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EFFECT OF LIGHT ON GERMINATION OF LIGHT-SENSITIVE SEEDS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 279

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Historical

Various explanations have been offered for the germination of light-sensitive seeds, and several conditions have been shown to favor or make possible the germination of such seeds. Rupture of coats, increased water supply, variation of quantity and intensity of light, reciprocal relation of heat and light, reaction of substratum and embryo, activation of enzymes, increased oxygen pressure, increased carbon dioxide pressure, and "certain inhibiting agencies" have been suggested as factors affecting the germination of light-sensitive seeds. Although quite possible, it seems hardly probable that no one of these is the fundamental or controlling factor. It would seem quite probable that one or two of these agencies are fundamental and the others are accessory means of setting in motion the processes that finally bring about germination. Enzyme action has been suggested repeatedly as a fundamental cause of germination, but no one has ventured to demonstrate the relation of enzymes to the germination of light-sensitive seeds.

An attempt has been made in this investigation to discover the fundamental relation of light to the germination of seeds, and to show just what light does to start germination. The effect of light on the germination of seeds has interested botanists for many years.

The first known publications on this subject were made by CASPARY (4) in 1860, when he announced that the seeds of *Bulliarda aquatica* are strongly light sensitive. In 1867 he (5) discussed the germination of seeds of *Pinguicula vulgaris*. In 1876 NOBBE (38) made the statement that germination was neither favored nor influenced by light. After WIESNER (51) had published the statement that the germination of seeds of *Viscum album* is favored by light, and STEBLER (48) had shown that *Poa pratensis* and *P. nemoralis* germinated up to 60 per cent in light and only up to 7 per cent in darkness, NOBBE (39) published results of experiments with grass seeds, including *Poa pratensis*, *Zea Mays*, and some other large seeds to uphold his earlier contention. PAUCHON'S (41) results supported NOBBE in the controversy. In 1883 CIESLAR (6) confirmed and extended STEBLER'S results, reporting *Agrostis stolonifera* and *Nicotiana macrophylla* as light sensitive. He made a careful study of the influence of temperature in connection with light, and showed that small seeds poor in reserve materials germinate better in white light, while large seeds are usually indifferent to light, and that seeds of *Poa nemoralis* germinate better in yellow light than in violet. LIEBENBERG (35) in 1894 confirmed STEBLER'S results, but referred to them as temperature effects.

In 1893 JÖNSSON (23) showed that after-ripening has a definite influence on the action of light in germination, that light increases the percentage of germination, that heat rays are unimportant, that intermittent light is as effective as continuous light, and that intermittent temperature may be substituted for light in the germination of such seeds as *Poa pratensis*, *P. nemoralis*, *Agrostis stolonifera*, and *Daucus Carota*.

In 1899 HEINRICHER (19) began publishing the results of his work on light germination. He (20) reported that seeds of *Pitcairnia maydifolia* germinate only in light, that the germination of *Veronica peregrina* seeds is hastened and several other small seeds are favored in germination by light. He considered the factors to be age, quickness of drying, moisture, illumination of parent plant, and light of different colors. He (21) concluded that the effect of light is a matter of activation of reserve materials, that the benefit of light is not due to its causing early carbon assimilation,

but rather to its effect on the enzyme activity in the production and digestion of stored foods. RACIBORSKI (43) found that tobacco seeds germinate in diffused light after 1–5 hours' illumination, a longer time being required if the intensity of light is low. In 1900 TAMMES (49) declared that the exposure of dry seeds to direct sunlight did not affect their later germination, and in 1902 LAURENT (29) made the same statement. REMER (44) reported that light hinders the germination of *Phacelia tanacetifolia*, but offered no explanation of the light relations. LASCHKE (28) confirmed earlier results with *Poa*, and stated that light cannot be replaced by higher temperatures. In the same year FIGDOR (10) made a report on the influence of light on the germination of seeds of Gesneriaceae. In 1912 he (11) reported that seeds of 12 species of this family are favored by light. In 1908 BESSEY (3) found that seeds of the epiphytic *Ficus aurea* and *F. populnea* germinate only in light.

KINZEL (24) in 1907 reported that the germination of freshly harvested seeds of *Nigella sativa* was prevented by light, while similar seed germinated up to 94 per cent in darkness. Even three minutes' illumination after 24 hours' incubation in darkness had a marked retarding effect. He considered the effect of light as photochemical, and designated such seeds as "light hard." The germination of some light-sensitive seeds in darkness was hastened by soaking in a solution of an enzyme such as papayotin (25). He (26) also published a long list of light-sensitive seeds, to which still others were added in his later work (27). He recognized as important factors in germination of seeds age of seed, character of seed coats, and color of light. LEHMANN'S work (30, 31, 32, 33, 34), begun in 1909, continued through 1915. Most of his experiments were conducted with seed of *Verbascum Thapsus*, *V. thapsiforme*, *Epilobium roseum*, and *Ranunculus sceleratus*. He showed the effects of substratum on germination in light, found the age of seeds to be an important factor, used Knop's nutrient solution as a stimulus instead of light, and found that salts favored germination of light-sensitive seeds in darkness. He claimed that light exerts its influence by starting or stopping some chemical changes in the seed, and established a relation between light and temperature. He also punctured seed coats as a substitute for light. LEHMANN

and OTTENWÄLDER (36) experimented with *Epilobium hirsutum* and other seeds and showed that acid solutions and proteolytic enzymes can be substituted for light. They referred the light effect to activation of enzymes, but did little to prove their hypothesis. PICKHOLTZ (42) connected light effects and temperature variations, and concluded that the influence of direct sunlight was mainly due to the heat rays which raised the temperature. Alternating temperatures helped the germination at different stages of maturity.

In 1912 a number of workers reported on the problem of light germination. BAAR (1) investigated seeds of several Amarantaceae and found that most seeds of this family (*Amaranthus*, *Celosia*, and *Blitum*) have an aversion to light. He considered the age of seeds generally important for the occurrence and intensity of the light effect, and also related the light effect to substratum and temperature variations. BECKER (2) brought forward a long list of examples of the light effect on germination of seeds. HAACK (18) in his work on the Scotch pines demonstrated the influence of heat, and reported that temperature variations act as a stimulus to light-sensitive seeds, and that blue light is more favorable to germination than darkness. SIMON (47) reported that the salts of iron hindered germination of seeds in darkness, but increased it in light. GASSNER (12) first reported on the germination of seeds of *Chloris ciliata* in 1910. He found three factors which may be substituted for light, namely, increased oxygen supply, after-ripening, and high temperature. He claimed that light offsets the effect of the limiting factor, and showed that the chaff of *Chloris ciliata* prevents easy germination. His later work (14, 15) took up the action of chemicals. He considered the latent influence of light as related to seed bed, temperature, and after-ripening, the influence of light on germination, the influence of desiccation, and the relations between light and media favoring or hindering germination. From a tabulation of tests with seeds of different families he concluded that in these cases nitrogen, variously combined in the media, shows the same favorable action as light, but he included contradictory results. He considered the favorable effect of Knop's nutrient solution as due only to the nitrates present. He reported the seeds

of *Ranunculus sceleratus* and *Oenothera biennis* as favorably influenced by light and by inorganic salts containing nitrogen, through a wide range of concentrations. OTTENWÄLDER (40), working with *Epilobium hirsutum* seeds, found that the light requirement as regards intensity is closely related to temperature, the former increasing as the latter is lowered. The illumination period is related also to the temperature, but more closely to light intensity. Light-sensitive seeds are also favorably and strongly influenced by weak acids. The hypothesis of a catalytic influence of light is said to have received support from these observations.

Materials

A preliminary examination of 115 samples of seeds collected from Shaw's Gardens, the Botanical Gardens of the University of Michigan, waysides, swamps, and fields indicated the following as available for the study of the effect of light in germination: *Daucus Carota, Nicotiana Tabacum, N. sylvestris, N. affinis, Nicotiana* hybrids, *Gentiana Saponaria, G. pannonica, Oenothera biennis, Verbascum Thapsus, Amaranthus caudatus, Rumex crispus, Phoradendron flavescens*, and *Datura Stramonium*. Of those mentioned, the writer has been unable to find any previous report of light sensitiveness of seeds of *Rumex crispus, Datura Stramonium*, and *Phoradendron flavescens*. Of the light-sensitive seeds not previously reported, seeds of *Rumex crispus* and *Phoradendron flavescens* are favored by light in germination, while seeds of *Datura Stramonium* are inhibited from germinating by light, as will be shown later.

JÖNSSON (23) in 1893 reported the seed of *Daucus Carota* as favored by light in germination. *Nicotiana Tabacum* seeds were first reported as light sensitive by RACIBORSKI (43) in 1900. The seeds of *Gentiana Saponaria, G. pannonica, Verbascum Thapsus*, and *Oenothera biennis* were reported as light sensitive by KINZEL (24) in 1907. BAAR (1) reported *Amaranthus caudatus* seeds as hindered in germination by light. The seeds of *Gentiana* are not conveniently suited to the purposes of this investigation on account of the longer incubation period. The seed of *Amaranthus caudatus* are not used because they are light-inhibited seeds. The seeds

of *Datura Stramonium* have been found by careful experiments to be light inhibited and to require total darkness and a temperature of about 30° C. for germination. They are accordingly reserved for a future study. Detailed study of the germination of *Phoradendron flavescens* seeds was deferred on account of the peculiar slimy ovary and the chlorophyll-bearing embryo. Seeds of *Nicotiana Tabacum, Rumex crispus, Oenothera biennis, Verbascum Thapsus,* and *Daucus Carota* were selected for this research because of their abundance and similar incubation periods.

Germination in light and darkness

Preliminary tests of *Rumex crispus* seeds on wet filter paper gave a germination of 84 per cent in light and 16 per cent in darkness after 8 days of incubation.

TABLE I

Treatment	Percentage germination in light	Percentage germination in darkness
Cleaned, dried, and soaked in flowing water for 24 hours and incubated	16	6
Cleaned, dried, and incubated	24	6
Cleaned, soaked for 4 days, and incubated	12	2

Preliminary tests of germination of seeds of *Phoradendron flavescens,* suggested by WIESNER'S (**51**) results with seeds of *Viscum album,* are given in table I. Seeds prepared as indicated in table I were counted into Petri dishes containing wet filter paper as substratum and placed in light and total darkness to incubate at room temperature, which ranged from 18–25° C. The incubation period was 27 days. These results indicate that light favors the germination of seeds of *Phoradendron flavescens.* On account of the sticky nature of the pulpy ovary and the succulence of the single fleshy green embryo, it was almost impossible to remove the mass of enveloping material without leaving a favorable substratum for molds and bacteria on the one hand, and without injury to the embryo on the other hand. Moreover, with the best of care many of the seeds failed to germinate and became moldy. With these

conditions we can understand the relatively low germination, and yet see that light favors the germination of these seeds.

Seeds of *Datura Stramonium* were treated as shown in table II. Seeds were allowed to incubate for 17 days, and the results indicate clearly an inhibitory action of light on their germination. The

TABLE II

Treatment	Percentage germination in light	Percentage germination in darkness
On soil, 26–30° C	6	98
On sand, 26–30° C	2	22
On filter paper, 20° C.	0	0
On filter paper, 24° C.	0	4
On filter paper, 30° C.	4	60

constituents of the soil solution seem to promote materially the germination of these seeds in darkness but not in light. *Datura Stramonium* seeds require different treatment from any of the other seeds tested, and accordingly are reserved for separate study.

Light sensitiveness, after-ripening, and viability

To establish standards for comparison with other data, and to indicate the degree of light sensitiveness, various light-sensitive seeds were incubated from time to time at room temperature (20–28° C.) on filter paper in light and darkness respectively. The results given in table III are fairly representative of these tests.

These data indicate what may be expected of the various light-sensitive seeds under investigation when subjected to germinating conditions at room temperature 20–28° C. on wet filter paper in Petri dishes. It appears that the seeds of some kinds of tobacco are less light favored than others (3. *Nicotiana affinis*, 108. *N. affinis*, 1. *Nicotiana* hybrid, and 117. Pennsylvania Havana tobacco). The results also indicate that not all of the seeds under investigation are entirely dependent on light for germination. A certain percentage of each lot of *Rumex crispus* and *Daucus Carota* seeds usually germinates in darkness. It is also noteworthy that seeds of *Oenothera biennis* do not always germinate, even in light. The seeds under investigation seem to retain their light sensitiveness

for long periods and to a rather high degree, especially those of *Verbascum Thapsus* and *Nicotiana Tabacum*. Attention should be called to low germination of newly harvested seeds of *Oenothera biennis*, *Daucus Carota*, and *Rumex crispus*. Tests for evidence of

TABLE III

Seeds	Incubated 9-18-15 to 9-29-15		Incubated 10-28-15 to 11-5-15		Incubated 5-30-16 to 6-6-16		Incubated 6-20-18 to 6-28-18	
	Light	Darkness	Light	Darkness	Light	Darkness	Light	Darkness
	Collected in 1914							
1. Nicotiana hybrid	91	56	87	65	87	60	84	45
3. Nicotiana affinis	79	57	87	45	74	43	82	19
8. Nicotiana hybrid	48	1	58	5	74	35	67	13
13. Nicotiana hybrid	84	0	89	2	89	6	80	0
22. Nicotiana hybrid	55	0	73	0	61	0	55	0
55. Verbascum Thapsus	81	1	80	0	87	0	53	0
61. Daucus Carota	63	19	78	10	71	20	32	7
66. Oenothera biennis	4	2	76	10	51	4	0	0
68. Rumex crispus	39	8	88	18	75	31	60	1
92. Nicotiana Tabacum	77	1	.....	.....	90	4	30	0
96. Daucus Carota	1	0	52	6	.....	.....	.....	.....
	Collected in 1915							
97. Verbascum Thapsus	94	0	94	0	82	0	.....	.....
98. Oenothera biennis	2	0	48	0	61	1	.....	.....
99. Rumex crispus	36	0	82	4	80	40	66	0
100. Verbascum Thapsus	74	1	73	0	39	0	60	0
101. Rumex crispus	61	0	94	0	92	2	30	0
102. Daucus Carota	.....	.....	45	1	44	11	.....	.....
103. Oenothera biennis	.....	.....	63	0	61	0	.....	.....
104. Rumex crispus	.....	.....	94	1	79	11	.....	.....
105. Pennsylvania seedleaf tobacco	.....	.....	.....	.....	75	24	.....	.....
111. Connecticut seedleaf tobacco	.....	.....	.....	.....	75	0	.....	.....
112. Honduras tobacco	.....	.....	.....	.....	49	0	.....	.....
113. Cuban tobacco	.....	.....	.....	.....	81	0	.....	.....
117. Pennsylvania Havana tobacco	.....	.....	.....	.....	100	71	.....	.....
118. Ohio seedleaf tobacco	.....	.....	.....	.....	86	2	.....	.....

a period of after-ripening in *Verbascum Thapsus* were quite negative. Newly harvested seeds of *V. Thapsus* germinate above 90 per cent in light and only about 2 per cent in darkness. Tests of still older seeds indicate that they retain light sensitiveness as long as they are viable.

Mechanical rupture

In 1906 CROCKER (7) succeeded in germinating a number of different kinds of seeds after breaking the seed coats. KINZEL (24) found that puncturing coats of some of his light sensitive seeds gave better germination in darkness. GASSNER (13) found that rupture of the coats of seeds of *Chloris ciliata* permitted good germination in darkness at 34° C. Thus it seemed quite possible that the seeds under investigation might be brought to germination by such treatment. Accordingly a more carefully controlled experiment was made to determine the rôle of the several seed coats in germination. Seeds of each kind were rubbed on fine sandpaper and placed on moist filter paper in Petri dishes. The Petri dishes were carefully wrapped in black cloth and placed in a dark room at 24–30° C. for 8 days. Concurrently, sets of unabraded seeds were placed to germinate in light and darkness.

TABLE IV

Seeds	Not abraded		Abraded	Not abraded
	Light	Darkness	Darkness	Darkness
Nicotiana Tabacum	60, 61	0, 0	0, 1	0, 1
Rumex crispus	30, 16	0, 1	40, 66*	0, ..
Verbascum Thapsus	75, 32	0, 8	6, ..	1, 3
Oenothera biennis	10, 39	3, 0	3, 5	7, 1
Daucus Carota	60, 20	27, 7	25, 15	21, 17

* Coats off.

Mechanical abrasion of seed coats for various periods in rotating cylinders containing coarse quartz sand gave similar results. An examination of the data in table IV reveals the beneficial effect of abrasion of seed coats in but one instance. In the case of *Rumex crispus* abrasion of the seed coats yielded a percentage of germination slightly exceeding that for light in the control, while the removal of the coats yields a percentage of germination even more than double that in light. This suggests that the seed coats of *Rumex crispus* inhibit or retard the entrance of some necessary factor, or perhaps retard the exit of some inhibiting factor, and that light in some way favors these movements.

Rupture by sulphuric acid

As long ago as 1896 ROSTRUP (45) of the Danish Seed Control found that concentrated sulphuric acid treatment hastened germination of hard seeds of *Lathyrus sylvestris*. TODARO (50) used concentrated sulphuric acid on red clover seed with beneficial results. He also reported that various weed seeds, including those of *Rumex crispus*, were all destroyed by a brief immersion in sulphuric acid. Accordingly, to determine more certainly the rôle of seed coats in the germination of the five kinds of seeds, they were treated with concentrated sulphuric acid for periods previously determined, carefully washed in carbonate of soda solution, then in distilled water, and placed in germinators as previously described.

TABLE V

Seeds	Treated with concentrated sulphuric acid				Untreated	
	Minutes in H_2SO_4	Germinated in darkness			In light 8 days	In darkness 8 days
		8 days (1)	10 days (2)	8 days (3)		
Nicotiana Tabacum.....	0.5	0	0	0	42	0
Nicotiana Tabacum.....	1	0	0	0	42	0
Verbascum Thapsus.....	0.5	0	0	0	72	0
Verbascum Thapsus.....	1	0	0	0	72	0
Daucus Carota.....	0.5	23	8	0	31	25
Daucus Carota.....	1	1	6	0	31	25
Rumex crispus.....	6	27	37	17	88	0
Rumex crispus.....	8	62	59	0	88	0
Oenothera biennis.....	8	38	34	41	78	0
Oenothera biennis.....	10	1	19	23	78	0

Table V indicates that treatment of seeds of *Rumex crispus* and *Oenothera biennis* with concentrated sulphuric acid yields an increased percentage of germination in darkness. Treatment with concentrated sulphuric acid for longer or shorter periods than indicated gives no better germination of the seeds in darkness. In *Daucus Carota* there is apparently an injury. This experiment indicates that light acts on the coat of *Rumex crispus* seeds, and points in that direction in case of seed coats of *Oenothera biennis*. These results in the main agree with those of the experiment on abrasion of coats. They confirm the results with seeds of *Rumex*

crispus and include the seeds of *Oenothera biennis* as being benefited in germination by acid treatment. Why the seeds of *Oenothera biennis* germinate better after treatment with H_2SO_4 and not by abrasion is unexplained.

Temperature and light

OTTENWÄLDER (40) claimed that within the range of temperatures which permit germination, light can be substituted for heat at low temperatures and heat for light at high temperatures. With *Verbascum Thapsus* and other seeds he found that germination occurred at high temperature in darkness and at low temperature

TABLE VI

Seeds	Temperature centigrade							
	10°	15°	19°	24°	27°	30°	35°	40°
	Light							
Nicotiana Tabacum	0	0	23	62	55	40	.....	.....
Verbascum Thapsus	0	0	0	77	82	84	.....	.....
Daucus Carota	0	12	21	36	33	25	.....	.....
Oenothera biennis	0	0	0	28	.....	6	.....	.....
Rumex crispus	0	1	42	65	22	5	.....	.....
	Darkness							
Nicotiana Tabacum	0	0	0	11	6	2	0	0
Verbascum Thapsus	0	0	0	7	14	15	3	1
Daucus Carota	0	4	7	16	12	12	4	0
Oenothera biennis	0	0	0	5	4	2	0	0
Rumex crispus	0	1	3	8	8	4	0	0

in light. In order to test this for American *Verbascum Thapsus* and to see whether it is generally true, the different seeds under investigation were placed to germinate in light and darkness at different temperatures. It was not possible to control closely temperature and prevent small fluctuations. These changes of temperature were never sudden, however, and had no effect except to increase slightly the percentage of germination in both light and darkness. The data reported represent results obtained from five different sets of determinations. Seeds were placed to germinate in Petri dishes on filter paper wetted with distilled water at temperatures indicated in table VI and allowed to incubate for 9 days.

These data offer no evidence of a reciprocal relation between heat and light as suggested by LEHMANN and OTTENWÄLDER, not even in the case of seeds of *Verbascum Thapsus*, nor have any of the various tests indicated this reciprocal relation in the seeds. Indeed, in each kind of seed under investigation the optimum temperature for germination in light is very close to that for germination in darkness. In germination in darkness the results show rather definite minimum and maximum as well as optimum temperatures. It is especially noteworthy that high temperature and darkness did not induce germination of *Verbascum Thapsus* seeds, as claimed by OTTENWÄLDER (40). No specific experiments were performed to determine the effect of light intensity on germination, although early in this investigation it became a very familiar fact that very little illumination would induce germination. Good germination in darkness was frequently the occasion for repetition of an experiment, only to find that germination had been induced by leaks in the light screens. A comparison of table VI with data given elsewhere indicates that highest germination of light-sensitive seeds does not occur at constant temperature, but at temperatures fluctuating between 20 and 27° C.

Effects of alternation of temperature, light, and darkness

As long ago as 1882 NOBBE (39) and his students used alternating temperatures to promote germination, and in 1884 LIEBENBERG (35) referred light effects to variations of temperature in the germination of seeds of *Poa pratensis*. As recently as 1911 PICKHOLTZ (42) referred the action of light in promoting germination to the effects of heat rays. In an attempt to distinguish the effects of light from those of temperature the following experiments were performed. Seeds of each kind were counted into Petri dishes with filter paper wetted with distilled water as substratum. One lot of cultures was placed in darkness on February 9 at 40° C., where it remained for 17 days. Another lot of cultures was placed in darkness at temperatures ranging from 0 to 12° C. for 17 days. Another lot was kept in darkness and subjected alternately to high temperature (40° C.) and low temperature (0–12° C.) for nearly equal periods throughout the 17 days. The low temperature and the

alternating temperature cultures were frozen on the morning of February 26. On this date observations were made and the cultures placed in light at room temperature to test viability.

As shown in table VII, the constant high temperature effectively inhibited the germination of all seeds except those of *Verbascum Thapsus*. The subsequent incubation in light at room temperature showed fatal injury to the embryos of *Daucus Carota*, *Oenothera biennis*, and *Nicotiana Tabacum*. The constant low temperature delayed germination, but seemed to induce increased germination in light in seeds of *Daucus Carota*, *Oenothera biennis*, and *Verbascum Thapsus*. This is especially noticeable in *Oenothera biennis* seeds.

TABLE VII

Seeds	Constantly at 40° C. in darkness for 17 days (*a*) and then at room temperature in light for 12 days (*b*)		Constantly at 0–12° C. in darkness for 17 days (*a*) and then at room temperature in light for 12 days (*b*)		Alternately at 40 and 0–12° C. in darkness for 17 days (*a*) and then at room temperature in light for 12 days (*b*)		Control at room temperature for 11 days	
	(*a*)	(*b*)	(*a*)	(*b*)	(*a*)	(*b*)	Light	Darkness
Verbascum Thapsus	14	78	0	86	6	90	76	2
Rumex crispus	0	54	0	58	0	56	82	30
Daucus Carota	0	1	0	60	0	8	32	6
Oenothera biennis	0	4	0	64	0	90	14	0
Nicotiana Tabacum	0	0	0	40	0	26	58	0

The alternating high and low temperature treatment delayed the germination in the same way as did constant low temperature. As shown by the subsequent incubation, *Daucus Carota* seeds were injured most. The germination of seeds of *Nicotiana Tabacum* and *Rumex crispus* was materially reduced by this treatment, while the germination of seeds of *Verbascum Thapsus* was favored, and the germination of seeds of *Oenothera biennis* very greatly increased.

In a further effort to distinguish effects of light and temperature an experiment was carried out as follows. Seeds were counted into Petri dishes, having filter paper wetted with distilled water for substratum, and placed under the following conditions: set *a* in light at 10° C. for 8 days and then at 25° C. for 8 days; set *b* in darkness at 10° C. for 8 days and then at 25° C. for 8 days; set *c*

in light at 10° C. for 8 days and then in darkness at 25° C. for 8 days; set *d* in darkness at 10° C. for 8 days, then at 40° C. for 4 days, followed by 4 days at 25° C. At the end of the 16 days' treatment all of the cultures were placed in light for 8 days at room temperature. The experiment was begun July 24.

Comparison of the data in *a* of table VIII with the control indicates that incubation in light at low temperature followed by incubation at room temperature results in reduction of percentage of germination of *Daucus Carota* seed. A comparison of *a* and the control indicates that alternating temperatures may in a measure replace light in the case of germination of *Verbascum*

TABLE VIII

Seeds	Constantly at 25° C. for 24 days		(a)			(b)			(c)			(d)		
	Light	Darkness	In light at 10° C. for 8 days	Then at 25° C. for 8 days	Then at 25° C. for 8 days	In darkness at 10° C. for 8 days	Then at 25° C. for 8 days	Then in light at 25° C. for 8 days	In light at 10° C. for 8 days	Then in darkness at 25° C. for 8 days	Then in light at 25° C. for 8 days	In darkness at 10° C. for 8 days	Then at 40° C. for 4 days, then at 25° C. for 4 days	Then at 25° C. in light for 8 days
Verbascum Thapsus	94	0	0	95	98	0	64	83	0	71	71	0	1	88
Rumex crispus....	89	2	0	98	98	0	2	63	0	94	97	0	31	34
Daucus Carota....	60	4	0	14	15	0	2	16	0	11	11	0	13	13
Oenothera biennis.	85	0	0	69	69	0	16	16	0	2	2	0	4	12
Nicotiana Tabacum	53	0	0	70	72	0	0	3	0	35	35	0	0	1

Thapsus seeds, that it is an important factor in the germination of *Rumex crispus* seeds, and further indicates the necessity of light in the early periods of incubation of *Daucus Carota*, *Oenothera biennis*, and *Nicotiana Tabacum*. A comparison of *b* with *a* points again to the necessity of light in *Rumex crispus*, *Daucus Carota*, *Oenothera biennis*, and *Nicotiana Tabacum*, and indicates that some inhibiting factor developed during the 8 days in darkness in the case of *Oenothera biennis* and *Nicotiana Tabacum*. A comparison of *c* with the control indicates that light does its work on such seeds as *Verbascum Thapsus*, *Rumex crispus*, and in a measure on *Nicotiana Tabacum* even at low temperature, and that as soon as heat is supplied germination occurs. Incubating *Daucus Carota* and *Oenothera biennis* seeds at low temperature for a period of 8

days, in light or darkness, produces a condition from which they do not recover when incubated at 25° C. in light or in darkness. A comparison of *d* with control *a*, *b*, and *c* indicates that sudden changes from extremes of temperature may delay germination of *Verbascum Thapsus* seeds, that such treatment inhibits the germination of a large percentage of *Rumex crispus* seeds, and that it almost entirely inhibits the germination of seeds of *Nicotiana Tabacum*. The results in *d* confirm the observations on *Daucus Carota* and *Oenothera biennis* made in connection with *b*, namely, that some limiting factor develops during incubation in darkness at low temperature which is not easily overcome. The most noteworthy result of this treatment is the complete inhibition of germination of seeds of *Nicotiana Tabacum*. This is in agreement with that found in *b*. Together these results when compared with the control indicate a light requirement for *Nicotiana Tabacum* seeds which is not replaced by any temperature combination tried.

To summarize, this experiment shows that alternating temperature may replace light in germination of *Verbascum Thapsus* seeds, that light is necessary for optimum germination of entire seeds of *Rumex crispus*, although change of temperature in a measure replaces light. The results of this experiment indicate that seeds of *Oenothera biennis* and *Daucus Carota* require light and medium temperature for optimum germination, and that incubation at low temperature in darkness permits a change which is not overcome by transfer to high temperature in darkness. Moreover, in *Daucus Carota* exposures to light at 25° C. did not bring about germination of these changed seeds. Incubation of *Nicotiana Tabacum* in darkness at 10° C. did not result in increased percentage of germination in darkness. Incubation of *Ncotiana Tabacum* seeds in light at 10° C. promoted subsequent germination in darkness.

Hot water treatment

In a further attempt to induce germination in darkness, an adaptation of the warm bath method of MOLISCH (37) was employed. The seeds were counted, wrapped in filter paper, inclosed in little bags of cheesecloth, and plunged into hot distilled water for 0.25 minute and 0.5 minute respectively. Great care

was taken to plunge them promptly into cold distilled water, when the hot water was squeezed out. The seeds were then placed to germinate for 7 days at room temperature under the usual conditions. Table IX indicates what may be expected of hot water treatment of seeds. Treatment at lower temperatures was ineffective and so was not tabulated. The experiment was begun March 4.

The results of the warm bath treatment are mostly negative. The percentage of germination of *Rumex crispus* in darkness is

TABLE IX

Seeds	Time (in minutes) in hot water	Treatment at 90° C.; germination at room temperature for 7 days		Treatment at 75° C.; germination at room temperature for 7 days		Treatment at 60° C.; germination at room temperature for 7 days		Untreated; germination at room temperature for 7 days	
		Light	Darkness	Light	Darkness	Light	Darkness	Light	Darkness
NicotianaTabacum	0.25	4	2	0	0	42	11	62	2
NicotianaTabacum	0.5	0	0	0	0	50	34	.....	.....
Daucus Carota...	0.25	22	0	24	6	20	3	52	2
Daucus Carota...	0.5	22	0	36	8	40	8	.....	.....
VerbascumThapsus	0.25	0	0	75	10	67	20	78	0
VerbascumThapsus	0.5	0	0	75	8	65	15	.....	.....
Oenothera biennis.	0.25	70	22	76	20	40	8	50	6
Oenothera biennis.	0.5	0	6	80	48	28	20	.....	.....
Rumex crispus....	0.25	68	40	34	12	58	35	66	18
Rumex crispus....	0.5	66	36	58	10	86	28	.....	.....

increased somewhat by treatment with hot water at 90° C., while that of *Oenothera biennis* is increased somewhat by treatment with hot water at 75° C. and 90° C. These results indicate the coat as the limiting factor in their germination. Treatment at 100° C. for short periods might furnish interesting information.

Water absorption

To determine the relation of water absorption to germination in light and darkness, 2 to 3 gm. of each of the different kinds of seeds were weighed separately and placed under favorable conditions for germination. As soon as the first germination in light was observed, the seeds were dried rapidly and weighed carefully, and the percentage of water absorbed was computed on the dry

weight basis. To confirm the results obtained a second series was treated similarly. Failing to obtain concordant results, two additional series of determinations were made. The variation in time of the appearance of the first hypocotyls and the uneven surfaces of the seed coats account for much of the variation in the amount of water absorbed. The results are given in table X.

In view of the small size of the seeds, their irregular surfaces, the difficulty of uniform drying, and the increase of weight on account of germination, the data of these determinations are not

TABLE X

Seeds	Series 1		Series 2		Series 3		Series 4	
	Percentage of imbibed water	No. of sprouts	Percentage of imbibed water	No. of sprouts	Percentage of imbibed water	No. of sprouts	Percentage of imbibed water	No. of sprouts
	Light							
Nicotiana Tabacum	92.9	13	82.3	10	108.5	3	65.5	8
Verbascum Thapsus	93.3	12	58.8	2	199.0	25	93.3	6
Daucus Carota	114.5	2	92.4	4	85.7	4	64.8	3
Oenothera biennis	40.7	1	48.5	4	61.7	7	39.4	11
Rumex crispus	48.4	3	43.4	2	54.3	5	52.7	29
	Darkness							
Nicotiana Tabacum	60.0	0	50.0	0	67.7	1	63.6	0
Verbascum Thapsus	81.2	0	68.0	0	90.0	2	76.9	0
Daucus Carota	90.7	0	46.1	1	97.3	1	64.0	6
Oenothera biennis	45.1	0	108.5	1	49.4	1	49.9	11
Rumex crispus	53.1	8	52.4	1	48.9	1	45.8	10

surprisingly discordant. In some cases the high percentages of water absorbed is accounted for by the many and large seedlings which could not be removed without more seriously changing the data. After eliminating the cases open to suspicion on account of the numerous sprouts, imperfect drying, etc., there appears to be relatively little difference in the percentage of moisture absorbed by seeds germinated in light and darkness. In *Nicotiana Tabacum* seeds of series 4 the imbibition is 65.5 per cent with 8 sprouts in light, while in series 3 the imbibition is 67.7 per cent with one sprout in darkness, from which it appears that germination may occur even though a smaller percentage of water is absorbed. A

comparison of determinations of absorption by *Verbascum Thapsus* seeds in light (series 2) and in darkness (series 4) indicates the same general relations. The data for the other seeds show similar results. From this experiment it appears that light is not necessary for the absorption of sufficient water for germination.

Injection of seeds with water

DE VRIES (8), having abandoned variation of temperatures, high temperatures (40–50° C.), and other treatments of seeds of *Oenothera biennis* as means of securing complete germination, injected soaked seeds with water under pressure of 6–8 atmospheres, after which he frequently secured germination of 100 per cent. The

TABLE XI

Seeds	Darkness for 17 days	Then in light for 7 days	Darkness for 15 days	Then in light for 7 days	Untreated	
	(*a*)		(*b*)		Light	Darkness
Verbascum Thapsus	1	4	0	24	81	0
Daucus Carota	6	10	8	12	61	21
Oenothera biennis	8	43	5	55	33	8
Rumex crispus	5	8	11	56	71	31
Nicotiana Tabacum	4	54	5	60	83	0

seeds (table XI, *a*) accordingly were soaked overnight at a temperature of 25–28° C., wrapped in filter paper, placed in water, exhausted of the air in their intercellular spaces by reducing the atmospheric pressure to 20 mm. for 1 hour, and then subjected to hydrogen gas pressure of 575–675 pounds per square inch for 24 hours. The seeds were then placed to germinate in darkness under the usual conditions. A second lot (*b*) was treated in the same way except that it was subjected to a pressure of 500–650 pounds per square inch for 48 hours. Both lots were germinated at room temperature. Evidently injection with water does not increase the germination of seeds of *Oenothera biennis*, *Nicotiana Tabacum*, *Daucus Carota*, or *Rumex crispus* in darkness. When the seeds are subsequently exposed to light, they germinate in one or both tests. These results confirm the conclusion arrived at in the weighing experiments, that impermeability to water is not the limiting factor in light germi-

nation. Perhaps better illumination of the injected *Oenothera biennis* seeds made possible the increased germination reported by DE VRIES.

Increased oxygen supply

In his investigation of the delayed germination of seeds of *Xanthium*, CROCKER (7) found that the seed coat excludes oxygen, while SHULL (46) found a very definite relation between the oxygen supply and the percentage of germination in seeds of *Xanthium*. In order to discover if increased oxygen supply would promote the germination of the light-sensitive seeds in darkness, the following experiment was performed. Counted seeds were placed on wet filter paper in open dishes and placed under water-sealed glass cylinders containing 40, 50, 60, 70, and 80 per cent oxygen respectively (table XII). Each cylinder was placed in a dark room at 23–28° C. and covered with a light-tight metallic cylinder.

TABLE XII

Seeds	Percentage of germination in oxygen				
	40	50	60	70	80
Nicotiana Tabacum	0	0	0	0	0
Verbascum Thapsus	0	0	0	0	0
Daucus Carota	3	3	9	15	24
Oenothera biennis	.....	1	1	1	3
Rumex crispus	8	.....	19	18	23

A comparison of the germination in darkness in the presence of different percentages of oxygen shows an increase of germination of seeds of *Daucus Carota* and *Rumex crispus* with an increase of oxygen supply. Other conditions in each of the cylinders being the same so far as known, this must be attributed to increased oxygen supply. A similar experiment with higher and lower percentages of oxygen would have been interesting, especially a test of germination in 20 per cent oxygen (ordinary air) under these conditions. It would probably have given results similar to those in 40 per cent oxygen and would have been rather more conclusive. The regularity of the increased percentage of germination, however, due to increased concentration of oxygen, indicates the reliability of the results. Clearly this experiment does not indicate an oxygen

deficiency in seeds of *Nicotiana Tabacum*, *Verbascum Thapsus*, and *Oenothera biennis*.

Substrata

LEHMANN (30) reported increased germination in darkness of light-sensitive seeds such as *Ranunculus sceleratus* with soil as substratum. BAAR (1) obtained an increased percentage of germination of seeds of *Amaranthus* when he substituted earth for filter paper as a substratum, but OTTENWÄLDER (40), who used soil and sand as well as filter paper as substrata for his *Epilobium hirsutum* seeds, found beneficial results in his experiments with only one lot of sand. Investigation showed that the sand had been treated with acid which had not been thoroughly washed out.

TABLE XIII

PERCENTAGE OF GERMINATION AFTER 18 DAYS

SEEDS	ON SOIL		ON SAND		IN SOIL	IN SAND	ON FILTER PAPER	
	Light	Darkness	Light	Darkness	Light	Light	Light	Darkness
Verbascum Thapsus.....	40	34	30	20	2	24	41	3
Daucus Carota.....	56	28	54	28	32	58	63	19
Oenothera biennis.....	2	0	2	2	0	6	3	2
Rumex crispus.....	68	2	46	10	12	38	42	8
Nicotiana Tabacum.....	70	6	78	0	56	76	71	1

In view of the divergent results with the different seeds, it was deemed desirable to determine the relation of sand and soil to germination in darkness of these light-sensitive seeds. Also the question arose as to whether light was as necessary under natural conditions as under laboratory conditions for the germination of light-sensitive seeds. The substrata were carefully sterilized, uniformly wetted, and prepared for the seeds. The seeds in "sand" and in "soil" were buried to a depth of 0.25 inch. All were put under the same temperature conditions (23–26° C.). The results are shown in table XIII. The experiment was begun May 13 and closed June 1.

The germination of seeds of *Verbascum Thapsus* on soil and sand in darkness is somewhat higher than that on filter paper. The substratum appears to have exerted a slightly favorable effect on the germination of seeds of *Daucus Carota*, but none on the other

seeds. Such results suggest a beneficial effect on some particular constituent contained only in seeds of *Verbascum Thapsus*. In light the percentage of germination seems to correspond roughly to the light intensity. Where the lighting is good, as on the filter paper or sand, the germination is good. Where it is diminished, as in the case of seeds buried in sand or soil, the germination is reduced. The increase of germination of seeds on sand or soil in darkness may be referred largely to the action of constituents of the soil and sand. The low germination of seeds of *Oenothera biennis* may be due to periodicity in dormancy, since seeds from the same lot gave a germination of 78 per cent in light and 0 per cent in darkness in October. From these results it appears that constituents of soil may only partially substitute for light with some seeds and not at all with others.

Effects of electrolytes

The effects of electrolytes on the germination of light-sensitive seeds have been variously reported. Beneficial effects on germination in darkness of *Ranunculus sceleratus* from hot water extracts of soils, Knop's nutrient solution, and salt solutions have been reported by LEHMANN (30). He reported no benefit from cold water extracts of soils. LEHMANN and OTTENWÄLDER (36) found that weak acid solutions promote germination in darkness of seeds of *Verbascum Thapsus*, *V. thapsiforme*, and *Lythrum Salicaria*. OTTENWÄLDER (40) reported that acids promote the germination of seeds of *Epilobium hirsutum* in darkness. GASSNER (15) reported that nitrogen compounds such as nitrates, nitrites, and ammonium salts through a wide range of concentrations favor the germination of seeds of *Chloris ciliata* in darkness. As many of the electrolytes reported by these investigators belonged to the lyophobe or lyophile series, a systematic study of their effects was undertaken, to discover, if possible, some relation between electrolytes and germination. Lots of 100 seeds each were counted into test tubes, about 2 cc. of a solution of an electrolyte added, and the tubes placed in darkness. After 24 hours most of the solution was drained from each test tube, which was promptly returned to the dark chamber for the seeds to germinate. The period allowed for germination was 7 days after soaking. The results are given in table XIV.

TABLE XIV

GERMINATION IN DARKNESS IN VARIOUS CONCENTRATIONS

SEEDS	N	0.1 N	0.01 N	0.001 N	0.0001 N	0.00001 N
Acetic acid						
Verbascum Thapsus	0	0	77	79	86	....
Rumex crispus	0	0	29	23	0	....
Daucus Carota	0	0	1	3	4	....
Oenothera biennis	0	8	48	42	53	....
Nicotiana Tabacum	0	0	27	46	23	....
Butyric acid						
Verbascum Thapsus	0	0	0	80	86	....
Rumex crispus	0	0	0	6	2	....
Daucus Carota	0	0	0	1	6	....
Oenothera biennis	0	0	0	45	50	....
Nicotiana Tabacum	0	0	0	38	10	....
Citric acid						
Verbascum Thapsus	....	0	40	95	98	....
Rumex crispus	....	6	11	8	13	....
Daucus Carota	....	0	10	5	12	....
Oenothera biennis	....	38	61	52	68	....
Nicotiana Tabacum	....	36	35	54	58	....
Tartaric acid						
Verbascum Thapsus	....	0	14	81	53	....
Rumex crispus	....	7	7	10	0	....
Daucus Carota	....	1	0	13	8	....
Oenothera biennis	....	2	52	75	67	....
Nicotiana Tabacum	....	21	59	50	27	....
Malic acid						
Verbascum Thapsus	....	5	64	100	91	....
Rumex crispus	....	1	22	13	6	....
Daucus Carota	....	0	3	7	1	....
Oenothera biennis	....	27	55	54	60	....
Nicotiana Tabacum	....	40	43	31	83	....
Potassium sulphocyanate						
Verbascum Thapsus	....	....	80	75	88	....
Rumex crispus	....	....	0	2	2	....
Daucus Carota	....	....	8	4	2	....
Oenothera biennis	....	....	70	65	61	....
Nicotiana Tabacum	....	....	3	1	7	....
Sodium sulphocyanate						
Verbascum Thapsus	....	....	0	93	52	....
Rumex crispus	....	....	0	0	1	....
Daucus Carota	....	....	2	4	0	....
Oenothera biennis	....	....	63	57	53	....
Nicotiana Tabacum	....	....	9	9	14	....

SEEDS	N	0.1 N	0.01 N	0.001 N	0.0001 N	0.00001 N
Sodium iodide						
Verbascum Thapsus	....	....	37	94	62	....
Rumex crispus	....	....	0	0	1	....
Daucus Carota	....	....	4	3	0	....
Oenothera biennis	....	....	42	72	49	....
Nicotiana Tabacum	....	....	1	2	3	....
Sulphuric acid						
Verbascum Thapsus	....	....	8	35	94	97
Rumex crispus	....	....	0	1	0	0
Daucus Carota	....	....	0	12	3	7
Oenothera biennis	....	....	25	36	34	40
Nicotiana Tabacum	....	....	2	24	9	2
Potassium sulphate						
Verbascum Thapsus	....	....	87	89	100	....
Rumex crispus	....	....	1	0	1	....
Daucus Carota	....	....	1	0	1	....
Oenothera biennis	....	....	44	58	38	....
Nicotiana Tabacum	....	....	12	5	8	....
Ammonium sulphate						
Verbascum Thapsus	0	51*	92	87	98	....
Rumex crispus	0	3	7	5	9	....
Daucus Carota	0	0	1	4	15	....
Oenothera biennis	0	44*	53	54	65	....
Nicotiana Tabacum	0	36*	44	40	23	....
Sodium sulphate						
Verbascum Thapsus	....	....	91	94	88	....
Rumex crispus	....	....	2	0	2	....
Daucus Carota	....	....	1	6	0	....
Oenothera biennis	....	....	58	48	41	....
Nicotiana Tabacum	....	....	4	9	10	....
Lithium sulphate						
Verbascum Thapsus	....	....	93	91	90	....
Rumex crispus	....	....	3	0	1	....
Daucus Carota	....	....	2	3	0	....
Oenothera biennis	....	....	46	53	37	....
Nicotiana Tabacum	....	....	17	12	9	....
Nickel sulphate						
Verbascum Thapsus	....	....	63†	62	86	....
Rumex crispus	....	....	3	2	0	....
Daucus Carota	....	....	5	28	21	....
Oenothera biennis	....	....	33	40	37	....
Nicotiana Tabacum	....	....	2	13	9	....

* Little more than swelling.

† Injured.

TABLE XIV—*Continued*

Seeds	N	0.1 N	0.01 N	0.001 N	0.0001 N	0.00001 N
Zinc sulphate						
Verbascum Thapsus	....	....	41	80	97	....
Rumex crispus	....	....	0	0	0	....
Daucus Carota	....	....	2	1	1	....
Oenothera biennis	....	....	37	40	51	....
Nicotiana Tabacum	....	....	8	3	2	....
Potassium nitrate						
Verbascum Thapsus	....	....	36	82	95	....
Rumex crispus	....	....	3	2	0	....
Daucus Carota	....	....	8	18	8	....
Oenothera biennis	....	....	55	61	55	....
Nicotiana Tabacum	....	....	50	23	23	....
Ammonium nitrate						
Verbascum Thapsus	0	62	100	82	93	....
Rumex crispus	0	3	14	11	8	....
Daucus Carota	0	0	0	1	1	....
Oenothera biennis	0	70	66	68	64	....
Nicotiana Tabacum	0	54	73	34	28	....
Sodium nitrate						
Verbascum Thapsus	0	64	100	98	91	....
Rumex crispus	0	14	26	4	15	....
Daucus Carota	0	6	1	6	4	....
Oenothera biennis	0	60	67	67	50	....
Nicotiana Tabacum	0	38	34	29	11	....
Aluminum nitrate						
Verbascum Thapsus	....	....	55	98	93	....
Rumex crispus	....	....	1	1	0	....
Daucus Carota	....	....	2	3	9	....
Oenothera biennis	....	....	12	57	55	....
Nicotiana Tabacum	....	....	0	10	14	....

Seeds	N	0.1 N	0.01 N	0.001 N	0.0001 N	0.00001 N
Cobalt nitrate						
Verbascum Thapsus	....	....	31*	99	93	....
Rumex crispus	....	....	1	4	2	....
Daucus Carota	....	....	4	2	8	....
Oenothera biennis	....	....	45	57	16	....
Nicotiana Tabacum	....	....	13	15	10	....
Potassium hydroxide						
Verbascum Thapsus	....	0	84	100	98	....
Rumex crispus	....	0	6	6	9	....
Daucus Carota	....	0	7	9	4	....
Oenothera biennis	....	0	44	55	42	....
Nicotiana Tabacum	....	0	17	34	53	....
Ammonium hydroxide						
Verbascum Thapsus	....	....	1	90	95	....
Rumex crispus	....	....	0	0	1	....
Daucus Carota	....	....	0	1	3	....
Oenothera biennis	....	....	31	....	56	....
Nicotiana Tabacum	....	....	1	15	12	....
Sodium hydroxide						
Verbascum Thapsus	....	0	81	96	83	....
Rumex crispus	....	0	1	2	2	....
Daucus Carota	....	0	3	11	6	....
Oenothera biennis	....	0	50	57	0	....
Nicotiana Tabacum	....	0	21	3	16	....
Hydrochloric acid						
Verbascum Thapsus	....	....	0	38	61	92
Rumex crispus	....	....	0	0	0	0
Daucus Carota	....	....	0	5	4	2
Oenothera biennis	....	....	5	61	56	60
Nicotiana Tabacum	....	....	0	10	22	10

* Little more than swelling.

From table XIV it appears that organic acids, bases, and salts of monovalent, bivalent, and trivalent ions induce germination in darkness of seeds of *Verbascum Thapsus* (80–100 per cent), *Oenothera biennis* (40–60 per cent), and *Nicotiana Tabacum* (10–50 per cent), while they inhibit the germination of seeds of *Rumex crispus* and *Daucus Carota*. These results were confirmed in an attempt to determine the minimum effective concentration of the electrolytes. In this attempt it was found that as good germination in darkness

occurs in ten-millionth normal solutions as in one-thousandth normal solutions. These results indicate no definite relation between the nature of the ion and germination. In another series of experiments on the relation of electrolytes to germination, with seeds from another crop, it was found that the germination of *Verbascum Thapsus*, *Oenothera biennis*, and *Nicotiana Tabacum* was inhibited, while the germination of *Rumex crispus* seeds was promoted in darkness by the action of the various electrolytes. This suggests that something in the conditions of growth, maturing, harvesting, or storage may have changed the sign of the charge of the ionizable constituents of the seeds. Further work on the effects of electrolytes on the germination of these seeds is highly desirable.

Soaking in solutions of electrolytes

It is generally believed that forcing agents of germination such as light, enzymes, and electrolytes are most effective during the early stages of incubation. KINZEL (24) by soaking seeds of *Nigella sativa* in a solution of papayotin and asparagin for 5 hours and then in water for 24 hours secured a 30 per cent increase of germination of "light hard" seed. OTTENWÄLDER (40) has reported that 24 hours is not sufficient time to secure the full effect of the acid on the germination of seed of *Epilobium hirsutum*, and that about 48 hours' soaking was necessary to get the best results from the action of the acid. An attempt was made, therefore, to determine whether soaking in solutions of electrolytes could promote the germination of light-sensitive seeds.

Seeds were soaked in the various solutions for 24–28 hours and washed in distilled water until all of the solution was removed. To avoid light effects, care was taken to work in very diffuse light. The seeds were spread on filter paper in Petri dishes and placed to germinate in light and darkness respectively, at room temperature, for 8 days.

An examination of table XV *A* shows that soaking in rather strong solutions of hydrochloric acid promotes the germination in darkness of seeds of *Nicotiana Tabacum*, *Verbascum Thapsus*, *Oenothera biennis*, and *Rumex crispus*, while it hinders the germination of seeds of *Daucus Carota* in light. The beneficial effects of solutions

of sulphuric acid appear only in the germination of seeds of *Daucus Carota* in darkness. Soaking seeds of *Nicotiana Tabacum* and

TABLE XV*A*

Seeds	Light				Darkness			
	N	0.1 N	0.01 N	∞	N	0.1 N	0.01 N	∞
	Hydrochloric acid (soaked 26 hours)							
Nicotiana Tabacum	83	70	70	65	16	35	22	11
Verbascum Thapsus	34	85	87	85	1	22	24	3
Daucus Carota	0	0	0	54	0	18	25	19
Oenothera biennis	76	92	87	78	37	33	.....	8
Rumex crispus	80	84	72	.....	23	35	35	15
	Sulphuric acid (soaked 28 hours)							
Nicotiana Tabacum	.....	76	76	71	.....	10	26	21
Verbascum Thapsus	.....	79	87	81	.....	2	10	0
Daucus Carota	.....	72	66	61	.....	12	38	21
Oenothera biennis	.....	83	51	62	.....	10	11	8
Rumex crispus	.....	69	73	71	.....	24	30	31
	Sodium sulphocyanate (soaked 28 hours)							
Nicotiana Tabacum	68	.....	.....	74	48	.....	.....	19
Verbascum Thapsus	57	.....	.....	83	0	.....	.....	5
Daucus Carota	36	.....	.....	75	12	.....	.....	26
Oenothera biennis	51	.....	.....	52	0	.....	.....	12
Rumex crispus	52	.....	.....	68	54	.....	.....	24
	Sodium hydroxide (soaked 24 hours)							
Nicotiana Tabacum	0	81	61	74	0	26	25	19
Verbascum Thapsus	0	63	86	83	0	10	19	5
Daucus Carota	0	77	63	75	0	30	45	26
Oenothera biennis	1	87	42	51	0	22	12	12
Rumex crispus	0	59	76	68	0	17	26	24

TABLE XV*B*

Seeds	Percentage of hydrogen peroxide (soaked 27 hours)									
	Light					Darkness				
	50	20	10	5	∞	50	20	10	5	∞
Nicotiana Tabacum	15	75	72	56	74	6	38	81	46	19
Verbascum Thapsus	12	70	75	68	83	0	0	6	2	5
Daucus Carota	76	83	94	78	75	26	48	61	53	26
Oenothera biennis	18	33	33	40	51	0	5	12	10	12
Rumex crispus	73	66	73	77	68	43	36	70	39	24

Rumex crispus in solutions of sodium sulphocyanate appeared to promote their germination in darkness. Soaking in potassium sulphocyanate gave similar results. Soaking in solutions of hydrogen peroxide promoted the germination of seeds of *Nicotiana Tabacum*, *Daucus Carota*, and *Rumex crispus* in darkness (table XV *B*). Germination in 0.001 N sodium hydroxide was about the same as in 0.01 N. Soaking in solutions of sodium hydroxide gave increased germination in darkness of seeds of *Verbascum Thapsus*, *Daucus Carota*, and *Oenothera biennis*. These results were confirmed by another set of tests.

The reaction of the seeds to the different electrolytes indicates that the ions of the electrolytes are acting on different constituents of different seeds. While, as shown in the preceding experiment, the use of hydrochloric acid, sodium sulphocyanate, and hydrogen peroxide as substrata yields no increase of germination of *Rumex crispus* in darkness, this experiment shows that soaking for a short period (24–28 hours) in solutions of these electrolytes does promote their germination in darkness. As *Rumex crispus* seeds were brought to germination in darkness by abrading and removing the coats, and by the action of concentrated sulphuric acid, their germination may naturally be referred to coat effects, the compounds acting on some constituent of the coat. The germination of *Daucus Carota* is not so easily accounted for. The germination in darkness was only slightly promoted by increased oxygen supply and on soil as substratum. The hydrogen peroxide may yield an increased oxygen supply and thus promote the germination of these seeds, but an explanation of the beneficial effects of sulphuric acid and sodium hydroxide on the same material is not easily made unless we refer to coat effects which have not been clearly indicated by other treatment. A longer period of soaking (48 hours) might have yielded data to settle this, as well as the failure of germination of the other seeds in certain solutions.

Lipoid solvents

Finding that lipoids occur in the coats and embryos of all the seeds and in the endosperm of four of them, it was thought desirable to determine the effect of acetone, alcohol, and ether on their

germination. Seeds of each kind were soaked in acetone for 15, 30, and 60 minutes respectively, air dried for two hours, and placed under favorable conditions for germination in darkness. At the end of 8 days of incubation (in darkness) the seeds were placed in light for 8 days, where none of them germinated. Other seeds were similarly treated with alcohol with similar results. A few seeds of *Daucus Carota* and *Oenothera biennis* survived the alcohol treatment and germinated in light and darkness. Other seeds were treated with ether, as indicated in table XVI.

The results show no promotion of germination of light-sensitive seeds in darkness when treated with lipoid solvents, but rather show inhibition or diminution of subsequent germination in light. This is especially true for acetone and alcohol. Ether inhibited germination in darkness of all seeds except *Daucus Carota*, and diminished the subsequent germination in light of seeds of *Verbascum Thapsus*, *Rumex crispus*, and *Nicotiana Tabacum*. Ether treatment did not affect the subsequent germination of seeds of *Oenothera biennis* in light.

TABLE XVI

Seeds	Soaked in ether 15 minutes		Soaked in ether 30 minutes		Soaked in ether 60 minutes	
	Darkness	Light	Darkness	Light	Darkness	Light
Verbascum Thapsus	0	28	0	30	0	0
Rumex crispus	0	35	0	3	0	2
Daucus Carota	15	17	17	17	12	12
Oenothera biennis	0	76	0	74	0	62
Nicotiana Tabacum	0	43	0	64	0	31

Microchemistry

In an attempt to find the substance responding to the action of light, an examination of the seeds was undertaken by microchemical methods suggested by Eckerson (9), and the nature and distribution of the different structural and nutritive materials were determined. Much of the information thus obtained has no evident bearing on the problem of light germination and may best be presented in a separate publication. Some of the substances and conditions in these seeds which may function in light germination are fat, suberin, starch, and reaction.

Starch occurs in the endosperm of seeds of *Rumex crispus* and *Daucus Carota*. It does not occur in the embryo of any seeds under investigation. Moreover, the hydrogen ion concentration is not likely to be materially changed by such hydrolysis of starch as may occur during their germination, and therefore we need not consider starch an important factor in their germination. Since suberin is found in the coats of *Oenothera biennis* and *Daucus Carota*, but not elsewhere, it can hardly be considered a common limiting factor in the germination of the light-sensitive seeds studied. Oily or fatty substances were found in the cell contents of coats, endosperms, and embryos of each of the seeds, appearing as small droplets of substance readily stained with Soudan III or Scharlach R. These lipoids almost never occurred as continuous layers which might obstruct the entrance of water or other substance necessary for germination, but usually as emulsions of fats in the cell sap. The results of the experiments on the absorption of water support the observation that there is no important obstruction of water by the constituents of the coats. Hence suberin and lipoids need no further consideration as limiting factors in absorption of water by these seeds.

Using neutral red as an indicator, seeds soaked in water in light and in darkness, as well as dry seeds, were tested for the reaction of the different parts with results shown in table XVII.

The outstanding result of the microchemical examination is the greater acidity of seeds incubated in light as compared with those incubated in darkness. This was found to be the case in each of the five kinds of light-sensitive seeds. The embryos incubated in light had a higher hydrogen ion concentration than those of the same kind incubated in darkness. This was especially noticeable in the hypocotyls. This result is contrary to HEINRICHER'S (21) unsupported assumpton that the effectiveness of the fat splitting lipase was favored by the increased acid formation in darkness in *Phacelia tanacetifolia*. Moreover, HEINRICHER offered no experimental evidence of increased acid formation in darkness. Having all known external factors, except illumination, alike for the seeds under investigation, we may properly conclude that the varying factor, light, in some way brings about increased acidity of their embryos.

TABLE XVII

Seeds	Coats	Endosperm	Embryo
	Verbascum Thapsus		
Dry	Acid	Cell walls acid; contents neutral	Cell walls acid; contents neutral
Soaked in darkness	Acid	Cell walls acid; contents neutral	Outer cell walls and contents alkaline to neutral; inner cell walls acid; contents alkaline
Soaked in light	Acid	Walls acid; contents acid	Walls and contents acid
	Rumex crispus		
Dry	Acid	Outer layer acid; cell contents neutral	Walls and contents neutral
Soaked in darkness	Acid	Outer layer acid; contents mostly acid	Walls acid; contents neutral
Soaked in light	Acid	Walls acid; contents acid	Walls and contents acid
	Daucus Carota		
Dry	Outer walls acid; inner alkaline	Cell walls acid to alkaline; contents neutral to alkaline	Alkaline
Soaked in darkness	Outer acid; inner alkaline	Walls acid to alkaline; contents neutral to alkaline	Alkaline except at tip of hypocotyl
Soaked in light	Outer acid; inner alkaline	Walls acid to alkaline; contents neutral	Walls and contents acid
	Nicotiana Tabacum		
Dry	Acid	Cell walls acid; contents neutral to alkaline	Cell walls acid; contents neutral to alkaline
Soaked in darkness	Acid	Walls acid; contents alkaline to neutral	Cell walls acid; contents partly alkaline, partly neutral
Soaked in light	Acid	Walls acid; contents neutral to acid	Walls acid; contents acid except at base of cotyledons

TABLE XVII—*Continued*

SEEDS	COATS	ENDOSPERM	EMBRYO
	Oenothera biennis		
Dry	Partly acid	Slightly acid or neutral	Cell walls acid; contents neutral
Soaked in darkness	Mostly acid	Slightly acid to neutral	Cell walls acid to neutral; contents alkaline to neutral
Soaked in light	Partly acid	Neutral to acid	Walls and contents acid

Quantitative determination of acidity

To verify the results of the microchemical examination, 5 gm. of each kind of seed incubated in light for five days and 5 gm. of each kind incubated in darkness for five days were separately ground, digested in neutral alcohol and ether, and then titrated with N/10 NaOH. The results obtained were as follows:

Seed	Light	Darkness
Rumex crispus	3.5 cc.	2.8 cc.
Daucus Carota	3.3	2.8
Verbascum Thapsus	4.8	4.4
Nicotiana Tabacum	3.9	2.8

These results show greater acidity of seeds incubated in light than of those incubated in darkness, and confirm the findings of the microchemical examination. While the increase of titratable acidity was not large, it was measurable and repeatedly obtained, and apparently was sufficient in each instance to determine germination. Since light is the variable factor in this and the preceding experiment, we may properly conclude that light initiates changes which produce the increased acidity of seeds incubated in light over those incubated in darkness. These results establish the fact that light functions in some way to bring about increased acidity in these light-sensitive seeds. There remains to show in the following experiment, if possible, how the acidity is increased.

Effect of germination on substratum

Having found that the embryos of these seeds become acid in reaction by incubation in light, it was thought that testing the

reaction of the substratum after a period of incubation might throw some light on what was happening in the seeds. A small quantity of each kind of seed was soaked in distilled water, 0.01 N NH_4NO_3 solution, and 0.01 N $NaNO_3$ solution at room temperature, and the substratum tested for reaction with neutral red. The results are shown in table XVIII.

TABLE XVIII

Seeds	Soaked 18 hours in distilled water		Soaked 24 hours in 0.01 N NH_4NO_3		Soaked 24 hours in 0.01 N $NaNO_3$	
	Light	Darkness	Light	Darkness	Light	Darkness
Verbascum Thapsus	Alkaline	Alkaline	Alkaline	Alkaline	Alkaline	Alkaline
Rumex crispus	Acid	Acid	Acid	Acid	Acid	Acid
Daucus Carota	Alkaline	Alkaline	Alkaline	Alkaline	Alkaline	Alkaline
Oenothera biennis	Neutral	Neutral	Alkaline	Neutral	Alkaline	Neutral
Nicotiana Tabacum	.....	.....	Alkaline	Alkaline	Alkaline	Alkaline

From the results it appears that seeds of *Verbascum Thapsus*, *Daucus Carota*, and *Nicotiana Tabacum* excrete an alkaline substance in darkness as well as in light; that seeds of *Rumex crispus* excrete an acid substance in darkness as well as in light; and that seeds of *Oenothera biennis* excrete an alkaline substance in light.

A quantitative experiment also verifies a part of these results. Weighed quantities of each of the five kinds of seeds were incubated in light for five days in tubes containing 2 cc. of 0.01 N $NaNO_3$ respectively. The results are given in table XIX.

TABLE XIX

Seeds	Weight of seeds (gm.)	Required to titrate
Verbascum Thapsus	0.7406	1.0 cc. 0.01 N HCl
Rumex crispus	1.3385	1.5 cc. 0.01 N NaOH
Daucus Carota	0.6510	0.9 cc. 0.01 N HCl
Oenothera biennis	0.7580	0.5 cc. 0.01 N HCl
Nicotiana Tabacum	0.8403	1.8 cc. 0.01 N HCl

The longer period of incubation evidently allowed time for the excretion of a measurable amount of acid or base by each kind of seed. From these results it appears that seeds of *Rumex crispus* excrete measurable amounts of acid substance during incubation, while the other kinds excrete alkaline substances.

Enzymes

The favorable effects of light on the germination of seeds of *Veronica peregrina* in the early work of HEINRICHER (19) was referred to its effect upon chemical actions connected with the reactivation of reserve materials, and later (21) to its effect upon enzyme activity in the production and digestion of stored foods. He (22) referred the retarding effect of light on the germination of seeds of *Phacelia tanacetifolia* to its photochemical action on reserve materials, and assumed that the effectiveness of the fat splitting lipase was favored by the increased acid formation in darkness, while the irrefrangible light or the rays of the first half of the spectrum interfered, neutralizing the acid and thereby checking the decomposition of fat.

It seemed possible that enzymes of some kind might be active agents, and light the stimulus or trigger in the germination of certain seeds. Just what kinds of enzymes function most in the germination of light-sensitive seeds has not been shown. To determine whether proteolytic enzymes were the important enzymes for the seeds, as LEHMANN and OTTENWÄLDER (36) believed for seed of *Epilobium hirsutum*, seeds of *Verbascum Thapsus* and *Nicotiana Tabacum* were incubated in light and darkness respectively for four days and promptly ground in a little 50 per cent water solution of glycerine to which a crystal of thymol had been added. Small drops of extract from each kind of seed were put on nutrient gelatin according to the method of GIESEN (16). After 30 minutes the extract was taken up with soft filter paper. There were very shallow pits formed where the extracts incubated in light had been. There were also shallow pits formed on the gelatin where the extract incubated in darkness had been. In fact, the pits produced by the extract germinated in darkness were deeper than those produced by the extract incubated in light. To verify these observations, the tests were repeated after allowing a more complete extraction of the enzyme. The extracts of seeds incubated in light were put in light, and the extracts of seeds incubated in darkness were put in darkness. The following day the same tests were repeated. Extracts from each lot of seeds were tested for their action by putting loopfuls on gelatin. After 30 minutes the extracts were removed separately, when it was

found that well defined pits had been formed in the gelatin. Moreover, where the extract was left on the gelatin for 24 hours, the pits became quite deep, even though there was abundance of thymol to inhibit bacterial action. These results were confirmed by the method of GRÜSS (17).

Since the activity on gelatin of enzymes of seeds incubated in darkness was equal to or greater than that of seeds incubated in light, the favorable effect of light on germination of *Nicotiana Tabacum* and *Verbascum Thapsus* cannot be referred to activation of proteolytic enzymes.

It has already been seen that starch does not occur in the embryos of any of these light-sensitive seeds, and that it occurs only in the endosperms of *Daucus Carota* and *Rumex crispus*. From these facts it is evident that hydrolysis of contained starch can increase the hydrogen ion of the embryos little if any. It has been seen that proteolytic enzymes develop equally well in darkness and light in these seeds, hence they can be rejected as important factors in determining light germination. Also, incubation in light does result in increased acidity of embryos over those incubated in darkness.

It has been shown that the embryos of these seeds all contain fatty substances. The generally accepted method of demonstrating the presence of lipolytic enzymes is by the increase of acidity in the presence of fats. Inasmuch as development of acidity in the presence of light and fatty substance has been clearly demonstrated, it may be concluded that light activates the lipolytic enzyme to split the fatty substance to yield an acid. The results obtained with enzymes of seeds of *Verbascum Thapsus* and *Nicotiana Tabacum* do not support HEINRICHER'S (22) assumption that light inhibited the action of lipase in seeds of *Phacelia tanacetifolia*. On the other hand, the results indicate that light favored the action of lipase in seeds of *Verbascum Thapsus* and *Nicotiana Tabacum*.

Discussion

COAT EFFECTS

The light relation of seeds of *Rumex crispus* is largely one affecting the coats, as is indicated by increased germination in darkness following abrasion and removal of coats, treatment with

concentrated sulphuric acid, and increased oxygen pressure. Light may bring about some change in the coats of *Rumex crispus* to admit oxygen or other required substance, or permit the escape of some inhibiting substance such as an organic acid. It may change the relation of the lipoids from the oil water phase to the water oil phase, or break up a nearly continuous oil layer in the coat, thus allowing entrance or escape of some limiting factor. The presence of lipoids in the coats and the excretion of an acid instead of an alkaline substance during germination suggest that an enzyme acting in the coats hydrolyzes the lipoids, thus yielding acid and making the coats permeable to some required substance, or permitting the elimination of some inhibitory substance.

There is some evidence of a coat effect in the germination of seeds of *Oenothera biennis*. While abrasion of the coats does not yield increased germination, hot water treatment and sulphuric acid treatment both yield considerable increases of germination in darkness. The presence of lipoids in the coats suggests the same explanation of the action of light as in the seeds of *Rumex crispus*, with the addition that the light may also have a beneficial effect on the constituents of the embryo.

In the seeds of *Nicotiana Tabacum*, *Verbascum Thapsus*, and *Daucus Carota* there is little evidence of coat effects, there being no increased germination caused by abrasion, sulphuric acid treatment, hot water treatment, or increased oxygen pressure. The only results suggesting coat effects are increased germination of *Daucus Carota* and *Nicotiana Tabacum* when soaked in hydrogen peroxide. This increased germination might be referred to the effects on the embryos.

The seeds of this investigation fall into three groups. The first is represented by the seeds of *Rumex crispus*, in which the coats must be made permeable to some external or internal substance by light, abrasion, or other agency before abundant germination occurs. The second group is represented by the seeds of *Oenothera biennis*, whose germination is partly dependent on the coats being made permeable, and partly on the activation of the embryos by light or chemical agencies. The third group is represented by seeds of *Nicotiana Tabacum*, *Daucus Carota*, and *Verbascum Thapsus*,

whose germination is not increased simply by making the coats permeable, but requires the action of light or a suitable substitute to induce good germination.

Microchemistry

The results of the various mechanical, physical, and chemical treatments of the light-sensitive seeds have offered few suggestions as to the nature of the effects of light on their constituents in inducing germination. The substitution of these various agencies for light has contributed little to an acceptable explanation of how light functions to bring about germination. These treatments, however, have served to localize the action of light and to determine the part of the seed affected. On the other hand, the microchemical examination yielded results which point to an acceptable explanation of the action of light on light-sensitive seeds. The outstanding findings of the microchemical studies were abundance of lipoids in each kind of seed and increased acidity of seeds incubated in light. Thus there are linked together light, lipoids, and increased acidity.

Enzymes

Since starch and other carbohydrates were not found in the embryos of these seeds and in the endosperms of but two of them, it is not necessary to give serious consideration to the probable reaction of the products of their hydrolysis. Moreover, since proteolytic enzymes were found to be equally active in light and darkness in *Nicotiana Tabacum* and *Verbascum Thapsus* seeds, they need not be considered as important causes of increased acidity of the seeds incubated in light. It remains to be considered whether the products of the hydrolysis of the lipoids are the cause of the increased acidity in light.

The development of acidity in the watery extract of an oily seed like that of the castor bean is generally considered evidence of the presence of lipase. Such development of increased acidity in light was demonstrated quantitatively for four of the five kinds of seeds, thus confirming the results of the microchemical examination, and giving reasonable ground for inferring that lipase splits the fats thus yielding fatty acids in seeds germinated in light.

Again, the presence of neutral or very faintly alkaline fats in the cells of the dry embryos and the development of acids in embryos incubated in light and no change or development of slight alkalinity when incubated in darkness is very significant. It is generally assumed that increased acidity of fatty substances indicates hydrolysis due to the action of enzymes. If this assumption be granted, the admission is necessary that light initiates processes which in some way result in increased acidity, which is followed by germination, and that where light is not admitted acidity does not develop sufficiently to cause good germination. Three explanations of how light acts may be offered: (1) light may act directly to split the fats to fatty acids and glycerine; (2) light may activate the lipolytic enzyme which splits the fats; (3) light may initiate some change that produces a little acid which may activate the lipolytic enzyme which splits the fats.

While it is possible and even probable that light can act directly on the inclusions of cells to produce such changes as the formation of acid, it is hardly necessary to make this assumption. The proteolytic enzymes become active in the absence of light when the seeds are put under the usual conditions for germination. A certain percentage germinate in darkness even though they have not been treated with electrolytes or other stimulating agencies. Moreover, the amount of acid produced, though small, is relatively more than might be expected from the chemical action of light on lipoids. The evidence of the existence of lipase rather indicates either of the other explanations as much more acceptable. There is little choice between light activating the enzyme and light initiating some change which produces a little acid which may activate the lipolytic enzyme which splits fats. In either case light is the stimulus which initiates the changes leading to germination.

Summary

1. The seeds of *Rumex crispus*, *Datura Stramonium*, and *Phoradendron flavescens* were found to be light sensitive. The germination of seeds of *Rumex crispus* and *Phoradendron flavescens* was promoted by light; the germination of seeds of *Datura Stramonium* was hindered by light.

2. Abrasion and removal of coats (ovary walls) of *Rumex crispus* seeds promoted their germination in darkness.

3. Treatment of seeds of *Rumex crispus* and *Oenothera biennis* with concentrated sulphuric acid caused an increase in the percentage of germination in darkness.

4. No reciprocal relation between the effects of light and temperature was found.

5. Light was not necessary for the absorption of sufficient water for germination.

6. Injection of water did not yield increased germination in darkness.

7. Almost all kinds of single electrolytes, regardless of the nature of the ions, seemed to promote germination of seeds of *Oenothera biennis*, *Nicotiana Tabacum*, and *Verbascum Thapsus* in darkness.

8. Embryos of seeds incubated in light became more acid than those incubated in darkness.

9. Light seemed to activate lipolytic enzymes which hydrolyzed fats to fatty acids.

10. The germination of seeds of *Rumex crispus* in darkness was promoted (increased) by hot water treatment, abrasion, treatment with concentrated sulphuric acid, increased oxygen pressure, fluctuating temperatures, and soaking in solutions of hydrochloric acid, sodium sulphocyanate, and hydrogen peroxide.

11. The germination of seeds of *Nicotiana Tabacum* in darkness was promoted by soaking in solutions of hydrochloric acid, sodium sulphocyanate, and hydrogen peroxide, as well as by the use of many single electrolytes as substrata.

12. The germination of seeds of *Verbascum Thapsus* in darkness was promoted by the action of light, fluctuation of temperature during incubation, alternating high and low temperatures, soil, and many single electrolytes as substrata.

13. The germination of seeds of *Oenothera biennis* in darkness was promoted during certain seasons by hot water treatment, sulphuric acid, preliminary incubation at low temperature, incubation in alternating high and low temperatures, and single electrolytes as substrata.

14. The germination of seeds of *Daucus Carota* in darkness was promoted by increased oxygen pressure and preliminary incubation at low temperature, while it was hindered by soaking in hydrochloric acid and by the use of single electrolytes as substrata.

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