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Volumetric Components of Seed Imbibition

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ABSTRACT

Swelling parameters were determined for 15 seed species; the swelling quotients were found to range from less than 1.1 (castorbean) to 2.8 (mungbean) and swelling coefficients ranged from 0.09 (castorbean) to 0.42 (cowpea). Swelling leads to a lowering of specific gravity of those seeds with high initial specific gravity, and an elevation of those with low initial specific gravity. The extent of swelling bears a linear relationship with moisture content. Redrying to air dryness only partially restores the original volume, but oven drying completely restores it. Temperatures alter both the rate and the extent of swelling. Solutes in the imbibing solution alter the dynamics of the volume increases, indicating several types of influences; these include osmotic effects, salt effects, valence effects, pH effects, and lyotropic effects. It is suggested that deformation resulting from imbibitional swelling may contribute to the stresses experienced by seed tissues during hydration.

Seed imbibition includes two simultaneous processes: the entry of water into the seed, and the swelling of seed polymers. The swelling of the seed, resulting from the expansion of polymers and the intercalation of water between polymers may be expected to create forces of deformation in the expanding tissues. The swelling component of seed imbibition has received very little experimental attention in half a century. The present experiments were undertaken as a part of a study of imbibitional events that may relate to limitations on seed vigor and viability.

MATERIALS AND METHODS

Volume determinations were made usually using 5 g of seeds (screened for uniformity of size), their volumes being measured by water displacement in a 25 ml Hubbard-Carmick specific gravity bottle. Although it is more usual to use xylene displacement for volume determinations (17), there were some undesirable after-effects attributable to the xylene; volume measurements with water displacement could be completed within 0.5 min and any changes in volume during that time were negligible. Time courses of weight and volumetric changes were obtained for seeds immersed in water. In cases where temperatures were controlled, the bottles were kept in a thermostatically controlled water bath during the imbibition time course. There was no radicle emergence during these experiments because of the continual immersion in water.

Swelling coefficients describe the rate of swelling in proportion to total swelling; they were determined using the integrated form of Pacheles' equation:

$$kt = -\log_e \frac{(a_{max} - a)}{a_{max}}$$

where a is the volume at time t, a_{max} is the volume at full hydration (24 h), and k is the swelling coefficient, distinctive for the material under investigation. Kühne and Kausch (8) used this equation in a study of pea imbibition, though the parameter they measured was weight gain rather than volume increase. We measured volume increases at various time intervals, and the calculated $\log_e \frac{(a_{max} - a)}{a_{max}}$ was plotted against t to yield a straight line of slope = $-k$.

For measurements of temperature effects on equilibrated volumes, 5 g of soybeans were fully imbibed (24 h) in specific gravity bottles filled with water, which were then immersed in a controlled temperature bath and held at each temperature for at least 1.5 h before the seed volume was determined. Temperatures were changed in a rising sequence in each experiment. The experiment was conducted 12 times, and the data are reported as volume means for the entire sample.

The soybean seeds used for the volumetric experiments were 1981 seeds of cv Cutler.

RESULTS

The progress of seed imbibition has been generally followed as an increase in weight; yet the volumetric increase may have markedly different dynamics (13, 16). To illustrate such differences in common seed species, the time course of weight gain and volume increases for seeds of soybean, corn, and castorbean are shown in Figure 1. It is evident that volumetric increases outstrip weight gains in soybean; the reverse is true for castorbean, in which weight gain exceeds the volume increases. Also there are striking differences in the extent of volumetric changes, with soybean showing enormous swelling during imbibition, corn showing intermediate swelling, and castorbean showing very little swelling.

The swelling of polymers upon hydration occurs at maximal rate during initial hydration, and slows as the swelling approaches a maximum. The changes in volume are described by Pascheles' equation

$$\frac{da}{dt} = k(a_{max} - a)$$

The dynamics of imbibition fit first order reaction kinetics (*e.g.* for soybean $R = -0.996$). In Table I, comparative data are reported for 15 species of seeds, comparing the quotient of weights for dry and imbibed seeds with the swelling quotient, and the swelling coefficient. The quotients indicate that swelling outstrips weight gain for most of the seeds tested. Furthermore, the seeds with high swelling quotients tend to have high swelling coefficients, such as soybean, limabean, kidney bean, pea, and cowpea, all of which swell to much more than double their initial volume. At the other extreme, cucumber, oat, and castorbean show lower swelling quotients than weight quotients. The swelling coefficients range from 0.421 for cowpea, which experiences

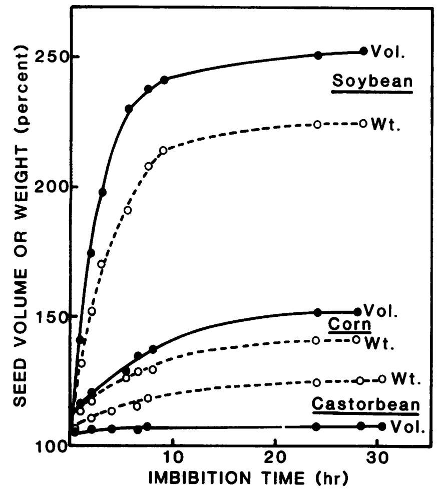

FIG. 1. Representative time course plots for volume and weight gains during imbibition of soybean, corn, and castorbean seeds.

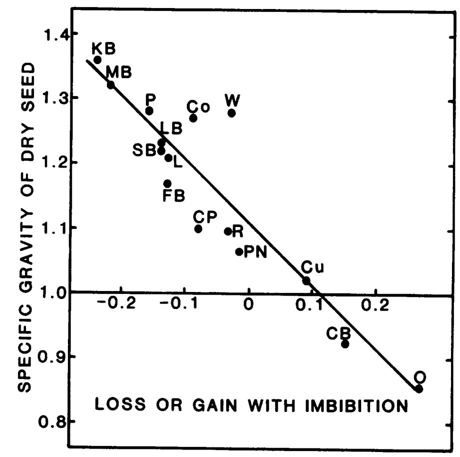

FIG. 2. The specific gravity of dry seeds of 15 species, and its relationship to loss or gain of specific gravity during imbibition. Slope = −0.077, $R = -0.923$. KB = kidneybean; MB = mungbean (Blackeye); P = Alaska pea; W = wheat (Little Club); Co = corn (Merit); LB = limabean (Fordhook); SB = soybean (Cutler); L = lettuce (Grand Rapids); FB = favabean; CP = cowpea (California Buckeye); R = radish; PN = peanut; Cu = cucumber (Straight Eight); CB = castorbean; O = oat (Astro).

Table I. *Parameters of Weight and Volume Increases during Imbibition of Various Seeds*

Weight and swelling quotients were calculated after 24-h imbibition; swelling coefficients were calculated from the volume changes. * indicates seeds in which seedcoats were nicked with a razorblade to facilitate imbibition.

Species	Weight Quotient (W/W_0)	Swelling Quotient V/V_0	Swelling Coefficient (k)
			h^{-1}
Soybean	2.28	2.60	0.325
Mungbean	2.18	2.82	0.180
Limabean*	2.12	2.54	0.293
Kidney bean*	2.04	2.47	0.411
Pea*	2.08	2.38	0.313
Lettuce	2.06	2.30	0.188
Cowpea	2.12	2.32	0.421
Fava bean*	2.06	2.22	0.235
Radish	1.75	1.82	0.255
Peanut	1.66	1.72	0.318
Corn	1.41	1.52	0.157
Wheat	1.46	1.49	0.113
Cucumber	1.37	1.28	0.225
Oat	1.64	1.25	0.153
Castorbean	1.24	1.09	0.092

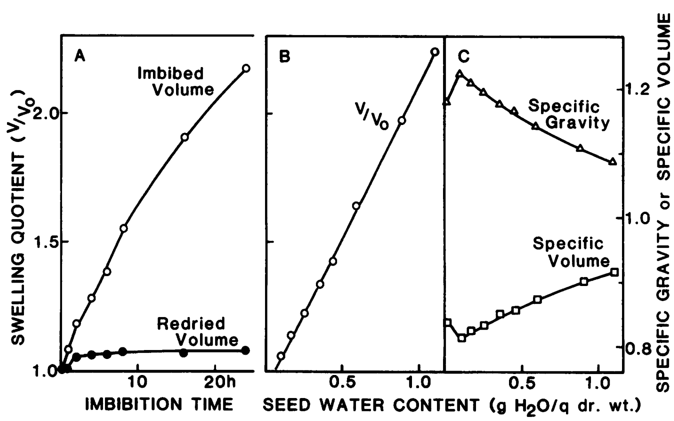

FIG. 3. Relationship between water content and soybean seed volume. A, Changes in the swelling quotient when the seeds are allowed to imbibe free water for differing periods of time, and the degree of recovery of original volume when the seeds were subsequently redried to air dryness. B, The relationship between the swelling quotient and the seed water content. C, Calculated specific gravity and specific volume in relation to seed water content. All data are from the same experiment.

a doubling of its volume in about 3 h, to 0.113 in wheat and 0.092 in castorbean, which experience very slow swelling. The high coefficients for soybean, limabean, kidney bean, and cowpea indicate that these species might suffer physical damage from the rapid volume increases.

Comparison of swelling quotients for isolated cotyledons and axes of soybean seeds reveals that the swelling of axes is almost one-third greater than that of cotyledons, the actual quotients being 3.20 ± 0.05% for axes, and 2.45 ± 0.01% for cotyledons. If stresses caused by swelling contribute to damage to the imbibing seed, the axis will be more acutely affected than the cotyledon.

The specific gravities of 15 seed species are shown in Figure 2, plotting the specific gravity of the dry seed on the ordinate, and the extent of loss or gain of specific gravity with imbibition on the abscissa.

The volume occupied by a polymer generally has a linear proportionality to water content (*e.g.* 11); a similar relationship is obtained for soybean seeds (Fig. 3B). The volumetric effect of a given increase in water is greater than the amount of water entering, indicating that the hydration event does not involve simple insertion of water between polymer plates in the seed; one can infer instead that there are configurational changes in the seed polymers with hydration which cause an exaggeration of volume increases over the amount of water entering. When the soybeans in Figure 3A were dried to their original dry weight, they did not fully recover their original volumes. However, if they were then oven-dried, the original volume was restored (data not shown).

Data from the same experiment were used to calculate the specific gravity and the specific volume of the seeds at the various water contents (Fig. 3C). It can be seen that specific gravity decreases as water content rises above about 0.1 g H_2O/g dry weight, and inversely the specific volume increases over the same range of water contents. The inflection of these two parameters at about 0.1 is common to protein volumetric changes, and has been interpreted as a widening of the packing characteristics of proteins when the tightest shell of bound water is removed (11).

The swelling of polymers shows a marked sensitivity to solutes

Table II. *Effects of Various Solutes on Volume Increases by Imbibing Soybean Seeds*

Each solute was present during imbibition at 1 M concentration.

Solute	Volume Increase (V/V_0)		
	8 h	24 h	48 h
NaOH	2.83	2.71	
Urea	2.53		
Water alone	2.40	2.50	2.52
NaI	1.90	2.43	2.71
KNO_3	2.00	2.40	
$NaNO_3$	1.85	2.27	2.54
NaCl	1.75	2.02	2.28
NH_4Cl	1.78	2.10	2.44
Sucrose	1.70	1.87	1.96
$MgCl_2$	1.60	1.83	2.10
Na_2SO_4	1.56	1.70	1.81

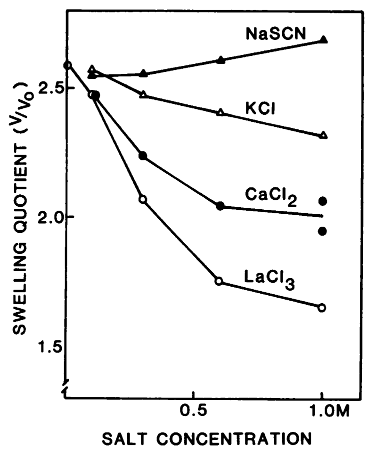

FIG. 4. Effects of various salts on the swelling quotient of soybean seeds. All data are for 24-h imbibition.

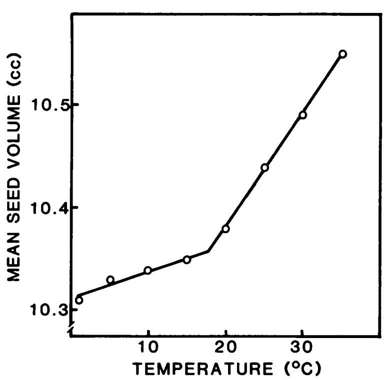

FIG. 5. Equilibration of fully imbibed soybeans at various temperatures (2 to 35°C) results in changes in the seed volume. Average of 12 experiments, each of which started with 5 g dry soybeans.

(*e.g.* 5, 18); among the features of the solute effects are substantial enhancement of swelling by alkali, and by iodide, inhibition by Na_2SO_4 and by other salts, increasing with valency, and with lyotropic effects. Some examples of solute effects on the swelling of imbibing soybeans are shown in Table II and Figure 4. Marked increases in swelling are obtained with NaOH, NaSCN, and urea, and by NaI in late stages of imbibition. Most of the salts tested have retarding effects on swelling, and greater inhibitions are obtained with cations with valences of 2 or 3.

Temperature has an expected effect on the rate and extent of swelling, and comparisons of seed swelling at 20 and 5°C indicate a temperature coefficient of between 1.2 and 1.6. The time required for completion of imbibition in soybeans is essentially doubled at the lower temperature. Temperature also affects the equilibrium volume of polymers (6); when fully imbibed soybeans were held for more than 1.5 h at various steps of elevated temperature, a volumetric increase at higher temperatures is likewise observed (Fig. 5). The equilibrium volume increases with temperature, but the slope of the temperature response is lesser for the temperature range between 1 and 15°C than for the range between 20 and 35°C. A similar change in slope was observed for fully imbibed pea seeds (data not shown).

DISCUSSION

The swelling of seeds with the entry of water is seen to have many of the characteristics of the swelling of polymers upon hydration (7). Among the characteristics shared by imbibing seeds and hydrating polymers are (a) an initial linear change in volume with water content, (b) a minimum specific volume at about 0.1 g H_2O/g dry weight, (c) a retardation of swelling at lowered temperatures and changed equilibrium volume with temperature, and (d) a sensitivity of swelling to solutes which either cover hydrophilic end groups (*e.g.* Na_2SO_4), which cause dissociation of hydrophilic end groups (*e.g.* NaOH), which have osmotic effects (*e.g.* sucrose), or valence effects (*e.g.* La^{3+}, Ca^{2+}, Na^+), or which alter some other polymer function through lyotropic effects (*e.g.* NaSCN, urea).

Using the analogy of seed swelling with polymer swelling, one would expect that increases in volume could exceed the increases in water content to the extent that polymeric seed constituents unfolded with hydration. A contrasting interpretation has been offered by Chung *et al.* (1) who calculated that the entering water simply took up spaces in 'cracks' between the tissue components. The data in Figure 1 and 3B indicate that the volume increases of soybeans with hydration are substantially greater than the weight of water imbibed.

Some interesting comparisons can be drawn between the data reported here and the pea imbibition data reported by Kühne and Kausch (10). Using weight increases, Kühne and Kausch calculated an imbibition rate constant of 0.52 h^{-1} for peas; this value is higher than the swelling coefficient of 0.31 which we obtained (Table I). The higher value reported by Kühne and Kausch may reflect in part their using a higher temperature (25°C), but a substantial part may be due to varietal differences which can have substantial effects on imbibition performance. Their experiments yielded temperature coefficients of 1.3 to 1.5 (9), which are not unlike the coefficients obtained in our experiments.

Kühne and Kausch were unable to detect differences in the equilibrium weight as a function of temperature, whereas our results for soybean and pea indicate a nonlinear alteration of equilibrium volume with temperature. The temperature effects on volume are so small that the limited sample size of the earlier work would not have revealed it. When we calculate temperature effects on equilibrium weight from our experiments, we get a similar nonlinear effect as for volume. The break in the temperature/volume curve suggests that a phase change occurs at temperatures slightly below 20°C; we have previously shown (15) that membrane lipids of soybean show no phase change in the biological temperature range which is detectable with differential scanning calorimetry, so the phase change inferred from the data in Figure 5 might be expected to reflect characteristics of bulk lipids or proteins in the soybean seed.

We have previously reported (17) that among the major components of the soybean seed, the cell walls show the highest degree of hydrophilicity, followed closely by proteins. It is reasonable to assume that the cell walls and proteins are the major components which contribute to the swelling of soybeans upon imbibition.

A linear deformation occurs during swelling of polymers which is proportional to the extent of volume increase (4). In imbibing seeds, such deformation should be expected to result sometimes in actual tissue rupture, especially in seeds which have the largest swelling quotients; in fact, rupture of cotyledons (2, 12, 14) and radicles (3) is known to occur in imbibing soybean, limabean, and kidney bean, each of which has a large swelling quotient (Table I). The large extent of imbibitional swelling in seeds, and especially in the radicles of seeds, is suggestive of a disruptive force which might logically contribute to the stresses experienced by the hydrating tissues during imbibition.

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