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Relationship Between Weathering Deterioration and Germination, Respiratory Metabolism, and Mineral Leaching from Cottonseeds[1]

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ABSTRACT

Germination of cottonseeds (*Gossypium hirsutum* L.) is reduced by preharvest exposure to weather, particularly if conditions are warm and humid. To identify some of the germination processes affected by weathering deterioration, seedlots of six cultivars were harvested before and after a period of field exposure and comparative germination studies were conducted. Seedling field emergence was reduced by 11 to 33% due to weathering. During imbibition, weathered seeds of sensitive cultivars released more K^+, Mn^{2+}, Mg^{2+}, and Ca^{2+}, but less Na^+ into the steep solution than did their unweathered counterparts. Leaching of individual minerals was a better indicator of seed quality than was the total release of electrolytes; release of K^+ and Ca^{2+} was significantly correlated with measurements of seed quality. Respiratory O_2 uptake was reduced in deteriorated seeds, with elevated estimates of the RQ after 7.5 h of imbibition. Aeration with 100% O_2 increased O_2 uptake and reduced the RQ, indicating that O_2 diffusion may have limited respiration during early germination. Weathering deterioration of membranes was confirmed by electron microscopy of cotyledonary lipid and protein bodies. These data indicate that the loss of membrane functional integrity is a major factor responsible for the reduction of germination potential by weathered seeds, and that the release of K^+ and Ca^{2+} into steep water during imbibition may be a good indicator of cottonseed planting quality.

***Additional index words:* Gossypium hirsutum L., Seed vigor, Membrane permeability, Emergence, Oxygen uptake.**

THE establishment of a stand of vigorously growing seedlings is one of the first requisites of cotton (*Gossypium hirsutum* L.) production (Christiansen and Rowland, 1981). Stand establishment, in turn, is significantly influenced by the quality of the seeds that are planted (Krieg and Bartee, 1975; Leffler, 1981). Many factors, including preharvest deterioration in the field (Christiansen and Justus, 1963; Halloin, 1981) and premature termination of seed maturation (Leffler, 1980) reduce the quality of cottonseeds. The potential severity of the problem with cottonseed quality is illustrated by observations in some cotton production regions that fewer than half the planted seeds produce seedlings that emerge and survive to give productive plants (Ferguson and Turner, 1971).

Because of the need to identify seeds with high planting quality (seed vigor), physiological criteria by which to assay seed quality are critical adjuncts to cotton production. Epigeous seedling growth rates (Christiansen, 1962) and radicle emergence rates (Dalianis, 1982) have been described as physiological manifestations of seed vigor. Physiological differences in seed performance have been based upon biochemical and ultrastructural differences. Positive relationships between seed vigor and O_2 uptake, and negative relationships between seed vigor and respiratory quotients, have been reported in other species (Anderson, 1970; Cantrell et al., 1972; Woodstock and Grabe, 1967).

Deterioration of cottonseeds has been related to the release of electrolytes (Anderson et al., 1964), while environmental stresses stimulated solute losses from radicles of cotton seedlings (Christiansen et al., 1970). Similarly, the release of electrolytes into steep water by germinating pea (*Pisum sativum* L.) seeds has been associated with seed deterioration (Matthews and Bradnock, 1968; Matthews and Carver, 1971; Nagy and Nagy, 1982). The removal of the testa from peanuts (*Arachis hypogea* L.) has been found to decrease viability and to increase the release of electrolytes (Samad and Pearce, 1978). In cotton, however, delinting procedures and post-delinting washing have been found to interfere with the use of electrolyte loss as a measure of cottonseed deterioration (Halloin, 1975). Even though there may be a general relationship between seed quality and electrolyte release, there may be an altogether different relationship between seed quality and the release of specific ions. In cabbage (*Brassica oleracea* L.), for example, the release of K^+ and Cl^- into steep water differed from that of Ca^{2+}, Mg^{2+}, and Mn^{2+} (Loomis and Smith, 1980). Therefore, analyses of the release of specific cations may provide more information about the physiology of seed quality than does the release of total electrolytic material.

In this paper, we describe the effects of mild preharvest field weathering on the field emergence of six cultivars of cotton. Three of these cultivars, which appeared from the field emergence tests to differ in sensitivity to weathering, were then examined for various physiological aspects of germination. Comparative evaluations were made on these seed lots for respiratory metabolism, steep water conductivity and the release of five cations into steep water during imbibition.

MATERIALS AND METHODS

Seed Production and Field Evaluations

Seeds used in these studies were produced in field plots at Stoneville, MS, in 1980. The cultivars used, 'Auburn 56', 'Coker 201', 'Coker 310', 'Delcot 277', 'PD 2165', and 'TH 149', represent a range of the germplasm adapted to the Southeastern USA. To eliminate potential physiological difficulties that might be associated with the presence of gossypol glands in the normal cultivars, each cultivar was genetically converted to a glandless derivative. Seed cotton was harvested twice during the production season. The first

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harvest was collected when the crop was mature, but before the crop had been exposed to adverse weather; the second harvest was collected approximately 5 weeks later, after exposure to weathering conditions. During this 5-week period, the weather station in Stoneville recorded 124 mm of rainfall, 89 mm of which fell during a cool 3-day period in late September. The mean daily high and low temperatures during this rainy period were 18.7 and 13.9 °C, respectively. Thus, although seed cotton was wet for an extended period, seed deterioration was reduced by the cool temperatures. Seeds were acid-delinted with concentrated H_2SO_4, rinsed with $NaHCO_3$, and dried prior to storage.

The planting quality of the seeds was evaluated in field plots at Stoneville in the 1981 and 1982 growing seasons. Seeds of each cultivar from each harvest were planted on 29 Apr. 1981 and on 4 May 1982. The number of emerged seedlings in each plot was recorded weekly during the 1st month after planting. Seedling emergence in 1981 was influenced by whether the plots were planted by an inside or outside hopper of the four-row planter. Therefore, the 1981 stand counts were adjusted by covariance to eliminate this potential bias from the data, and adjusted means are presented. No such difficulty was encountered in 1982, so unadjusted means are presented. From the results of these field evaluations, three cultivars, Coker 201, Delcot 277, and PD 2165, were selected for further examination at Beltsville, MD.

Seed Respiration

Oxygen uptake and CO_2 evolution from seeds of each of the three cultivars were measured at 25 °C in a Gilson differential respirometer[3], with 10 seeds in each 15-mL reaction vessel. For the readings taken 6 and 7.5 h after the start of imbibition, 1.5 mL of double-distilled water was placed in each vessel with dry seeds. For the readings taken 24 and 25.5 h after the start of imbibition, 1.0 mL of water was used; seeds were imbibed for 23 h on moist germination paper at 25 °C before they were transferred to the vessels. As the seeds were transferred, the number of sprouted seeds (visible radicle) was recorded. Carbon dioxide was absorbed on filter paper wicks, saturated with KOH (200 g L^{-1}), in the center well of the flasks. Evolution of CO_2 was calculated by difference, using flasks without KOH in the center well. Respiratory quotients were calculated as μL CO_2 evolved/μL O_2 absorbed. There were two replicates in a randomized complete block design.

Conductivity

The conductivity of imbibitional water was measured after 4 and 24 h at 25 °C with an ASA 610 seed analyzer[3]. Single seeds were incubated in 4 mL of double-distilled water in individual cells of the analyzer. The conductivity of the water in each cell was measured at the indicated times. Values reported are the means from 100 determinations in each of two replications in a randomized complete block design.

Mineral Analysis

Oven-dry samples of 20 seeds were ground in a Wiley mill to pass a 30 mesh screen; a 1 g subsample was ashed at 500 °C for 12 h. Both weathered and unweathered seed samples were ashed at the same time. After the ashing, the samples were cooled and 4 mL of concentrated HNO_3 was added; samples were then placed on a hot plate at 80 °C. Once the samples had dried, 10 mL of 3.0 M HCl was added to each and they were covered with a watch glass and refluxed for 2 h at 80 °C. The samples were then filtered through Whatman 42 filter paper (pre-rinsed with 0.1 M HCl), rinsed and brought to a final volume of 25 mL with 0.1 M HCl. The seed coats were removed from 50 to 60 seeds of both control and weathered Coker 201 and Delcot 277 samples. The seed coats were then ground, ashed, and extracted as described above. The mineral contents of the extracts, each adjusted to 0.1 M HCl, were determined with an Instrumental Laboratories 275 atomic absorption/emission spectrophotometer[3] or a Varian Techtron AA6 atomic absorption spectrophotometer[3]. Standards were made with 0.1 M HCl. Determinations on whole-seed samples were replicated three times in a completely randomized design; those on the seed coats were replicated twice.

[3] Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the USDA and does not imply its approval to the exclusion of other products that may be suitable.

Table 1. Reduction in field emergence, germination, and growth by weathering exposure of cottonseeds.

Cultivar	Seedlot	Field emergence 1981	Field emergence 1982	Standard germination†	Seedling growth†
		%	%	%	mm
Auburn 56	Control	72	83	–	–
	Weathered	47**	70*	–	–
Coker 201	Control	69	79	91	196
	Weathered	65	68	82*	176*
Coker 310	Control	75	77	–	–
	Weathered	50*	62*	–	–
Delcot 277	Control	69	75	95	193
	Weathered	34**	63*	73**	142*
PD 2165	Control	79	76	93	190
	Weathered	43**	63*	64**	133**
TH 149	Control	71	78	–	–
	Weathered	53*	70*	–	–
Means	Control	72	78	93	193
	Weathered	49	66	73	150

*,** Indicate significant difference from the control, p = 0.05 and 0.01, respectively.

† Standard germination and seedling growth were measured after 4 days at 25°C in dark germinators.

Leaching of Cations

Samples of control and weathered seeds were imbibed at 30 °C; for each sample, 100 seeds were placed in 50 mL of double-distilled water. At 4 and 24 h, the steep water was drawn off with a syringe and adjusted to 0.1 M HCl with concentrated HCl. Mineral contents were determined by atomic absorption spectrophotometry as described above on two replicates in a randomized complete block design.

The kinetics of mineral leaching from control and weathered seeds of Delcot 277 were determined over a 24 h period at 24 to 27 °C. In this experiment, 100 seeds of each seedlot were placed into 100 mL of double-distilled water. At predetermined times during imbibition, duplicate 0.5 mL aliquots were removed; K^+ and Na^+ contents of the aliquots were determined by atomic absorption spectrophotometry. At each sampling, the volume of water removed in the aliquots was replaced with double-distilled water to maintain a constant volume of solution. This experiment was replicated twice.

Electron Microscopy

Cotyledons from dry unweathered and weathered Delcot 277 seeds were prepared for electron microscopic examination by the procedure outlined by Yatsu (1983). Seeds were soaked in hexane for 2 h, then fixed in 1% OsO_4 in hexane. After fixation, the samples were rinsed repeatedly with acetone and then transferred incrementally into Spurr's

Table 2. Effect of field weathering on respiratory metabolism in cottonseeds.

		Oxygen uptake atmosphere				Respiratory quotient atmosphere				
		Air		Oxygen		Air		Oxygen		
Cultivar	Seedlot	6 h	24 h	7.5 h	25.5 h	6 h	24 h	7.5 h	25.5 h	Sprouted seeds
		$\mu L\ O_2\ h^{-1}\ seed^{-1}$				$\mu L\ O_2/\mu L\ CO_2$				
Coker 201	Control	2.0	32.8	12.9	50.0	4.8	1.4	1.6	0.9	7
	Weathered	2.3	23.8	8.3	39.5	4.8	1.5	1.7	6.0	7
Delcot 277	Control	2.9	23.8	17.2	58.5	3.9	1.1	1.4	0.7	6
	Weathered	3.4	26.9	13.5	46.8	5.9	1.5	1.7	1.0	4
PD 2165	Control	2.5	12.2	7.5	21.3	3.9	2.2	1.7	1.3	4
	Weathered	2.6	11.2	7.0	21.7	4.8	2.8	2.3	1.8	3
$LSD_{0.05}$		NS	8.36	10.54	13.2	NS	0.96	0.42	0.39	

resin. Tissue sections were cut on a Reichert Ultracut ultramicrotome, stained with uranyl acetate and lead citrate, and viewed with a Hitachi HU-11 C electron microscope.

RESULTS AND DISCUSSION

Germination, Growth and Field Emergence

Even though the environmental conditions were relatively mild and dry during the exposure period in the fall of 1980, evaluations of seedling emergence in both 1981 and 1982 showed significant reductions due to the weathering exposure of the mature seeds (Table 1). Emergence and survival of seedlings were generally higher in 1982 than in 1981, even though the seeds had been stored for an extra year. This contrast illustrates the significant impact that the evaluation environment can have on measurements of seedling emergence and stand establishment. In 1981, the weather was dry immediately after planting, then it turned cool and wet about 2 weeks later; these conditions reduced the emergence and survival of all seed lots. In 1982, however, conditions were nearly ideal throughout the evaluation period, and both emergence and survival were favored. Because of these environmental conditions, the contrast between weathered and unweathered seed lots was much more pronounced in 1981 than in 1982, indicating that seed deterioration is likely to be much more of a factor in stressing environments. These contrasting environments produced remarkably similar estimates of weathering sensitivity, however, in that no major reversals or contrasts were observed between the 2 years.

In both field evaluations seeds of Coker 201 appeared to be least affected by the exposure to weathering, while seeds of Delcot 277 and PD 2165 were among those most severely affected by the weathering. Since they represented the range of responses that might be expected from such a study, these three cultivars were selected for further evaluations in the laboratory. Germination and growth measurements agreed with the field emergence data in indicating greater deterioration in Delcot 277 and PD 2165 than in Coker 201 (Table 1).

Seed Respiration

Gas exchange by cottonseeds was measured twice each in atmospheres of air and 100% O_2 (Table 2). At 6 h of imbibition in air, O_2 uptake by weathered seeds was slightly greater than that by unweathered seeds. By 24 h, however, O_2 uptake from air by weathered seeds was less than that by unweathered seeds. When similar measurements were made in an atmosphere of 100% O_2, weathered seeds absorbed less O_2 than did the unweathered seeds at both 7.5 and 25.5 h. The measured differences for the respiratory quotients reflected these differences in O_2 uptake. Although similar differences between respiratory rates and seed vigor were observed between seed lots of each cultivar, the relationship did not hold up when comparisons were made between cultivars (Table 2). The above differences in respiratory metabolism between weathered and control lots were not always statistically significant. However, similar trends, i.e., decreases in O_2 uptake and increases in RQ were observed in the weathered lots of each cultivar, indicating that field weathering may, in fact, have influenced respiratory metabolism during early germination.

The stimulation of O_2 uptake and reduction of RQ by exposure of the seeds to 100% O_2 indicate a restriction of diffusion that may have limited respiration during early imbibition. The highly fermentative nature of this early respiration might also indicate that these seeds endured a period of anaerobic stress during the first few hours of germination. Woodstock and Taylorson (1981) reported that periods of submergence as short as 60 min could be injurious to soybean [*Glycine max* (L.) Merr.]. The high RQ values we observed at 6 h indicate that cotton seeds may be similarly quite susceptible to soaking injury.

Although these data agree with earlier descriptions of the relationship between respiration and vigor (Cantrell et al., 1972; Kittock and Law, 1968; Woodstock and Grabe, 1967), they also illustrate the potentially confounding effects of gas diffusion. The slight enhancement of O_2 uptake from air by weathered seeds at 6 h suggests than an initial barrier to diffusion was made slightly less effective by the preharvest field exposure.

Electrolyte Release

Except for Coker 201, the relatively weathering-tolerant cultivar, weathered seeds released more electrolytes during a 24-h soaking period than did unweathered seeds (Table 3). Only the seeds of Delcot 277, one of the sensitive cultivars, showed any differences due to weathering at 4 h. Generally, the conductivity data supported the conclusions from the field measurements, that both Delcot 277 and PD

Table 3. Effect of field weathering on the conductivity of leachates from cottonseeds.

Cultivar	Seedlot	Conductivity measured at 4 h	24 h
		μAmp†	
Coker 201	Control	25	44
	Weathered	23	44
Delcot 277	Control	31	57
	Weathered	40	67
PD 2165	Control	31	53
	Weathered	31	61
$LSD_{0.05}$		5.8	9.0

† μAmps for 1 seed in 4 mL double distilled water at 25°C. Values are the means of two experiments of 100 seeds in the ASA-610.

2165 were more sensitive to weathering deterioration than was Coker 201. The means of the conductivity measurements, however, were not as markedly different as were the measurements of field germination and emergence. When the conductivity values for the individual seeds were plotted into frequency histograms, however, the data indicated that weathered seeds produced both a wider range and a broader distribution than did the unweathered control seeds (Fig. 1).

That the leachate conductivity differences between weathered and unweathered seed lots was greater at 24 h than at 4 h likely indicates the temporal effects of imbibition and electrolyte diffusion. It may also indicate, however, differential sensitivity of the tissues to the extended period of anaerobiosis in the cells of the analyzer. Christiansen et al. (1970) demonstrated increased solute losses from cotton seedling roots subjected to anaerobiosis. Which of these possible explanations, if either, might be correct will be subject of further analyses; the results do, however, indicate differences between weathered and unweathered seed lots for electrolyte release.

Leaching of Cations

The cation concentrations in these seeds (Table 4) were similar to previously reported values (Bartee and Krieg, 1974; Leffler and Tubertini, 1976). No major differences were found between the weathered and unweathered samples of these cultivars, although the weathered seeds of both Coker 201 and Delcot 277 were considerably lower in Na^+ than were the unweathered controls. Since this pattern did not hold for PD 2165, these apparent differences between samples of the other cultivars are likely to be independent of the weathering deterioration of germination capability. The distribution of these cations between the embryo and seed-coat fractions varied markedly among the ions, but not between cultivars. Most significantly for sensitivity to leaching, over 90% of the seed Na^+ content was localized within the seed coat. Relatively more of the seed Mg^{2+} was contained within the embryo, but the distribution of the other cations between the embryo and seed coat essentially reflected the weight fractions of the seed.

At both 4 and 24 h, the total release of each cation, except Na^+, was greater for the weathered than for the unweathered seeds (Table 5). When the quantities leached were normalized as percentages of the initial contents, however, more of all cations—including Na^+—was leached from the weathered seeds. Among the measured cations, there was considerable variation in the quantities leached in 24 h, ranging from the low values shown by Mg^{2+} and Ca^{2+} to the high losses shown by K^+ and Na^+.

The leaching kinetics of K^+ and Na^+ from weathered and unweathered seeds of Delcot 277 were compared over a 24-h period (Fig. 2). Within the first 10 min of imbibition, unweathered seed lost 620 mg/kg of K^+, or 5.2% of their total. During this same interval, the weathered seeds lost nearly half-again as much K^+, 970 mg/kg, or 8.2% of their initial con-

Table 4. Distribution of cations in weathered and unweathered cottonseeds.

Cation	Coker 201 Control	Coker 201 Weathered	Delcot 277 Control	Delcot 277 Weathered	PD 2165 Control	PD 2165 Weathered	$LSD_{0.05}$
	mg/kg						
K^+	11 778†	12 600	11 750	11 790	11 636	11 875	471
Mg^{2+}	4 000	4 286	4 500	4 263	3 500	3 667	162.6
Ca^{2+}	1 500	1 675	1 667	1 525	1 150	1 420	409.3
Na^+	410	343	439	207	781	792	136.6
Mn^{2+}	18	18	18	17	–	–	1.2
	Seedcoat fraction, %‡						
K^+	26	23	32	30	–	–	
Mg^{2+}	12	13	12	13	–	–	
Ca^{2+}	26	30	24	27	–	–	
Na^+	95	95	98	91	–	–	
Mn^{2+}	36	35	40	37	–	–	

† Values are the means of three reps.
‡ The seedcoat comprised approximately 31% of the dry weight of the seeds. Values presented are the proportion of the total seed complement of each cation that was accounted for by the content of the seedcoats.

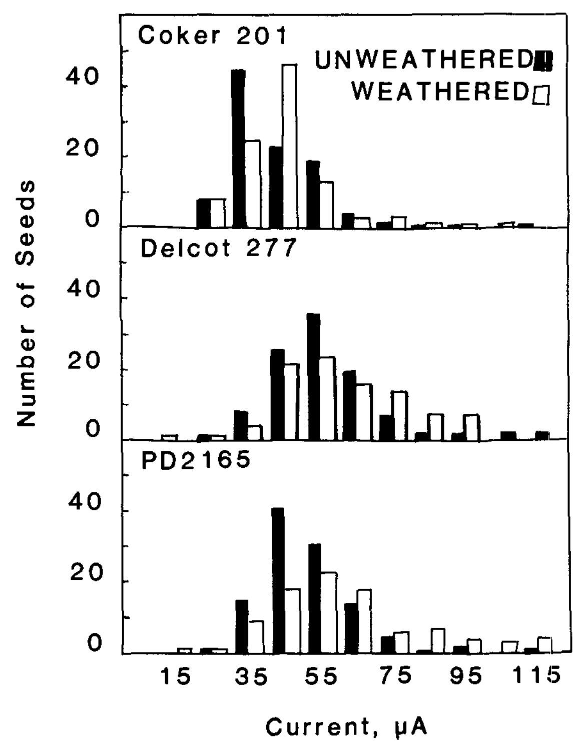

Fig. 1. Effect of field weathering on the frequency distribution of electrolyte release (μA current) from Coker 201, Delcot 277, and PD 2165 cottonseeds imbibed for 24 h. Solid bars, unweathered seeds; open bars, weathered seeds.

Table 5. Leaching of cations from weathered and unweathered cottonseeds.

		Cation									
		K^+		Na^+		Mg^{2+}		Ca^{2+}		Mn^{2+}	
Cultivar	Seedlot	4 h	24 h	4 h	24 h	4 h	24 h	4 h	24 h	4 h	24 h
		mg/kg									
Coker 201	Control	1060	1930	168	228	16	35	3.0	7.5	0.11	0.19
	Weathered	1260	2750	151	200	30	107	6.7	25.5	0.20	0.61
Delcot 277	Control	1410	3030	202	275	27	69	5.0	12.0	0.23	0.44
	Weathered	2180	4850	145	184	81	244	12.2	42.6	0.52	1.32
PD 2165	Control	1280	2350	250	343	7	22	2.3	6.3	-	-
	Weathered	1900	3200	301	380	22	64	7.1	19.1	-	-
$LSD_{0.05}$		402	390	23.9	67.1	11.8	26.7	2.93	6.90	0.063	0.135
		% of initial									
Coker 201	Control	9	16	41	55	0.4	0.8	0.2	0.5	0.6	1.1
	Weathered	10	22	44	58	0.7	2.6	0.4	1.7	1.1	3.5
Delcot 277	Control	12	26	46	63	0.6	1.7	0.3	0.8	1.3	2.5
	Weathered	18	41	70	89	1.9	5.7	0.8	2.6	3.0	7.5
PD 2165	Control	11	20	32	44	0.2	0.6	0.2	0.4	-	-
	Weathered	16	27	38	48	0.6	1.7	0.5	1.3	-	-

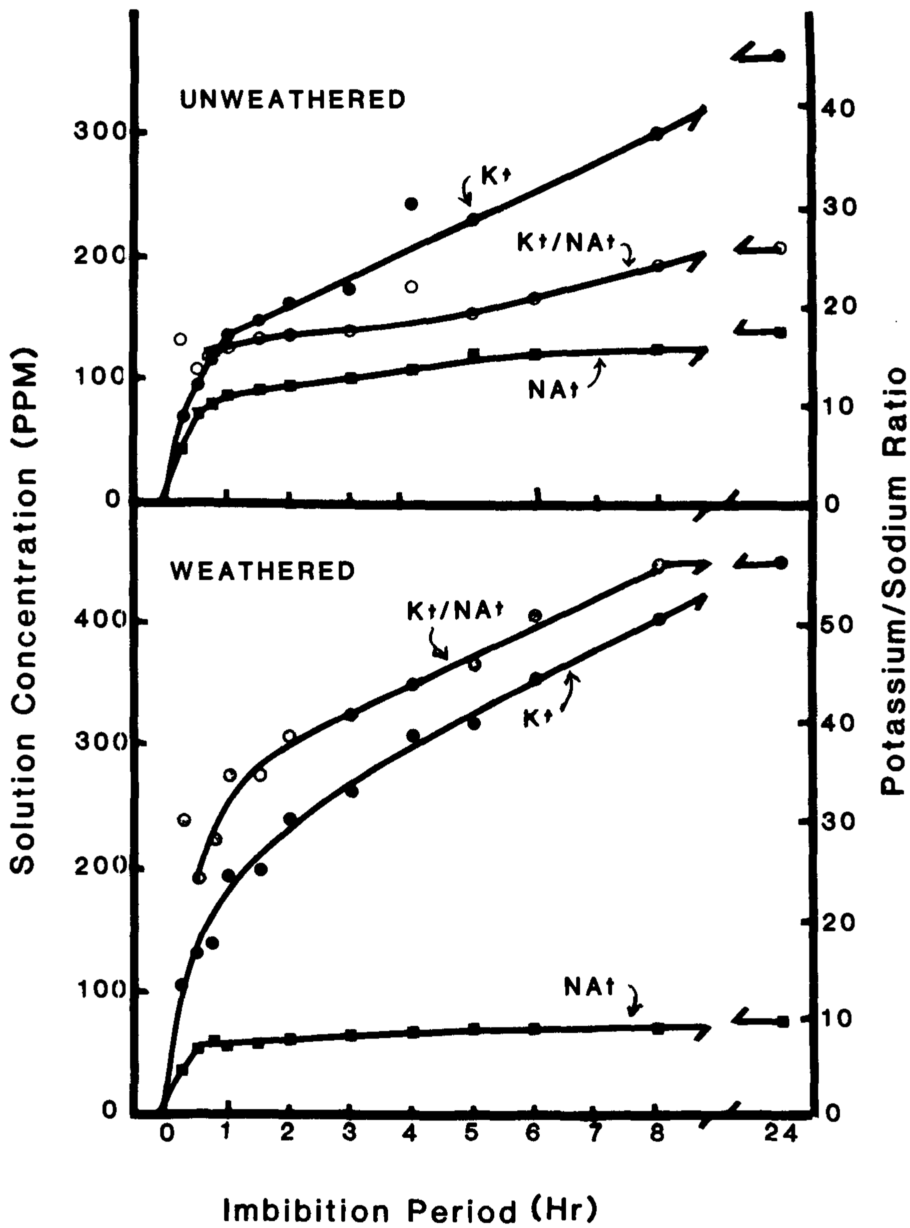

Fig. 2. Time course of the release of K^+ and Na^+ into steep water during imbibition of weathered and unweathered Delcot 277 cottonseeds. ●, Na^+; O, K^+; +, K^+:Na^+ ratio.

Table 6. Correlations of seedling growth, % germination, field emergence and vigor index with selected seed quality measurements.

		Correlations with†					
				Field emergence			
Quality measurement	Time period	Growth	% Germ.	1981	1982	Mean	Vigor index‡
	h	r					
Leachate conductivity	4	−0.54	−0.50	−0.64	−0.49	−0.61	−0.52
	24	−0.70	−0.62	−0.73	−0.62	−0.72	−0.68
	Δ4-24	−0.78*	−0.66	−0.72	−0.68	−0.74	−0.76
K^+ released	4	−0.86**	−0.80*	−0.93**	−0.86*	−0.93**	−0.89*
	24	−0.75*	−0.67	−0.85*	−0.83*	−0.86*	−0.74*
	Δ4-24	−0.61	−0.52	−0.73	−0.73	−0.73	−0.56
Na^+ released	4	−0.09	−0.08	0.03	0.02	0.01	−0.21
	24	0.21	0.23	0.16	0.14	0.13	−0.08
	Δ4-24	0.41	0.43	0.53	0.49	0.51	0.33
Ca^{2+} released	4	−0.80*	−0.76*	−0.91*	−0.91*	−0.92**	−0.81
	24	−0.74*	−0.69	−0.84*	−0.89*	−0.86*	−0.74
	Δ4-24	−0.70	−0.66	−0.80*	−0.87*	−0.82*	−0.70
Ca^{2+} % of total	4	−0.86*	−0.80*	−0.94*	−0.92**	−0.95**	−0.86*
Ca^{2+} % of total	24	−0.75*	−0.72*	−0.84*	−0.91*	−0.86*	−0.75
Mg^{2+} released	4	−0.61	−0.54	−0.79	−0.72	−0.77	−0.60
	24	−0.62	−0.55	−0.78	−0.77	−0.78	−0.62
	Δ4-24	−0.61	−0.55	−0.77	−0.79	−0.78	−0.62
Growth		–	0.97**	0.92**	0.91**	0.95**	0.99**
% Germination		0.97**	–	0.88*	0.87*	0.91*	0.98**

*,** Significant at the 0.05 and 0.01 levels, respectively.

† Correlations with growth and germination involve four cultivars and eight samples; all others involve three cultivars and six samples.

‡ Vigor index = (Growth + % Germination + Mean field emergence)/3.

tent. In contrast, more Na^+ was leached from unweathered than from weathered seeds; this probably reflects the higher initial Na^+ content of the unweathered Delcot 277 seeds. While the leaching of K^+ was sustained throughout at least 8 h, nearly all of the leaching of Na^+ occurred in the 1st hour. This contrasting pattern is consistent with the relative distributions of the ions between the embryo and the seed coat.

Although the differential loss of Na^+ from weathered and unweathered seeds largely reflected the initial Na^+ contents of the seed coats, that of K^+ did not. Substantially more K^+ was leached from weathered than from unweathered seeds, suggesting an increased membrane permeability in the weathered embryos. Further, there was a substantial difference between weathered and unweathered seeds for the ratio between K^+ and Na^+ lost to leaching. The higher K^+:Na^+ ratio in the leachate from weathered seeds would be consistent with embryonic membrane damage due to weathering.

Loomis and Smith (1980) observed no significant losses of Ca^{2+}, Mg^{2+}, or Mn^{2+} from artificially aged cabbage seeds after 16 h of leaching, although they did measure significant leaching of both K^+ and Cl^-. We found that significantly more of each of the divalent cations was leached from weathered than from unweathered seeds (Table 5), although very low percentages (between 0.5 and 7.5%) of the divalent cations were leached during 24 h. Our procedure, wherein we measured the ions in the leachate rather than those remaining in the seeds, may have provided greater sensitivity for distinguishing small differences. Since the divalent cations were initially present in the seeds at relatively low concentrations, relative sensitivities may be critical.

The release of K^+ and Ca^{2+} was negatively correlated with measures of seedling growth across all cultivars (Table 6). The increased imbibitional loss of these cations from weathered seeds, and the significance of these correlations, provide further indication of a loss of tissue organizational integrity, such as a destruction of cell wall and membrane structure and/or function. The significance of the correlations between the leaching of K^+ and Ca^{2+} and the vigor measurements indicates that the rates at which these cations are leached from seeds may have promise as indices of vigor. Expressing the leaching of Ca^{2+} as a percentage of the initial content increased the correlation, but similar normalization of the leaching of the other cations did not improve the correlations with vigor measures (data not shown). Leaching of Na^+, even if significantly correlated with seedling vigor measures, would probably not be a desirable index, because nearly all of the cottonseed Na^+ is contained within the seed coat, and because Na^+ salts are often used to neutralize seeds after acid delinting. Each of these factors may confound measurements based upon the release of Na^+.

Electron Microscopy

The ultrastructure of Delcot 277 cottonseeds was strikingly altered by the field weathering (Fig. 3). The cytoplasm of unweathered seeds contained lipid bodies with darkly stained outer surfaces and it was fully packed with ribosomes. In contrast, the outer surfaces of lipid bodies from weathered seeds appeared to be diffuse rather than discrete, and no ribosomes were detected in the cytoplasm. Weathering deterioration was also exhibited by the protein bodies, which lost uniformity of staining intensity and developed many small areas of electron translucency upon weathering. The loss of membrane integrity, described above in the discussion of cation leaching, is consistent with the disappearance of the distinct outer surface of the lipid bodies.

That the lipid bodies appear to be the principle exhibitors of weathering deterioration is consistent with previous reports. Halloin (1981) indicated that high levels of free fatty acids were primary indicators of weathering, and the morphological alteration of the lipid bodies might well be associated with the formation of lipid breakdown products such as free fatty acids. Further, Krieg and Carroll (1978) showed that lipid consumption was particularly important during early germination of cottonseeds, so reactions in the lipid bodies would be expected to develop rapidly once the seed became hydrated. These ultrastructural contrasts support the conclusion that the loss of membrane integrity accompanied the weathering deterioration of these cottonseeds.

CONCLUSIONS

We found that germination, early seedling growth, and field emergence of seedlings were reduced by

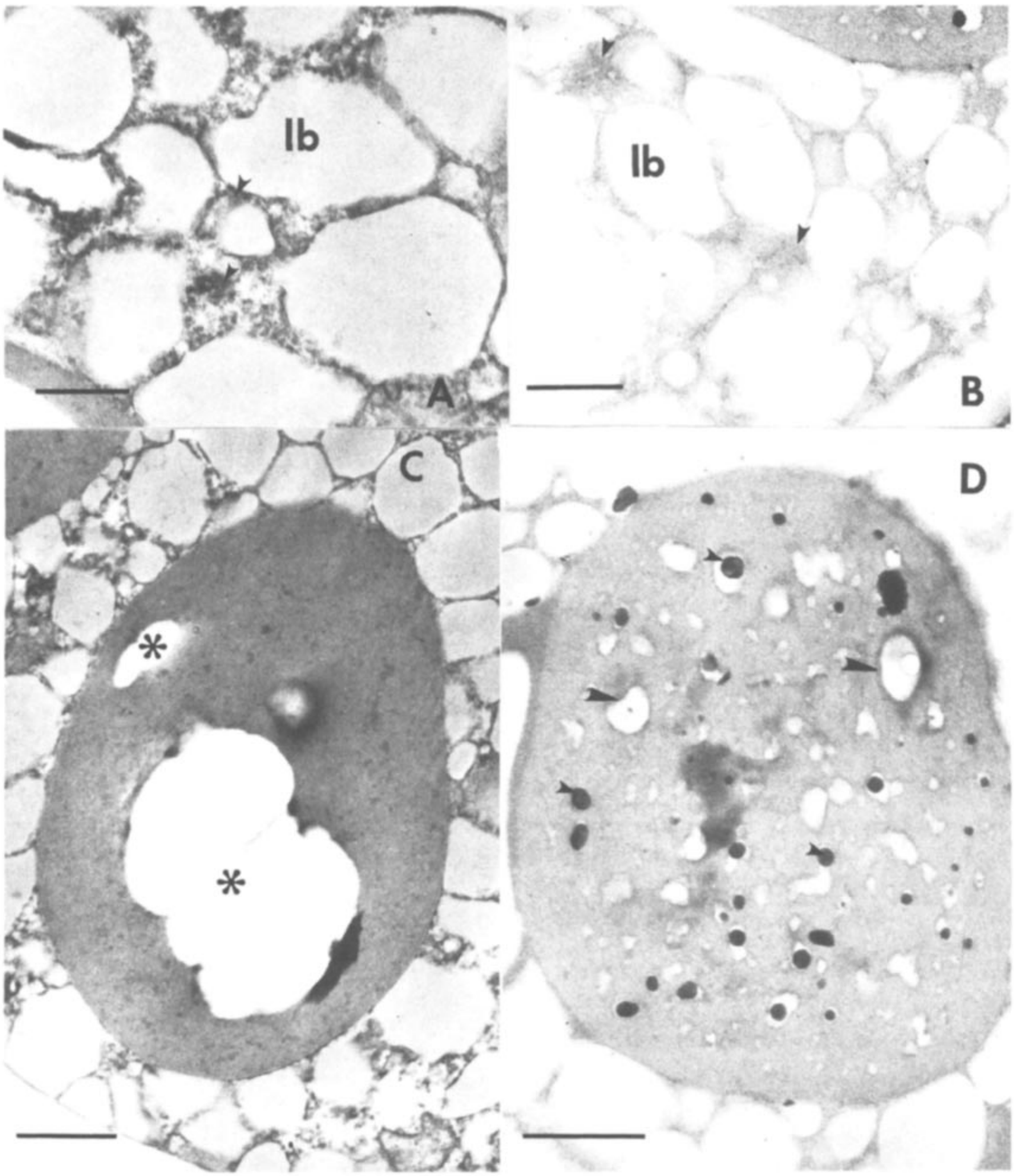

Fig. 3. Electron microscopy of cotyledons from Delcot 277 cottonseeds. A. In an unweathered seed, lipid bodies (lb) exhibit distinct borders and the cytoplasm (arrows) is dense with ribosomes. B. In a weathered seed, the distinct borders of the lipid bodies (lb) are missing and the cytoplasm (arrows) appears pale and lacks structurally distinguishable ribosomes. C. Protein bodies in an unweathered seed appear uniformly electron opaque and contain globoids of phytic acid (*) that are lost when the tissue section is exposed to the electron beam. D. Protein bodies in a weathered seed are much less uniform, and contain areas that are relatively electron translucent (large arrows) and areas that are much more electron dense (smaller arrows). Bar = 0.5 μm in A and B, 1.0 μm in C and D.

preharvest weathering of cottonseeds. Cultivars appeared to differ in sensitivity to this weathering deterioration, which affected respiratory metabolism and membrane permeability of the seeds during early germination. Increased membrane permeability was shown by greater leaching of K^+, Ca^{2+}, Mn^{2+}, and Mg^{2+} during early imbibition. The imbibitional leaching of K^+ and Ca^{2+} were better correlated with germination performance than were other physiological measurements. Over 90% of the Na^+ present in the seeds was contained in the seed coats, perhaps as a result of the use of sodium bicarbonate to neutralize the seeds after acid delinting. Leaching of K^+ and Ca^{2+} could be measured with adequate precision to distinguish between weathering sensitive and insensitive cultivars, and may warrant consideration as practical measures of seed vigor. Respiratory metabolism which has been associated with seed vigor in other species (Anderson, 1970; Cantrell et al., 1972; Woodstock and Grabe, 1967) did not prove to be as sensitive an indicator of seed quality as the leaching of specific cations.

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