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INTERACTION OF TEMPERATURE AND LIGHT IN GERMINATION OF SEEDS¹

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Fully imbibed, viable seeds of many plants may fail to germinate. One limitation on germination may be a light requirement which can be removed by the correct poisoning of a reversible photoreaction (1, 2, 8). This reaction which can be repeatedly reversed by alternate exposures to red and far-red radiation also controls photoperiodic flowering responses, etiolation, bulbing, and a number of other growth responses (5). That the photocontrol is ubiquitous in all higher plants is shown by the etiolation response of seedlings (6).

The temperature, too, must be in a favorable range for seed germination which, for many kinds of seeds, is well below that best suited for subsequent growth of the seedling. A change in temperature is often effective in promoting germination. For limited periods, the temperature may greatly exceed the favorable constant range. A response to a change in temperature is also evident in vernalization, tillering, and dormancies of plant buds (9) as well as in diapause of animals. It is a phenomenon with broad significance.

Germination requirements are examined here with reference to the results of a few simple physiological experiments involving the effects of light and temperature conditions and to some extent the effects of nitrate solutions. The results suggest a pattern for the control of the germination process as well as for the growth of all organisms that must follow the temperature of the environment.

MATERIALS AND METHODS

The experiments reported here were chiefly with seeds of *Lepidium virginicum* L. collected from plants grown in the greenhouse. Other seeds were obtained from various sources and all were held in dry storage at -18°C or $+5^{\circ}\text{C}$. The seeds were placed in Petri dishes on two layers of standard germination blotters saturated with water or 0.2% KNO_3 solution. The dishes were immediately covered with two or more layers of black cloth throughout all tests except when irradiations were in progress. That the cloth excluded visible light was indicated by failure of exceedingly light-sensitive seeds to respond to visible radiant energy when so protected. Durations of germination experiments varied with the kinds of seeds and with the purposes of the experiments. They ranged mainly

from 2 to 5 days after the final experimental variable was introduced. Seeds with emerged radicles were counted as germinated.

Radiation sources were incandescent and standard cool white fluorescent lamps. The incandescent-filament lamp, with a filter of two layers of red and two layers of dark-blue cellophane, either with or without a supplemental water filter, was an effective source of far-red radiant energy in the region of wavelengths greater than 7000 \AA for inhibiting germination. When provided with a two-layer red cellophane filter to eliminate the blue part of the visible spectrum the fluorescent lamp, because of its relatively low emission between 7000 and 8500 \AA , was an adequate source of red radiant energy in the region of 5800 to 7000 \AA for promotion of germination. The sources used each gave about 0.3 milliwatts/cm² power in the wavelength regions of maximum effectiveness. Blue cellophane filters have been found to fade when subjected to the full intensity of 300-watt flood lamps at a distance of a foot or 18 inches, but only after several hundred hours of use. Care was accordingly exercised to replace them before significant change in transmission occurred.

During germination tests in seed germination cabinets, temperatures were usually controlled within $\pm 1^{\circ}\text{C}$. Some of the experiments involved several different constant temperatures; others made use of daily temperature alternations, an 8-hour period at one temperature being followed by 16 hours at a lower temperature. In still other experiments, a single change was made from the temperature employed during imbibition to one used for a short period or during the remainder of the germination period. Exposures to radiant energy and in some cases applications of KNO_3 or other solutions were made concurrently with, before, or after the temperature change.

In all experiments with *Lepidium virginicum* L. and *Nicotiana tabacum* L. four lots of 100 seeds each were used for each treatment. Results for *Fragaria virginiana* Duchesne and *Verbascum thapsus* L. in table II were based on single lots of 100 seeds and those used for other species on two lots each. Control lots of seeds were held in darkness in all experiments and under special conditions in some experiments as required by their particular designs. All seeds of *L. virginicum* in the dark control lots almost invariably failed to germinate.

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Conditions such as irradiation time and designs of particular experiments were established by numerous preliminary trials. The experiments reported were selected, because they have some common theme, from some 200 that have not been described. Upon repetition each gave essentially the same results in the course of several years, but the detailed results varied because the stored seeds had undergone gradual changes.

RESULTS AND PRELIMINARY DISCUSSION

When imbibed in water and germinated on blotters at a constant temperature of 20° C, seeds of *Lepidium virginicum* are limited to about 30% germination as a maximum response to red radiation (table I). The 70% of the seeds that fail to respond can, for the greater part, be made to germinate by a temperature alternation in the presence of a solution of 0.2% KNO₃. In fact, application of either the temperature alternation or nitrate alone is largely effective. These simple observations indicate the presence of factors in addition to radiant energy which limit germination. These additional limitations are also evident in many other light-sensitive seeds, as shown by results in table II, which indicate that an alternation in temperature often enhances germination. The response to a temperature change as well as to nitrate solution is well known to workers in seed-testing laboratories.

A further distinct limitation on germination is an unfavorably high constant temperature. In fact, germination of 5 of the 9 kinds of seeds listed in table II was prevented by holding them imbibed at 30° C

TABLE I

GERMINATION OF SEEDS OF *LEPIDIUM VIRGINICUM* L. EXPOSED TO RADIANT ENERGY IN THE RED FOR VARIOUS PERIODS AFTER IMBIBITION FOR 24 HRS

GERMINATION TEMP	IMBIBITION LIQUID	RADIATION PERIOD	GERMINATION	
			INDIVIDUAL LOTS	AVERAGE
° C		hrs	%	%
20	H ₂ O	0	0, 0, 0, 0	0
20	H ₂ O	0.25	31, 29, 18, 25	26
20	H ₂ O	0.50	26, 22, 31, 26	26
20	H ₂ O	1.0	32, 25, 32, 34	31
20	H ₂ O	2.0	26, 43, 28, 26	31
20	H ₂ O	4.0	39, 30, 39, 35	36
20	H ₂ O	8.0	36, 17, 29, 21	26
20	0.2% KNO ₃	1.0	93, 92, 95, 93	93
15-25	H ₂ O	1.0	89, 87, 86, 88	88
15-25	0.2% KNO ₃	1.0	91, 88, 92, 87	90

either after exposure to red radiation or in darkness. Germination of the other 4 kinds was reduced but not completely inhibited at 30° C. Such facts, well known to workers with seeds who use them to determine the temperatures for seed germinating chambers, are presented here merely to illustrate the essential point of a definite germination limitation. All the kinds of seeds selected were known to be light-sensitive, but they were a random sampling for temperature requirements except that all were from temperate-zone plants.

TABLE II

GERMINATION OF WATER-IMBIBED SEEDS OF VARIOUS SPECIES IN DARKNESS AND AFTER A SATURATING EXPOSURE TO RED RADIANT ENERGY AS INFLUENCED BY CONSTANT AND DAILY ALTERNATING TEMPERATURES

SEED SPECIES	LIGHT CONDITION	GERMINATION AT INDICATED TEMPERATURES (° C)					
		15	20	25	30	35	20-30
		%	%	%	%	%	%
<i>Brassica juncea</i> (L.) Coss. hort. var. Giant Southern Curled hort. var. Chinese Broadleaf	Red	90	48	18	2	..	40
	Dark	53	20	6	0	..	34
	Red	58	46	47	31	..	58
	Dark	25	30	30	20	..	58
<i>Fragaria virginiana</i> Duchesne	Red	16	17	70	4	..	*
	Dark	2	1	0	0	..	*
<i>Lepidium campestre</i> (L.) R. Br.	Red	15	12	4	1	0	29
	Dark	1	1	1	0	0	1
<i>L. virginicum</i> L.	Red	21	32	33	0	0	73
	Dark	0	0	0	0	0	0
<i>Nicotiana Tabacum</i> L. hort. var. Maryland Mammoth hort. var. Kentucky 16	Red	94	96	94	84	8	97
	Dark	2	5	6	< 1	0	97
	Red	87	90	92	86	35	93
	Dark	8	27	20	4	0	86
<i>Sisymbrium officinale</i> (L.) Scop.	Red	15	27	40	26	5	53
	Dark	1	< 1	9	20	1	18
<i>Verbascum Thapsus</i> L.	Red	0	18	45	16	..	65
	Dark	0	0	0	0	..	7

* At 15 to 25° C germination: red—87%, dark—7%.

TABLE III

GERMINATION OF SEEDS OF *LEPIDIUM VIRGINICUM* WITH A SINGLE CHANGE IN TEMPERATURE FROM 15 TO 25° C AT THE INDICATED NUMBER OF HRS BEFORE OR AFTER A SATURATING AMOUNT OF RED RADIANT ENERGY

PERIOD BETWEEN TEMP CHANGE AND IRRADIATION	GERMINATION WHEN TEMP CHANGE OCCURS *	
	BEFORE IRRADIATION	AFTER IRRADIATION
hrs	%	%
0	87	87
4	70	89
8	65	93
12	60	86
16	71	90
20	72	82

Seeds imbibed in water for 24 or 28 hrs at 15° C prior to temp change.

* Germination 42 % at constant temperature of 20° C.

The optimum constant temperatures for germination, while not determined precisely, were in the region of 15° to 25° C for all the seeds listed in table II, with 5 of the 9 kinds being in the narrow region of 20° to 25° C. This optimum temperature range for germination is well below that for optimum growth of many plants and the limiting temperature for germination (near 30° C) is below the limiting temperature for growth.

Two features of the enhanced germination response to a change in temperature are shown by results in table III. The first of these is that a single change in temperature is effective and the second is that this change may either precede or follow the exposure to radiation by as much as 20 hours. Other experiments, the details of which are not presented, show that with longer periods of imbibition this interval during which the temperature change is effective is more restricted as also is the enhancement of germination by the temperature change. The results in the last column of table II show that the enhancement is attained even when the higher temperature is above that which suppresses germination when it is held constant. In fact, an alternating temperature of 20° to 35° C after exposure to red radiant energy increased germination of seeds of *Sisymbrium officinale* to 86 %. Under these conditions, seeds of *Lepidium virginicum* and *L. campestre* gave 36 and 14 % germination, respectively, about the same as the values at the optimum constant temperature.

Changes effected by holding imbibed seeds at temperatures as high as 35° C can be both drastic and rapid as illustrated by results in table IV. Seeds of *Lepidium virginicum* placed for 2 hours in a chamber at 35° C after imbibition at 20° C for 24 hours germinated 99 % in response to a saturating exposure to red radiant energy. The 2 hours included the interval over which temperature equilibration was being attained so that the actual time at 35° C was considerably shorter. Seeds held for 2 hours at 35° C require about one-eighth the energy for 50 % promotion of

germination if the period at 35° C precedes rather than follows exposure to red radiation. The sensitivity of seeds held at 35° C either before or after radiation exposure, however, is much greater than that of seeds held constantly at 20° C. A change of this type had previously been noted by Siegel (7) for seed of *Digitaria sanguinalis* (L.) Scop. heated to 75° C for 60 minutes while unimbibed.

The percentage germination and the sensitivity to red radiant energy of *Lepidium virginicum* seeds held at 35° C for 24 hours depend on whether they receive an essentially saturating irradiation near the beginning of the 24-hour period of high temperature. Total germination and sensitivity both were lower if the seeds did not receive the initial exposure to red radiant energy (table V). The seeds given red before the period at 35° C were also given an essentially saturating amount of far-red immediately after the period at 35° C to convert the pigment to the red-absorbing form to permit measuring sensitivities to red and then far-red radiant energy.

Respiration is an essential process in germination, for seeds do not germinate in absence of oxygen. The oxygen consumptions of non-germinating seeds of *Lepidium virginicum* in darkness in either water or potassium nitrate solution at 20° C are shown in figure 1. Measurements were not made until 2½ hours after the seeds were placed in the flasks since the process of

TABLE IV

GERMINATION OF SEEDS OF *LEPIDIUM VIRGINICUM* IRRADIATED BEFORE OR AFTER BEING HELD AT 35° C* FOR 2 HRS

DURATIONS OF IRRADIATION		GERMINATION OF SEEDS	
RED	FAR-RED	BEFORE 2-HR PERIOD AT 35° C	AFTER 2-HR PERIOD AT 35° C
sec	sec	%	%
0	0	3	3
5	0	..	32
10	0	..	56
20	0	26	74
40	0	34	90
80	0	52	95
160	0	73	98
320	0	96	..
10 × 60	0	95	..
16 × 60	0	..	99
64 × 60	0	98	..
Saturation **	5	99	99
"	10	97	98
"	20	84	91
"	40	39	46
"	80	8	14
"	160	5	14
"	16 × 60	2	7

Seeds imbibed in water at 20° C for 24 hrs before placing at 35° C; returned to 20° C after treatments.

* Germinations 0, 27 and 55 %, respectively, for seeds held continuously at 20° C in the dark or with prior 4 or 64 min of red.

** Time 64 and 16 min, respectively, for lots exposed to irradiation before and after 2-hr period at 35° C.

TABLE V
SENSITIVITY OF SEEDS OF *LEPIDIUM VIRGINICUM* TO RED AND FAR-RED IRRADIATION AFTER BEING HELD FULLY IMBIBED AT 35° C FOR 24 HRS WITH AND WITHOUT PRIOR EXPOSURE TO RED

DURATIONS OF IRRADIATION AFTER 24 HRS AT 35° C		GERMINATION OF SEEDS	
RED	FAR-RED	SATURATED WITH RED AT BEGINNING OF 35° C TREATMENT *	NOT SATURATED WITH RED AT BEGINNING OF 35° C TREATMENT
sec	sec	%	%
0	0	5	0
20	0	34	..
30	0	..	4
40	0	42	..
60	0	..	8
80	0	58	..
120	0	..	14
160	0	68	..
240	0	..	25
320	0	78	..
480	0	..	24
64 × 60	0	87	59
64 × 60	10	66	33
64 × 60	20	49	25
64 × 60	40	31	11
64 × 60	80	20	1
64 × 60	16 × 60	24	0

Seeds held 4 days at 20° C in dark after final irradiation.

* Given 16 min of far-red at end of 35° C treatment before irradiations shown in columns 1 and 2.

imbibition occupied part of this period. The rate of oxygen consumption was not essentially influenced by the presence of nitrate. The rate for the seeds held in darkness was constant for the first 23 hours and then changed to a lower constant rate, which indicates a steady state condition for respiration of the non-germinating seeds. These results are closely similar to those previously reported for lettuce seed (4). The results with lettuce seed show in addition that respiration increases as germination starts. The enhanced respiration is the earliest physiological expression of germination that has been found (3). Its onset, in fact, takes place during a period in which germination can still be stopped by high temperature or by far-red irradiation of light-sensitive seeds.

GENERAL DISCUSSION

The ways in which the several factors mentioned in the previous section may be involved in the onset of germination are now considered. By "onset" is meant initiation of cell enlargement that results in emergence of the root. The discussion is restricted to initiation with the hope of circumscribing a single process.

The factors are: 1) imbibition of water, 2) a reversible photoreaction, which has been treated in greater detail elsewhere (2, 7), 3) a favorable temperature, with a distinct limitation at high temperatures, and 4) any changes induced by the presence of nitrate or by alternations in temperature.

Respiration, which might be treated as a primary process for germination, is considered first. Respiration, involving the reduction of oxygen and the ultimate appearance of carbon dioxide, can be considered as producing three essential types of substances: substrate compounds for reactions supplying free energy, hydrogen-transferring intermediates and intermediates for synthesis. These three types of materials, of course, are cyclically involved in respiration itself. In viable non-germinating imbibed seeds these materials are in a steady state which might be constant for periods of many hours but which would change over longer periods as substrates for respiration change, i.e., periods of a week for seeds of *Lepidium virginicum*. If the temperature is increased the rate of oxygen consumption quickly responds and a new steady state of the essential materials is established.

Germination is connected with respiration through one or more of the three essential types of materials, and can be considered as utilization of some of these materials. In a sense, it is the coupling of a particular system into the respiration cycles. When germination starts an added demand is made for the essential materials and respiration at a constant temperature is enhanced by the tendency of the added demand to reduce the steady state level of the intermediate.

The general way in which the reversible photoreaction is effective can now be considered. The pigment in the far-red-absorbing form promotes germination by directly controlling the levels of two compounds concerned in the germination process. The pigment may act as an enzyme in a reaction

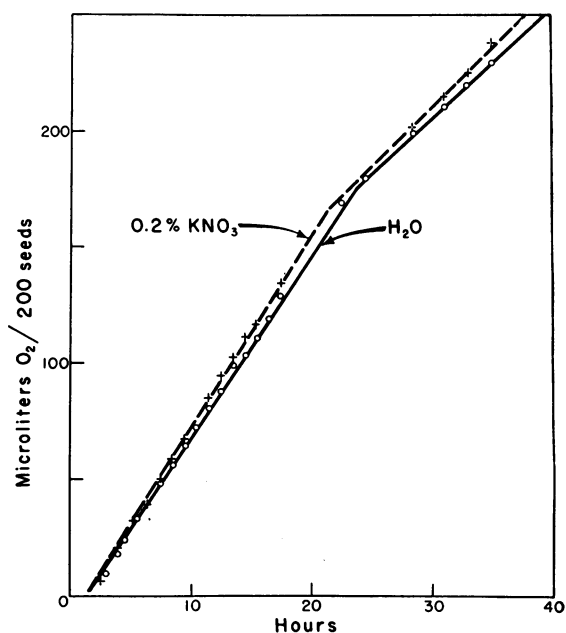


FIG. 1. Oxygen consumption at 20° C of imbibed seeds of *Lepidium virginicum* in darkness in water and in 0.2% KNO_3 solution. (Courtesy of C. E. Hagen.)

between the two compounds, may serve as an inhibitor of this reaction or may be one of the compounds. The first case, however, is the more likely and will be used as the basis for further discussion. With a favorable level of the two compounds germination will proceed in some seeds.

An important feature of the photoreaction is that, although it is present, its participation in the germination process is not necessary for all seeds. This is true not only for all of the seeds of many kinds of plants such as maize but also for some of the seeds, the dark-germinating ones, in lots that in general are light-sensitive, such as those of some varieties of lettuce. The deduction is that in such seeds the levels of the two compounds intermediated by the photoreaction are satisfactory for germination independent of the photoreaction. This implies that the levels of the compounds are controlled through other reactions. Absence of physiological evidence for photocontrol, however, does not imply that the photoreaction is absent.

Next to be considered are those imbibed light-sensitive seeds in which the pigment was placed in the far-red-absorbing form without causing germination. About 70 % of the seeds in several lots shown in table I are of this type. That the pigment is in the far-red-absorbing form in these non-germinating seeds is indicated by results such as those in table III in which a change of temperature that follows the exposure to red radiation greatly enhances germination. A change in temperature without exposure to radiation did not effect germination of *Lepidium virginicum*. Two possible reasons exist for failure of such seeds to germinate even after the necessary photoreaction: first, the necessary levels of the two compounds directly controlled by the photoreaction were not reached in these seeds, or second, there were control points in the germination process other than the photoreaction. Both are considered tenable. The first holds in some seeds that have relatively low respiration levels, either inherently as individuals of the lot or are brought to such a state by being held imbibed but non-germinating for long times. These seeds often are intractable to bring to germination.

A control of germination other than the photoreaction is best illustrated by the effect of nitrate which, as shown in table I, enhances germination after the photoreaction is removed as a limiting factor. Its value as an illustration rests upon the fact that nitrate does not influence respiration of non-germinating seeds (fig 1), which fact eliminates the confusion of a change in level of respiratory-derived materials. Nitrate does not replace the photoreaction for it has no effect on germination of seeds of *Lepidium virginicum* in darkness, nor does the photoreaction satisfy the requirement met by presence of nitrate. At least two controls, then, are present and they must enter at different places in the germination process. The process in which nitrate participates directly influences the amount of three compounds: one the nitrate itself and the others, the reacting compound and its

product with nitrate. Involvement of this process in the course of germination follows from this nitrate reaction.

The contrasting effects on germination of light-sensitive seeds resulting from either holding them constantly at a temperature such as 30° C or holding them for 8 to 12 hours at this temperature before returning them to, say, 15° or 20° C, might now be approached. Results of the two conditions are strikingly different in that germination is prevented by the first and enhanced by the second. The high temperature increases the rate of oxygen consumption; so it must change the steady state levels of the three types of materials furnished by respiration. But the change in temperature also influences the rates of all the component reactions in the germination process. Temperature change very quickly affects rates of the various reactions and more slowly alters the amounts of reactants and products toward a new steady state. If the high temperature is held for a time and then lowered, a new balance of reactants for germination is afforded at the lower temperature. The most general conclusion is that this new balance of reactants, while unfavorable for germination at the high temperature, is favorable at the reaction rates involved at the lower temperature. The germination process, whatever its nature, would appear to require a delicate balance among its component reactions if for no other reason than that it can be controlled by so many factors.

Temperature changes made after the pigment is changed to the far-red-absorbing form not only influence the number of seeds that can be induced to germinate, but also, as shown by the results in tables IV and V, influence the sensitivities of the seeds to red and far-red radiation. The change alters the two sensitivities in opposite directions, a finding consistent with the participation of a reactant other than the pigment in the photoreaction. This is an indication that at a point in the germination process other than that of its coupling to respiration, the whole balance of reactions is influenced by the change in temperature. The altered sensitivities in the photoreaction, for example, could occur if the reactant other than the pigment were a hydrogen donor or acceptor involved in other hydrogen-transferring reactions either in respiration or in the germination process.

While temperature change and presence of nitrate are equally effective in causing some seeds to respond to light, they need not be equivalent in the mode of action. Nitrate probably controls more specifically through a single reaction than does temperature. Light is obligatory for the germination of each seed of *Lepidium virginicum*, but some of the seeds have a further requirement. A temperature change and the presence of nitrate are two alternative means for initiating further necessary adjustment among component reactions of germination. The conditions could be different in other seeds, such, for instance, as tobacco (table II). Some of the tobacco seeds that could be induced to germinate by exposure to light

germinated in darkness following temperature alternation; for them light was not obligatory if the temperature was satisfactory.

SUMMARY

Effects on germination of light and of holding at several temperatures were measured on seeds of *Lepidium virginicum*, *L. campestre*, *Sisymbrium officinale*, *Fragaria virginiana*, *Verbascum thapsus*, *Nicotiana tabacum* vars. Maryland Mammoth and Kentucky 16, and *Brassica juncea* vars. Chinese Broadleaf and Giant Southern Curled. Germination of each seed species is enhanced by exposure to red radiation and by a diurnal alternation of temperature. A constant temperature in excess of 30° C is very unfavorable for germination.

Seeds of *Lepidium virginicum* imbibed in water and exposed to a high red irradiance for a period adequate for maximum displacement of the photoreaction gave about 30 % germination. This value was increased to 88 % or more by a temperature change from 15° to 25° C or by imbibing in 0.2 % KNO₃ solution. The effective temperature change could precede or follow the exposure to light by as much as 20 hours. Holding imbibed seeds at 35° C for 2 hours enhanced germination and affected the sensitivity of the photoreaction to radiation.

The germination process, which depends upon respiration, is controlled at different points by the several factors. The photoreaction, while possibly present in all seeds, is not obligatory for germination of all seeds. It controls the levels of two compounds which are also under control by other reactions subject to influence by temperature.

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CULTURE STUDIES ON SPHAEROCARPOS. III. THE UTILIZATION OF NITROGEN BY SPHAEROCARPOS TEXANUS^{1,2}

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The literature dealing with nitrogen utilization by plants (1, 7, 8) indicates that nitrate is the principal form of nitrogen taken from the soil by most species of higher plants. Under customary field conditions, ammonium and amino nitrogen may not be available because they are converted into nitrate nitrogen by soil microorganisms. Nitrites, although they may be utilized by some plants, are rarely if ever an impor-

tant source of nitrogen for plants in nature. There is evidence that some plants can absorb and utilize amino acids and other nitrogenous compounds.

Nightingale (8), reviewing utilization of ammonium and nitrate by higher plants, noted in summary: "If a liberal supply of oxygen is available in the nutrient substrate, and of cations, including particularly potassium, and if there is adequate absorption of other essential elements, then ammonium is more rapidly utilized by plants than nitrate." However, in none of the experiments cited were ammonium and nitrate simultaneously available to the plants. On the other hand, it has been demonstrated (9, 10) that when the unicellular green alga *Chlorella vulgaris* is grown in a medium containing ammonium nitrate, it preferentially absorbs ammoniacal nitrogen.

In contrast to the numerous papers on nutrition of algae, there are relatively few studies on nutrient

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