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PHOTOCONTROL OF LEPIDIUM SEED GERMINATION¹

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The same reversible photoreaction has been found to control seed germination of some lettuce (*Lactuca sativa* L.) varieties and floral initiation of *Xanthium saccharatum* Wallr. (1, 2). Observations are extended here to the light response of seed germination of *Lepidium virginicum* L. and *L. densiflorum* Schrad. Seed of the former had earlier been noted to be light-sensitive (10). The results permit a closer definition of the reversible photoreaction and give some knowledge about the immediate reactions with which it is associated. A better understanding of the manner of action of several factors such as varying temperature and presence of nitrate or coumarin, known to affect germination of some seeds, is afforded.

METHODS AND PRELIMINARY OBSERVATIONS

Methods used were similar to those described for lettuce seed (3). In short, seeds were placed in glass Petri or square plastic dishes on blotters that had been wetted with water or solutions. They were held in darkness between black cloths in germination chambers at definite controlled temperatures for various periods and then subjected to the experimental treatment such as irradiation and shift of temperature. Three or more days after treatment the germinated seeds were counted. Irradiation was with either incandescent or fluorescent lamp sources equipped with filters to give radiation in the desired regions or with the spectrograph previously described and used in studies of photoperiodic responses (8). When the spectrograph was used the spectrum was reflected downward by a mirror and dishes containing seeds were placed at various positions in the spectrum for various periods as required.

A mixture of seeds of the two *Lepidium* species was collected locally and increased in the greenhouse. Here the two species were separated on the basis of leaf shape, type of inflorescence, and orientation of embryo within the seed. Lots of seeds of each species were collected and held in an icebox at about 5° C prior to use which extended over about 1 year. Pre-

liminary experiments were performed to determine temperatures, times of imbibition, effective concentrations of solutions and other factors necessary for design of experiments.

Initial populations of 400 seeds in 4 lots of 100 each were used for each individual measurement unless otherwise stated. Precision was accordingly low for lots with less than about 10% germination or greater than about 90%. Values were transformed through the use of probits (5) plotted against log energy.

Preliminary experiments indicated that seeds of *Lepidium densiflorum* resembled those of *Lactuca sativa* in their irradiation requirements for promotion and inhibition of germination. They changed in sensitivity to radiation, however, over a 4-month period after harvest and their germination in the dark at 15° C increased to more than 50%. For these reasons observations on germination of *Lepidium densiflorum* seeds were limited to crucial comparisons with *L. virginicum*.

THE ACTION SPECTRUM: Experiments with seeds of *Lepidium virginicum*, involving constant and alternating temperatures and presence or absence of KNO₃ solutions and light, were performed to find suitable germination conditions for subsequent action-spectrum and other experiments. Not a single seed germinated in any of these lots without irradiation. With standard cool white fluorescent lamps with a red filter, which gave radiation from 5800 to 7000 Å and a biological effect equivalent to that obtained with about one joule/cm² in the region 6300 to 6600 Å, germination was measured at constant temperatures of 15°, 20°, 25°, and 30° C and at alternations of 16 hours at 15° and 8 hours at 25° C. At constant temperatures maximum germination occurred at 20° C in the presence of KNO₃ solution (table I). With water germination was much greater with temperature alternations than with any constant temperature and when KNO₃ solutions were used it was rather close to 100%.

The increased germination resulting from alternations of temperature between 15° and 25° C (table I)

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TABLE I
GERMINATION OF *LEPIDIUM VIRGINICUM* SEEDS *

TEMP. °C	% GERMINATION **	
	WATER	KNO ₃
15	21	84
20	32	80
25	33	60
30	0	0
15-25 †	90	97

* Germinated on blotters wetted with water or 0.2% KNO₃ at various temperatures at a radiant energy of about 1 joule/cm² in the region 5800 to 7000 Å (irradiation for 1 hr with 1000 fc from a standard cool white fluorescent lamp with a red filter) at the end of the second day.

** 200 seeds per treatment. Under all conditions dark controls gave no germination.

† Alternating.

was attained by a single shift from 15° to 25° C when irradiation occurred at the time of the shift (table II). These results naturally raise a question as to the time required at 25° C for enhancement of germination to be realized after a shift from 15° C with irradiation at the time of the shift. After 0, 4, 8, 12, 16 and 24 hours at 25° C germination percentages were 10, 37, 53, 51, 63 and 63. The full effect was found to result from about 12 to 16 hours at the higher temperature.

The germination resulting from a range of energies of promotive or inhibitory radiation can be expressed graphically as straight lines by transformation of the values. Such lines are obtained if the probits (5) corresponding to the germination percentages are plotted on a linear scale against energy on a logarithmic scale (fig 1). These lines were used, as illustrated in the next paragraph and in the figure, to estimate energies for construction of action-spectrum curves expressing 50% effect.

For measurement of the action spectrum seeds were placed along the spectrum for definite periods. Radiant energies thus were not constant for the various wave-length regions and various percentages of germination were observed. The energies and percentages of germination fix points in the probit-log-

TABLE II
GERMINATION OF *LEPIDIUM VIRGINICUM* SEEDS *

TEMP. °C FIRST 2 DAYS	TEMP. °C 3RD-6TH DAY	% GERMINATION	
		IRRADIATED SEEDS	NON-IRRADIATED SEEDS
15	15	37	0
25	25	41	0
15	25	92	0
25	15	32	0

* Germinated on blotters with water as affected by temperature regimen at a radiant energy of about 1 joule/cm² in the region 5800 to 7000 Å at the end of the second day.

energy field. The estimated energy required to obtain 50% germination, that is a probit of 5.0, is found by displacing each point to the level of a probit of 5 along a parallel to the lines shown in figure 1. The abscissa of this latter point gives the estimated energy. In each case an effort was made to give radiant energy that would result in about 50% germination so that the energy conversion factor would not be great. For example, a given radiation treatment having a relative energy of 0.5 resulted in a germination of 31%. This fixes the point a of figure 1. Displacement to 50% parallel to the lines for promotion leads to the

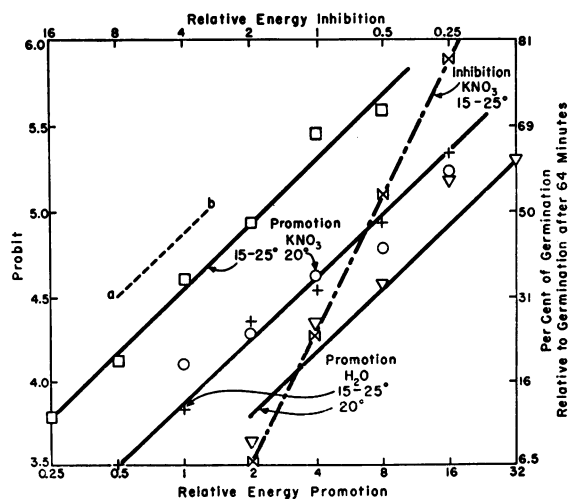


FIG. 1. Variation in germination of *Lepidium virginicum* seeds with radiant energy (fluorescent, 1000 fc red filter) at a constant temperature of 20° C and with a shift at time of irradiation from 15 to 25° C in presence of H₂O or 0.2% KNO₃ solution. The longer broken line shows inhibition of germination at 7300-7500 Å. Broken line a-b illustrates method of reducing data described in text. Relative energies on abscissas correspond to exposures in minutes with about 3×10^{-4} watts effective irradiance.

point b, which has an abscissa of 1.26. Accordingly, 50% germination requires a relative energy of 1.26.

This method of converting energies that gave observed germination percentages to those required for 50% germination has been used to construct action-spectrum curves for promotion at wave lengths less than 6800 Å and for inhibition at wave lengths greater than 7250 Å (fig 2). Between these limits promotive and inhibitory effects are simultaneously involved to such a degree that attempts at conversion lead to ambiguous values. If very high energies are given at wave-length stations between 6800 and 7250 Å to either fully promoted or fully inhibited seed equilibrium values are obtained. These values range from appreciably less than 100 for wave lengths just above 6800 Å to values considerably greater than 0 for wave lengths shorter than 7250 Å. At some position between these limits an equilibrium value of 50% occurs. For *Lepidium virginicum* the wave-length

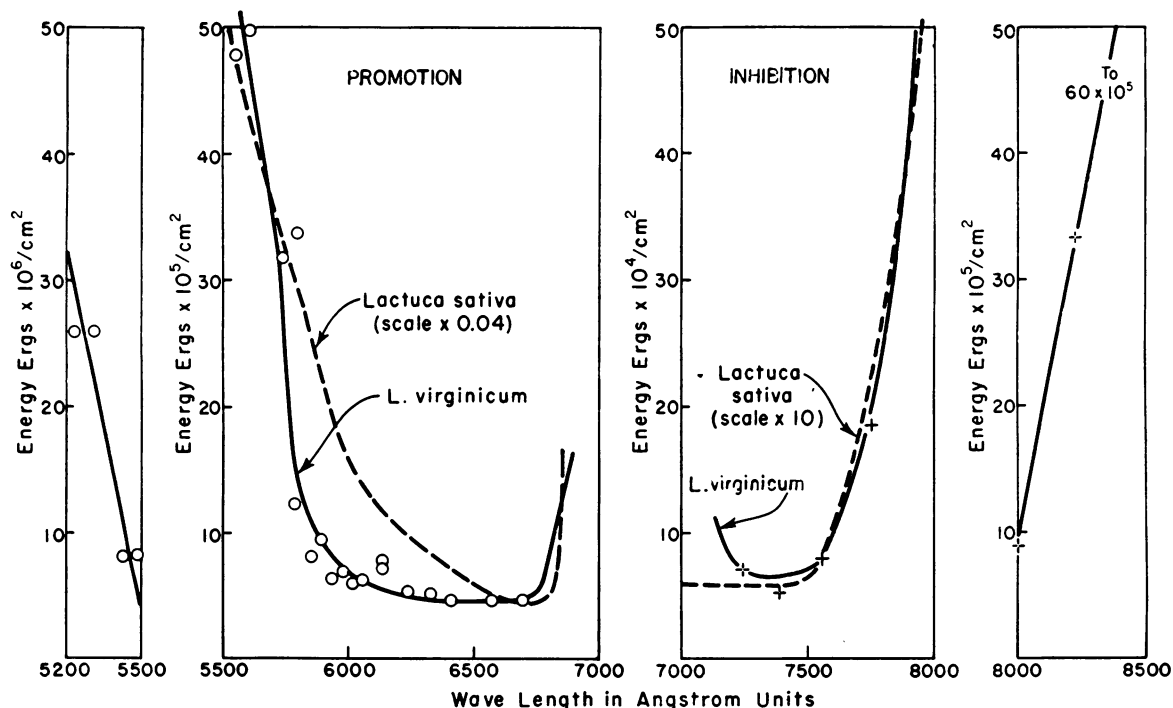


FIG. 2. Action spectra for promotion and inhibition to 50% germination of *Lepidium virginicum* seeds at 20° C in 0.2% KNO₃ solution (solid lines). Action spectra for promotion and inhibition of germination of seeds of *Lactuca sativa* shown for comparison.

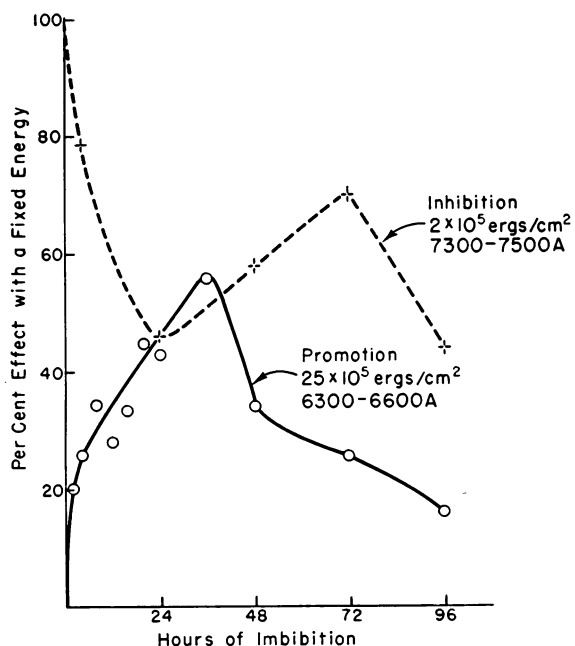


FIG. 3. Variation in sensitivity to promotion and inhibition of germination at 20° C of *Lepidium virginicum* seeds imbibed in 0.2% KNO₃ solution with time of imbibition prior to irradiation.

region for the 50% value of germination with 0.2% KNO₃ at 20° C was between 6875 and 6900 Å.

Significant points were determined for *Lepidium densiflorum* seeds irradiated after 24 hours of imbibition in water at 15° C. Minimum energy for inhibition to 50% germination was found at 7400 Å, the required incident energy under the particular conditions being 1.8×10^5 ergs/cm², and for promotion to

TABLE III

ENERGIES REQUIRED (ERGS $\times 10^6$ /CM²) FOR 50% GERMINATION OF LEPIDIUM VIRGINICUM AND L. DENSIFLORUM WHEN PROMOTED AT 6300 TO 6600 Å OR INHIBITED AT 7300 TO 7500 Å IN THE PRESENCE OF WATER OR SOLUTIONS

KIND OF SEED AND IMBIBED LIQUID	ENERGY FOR INHIBITION			ENERGY FOR PROMOTION		
	20° C	15° C	15-25° C*	20° C	15° C	15-25° C*
	<i>L. virginicum</i>					
H ₂ O	0.3	...	0.4	14.0	...	5.8
0.2% KNO ₃	0.5	...	0.55	5.8	...	1.7
0.5% CS(NH ₂) ₂	0.75	2.0
<i>L. densiflorum</i>						
H ₂ O	...	1.8	0.1	...

* Alternating.

50 % germination at 6650 Å, the required incident energy being 6.0×10^3 ergs/cm². The wave length for 50 % germination with very high energies was between 7000 and 7150 Å. Precision of these values was low because of the high germination of dark controls.

FACTORS CONTRIBUTING TO CHANGES IN SENSITIVITY OF SEEDS TO RED AND FAR-RED RADIATION: The energies required for promotion or inhibition of germination by a given amount depend on factors such as duration of imbibition at the time of irradiation, the presence in the water of such substances as coumarin, thiourea or potassium nitrate and occurrence of shifts in temperature during germination (fig 3 and tables III and IV). The radiant energies given for 50 % germination in table III were obtained from the probit-log-energy lines. The slopes and positions of these lines depend upon all the observed germination values between about 10 and 90 %. Every experimental result between these limits thus contributes to the precision of estimates of the energy required for a desired result such as 50 % germination.

The interaction of various energies in the red with coumarin concentration on the germination of seed of *Lepidium virginicum* at 15 to 25° C was physiologically significant. Low concentrations of coumarin resulted in a promotion of germination above that of control lots, while slightly higher concentrations of coumarin were inhibitory (table IV). Sensitivity of germination to inhibition by far-red radiation was also measured, but less minutely. Its change was the inverse of that for the red.

REVERSIBILITY: Germination of seed of *Lepidium densiflorum* and *L. virginicum* is repeatedly photo-reversible by red and far-red radiation (table V). The red energy was the same for both, but the far-red energies differed somewhat. In the case of *L. virginicum* a 5 cm water filter was placed between the seed and the source to reduce heating of the seed during the 8-minute exposures.

DISCUSSION

Action spectra for promotion and inhibition of seed germination of *Lepidium virginicum*, *L. densiflorum* and *Lactuca sativa* (1) are closely similar on

TABLE IV

EFFECT OF COUMARIN AT VARIOUS CONCENTRATIONS ON GERMINATION SENSITIVITY OF LEPIDIUM VIRGINICUM SEEDS TO RED RADIATION *

COUMARIN CONC. 10 ⁻⁵ MOLAL	% GERMINATION AFTER IRRADIATION WITH RED FOR MINUTES INDICATED					
	0	2	4	8	16	64
0.0	0	19	37	44	55	59
0.5	0	25	35	48	58	70
1.0	0	32	49	56	63	64
2.0	0	24	39	50	54	69
4.0	0	22	43	57	60	63
6.8	0	2	4	11	10	24
10.3	0	..	0.2	..	0.5	2.5

* Radiation about 0.3×10^{-4} watt effective.

TABLE V

GERMINATION OF LEPIDIUM DENSIFLORUM AND L. VIRGINICUM SEEDS IN DARKNESS FOLLOWING ALTERNATING FIXED RADIANT ENERGIES OF RED AND FAR-RED

NUMBER OF IRRADIATIONS		CHARACTER OF FINAL RADIATION	% GERMINATION	
RED	FAR-RED		L. DENSI- FLORUM	L. VIRGINI- CUM
1	0	Red	92	45
1	1	Far red	22	0
8	7	Red	95	48
8	8	Far red	24	0
0	0	8	0

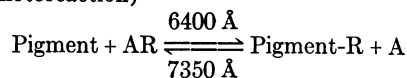
a relative scale. These action spectra are, moreover, closely similar to those for floral initiation (2), cuticle coloration in tomato (9), and internode and leaf elongation (4). The minimum energy requirement for action in one direction for each of these responses is at a wave length near 6400 Å and that for action in the opposite direction is near 7350 Å. The phenomena are certainly controlled by the same causal photo-reaction.

Incident energy requirements for 50 % effect at the wave lengths for maximum effectiveness are shown in table VI for the several responses. The narrow range of energy values for the response to red and far-red radiation for germination of two of the three kinds of seeds, for floral initiation of *Xanthium*, and for cuticle coloration of tomato indicates that the growth-controlling reaction is operating at about the same level in many tissues.

The discussion can most readily be based on the scheme of reactions deduced from the results of seed germination and floral control by radiation.

This scheme is as follows:

(Photoreaction)



+
Molecule

(Subsequent
reaction)

[Pigment-R-Molecule]

The photoreaction might be the reverse of the one indicated, that is, A might be on the left side and AR on the right.

One basis of the reaction scheme involves a feature of the response of a population to stimuli. This has been thought out particularly well in the probit analysis for insect toxicants, but has not been used widely in plant physiology (5). The population is formed of individuals of differing sensitivities, some very responsive and others insensitive to the stimuli. If two stimuli have different values for the mean response but the same variance then it is likely that the action of the stimuli has the same limiting factors. The variance is the reciprocal of the slope of the probit vs energy line. So if these lines are parallel for response

to radiation in the presence of different stimuli the stimuli probably affect the same system at the same limiting point.

Thus, treatment of *Lepidium* seeds with 0.2% KNO_3 solution has two distinct effects. First, it promotes total germination without change of variance of the population. In other words, the additional seeds that germinate in presence of KNO_3 and radiation are distributed in the same normal population for response to radiation as are those imbibed in water. Secondly, the mean radiant energy required for a given percentage germination of the seeds that can be influenced if saturating energies of this radiation are used is shifted. Thiourea has this same action. These results are obtained even though the concentrations of nitrate and thiourea used are such as to inhibit markedly seedling growth—presumably through reactions unrelated to the one being discussed.

If a factor such as imbibition in the presence of KNO_3 solution acts to change the sensitivity to red radiation, that to far red generally changes in the opposite direction. This can be seen from results in table III and from the change of sensitivity with time of imbibition prior to irradiation as shown in figure 3. A further fact bearing on the nature of the photoreaction is that the variances of the populations for promotion and inhibition of germination for *Lepidium virginicum* differ as shown by different slopes of the lines in figure 1.

To complete the argument, which can be done in several ways, the close agreement of results following one and eight reversals, as shown in table V, requires total pigment to be invariant during the course of the reversals. The coupled change in sensitivities then must be operating through other molecules than the pigment in the reversible photoreaction. These molecules are shown as AR and R, and this notation includes the possibility of R being hydrogen (H or 2H) or that AR is an electron donor and A an electron acceptor. Thiourea and nitrate affect the relative amounts of AR and A.

The absence of a temperature coefficient for the photoreaction requires AR and A to be non-limiting on the rate of the reaction. This indicates that they

TABLE VI

INCIDENT ENERGIES REQUIRED FOR 50% EFFECT BY PROMOTION AND INHIBITION FOR VARIOUS PLANT RESPONSES

RESPONSE	SPECIES	INCIDENT ENERGY (ERGS/CM ² × 10 ⁴)	
		RED	FAR-RED
Seed germination	<i>Lepidium virginicum</i>	140	3
	<i>L. densiflorum</i>	0.6	18
	<i>Lactuca sativa</i>	2	60
Floral initiation	<i>Xanthium saccharatum</i>	4	30
Cuticle coloration	<i>Lycopersicon esculentum</i>	2	..

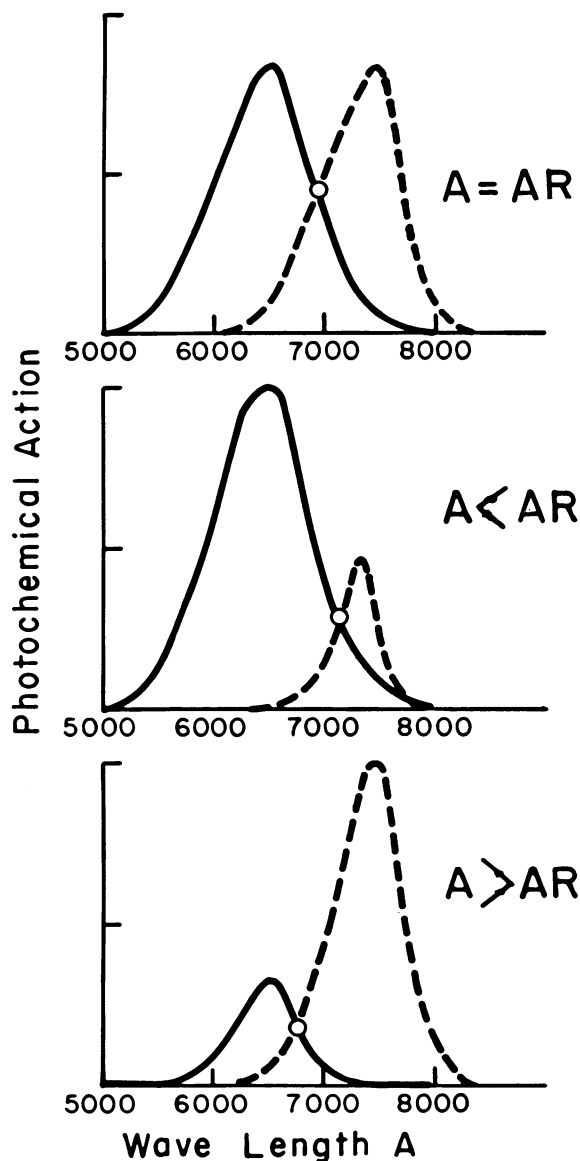


FIG. 4. A schematic diagram illustrating several relations between photochemical action in the red and far-red for the controlling photochemical reaction.

are closely associated with the pigment. The photosensitivity in the two directions then depends upon the relative amounts of AR and A present. The consequences are shown in figure 4, which explains the fact that the wave lengths of greatest sensitivity are constant but that the wave length for 50% response with high energy varies over a range of 300 Å. The possibilities illustrated in figure 4 are also in accord with the results listed in table VI. Thus, *L. virginicum* seeds correspond to $A > AR$, while for the other examples $A < AR$.

A similar reaction from photochemical literature is the reversible photoreduction of methylene blue or thionine in an anaerobic system in the presence of ascorbic acid, stannous chloride and other chemicals,

as recently reported (7). This, too, was shown to be repeatedly photoreversible, the effective wave lengths for reduction of the dye being in the visible and for its oxidation in the ultraviolet. The quantum efficiencies self-evidently depend upon the concentration of ascorbic acid although this dependence was not examined. Essentially the excited dye reacts with the reductant to form the leuco base and the excited leuco base reacts with the oxidant to regenerate the dye.

The question has existed as to whether the right or the left side of the photoreaction is further coupled with biological response. That it is the right side, the condition resulting from the action of red radiation, is shown by failure of the inhibition of flowering response in *Xanthium saccharatum* to be reversed after this condition has been established for 30 minutes by an irradiance in the red as shown in table VII taken from the thesis of Downs (4). Germination of light-sensitive seed is reversible in either direction for at least 4 hours which fact is indicative of another factor

TABLE VII

EFFECT OF TEMPERATURE DURING INTERVAL BETWEEN IRRADIATIONS ON LOSS OF REVERSIBILITY FOR FLORAL INITIATION OF *XANTHIUM SACCHARATUM*

INTERVAL BETWEEN RED AND FAR-RED RADIATIONS	FLOWERING CONDITION * WITH VARIOUS TEMPERATURES DURING THE INTERVAL	
	20° C	4° C
<i>min.</i>		
0	7	..
20	4	6
30	2	4
40	1	4
60	0	3

* Flowering condition was estimated on a scale of 8 stages of primordium development at time of dissection of 4 plants in each group, stage 8 being most advanced.

limiting the rate of the subsequent reaction. It is probably this factor that is associated with loss of light sensitivity when the seed coat is disturbed. The factor could, for example, simply be spatial separation of the active molecule from the photoreaction system in seeds. It could also be the introduction of a molecule different from A or AR that reduces or oxidizes Pigment or Pigment-R in darkness.

As seen from the values in table IV, coumarin at low concentrations promotes germination, reaching a maximum effectiveness at a concentration of 1×10^{-5} molal and inhibits germination at higher concentrations. Indoleacetic acid also has its maximum effectiveness on elongation of a coleoptile segment at a concentration of 1.1×10^{-5} molal (6). Coumarin would appear to be functioning by "two-point attachment" to another molecule (6). Coumarin probably does not act on the reversible photoreaction because it changes the variance of the population for germination on irradiation. A different variance obtains at each concentration.

When the temperature of imbibed *L. virginicum* seeds is quickly raised to about 40° C the ratio of AR to R changes rapidly. After some minutes the seeds reverse their relative sensitivities to irradiations in the region of 6500 and 7400 Å. If imbibed seeds are irradiated enough for about 50 % germination, then the radiation from the sun or an incandescent filament at about 3000° K should at high irradiances suppress germination but as the seeds warm, germination should be enhanced. This is observed.

The subject of this photoreaction controlling growth is now at the point where progress would be greatly aided by answers to three questions. (1) What is the pigment? (2) With what does auxin combine to be physiologically effective? (3) What reaction is controlled by the [Pigment-R-Molecule] combination?

SUMMARY

Action spectra for germination control of *Lepidium virginicum* and *L. densiflorum* seeds were measured to establish features of the reversible photoreaction that is a general factor involved in growth of higher plants. The maximum sensitivity for inhibition is near 7400 Å as previously found for lettuce seed germination and other responses. The sensitivity decreases monotonously with wave length by about 100 fold from 7400 Å to 8500 Å. The maximum sensitivity for promotion is near 6400 Å and this decreases monotonously by 100 fold from 6400 Å to 5260 Å. The seeds were unresponsive to inhibition or promotion of germination by radiation of wave lengths between 4000 and 5260 Å at 50 fold the energy required for 50 % germination at 6400 Å.

Interactions of the photoreaction with a shift of temperature and with nitrate and thiourea, factors known to affect germination of light-responsive seed, were measured. These are coupled in such a way as to cause opposite changes in sensitivity for promotion and inhibition of germination by the photoreaction. The relative sensitivity for promotion and inhibition of germination of *L. virginicum* was varied by more than 15 fold. This indicates that a molecule other than the pigment is involved in the photoreaction.

Coumarin promotes germination of *L. virginicum* at concentrations of 0.5 and 1.0×10^{-5} molal and inhibits germination at higher concentrations. This indicates that it effects control by two-point attachment in a rate-limiting reaction for germination as does auxin for cell elongation. Germination was almost completely inhibited by 1.0×10^{-4} molal coumarin.

A single shift in temperature of imbibed seeds of *L. virginicum* from 15° to 25° C at the time of irradiation in the red enhanced the population for germination without change of variance. A shift from 25° to 15° C was without effect on germination.

Reversible control of floral initiation in *Xanthium saccharatum* with radiation in the region of 7400 Å can only be accomplished for a period of 30 minutes after irradiation in the region of 6500 Å. This indicates that the pigment in the form with an absorption

maxima near 7400 Å undergoes a reaction with another molecule in darkness.

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INHIBITION OF ION ABSORPTION AND RESPIRATION IN BARLEY ROOTS^{1,2}

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Since most current theories of ion absorption postulate the existence of metabolically produced ion carriers (18), studies of the effect of respiratory inhibitors on ion absorption have assumed increasing significance. Machlis (13) found that cyanide, azide, malonate and iodoacetate inhibited bromide absorption by barley roots. Lundegårdh (10) inhibited chloride absorption in wheat roots with iodoacetate. He also inhibited chloride and nitrate absorption with fluoride. Lundegårdh (11) and Milthorpe and Robertson (16) inhibited salt absorption with cyanide. Weeks and Robertson (27) found salt absorption in carrot tissue to be sensitive to carbon monoxide and the inhibition to be photoreversible. Robertson et al (20) linked salt absorption in carrots to oxidative phosphorylation by inhibition of absorption with 2,4-dinitrophenol (DNP). The literature cited strongly suggests the involvement of the Krebs cycle in ion absorption.

Most of the studies of the effect of inhibitors on ion absorption have been carried out by investigating the effect on either anion absorption or total salt absorption as measured by the conductivity method (16). Few, if any, studies have been undertaken to investigate the effect of inhibitors on cation and anion absorption simultaneously.

Differences or similarities in the metabolism as-

sociated with the absorption of K and Br ions in excised barley roots were examined by studying the effect of various inhibitors on K and Br absorption and on respiration. This was carried out in order to determine how closely glycolytic and Krebs cycle activity could be linked to the two ion absorption mechanisms. The roles of terminal oxidases and of phosphorylation also were studied by the use of inhibitors.

MATERIALS AND METHODS

All experiments were carried out with excised roots from 6-day-old barley seedlings (variety Atlas 46). Seedlings were grown in a very dilute nutrient solution in the dark as described earlier (8) with the omission of the peroxide treatment. The excised roots were rinsed in distilled water and were centrifuged for 5 minutes at 65 × g. Weighed portions were then placed into various solutions.

Except where indicated otherwise, the temperature of growth and experiment was 26° C. The temperature of the bath in which respiration was measured was 25.5° C. All solutions used for absorption studies were aerated by compressed air except as indicated.

For pretreatments, root to solution ratios of 3 to 5 gm/l were used. In KBr solutions, 2 gm/l were used. In Warburg vessels 0.5 gm of root material was placed into 2.5 ml of water.

Potassium salts were used throughout this work, both in pretreatments and in subsequent treatments. After each treatment, roots were rinsed and centrifuged before being weighed out for the next treatment.

At the conclusion of the experiment, the roots

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