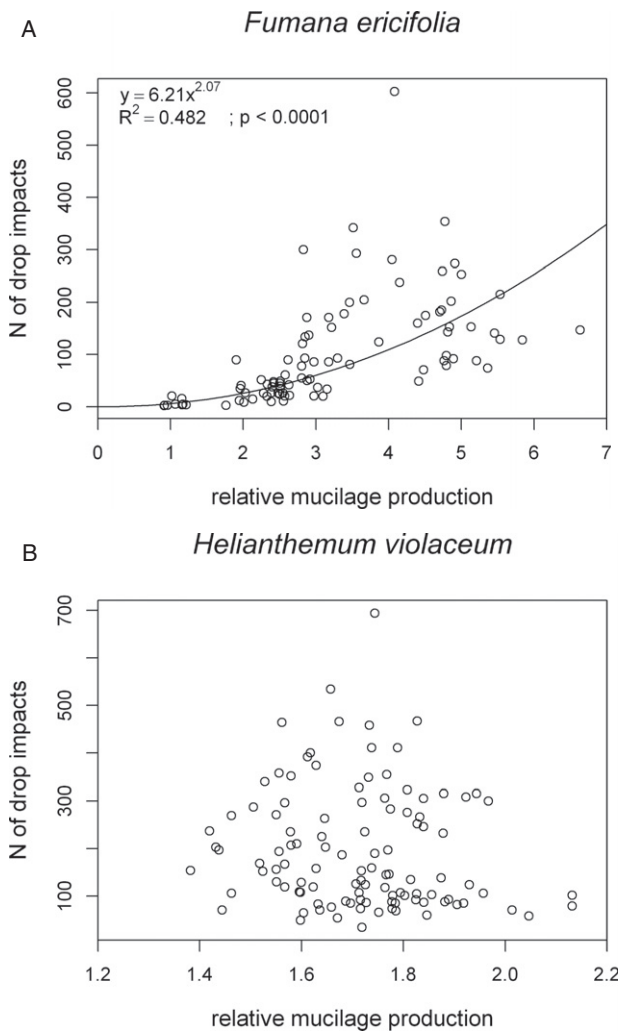
## RESULTS

### TYPE OF SEED MUCILAGE

We found differences in the type of seed mucilage. Ruthenium red stained the mucilage of *F. ericifolia* and *H. violaceum* seeds homogeneous pink, revealing the pectin matrix of the mucilage in both species (Fig. S1). After staining the seeds with methylene blue, dark blue cellulose strands were identified in the mucilage of *F. ericifolia* but not in the seeds of *H. violaceum* (Fig. S2). Seeds of *H. violaceum* seeds stained slightly blue in the outermost surface of the mucilage but no cellulose fibres were coloured. The mucilage of *F. ericifolia* seeds therefore present a heterogeneous system made of pectin matrix with cellulose strands.

### SEED RESISTANCE TO DROP IMPACT

The number of water drop impacts needed to detach the *F. ericifolia* seeds is directly related to their relative mucilage production. After applying different regression models, the power relationship was the best fit between the relative mucilage production of a seed and the number of drop impacts needed to detach it ( $r^2 = 0.482$ ,  $F_{1,82} = 75.49$ ,  $P < 0.0001$ ) (Fig. 1A), thereby indicating an exponential effect of mucilage secretion on the seed resistance to drop impacts. For *H. violaceum*, this relationship follows a bell shape distribution (Fig. 1B), indicating the lack of a relationship



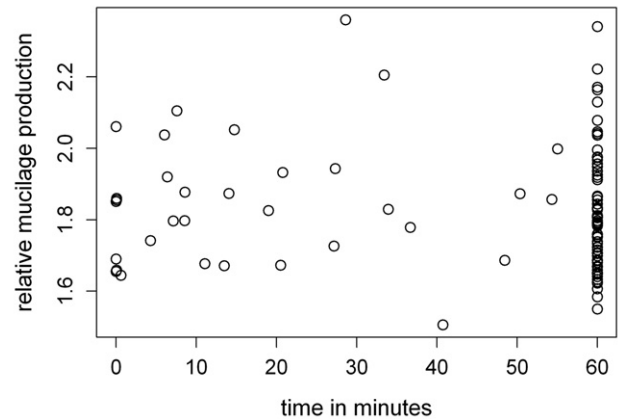
**Figure 1.** Resistance of adhered seeds to detachment by drop impact (0.05 g drops of water from a height of 1 m). Seeds had been glued to filter paper with their own previously secreted mucilage and air dried. Relative mucilage was calculated as the total mucilage (in mm<sup>2</sup>) divided by the seed area (in mm<sup>2</sup>). Each point reflects one separately tested seed. A, *Fumana ericifolia* ( $N = 83$ ). B, *Helianthemum violaceum* ( $N = 104$ ).

between seed mucilage secretion and the susceptibility of a seed to be moved by drop impacts.

The number of water drop impacts needed to detach the seeds of both species was not related to variation in seed size. Both species showed a cloud of points of seed size and numbers of drop impacts needed to detach for which no regression could be fitted (data not shown).

#### SEED RESISTANCE TO RUN-OFF

All the seeds of *F. ericifolia* remained glued to the ground until the end of the water flux experiments (60 min), irrespective of the amount of mucilage the



**Figure 2.** Resistance of adhered seeds of *Helianthemum violaceum* to detachment by run-off in a hydraulic flume with an inclination of 25° and flow shear stress of 5 Pa for a maximum of 60 min. Seeds were glued to the bottom of the flume channel with their own previously secreted mucilage and air dried. Relative mucilage was calculated as the total mucilage (in mm<sup>2</sup>) divided by the seed area (in mm<sup>2</sup>). Each point reflects one separately tested seed ( $N = 104$ ).

seeds produced; and 66.4% of the *H. violaceum* seeds remained glued until the end of the experiment. However, we found no relationship between the time that these seeds resisted run-off and their relative mucilage production (Fig. 2). Also, when comparing the seed size of both species with the time that seeds resisted to run-off, we did not find any relationship (data not shown). When we performed the experiment with demucilaged seeds of *H. violaceum*, most of the seeds were removed from the ground by run-off in just a few minutes and, again, we did not find a relationship with the amount of mucilage, indicating that mucilage secretion *per se* helps seeds of this species resist removal by run-off, although there is no direct relationship with the amount of mucilage secretion. We could not perform the experiment with demucilaged seeds of *F. ericifolia* because the mucilage layer of the seeds cannot be eliminated without strongly damaging the seed coat, thus influencing the condition of the seed to resist run-off.

#### DIFFERENCES IN AMOUNT OF SEED MUCILAGE FROM PLANTS GROWING UNDER CONTRASTING EROSION REGIMES

The relative mucilage production of *F. ericifolia* seeds was lower ( $3.329 \pm 0.031$ ) for seeds collected from 'no erosion' plant populations than for seeds from 'severe erosion' plant populations ( $3.697 \pm 0.033$ ; 95% CI =  $-0.5805$  to  $-0.1875$ ). Seeds of *H. violaceum* from plants in 'no erosion' and 'severe erosion' populations produced very similar amounts of mucilage

( $1.744 \pm 0.006$  versus  $1.727 \pm 0.006$ ; 95% CI =  $-0.0113$  to  $0.0469$ ).

When we evaluated the mixed models using the amount of mucilage secreted by each seed as the response variable, the model with the lowest AIC for *F. ericifolia* was that using the individual plant from which the seed was collected as a random term (AIC = 903.74). However, for models using the individual plant nested within a population, those with the population as random terms, and the model using no random term, obtained higher AIC values (905.74, 1104.17, and 1151.36, respectively). For the *H. violaceum* species, the best adjusted model was also the one where individual plants from which the seeds were collected were used as a random effect (AIC =  $-1038.47$ ). The models with the individual plant nested within the AIC population ( $-1036.47$ ) and those using only population as random terms, or no random terms, gave poorer fitted models (AIC =  $-924.59$  and  $-920.99$ , respectively). For both species, analysis of variance indicated that the model with the lowest AIC was significantly better than the next lowest AIC model ( $P < 0.0005$  in all cases).

Our results also show that the effect of the treatment (fixed term) was significant only for *F. ericifolia* and not for *H. violaceum* (likelihood ratio test:  $P = 0.0109$  and  $0.3149$ , respectively), indicating a significantly higher mucilage amount in seeds collected from plants living in the severe erosion site for *F. ericifolia* (95% CI =  $-0.5805$  to  $-0.1875$ ) but not for *H. violaceum* (95% CI =  $-0.0113$  to  $0.0469$ ).

The selected terms in the mixed models were the same when we used seed weight and seed area as response variables. For both species, the lowest AIC was found when the plant individual from which the seeds were collected was used as random effect, whereas the models with the individual plant nested within the population, models using only population as random terms, and models with no random term gave poorer fitted models (see Supporting information, Table S1). We found significantly heavier seeds in the 'severe erosive' treatment than in the 'no erosion' treatment for both species (*H. violaceum*:  $0.86 \pm 0.0083$  mg versus  $0.72 \pm 0.0069$  mg and *F. ericifolia*:  $2.22 \pm 0.049$  mg versus  $1.74 \pm 0.081$  mg, respectively). However, we found significant differences in seed area between the two erosive sites in *H. violaceum* (95% CI =  $-0.206$  to  $-0.123$ ;  $1.33$  and  $1.16$  mm<sup>2</sup>, respectively) but not for *F. ericifolia* (95% CI =  $-0.152$  to  $0.137$ ).

## DISCUSSION

The results of the present study incompletely support the hypothesis that myxospermy can be considered as an adaptation to soil erosion. The results clearly indicate

that myxospermy reduces the probability of seeds of both species being removed by mechanisms of soil erosion (drop impact and run-off). However, the quantifiable effect of mucilage appears to be mechanism- and species-dependent.

Thus, in *F. ericifolia*, the relative amount of mucilage produced per seed is positively and strongly related to the reduction of seed removal by rain drop impact (mechanism selection) and, at the same time, the phenotypic variation of mucilage secretion is related to the intensity of soil erosion experienced by mother plants (character selection). However, no relationship was found between the amount of mucilage released by the seeds and the time they resisted run-off.

For *H. violaceum*, despite the close phylogenetic relationship between both species (Guzmán & Vargas, 2009), the results did not support any of the assumptions of mechanism and character selection that we found in *F. ericifolia*. Although we found a clear difference in *H. violaceum* between intact and demucilaged seeds in relation to removal rate by drop impact and run-off, we did not find any relationship between the amount of mucilage secretion of intact seeds and the removal rate by these erosive mechanisms. Consistently, the erosive environment experienced by mother plants had no influence on the amount of mucilage secreted by the seeds.

Differences in the kind of mucilage substances could be at the basis of the variation in adherence ability between both species, as stated by Grubert (1974), Werker (1997) and Kreitschitz (2009). The mucilage of *F. ericifolia* seeds has cellulosic fibrils embedded in the pectin layer, whereas the mucilage of *H. violaceum* seeds is composed only of a pectin layer. Cellulosic strands in the matrix of the pectin mucilage are assumed to supply stronger adherence to seeds than simple pectin mucilage (Gutterman *et al.*, 1967; Witzum *et al.*, 1969; Grubert, 1974). The seeds of *F. ericifolia* stayed attached to the soil surface under heavy run-off at a higher rate than that of *H. violaceum*. In addition, the mucilage of *F. ericifolia* seeds cannot be removed from the seed coat but it is easily removed from *H. violaceum* seeds. We propose that the mucilage composed only of pectins, such as that of *H. violaceum*, has not been moulded by the forces of erosion but has sufficient adhesion power to resist erosion events of average intensity. Therefore, we consider that other factors not tested in the present study could influence their phenotypic variation.

The results also show that mother plants of both species exert considerable control over seed size and the amount of mucilage secretion, as indicated by the fitted mixed models. Seed-related traits have often been found to be more strongly controlled at an

individual than at a population level (e.g. with seed output, size, and dormancy). Adaptive and non-adaptive explanations have been proposed to explain this pattern. Intraspecific variability of reproductive traits can help species recruit under variable temporal and spatial environmental conditions, as may be the case for patchy vegetation in semi-arid environments (Aguiar & Sala, 1999; Halpern, 2005; Alados *et al.*, 2010; Baraza, Arroyo & Garcia, 2010; Moreira & Pausas, 2012). Under the erosive conditions that prevail in these environments, a quantitative increment in relative mucilage production may benefit the seeds only if longer or stronger adherence translates to a greater chance of the seeds germinating and establishing themselves under the environmental conditions of the sites where they were primarily dispersed. We did not obtain direct empiric or experimental evidence in the studied populations, although the spatial pattern of seedling recruitment in the *Fumana* species strongly suggests that it is the case. Arnan *et al.* (2010) found that 88% of all new individuals that established themselves over 3 years did so at distances of less than 0.5 m from the adult individuals. In the closely-related phylogenetic species *Fumana thymifolia*, Jump *et al.* (2009) found a very limited seed dispersal within the population after studying the within-population genetic structure using amplified fragment length polymorphism. We cannot exclude the possibility that individual variation in the amount of mucilage secretion may also be the result of differences in resource acquisition among plants as a direct consequence of the spatial heterogeneity of soil-related resources in these environments, which can translate directly or indirectly to the mucilage secretion ability (through seed size).

To consider erosion as an agent of selection, plants under selection need to experience sufficient erosion events that result in different seed survivals. Because rainfall with drop sizes as used in the present study has an approximately 5-year recurrence and *Fumana* species have a mean life span of 15–20 years (Kovács, Kovács-Láng & Babos, 2002), *Fumana* plants should experience three or four events of that magnitude during their lives. By contrast, superficial water flow, with intensities such as that used in the present study, usually occurs only once in the life of the studied plants, and so selection pressure would be weaker than in the case of raindrop impact. In the run-off experiments, we found that the adhesive power of dried mucilage was sufficiently strong to make seeds resistant to high intensity run-off events because all *F. ericifolia* seeds and 66.4% of *H. violaceum* seeds stayed glued until the end of the experiment. Selective pressure towards stronger adherence might only work for very extreme events, although those events have return periods that exceed the life of these plants.

Moreover, during these extreme events, the entire soil may be eroded as a result of the formation of rills and gullies (Poesen *et al.*, 2003) meaning that seeds would be removed together with the eroded soil layer, independently of the relative amount of mucilage produced.

In conclusion, the results of the present study show that mucilage secretion reduces seed removal caused by the erosive mechanisms that operate under natural conditions such as rain drop detachment and removal by water run-off. However, the results also indicate that the adaptive value of mucilage secretion appears to depend on the species and the erosion mechanisms involved. Only in one of the two species that we studied (*F. ericifolia*) did we find that the reduction in seed removal as a result of raindrop impact is proportional to the amount of mucilage secreted, and also that the greater the strength of the erosion processes that plants experience, the more mucilage is produced by their seeds. However, the closely-related species *H. violaceum* did not fit any of the assumptions of our hypothesis and it is possible that, in this species, mucilage secretion has been shaped by selective pressures unrelated to erosion.

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## SUPPORTING INFORMATION

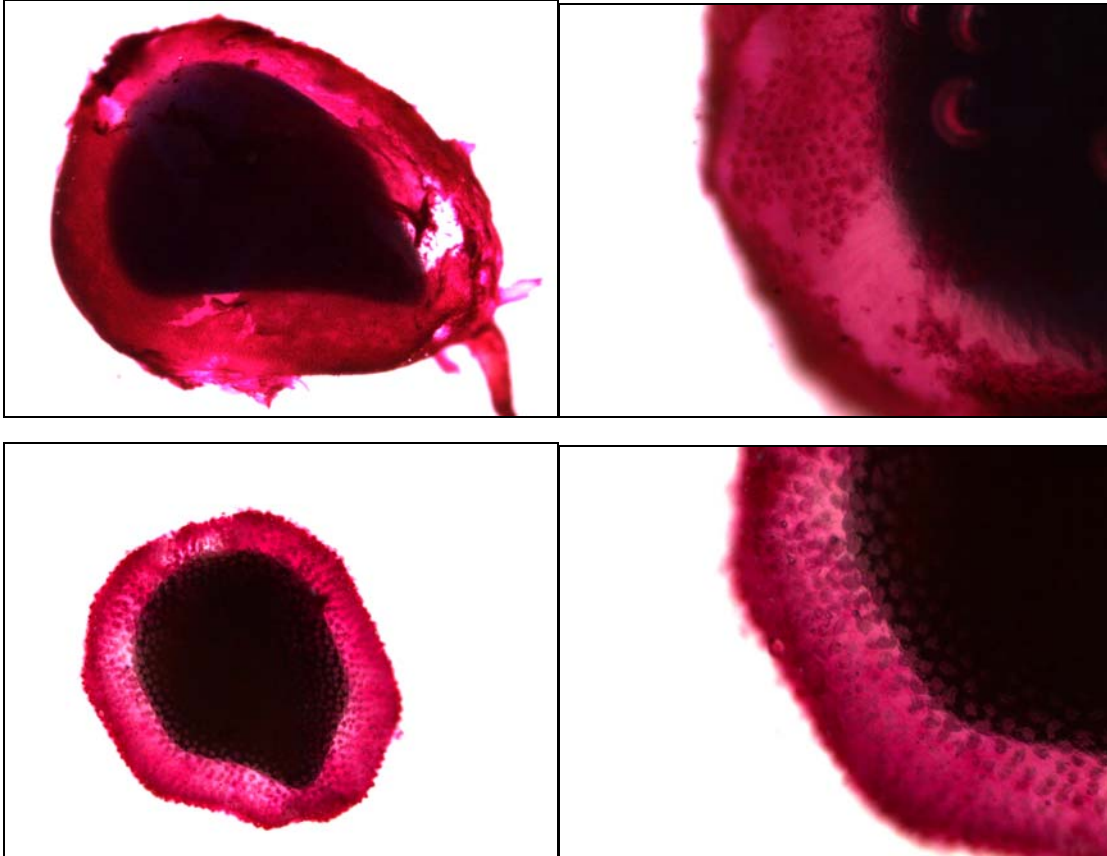
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**Figure S1.** Structure of the mucilage stained with ruthenium red. Intense pink stained polysaccharides are visible in the mucilage layer around the seed. A, *Fumana ericifolia* seed at 1.25 × 80 amplification. B, *Fumana ericifolia* seeds at 6.3 × 80 amplification. C, *Helianthemum violaceum* seeds at 1.5 × 80 amplification. D, *Helianthemum violaceum* at 6.3 × 80 amplification.

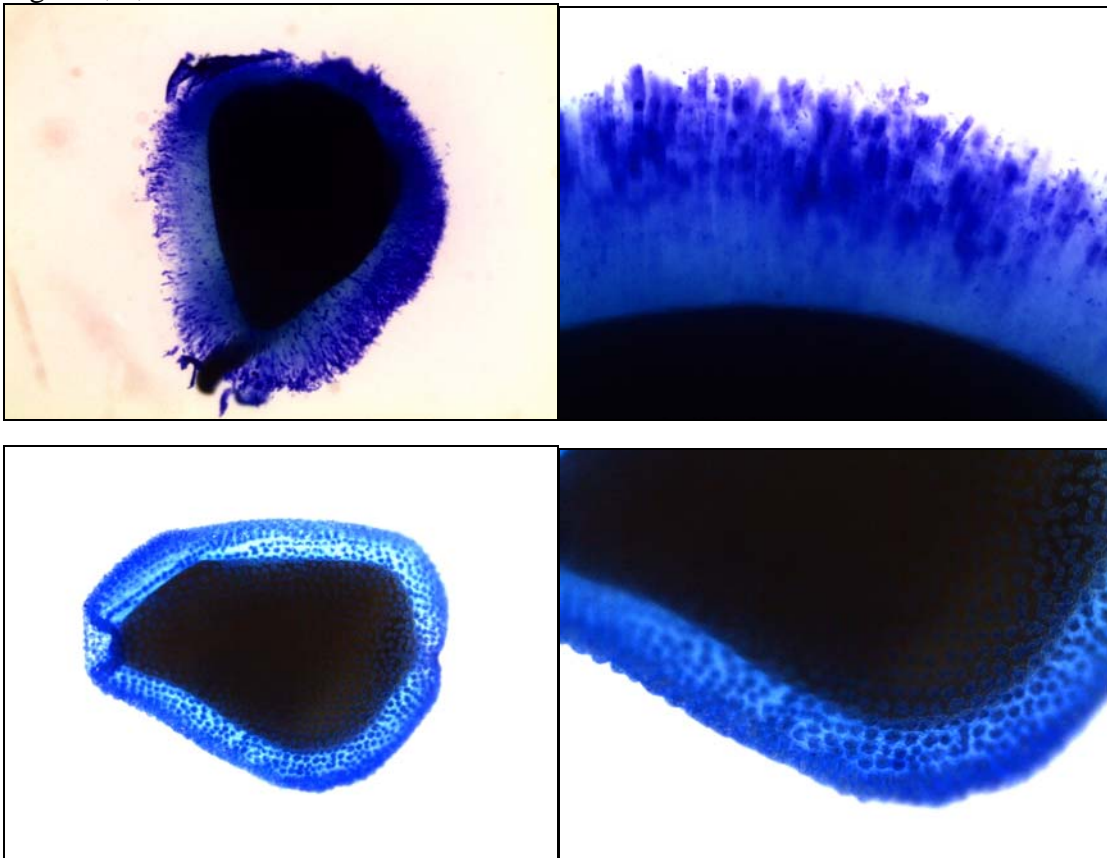
**Figure S2.** Mucilage formation and staining results with methylene blue. The outermost surface is stained blue and cellulose fibrils are stained dark blue. A, *Fumana ericifolia* seed with cellulosic fibrils clearly stained dark blue ( $1.25 \times 80$  amplification). B, *Fumana ericifolia* seed mucilage at  $8 \times 80$  amplification. Thick helicoidal strands of cellulosic fibrils, partially uncoiled, are stained dark blue. C, *Helianthemum violaceum* seed mucilage without cellulose fibrils; only the outer layer of the mucilage is stained blue ( $1.5 \times 80$  amplification). D, *Helianthemum violaceum* without cellulosic mucilage; only the surface is stained blue ( $6.3 \times 80$  amplification).

**Table S1.** Effects of erosive pressure on seed mucilage secretion, seed weight, and seed size derived from generalized linear mixed-effects model with individual plant and populations from which seeds were collected fitted as random effects for both studied plant species.

Figs 1a, b, c & d



Figs 2 a, b, c & d





Supplementary Table 1

Effects of erosion pressure on seed mucilage secretion, seed weight and seed size derived from generalized linear mixed-effects model with individual plant and population from which seeds were collected fitted as random effects for both studied plant species.

*F. ericifolia*

| Relative Mucilage | Random effects           | AIC     | p-value for the tested model |
|-------------------|--------------------------|---------|------------------------------|
|                   | No random effect         | 1151.36 | 1                            |
|                   | Individual               | 903.74  | <0.0005                      |
|                   | Population               | 1104.17 | 1                            |
|                   | Individual in Population | 905.74  | 1                            |
| Seed weight       | No random effect         | -1730.5 | 1                            |
|                   | Individual               | -1819.7 | <0.0005                      |
|                   | Population               | -1730.0 | 1                            |
|                   | Individual in Population | -1817.7 | 1                            |
| Seed size         | No random effect         | 817.47  | 1                            |
|                   | Individual               | 523.24  | <0.0005                      |
|                   | Population               | 756.71  | 1                            |
|                   | Individual in Population | 525.24  | 1                            |

| Fixed effect      | 95% confidence intervals |               |
|-------------------|--------------------------|---------------|
| Erosion pressure  |                          |               |
| Relative Mucilage | -0.5804812               | -0.1874672    |
| Seed weight       | -0.0007930178            | -0.0002128707 |
| Seed size         | -0.1544857               | 0.1289406     |

*H. violaceum*

| Relative Mucilage | Random effects           | AIC      | p-value for the tested model |
|-------------------|--------------------------|----------|------------------------------|
|                   | No random effect         | -920.99  | 1                            |
|                   | Individual               | -1038.47 | p< 0.0005                    |
|                   | Population               | -924.59  | 1                            |
|                   | Individual in Population | -1036.47 | 1                            |
| Seed weight       | No random effect         | -8344.6  | 1                            |
|                   | Individual               | -8499.9  | p< 0.0005                    |
|                   | Population               | -8347.8  | 1                            |
|                   | Individual in Population | -8497.9  | 1                            |
| Seed size         | No random effect         | -417.78  | 1                            |
|                   | Individual               | -683.15  | p< 0.0005                    |
|                   | Population               | -443.34  | 1                            |
|                   | Individual in Population | -681.15  | 1                            |

| Fixed effect      | 95% confidence intervals |               |
|-------------------|--------------------------|---------------|
| Erosion pressure  | Lower                    | Upper         |
| Relative Mucilage | -0.01130278              | 0.04694915    |
| Seed weight       | -0.0001785173            | -0.0001048075 |
| Seed size         | -0.2078965               | -0.1242132    |