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Subcuticular Secretion by Cactus Seeds Improves Germination by Means of Rapid Uptake and Distribution of Water

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We report a formerly unknown mechanism in seeds which improves germination under relatively dry conditions. During seed development of several South American cacti, epidermal cells produce proteinaceous material that appears to pass through ectosedmata in the outer cell wall and which accumulates under the cuticle. Once moistened, this secretory layer readily absorbs water and distributes it over the seed surface. It thus improves water uptake and ensures germination with the minimum amount of available water, which may be advantageous in (semi-)arid regions. In experiments with seeds of *Echinopsis thionantha* (Speg.) Werd. and *Gymnocalycium gibbosum* (Haw.) Pfeiff. under water stress, intact seeds took up significantly more water and germinated better than seeds from which the hydrophilous layer had been artificially removed.

Key words: Cactaceae, seeds, ectosedmata, imbibition, germination.

INTRODUCTION

The germination of seeds is one of the most risky stages in the reproduction of higher plants. Many seeds do not germinate because they are killed by animals or pathogens, or because environmental conditions are—whether or not temporarily—unsuitable for the plant species in question. The dominant factor usually depends on local circumstances.

In deserts and (semi-)arid regions, viable seeds have to overcome another difficulty, viz. the uptake of a sufficient quantity of water. Rainy periods are usually of short duration and the top soil layers quickly desiccate and salt up (Kigel, 1995). Therefore, seeds of desert plant species must not only react rapidly to moisture by making the seed coat permeable so as to leach out any germination-inhibiting substances, but they must also be capable of taking up the water necessary for germination and growth. Therefore, it is not surprising that several different adaptive features to improve water uptake have evolved in many desert plants.

From the literature on this subject (e.g. Harper and Benton, 1966; Harper, Lovell and Moore, 1970; Gutterman, 1994; Kigel, 1995), it is apparent that in most cases better uptake of water is achieved by increased contact between the seed and the soil. Large and globose seeds that are shed on the soil surface make a particularly poor seed-soil contact due to their relatively low surface-to-volume ratio, which hinders the uptake of sufficient amounts of water. Such seeds need to be buried (for example by the action of animals) to enhance the seed-soil contact. Other adaptations in seeds to achieve a better uptake of water include the presence of hygroscopic hairs (Gutterman, Witztum and Evenari, 1967; Mott, 1974), papillae (New and Herriott, 1981) and, most frequently occurring in desert plants, the production of mucilage around the seed (Harper *et al.*, 1970).

Many seeds of desert plant species become slimy soon after they have been moistened, either by rain or by a wet soil. The mucilage is mostly produced by the seed coat and envelops the seed almost entirely. Due to its ability to retain water for some time, it increases the area of the seed that is in contact with water. Moreover, mucilage has been reported to promote seed dispersal and seed establishment, as it glues the seeds to passing animals and to the soil, respectively (Grubert, 1974).

Recently, we found a formerly unknown mechanism in seeds to overcome the problem of water scarcity which has much in common with the mucilage production mentioned above. In a number of genera of cacti, e.g. *Gymnocalycium*, *Echinopsis*, *Matsucana*, *Parodia*, *Pyrrhocactus* [nomenclature according to Hunt (1992)], the ripe seed is surrounded by a paper-like, brownish, often irregularly folded and cracked layer, such as in *Echinopsis thionantha* (Speg.) Werd. (Fig. 1). This layer appears to be hydrophilous; water is readily absorbed and distributed all over the seed grain. It can be peeled off rather easily, revealing (in this particular species) the smooth, black surface of the seed coat (Fig. 2). This feature occurs almost exclusively in genera that belong to the tribe Notocacteae Buxbaum, and is possibly indicative of a common descent of these taxa. Their distribution is restricted to South America, predominantly in the seasonally dry regions of Peru, Bolivia, Argentina and Chile.

The nature and function of this epidermal layer has long been controversial in that different terms are still being used for it: Buxbaum (1958) and Leuenberger (1974) named it ‘aril’, and Buxbaum (1977) the ‘third integument’. Barthlott and Ehler (1977) described it as a granular substance between the seed coat and the cuticle, probably consisting of...
pectin-like compounds. Rather than trying to unravel the chemistry of this layer, we focused our attention on its mode of development and its probable biological significance.

MATERIALS AND METHODS

Plant material

Fruits and seeds of Gymnocalycium gibbosum (Haw.) Pfeiff., a globose cactus from the Monte desert in East Argentina, were taken from field-collected specimens in cultivation under field number P 94. Fruits of Rebutia steinbachii Werd., a clustering dwarf species from the eastern Andes of Bolivia, were taken from cultivated specimens raised from commercially available seed distributed as Sulcorebutia camacho i.n.n. (field number KK 1801). Fruits of Matucana paucicostata Ritter and M. intertexta Ritter, both globose species from the northern Peruvian Andes, were obtained from cultivated specimens raised from habitat-collected seed in the first author’s collection. Seeds of Echinopsis thionantha (Speg.) Werd., a globose species from northern Argentina, were obtained from habitat-collected specimens. These seeds are being sold as Acanthocalycium glaucum P 143.

Seed coat development

The development of the outer seed layer was monitored in Gymnocalycium gibbosum, Matucana paucicostata and Rebutia steinbachii. Immature fruits were harvested 1, 2, 3 and 4 weeks after pollination. After fixation in FPA, the fruits were dehydrated by a series of n-butanol/water mixtures to be imbedded in metacrylate plastic. The fruits were subsequently sectioned at 7 and 15 µm and mounted on glass slides. The 7-µm slides were stained with toluidin blue and Schiff’s reagent to serve as permanent slides, whereas the 15-µm slides were left unstained and used for qualitative histochemical tests (including cellulose, pectin, tannin, proteins, lipids, polyphenols) as described by Gahan (1984).

For SEM studies immature and mature seeds were photographed under a ISI-300 scanning electron microscope at 9 kV. In order to improve resolution, the backscatter technique (ISI-Robinson RBSE 130R detector) at 19 kV was used. The seeds were prepared in different ways: fresh, fixed in FPA or fixed in FPA followed by dehydration in an acetone series and tetramethylsilane. Finally, the seeds were mounted on double-adhesive tape and gold-sputtered.

An immature fruit of Gymnocalycium gibbosum was used in a transmission electron microscopic study. After fixation with 2.5% glutaraldehyde, post-fixation with 1% osmium tetroxide, dehydration with ethanol and embedding in LRWhite, ultra-thin sections were observed with a Philips EM 300 instrument.

For mass spectrometric analysis of the secretory material, approx. 30 seeds of Echinopsis thionantha were stripped by carefully peeling off the outer seed layer with a clean razor. In doing so, the cuticle and the underlying secretory layer are removed together, without any possibility of separating these two constituents. The peeled-off material was examined with ion-source pyrolysis mass spectrometry in a JEOL JMS-SX 102 mass spectrometer. Both chemical (ammonia) and electron impact ionization were applied. Curie-point pyrolysis mass spectrometry under EI conditions was conducted in a HRGC MEGA 2 series FISON Instruments gas chromatograph connected to a JEOL DX-303 double focusing mass spectrometer. Further mass spectrometry details are given by Graven et al. (1996).
Imbibition and germination

Imbibition and germination experiments were conducted with fresh seeds of *Echinopsis thionantha*, *Gymnocalycium gibbosum*, *Matucana paucicostata* and *Matucana intertexta*. Both stripped and intact seeds of *Echinopsis thionantha* and *Gymnocalycium gibbosum* were subjected to imbibition tests. Imbibition was estimated by soaking a variable number of
seeds (usually 20 or 25) in water, taking the seeds out after a certain period of time, allowing the seeds to dry on filter paper and to evaporate external water for exactly 10 min and weighing the seeds individually or in portions of 10 seeds on an M 500 P Sartorius micro-balance. Tests were also carried out to determine whether the seeds were capable of taking up water through the testa by blocking the hilum-micropylar zone with a drop of nail polish.

Germination experiments were carried out as follows: samples of ten fresh intact or stripped seeds were sown on the surface of 7 cm plastic pots filled with fine (particle size < 1 mm) or coarse sand (particles between 1 and 2 mm). The pots were placed in a box filled with water, the level of which was kept 1 cm below the edge of the pots. The seeds were sprayed with water every 2 d. During the day (0600–1800 h) the temperature was kept at approx. 25 °C and extra lamp light was given from above, whereas during the night (1800–0600 h) the temperature was 15–20 °C without additional light. The germination results of seeds from the same source under optimal conditions (on moist filter paper in petri dishes, temperature and light regimes similar) were taken as controls; these were found to be around 90%.

RESULTS

Outer seed layer development

In all three species studied (Gymnocalycium gibbosum, Matucana paucicostata and Rebutia steinbachii) the formation of the outer seed layer proved to proceed in a very similar way. Only the speed of seed development may vary, not only between different species but also within a single species, depending on external circumstances. The development of the outer seed layer was most clearly observable in the Gymnocalycium gibbosum slides so the results described below refer to this species.

Two weeks after pollination, a mass of granular bodies was observed packed inside the lumina of the epidermal cells (Fig. 3). At this stage the secondary thickening of the outer cell wall had just begun. Moreover, a reticulate network of channels known as ectodesmata (Frey-Wyssling, 1976) became visible, through which the granular particles were transported from the inside to the outside of the cells of the seed coat, only to be covered by the cuticle (Fig. 4). These particles were membraneless, as was shown by transmission electron microscopy. Locally, on the seed surface, the number of particles apparently became so high that vesicles were formed (Fig. 5). A week later the outer cell wall had considerably thickened and the secretion process had come to an end (Figs 6 and 7). Four weeks after pollination, the particles had somehow fused and seemed to have ‘melted’, because the original granular structure could no longer be discerned. The cuticle had also sunk in, together with the particulate layer underneath it (Fig. 8). Accordingly, the irregular folding pattern of the surface of the ripe seed of Gymnocalycium gibbosum begins to form approximately in the fourth week of development.

Considering the chemical composition of the secretory layer, the qualitative histochemical tests did not yield good results. For example, two different protein reagents gave

![Fig. 9. Pyrolysis (EI) mass spectrum of the secretory layer (including the cuticle) of Echinopsis thionantha showing proteins (arrowheads), tannin derivatives (t), fatty acids (●) and some β-sitosterol (◇).](image-url)
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Fig. 10. Germination of both intact and stripped seeds of *Echinopsis thionantha* and *Gymnocalycium gibbosum* under controlled conditions of water scarcity (see text). *E. thionantha* on fine (A) and coarse sand (B). *G. gibbosum* on fine (C) and coarse sand (D).

different reactions: the Coomassie Blue reagent gave a positive reaction, nitrosine a negative one. Most tests on both cellulose and polyphenols were negative.

Mass spectrometric analysis of the outer seed layer revealed the presence of mainly proteins. In the pyrolysis electron impact mass spectrum (Fig. 9) the peaks at m/z 69, 81, 91, 94, 100, 117, 135, 149, 154, 167, 194 and 279 are considered to represent proteinaceous pyrolysis fragments (Niemann et al., 1992; Boon pers. comm.). Mass peaks for phenolic compounds were also observed (m/z 110, 124), which may indicate the presence of vegetable polyphenols such as tannins (Graven et al., 1996). The presence of all these compounds was confirmed by pyrolysis gas chromatography mass spectrometry. Peaks that indicate fatty acids (m/z 252, C_{16-2}; 256, C_{16-0}; 264, C_{18-1}) and β-sitosterol (m/z 397–415) were also found in the spectrum, and are probably due to cuticular remains. It was remarkable that there was no evidence suggesting the presence of carbohydrates and pectin.

**Imbibition and germination**

The biological significance of the secreted material was demonstrated in imbibition and germination tests with seeds of *Echinopsis thionantha*, *Gymnocalycium gibbosum* and *Matucana intertexta*. In all species the results were very similar.

In free water the seeds of the species studied took up water very quickly: within 24 h all seeds had become almost fully imbibed. The average increase in fresh weight varied from 29 to 44%, depending on the species. The seeds were capable of imbibing water through the seed coat: seeds whose hilum had been blocked took up water as quickly as untreated seeds.

In a subsequent experiment, the imbibition of both stripped and intact seeds of *Echinopsis thionantha* was estimated. The stripped *Echinopsis* seeds took up significantly less water than the untreated ones (Student’s *t* test = 2·992, d.f. = 38, *P* < 0·005), averaging 23·7 and 29·3%, respectively. On the other hand, when fully imbibed seeds were allowed to desiccate, both seed types reacted in the same way: stripped seeds and intact seeds reached their original dry weight in 80 and 81 min respectively.

The ability of the secretory layer to absorb additional water was mirrored by the germination responses of the seeds. When seeds of *Echinopsis thionantha* were sown on moist fine sand, only 10% germinated; stripped seeds did not germinate at all (*χ^2* = 0·55, d.f. = 1, *P* > 0·30). If seed-soil contact was improved by pressing the seeds down in the
sand, most seeds of both types did germinate [60% of the stripped seeds and 70% of the intact seeds ($\chi^2 = 0.22$, d.f. = 1, $P > 0.50$)]. However when seeds lying on fine sand were watered all over by spraying them every 2 d differences between intact and stripped seeds were conspicuous (Fig. 10A). In this case 65% of the intact seeds germinated, compared to only 20% of stripped seeds ($\chi^2 = 8.491$, d.f. = 3, $P \leq 0.05$).

Germination of seeds of the same species in moist coarse sand yielded different results with 55% of the intact and compared to only 20% of stripped seeds ($\chi^2 = 0.491$, d.f. = 3, $P \leq 0.50$). However when seeds lying on fine sand were watered all over by spraying them every 2 d differences between intact and stripped seeds were conspicuous (Fig. 10B; $\chi^2 = 0.800$, d.f. = 3, $P \geq 0.80$).

Similar experiments with seeds of Gymnocalycium gibbosum yielded comparable results (Fig. 10C and D). Thus for intact and stripped seeds of this species, sown on moist fine sand and sprayed every 2 d, germination percentages of 89 and 30%, respectively, were recorded ($\chi^2 = 6.739$, d.f. = 1, $P \leq 0.01$). On coarse sand germination was 75 and 55%, respectively ($\chi^2 = 0.701$, d.f. = 1, $P \geq 0.30$).

**DISCUSSION**

Hydrophilous properties in cuticles are commonly ascribed to pectin (Barthlott and Ehler, 1977; Lyshede, 1978), which usually occurs in cuticles in large quantities (Martin and Juniper, 1970; Lyshede, 1982). However, the present mass spectrometric data indicates that the hydrophilous nature of the secretory layer examined here must be ascribed to proteins rather than to pectin.

As the cuticle in the ripe seed of the species studied is irregularly folded and cracked (Fig. 1), water can easily penetrate underneath the cuticle. The secretory layer of the seeds studied then serves to improve both water uptake and germination. Compared to stripped seeds, the secretory layer of the intact seeds not only enables the seed to take up more water, but also it appears to retain the water long enough for it to be absorbed by the seed. This becomes particularly clear in the germination experiments with the seeds lying on fine sand. Apparently, in the stripped seeds, the poor seed-soil contact prevents the uptake of sufficient water from the substrate. Furthermore, most of the sprayed water had probably evaporated before it could be absorbed. In such conditions the hydrophilous layer around the intact seed ensures better water uptake and thus better germination. Recent experiments have shown that fully imbibed (both intact and stripped) seeds of Echinopsis thionantha lose their water equally fast, so the absence of the cuticle, which may influence the rate of water loss, does not account for the observed difference in germination.

The advantageous effect of the secretory layer was lost in coarse sand as the seeds had the opportunity to sink in between the larger sand particles resulting in better contact with the substrate.

Our germination tests have shown that for seeds under water stress the secretory layer substantially improves germination, especially for seeds lying on the surface of a fine sandy or loamy soil which suffer from poor contact with the soil. Indeed, in the wild many species producing seeds with a secretory layer are to be found on loamy soils, where water deficiency during germination is likely to occur. In many of these species seed dispersal takes place by runoff water (Bregman, 1988), which often deposits seeds on a loamy substrate. Another factor which could play a role in the evolution of the secretory layer is a very low amount of precipitation. In extreme arid regions, such as the coastal desert of N. Chile and S. Peru, there is often so little rain, even in the ‘wet’ season, that a more efficient utilization of the little water available is essential for seeds to germinate. The occurrence, in such areas, of a considerable number of species of Cactaceae producing seeds with a secretory layer is probably not accidental.

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**LITERATURE CITED**


