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Changes in the Endosperm Cell Walls of Two *Datura* Species before Radicle Protrusion

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ABSTRACT

The possibility of an association between changes in cell walls of the micropylar portion of the endosperm and the induction of germination was explored in seeds of *Datura ferox* and *Datura stramonium*. The structure of the inner surface of the endosperm was studied by scanning electron microscopy and the composition of cell wall polysaccharides analyzed by gas chromatography and gas chromatography-mass spectrometry. Both scanning electron microscope images and chemical analysis showed changes in the micropylar portion of the endosperm in induced seeds before radicle protrusion. The inner surface of the endosperm appeared eroded, and in some areas, wall material seemed to be missing. The content of the main component of the cell wall polysaccharides, containing predominantly 4-linked mannose, decreased well before the emergence of the radicle through the endosperm. We propose that the degradation of a mannan type polysaccharide is an important factor in the reduction in mechanical strength of the endosperm, thus facilitating germination.

The seeds of *Datura ferox* are deeply dormant at ripening (21, 25). After partial reduction of the innate dormancy by after-ripening germination can be induced by light, through the phytochrome system (7, 22, 25). In this species, dormancy is imposed by the tissues surrounding the embryo (22). One of the important roles of the enveloping tissues in seeds with 'coat-imposed' dormancy is presentation of a physical barrier restricting embryo growth (3).

In these cases, induction of germination by light requires overcoming this physical constraint and could involve either an increase in the expansion force of the embryo, a reduction in the mechanical strength of the enveloping tissues, or both. Generally, larger embryo growth potential has been observed after induction of germination in seeds with this type of dormancy (9, 16, 27). On the other hand, changes in the mechanical strength of the covering tissues have also been suggested to be associated with germination (14, 19). In *D. ferox* R[2] can induce both an increase in growth potential of the embryos and a softening of the endosperm (the main physical barrier in this species) and both of these changes occur several hours prior to radicle emergence (22, 23). Endosperm softening has been observed in other species (10, 29). In lettuce, a decrease in endosperm mechanical resistance has been shown (28) but doubts have been raised about its significance (3). In pepper, however, endosperm softening clearly precedes radicle protrusion and is well correlated with the promotive effects of temperature and gibberellin on germination (29). The use of gibberellin-deficient mutants in tomato has also led to the demonstration that gibberellin promotion of germination involves endosperm softening (10). The nature of the changes leading to endosperm softening is not completely understood. Psaras *et al.* (20) have shown pregerminative ultrastructural changes in lettuce seeds which these authors suggest may be related to a decrease in mechanical strength. In this system there is no agreement about the possibility of modifications in the cell walls (15, 20). Although the demonstrated decrease in resistance to puncturing of the pepper seed endosperm may be related to the activity of cell wall degrading enzymes (29) the composition of endosperm cell walls in these seeds was not reported.

The purpose of this work was to determine whether changes in *Datura* spp. endosperm cell walls could be part of the softening observed (23) before radicle protrusion. This question was addressed by combining GLC analysis of cell wall polysaccharides and SEM observations of the inner surface of the endosperm. Seeds of *D. stramonium* were used in addition to those of *D. ferox*, because *D. stramonium* seeds do not require light for germination and the endosperm restricts embryo growth (6). Thus, an interesting comparison might be provided.

METHODS AND MATERIALS

Seeds and Incubation Conditions

The *Datura ferox* seeds were collected in the summer of 1985 in a soybean crop in Rojas, Province of Buenos Aires, Argentina, and stored air dry at 20°C until June 1986. They were then shipped by air to Davis, CA, and kept at room temperature until used in the period July 1986 to March 1987. The seeds of *D. stramonium* were collected by Dr. Thomas Rost in 1975 near Davis, then kept at room temperature.

Incubations were carried out in glass Petri dishes. Seeds were placed on top of one layer of cotton wool and one disc of Whatman No. 1 filter paper saturated with distilled water. The *D. ferox* seeds were decoated after 24 h of incubation to achieve a good response to light (25) and immediately irradiated with either FR or FR followed by R. Dark conditions

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[2] Abbreviations: R, red light; FR, far-red light; GermI, germination stage 1; GermII, germination stage 2; Cell, cellulose.

were obtained by enclosing the Petri dishes in black plastic canisters and handling the imbibed seeds only under a dim green safe light. The green, R and FR sources were described previously (8). Irradiations with R and FR were of 20 min and carried out at 25°C. The rest of the time the *D. ferox* seeds were cycled through the following temperature regime: 8 h/d 33°C; 16 h/d 20°C. All *D. stramonium* seeds were kept for 24 h at constant 20°C in the dark. Then the control seeds remained at constant 20°C, while the seeds to be induced were transferred to 33°C to initiate a daily cycle of 16 h at 33°C and 8 h at 20°C.

Cell Wall Preparation

The endosperm portions (either as 0.5 mm micropylar cone tips or the whole endosperm separated from the embryo) were dissected and collected in ice-cold 80% ethanol. They were processed after all samples were collected on the same day, or stored in the freezer until processing was possible. In no case, was the storage period longer than a week. For wall preparations, the ethanol was decanted and the tissue was ground first with a mortar and pestle and then a glass homogenizer in 100 mM potassium phosphate (pH 7.0).

The homogenate was centrifuged for 10 min at 1000*g* and the pellet was suspended in 2 M NaCl; after centrifugation the pellet was washed successively with distilled water and methanol and then suspended in chloroform:methanol (1:1, v/v). This suspension was kept on ice for 15 min with occasional shaking and then centrifuged again. The pellet was washed with methanol and water and freeze-dried. The dried wall material was stored in a vacuum oven at 35°C until analyzed. Analysis started usually the next day and in no case was storage longer than one week.

Cell Wall Analysis

The dry samples (0.5–1 mg) were hydrolyzed in 500 μL of 2 M TFA (containing 50 μg myoinositol as internal standard) for 1 h at 121°C. After centrifugation the TFA-insoluble pellet was washed with 80% ethanol. The TFA-soluble material, after removal of the TFA in an air stream, was resuspended in 1 mL of water and separated into two aliquots: one for neutral sugars GLC analysis (1) and the other for colorimetric uronic acid assay (5). The TFA-insoluble material was used for cellulose analysis.

Gas chromatographic analysis of the neutral sugar alditol acetate derivatives was achieved using a fused silica capillary column [30 m × 0.25 mm (i.d.)] of bonded OV-225 (DB-225, J&W Scientific). The carrier gas was H_2 and the oven was held at 210°C. Peak areas were integrated by a Perkin Elmer Sigma-10 Chromatography Data System.

For cellulose analysis the pellet insoluble after TFA hydrolysis was dissolved in 1 mL of 67% H_2SO_4. A 100 μL aliquot of the H_2SO_4 was diluted with 3 mL of water containing 50 μg of myoinositol and hydrolyzed 1 h at 121°C. After neutralization of the hydrolysate with $BaCO_3$ the samples were reduced, acetylated, and analyzed with the GLC system as described above. Cellulose was quantitated from the glucose measured in these samples.

For methylation analysis, wall preparations (0.5–1 mg dry weight) were dissolved by sonication in 0.5 mL of DMSO. After the addition of 50 μL of DMSO anion (11), the sample was sonicated for 1 h, 6 μL of methyl iodide added and the sample stirred for 1 h. This cycle was repeated three times. After a final 25 μL addition of methyl iodide the sample was stirred overnight. The sample was cleaned of contaminating reactants by passing through a Sep-Pak C-18 cartridge (30) and then hydrolyzed with TFA, reduced (with $NaBD_4$ rather than $NaBH_4$) and acetylated as described.

The GLC separation of the methylated alditol acetates used the DB-225 column and the following temperature program: 5 min at 150°C, then 1.5°C/min to 210°C, followed by a 10 min hold. Quantitation of permethylated derivatives was based on the effective carbon response values described by Sweet *et al.* (26). Combined GC-MS analysis was performed at the Facility for Advanced Instrumentation, U.C. Davis, with a VG Analytical ZAB-HS-2F mass spectrometer and with a Hewlett Packard model 5890 gas chromatograph interfaced to a model 5970 Mass Selective Detector.

Scanning EM Observations

The endosperm samples consisted of the conical 0.5 mm micropylar ends or longitudinally sectioned half-cones of about 1 mm length. Samples were processed according to three different methods: fixation in glutaraldehyde and osmium, freezing, or coated fresh. Fixation was carried out overnight in 5% glutaraldehyde in 100 mM potassium phosphate (pH 7), and then 1 h at room temperature in 2% OsO_4. After being washed with distilled water, the samples were dehydrated by an ethanol series, and kept in 100% ethanol until dried with the critical point apparatus (Bomar). The dry samples were mounted on aluminum stubs with sticky tabs and then sputter coated with Au-Pd. Fresh samples were similarly mounted and coated.

For low-temperature SEM observations, the samples were prepared in the Emscope SP-2000 sputter-cryodevice. The sections were affixed to copper specimen holders using Tissue-Tek compound and frozen by plunging into a liquid nitrogen slurry. The frozen-hydrated samples were gold sputter-coated and placed on the SEM stage cooled by liquid nitrogen.

The observations on the fresh and glutaraldehyde-osmium fixed endosperms were done on samples from three different experiments for *D. stramonium* and two experiments for *D. ferox*. For the observation of frozen samples two experiments were carried out with *D. stramonium* and one with *D. ferox*. In total about 150 seeds were examined. All samples were studied with an S-800 Hitachi field emission SEM using a 15 kV acceleration voltage and 10 μA beam current.

RESULTS AND DISCUSSION

Germination Behavior

The time of radicle protrusion through the endosperm, GermI (23), was determined for both species under inductive and noninductive conditions. *D. ferox* seeds were decoated after 24 h of incubation and induced to germinate by a R treatment; GermI started at about 45 h after R and reached a final value of 80%. FR-treated seeds, on the other hand,

showed very little germination (Fig. 1a). Germination of intact *D. stramonium* seeds was induced by incubation for 16 h/d at 33°C and 8 h/d at 20°C. GermI started at about 72 h of incubation and 85% of the seeds finally germinated whereas control seeds, incubated continuously at 20°C, did not enter GermI before 96 h (Fig. 1b). Complete germination of *D. stramonium* seeds, that is radicle protrusion through the seed coats, GermII (23) followed the onset of GermI by approximately 24 h.

Endosperm Cell Wall Polysaccharide Composition

Changes in the mechanical properties of the micropylar endosperm prior to germination have been shown in *D. ferox* (23). It is likely that a change in only that portion of the endosperm can be decisive for germination (3, 20, 23). Therefore, micropylar and bulk endosperms of both species were analyzed separately.

The sugar compositions of the cell walls of the bulk endosperms of the two *Datura* species are similar (Table I). Man is, by far, the most abundant neutral sugar, accounting for about 60% of total sugar weight. Gal and cellulosic Glc account for another 10% each. The presence of Rib in small amounts has been noted in endosperm cell wall preparations

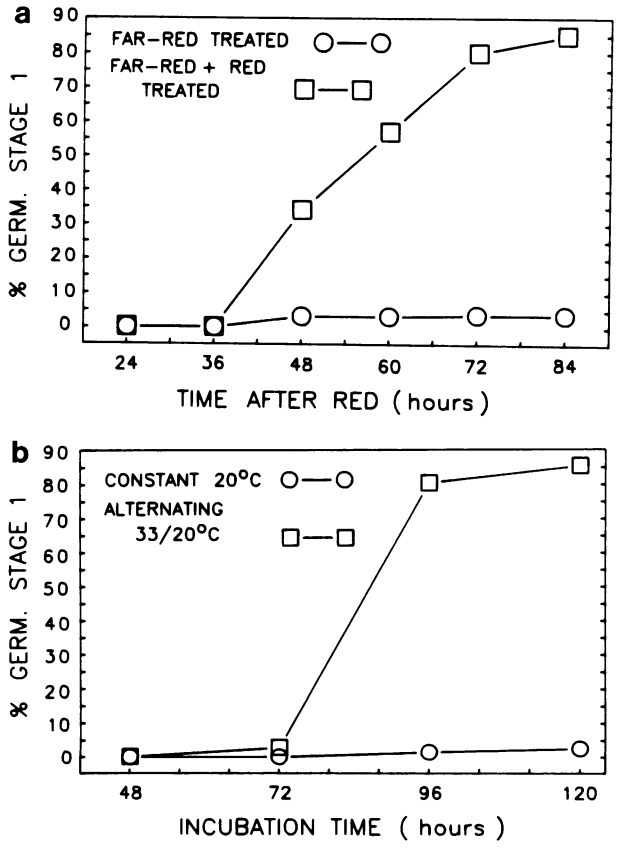

Figure 1. a, Time course of radicle protrusion through the endosperm (GermI) in decoated *D. ferox* seeds, irradiated with FR or FR + R; b, time course of radicle protrusion through the endosperm (GermI) in intact *D. stramonium* seeds incubated at constant 20°C or alternating 16 h 33°C/8 h 20°C.

Table I. *Sugar Composition of Polysaccharides from Cell Walls of Bulk and Micropylar Endosperm of D. stramonium and D. ferox Seeds Incubated for 24 h in Noninducing Conditions*

Component	*D. ferox*		*D. stramonium*	
	Micropylar	Bulk	Micropylar	Bulk
	% of total sugars			
Rha	0.9[a]	0.6	1.1	0.4
Rib	0.9	1.0	1.3	1.4
Ara	16.6	5.2	13.2	4.6
Xyl	2.3	0.8	2.4	0.6
Man	43.0	61.0	56.0	67.0
Gal	7.0	12.3	6.0	9.5
Glc	3.3	1.8	2.4	2.1
Cell	16.0	12.0	11.5	10.0
Uronic acid	8.5	5.0	5.9	4.6

[a] The figures are averages from three different preparations.

of other seeds and considered to be a cytoplasmic contaminant (12).

The compositions of the micropylar portions of the endosperms are similar to those of the bulk endosperm. Man is also the predominant sugar in the micropylar endosperm cell walls although its contribution is slightly less, particularly in *D. ferox* (43% instead of 61%, Table I). The second most abundant sugar in the micropylar cell walls is Ara (15%) followed by cellulosic Glc. The ratio Man/Gal is slightly larger in the micropylar than in the bulk endosperm: 6.1 and 4.5, respectively, in *D. ferox*, and 9.5 and 7.1 in *D. stramonium*.

Polysaccharide Linkages by Methylation Analysis

To gain information about the nature of the glycosidic linkages involved in the insoluble polysaccharides, cell wall preparations from both portions of the endosperm were subjected to methylation analysis. The methylated polysaccharides were reduced with borodeuteride and after acetylation the permethylated alditol acetates were analyzed by GLC and combined GC-MS. The GLC analysis of the methylated preparations of bulk and micropylar endosperm from both species gave similar chromatograms. The GLC analysis of the permethylated alditol acetates from the cell wall of *D. stramonium* micropylar endosperm (Fig. 2) is typical of the samples and gives an example of the glycosidic linkages (and, hence, polysaccharide types) found in these walls. In this preparation over 70% of the residues identified are 4-linked Man and Glc. Most of this is 4-linked Man. Derivatives indicative of branched hexosyl residues (4,6-linked Man and Glc) account for 5% of the preparation. Another 5% is accounted for by terminal galactosyl and terminal-glucosyl or -mannosyl residues. (GLC separation of the glucosyl and mannosyl derivatives is insufficient to allow us to distinguish between them.) Several derivatives involving arabinosyl residues (terminal, 5-linked, and 2-linked) were identified. These accounted for another 1% of the sample. Some minor peaks could not be identified by the GC/MS analysis and accurate quantitation of these samples is difficult. Nevertheless the same derivatives in roughly similar amounts were identified in all samples analyzed.

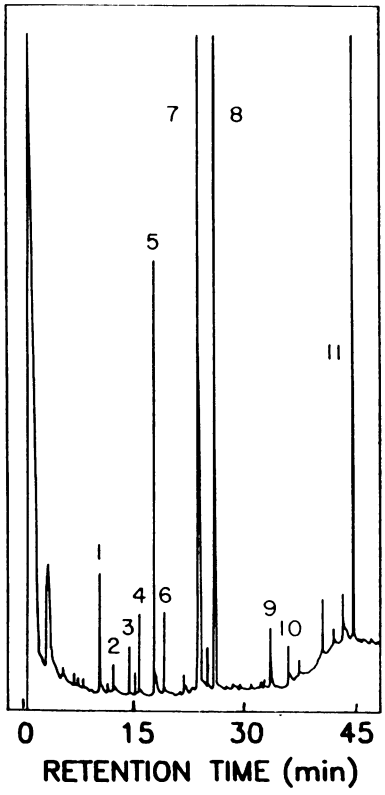

Figure 2. Gas chromatogram of the methylated alditol acetates from the polysaccharides of methylated cell wall preparations of micropylar endosperm of *D. stramonium* seeds harvested after 64 h incubation at constant 20°C. The numbers indicate peaks representing derivatives of: 1, terminal Ara; 2, terminal Xyl; 3, 2-linked Ara; 4, terminal Glc or Man; 5, 5-linked Ara; 6, terminal Gal; 7, 4-linked Man; 8, 4-linked Glc; 9, 4,6-linked Man; 10, 4,6-linked Glc; and 11, inositol (internal standard).

These linkage types are indicative of several polymer types that are typical of dicot cell walls (17). Xyloglucan is suggested by the presence of 4-Glc, 4,6-Glc, and t-Xyl. Cellulose is also suggested by the presence of 4-Glc residues. The variously-linked arabinosyl residues (in combination with the uronosyl residues reported in Table I, but not accounted for in the methylation analysis) are suggestive of pectic arabinans. The analysis suggests that by far the predominant polymer type in these cell walls is a mannan as indicated by the 4- and 4,6-linked mannosyl residues. However, the presence of 4-linked Glc could be attributed to the possible presence of a glucomannan. The terminal Gal could be ascribed to the presence of a galactomannan component. These types of polysaccharides have often been described as cell wall-localyzed (structural and/or storage) polymers in several seed types (4). These identifications of polymer types based on methylation analysis are preliminary suggestions and await the isolation of sufficient endosperm wall material to permit extraction and purification of specific polysaccharides. However, the composition of that portion of the wall materials disappearing during germination provides further evidence for a major mannan component.

Changes in the Micropylar Endosperm Cell Wall Polysaccharides Prior to Radicle Protrusion

Seeds of both *Datura* species were incubated in conditions conductive to germination (Fig. 1, a and b) and micropylar portions of the endosperm were dissected just prior to radicle protrusion. This was at 72 h after the beginning of incubation for *D. stramonium* and 36 h after R for *D. ferox*. Similar samples were harvested from control seeds incubated under noninductive conditions. Induction of germination brought about changes in the composition of cell wall polysaccharides of both species (Table II; Fig. 3). There was a substantial decrease in the cell wall Man content. In the micropylar endosperm of induced seeds nearly 70% of the Man was lost from the wall before germination. There were reductions in amounts of other sugars but these differences from controls were generally smaller. The ratios of Man to Glc disappearing averaged 40 and 31 for *D. stramonium* and *D. ferox*, respectively. This argues against the idea that the *Datura* wall component that is lost is a glucomannan because this polymer type generally has equal amounts of Glc and Man (4). The

Table II. *Sugar Composition of Cell Wall Polysaccharides in the Micropylar Portion of the Endosperm of D. stramonium and D. ferox Seeds Induced to Germinate or Noninduced Controls*

For incubation conditions see Figure 1a and 1b. Sampling of *D. stramonium* at 72 h of incubation and of *D. ferox* 38 h after irradiation.

Component	*D. stramonium*[a]		*D. ferox*[b]	
	20°C	33°/20°C	FR	R
	μg/micropylar portion			
Rha	0.21 ± 0.08	0.19 ± 0.09	0.5	0.35
Rib	0.23 ± 0.06	0.05 ± 0.03	0.25	0.04
Ara	2.8 ± 0.3	2.0 ± 0.5	4.4	3.2
Xyl	0.5 ± 0.1	0.4 ± 0.13	0.6	0.6
Man	10.4 ± 0.4	2.3 ± 0.9	11.2	3.3
Gal	1.2 ± 0.2	0.6 ± 0.2	1.7	1.1
Glc	0.6 ± 0.2	0.4 ± 0.1	0.9	0.65
Uronic acid	1.5 ± 0.3	0.8 ± 0.07	2.3	1.75
Cell	2.8 ± 0.7	1.9 ± 0.65	4.2	3.3

[a] The *D. stramonium* data are averages of five determinations ± the SD. [b] *D. ferox* data are averages of two determinations.

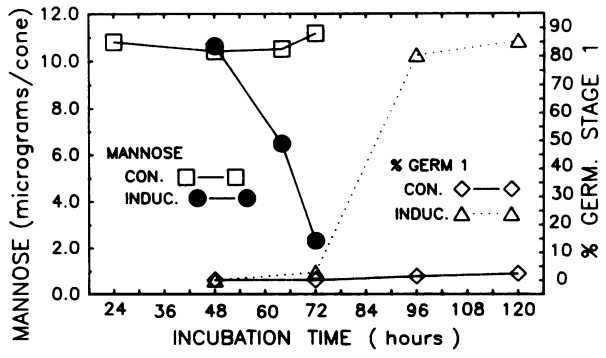

Figure 3. Mannose content in cell wall preparations of micropylar endosperm of *D. stramonium* seeds at different incubation times under inducing or noninducing conditions as in Figure 1b. Data of Figure 1b included for comparison.

ratio of Man to Gal loss averaged 13 for both species, thus arguing against the idea that a galactomannan (a polymer generally having >20% Gal substitution of a 4-mannan backbone [4]) is involved. We conclude that a 4-linked mannan is the wall component lost.

In *D. stramonium* seeds there was no change in the Man content of the micropylar endosperm after 48 h of incubation in either inductive or noninductive conditions (Fig. 3). From that time on, however, there was a steady decrease in the Man content of induced seeds. At 64 h of incubation there was nearly 50% less Man than at 48 h showing that wall Man mobilization begins well before radicle protrusion; even by 72 h Man was the only sugar showing a significant change (Table II).

Methylation analysis of wall preparations of micropylar portions harvested from induced *D. stramonium* seeds at 64 h of incubation shows that the only components whose percent contribution decreased in induced seeds were 4- and 4,6-linked Man. The content of several other derivatives showed relative increases, presumably because their presence in the wall did not change.

Degradation in Isolated Micropylar Portions

The possibility that, once initiated, mannan degradation could proceed in isolated endosperms of *D. stramonium* was explored. Micropylar portions were dissected from induced and noninduced seeds at 64 h of incubation, suspended in 100 mM acetate buffer (pH 6.0) and incubated for an additional 8 h, either at 33 or 0°C. The sugar composition of the walls prepared from endosperms dissected from control seeds showed no change after the 8 h incubation at 33°C. In contrast, the Man content in the walls of endosperm portions from induced seeds decreased during incubation at 33°C (although to a lesser extent [about 50%] than in the endosperms of intact, induced seeds during the same period of time at 33°C, Table III). Therefore once Man mobilization begins it can continue, to a limited extent, in the micropylar endosperm even if this tissue is isolated from the rest of the seed. Apparently, for a maximum degrading activity a close contact between endosperm and the radicle tip is necessary although some influence of excision and wounding on the micropylar endosperm itself could account for reduced mobilization of cell wall mannan.

Table III. *Changes in Composition of Cell Wall Polysaccharides of Micropylar Endosperm Portions Dissected from D. stramonium Seeds at 64 h of Incubation under Either Inducing or Noninducing Conditions*

After dissection samples were kept for 8 h at either 33 or 0°C. As control, a sample was dissected from intact induced seeds after they were held the same 8 h at 33°C.

Component	Noninduced		Induced		Control
	0°C	33°C	0°C	33°C	33°C
	μg/micropylar portion				
Rha	0.3[a]	0.25	0.3	0.27	0.25
Rib	0.23	0.22	0.14	0.08	0.03
Ara	2.7	2.7	2.7	2.4	2.1
Man	10.5	11.0	6.5	4.4	1.6
Gal	1.2	1.1	1.1	0.9	0.6
Glc	0.4	0.5	0.4	0.45	0.4
Cell		3.2	3.0	2.3	2.4

[a] Averages from two experiments.

Structural Changes in the Micropylar Endosperms Prior to Radicle Protrusion: SEM Observations

The SEM images of the micropylar endosperm of both *Datura* species are similar. The cells in the micropylar endosperm are smaller than those of the bulk endosperm, and there are five to six cell layers in front of the radicle tip. These cells form a cone-shaped structure covering the radicle tip. This cone has a height of 300 to 350 μm and the total number of cells in this portion of the endosperm is about 800. The internal surface of the endosperm, that in contact with the embryo, has a smooth appearance and in cross sectional view, the inner surface appears to be essentially a smooth sheet, (Figs. 4, a and c; 5a). The cells are barely discernible beneath a layer of what seems to be remnants of wall material of cells compressed during seed development. The smooth appearance of the inner surface of the endosperm remains unchanged in seeds incubated under noninducing conditions up to 48 h after FR irradiation in *D. ferox* and 72 h of incubation at continuous 20°C in *D. stramonium* (Figs. 4, a and c; 5, a, c, and e). In contrast, sharp differences are seen in endosperm sections dissected from seeds induced to germinate at incubation times close, but prior, to radicle protrusion. The material lining the inner surface of the endosperm appears eroded; in many areas substantial amounts of wall material seem to be missing and the contours of the cells beneath are clearly discernible (Fig. 4, b, d, and e,). Similar changes were observed in samples coated fresh or frozen (data not shown). The erosion was not uniform, in many sections there were areas more affected than others and there was variation between sections. Eroded areas were not found in all induced seeds, but the proportion of samples from induced seeds not showing much alteration (about 30%) is in agreement with the fact that not all seeds germinate and all those which do germinate do not do so at the same time. In any case, the modified areas are not found in control seeds. All observations were made on seeds not showing radicle protrusion, as demonstrated by the fact that the micropylar cones dissected from induced seeds, even those extensively eroded, are still without fractures (Fig. 4b). Even when the loss of material covering the cells is extensive the cells appear intact, with no obvious signs of being collapsed or damaged (Figs. 4e and 5f). Most of them look turgid and some remaining surface layer material still connects neighboring cells. The general appearance of the micropylar endosperm inner surface near the time of germination was similar in both species, (Figs. 4 and 5) and the most relevant features indicating alterations in the wall material were visible in samples processed following each of the three different methods for SEM observation described. Therefore, the interpretation that there are pregerminative changes in the cell wall material seems to be well substantiated.

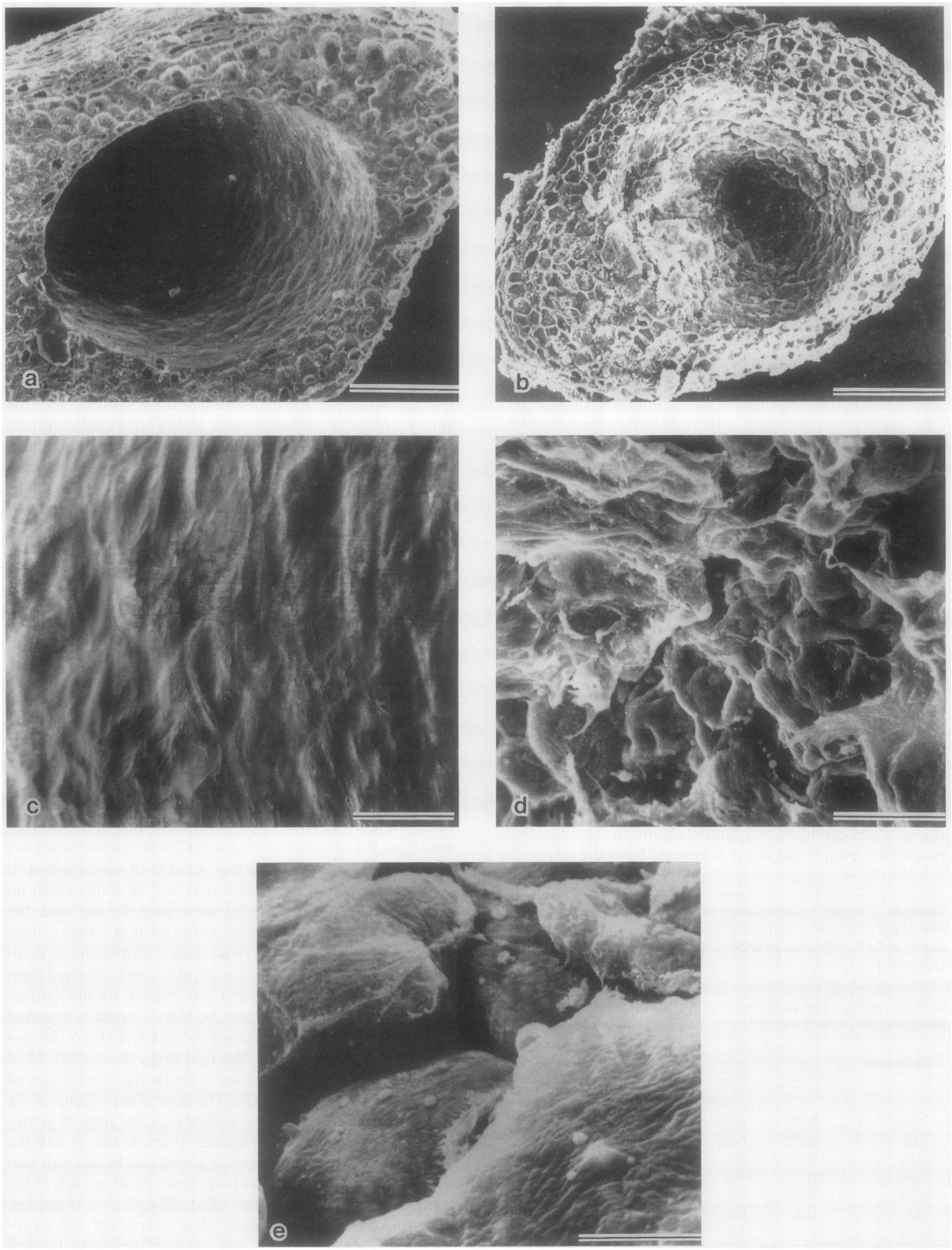

Figure 4. Scanning electron micrographs of micropylar endosperm cones of *D. stramonium* seeds glutaraldehyde-osmium fixed and critical point dried. a, c: control seeds; b, d, and e: induced seeds. Sampling at 72 h of incubation. Bars: a, b = 200 μm; c, d = 25 μm; e = 10 μm.

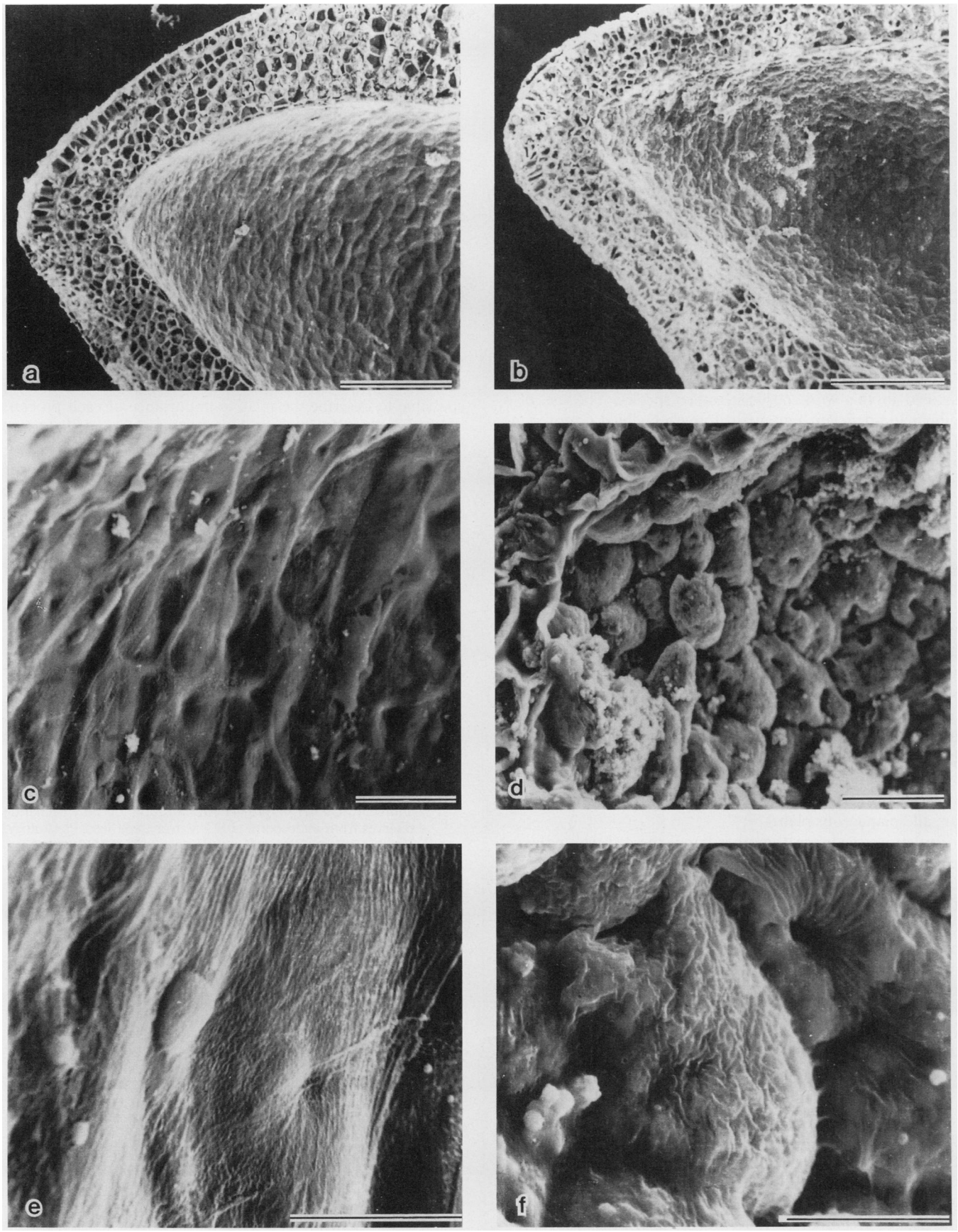

Figure 5. Scanning electron micrographs of median sections of micropylar and adjacent bulk endosperm of *D. ferox* seeds, glutaraldehyde-osmium fixed and critical-point dried. a, c, e: FR irradiated seeds; b, d, f: FR + R irradiated. Sampling at 38 h after irradiation. Micrographs d and f are enlargements of the most affected areas of section b. Bars: a, b = 200 μm; c, d = 25 μm; e, f = 10 μm.

Relationship to Germination-Regulating Processes in Other Species

The walls of *Datura* endosperm cells appear to contain a complement of polysaccharide species that is typical of dicots. The most prevalent of the polymers present is rich in 4-Man like the mannan B of palm seeds and the mannans found in seeds of several other species (2, 4). It is primarily this wall component that is lost from the wall following induction of germination, but prior to the onset of radicle protrusion. The timing of mannan loss coincides with a pronounced erosion of the endosperm cell wall (as shown by SEM observations, Figs. 4 and 5). Thus it is clear that in *D. ferox* and *D. stramonium* a weakening of the tissue barriers which limit radicle growth preceeds the onset of that growth.

There are indications that processes similar to those described in this work occur in other species. The use of a gibberellin-deficient mutant in tomato enabled Groot *et al.* (10) to block germination and to restore the ability to germinate by addition of gibberellin. The GA treatment of isolated endosperms resulted in the appearance of endo-β mannanase and other hydrolases correlated with the release of monosaccharides, mainly Man. Weakening of the endosperm by enzymatic degradation of mannan-rich walls appears to precede germination in both tomato and *Datura*. Watkins *et al.* (29) have found a pregerminative decrease in the resistance of the endosperm of pepper seeds to puncturing. This is accompanied by an increase in enzymatic activity capable of releasing reducing sugars from wall preparations and from galactomannan solutions. The composition of the pepper endosperm walls was not reported and the location of the enzyme activity has not been determined. Should these walls contain a considerable amount of mannan and the degradative activity be found in the micropylar part of the seed the situation would be similar to that in both *Datura* species. It may be noted that tomato, pepper (*Capsicum annuum*), and Datura are all Solanaceous plants.

In lettuce seeds, on the other hand, the cell wall polysaccharide composition is similar to that of *Datura* but no changes have been detected before germination (12). Reductions in endosperm mechanical strength as part of the processes leading to germination have been proposed by several authors (10, 14, 19) but the possibility that these are an essential part of the germination process in this species has not been fully accepted (3). Cell wall alterations in induced lettuce seed endosperm have been reported (15) but not confirmed by more recent work (20). It is possible that endosperm softening might be less important in lettuce than in *Datura*, pepper and tomato seeds.

The enzymatic system involved in pregerminative mannan degradation in *Datura* might be similar to that found after germination in other species with cell wall mannans such as palm and lettuce seeds (2, 10, 12, 13, 18). Preliminary experiments (24) indicate greater mannanase and mannosidase activities in seeds of both *Datura* species which have been induced to germinate. An increase in cellulase activity in *D. ferox* seeds induced to germinate has been reported (23). However the change in cellulose content observed in this work is not significant and it therefore does not seem likely that this cellulase activity plays an important role in endosperm softening.

Once the mannan degrading process is induced in *D. stramonium* seeds it can proceed even if the micropylar endosperm is isolated from the rest of the seed. The fact that mannan utilization in isolated tissue is less than that in the intact seed suggests the need for close contact between the endosperm and the radicle for maximum expression of wall digestion.

Endosperm softening is not the only pregerminative change observed in induced *Datura* seeds. In both species there are reports of an increase in embryo growth potential (22). The information available suggests that in these species the induction of germination may require the concomitant reduction in the mechanical resistance of the endosperm and an enhanced capacity for elongation of the embryo. It is not yet possible to describe the relative importance of each process. Although the environmental signals are different: light and alternating temperatures for *D. ferox*, only alternating temperatures for *D. stramonium*, the processes affected are the same. Whether the sequence of events between the perception of the external signal and the final effects on endosperm softening and embryo elongation are also the same is the matter for future work.

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