Effect of Light, Temperature, and Their Interactions on Germination of Seeds of Kentucky Bluegrass (*Poa pratensis* L.)[1]

Vivian K. Toole[2] and H. A. Borthwick[3,4]
U. S. Department of Agriculture
Beltsville, Maryland

Abstract. The germination of Kentucky bluegrass, (*Poa pratensis* L.), seeds is profoundly influenced by two light reactions. One, the phytochrome reaction (P), is promotive, and the other, the so-called "high-energy reaction (HER)", is inhibitory to germination. The level of germination displayed in 14 days as the resultant of these two opposing reactions is appreciably influenced by temperature.

In darkness, the seeds germinate well at certain temperature alternations but not at constant temperatures. At 15-25°C, the promotive effects of temperature alternation are accomplished in the first 5 to 6 cycles. The promotive effects of alternations are displayed in darkness when the daily period at 25° is between 4 and 14 hours.

Brief daily high-intensity fluorescent illuminances (approximately 4,000 ft-c) during otherwise continuous darkness at constant 20°C induce high germination in most lots. Continuous medium-to-high intensity illumination (approximately 1,200 ft-c) very weakly promotes germination and in potentially promoted seeds inhibits germination to about the level of the dark controls.

Inhibitory effects of continuous light on potentially promoted seeds are best displayed at 20°C constant although, in 'Newport' they are observable at 15-25°. The inhibition of 'Newport' at 15-25° is to a level below that of the dark controls but above that caused by prolonged illumination at 20°.

Thus conditions most promotive to germination are 15-25°C alternations and brief daily illuminances of high intensity. Simultaneous application of these 2 conditions causes higher germination than when either is applied alone and in one lot of 'Newport' the effects are strikingly additive.

Germination of seeds of Kentucky bluegrass is influenced by light and temperature. Toole (7) showed that they germinated low at all constant temperatures and high in darkness at a suitable alternation of temperature. It has been known for almost 100 years that light promotes the germination of some seed kinds (3) and for about 60 that light inhibits the germination of others (5), but the mechanism of control through the phytochrome reaction was not known until comparatively recently (1). Germination control through 2 opposing light reactions within the same seed was recently shown in the germination of Kentucky bluegrass seed (6). The typical phytochrome reaction (P) causing promotion of germination was initiated by brief exposures to light. The so-called high-energy reaction (HER) causing inhibition of germination was initiated by prolonged illuminations.

The objectives of this study were to examine the effects of light, temperature, and their interactions on the germination of Kentucky bluegrass seeds and to identify combinations of these conditions that would lead to maximum display of the 2 respective light reactions.

Materials and Methods

Foundation seed of Kentucky bluegrass representing 2 lots each of cultivars 'Newport' and 'Cougar' from the 1965 and 1966 crops were received soon after harvest. After removal of light seeds and empty florets, mature seeds were stored in sealed glass jars at -11°C to -13° a few days after receipt.

Seeds were germinated in petri dishes on 2 filter papers moistened to a slightly shiny appearance with tap water. Dishes were equilibrated to temperature before planting. The planted seeds, 100 per dish, were held at a given temperature, ± 1°C, in total darkness except for specified light treatments. Light was excluded by placing the dishes with seeds between several folds of black sateen cloth.

Alternating temperatures involving darkness were obtained by manual transfers from one cabinet to another. Experiments involving alternating temperatures and 8-hr light periods were performed within a single chamber controlled by dual thermostats. Unless otherwise stated, the low and high parts of the temperature cycle were 16 and 8 hr, respectively, each day. Tests were begun on the low part of the cycle.

For brief light exposures, seeds were taken to the given light source and then returned to the temperature-controlled chamber. Red light was obtained by filtering the light from a bank of eighteen, 96-inch, slimline, T8, cool-white fluorescent lamps through 2 layers of red cellophane. Far red was obtained by filtering light from 3, 300-W, incandescent-filament, flood lamps through 2 layers each of red and blue cellophane and 10 cm of water. At distances of 1 meter the power of the red source was 0.6 mw/cm^2 in the wave band 600 to 680 nm and of the far red was 0.75 mw/cm^2 in the wave band 700 to 750 nm. Unfiltered cool-white fluorescent light was used in most experiments. The 10-min unfiltered illuminations were 4,000 ft-c and were given each 24 hr for the days indicated after the seed had been held imbibed in darkness for an initial 24-hr or 72-hr period. This intensity was obtained from a bank of 16, 4-ft lamps equipped with a dimming device that permitted wide variation of intensity without change in quality.

It was impractical to operate this source unattended for long periods, therefore prolonged irradiations were performed at lower intensities with lamps of fixed output in a temperature-controlled cabinet or a growth room. In the growth room short and long illuminations of 1,800 ft-c and lower intensities were obtained by variation of distances from a 2-lamp cool-white fluorescent source. In the controlled cabinet illumination from top-mounted cool-white fluorescent lamps was approximately 1,200 ft-c at seed level. Eight-hr daily illuminations in these cabinets were given during the 25°C parts of the daily 15-25° cycles. Various prolonged illuminations at 20° constant were also obtained in these cabinets.

To obtain various periods of darkness preceding the light treatments, planting dates were staggered so that all lots were illuminated simultaneously. The period of darkness during which the seeds were held imbibed is given in each experiment.

[1]Received for publication September 11, 1970.

[2]Research Plant Physiologist, Market Quality Research Division, ARS.

[3]Collaborator, Crops Research Division, ARS.

[4]The authors wish to thank J. D. Maguire, Dept. of Agronomy, Washington State University, Pullman, who kindly furnished foundation seed from the 1965 and 1966 harvests.

This period included the imbibition period *per se.*

Emergence of radical, regardless of whether seedling structure was normal or not, was taken as a measure of germination. Results 14 days from planting are given unless otherwise noted. In all experiments 2 or more replicates of each treatment were included and in most cases conclusions were based on 2 or more identical or similar experiments. Since only relatively large differences between treatments were of interest, detailed statistical analyses were not included.

Results

Temperature effects

Constant temperature. Few imbibed seeds germinated in total darkness at constant temperatures ranging from 15°C to 30° (Table 1). Imbibed seeds irradiated 10 min with red light each 24 hr throughout the 14-day period germinated appreciably higher than their respective dark controls at 15° and 20° but differences were not so great at 25° and 30°.

Table 1. 14-day germination of 2 lots each of 2 cultivars of 'Kentucky' bluegrass seeds after indicated minutes of red light and various temperature conditions.

Temperature conditions	Red light[a]	Germination			
		Newport 16	Newport 50	Cougar 13	Cougar 51
Degrees C	min.	%			
Constant					
15	2	80	83	94	86
20	2	24	80	96	84
25	2	0	34	72	38
30	2	0	2	14	12
15	0	6	8	34	12
20	0	0	8	16	9
25	0	0	4	2	8
30	0	0	0	0	8
Alternating[b]					
10-25	0	60	72	77	74
15-25	0	68	77	85	80
10-30	0	55	64	75	57
20-30	0	1	79	85	58

[a]Each 24 hr.

[b]Daily 16-8 hr temperature cycles.

Alternating temperature. Seeds germinated higher in total darkness at alternating temperature on daily 16-8-hr cycles than at constant temperatures. Results at 15-25°C alternation were generally higher than those of the other 3 alternations (Table 1).

In a later experiment, the daily duration of the 15°C and 25° parts was varied reciprocally between the limits of 2 and 22 hours (Fig. 1). High germination of the 2 lots tested was obtained in total darkness when the 25° part of the cycle was maintained 4 to 14 hr per day.

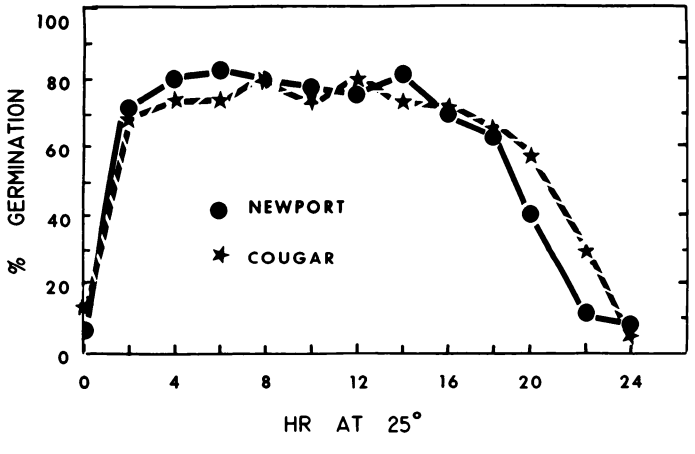

Fig. 1. 14-day germination in darkness of seeds of cvs. Newport, Lot 50, and Cougar, Lot 13, at 15-25°C daily alternation with periods at 25° varied between 2 and 22 hr.

The number of 15-25°C, 16-8-hr cycles required to promote germination in darkness was also investigated. After completion of each of the 1 to 9 cycles, dishes were returned to 15° constant (Fig. 2). Five to 6 cycles were as effective as 9.

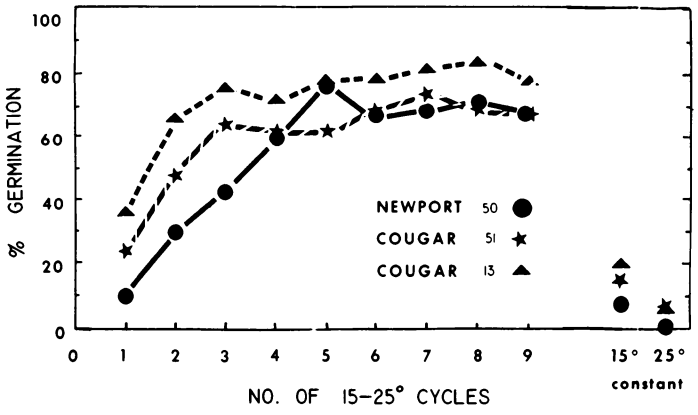

Fig. 2. 10-day germination in darkness of seeds of cvs. Newport, Lot 50, and 'Cougar', Lots 13 and 51, at 15-25°C on daily 16-8hr cycles for 1 to 9 days and then at 15° constant.

Light effects

Two light reactions. The duality of light action on Kentucky bluegrass seeds is shown in Fig. 3 where short illuminances of 1,800 ft-c each 24 hr for 4 successive days were promotive of germination at 20°C but prolonged ones were inhibitory. At lower intensities both promotive and inhibitory effects were also evident but of lower level.

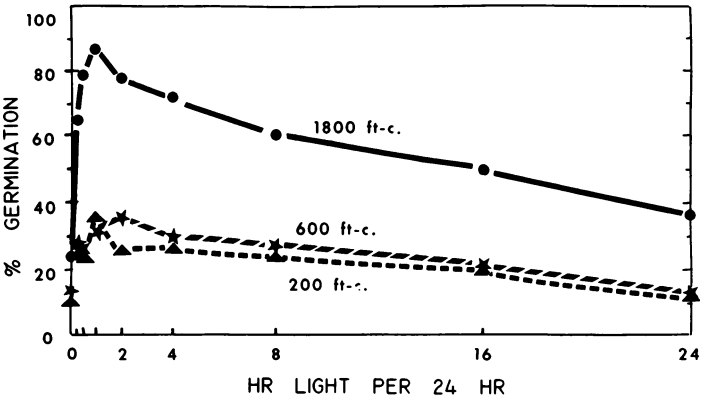

Fig. 3. 14-day germination of seeds of cv. Newport, Lot 50, at 20°C in reponse to various daily illuminations of indicated duration and intensity for 4 successive days begun after 72-hr dark imbibition.

Evidence for promotion through phytochrome

Reversibility. Promotion of germination by brief light exposures is under phytochrome control as shown by repeated reversibility of response following alternate exposures to red and far-red irradiances (6). Such reversibility was observed in all lots studied here.

Light energy. Results in Fig. 3 show that maximum promotive effects are to be had when irradiation times are kept as brief as possible to avoid inhibitory effects of HER. A 4,000-ft-c fluorescent source was accordingly used instead of the 1,800-ft-c one to provide a range of radiant energies. Germination increased with duration of exposure showing that the response was either energy-or time-dependent (Fig. 4). To distinguish between these 2 alternatives reciprocity experiments were performed in which several light energies were each obtained by reciprocal variation of duration and intensity of light exposure. One such experiment involved illuminances of ¼ to 4 min at 2,000 ft-c and 1 to 16 min at 500 ft-c (Fig. 5). Reciprocity held reasonably well over this range of intensities

and times as shown when germinations were plotted against ft-c-min.

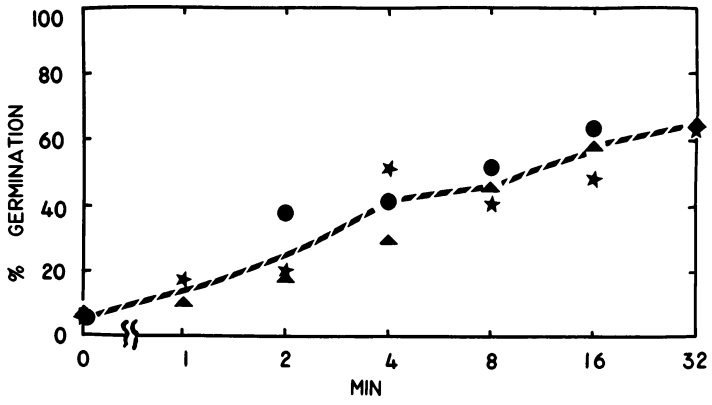

Fig. 4. 14-day germination of seeds from 3 experiments of cv Newport, Lot 50, at 20°C in response to a single 4,000-ft-c illumination of indicated duration after 72-hr dark imbitition.

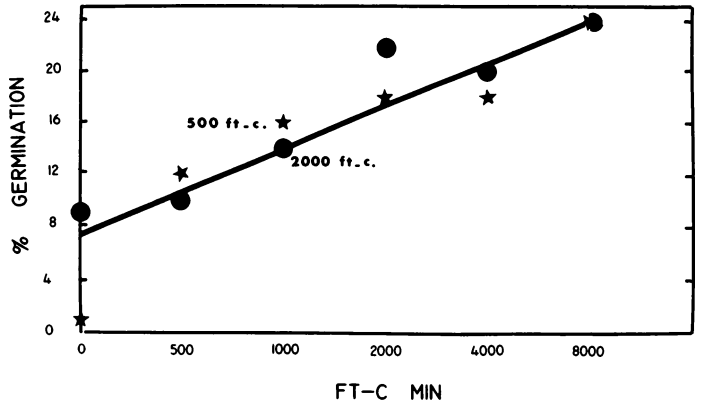

Fig. 5. 14-day germination of seeds of cv. Newport, Lot 50, at 20°C in response to indicated paired illuminances (ft-c-min) at intensities of 500 and 2,000 ft-c.

Daily brief illuminances. Daily 10-min illuminations at 4,000 ft-c unfiltered fluorescent light for 1 to 10 days after seeds were held imbibed 24 and 72 hr showed that the first several light cycles at 20°C markedly increased germination (Fig. 6). More than 5 or 6 light cycles gave essentially no further change.

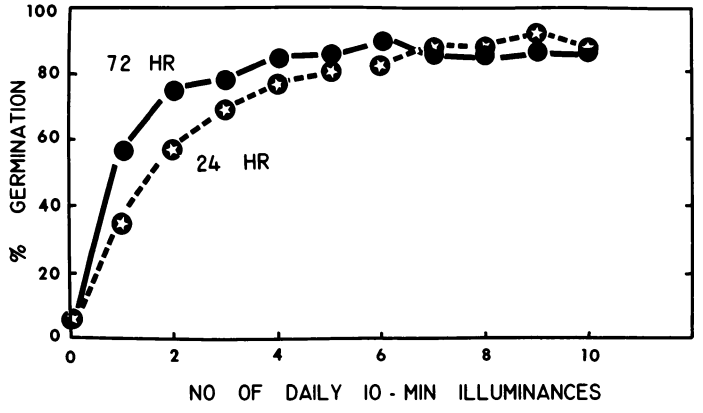

Fig. 6. 14-day germination of seeds of cv. Newport, Lot 50, at 20°C in response to indicated number of daily 10-min, 4,000-ft-c illuminations after an initial 24- and 72-hr dark imbibition period.

Dark periods preceding light. Seeds held imbibed in darkness for 1 to 10 days at 20°C were given a simultaneous 10-min illuminance at 4,000 ft-c followed by 10 days of darkness before counting. Germination results were highest in lots that received 1 to 4 days of darkness before the single illuminance. Seeds held for longer periods were less responsive to light. In connection with these studies water uptake (% increase in fresh weight) was determined. After 12 hr of imbibition at 20° water uptake was approximately 45%. Water uptake was approximately 65% at 72-hr when a few seeds had developed attachment hairs.

Evidence for inhibition through HER

Continuous illumination from planting. When seeds were placed in unfiltered fluorescent light of 1,200 ft-c at 20°C immediately after planting and left 1 to 3 days before transfer to darkness, germination was about 10% higher than in the dark controls. This slight promotion was not evident, however, for those illuminated longer than 3 days (Table 2).

Table 2. Germination at 20°C of seeds of cv. Newport, Lot 50, illuminated with unfiltered fluorescent light, 1,200 ft-c, various numbers of days.[a]

Days illuminated	Germination	
	10 days	21 days
	%	%
1	17	18
2	14	15
3	16	17
4	8	10
5	6	8
14	1	4
Dark controls	6	7
Light controls[b]	26	-

[a]Begun immediately after planting.

[b]10 min light of 1,200 ft-c each 24 hr for 4 successive days.

Continuous illumination after darkness. Seeds held imbibed in darkness at 20°C for 0, 3, 6, 9 and 12 days preceding continuous illumination with unfiltered light of 1,200 ft-c at 20° germinated 6, 8, 10, 12 and 11% respectively. The dark control germinated 6%.

Continuous and intermittent illumination on promoted seeds. At 20°C imbibed seeds held 72 hr in darkness were promoted to approximately 50% by a single 10-min illumination at 4,000 ft-c. Seeds potentially promoted to the same level were subjected to intermittent and continuous illuminations immediately thereafter for 5 successive days (Table 3) and then were returned to darkness for an additional 5 days. When illuminated continuously following the 10-min illuminance approximately 2/3 of the potentially promoted seeds failed to germinate even at 15 ft-c. However germination was not reduced to the level attained by controls illuminated with 1,000 ft-c only (Table 3). Fifteen and 60 sec of illumination each 15 min were less inhibitory to potentially promoted seeds than continuous except at the highest intensity.

Table 3. 14-day germination at 20°C of seeds of cv. Newport, Lot 50, given a single 10-min illumination at 4,000 ft-c after 72 hr dark imbibition and followed by 5 days of continuous or intermittent light of indicated intensity.

Intensity of illumination after promotive treatment	Germination when promotive treatment was followed by:		
	Continuous illumination	Light cycles	
		60 sec per 15 min	15 sec per 15 min
ft-c	%	%	%
0	51	54	38
15	19	37	42
30	14	32	34
60	19	23	38
125	17	25	34
250	21	27	31
500	18	20	26
1000	19	14	16
1000[a]	10	-	-
500[a]	4	14	16
Dark controls	4	6	12

[a]Controls not given preliminary promotive treatment.

Various periods of prolonged illumination immediately after the single 10-min illuminance indicated that a 2-hr illuminance at 1,200 ft-c, the shortest one tested, was sufficient to reduce germination greatly (Table 4).

Table 4. 14-day germination at 20°C of seeds of cv. Newport, Lot 50, given a single 10-min illumination at 4,000 ft-c after 72 hr dark imbibition and followed by various periods of illumination at 1,200 ft-c.

Periods at 1,200 ft-c following promotive treatment	Germination
	%
0	51
2 hours	30
4 "	18
8 "	24
16 "	13
1 day	22
2 "	13
4 "	5
8 "	5
Dark controls	3

Continuous illumination between promotive treatments. After an initial 24-hr dark period, imbibed seeds of cultivar 'Newport', Lot 50, were illuminated 10 min at 4,000 ft-c each 24 hr for 4 successive days. Seeds returned to darkness at 20°C between these 10-min promotive treatments germinated 70% whereas those returned to 1,200-ft-c continuous unfiltered fluorescent illumination germinated 5%. Control seeds that did not receive the 10-min illuminances germinated 4% in darkness and 2% in continuous 1,200-ft-c illumination.

Interaction of light and alternating temperature

Promotive response. Two conditions are shown to be highly promotive to germination in most cases: daily 10-min illuminations at 4,000 ft-c for 5 or more consecutive days at 20°C and 15-25° daily alternations in total darkness. To observe possible interactions between these promotive treatments columns 3, 5, and 6 in Table 5 are to be compared. One lot of 'Newport' germinated almost twice as much at 15-25° when given repeated 10-min illuminances as when held in darkness at that temperature or when given repeated 10-min illuminances and germinated at 20°. Differences were not so great for the other seed lots because they showed rather high germination when either condition was applied alone.

Inhibitory response. Prolonged illumination at 20°C suppressed germination. Therefore the interaction of prolonged illumination and 15-25° was also measured (Table 5, Col 7).

Table 5. 14-day germination of 4 lots of Kentucky bluegrass at constant and alternating temperature in response to brief and prolonged illuminations of unfiltered fluorescent light.

Cultivar & Lot No.	Germination at indicated temperature °C after indicated light treatment					
	20 constant			15-25 alternation		
	0 min[a]	10min[b]	8 hr[c]	0 min[a]	10 min[d]	8 hr[e]
Newport		%			%	
16	0	41	1	50	86	24
50	8	80	6	82	84	58
Cougar						
13	10	86	52	78	88	82
51	10	88	46	74	92	83

[a]Dark controls.

[b]10 min at 4,000 ft-c each 24 hr for 5 successive days after 3 days dark imbibition.

[c]8-hr daily at 1,200 ft-c begun after 3 days dark imbibition.

[d]10 min at 4,000 ft-c each 24 hr at the 2nd hr of the 25° part of the cycle for 5 successive days after 3 days dark imbibition.

[e]8-hr daily at 1,200 ft-c begun on the 25°C part of the temperature cycle after 3 days dark imbibition.

Results are to be compared with the dark controls at 15-25° (Col 5) and prolonged illumination at 20° (Col 4). Both cultivars germinated higher at 15-25° than at 20° when illuminated 8 hr daily. However at 15-25°, seeds of 'Newport' germinated appreciably higher in darkness than when illuminated 8 hr daily.

Discussion

Seeds continually undergo physiological change and thus their condition never remains constant. Upon imbibition the net effect of the interacting environmental conditions is therefore either promotive or inhibitory to germination.

In darkness, germination of Kentucky bluegrass seeds is low at constant and relatively high at alternating temperatures. This corroborates previous findings (6). The high germination in darkness resulting from as much as 14 hr daily at 25° in a 15-25° daily alternation can be attributed to the temperature change. Only 5 to 6 daily cycles are required indicating that the effects of the temperature shifts are on early germination processes.

Conditions for maximum display of the promotive light reaction are brief daily illuminances and germination at 20°C. The advantages of the daily illuminances can be explained on the basis of keeping P active. An additive effect of brief daily illuminances and alternating temperature is sometimes observed in certain dormant lots that are not promoted to a high level by alternating temperature alone.

The reversible responses to brief irradiances of red and far-red identify the phytochrome reaction. The responses were energy-, and not time-dependent within the limits of time and intensity tested. In measuring reciprocity, care was used to avoid periods in which inhibitory effects of HER would be approached.

Although intensities of fluorescent illumination of 200 to 600 ft-c are known to convert sufficient P to the active form (P_{fr}) for pronounced responses in many biological systems (2,4), these seeds increased in germination with increased intensity up to 4,000 ft-c. This was unexpected but might be due to the added seed coverings present in such grasses.

Conditions for maximum display of the inhibitory effects of HER on non-light-promoted seeds are 15-25°C alternation with prolonged illuminances. HER is best displayed in potentially light-promoted seeds at 20° when a brief illuminance precedes prolonged illumination or when brief daily illuminances are given alternately or simultaneously with prolonged illuminances. In all cases inhibitory effects of the HER override promotive effects of an alternating temperature and of brief, daily illuminances. Inhibition from prolonged illumination depends on the intensity of the 700-750 nm component and is apparently independent of the red light intensity (6).

The opposing responses, displayed when an increase in germination resulted from brief illuminances and decreased germination from prolonged illuminances, were observed in both cultivars. However, the HER was exhibited to a greater degree in the 'Newport' lots than in the 'Cougar' lots which were observed to be less dormant.

Literature Cited

1. Borthwick, H. A., S. B. Hendricks, M. W. Parker, E. H. Toole, and Vivian K. Toole. 1952. A reversible photoreaction controlling seed germination. *Proc. Nat. Acad. Sci.* 38:662-666.
2. _____ , _____ , E. H. Tolle, and V. K. Toole. 1954. Action of light on lettuce seed germination. *Bot. Gaz.* 115:205-225. 115:205-225.
3. Caspary, ..Robert. 1861. Schriften Königlichen Physikalisch-Ökonomischen Ges. Königsberg, 1, (1860):66-91.
4. Cathey, H. M., and H. A. Borthwick. 1964. Significance of dark reversion of phytochrome in flowering *Chrysanthemum morifolium. Bot. Gaz.* 125:232-236.
5. Heinricher, E. 1903. Notwendigkeit des Lichtes and befördernde Wirkung desselben bei der Samenkeimung. *Botan. Centr. Beih.* 13:164-172.
6. Hendricks, S. B., V. K. Toole, and H. A. Borthwick. 1968. Opposing actions of light in seed germination of *Poa pratensis* and *Amaranthus arenicola. Plant Physiol.* 43:2023-2028.
7. Toole, E. H. 1923. Problems of germinating the various bluegrasses. *Seed World.* 14:23,30.