Role of Seed Coats in Delayed Germination

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RÔLE OF SEED COATS IN DELAYED GERMINATION.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY. LXXXV.

WILLIAM CROCKER.

(WITH FOUR FIGURES)

I. Historical.

IT is well known that in many species of plants not all the seeds of a given crop germinate promptly after being subjected to so-called germination conditions; instead they germinate at irregular intervals through a period of weeks, months, or even years. It happens in many species that none of the seeds of a crop will germinate until they have been subjected to germinative conditions for a year or more, and that in these cases of marked delay germination is distributed through a further period of greater or less length.

Delayed germination is well illustrated in the results of the researches of NOBBE and HÄNLEIN (**8**, *a*, *b*). Table I shows their observations on thirty-one species of common weeds. They began with 400 seeds of each species and continued their experiments 1,173 days.

KIENITZ (**4**) found marked distribution in the germination of crops of the beech, white fir, ash, hornbean, and pine; and WINKLER (**15**) in sowings of *Euphorbia cyparissias*, *E. exigua*, Cuscuta, etc. WIESNER (**14**) found that the seeds of *Viscum album* germinate only sparingly in the fall after ripening, but readily the following spring. KUNTZE (**6**) in reviewing the literature on germination mentions a large number of cases of delayed germination. The hawthorn, he states, will grow only after being in the ground one to three years.

One of the most interesting cases of delayed germination is that of the cocklebur (Xanthium) reported by ARTHUR (**1**). He found that the two seeds in the bur are not the exact counterparts of each other, but can be distinguished readily by their form and position in the bur. One seed, which he terms the upper because it is borne nearer the apical end of the bur, is convex on the outer face and con-

TABLE I.

AFTER NOBBE AND HÄNLEIN; 400 SEEDS OF EACH SPECIES.

Name/No. of seeds germinated on day	4	5	6	7	8	9	10	16	36	72	145	351	519	714	874	1082	1173	Total	Per ct.
1 Aquilegia vulgaris	..	..	..	...	...	...	..	3	..	..	..	..	...	...	...	...	...	3	0.75
2 Campanula rotundifolia	..	..	..	11	8	1	1	1	1	..	...	..	11	13	10	1	...	58	14.50
3 Campanula persicifolia	..	..	..	...	3	8	14	112	56	1	..	11	9	2	8	2	6	232	58.00
4 Campanula Trachelium	..	..	..	...	...	...	..	...	..	..	..	..	1	...	...	...	...	1	0.25
5 Chaerophyllum temulum	..	..	..	...	...	...	..	...	..	..	..	..	...	...	...	...	3	3	0.75
6 Chenopodium album	..	..	2	...	...	2	3	2	..	1	19	53	27	...	2	3	3	117	29.25
7 Capsella Bursa-pastoris	..	3	..	3	...	...	..	...	..	..	4	4	20	...	24	10	7	75	18.75
8 Chelidonium majus	..	..	..	...	...	...	..	...	59	8	2	..	...	3	90	1	32	195	48.75
9 Digitalis purpurea	..	..	..	9	45	102	47	159	24	1	..	..	...	...	rest	eca	yed	387	96.75
10 Hypericum hirsutum	..	..	..	2	1	4	13	132	58	..	..	2	...	...	...	...	...	212	53.00
11 Hypericum montanum	..	..	..	3	19	61	31	171	46	..	..	..	...	1	...	...	...	332	83.00
12 Hypericum perforatum	..	..	..	1	...	...	2	20	28	2	..	..	...	1	3	1	...	58	14.50
13 Jasione montana	..	..	17	98	141	81	30	28	2	..	..	..	...	...	rest	deca	yed	397	99.25
14 Lithospermum arvense	2	12	32	36	52	18	8	52	42	28	22	12	22	6	rest	deca	yed	344	86.00
15 Lysimachia vulgaris	..	..	..	...	...	...	..	...	..	..	..	..	...	1	...	...	...	1	0.25
16 Myosurus minimus	..	..	16	160	103	19	2	5	..	1	..	11	11	4	13	...	2	347	86.75
17 Oxalis corniculata	..	..	..	...	...	...	..	3	5	1	..	1	...	...	2	...	...	12	3.00
18 Papaver Argemone	..	..	5	65	116	16	8	34	30	15	4	26	17	...	...	...	...	336	84.00
19 Papaver dubium	..	..	2	216	169	...	..	1	..	..	..	..	...	...	...	...	...	388	97.00
20 Phyteuma spicatum	..	..	..	...	...	...	..	...	..	..	..	..	...	...	...	...	...	0	0.00
21 Plantago major	..	..	..	...	...	...	..	...	..	..	..	..	...	...	...	...	1	1	0.25
22 Plantago media	..	5	8	4	4	2	1	...	..	..	..	1	...	9	1	6	2	43	10.75
23 Polygonum persicaria	..	3	33	15	2	...	..	2	..	..	..	..	...	...	...	...	...	55	13.75
24 Primula elatior	..	..	..	...	...	...	..	...	..	..	..	..	...	...	...	...	...	0	0.00
25 Potentilla argentea	..	..	3	15	57	67	58	74	7	..	..	2	3	3	3	2	7	301	75.25
26 Scrophularia nodosa	..	..	1	5	27	96	42	47	6	..	..	..	...	...	7	...	...	231	57.75
27 Thlaspi alpestre	..	..	..	...	...	...	..	...	..	..	..	..	all	deca	yed	...	...	0	0.00
28 Thlaspi arvense	..	..	..	...	1	...	..	2	..	..	..	2	10	11	11	15	35	87	21.75
29 Verbascum nigrum	..	..	..	...	...	...	..	...	..	..	..	..	...	...	...	...	...	0	0.00
30 Veronica Beccabunga	..	..	..	...	...	...	..	1	1	1	6	25	42	103	...	4	...	183	45.75
31 Veronica officinalis	..	..	..	...	...	8	56	332	..	..	..	..	...	...	rest	deca	yed	396	99.00

cave on the inner. The lower seed, lying nearer the base of the bur, is concave on the outer face and convex on the inner. ARTHUR found that plantings of burs of the cocklebur (mainly *Xanthium canadense*) in a garden bed resulted in the germination of all the lower seeds in the first year after ripening, and of only a very small per cent. of the upper seeds. The second year a great majority of the upper seeds grew. A few of the upper ones, however, grew in the third and fourth years.

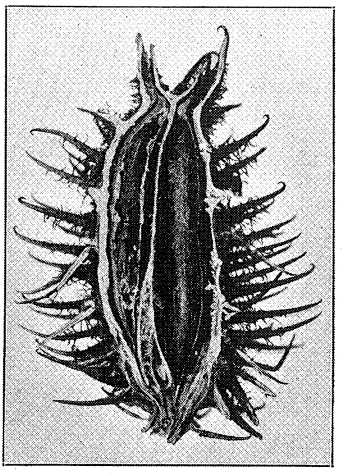

FIG. 1.—Cocklebur cut away to show upper and lower (dimorphic) seeds.

MASTERMAN (7) claims that both seeds grow the first year after they are planted. His methods, however, were not at all adapted for detecting whether the two seeds of the bur have markedly different germinative characters. Both ARTHUR'S work and the experimental results of this paper show that such differences in the germinative characters undoubtedly exist.

Delayed germination was found by NOBBE and HÄNLEIN to be due in many cases to the impermeability of the seed coat to water. As to the seeds listed in Table I, however, they say that in every case the seeds absorbed the water readily and yet lay in the germinator in a saturated condition for long periods, either not germinating at all or scattering their germination over a long period. They maintain that in cases where water is admitted the growth after long exposure to germinative conditions must be due to some change going on within the embryo during the period of rest. WINKLER (15), WIESNER (14), KIENITZ (4), and PFEFFER (9) expressed similar views.

ARTHUR found that both seeds of the cocklebur take up water readily while in the bur. The bur, therefore, does not account for the delay. He also believes that the extremely delicate seed coats are in no way different in the two seeds and that the structure of the seeds, therefore, offers no explanation of their germinative difference.

He suggests that enzymes are produced readily in the lower seeds and that, therefore, they have foods at hand to begin their growth immediately; whereas the upper seeds are able to develop digestive ferments only after a long period of rest, and hence their germination is delayed one or more years. This theory has its experimental basis in the fact that if both sorts of seeds are exposed to germinative conditions for some time the lower ones show much reducing sugar, while the upper ones have only a trace. My interest in this problem was aroused by ARTHUR'S paper on the cocklebur, and the work was begun for the purpose of testing this enzyme theory and determining definitely the cause of the delayed germination of the upper seed.

II. Materials and methods.

Most of the germinative tests reported in this paper were made between moist filter papers, but in all these cases corresponding tests with very similar results were made in fine quartz sand and in garden soil. For *Avena fatua*, Iris, and cocklebur seeds in the bur, all tests were made in sand and garden soil on account of the great liability of these structures to be attacked by fungi.

Five species of cocklebur were used: *Xanthium canadense* Mill., *X. echinatum* Murr., *X. glabratum* (DC.) Britton, *X. glanduliferum* Greene, and *X. speciosum* Kearney. In each species similar conditions gave similar results whether the seeds were in the bur or removed from it; but for convenience in handling and accuracy of the records the seeds were generally removed from the bur. For testing increased oxygen pressures the soaked seeds were allowed to rest on the walls of flasks containing oxygen or (in the checks) air. Germinative tests at high temperatures were made in ordinary paraffin ovens regulated to the desired temperature. The effect of temperature on the rate of diffusion of oxygen through the seed coats of the cocklebur was determined in a large water bath (such as is used in chemical laboratories for determining solubility, etc.) regulated to 0.01°.

The seeds used were collected when thoroughly ripe from various parts of the United States and Europe,[1] stored in a dry room, and used in experimentation within six months after collected. Since a year of dry storage and the region from which the seeds were

[1] I am indebted to M. P. DIEUDONNÉ for collections from Belgium.

gathered gave no marked germinative variations in the species used, the time of storage and the region in which they were collected need no further consideration. A seed was considered germinated when root hairs appeared, except in Xanthium and Iris, where late development of root hairs made this test worthless and the lengthening of the radicle 5^{mm} was considered the criterion of germination.

III. Experiments.

1. Cocklebur.

Effect of enzymes.—When work was begun on the seeds of the cocklebur, Arthur's enzyme theory was adopted as a working hypothesis, on the supposition that it would involve a study of the difference in the development and action of the enzymes in the two seeds. As Waugh (**13**), Stone (**10**), and Thompson (**11**) were able to increase markedly the germination of old seeds by soaking them in solutions of various enzymes, it was thought that perhaps the upper seeds of the cocklebur could be made to germinate without delay by merely soaking them in solutions of pepsin, or plant trypsin, or in filtered extracts from the germinated lower seeds. Experiments in this line gave only negative results, but led to the discovery that high temperatures cause the immediate germination of some of the upper seeds. The enzyme theory was abandoned after failures to detect any differences in the digestive activities of extracts of the upper and lower seeds. At this point the results showed that the difference in the germinative characters of the two seeds had other causes; hence a new line of experiments was begun.

Effect of high temperatures.—High temperatures bring about the immediate germination of the upper seeds of *X. echinatum*. Table II shows the results of temperature experiments with this species. Results were similar whether soil or filter paper was used for germinators and whether the seeds were in the burs or removed from them.

As Table II shows, the lower seeds of *X. echinatum* germinate readily at 22–24°, but even more readily at 32–34°. The upper seeds do not germinate at all at 22–24°, but respond readily at 32–34°. The lowest temperature at which any considerable per cent. of the upper seeds grow is at 33°, and the similar point for the lower seeds

TABLE II (*X. echinatum*).

TEMP. °C.	SEEDS	PER CENT. GERMINATED AFTER			
		1 day	2 days	5 days	8 days
22–24.....	upper	0	0	0	0
	lower	3	31	87	99
32–34.....	upper	8	55	99	99
	lower	23	100	100	100

is 23°. In *X. canadense*, *X. glabratum*, and *X. speciosum* the upper seeds germinate only sparingly at a constant temperature of 35°, but to a considerably larger per cent. at a temperature fluctuating between 25 and 40°. The lower seeds of *X. canadense* germinate readily at 18–21°, while the lower seeds of *X. glabratum* have a minimum germinative temperature of about 23°. The highest minimum germinative temperature yet reported, 15.6–18.5°, is recorded by DETMER (**2**) for the cucumber and watermelon.

From the above data it may be seen that in the cocklebur there are remarkably high minimum germinative temperatures. *X. echinatum*, the least remarkable of the four species studied in this respect, has this critical temperature 15° higher in the upper seed and 5° higher in the lower one than that of the watermelon and cucumber.

Effect of wounding.—In removing seeds from the burs the knife often clipped off a small portion of the distal ends of the cotyledons. It was observed that the upper seeds so wounded begin a marked growth in the wounded region even at the temperature of 20–22°. The growth gradually moves down the cotyledons until it reaches the radicle. This reverses the normal method of germination. Normally the radicle first pushes out, sets itself in the ground, and lifts the cotyledons above the soil, after which they begin their growth. When the upper seeds are wounded at the radicle end, either by a slight cut or a pin prick, germination takes place in the normal way. This observation suggested complete removal of the seed coat.

Effect of removing the seed coat.—After the seeds have been soaked six hours the extremely delicate seed coats can be removed, without the least injury to the embryo, by merely pinching the seed between thumb and finger. The coat-free upper and lower seeds of any one of the four species studied germinate with almost equal readiness at any

point within their temperature limits, and the two seeds have almost identical temperature limits. Table III shows the relative speed of

TABLE III.

X. canadense; COATS REMOVED; TEMP. 18–22°.

SEEDS	PER CENT. GERMINATED AFTER			
	3 days	4 days	6 days	9 days
Upper.....	47	75	84	100
Lower.....	51	77	89	100

germination of the upper and lower seeds of *X. canadense* at 18–22°. The minimum germinative temperature for the upper and lower seeds of this species with the seed coats removed is about 18°. The same point for other species mentioned is 2–3° higher. Each seed of Xanthium has then two minimum germinative temperatures: one with the seed coat intact, and a lower one with the seed coat removed. In the upper seeds these two temperatures differ by fifteen or more degrees and in the lower ones by two or more degrees. Table IV gives the approximate germinative minimum temperatures for each

TABLE IV.

MINIMUM GERMINATIVE TEMPERATURES.

Species	Seeds	Minimum temp. °C. coats intact	Minimum temp. °C. coats removed
X. canadense.....	upper	fluctuating 25–41	18
	lower	21	18
X. echinatum.....	upper	32–33	19–20
	lower	23	19–20
X. glabratum.....	upper	fluctuating 25–41	20
	lower	23	20
X. speciosum.....	upper	fluctuating 25–41	20
	lower	22	20

of the four species with seed coats intact and seed coats removed.

It is evident from the results so far given that ARTHUR'S statement that the difference between the two seeds does not lie external to the embryo, but in the embryos themselves, is entirely wrong. He overlooked the real point of difference, the seed coats, because of their extreme delicacy and because of the ease with which they admit water.

Effect of increased oxygen pressures.—Since the delay is secured by the seed coat, it must exclude either water or oxygen. As ARTHUR states, both seeds seem to take up water with equal readiness. I found that in eighteen hours of soaking, the upper seeds of *X. canadense* imbibed 51 per cent. of their dry weight, while the lower ones imbibed 62 per cent. In the same time the upper seeds of *X. echinatum* imbibed 48 per cent. and the lower ones 47 per cent. The difference then in water imbibition will not serve to explain the difference in the germinative characters of the two seeds.

It was found best in testing increased oxygen pressures to soak the seeds 12–18 hours and then allow them to stick to the walls of a flask or bottle containing the oxygen or (in the check) air. After being thus treated and kept at 21–23° for six days, the upper seeds of *X. canadense* gave 100 per cent. of germination in pure oxygen and 0 per cent. in air. The growth in the seeds germinated in oxygen at these relatively low temperatures does not take place in the normal way. It begins in the distal region of the cotyledons and works down toward the radicle, as was described for seeds wounded at the distal end of the cotyledons. This peculiarity seems to be related to the structural character of the seed coat.

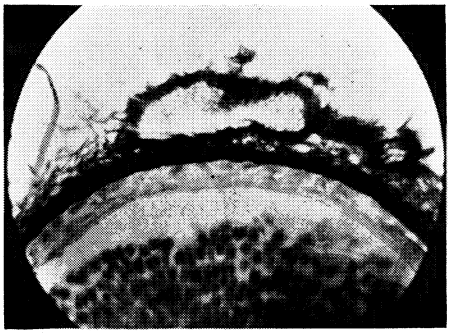

FIG. 2.—Cross section of the seed coat of Xanthium, showing the three layers.

The seed coat consists of three distinct layers (*fig. 2*). The outer layer consists of shell-like cell walls which are more and more collapsed as the inner portion of the layer is approached. This layer is traversed by several groups of tracheae which are parallel with the long axis of the seed. The middle layer is very dense, apparently consisting of collapsed cell walls and staining very deeply with safranin. The inner layer consists of 1–5 layers of cells containing protoplasm and nuclei. Each layer is thickest at the radicle end and gradually becomes thinner toward the distal end of the cotyledons.

The measurements of the three layers are approximately as follows: the outer layer, at the radicle end 100 μ, at the distal end 20 μ; the middle layer, at the radicle end 15 μ, at the distal end 8 μ; the inner layer, at the radicle end 30 μ, at the distal end 4 μ. The middle layer is somewhat thicker in the upper than in the lower seed. In both seeds it is less dense at the distal end. It seems probable that the denser middle layer is only partly permeable to oxygen, less so in the upper than in the lower seed, and less so at the distal than at the radicle end. This accounts for the increased oxygen pressure initiating the growth at the distal end.

Gaseous exchanges in respiration.—The two sets of experiments on the gaseous exchanges of the seeds in respiration throw some light on the effect of the seed coats. The first set, reported in Table V, shows the ratio of the oxygen taken up with the seed coats removed to that taken up with the seed coats intact. Only the ratios can be compared here, for different weights of seeds were used in the four different determinations. These measurements were made with eudiometers.

TABLE V.

ABSORPTION OF OXYGEN; SEED COATS INTACT AND REMOVED; TEMP. 23°.

SEEDS USED	COATS	CC. OF OXYGEN TAKEN UP AFTER		RATIO OF I TO II	
		6 hrs.	22 hrs.	6 hrs.	22 hrs.
Lower seeds, X. canadense	removed I	2.5	12.6		
	intact II	1.6	6.9	1.6	1.8
Lower seeds, X. echinatum	removed I	12.2			
	intact II	7.1		1.7	
Upper seeds, X. glanduliferum	removed I	4.4	11.0		
	intact II	2.1	3.6	2.1	2.4
Upper seeds, X. echinatum	removed I	9.4	13.4		
	intact II	4.5	6.7	2.1	2.0

As Table V indicates, the lower seeds at 23° take up 1.6 to 1.7 times as much oxygen with the seed coats removed as with the seed coats intact; while the upper seeds take up 2 to 2.4 times as much oxygen with the seed coats removed as with them intact. The ratios are probably much smaller than would obtain in the soil or between wet filter papers, for the coats of the seeds lying on the walls of the eudiometer, though in a theoretically saturated atmosphere,

became relatively dry. As is found by actual measurement, oxygen diffuses through the relatively dry seed coats much more rapidly than through those saturated with water. This is indicated again by the fact that the soaked upper seeds of *X. echinatum* germinate readily at 25–27° when resting on the walls of corked bottles containing air, while in soil or between saturated filter papers a temperature of 33° is necessary for any considerable germination. The upper seeds of every species with seed coats intact can be most easily germinated by allowing the seeds to lie on the walls of corked bottles of such size that a good oxygen supply is given. In this condition the relatively dry coats allow the passage of considerably more oxygen, hence germination comes about at lower temperatures.

The second set of experiments on gaseous exchange is for the purpose of determining why high temperatures bring about the germination of the upper seeds with the seed coats intact. It immediately suggests itself that this may be due to one or both of two things: the amount of oxygen diffusing through the seed coat may rise with the rising temperature, or the amount of carbon dioxide evolved may become greater in proportion to the amount of oxygen consumed as the temperature rises.

It is seen in the 6-hour 50-minute column, as well as in the 12-hour column of Table VI, that the seeds with the coats intact at 19°, whether upper or lower, take up less than half as much oxygen as is taken up by the seeds at 33°. This indicates that the diffusion of oxgyen through the seed coats is much slower at 19° than at 33°. This conclusion, however, needs more direct evidence. Especially is this apparent when it is remembered that 19° is slightly below the minimum germinative temperature, even with the coats removed, and that the amount of oxygen consumed, therefore, may not represent the full amount that can diffuse through the coats at 19°, provided the consumption on the inside is complete.

The apparatus in *fig. 3* was used for determining accurately the effect of high temperatures on the rate of diffusion of oxygen through the seed coats. *S* is a storage bottle for potassium pyrogallate; *f*, a flask from which the oxygen is to be absorbed by potassium pyrogallate; *t*, a seed coat fastened on the end of a glass tube; *w*, a vial of water from which a thread reaches the seed coat to keep it wet; *c*, a capil-

lary tube sealed at the upper end with wax; c', a small graduated tube by which the rate of diffusion of oxygen is to be ascertained; and a, an air chamber. After s is furnished with 300^{cc} potassium pyrogallate the whole apparatus, excepting pinchcock 1 and the portions of tubes c and c', is immersed in a water bath regulated to the desired temperature within .01°. After the apparatus has had sufficient time to attain the temperature of the bath, pinchcocks 1 and 2 are loosened; the plugged end of c filed off; and the pryogallate forced into f. Now c is resealed and the pinchcocks reclamped; a drop of water is allowed to enter the graduated tube c'; and the rate of diffusion of the oxygen is read by the rate of the movement of the drop in tube c'. The same seed coat can be used repeatedly at temperatures to be compared. Numerous measurements made in this way showed the rate of diffusion 1.4 to 1.6 as fast at 33° as at 19°.[2]

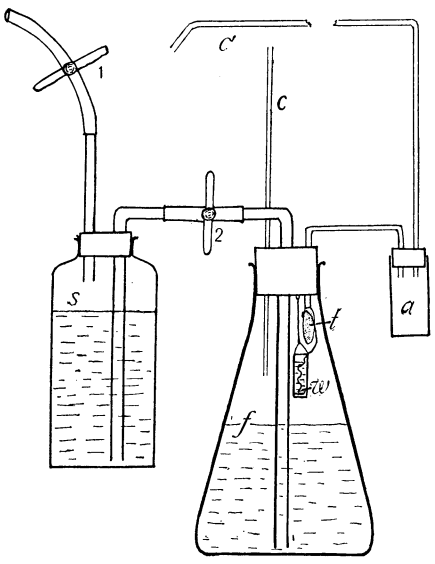

FIG. 3.—Apparatus for testing permeability of seed coats of cocklebur to oxygen. For description see text.

The amount of CO_2 evolved is considered the best measure for the amount of respiration occurring. It is evident from the measurements recorded above, that if the ratio $CO_2:O_2$ remains constant with the rise in the available oxygen due to the rise in temperature, the amount of carbon dioxide evolved will increase from 1 to 1.4–1.6 as the temperature rises from 19° to 33° with the seed coats intact. If it happens, however, that the ratio $CO_2:O_2$ rises in value along with the rise in the rate of the diffusion of oxygen, then the increase in respiration with this rise in temperature will be still more marked.

[2] When the coat was allowed to dry somewhat the rate of diffusion was also greatly increased.

This involves the necessity of making a study of the respiratory ratios at 19° and at 33°, both with the coats intact and removed.

Table VI shows the effect of high temperatures on the respiratory ratios when the seed coats are intact. In this experiment equal weights (2.68gm) of seeds of *X. echinatum* were used in each case. The results are therefore comparable in every way. It is seen that at 33°, up to the 6-hour 50-minute reading the respiratory ratio for both upper and lower seeds is 1. After this reading the ratio falls rapidly, reaching at the 12-hour reading 0.82 in the case of the lower seeds and 0.87 in the case of the upper ones. It was observed that soon after the 6-hour 50-minute reading the radicles began breaking through the seed coats. In a large number of experiments at 33° the respiratory ratio always fell rapidly after the radicles broke the seed coat. At 19°, with the seed coats intact, the respiratory ratio is 0.6 in the lower seeds and 0.64 in the upper ones. Many measurements at 33° with seed coats removed showed a respiratory ratio of 0.7–0.8 in both the upper and lower seeds, while numerous measurements at 19° with seed coats removed always gave a respiratory ratio of about 0.6.

The facts may be summarily stated thus: the respiratory ratio with the seed coats removed is about 0.6 at 19° and 0.7–0.8 at 33°; with the seed coats intact it is 0.6 at 19° and 1.0 at 33°. From these results two conclusions are plain: the respiratory ratio rises considerably (from 0.6 to 0.8) with a rise in temperature from 19° to 33° under the free oxygen supply secured by the removal of the seed coats; but it rises much more (from 0.6 to 1.0) with the same

TABLE VI.

X. echinatum—SEED COATS INTACT; 119 UPPER SEEDS; 88 LOWER; 2.68 GM.

SEEDS	TEMP. °C.	CC. INTERCHANGE OF GASES AFTER											
		4 hrs. 50 min.			6 hrs. 50 min.			10 hrs.			12 hrs.		
		O_2	CO_2	$\frac{CO_2}{O_2}$	O_2	CO_2	$\frac{CO_2}{O_2}$	O_2	CO_2	$\frac{CO_2}{O_2}$	O_2	CO_2	$\frac{CO_2}{O_2}$
Lower	33	3.3	3.3	1.	4.9	4.9	1.	7.9	6.5	.83	9.9	8.3	.82
Upper	33	2.8	2.8	1.	4.1	4.1	1.	6.	5.3	.88	7.1	5.9	8.7
Lower	19				1.8	1.1	.61				4.0	2.4	.60
Upper	19				1.5	.94	.63				3.3	2.1	.64

rise in temperature when the oxygen supply is diminished by the presence of the seed coats.

This rise in the respiratory ratios with the rise in temperature is measured for the entire seed (more than 95 per cent. of which is storage material) and not for the actively growing radicle. It is probable that for the radicle the respiratory ratio rises far above 1. This becomes more evident when it is remembered that at 33° the radicle grows first, as is normal; but that at 22°, in increased oxygen pressures, though it be five atmospheres, the growth of the cotyledons first takes place. Here, too, it should be recalled that the seed coat is much thicker and more dense over the radicle than over the cotyledons.

From the data of this section two effects of a rise in temperature are evident. It increases the rate of diffusion of oxygen through the seed coats; and it increases the respiratory ratio somewhat with the seed coats removed and markedly with the seed coats intact. If CO_2 be taken as the criterion, it is possible from the data above to calculate quantitatively the increase in respiration with a rise in temperature from 19° to 33°, when the seed coats are intact. The increase in the rate of diffusion of oxygen (if there were no increase in the ratio $CO_2:O_2$) equals an increase in respiration from 1 to 1.5 (the average of 1.4–1.6). But the increase in the respiratory ratio is from 0.6 at 19° to 1.0 at 33°. This then increases the respiration from 6 units to 10 units. When the two facts that indicate an increase in respiration are considered together, it is evident that a rise in temperature from 19° to 33° with the coats intact causes a rise in the amount of respiration from 0.6 to 1.5, or from 1 unit to 2.5 units.

It is evident from Table V that the seed coats of the lower seeds, as well as those of the upper, greatly restrict the amount of oxgyen used by the seeds, and that this restriction, though considerable, is not markedly greater in the upper seeds than in the lower ones. In *X. echinatum* this rather slight difference in the rate of diffusion of oxygen through the seed coats of the upper and lower seeds is yet sufficient to give the upper seeds a minimum germinative temperature of 32° with seed coats intact, and the lowers 22°; while both seeds with the seed coats removed have a minimum germinative

temperature of 19°. Since the difference of the two seed coats in the matter of oxygen diffusion is rather slight, it is not remarkable that the structural difference is not radical. This slight difference, however, is sufficient to raise the minimum germinative temperature and secure the delay of the upper seed.

Growth of upper seeds.—It is now obvious how the *delay* of the upper seeds is secured. But why do they grow at all in nature? This comes about by a partial disintegration of the seed coats, which is clearly shown by a change in appearance from shiny brown to a dull black or in some cases to colorless, and results in the admission of more oxygen. The length of the delay depends upon the ability of the seed coat protected by the surrounding bur to resist the factors of disintegration in the soil. The portion of the bur covering the lower seed decays within a few months after burial, while the portion covering the upper seed is always far more persistent. A variation in the persistence of the portion of the bur covering the upper seed, as well as the variation in the ability of the seed coat to resist the factors of disintegration independent of the bur, gives considerable variation in the length of delay of the upper seed. These facts show why only a few of the upper seeds grow the first year after ripening, the vast majority the second year, and a few not until the third and fourth year.

Table VII shows the effect of a period in the ground upon the seed coats and upon the vitality of the embryos of the upper seeds of *X. canadense*. Burs produced in 1904 were gathered in November of that year and stored in the laboratory until March 1905. At this time half these burs were buried and the other half kept in the laboratory. In November 1905 the upper seeds of 1904 burs stored in the laboratory, of 1904 burs buried eight months, and of 1905 burs gathered from the same patch, were removed and put to germinate. At 28–33°, with coats intact as Table VII shows, upper seeds of 1904 buried eight months gave 96 per cent. germination, upper seeds of 1904 stored in the laboratory gave 0 per cent., and upper seeds of 1905 gave 3 per cent. As shown in the same table, similar seeds with the coats removed in a germinator at 18–22° (near the minimum germinative temperature with the seed coats broken) gave in upper seeds of 1905 prompt germination, in upper seeds of 1904 stored

TABLE VII.

X. canadense; UPPER SEEDS.

SEEDS	COATS	TEMP.	PER CENT. GERMINATED AFTER		
			3 days	6 days	33 days
1905 just gathered	on	28–33	0	0	3
1904 stored in laboratory 1 yr.	on	28–33	0	0	0
1904 in lab. 4 mos., buried 8 mos.	on	28–33	44	88	96
1905 just gathered	off	18–22	48	100	100
1904 stored in lab. 1 yr.	off	18–22	20	42	100
1904 in lab. 4 mos., buried 8 mos.	off	18–22	0	4	94

in the laboratory less prompt germination, in upper seeds of 1904 buried eight months much less prompt germination. From this table two things are evident. A period in the ground causes a partial disintegration of the seed coats which lowers the germinative minimum temperature with the seed coats intact. This accounts for the results in Table VII with the temperature at 28–33° and the coats intact. The vitality (if we mean by vitality the readiness with which seeds will germinate at a given temperature) of the embryos falls somewhat with a year of dry storage and markedly with eight months in the ground.

2. AXYRIS AMARANTHOIDES.

L. R. WALDRON of the North Dakota Agricultural College informed me that *Axyris amaranthoides* bears two kinds of seeds. One grows soon after being subjected to germinative conditions and the other fails to grow under similar conditions. The former, which is flattened and winged (*fig. 4, a*) he designated as *a*; the latter, which is almost spherical (*fig. 4, b*), as *b*. From material kindly furnished by him I have found that the distal portions of the branches bear entirely form *a*, and the proximal portions entirely form *b;* while the intermediate zone bears both forms even within the same seed cluster. Every seed is either one form or the other, there being no intergrading. Seeds of form *a* and *b* are about equal in number. It is found that form *b* fails to grow because the seed coat is only very slowly permeable to water. Form *a* soaked in water at 23°

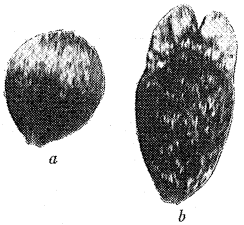

FIG. 4.—Dimorphic seeds of *Axyris amaranthoides.*

absorbed after 24 hours 39 per cent. of dry weight, after 48 hours 70 per cent. Form *b* absorbed after 24 hours 4 per cent., after 48 hours 5 per cent., after 120 hours 6.5 per cent., and after 174 hours 9.5 per cent. After 174 hours soaking, 2 per cent. of form *b* had swollen up and germinated. At 23° with seed coats broken, as shown in Table IX, form *b* germinates much more readily than form *a* with seed coats intact, and somewhat more readily than form *a* with seed coats broken. It is evident from these tests that the embryo of form *b* is more vigorous than the embryos of form *a*.

It should be pointed out that the embryo of form *b* has an ideal storage condition. The water-excluding seed coat keeps it dry as it lies buried in the ground with the temperature relatively low. With the partial disintegration of the coat comes the admission of water and the growth of the embryo. The length of the delay in germination thus secured will vary greatly with water and temperature conditions, and with the different individuals of form *b*. It is probable that it will amount to many years in some cases.

In Axyris, as in the cocklebur, the same plant bears two sorts of seeds. One sort grows rapidly in nature and the other only after a considerable delay. Unlike the cocklebur, the seeds are not paired, and the delay is secured by the seed coat shutting out water rather than oxygen.

3. Abutilon Avicennae and Chenopodium album.

Agriculturists claim that the seeds of *Abutilon Avicennae* lie in meadows and pastures for twenty years without growing, but upon breaking the soil grow in great abundance. When these seeds are soaked in water for forty-eight hours about 13 per cent. swell up, while the embryos of 87 per cent. remain extremely dry and can be pulverized. After weeks of soaking only a small per cent. additional will swell, a few at a time. For the relative per cent. of germination of these seeds with seed coats intact and seed coats broken see Table IX.

In *Chenopodium album*, mentioned in the Nobbe-Hänlein table, about 16 per cent. of a crop of seeds swell up after twenty-four hours soaking. By continual soaking the remaining seeds gradually swell a few at a time, but much more readily than is the case with Abutilon.

Contrary to NOBBE AND HÄNLEIN's conclusion, the distributed germination shown by this species is secured by the slowness with which water penetrates the seed coats.

While in Axyris and Xanthium delayed or distributed germination is secured by peculiar seed coat characters of one form of the dimorphic seeds, in *Abutilon Avicennae* and *Chenopodium album* the distributed germination is secured by a variation in the seed coat characters of similar seeds.

4. IRIS.

Dr. C. J. CHAMBERLAIN informed me that he had never succeeded in germinating seeds of various species of Iris, although he had often attempted it in order to have root tips for cytological purposes. The bulk of the seed consists of the horny endosperm with food stored on the walls as hemicellulose. On one side of the endosperm is a cylindrical cavity in which the embryo is borne. The cavity is covered by a cap, thus entirely closing in the embryo. When the seed is dry, the embryo only partially fills the cavity, but after twenty-four hours soaking it completely fills it. In this condition, however, the seeds will lie for weeks without germinating. If now the caps are removed and the seeds still kept in the water, the embryo protrudes 3–7^{mm} within an hour. Seeds with caps removed germinate very readily, while those with caps intact do not germinate at all. For the effect of removing the cap in *Iris sibirica* and *I. Pseudacorus*, see Table IX. Increased oxygen pressure and high temperatures with the caps intact did not cause germination. With the caps removed, the most successful germination was secured by using sterilized sand as a germinator at 28–33°.

The amount of moisture absorbed by the embryo within the limiting wall of the endosperm is not sufficient to permit growth to begin. By taking away this limit to water absorption by removal of the cap or a portion of the endosperm in the region of the embryo, absorption is resumed and growth soon begins. Judging from a number of observations, it appears that in nature long soaking and accompanying disintegration lead to the loosening of the cap, or more frequently to the decay of the endosperm at one side of the embryo.

TABLE VIII.

Species	Temp. °C.	Coats	Per cent. germinated after				
			2 days	3 days	5 days	9 days	38 days
Plantago major	28–33	entire	20	28	44	54	60
		broken	50	81	92	96	96
	18–22	entire	0	0	0	0	0
		broken	0	0	0	0	0
Plantago Rugelli	28–33	entire	2	2	9	15	38
		broken	91	98	98	98	98
	18–22	entire	0	0	0	0	0
		broken	0	0	0	12	12
Thlaspi arvense	28–33	entire	32	38	41	41	41
		broken	94	100	100	100	100
	18–22	entire	0	0	0	0	0
		broken	48	75	86	93	96

I was obliged to abandon work on these seeds, for on account of handling them I had repeated and severe attacks of dermatitis from contact with the syrupy covering of the endosperm. The symptoms were identical with those of Rhus poisoning.

5. The Nobbe-Hänlein Table.

Beside *Chenopodium album*, I have studied the following seeds mentioned in the Nobbe-Hänlein table: *Aquilegia vulgaris*, *Capsella Bursa-pastoris*, *Lysimachia vulgaris*, *Plantago major*, *P. Rugelii*, and *Thlaspi arvense*. It was found, in agreement with Nobbe and Hänlein, that all these seeds absorb water readily.

In *Aquilegia vulgaris* Nobbe and Hänlein obtained a germination of only 0.75 per cent. after sixteen days, and no more during the remaining three years. In all tests at 23° I found over 50 per cent. germinating within thirty days. None generally germinated short of sixteen days because of the rudimentary state of the embryo. Breaking the seed coats cut down the percentage of germination by allowing infection by fungi which the slow growing embryos were unable to resist.

Table VIII shows the germination of seeds of *Plantago major*, *P. Rugelii*, and *Thlaspi arvense* at 18–22° and 28–33° with seed intact and seed coats broken. It is seen that with seed coats broken and with favorable temperatures over 95 per cent. germinate in every case. These results should be compared with Table I, in which

NOBBE and HÄNLEIN show the germination of only 0.25 per cent. of *P. major*, 10.75 per cent. of *P. media*, and 21.75 per cent. of *Thlaspi arvense* after 1,173 days. In the last species the germination is late in that period.

From Table VIII several important facts are evident. The temperature of 18–22° is below the minimum germinative temperature of seeds of *P. major* with seed coats intact or broken, and below that of *P. Rugelii* and *Thlaspi arvense* with seed coats intact. It is very near the germination minimum of *P. Rugelii* with seed coats broken and well above that of *Thlaspi arvense* in similar condition. A very marked increase in germination is secured by rupturing the coats even when the most favorable temperatures are used. High temperatures, then, will overcome only in part the seed coat effects. High temperatures are more efficient in overcoming the seed coat effects in *P. major* than in *P. Rugelii*.

In Table IX a similar effect of rupturing the seed coat is shown for *Capsella Bursa-pastoris*, *Lysimachia vulgaris*, and *Euphorbia Cyparissias*. It should be mentioned that the region and extent

TABLE IX.

TEMP. 18–22° EXCEPT FOR IRIS, WHICH WAS 28–33°.

SPECIES	COATS	PER CENT. GERMINATED AFTER					
		1 day	3 days	6 days	14 days	20 days	30 days
Abutilon Avicennae	entire	0	13	13	13	13	13
	broken	48	98	98	98	98	98
Avena fatua	entire	0	0	8	8	8	8
	broken	0	4	92	96	96	96
Capsella Bursa-pastoris	entire	0	8	14	15	15	15
	broken	0	80	100	100	100	100
Chenopodium album	entire	0	10	16	16	16	16
	broken	0	80	100	100	100	100
Euphorbia Cyparissias	entire	0	0	2	20	20	20
	broken	0	20	84	84	84	84
Lysimachia vulgaris	entire	0	0	0	0	0	0
	broken	0	44	60	64	64	64
Axyris amaranthoides A	entire	0	76	92	94	96	96
	broken	14	100	100	100	100	100
A. amaranthoides B	entire	0	0	0	0	0	0
	broken	55	100	100	100	100	100
Iris sibirica	cap on	0	0	0	0	0	0
	cap off	0	6	18	75	98	98
Iris Pseudacorus	cap on	0	0	0	0	0	0
	cap off	0	14	32	87	97	97

of the rupture makes no difference so long as the embryo is not injured.

NOBBE and HÄNLEIN make no mention of the temperature maintained in the course of their experiments and seem unconscious of the fact that the temperature plays an important part. Judging from the results in Table VIII as compared with their results in Table I, they must have run their germinators at relatively low temperatures. These investigators, as well as WINKLER, were likewise entirely unaware of the effect of the seed coats upon germination.

6. AVENA FATUA.

Avena fatua has some germinative characters which are interesting and which show that the seed coat characters just described for other seeds appear in the grasses. Table IX shows that at 18–22°, 8 per cent. grow with seed coats entire and 96 per cent. with coats broken. At 33°, 50–60 per cent. grow with coats entire and 97 per cent. with the coats broken. This seed coat character probably distributes the germination of a given crop over a period of years. It probably accounts for the claim of farmers that these grains will lie in pastures and meadows for twelve to fifteen years and then grow abundantly when land is plowed. WALDRON (12) believes this idea is wrong, but it is easy to see how his vitality tests might be entirely misleading, for the seed coat character just described was not taken into account.

7. HAWTHORNS.

I found, as is popularly believed, that no hawthorn seeds will grow immediately after ripening. The seeds of various species were tested by removal of seed coats and subjection to high temperatures and high oxygen pressures; but none of these conditions sufficed to cause germination. Seeds that lay in the soil for a year or more germinated to some extent; while seeds stored in the dry for a similar period did not germinate at all, although the tests were made with naked embryos as well as with seeds bearing the coats. It is evident, therefore, that the change that must precede germination is in the embryo itself rather than in the seed coat; but it is also more or less a matter of disintegration, as is true in seeds whose germination is delayed by seed coats.

IV. General considerations.

Two statements of ARTHUR concerning the cocklebur need special consideration. He says: "Seeds in the bur retain their germinative power, when kept in a dry room, for two years or more; but seeds removed from the bur dry out within a few days and will no longer grow. Seeds removed from the bur and placed in a germinator retain their bright polished appearance as long as they are alive; when dead they turn dull and lusterless." I find that the seeds retain their vitality fully as well when removed from the bur and allowed to dry as when in the bur. In fact, a dry cool place is the best for storage of these seeds whether in or out of the bur, as is true for most seeds. The seeds removed from the bur and kept in a dry place retain their vitality much more than five years. I found the condition of the seed coat no indicator of the vitality of the embryo. The coat through disintegration loses its luster and turns black or sometimes colorless, which means that more oxygen is admitted and that the minimum germinative temperature of the seed has fallen. It is not surprising that ARTHUR drew these conclusions, for his work gave him no idea of the germinative conditions of the upper seeds or of the significance of the seed coat.

As DUVEL (**3***a*) states, seeds retain their vitality longest in conditions that permit of least respiration. KOLKWITZ (**5**) has shown that respiration is extremely slight in dry seeds at low temperatures. The embryos of seeds whose germination is delayed by coats that exclude oxygen, such as Abutilon, Axyris, and Chenopodium, are kept very dry by the coats. As they lie in the ground they are likewise relatively cool. In nature, in short, they have the most favorable storage conditions up to the time when the coats, through partial decay or long exposure to water, admit moisture and germination begins. It is not wonderful that such seeds lie in the ground twenty to twenty-five years and yet retain their vitality. While the reduction in the oxygen admitted to the upper seed of the cocklebur cuts down the respiration considerably, it does it to no such extent as does the exclusion of water. The coats that exclude water are undoubtedly much better adapted to securing a long delay than are the coats that merely exclude oxygen. In nature the longer delays are certainly secured by the former method.

PFEFFER (9) says: "The conditions which lead to certain seeds resting under the soil for as long as fifty years and germinating when dug up have not as yet been determined." This, as well as the sudden appearance of weeds in forests after fires and in meadows of many years standing immediately upon plowing, is probably explained by a few simple facts. Weed seeds are produced in great abundance. Because of variation in seed coat characters or in some cases of embryo characters, a given crop distributes its germination over a period of years. Seeds deep in the soil germinate less readily because of lack of oxygen or water, and those that do grow exhaust the stored food before reaching the surface. Bringing such seeds to the surface greatly increases their germination and removes the danger of exhaustion of the stored food. The plants of meadows and forests keep the water supply reduced and thereby cut down the chances for the germination and later growth of the weed seeds present. With the destruction of the plants of the forest or meadow comes a great increase in the germination of the weed seeds and a removal of the opposition to their future growth. These phenomena, then, will probably all be explained by a study of the germinative characters of the seeds such as is described in the experimental portion of this paper, along with certain other well established facts on germination.

It is undoubtedly true that many of the tests that have been made for the vitality of weed seeds are untrustworthy, because the significance of the seed coats has been overlooked. This is clearly shown by the results of DUVEL (3*b*) and WALDRON (12), who have carried on extensive experiments to determine the length of time weed seeds must be buried in order to lose their vitality. In column I of Table X is shown the percentage germination determined by me with seed coats broken and with favorable temperatures. In column II appear DUVEL'S results, in which he uses what he terms the "most favorable temperatures," but overlooks of course the seed coat effects. The figures quoted from DUVEL are from the column "original samples," which means fresh seeds, as were the seeds for determining the percentages of column I.

The effect of rupturing the seed coats, as is shown in this table, is very evident, although DUVEL has in part overcome the seed coat effects by high temperatures. The average percentage of germina-

TABLE X.

	I	II
1. Axyris amaranthoides	100	0
2. Xanthium pennsylvanicum	98	50
3. Thalaspi arvense	100	57
4. Plantago Rugelii	96	4
5. Avena fatua	96	75
6. Plantago major	96	24
7. Chenopodium album	100	67

tion for the seven species tested is 98 per cent. with seed coats ruptured, 40 per cent. with seed coats intact. It may be urged that the seeds used by DUVEL are of low vitality. This, however, does not seem at all probable, for I obtained only slightly higher percentages, as shown in Tables VIII and IX, with the coats intact and with favorable temperatures, than those reported by DUVEL. These slightly higher percentages can be accounted for by the fact that the temperature used by me was 28–33°, while the temperature used by DUVEL was 20–30°. Two species of seeds mentioned in this table need special consideration. In *Axyris amaranthoides*, DUVEL determined the vitality as 0 per cent. This is exactly what would be expected if form *b* alone (as shown in Table IX) were used, and if the effect of the seed coat were overlooked. In *Xanthium pennsylvanicum* he finds 50 per cent. germinating. This, too, is what would be expected if the upper and lower seeds of the cocklebur were put in a germinator at 20–30°. The lower seeds would germinate in this condition and the others fail to do so. It seems probable, then, that in Xanthium and Axyris DUVEL overlooked the dimorphic character of the seeds, as well as the effect of the coats on germination. Vitality tests of this kind, that neglect the effect of the seed coats, are tests of the condition of the seed coats rather than tests of the real vitality of the embryos themselves. It is evident that if these errors appear in the original tests for vitality they will likewise appear in the tests made after different periods of burial. If vitality tests, looking to the extermination of weeds, are to be of real value, the exact germinative character of each species must first be determined, and all vitality tests must then be made on the basis of these germinative characters.

It is obvious that the seeds which fail to grow in the ordinary grain tests often do so because of seed coat characters rather than because of lack of vitality of the embryos. This, however, does not in any wise invalidate the ordinary methods of testing grains to be used for seeding, since seeds that are delayed a month or more in germination are of no value in producing the crop. On the other hand, when it comes to testing weed seeds, looking toward extermination, it is highly important that these seed coat characters be taken into consideration.

I am impressed by the high vitality of weed seeds. This is especially true of the more noxious weeds and those in which the seed coat secures a long delay. The high vitality is not shown alone by a quick response to germinative conditions. The percentage of germination in noxious weeds, provided real germinative conditions are given (the seed coat hindrance removed), is very close to 100; and a marked growth of the embryo generally takes place within two days after being subjected to germinative conditions. After recognizing this fact, one is led to suspect that many other cases of low vitality in weed seeds mentioned by DUVEL and others (not examined in this paper) must be due to seed coat characters rather than to lack of vitality in the embryos.

While this paper indicates, exactly contrary to the conclusions commonly held, that delayed germination is in most cases secured by seed coat characters, it yet recognizes that in the hawthorns delay is secured by embryo characters. It is probable that a number of other seeds will be found to belong to the same category as the hawthorn. It is of great interest to know just the changes which take place in the seeds of the hawthorns and finally lead to germination through long exposure to germinative conditions. This subject is now under investigation. It must be urged that, until these changes are understood, any attempt to determine the vitality of such seeds is futile.

The methods by which seed characters that secure delayed germination have come about naturally deserves consideration. It may be adaptation coming through natural selection, but an attempt to prove this would end in failure. This delay in many cases, however, is of undoubted advantage to the species. ARTHUR urges that in the cocklebur the two seeds are borne in an indehiscent structure,

the bur, and that it is impossible to have the two seeds distributed in space, so a distribution in time is substituted. Why such an indehiscent involucral structure should be developed instead of such a bur as appears in the burdock needs answer. With the indehiscent bur already in existence the advantage is plain. It is clear that such germinative characters as appear in the seeds of *Axyris amaranthoides*, *Abutilon Avicennae*, etc., insure that the soil will always be supplied with these seeds in process of germination. The destruction of existing vegetation, by fire or otherwise, is followed by a quick appearance of these weeds. In species where none of a given crop of seeds grow until a year or more after falling, it would seem that the adaptive characters, if they be such at all, had overstepped the line of greatest advantage.

V. Summary.

1. Delayed germination is reported in the seeds of many plants and, exactly opposite to the common view, its cause generally lies in the seed coats rather than in the embryos; but in the hawthorns, as perhaps in some other seeds, it is due to embryo characters.

2. In the upper seed of the cocklebur the delay is secured by the seed coat excluding oxygen, while in *Axyris amaranthoides*, *Abutilon Avicennae*, and many other seeds, it is secured by the coats excluding water.

3. In Iris seeds the failure to germinate is due to the endosperm and cap stopping water absorption before the quantity necessary for germination is obtained by the embryo.

4. In *Plantago major*, *P. Rugelii*, *Thlaspi arvense*, *Avena fatua*, and others, the real method by which the coats secure the delay is not yet determined, but there is no doubt that the delay is due to the coats.

5. Seed coats which exclude water are much better adapted to securing delays than are seed coats which exclude oxygen, because of the much greater reduction of respiration in the first case.

6. In nature growth of the delayed seeds comes about through the disintegration of the seed coat structures by a longer or shorter exposure to germinative conditions, and the length of the delay depends upon the persistence of the structure securing it.

7. In the cocklebur the bur aids in preserving the seed coat of the upper seed by being most persistent over it.

8. Even in the hawthorns, where the delay is secured by embryo characters, the germination finally comes about in the course of long exposure to germinative conditions and not in dry storage.

9. In the cocklebur the seed coats of both the upper and lower seeds cut down the oxygen supply, but the first the more markedly. This gives the upper seed a much higher minimum germinative temperature and the lower seed a somewhat higher one. Hence we have in the cocklebur seeds two minimum germinative temperatures; one with the seed coats intact and a much lower one with the coats removed. In the upper seeds these differ by fifteen or more degrees; in the lower seeds by three to five degrees.

10. High temperatures bring about the germination of the upper seeds of the cocklebur with coats intact by increasing the rate of diffusion of oxygen through the seed coat and by raising the respiratory ratio.

11. The minimum germinative temperatures of the seeds of the cocklebur, *Plantago major*, *P. Rugelii*, *Thlaspi arvense*, and various other seeds, with the seed coats intact, is far above the highest minimum germinative temperature reported; while in the cocklebur and *Plantago major* with coats removed this critical temperature is considerably above the highest reported.

I am indebted to Professor CHAS. F. HOTTES, of the University of Illinois, for suggesting the problem in reference to the cocklebur, and to Professors JOHN M. COULTER and C. R. BARNES for kind suggestions and assistance during the progress of the work.

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