RESPONSES TO GIBBERELLIN OF LIGHT-REQUIRING SEEDS OF LETTUCE & LEPIDIUM VIRGINICUM [1]

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Many workers have shown that lettuce (7, 9, 12, 13, 14, 15, 17) and other photosensitive seeds (8, 16, 20, 23) can be caused to germinate in the dark by treatment with gibberellic acid. Previous work (24) indicates how light and temperature, singly and in combination, can control germination of seeds. The present studies were made to determine whether gibberellin could modify the response to light or temperature or to light and temperature interactions.

The response to light of lettuce (*Lactuca sativa* L.) and *Lepidium virginicum* L. seeds used in these studies is known (3, 25, 26). Reference to other pertinent gibberellin studies is made later.

MATERIAL & METHODS

Seeds of *Lepidium virginicum* L. were harvested from plants grown in 1957 in the greenhouse at Beltsville, Md.; Grand Rapids variety of lettuce[2] of the 1952 harvest, and Great Lakes variety of lettuce[3] of the 1954 harvest also were used in these studies. These seed bulks were stored dry in sealed jars at −12 C. These seeds were selected because the germination responses to light and temperature were known; Lepidium was the same species and Grand Rapids was the same sample as that used in the light studies reported previously (3, 25, 26).

Seeds were planted in petri dishes on a double thickness of Whatman No. 2 filter paper wetted with 7.5 ml per dish of gibberellin solution, other indicated chemicals, or distilled water. Water controls were included in all experiments. The dishes were immediately placed between several layers of black cloth on trays in germination chambers controlled within ±0.5 C. The 100 seeds planted per dish are referred to in this paper as seed "lot". The imbibed seeds were held in darkness except for the period of exposure to light and/or during transfer of seeds from one substratum to another. The imbibed seeds were transferred with a spatula. Transfer was done approximately three feet from a safe lamp consisting of a single 20-w fluorescent tube covered with a green gelatin filter (29).

Except in two experiments involving temperature studies, the Lepidium seeds were maintained at 20 C for approximately 22 hours followed by 2 hours at 35°, and then were returned to 20° (designated as 20°/35°/20°). In some experiments the period at 20° before the high temperature was longer than 22 hours and this fact is then mentioned. The 20°/35°/20° temperature combination gave full promotion of Lepidium seeds exposed to red light immediately preceding or following the 2-hour period at 35° (26). The lettuce seeds were germinated at 25°. This temperature was selected instead of 20° reported in the light studies (3) because the seeds had changed sufficiently during the 4 years of storage since those studies were made to give a rather high germination in darkness at the latter temperature.

The seeds were exposed to red light obtained by filtering the light from a bank of 2 or 18 ninety-six-inch T8 slimline cool white fluorescent tubes through two layers of red cellophane. The seeds were placed 1.1 or 2 m from 2 tubes or 1.1 m from 18 tubes. The intensities in the region 5,800 to 6,950 A, at the level of the irradiated seeds were 700, 300, and 6,000 ergs/cm²/second, respectively.

Short exposures to far-red radiant energy were obtained by filtering the light from three 300-w internal reflector incandescent-filament lamps through two layers each of red and of blue cellophane and about six centimeters of water. The seeds were placed 1.1 m from the radiation source. The intensity in the region 6,950 to 7,900 A was 7,500 ergs/cm²/second at the level of the irradiated seeds. Continuous exposure to low intensity far red was obtained by filtering the light from a bank of 12 cool white fluorescent tubes through two layers of blue cellophane which transmitted far-red and blue radiations of the source. Far-red and blue radiations were thus available, but previous light studies on seeds (3) had shown that the blue light was relatively ineffective.

In general, the germinated seeds of Lepidium were counted 5 to 7 days after the shift to high temperature and those of lettuce were counted 3 days after planting.

[1] Received March 3, 1961.

[2] Secured through the courtesy of Mr. F. G. Cuthbertson of the Ferry-Morse Seed Co., Mountain View, Cal.

[3] Secured through the courtesy of L. R. Hawthorn, Crops Research Division, Logan, Utah.

Gibberellins A_1, A_2, and A_4[4] were applied as the acid and gibberellin A_3 (gibberellic acid or GA) as the acid[5] or the potassium salt[6] in aqueous solutions. In preliminary experiments with gibberellin A_3, ethanol (0.01 M in the final solution) was used to dissolve crystals of gibberellin. The germination of seeds of both lettuce and Lepidium in the ethanol controls, however, was greatly enhanced over that in the water controls. Because of this promoting effect, ethanol was not used to prepare the gibberellin solutions. Aqueous solutions were prepared by continuously agitating the crystals for several hours in warm distilled water. Solutions were used on the day of preparation or were held at 5 C and used within 1 or 2 days after preparation. The concentrations of the solutions are indicated for each experiment.

Citrate-phosphate-buffered solutions were prepared by combining different volumes of 0.005 M citric acid and 0.01 M disodium phosphate. The pH 3.2 buffer was prepared by combining 3 volumes of 0.005 M citric acid and 1 volume of 0.01 M disodium phosphate. The resulting solutions, although weakly buffered, were adequate for the experiments. Buffers prepared from solutions more concentrated than 0.01 M were toxic to the seeds.

[4] Obtained from Y. Sumiki, Dept. Agr. Chem., Univ. Tokyo, Tokyo.

[5] Obtained from Eli Lilly and Co., Indianapolis, Ind.

[6] Obtained from Merck and Co., Inc., Rahway, N. J.

Results

Forms of Gibberellin: Gibberellins A_1, A_2, A_3, and A_4 at concentrations of 10^{-7} to 10^{-3} M were tested for their effects on the germination of Grand Rapids lettuce seeds. Gibberellins A_4, A_3, A_1, and A_2 were effective for promoting germination in total darkness in the order listed (fig 1). Gibberellin A_3 was used, however, in all the remaining experiments reported because of the limited supply of A_4.

Concentrations of Gibberellic Acid: When treated with aqueous solutions of gibberellic acid, light-requiring seeds of Grand Rapids lettuce, and of Lepidium germinated completely in total darkness. The curves (fig 2) show that individual seeds within the lettuce population varied more in their requirement for gibberellin than did seeds of Lepidium. The optimum concentration for maximum germination did not differ greatly for the two seed kinds: 10^{-3} M for lettuce and 2.5×10^{-3} M for Lepidium. At concentrations immediately above these levels, radicle lengths of the seedlings were reduced. At still higher levels very abnormal development occurred; the embryos of all seeds broke through the seed coats with no development of the radicles. Such abnormal development is common for toxic concentrations of chemicals.

Duration of Gibberellin Treatment: Lepidium seeds were held in darkness at 20 C on distilled water for 22 or 46 hours plus 2 additional hours at 35°. At the end of the high-temperature treatment, the seeds were transferred to a substratum wetted

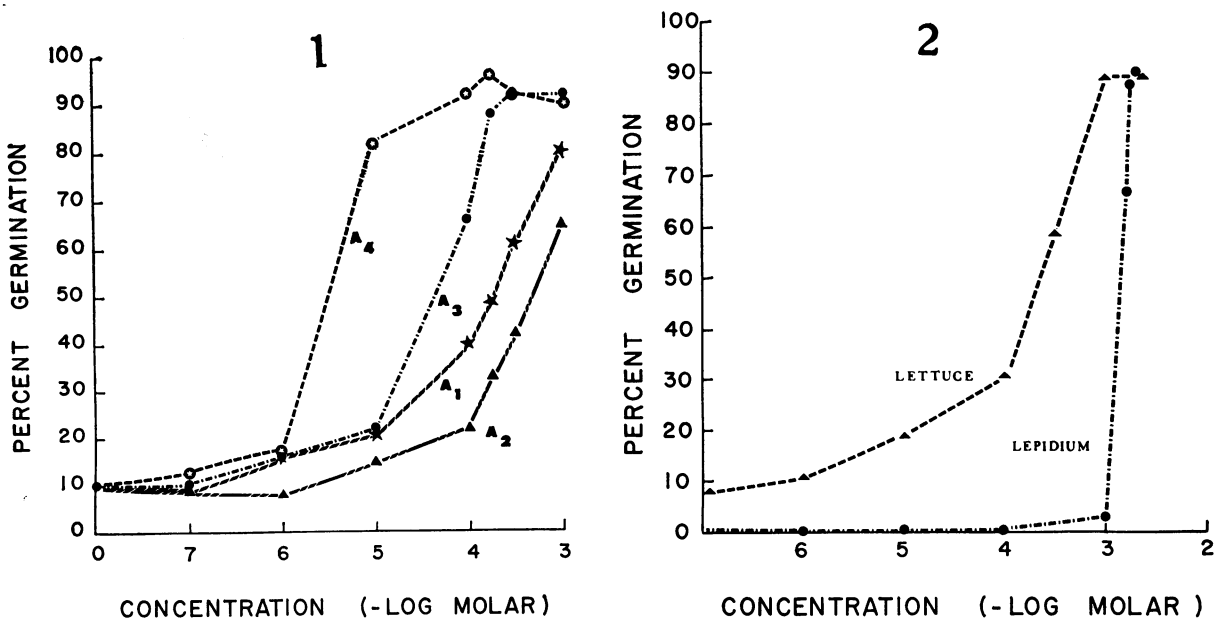
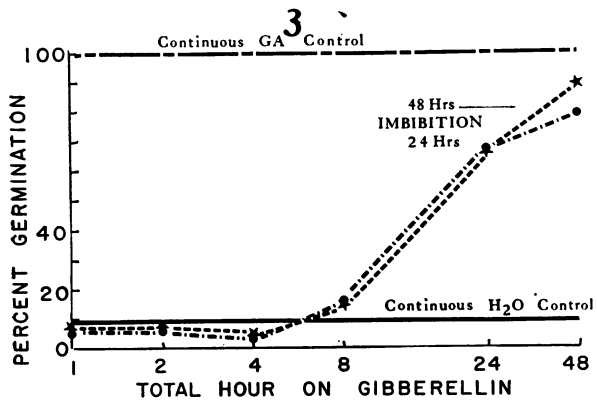


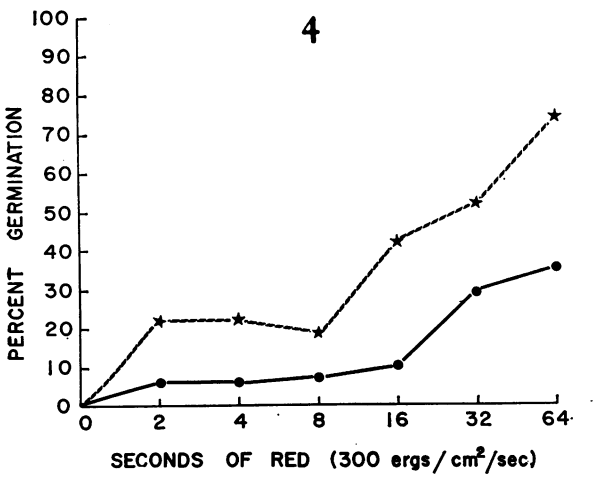
Fig. 1. Percentage germination in total darkness at 25 C of Grand Rapids lettuce seeds imbibed with different forms of gibberellins at various concentrations.

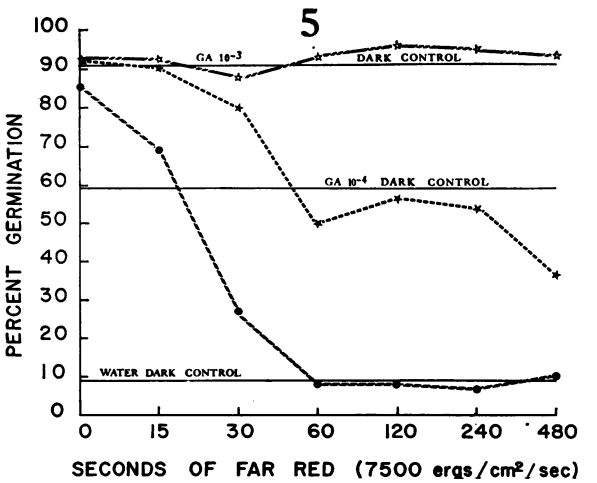
Fig. 2. Percentage germination of Lepidium and of Grand Rapids lettuce seeds in total darkness at 20/35/20 and 25 C, respectively, with indicated concentrations (—Log M) of aqueous solutions of gibberellic acid.

with gibberellic acid (2×10^{-3} M)for 1 to 48 hours (fig 3), and then returned to distilled water.

Seeds treated 8 hours with gibberellic acid germinated above those in the continuous water controls. The amount of promotion was greater as the total hours on gibberellic acid were increased from 8 to 24 to 48 hours.







INTERACTION OF GIBBERELLIN & TEMPERATURE: For two experiments of the effects of gibberellic acid on response to temperature, Lepidium seeds were held in darkness on a substratum moistened with 2×10^{-3} M gibberellic acid or with distilled water.

In the first experiment seeds were held at 20 C on moistened substratum in darkness 24 hours before half of the gibberellin lots and half of the water lots were exposed 10 minutes to red light (6,000 ergs/cm²/sec). All lots were then transferred to 35°. After 2 to 120 hours, two lots of light-promoted seeds and two lots of non-light-promoted seeds were returned to 20°.

Water-imbibed Lepidium seeds held 2 hours at 35 C either before or after exposure to light germinate 100 % when transferred to 20° (26). Germination was reduced to 4 % if the seeds were held at 35° for 24 hours. Seeds held longer at 35° retained their viability but failed to germinate (table I). When the temperature was lowered to 20° high percentages of the seeds treated with gibberellin germinated even though they had been held as long as 120 hours at 35°.

In the second experiment, Lepidium seeds were held in darkness at constant temperatures of 15, 20, 25, and 30 C. Controls were run at 20°/35°/20°. After 24 hours half of the gibberellin lots and half of the water lots were exposed 64 seconds to red light (700 ergs/cm²/sec). The other lots were maintained as dark controls.

Lepidium seeds germinated over a broader temperature range either in total darkness or after promotion by red light if treated with 2×10^{-3} M gibberellic acid rather than with water (table II). Lepidium seeds on a substratum wetted with water germinated below 5 % at constant temperatures of 15, 20, 25, and 30 C when given the same energy of light that caused approximately 50 % of the seeds to germinate at 20°/35°/20°. Gibberellin-treated seeds germinated completely or practically so in 14 days at 15° and 20° and much higher than those wetted with water at 25°.

INTERACTION OF GIBBERELLIN & RADIATION: High percentages of Lepidium seeds saturated with

FIG. 3. Percentage germination of Lepidium seeds in total darkness at 20/35/20 C after exposure to gibberellic acid (2×10^{-3} M) for periods indicated following 22 or 46 hours of imbibition at 20° plus 2 hours at 35°.

FIG. 4. Percentage germination of Lepidium seeds at 20/35/20 C with 10^{-3} M gibberellic acid (*broken line*) and with water (*solid line*) following various seconds of exposure to red light.

FIG. 5. Percentage germination of Grand Rapids lettuce seeds at 25 C with gibberellic acid and with water following 240-second exposure to red light (6,000 ergs/cm²/sec) and then seconds of far red as indicated. *Open star*, gibberellic acid 10^{-3} M; *closed star*, gibberellic acid 10^{-4} M; *closed circle*, water. *Solid lines* serve as the appropriate controls.

TABLE I

INTERACTION OF GIBBERELLIC ACID, RED LIGHT, & DURATION OF HIGH TEMPERATURE ON GERMINATION OF LEPIDIUM VIRGINICUM SEEDS AT 20/35/20 C

SOLUTION & LIGHT TREATMENT	% GERMINATION OF SEEDS HELD FOR INDICATED NUMBER OF hours AT 35 C					
	2	24	48	72	96	120
Water:						
Dark	0–0	0–0	0–0	0–0	0–0	0–0
Light*	100–100	3–5	0–0	0–0	0–0	0–0
2×10^{-3} M GA:						
Dark	98–99	99–99	93–96	100–100	85–86	88–89
Light*	100–100	100–94	99–100	99–100	82–67	87–78

* 10 minutes red (6,000 ergs/cm²/sec).

red light (25, 26) or treated with 2.5×10^{-3} M gibberellic acid germinated in total darkness. Therefore to measure any interaction, these two factors had to be reduced below the levels independently causing high germination. Seeds held on a substratum wetted with 10^{-3} M gibberellin or water were exposed for various seconds to red light (300 ergs/cm²/sec) at the end of the high-temperature treatment. At any given energy of irradiation a higher proportion of gibberellin-treated than of water-treated seeds responded to red light but the data are inadequate to indicate whether the effects are synergistic or additive (fig 4).

On the 7th day some of the seeds treated with a sub-optimal concentration of gibberellin and irradiated at a sub-optimal energy level had split the seed coats, but the radicles had not emerged. This delay in germination does not exist if seeds are saturated with light or treated with an optimum concentration of gibberellin.

Far-red radiation can nullify the promoting action of red on water-imbibed seeds (3, 25, 26). The effects of far red on germination of gibberellin-treated seeds were determined by treating lettuce seeds with 10^{-4} and 10^{-3} M gibberellic acid and Lepidium seeds with 10^{-3} and 2×10^{-3} M. After 2 hours in darkness for lettuce and the high temperature treatment for Lepidium, gibberellic acid and water-treated seeds were exposed 4 minutes to red light (6,000 ergs/cm²/sec). Immediately thereafter the seeds were exposed, as indicated in figure 5 for lettuce, 0 to 480 seconds to far-red radiation (7,500 ergs/cm/²sec). They were returned to 25 and 20 C and the ones germinated were counted 5 and 8 days later (lettuce & Lepidium, resp.).

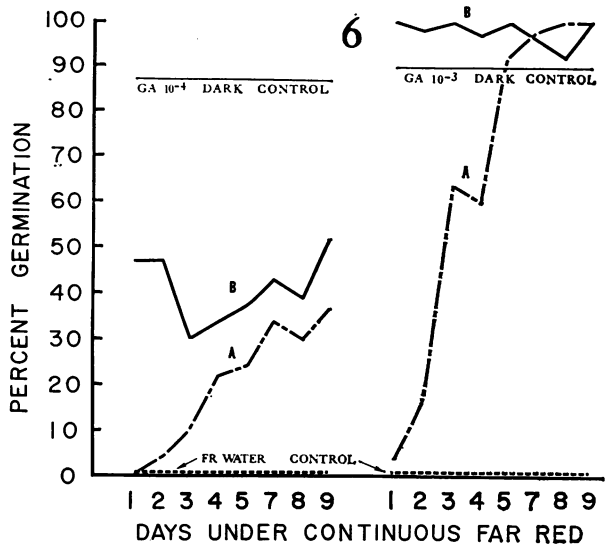
The lettuce seeds partially promoted by gibberellin were inhibited to or below their dark controls (fig 5) and those of Lepidium almost to their dark controls (not shown). The difference in germination between 480 seconds' exposure and the dark controls is significant at the 5 % level. We do not attach biological significance to this difference, however. The lettuce and Lepidium seeds fully promoted by gibberellin were not inhibited by short exposures to far red.

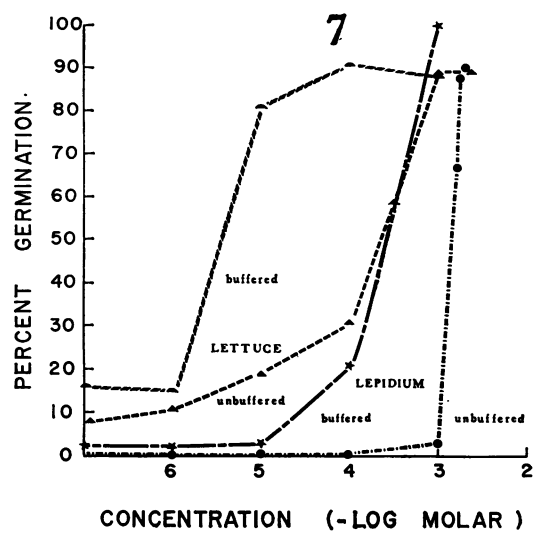
TABLE II

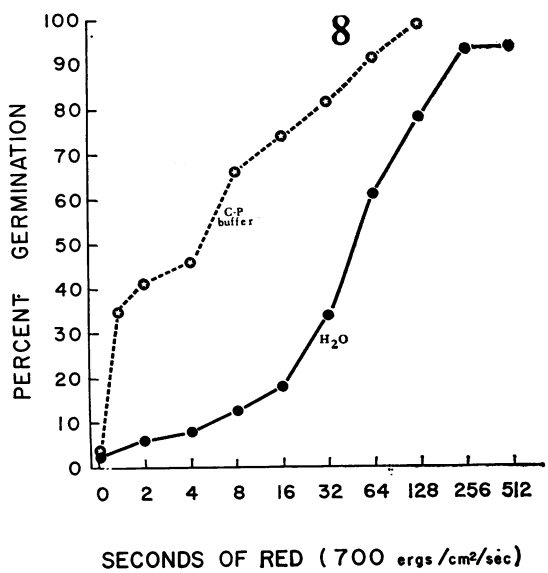
INTERACTION OF GIBBERELLIC ACID, RED LIGHT, & TEMPERATURE ON GERMINATION OF LEPIDIUM VIRGINICUM SEEDS

SOLUTION & LIGHT TREATMENT	DAYS OF GERMINATION	% GERMINATION OF SEEDS HELD AT INDICATED temp (°C)				
		15	20	25	35	20/35/20
Water:						
Dark	7	0–0	0–0	0–0	0–0	0–0
Light*	7	2–3	3–1	1–1	0–0	54–44
2×10^{-3} M GA:						
Dark	7	53–50	83–89	40–34	0–0	99–99
Light*	7	77–72	88–95	43–46	0–0	100–98
Water:						
Dark	14	0–0	0–0	0–0	0–0	0–0
Light*	14	0–1	1–7	0–1	0–0	44–41
2×10^{-3} M GA:						
Dark	14	93–99	99–100	30–30**	0–0	100–100
Light*	14	98–94	100–100	56–61**	0–0	100–100

* 64 seconds red (700 ergs/cm²/sec).
** Most of remainder with split seed coats.







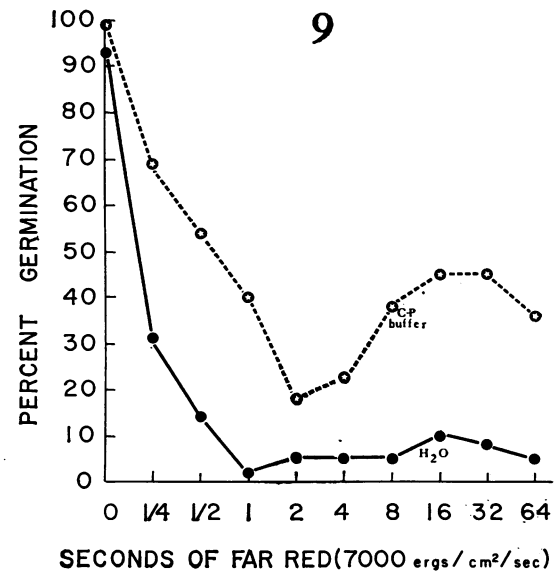


FIG. 6. Percentage germination of Grand Rapids lettuce at 25 C under continuous far red (A) and during 2 additional days in darkness (B) with 2 concentrations of gibberellic acid (10^{-4} M and 10^{-3} M). The appropriate controls are indicated.

FIG. 7. Percentage germination of Lepidium and of Grand Rapids lettuce seeds in total darkness at 20/35/20 and 25 C, respectively, with indicated concentrations (—Log M) of buffered (citrate-phosphate buffered to pH 3.2) and unbuffered aqueous solutions of gibberellic acid.

FIG. 8. Percentage germination of Lepidium seeds at 20/35/20 C with citrate phosphate buffer (pH 3.2) and with water following indicated seconds of exposure to red light (700 ergs/cm²/sec).

FIG. 9. Percentage germination of Lepidium seeds at 20/35/20 C with citrate phosphate buffer (pH 3.2) and with water after exposure for 512 seconds of red (700 ergs/cm²/sec) followed by indicated seconds of far red (7000 ergs/cm²/sec).

We tested the influence of continuous exposure to low intensity far red on lettuce seeds treated with the same two concentrations of gibberellin. Seeds of the variety Great Lakes were included because water-imbibed seeds of this variety behave like gibberellin-promoted Grand Rapids seeds—they germinate in the dark. The Grand Rapids water controls were exposed 4 minutes to red light (6,000 ergs/cm²/sec) after a dark period of 1 hour. They were then placed under continuous far red. All other lots except the dark and unfiltered light controls were placed under continuous far red immediately after planting. Four replicates for each solution treatment for each variety were left under continuous far red for 1 to 9 days as indicated for Grand Rapids in figure 6. At the end of these continuous far-red periods, two replicates were withdrawn and the germinated seeds counted immediately. At the same time two replicates were withdrawn and placed in total darkness and the number germinating after two additional days recorded.

Light-promoted Grand Rapids (fig 6) and non-light-promoted Great Lakes lettuce seeds (not shown) imbibed on a substratum wetted with water were completely inhibited in germination by continuous far red. Less than 5 % of the seeds germinated after removal to darkness (not shown). Grand Rapids and Great Lakes lettuce seeds treated with 10^{-3} M gibberellin were prevented by continuous far red from germinating during the first 2 days. After removal from far red, seeds of Grand Rapids lettuce germinated completely, and those of Great Lakes increased markedly in germination. Such gibberellin-treated seeds, given far red for 7 to 9 days, germinated completely or practically so while under the continuous far red. Thus, far red delays but does not prevent germination in the presence of gibberellin.

The germination of seeds from these same varieties treated with 10^{-4} M gibberellin was inhibited by continuous far red much below their dark controls (fig 6).

EFFECT OF BUFFERS: The acid or the potassium salt of gibberellin was effective on Grand Rapids lettuce seeds, but the acid only was effective on Lepidium seeds (results not given). The effect of pH on the activity of the potassium salt was investigated. Citrate phosphate was used to give buffered solutions of the potassium salt of gibberellin with various pH values. Controls were run with water, with unbuffered potassium salt, and with the acid of gibberellin. The concentration of 10^{-3} M of gibberellin was used. Tests were made in total darkness and with an exposure to light (700 ergs/cm²/sec).

When buffered to pH 3.5, the potassium salt of gibberellic acid acted in the same manner as gibberellic acid (table III). With a higher pH the number of seeds promoted to germinate was lowered. Solutions of lower pH were not used because they prevented elongation of the radicles. The light energy used was calculated to give approximately 50 % germination of water controls. With the same light intensity, citrate phosphate buffer alone caused an additional 36 % to germinate. In total darkness citrate phosphate buffer had no effect unless combined with gibberellin.

These results with buffered solutions of the potassium salt of gibberellin suggested that a solution combining citrate phosphate buffer and gibberellic acid might be more effective than gibberellic acid alone. Such combined solutions at pH 3.2 were prepared with concentrations of gibberellic acid from 10^{-6} to 10^{-2} M. Buffered solutions of gibberellic acid were effective at lower concentrations of gibberellin than unbuffered ones in causing lettuce and Lepidium seeds to germinate in the dark (fig 7).

The results given in table III also suggested that citrate phosphate itself at pH 3.2 modified the light action. The effect of light on the germination of Lepidium seeds treated with distilled water or citrate phosphate buffer (pH 3.2) was determined. After the 35 C treatment, two lots each from the water and buffer treatments were irradiated with red light (700 ergs/cm²/sec) as indicated in figure 8. The remaining seeds were all given 512 seconds of red light and

TABLE III

GERMINATION OF SEEDS OF LEPIDIUM VIRGINICUM WITH DIFFERENT FORMS OF GIBBERELLIC ACID WITH pH ADJUSTMENT OF POTASSIUM SALT SOLUTION WITH CITRATE PHOSPHATE BUFFER

FORMS GIBBERELLIC ACID*	BUFFER**	pH	% GERMINATION OF SEEDS	
			HELD IN TOTAL DARKNESS	PROMOTED WITH RED LIGHT***
0 (water control)	0	5.7	0–0	56–55
Gibberellic acid	0	3.2	83–82	99–95
Potassium salt of gibberellin	0	6.0	0–0	65–71
" " "	C–P	3.5	100–100	100–100
" " "	C–P	4.1	32–35	99–99
" " "	C–P	4.4	0–4	78–76
0 (buffer control)	C–P	3.2	0–0	92–91

* 2×10^{-3} M, optimum concentration.

** Citrate phosphate 0.01 M.

*** 64 seconds, 700 ergs/cm²/second, estimated to give 50 % germination of H_2O control.

TABLE IV

GERMINATION OF SEEDS OF LEPIDIUM VIRGINICUM WITH COMPONENTS OF BUFFER SOLUTION

WETTING AGENT FOR SUBSTRATA	MOLAR CONC	pH	% GERMINATION OF SEEDS	
			HELD IN TOTAL DARKNESS	PROMOTED WITH RED LIGHT*
Citrate phosphate buffer		3.45	0–1	94–95
Citric acid	0.001	3.10	0–1	97–98
Na_2PO_4	0.001	8.25	0–0	85–80
Distilled water			0–0	67–57

*64 seconds red (700 ergs/cm²/sec).

then two lots each from the water and buffer treatments were exposed to far red as indicated in figure 9. The germinated seeds were counted 4 days after the light treatment. The red light energy required for a given germination response was less for seeds treated with the buffer solution than for those treated with water. As with other stimuli (3, 25), when the germination response to red is increased by the presence of buffer, the germination response to far red is decreased.

The increased germination of light-treated seeds on citrate phosphate buffer over that of seeds held on water is not a direct effect of pH of the solution. Solutions of citric acid at pH 3.10 and of sodium phosphate at pH 8.25 used separately (at 10^{-3} & 5×10^{-3} M, resp.) increased germination above the water controls of light-promoted seeds (table IV). Other solutions such as the phthalate–HCl buffer, glycine–HCl buffer, and aconitate buffer increased the level of germination of light-promoted seeds over that of seeds held on water but had no effect on seeds held in darkness (results not given). In all cases the seed coats were bleached to a straw color by these chemicals.

DISCUSSION

The optimum concentration of gibberellic acid for promotion of germination of Grand Rapids lettuce seeds in darkness was reported by Evenari et al. (7) to be 2.9×10^{-5} to 2.9×10^{-4} M and by Haber and Tolbert (9) to be 3×10^{-4} M. The optimum concentration we found was 10^{-3} M. These differences in reported optimal concentrations are not surprising because both the physiological condition of the seed and the purity and stability of the gibberellin preparations differ.

The differences in the range of effective concentrations of gibberellic acid for stimulation of germination of seeds of lettuce and Lepidium in the dark could be due to differences in coat structure of these two kinds of seeds. The Lepidium seed has a harder seed coat than lettuce and becomes mucilagenous on wetting.

Lettuce and Lepidium seeds may be fully promoted by light and still be blocked from germinating unless certain temperature conditions are provided (3, 25, 26). We found that Lepidium seeds treated with an optimum concentration of gibberellin germinated over a much wider range of temperatures than water-imbibed, light-promoted seeds. Gibberellin also prevented dormancy induced by high temperatures. This is in agreement with other workers (7, 14, 15, 21) who showed that gibberellin prevented high temperature induced dormancy in lettuce seed. Thus gibberellin not only allowed the germination processes to by-pass the light requirement, but it also overcame some of the temperature blocks to germination. Black and Naylor (1) reported that applied gibberellin prevented the onset of dormancy during maturation of the seed. The physiological condition of the gibberellin-promoted seeds, expressed by lack of need for light and tolerance to a wide temperature range, suggests a similarity to the physiological state of seeds afterripened in normal dry storage.

With both lettuce and Lepidium seeds, gibberellin had two effects, as also observed by Evenari et al. (7). Optimum concentrations of gibberellin allowed otherwise light-requiring seeds to germinate in darkness. Also, gibberellin concentrations below those that stimulated germination in darkness increased the number of seeds that responded to a given light exposure.

In agreement with Ikuma and Thimann (12) the inhibitory action of far red was influenced by the concentration of gibberellin. If the concentration of gibberellin was such that the seeds were not fully promoted, a short exposure to far red inhibited germination to or slightly below that in the gibberellin dark controls. Continuous far red inhibited such seeds far below the gibberellin dark controls. A short exposure to far red had no inhibitory effect on seeds fully promoted by gibberellin. And continuous far red retarded rate but not total germination of these seeds. Kahn (13) also found continuous far red delays germination. He pointed out that seeds treated with gibberellin still contained it, probably in the active form.

Various buffered solutions and the components of the citrate phosphate buffer had no influence on germination of Lepidium seeds in darkness, but they did markedly influence the sensitivity of seeds to gibberellin and to light.

Evenari et al. (7) and Miller (18) reported that kinetin caused light-requiring lettuce seeds to germinate in total darkness. Contrariwise, Weisz (28) reported that at 26 C kinetin was ineffective in darkness. Raleigh (22) stated that the light requirement in lettuce seeds was sometimes removed by thiourea. Certain dilute concentrations of potassium nitrate, thiourea, and coumarin (25), as well as citric acid, sodium phosphate, and various buffers, reported here increased the number of Lepidium seeds that responded to red light, but none of these chemicals induced any germination in darkness. Gibberellin is the only factor found thus far to bring about germination of seeds of Lepidium in total darkness.

It is not clear whether the increased stimulus to germination in darkness from buffering the solutions of the potassium salt of gibberellic acid was due to changed pH of the solution or to specific influence of the added ions. Since the solution was buffered below 3.8, the pKa of the gibberellic acid as reported by Cross (6), most of the gibberellic acid was in the undissociated state. Solutions of gibberellic acid more dilute than 2×10^{-3} M, when prepared in a buffer at pH 3.2, were more active physiologically than similar unbuffered solutions. These experiments suggested that the undissociated form penetrated and was actually the active form of gibberellin. The gibberellin solution did not undergo degradation in the acid solution since allogibberic acid was inactive in all tests with seeds as reported by Brian et al. (4) and growing plants as reported by Halevy and Cathey (10). Similar observations have been made with auxin (27), at the optimum pH 4 to 5, where the activity was correlated with the undissociated form rather than total auxin concentration. The acid form of 2, 4-dichlorophenoxyacetic acid was shown to be physiologically more active for and more readily translocated in plants (27) than the salt form.

Yamaki et al. (30) reported that a diffusate from leaf discs of bean that had been illuminated with red light induced germination of tobacco seed. One of the substances from the diffusate had the same Rf value as the gibberellins in several solvent systems. Ikuma and Thimann (12) were unable to demonstrate that even a trace of gibberellin A_3 and A_5, as assayed on dwarf maize, was produced by light treatment of lettuce seed. Dwarf maize, used by Ikuma and Thimann, was insensitive to applications of gibberellin A_4, but was responsive to gibberellins A_1, A_3, and A_5 (5). Gibberellin A_4, however, was much more active than A_3 in promoting germination of tobacco (11) and lettuce seeds (reported herein) and in expansion of bean leaf discs (30). Some workers (9, 12) concluded that light does not induce germination of lettuce seeds by liberating a gibberellin although others (2, 19) reported gibberellin in lettuce seeds. It is thus not possible to state the relationship of light to the liberation of gibberellin in the seed.

Summary

The mode of action of gibberellin in the germination of light-requiring seeds of *Lepidium virginicum* and Grand Rapids lettuce was studied. The response to four forms of gibberellin, the effects of gibberellin concentration and of buffering the acid and the potassium salt of gibberellin, and the interaction between gibberellin and temperature and gibberellin and the phytochrome system were determined.

Gibberellin had three separate effects. A: Gibberellin caused light-requiring seeds to germinate in total darkness. B: It removed certain temperature blocks to germination and prevented the onset of dormancy imposed by high temperature. C: When suboptimal levels of light and gibberellin were used, gibberellin caused a higher percentage of seeds to germinate at a given energy of red light.

Seeds fully promoted by gibberellin were not inhibited by short exposures to far red but were retarded in rate of germination by continuous exposure. Seeds partially promoted by gibberellin were inhibited to or slightly below the gibberellin dark controls by short exposures to far red and much below the gibberellin dark controls by continuous far red.

Gibberellin solutions buffered below the pKa value were more active than unbuffered solutions in promoting germination.

Literature Cited

1. Black, M. & J. M. Naylor. 1959. Prevention of the onset of seed dormancy by gibberellic acid. Nature 184(4684): 468–469.
2. Blumenthal-Goldschmidt, S. & A. Lang. 1960. Presence of gibberellin-like substances in lettuce seed. Nature 186(4727): 815–816.
3. Borthwick, H. A., S. B. Hendricks, E. H. Toole, & V. K. Toole. 1954. Action of light on lettuce seed germination. Botan. Gaz. 115: 205–225.
4. Brian, P. W., J. F. Grove, H. G. Hemming, T. P. C. Mulholland, & M. Radley. 1958. Gibberellic acid, Part VI. The Biological activity of allogibberic acid & its identity with gibberellin B. Plant Physiol. 33: 329–333.
5. Cathey, H. M., N. W. Stuart, V. K. Toole, & S. Asen. 1961. Enhancement of gibberellin-induced phenomenon. Adv. Chem. Series. 28: 135–141.
6. Cross, B. E. 1954. Gibberellic acid, Part I. J. Chem. Soc. (London) 1954, 4670 pp.
7. Evenari, M., G. Neumann, S. Blumenthal-Goldschmidt, A. M. Mayer, & A. Poljakoff-Mayber. 1958. The influence of gibberellic acid & kinetin on germination & seedling growth of lettuce. Bull. Res. Coun. Israel 6D: 65–72.
8. Fujii, T., S. Isikawa, & A. Nakagawa. 1960. The effects of gibberellin on the germination of the seeds of *Sedum kamtschaticum* Fisch. Botan. Mag., Tokyo, 73(868): 404–411.
9. Haber, A. H. & N. E. Tolbert. 1959. Effects of gibberellic acid, kinetin, & light on the germination of lettuce seeds. In: Photoperiodism & Related Phenomena in Plants & Animals, R. B. Withrow,

ed. Pp. 197–206. Am. Assoc. Advancement Sci., Washington, D. C.

10. HALEVY, A. H. & H. M. CATHEY. 1960. Effects of structure & concentration of gibberellins on the growth of cucumber (*Cucumis sativus* L.) seedlings. Botan. Gaz. 122: 63–67.
11. HASHIMOTO, T. & T. YAMAKI. 1959. On the physiological effects of gibberellins A_1, A_2, A_3 & A_4. Botan. Mag., Tokyo, 72(850): 178–179.
12. IKUMA, H. & K. V. THIMANN. 1960. Action of gibberellic acid on lettuce seed germination. Plant Physiol. 35: 557–566.
13. KAHN, A. 1960. Promotion of lettuce seed germination by gibberellin. Plant Physiol. 35: 333–339.
14. KAHN, A., J. A. Goss, & D. E. SMITH. 1956. Light & chemical effects on lettuce seed germination. (Abstr.) Plant Physiol. 31 suppl.: xxxvii.
15. KAHN, A., J. A. Goss, & D. E. SMITH. 1957. Effect of gibberellin on germination of lettuce seed. Science 125(3249): 645–646.
16. KRIBBEN, F. J. 1957. Die Abkürzung der Samenruhe bei Arabidopsis durch Gibberellinsäure. Naturwissenschaften 44(10): 313.
17. LONA, F. 1956. L'acido gibberellico determina la germinazione dei semi di *Lactuca scariola* in fase di scoto-inhibizione. L'Ateneo Parmense 27(4): 641–644.
18. MILLER, C. O. 1956. Similarity of some kinetin & red light effects. Plant Physiol. 31: 318–319.
19. MURAKAMI, Y. 1959. The occurrence of gibberellins in mature dry seeds. Botan. Mag., Tokyo, 72(Nos. 857–858): 438–442.
20. OGAWARA, K. & K. ONO. 1958. Effect of light on the germination of Petunia hybrida seeds. Hort. Assoc. Japan J. 27(4): 276–281.
21. POLJAKOFF-MAYBER, A., M. EVENARI, & G. NEUMANN. 1958. Effect of red light & gibberellic acid on the temperature-inhibited germination of lettuce seeds. Bull. Res. Counc. Israel 6D(2): 99–102.
22. RALEIGH, G. J. 1943. The germination of dormant lettuce seed. Science 98(2555): 538.
23. ROLLIN, P. 1959. Influence de l'acide gibberellique sur la germination des semences photosensibles. Bull. Soc. Franc. de Physiol. Vég. 5(1): 24–26.
24. TOOLE, E. H., S. B. HENDRICKS, H. A. BORTHWICK, & VIVIAN K. TOOLE. 1956. Physiology of seed germination. Ann. Rev. Plant Physiol. 7: 299–324.
25. TOOLE, E. H., V. K. TOOLE, H. A. BORTHWICK, & S. B. HENDRICKS. 1955. Photocontrol of Lepidium seed germination. Plant Physiol. 30: 15–21.
26. TOOLE, E. H., V. K. TOOLE, H. A. BORTHWICK, & S. B. HENDRICKS. 1955. Interaction of temperature & light in germination of seeds. Plant Physiol. 30: 473–478.
27. VAN OVERBROOK, J. 1956. Adsorption & translocation of plant regulators. Ann. Rev. Plant Physiol. 7: 355–372.
28. WEISZ, J. 1960. Action des températures relativement basses sur la germination, dans la kinétine, des semences de Laitue, variete (Grand Rapids). Acad. sci., Paris. Compt. rend. 251(1): 125–127.
29. WITHROW, R. B. & L. PRICE. 1953. Filters for the isolation of narrow regions in the visible and near-visible spectrum. Plant Physiol. 28: 105–114.
30. YAMAKI, T., T. HASHIMOTO, T. ISHII, & M. YAMADA. 1958. Some physiological effects of gibberellin on seed germination, leaf expansion, dehydration of mitochondria, & probable formation of gibberellin in the leaf. In: Proc. Meeting on Gibberellin Res. in Japan. Pp. 51–52 (Jap. Gibberellin Res. Assoc., Tokyo). 80 pp.