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Water Uptake and Heat Evolution by Germinating Cotton Seed

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If the rate at which water is taken up during the early stages of germination has been studied for the seeds of many plants, the information on the cotton seed is not very abundant in that respect. On the other hand, no parallel studies on the kinetics of water uptake and heat evolution have been made. The purpose of this investigation was to gather information about the kinetics of both processes during the first day of germination of the cotton seed.

Materials and Methods

Cotton seeds (*Gossypium hirsutum*, L. var. 1021-849) from the 1961 harvest at the Lubarika Agricultural Station of the Ruzizi Valley (Congo) were used. They were delinted by treatment with concentrated sulfuric acid for about 5 minutes followed by rinsing with water. During rinsing, the floating seeds were eliminated. After wiping off excess moisture, they were stored at 25° and 40% relative humidity. Water uptake was made to occur in a cylindrical glass vessel with a fritted glass bottom plate through which a stream of water-saturated air was passed. This vessel with a shallow layer of distilled water was kept in a water thermostat, the temperature of which was kept constant within 0.1° from 20° to 47.5°. Water uptake was measured on a 10 seed lot, after wiping with blotting paper, by weighing after a given period of time. The wet weight determination was followed by oven drying at 105°.

Heat evolution was measured in a Calvet Microcalorimeter (2, 3) on groups of 5 seed (about 0.4 g dry matter) to which 0.5 ml water was added. The observation of the heat evolution was made during periods of time ranging from 3 to 24 hours and the water uptake was determined at the completion of each experiment. The initial moisture content of the seeds which had been kept under the conditions indicated above namely 25° and 40% relative humidity varied from 7 to 10% and their viability was about 90%.

Results

When the water uptake during the first 12 hours is plotted against time, fairly smooth sigmoid curves are obtained (fig 1). It should be observed that the variability of the results is fairly low even though each experimental point corresponds to a different 10 seed lot. The sigmoid aspect of the curves sug-

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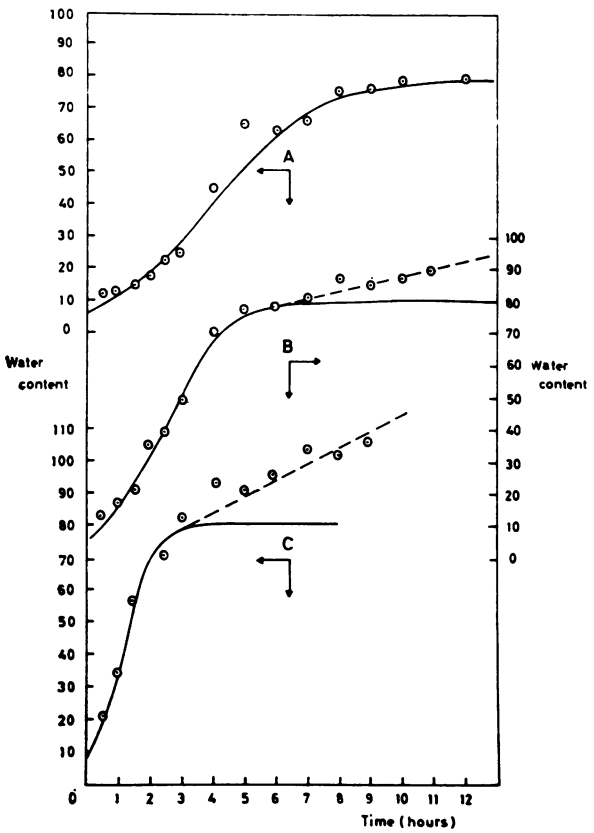

FIG. 1. Time course of water uptake (on a dry weight basis) of the cotton seed. The full lines are calculated from equation I with $k_A W_s = 0.6$ hour^{-1} at 20° (A), $k_A W_s = 1.0$ hour^{-1} at 30° (B), and $k_A W_s = 2.11$ hour^{-1} at 47.5° (C). Common asymptotic value at the 3 temperatures: 80% water on a dry weight basis.

gest that the kinetics of the initial water uptake might follow an autocatalytic rate law.

If W and W_s are water contents at time t and after an infinite time, no uptake processes other than those governed by the autocatalytic rate law being operative, then we should have the following differential equation for the rate of water uptake, where k_A is a constant at a given temperature:

$$\frac{dW}{dt} = k_A W(W_s - W) \qquad \text{I}$$

with the initial condition,

$$W = W_o \text{ for } t = o, \qquad \text{II}$$

integration of equation I gives

$$W = \frac{W_o W_s}{W_o + (W_s - W_o) \exp(-K_A W_s t)} \qquad \text{III}$$

According to equation III a plot of log $[W/(W_s - W)]$ against the time t should give a straight line from the slope of which the value of k_A, the autocatalytic water uptake constant may be calculated.

Since the slope represents actually the product $k_A W_s$, a common asymptotic value W_s has to be assumed. We have taken 80% water by weight at all temperatures studied. The semilogarithmic plots are not shown since the constants k_A were used to calculate the curves of figure 1 using formula III and the common limiting value of W_s mentioned above.

The agreement between the calculated curve and the experimental points is satisfactory, especially at the lowest temperature. At higher temperatures, the steepness of the autocatalytic rate law could indicate that the water uptake can be represented by a rapid linear phase followed by a slower linear phase. This is unlikely since one parameter only, k_A, is adjusted for fitting the theoretical curves to the experimental results at various temperatures and the deviation from the linearity at lowest temperatures are apparent.

When the values of the water uptake coefficient k_A are plotted in an Arrhenius graph a straight line relationship is obtained which allows the calculation of an apparent activation energy of 8.2 Kcal/mole (fig 2).

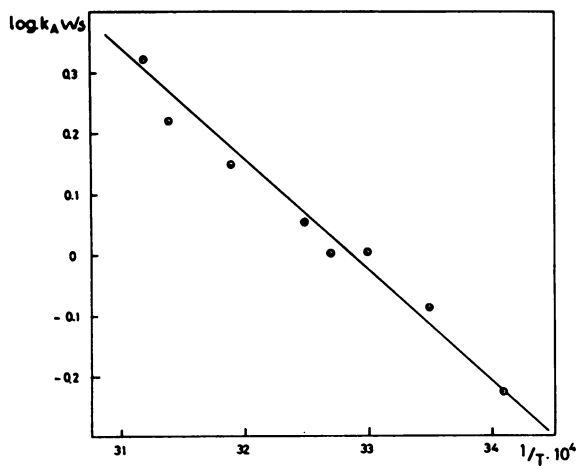

FIG. 2. Arrhenius graph of the parameter $k_A W_s$ of equation I.

After the water uptake has followed the autocatalytic law up to 80% water (on dry matter basis), it proceeds then linearly (broken lines of fig 1). The time lag of the linear water uptake may be defined as the abcissa of the intercept of the straight line representing the constant rate of water uptake and the horizontal line $W = W_s$. The water uptake at constant rate and its time lag are closely related to temperature as shown in table I.

The apparent activation energy of the linear water uptake coefficient is found to be 7.1 Kcal/mole as calculated from the slope of a straight line drawn through the points of an Arrhenius graph (fig 3). The kinetics of water uptake are very similar to those of the heat evolution measured at 30° in the Calvet microcalorimeter.

Table I

Linear Water Uptake Coefficient K_L and Its Time Lag

Temperature	K_L*	Time lag Hours
20.0	.....	> 12
22.5	1.85	9
25.0	2.20	7
27.5	2.50	8
30.0	2.36	6
32.5	2.83	6
35.0	2.57	5
40.0	3.50	4
45.0	4.33	3
47.5	4.31	2.5

* Grams of water per 100 g dry matter per hour.

Figure 4A shows the uncorrected rate of heat evolution as a function of the time during the first 24 hours of germination. In agreement with the data presented in table I, heat evolution ceases after about 4 to 5 hours. The exothermal phase is followed by a fairly long period where no heat is evolved or where a very slight but reproducible heat uptake takes place. Although it is very slight, less than 0.05 cal per hour per gram dry weight, this endothermal phase is always found to occur.

Figure 4B shows another heat evolution curve which has been corrected for the time constant of the calorimeter. In this experiment the heat evolution ends somewhat earlier than in curve A, roughly 3.5 hours instead of 4 to 5. Figure 4C shows the cumulative heat evolution during the first 3 hours, calculated by graphical integration of the differential curve B. Here again the sigmoid aspect of the curve is apparent. This was to be expected since the heat of wetting of the seed tissue must follow a time course similar to that of the water uptake.

We may admit that the differential rate law for

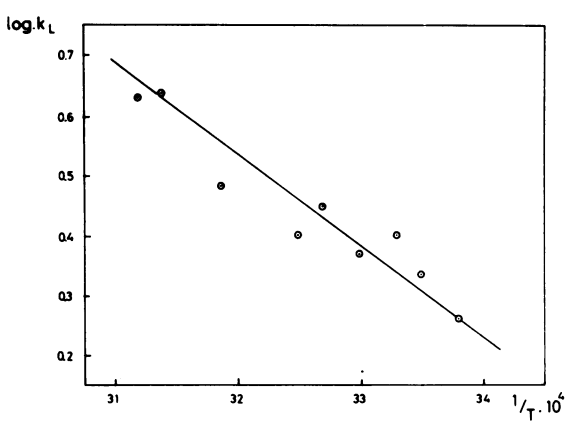

FIG. 3. Arrhenius graph of the slopes of the non-autocatalytic water uptake (shown by broken lines in fig. 1 B and C).

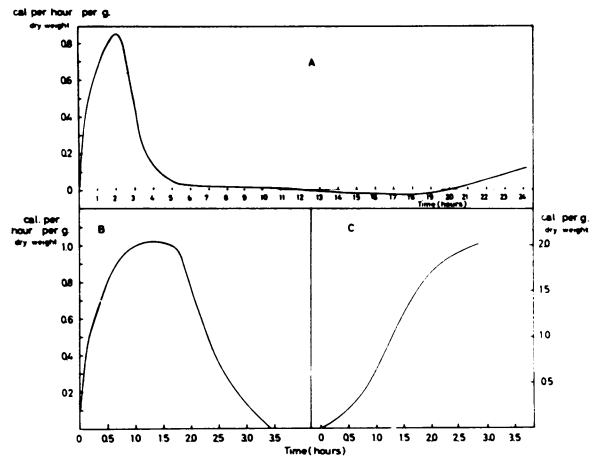

FIG. 4. A, uncorrected rate of heat evolution (calories per hour per gram dry wt) during the first day of germination of the cotton seed. B, same curve for the first 3.5 hours but corrected for the time constant of the calorimeter. C, integrated from (b) giving the total amount of heat evolved in calories per gram dry weight (measurement temperature 30°).

the heat evolution is similar to the one expressed by equation I for water uptake. If Q is the amount of heat evolved after time t, and Q_s the total amount of heat evolved, we should have (where k is a constant),

$$\frac{dQ}{dt} = kQ(Q_s - Q) \qquad \text{IV}$$

Integration gives

$$\ln [Q(Q_s - Q)] = k Q_s t + \text{constant} \qquad \text{V}$$

The adaption of the integration constant is less direct here than it was for water uptake. The rate of heat evolution at $t = 0$ is finite although very small and not measurable experimentally. Therefore, a somewhat artificial determination of the integration constant is made by noting that after a certain time $t_{1/2}$ an amount of heat $Q_s/2$ has been evolved. This gives then

$$Q = \frac{Q_s}{1 + \exp [-kQ_s (t - t_{1/2})]} \qquad \text{VI}$$

If equation VI is followed, plotting the curve of figure 4C in semilogarithmic fashion namely log $(Q/(Q_s - Q))$ vs. time should have given straight lines. This is apparent from figure 5 where 2 integrated heat evolution curves have been plotted in this manner. The slope of the straight line allows the calculation of the parameter kQ_s which has a value of about 2.3 hour^{-1}. The time course of heat evolution at 30° is thus more rapid than the water uptake at the same temperature. The asymptotic values which have been chosen by inspection for fitting the experimental sigmoid curves to the semilogarithmic plots of figure 5 were 2.08 and 1.60 cal per gram dry matter. It may thus be inferred that the average heat

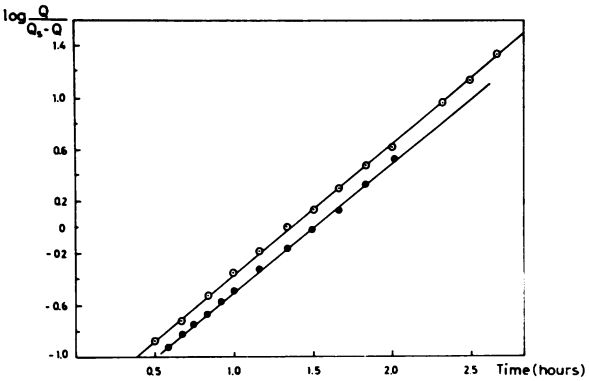

FIG. 5. Plot of the heat evolution from 2 different experiments according to equation (2).

of wetting of the cotton seed tissue is of the order of magnitude of 2 cal per gram dry matter.

Discussion

Qualitatively the results presented here agree with most other observations on the water uptake by germinating seeds. A period of rapid uptake usually attributed to a simple physical wetting of the seed tissues is followed by a slower uptake which is metabolic in nature. The slower uptake which occurs at constant rate may be thought to pertain to a quasi-stationary state of water uptake, the kinetics of which are of zero order. This state will obviously be continuously modified by changes induced by the growth process.

Between these 2 steps a lag may occur during which water is absorbed very slowly (6, 7, 9, 12,14). Since the cotton seed germinates very rapidly, there is obviously no lag between the purely physical and the metabolic water uptake. This is apparent from the data of table I which shows that at temperatures higher than 20° a metabolic water uptake occurs less than 10 hours after the seed has been brought into contact with water.

Quantitatively, the attempts which have been made in the past for describing the kinetics of the first step of water uptake were based either on purely empirical formulations (5, 11), on solutions of Fick's second law of diffusion (4) or a combination of both (10). The latter were made under the assumption that the diffusion coefficient of water was not concentration dependent.

This is unlikely in an unsaturated medium with colloïdal properties such as seed tissues, especially when water uptake is initiated in fairly dry seed material. This may be the reason why water uptake may be described satisfactorily by solution of the diffusion equation when not too dry seeds are used. This results, in the case of the cotton seed, in the disappearance of the inflection point of the water-uptake time curve. The formulation which has been presented here amounts to the mathematical translation of the observation that water uptake proceeds at a rate which is proportional to the water content and to the difference between it and that at saturation of the material.

It seems reasonable to admit that the rate of water uptake is proportional to a driving force resulting from the difference between the water content at saturation and that at a given time. Further the diffusivity of water in the seed tissues must increase with their water content. Hence the autocatalytic rate law expressed by equation I.

The agreement of the theoretical curve deduced from this assumption and the experimental points offers the advantage that the first stage of water uptake may be characterized by a single temperature-dependent parameter which has been termed k_A above. This greatly facilitates the study of the effect of temperature on the water uptake. The apparent activation energy 8.2 Kcal/mole which has been found is comparable to the values which have been observed by other authors for the influence of temperature on the diffusivity of water into seed or other plant material.

Fan et al (4) found for the water uptake by wheat kernels, activation energies which varied from 10.2 to 11.2 Kcal/mole up to 65°. For the same material Becker (1) found 12.3 Kcal. The activation energies for water transport were found to be 8.3 and 9.4 for whole tomato and sunflower plants (8).

It is to be expected that a metabolic water uptake proceeds at a constant rate at least at the quasi-stationary state. This may be more precisely defined as the state where increase of the rate of water uptake due to the growth process is negligible within the experimental error. The effect of the temperature on the rate of this metabolic water uptake can be expressed by an activation energy of 7.1 Kcal/mole. This is a value which is often found for many enzyme catalyzed reaction. Since this value is fairly low it might be concluded that temperature does not exert a very marked effect on the rate of water uptake by the cotton seed. There is however the fact to be considered that the lag before the metabolic water uptake is initiated is also temperature dependent, and consequently the total amount of water which has been taken up by a seed during the first day of germination is more strongly temperature dependent than the temperature characteristic of the constant rate of water uptake would suggest.

The calorimetric study of the first step of water uptake supplies a confirmation of the kinetics of this process and at the same time an estimation of the heat of wetting of the material.

Summary

The water uptake of dry cotton seeds (*Gossypium hirsutum* L. var. 1021–849) can be represented by an autocatalytic rate law followed by a metabolic uptake which proceeds according to zero order kinetics.

The temperature-dependent parameters which occur in the autocatalytic rate law and the zero order kinetics have apparent activation energies of 8.2 and 7.1 Kcal/mole respectively.

The time course of the evolution of the heat of wetting can be expressed in the same way as the first step of water uptake. The heat of wetting of the cotton seed tissue is of the order of 2 cal per gram dry matter.

Acknowledgments

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Biosynthesis of Ricinine in Excised Roots of Ricinus communis [1, 2]

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The alkaloid, ricinine, has been used to study the biosynthesis of the pyridine nucleus in plants. The degradation of this compound following the in vivo incorporation of labeled precursors has made possible the tentative designation of compounds which contribute to the pyridine ring (3, 5, 7, 8, 9). Plant biosynthesis studies of this nature can be facilitated by utilizing the tissue with the highest alkaloid synthesizing activity.

Solt and co-workers (6) have found that sterile root cultures of *Nicotiana glauca* provided a uniform, controlled system capable of producing alkaloids in the same manner as the intact plant.

The work reported here was initiated primarily to investigate the possibility of ricinine biosynthesis in root tissues independent of leaves and stem. The biosynthetic activity of root tissue was compared with that of the cotyledons and embryos reported previously (10). In addition, the root cultures under controlled conditions provided an isolated system to study further the effect of precursor levels on ricinine production. Preliminary evidence is presented for a control mechanism in ricinine biosynthesis, which may originate in root tissue. It is hoped that these results may stimulate further research on the role of alkaloids in plant metabolism.

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Materials and Methods

Excised Root Cultures. Sterilized castor seed, free of the seed coat were germinated at 30°. After 6 days, 5 roots each approximately 2 cm in length were excised and grown in the dark in 50 ml of modified Whites medium (4), supplemented with 5.0 mg kinetin, 0.1 mg thiamine HCl, and 1.0 mg glycine per liter. In certain experiments, this medium was further fortified with $NaNO_3$, NaH_2PO_4,