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Short soaking in water inhibits germination of *Datura ferox* L. and *D. stramonium* L. seeds

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Summary: Résumé: Zusammenfassung

Seed coat structures may play a major role in determining the germination of seeds. The involvement of seed coat characteristics in the inhibition of germination observed when dry seeds of *Datura ferox* L. and *D. stramonium* L., were rinsed or soaked in water for 1–5 min ('soaking effect') was investigated. Germination was inhibited by soaking *D. ferox* seeds even at late stages of the germination process up to one day before root protrusion. Simulation of the 'soaking effect' was achieved by blocking the hilum with lanolin, enamel lacquer or agar during imbibition. Blocking the hilum did not prevent water uptake by the seeds. Seed coat integrity was necessary for the 'soaking effect' since germination inhibition was eliminated by removing or cracking the seed coat. Drying the seeds up to 12 h after soaking reversed the inhibitory effect. SEM observations of both species revealed a loose outer parenchymatous tissue covering the hilum, an inner spongy tissue between the nucellus and the hilum and a space between the seed coat and the nucellus. We concluded that during soaking, water is trapped in the space between the seed coat and the nucellus, and in the intracellular spaces of the spongy tissue within the hilum. This water 'plug' may limit the diffusion of gas(es) to and from the embryo through the hilum, thus inhibiting germination.

Un court trempage dans l'eau inhibe la germination des graines de Datura ferox *L. et* Datura stramonium *L.*

La structure de l'enveloppe des graines peut jouer un rôle majeur dans la détermination de la germination des graines. On a étudié l'influence des caractéristiques de l'enveloppe des graines sur l'inhibition de la germination observée quand des graines sèches de *Datura ferox* L. et *D. stramonium* L. ont été rincées ou trempées dans l'eau pendant 1 à 5 minutes ('effet trempage'). La germination est inhibée par trempage chez les graines de *D. ferox* même aux stades tardifs du processus de germination jusqu'à un jour avant la saillie racinaire. La simulation de 'l'effet trempage' est obtenue par blocage du hile avec de la lanoline, de la laque vernis ou de l'agar pendant l'inhibition. Le blocage du hile n'empêche pas l'absorption d'eau par les graines. L'intégrité de l'enveloppe est nécessaire pour 'effet trempage' tandis que l'inhibition de la germination est éliminée par épluchage ou scarification. Un séchage de 12 h après le trempage supprime l'effet inhibiteur. Une observation chez les deux espèces a montré un flottement à l'extérieur du tissu parenhymateux couvrant le hile, un tissu intérieur spongieux entre la nucelle et le hile et un espace entre le tégument et le nucelle. Nous en concluons que pendant le trempage, l'eau est prise dans l'espace entre le tégument et le nucelle et dans les espaces intracellulaires du tissu spongieux à l'intérieur du hile. Ce 'tampon' aqueux doit limiter la diffusion du (des) gaz vers et depuis l'embryon à travers le hile, et ainsi empêcher la germination.

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Kurzzeitiges Quellen in Wasser verhindert die Keimung der Samen von Datura ferox *L. und* D. stramonium *L.*

Die Struktur der Samenschale kann eine erhebliche Rolle bei der Keimung spielen. Dieses Phänomen wurde an trockenen Samen von *Datura ferox* L. und *D. stramonium* L., die zwischen 1 und 5 Min. in Wasser quellen konnten ('Quell-Effekt'), untersucht. Die Keimung von *Datura-ferox*-Samen wurde sogar verhindert, wenn sie in späten Stadien des Keimungsprozesses bis zu 1 Tag vor dem Durchburch der Wurzel einer Quellung in Wasser ausgesetzt wurden. Eine Blockierung des Hilums mit Lanolin, Emaille-Lack und Agar-Agar während der Quellung verhinderten den 'Quell-Effekt' nicht. Für diesen Effekt war die vollständige Samenschale notwendig, denn die Verhinderung der Keimung wurde durch Entfernen oder Brechen der Samenschale aufgehoben. Raster-elektronenmikroskopische Untersuchungen zeigten bei beiden Arten ein lockeres parenchymatisches Außengewebe, welches das Hilum bedeckt, ein schwammiges Innengewebe zwischen dem Nucellus und dem Hilum und einen Zwischenraum zwischen der Samenschale und dem Nucellus. Es wird angenommen, daß während der Quellung (in überschüssigem Wasser) in diesen Zwischenraum und das schwammige Hilumgewebe Wasser eindringt. Dieser 'Wasserpfropfen' kann die Diffusion von Gasen in oder aus dem Embryo durch das Hilum einschränken und so die Keimung verhindern.

Introduction

Datura ferox and *D. stramonium* are noxious weeds that thrive in irrigated summer crops. They have a persistent soil seedbank due to prolonged dormancy and viability of the seeds (Toole, 1946; Adamoly, Goldberg & Soriano, 1974). In this weed species, the seed coat plays a major role in the regulation of dormancy and germination (Soriano, Sanchez & deEilberg, 1964; Sanchez, Soriano & Slabnik, 1967; Burkart & Sanchez, 1969; deMiguel & Soriano, 1974; Guglida, Soriano & Burkart, 1976; Monagham & Felton, 1979; deMiguel, 1980).

Restricted permeability of the seed coats to gases and water may inhibit seed germination in many species (Taylorson & Hendricks, 1977; Werker, 1980/81). This impermeability is mostly due to the deposition of hydrophobic substances on the cell walls of the seed coat, and to the blockage of the hilum, the micropyle, and the chalaza (Werker 1980–81). In other species the seed coat is water permeable but impermeable to gases. In several plant species, the seed becomes surrounded with a thick mucilage layer during imbibition (Grubert, 1974), that may restrict gas exchange as in *Hirschfeldia incana* (Negbi, Rushkin & Koller, 1966) and in *Blepharis persica* (Witztum, Gutterman & Evenari, 1969). In *Sinapis arvensis* limited oxygen supply to the embryo elicited the synthesis of cell elongation inhibitors (Edwards, 1969). In *Xanthium pennsylvanicum* lack of oxygen may reduce the oxidation of germination inhibitors (Porter & Wareing, 1974). Moreover, the moistened seed coat may limit the leaching of inhibitors from the embryo, causing further inhibition of germination (Porter & Wareing, 1974). On the other hand, hypoxia or anoxia caused by flooding or prolonged soaking of seeds may result in irreversible injury due to accumulation of toxic metabolites (Norton, 1986).

In the present study we describe a phenomenon in which germination of *D. ferox* and *D. stramonium* is inhibited by rinsing dry seeds or by soaking them in water for very short time periods (1–5 min). The role of seed coat structures in the inhibition of germination by soaking in water was examined in this species.

Materials and Methods

Ripe fruits of *Datura ferox* L. and *D. stramonium* L. were harvested in cotton fields near Palmachim and Kvutzat Yavne (Coastal Plain of Israel), during November 1984. Mature (black) seeds were stored in open containers at room temperature.

Seeds were germinated in 9-cm glass Petri-dishes on Whatman filter paper (No. 1) and 7 ml of deionized water (pH 7) unless otherwise mentioned. The dishes were covered with two layers of polyethylene to reduce evaporation and placed in a continuously illuminated chamber with a thermoperiod of 30/20°C (12/12 h). Light sources were fluorescent lamps (Cool White, Sylvannia), providing 4×10^3 erg cm^2 s^{-1} at the Petri-dish level. The duration of each experiment was 10 days. In some experiments seeds were germinated on 1% agar (Bacto-Agar, Difco) in similar Petri-dishes.

Soaking of seeds was performed in deionized water (pH 7) at room temperature. Immediately after soaking, the seeds were germinated as described above. Drying the soaked seeds was carried out under an air stream at room temperature for at least 15 min. Dehydration was terminated when the seeds reached their initial water content (6%). Seed coat cracking was carried out using a blender operated at full speed for short pulses. Seed coats were removed after cracking. The treated seeds were examined under a light microscope before use in order to assure that they were not damaged.

Electron scanning microscopy (SEM) observations were made with Jeol JSN 35C-SEM at 25 KV acceleration voltage. Seeds and seed sections were gradually fixed by transferring them to ethanolic solutions of increasing concentrations, and dried for 2 h in CO_2 enriched atmosphere using a Critical Point Drier (Druva). The seeds were then coated with gold, studied with the SEM, and representative photographs were taken.

Experiments were conducted at least two times in a completely randomized design, with 4–8 replicates, 25 seeds in each Petri-dish. Germination percentages are the average of the total sum of replicates. Data were analysed using the Duncan multiple-range test. A multiple non-linear regression analysis was used when necessary. Germination percentages were arc sine transformed before statistical analysis, and tabulated as the original data.

Results

'Soaking effect'

Pre-treatment of dry *D. ferox* and *D. stramonium* seeds by rinsing them in running water for 30 min, or by soaking them in water for 1 or 5 min resulted in a complete inhibition of their germination as compared to untreated seeds (0 *vs* 80 ± 3% and 0 *vs* 90 ± 2% for *D. ferox* and *D. stramonium*, respectively). Moreover, no germination was observed even if the seeds were soaked for 1–5 min following 24 or 72 h of imbibition on filter paper. Thus, soaking was inhibitory even at late stages of germination up to one day before root emergence.

A rapid influx of water into the seed during initial stages of imbibition has been reported to inhibit seed germination in several species (Simon & Raja, 1972; Khan, Varade & Pal, 1982). In *D. ferox* the amount of water taken up by the seeds during the first 5 min of soaking, was similar to that absorbed during the same period of time by seeds laid on wet filter paper (Table 1). However, the amount of water absorbed was higher in the soaked seed after 30 and 60 min. As the inhibitory 'soaking effect' was evident even after 1 min of soaking, it is clear that a fast initial water uptake is not the factor involved in germination inhibition of *D. ferox*.

Germination of *D. ferox* seeds laid with their flat face on moist filter paper, was greatly influenced by the water level in the Petri-dish (Fig. 1). As the water level was raised and a greater part of the hilum opening was covered with water, the germination was inhibited further. Germination was maximal (77%) when the water volume was 6 to 7 ml, and only one third of the seed height was covered with water, without reaching the hilum. About 25% inhibition was observed when 11 ml of water were added resulting in a thin layer of water on the hilum opening. Germination was

Table 1 Water uptake by *Datura ferox* seeds soaked in water or imbibed on moisten filter paper at 20°C

Time (min)	Weight increase (%) Imbibition	Soaking
5	6	6
30	7	14*
60	9	20*

* Significant differences ($P < 0{\cdot}05$) between soaking and imbibition at the same time.

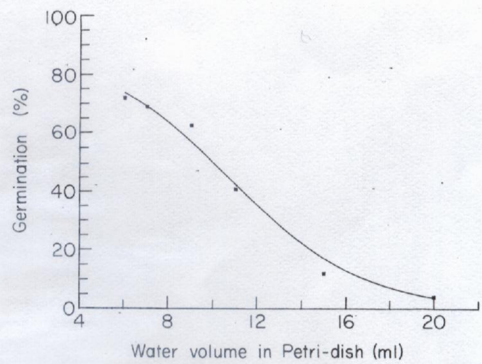

Fig. 1 Effect of water volume in Petri-dish (9 cm) on the germination of *D. ferox* seeds. Data were fitted according to the logistic equation: $Y = a/1 + b^{*}e^{-kX}$ (a = 89, b = 0·027, k = −0·33) ($P < 0{\cdot}05$); $r^2 = 0{\cdot}987$.

Table 2 Effect of blocking the hilum on the germination of *D. ferox* seeds

Treatment	Germination (%)
Hilum facing down, immersed in agar	0 d*
Hilum facing up above the agar	71 b
Seed laid on its flat face	88 a
Hilum blocked with lanolin	0 d
Seed coat covered with lanolin (except the hilum)	53 c

* Numbers followed by a similar letter are not significantly different at $P<0{\cdot}05$ according to Duncan's multiple-range test.

Table 3 Effect of removing or cracking the seed coat on the germination of soaked *D. ferox* seeds

Treatment	Germination (%)
Soaking intact seeds	0 c*
Soaking seeds—seed coat removed	56 b
Soaking seeds—seed coat cracked	61 b
Soaking intact seeds, the seed coat cracked after soaking before germinating	87 a
No soaking—seed coat removed	88 a
No soaking—intact seeds	83 a

* Numbers followed by a similar letter are not significantly different at $P<0{\cdot}05$ according to Duncan's multiple-range test.

strongly inhibited when the hilum was fully submerged following the addition of 20 ml water.

In order to study the role of the hilum in the germination process of *D. ferox*, four treatments were examined as compared to the standard germination procedure:

(a) Seeds were germinated while their narrow side was immersed in agar and the hilum faced up above the agar;

(b) as above, but with the hilum immersed in the agar, facing down;

(c) the seeds were laid on their flat face on moist filter paper with the hilum blocked with lanolin;

(d) with the hilum unblocked, but the rest of the seed coat covered with lanolin.

Similar to the 'soaking effect', blocking the hilum with agar or with lanolin resulted in a complete inhibition of germination (Table 2), even though, the rate of water uptake was similar in all treatments (data not shown). Thus, the seed coat of *D. ferox* does not impair water uptake.

Elimination of the 'soaking effect' in D. ferox

Protection of the hilum from rapid hydration by covering it with enamel lacquer prior to soaking, and removing it just before laying the seeds on wet filter paper for germination, prevented the inhibition induced by soaking. This treatment resulted in 51±7% germination. The observed lower germination, compared to 75% of the untreated control, can be attributed to the toxic effect of the enamel lacquer, since covering other regions of the seed with a point of enamel lacquer caused a similar decrease in germination.

Removing or cracking the seed coat before soaking, eliminated the 'soaking effect' (Table 3). Cracking the seed coat following the soaking treatment, also eliminated the effect. Therefore,

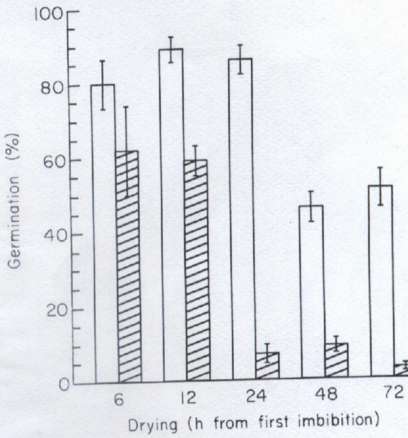

Fig. 2 Effect of drying at different times after the start of imbibition on the germination of *D. ferox* seeds pretreated with soaking in water for 5 min. The seeds were dried to their initial water content (6%). After drying seeds were reimbibed. (■ soaked; □ control).

seed coat integrity seems to be necessary for the inhibition of germination caused by soaking.

Drying the soaked seeds can eliminate the soaking effect. Soaked seeds were imbibed on wet filter paper for various periods of time, subjected to drying and then reimbibed. Dehydration of soaked seeds was effective only if carried out during the first 12 h of imbibition after soaking. Later on, the inhibition was irreversible (Fig. 2). On the other hand, dehydration of unsoaked seeds that were imbibed on moist filter paper up to 24 h (control), did not affect germination. Moreover, even if the dehydration was carried out 24 h before root protrusion, 50% of the seed population were still able to germinate.

Anatomical structure of D. ferox *and* D. stramonium *seeds*

The anatomical structure of the hilum was stu-

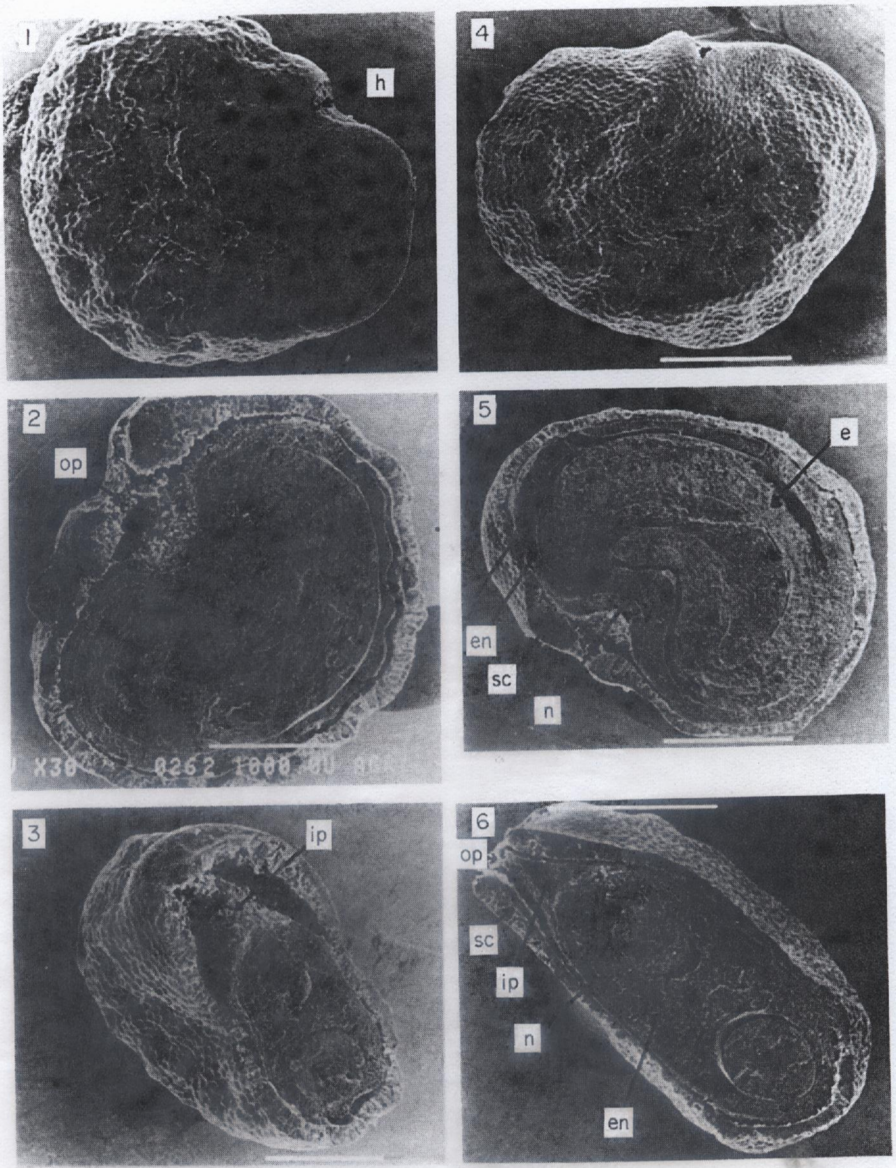

Fig. 3 Scanning electronic micrographs of *D. ferox* (1, 2, 3) and *D. stramonium* (4, 5, 6) seeds: 1, 4 = Whole seed; 2, 5 = Longitudinal section; 3, 6 = Cross section through the hilum. Note the space between the seed coat and the nucellus. h = hilum; op = outer parenchymatous tissue; n = nucellus; en = endosperm; sc = seed coat; ip = inner parenchymatous 'plug'; e = embryo. Bar represents 1 mm.

died using SEM. Two well defined tissues were observed in longitudinal and transverse sections of the hilum. The outer one which appears to be part of the seed coat, is a very loose parenchymatous tissue covering the hilum (Fig. 3-2 and 3-6). The inner tissue, has a spongy texture which creates a 'plug' in the hilum space, and appears to be part of the nucellus that covers the endosperm and the embryo (Fig. 3-3 and 3-6). In addition, a clear space between the seed coat and the nucellus envelope was evident.

Discussion

The poor seed germination as a result of inhibition induced by soaking seeds for a short period of time ('soaking effect'), is completely different from the well known injury induced in many species by soaking their seeds in water for long periods (Norton, 1986; Crawford, 1977). First, as germination is inhibited even by very short period of soaking in water, one may conclude that oxygen deficiency during soaking itself is not involved in the inhibition of germination. Moreover, unlike long-term soaking that results in an irreversible decline of germination, the inhibition described in this study is reversible, and the seed remain viable.

The response of *Calligonum comosum* fruits to soaking (Koller, 1956), is similar to the 'soaking effect' observed in *Datura* seeds. Their germination is inhibited following half an hour of submersion and this effect is reversed by subsequently drying of the fruits. Koller (1956) suggested that during rinsing water may block air inlet into the embryo or that compounds essential for germination are leached out the fruits. In *D. ferox* however, such a leaching could not explain the observed inhibition, since soaking of seeds whose seed coats was removed or cracked did not inhibit germination (Table 3).

Our results indicate that the seed coat is an effective barrier for gas penetration, and the hilum opening serves as an avenue for gas diffusion into the seed. It is possible that during soaking, a layer of water is trapped in the space between the seed coat and the nucellus. The water may also fill the intracellular spaces of the inner spongy tissue within the hilum. This 'plug' of water may limit the diffusion of gas(es) to and from the embryo, thus inhibiting germination.

Cracking or removing the seed coat of *D. ferox* before or immediately following soaking, prevents the detrimental effect of the water 'plug', allowing gas(es) diffusion to and from the embryo. When intact seeds are imbibed on filter paper and the hilum is not immersed in water, the creation of the water plug in the spongy tissue within the hilum is prevented. Thus, water may penetrate through the seed coat while an open route to gas(es) diffusion through the hilum is maintained (Table 2). Light microscope observations showed that following soaking, the spongy tissue within the hilum is saturated with water. This was not observed following imbibition on filter paper (data not shown).

Seeds of both, *D. ferox* and *D. stramonium* are dormant when the fruit ripens and they emerge gradually from dormancy with time (Soriano *et al.*, 1964; Stoller & Wax, 1974). It is known that secondary dormancy can be induced under stress conditions (Karssen, 1980/81; Bewley & Black, 1982; Côme & Thevenot, 1982). As the soaked seeds are viable but do not germinate under optimal conditions, we assume that secondary dormancy is induced. Restricted oxygen diffusion may prevent the oxidation of the endogenous inhibitors reported to be present in *D. ferox* seeds (Soriano *et al.*, 1964; deMiguel, 1980), or may promote the synthesis of others. Burkart & Sanchez, (1969) have found that the level of inhibitors is increased during the first 24 h of imbibition. Thus, the fact that the 'soaking effect' can be cancelled by drying seeds during the first 12 h of imbibition (Fig. 2), might be explained by low levels of inhibitors accumulated during this period.

During the imbibition of seeds, we observed that dark coloured compounds were released from the seed coat. The presence of dark coloured phenols in the seed coat is known to reduce the amount of oxygen that reaches the embryo (Côme & Tissaoui, 1973). Restriction of gas diffusion due to seed soaking combined with the presence of phenols in the seed coats may lead to increased internal hypoxia in the seed. In turn, this hypoxia may cause the synthesis of inhibitors or may limit the oxidation of inhibitors already present in the seed, resulting in inhibition of germination.

Inhibition of germination in submerged seeds without impairment of viability may be considered as a protective adaptation in species growing in habitats in which flooding is a frequent event. Further research is needed in order to determine if the 'soaking effect' is a common phenomenon among other species of *Datura*, or whether it is a trait characteristic to those species (or populations) growing as weeds in irrigated crop fields.

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