PREVENTION OF SKOTODORMANCY IN LACTUCA SATIVA VARIETY GRAND RAPIDS LETTUCE SEEDS

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PREVENTION OF SKOTODORMANCY IN *LACTUCA SATIVA* VARIETY GRAND RAPIDS LETTUCE SEEDS

An Abstract

Presented to

the Graduate Council of

Austin Peay State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

James Edward Conatser Jr.

August 1975

ABSTRACT

This study was undertaken to determine the following: (1) if red light, gibberellic acid, thiourea, or punching could overcome the effects of skotodormancy in Grand Rapids variety lettuce seeds, (2) if skotodormancy involves a decrease in permeability of the endosperm, and (3) if skotodormancy or any of the experimental treatments involves a change in net protein synthesis.

Red light, gibberellic acid, and thiourea were only effective after 4 or less days of dark storage. Six or more days of dark storage caused a rapid loss of responsiveness. The results show that punching did effectively overcome the effects of skotodormancy after all lengths of dark storage tested. Red light, gibberellic acid, and thiourea each effectively raised the level of dark germination of punched seeds. Vacuum-treatment of seeds to facilitate the entrance of chemicals through the endosperm had no effect on germination. Experiments involving leucine-^{14}C indicate that endosperm permeability actually increased during the onset of skotodormancy. The leucine-^{14}C soluble pool increased as the length of dark storage increased. Skotodormancy does not appear to be associated with decreased permeability of the endosperm to chemicals. Skotodormancy was not found to be

related to net protein synthesis. Red light and gibberellic acid had no effect on net protein synthesis. Thiourea increased protein synthesis on day 4 and caused a small decrease on day 6.

PREVENTION OF SKOTODORMANCY IN *LACTUCA SATIVA* VARIETY GRAND RAPIDS LETTUCE SEEDS

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August 1975

To the Graduate Council:

I am submitting herewith a Thesis written by James Edward Conatser Jr. entitled "Prevention of Skotodormancy in Lactuca sativa Variety Grand Rapids Lettuce Seeds." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Biology.

Benj. L. Stone
Major Professor

We have read this thesis and recommend its acceptance:

Davis L. Findley
Second Committee Member

Floyd M. Ford
Third Committee Member

Accepted for the Council:

Wayne E. Stamper
Dean of the Graduate School

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CHAPTER I

INTRODUCTION

Borthwick et al. (1952) found that germination of Grand Rapids variety lettuce seeds can be regulated by different wavelengths of light. Red light promotes germination and far red light inhibits germination. The red and far red effects are reversible. Khan et al. (1957) reported that gibberellic acid could replace red light in stimulating germination. The effect of gibberellic acid was not reversible by far red light. Mayer and Poljakoff-Mayber (1963) reported that thiourea could replace the red light requirement. Scheibe and Lang (1965) reported that removal of the endosperm allowed complete dark germination that was not affected by far red light. Ikuma and Thimann (1960) reported that punching increased dark germination. After extended dark storage, imbibed seeds lose their responsiveness to light treatment (Black and Richardson 1965). This phenomena is known as skotodormancy. Speer et al. (1974) reported that dark-stored imbibed seeds also lose their responsiveness to other germination promoters and suggested that decreased permeability of the endosperm might be the cause.

This study was undertaken to determine the following: (1) if red light, gibberellic acid, thiourea, or punching could overcome the effects of skotodormancy in Grand Rapids

variety lettuce seeds, (2) if skotodormancy involves a decrease in permeability of the endosperm, and (3) if skotodormancy or any of the experimental treatments involves a change in net protein synthesis.

CHAPTER II

LITERATURE REVIEW

Several varieties of seeds, such as Lactuca sativa variety Grand Rapids (Borthwick et al. 1952), Betula pubescens (Wareing and Black, 1957), Rumex obtusifolius (Staden and Wareing, 1972), Lepidium virginicum (Toole et al., 1955), Rheum rhaponticum and Agrostis alba (Boucher, 1956) and Daucus carota, Nicotina tobacum and Rumex crispus (Mayer and Poljakoff-Mayber, 1963), require light for germination. Others, such as Nemophila insignis (Wareing and Black, 1957) and Lamium amplexicaule (Hendricks et al., 1959) are inhibited from germination by light.

Borthwick et al. (1952) reported that Grand Rapids variety lettuce seeds are stimulated to germinate by red light in the spectral region of 590 to 680 nanometers (nm.). Far red light in the region of 700 to 800 nm. depressed germination. These two effects are reversible and the final effect on germination is dependent on which light treatment was given last. The far red inhibition requires 4 times as much energy as the red light promotion. This is why the net effect of white light is promotion of germination (Black, 1969).

The light absorbing pigment responsible for the red-far red effect is known as phytochrome (Butler et al., 1959). Phytochrome exists in two different forms, (P_r) and (P_{fr}), (Borthwick et al., 1952). The form of phytochrome which induces germination, P_{fr}, has an absorption maximum near 730 nm. The inactive form of phytochrome, P_r, has an absorption maximum near 660 nm. (Hendricks and Borthwick, 1967). Red light (660 nm.) converts the inactive P_r to the active P_{fr} and far red light (730 nm.) converts it back to P_r (Borthwick et al., 1952). Phytochrome has been extracted and purified by Butler et al. (1959). Hsiao et al. (1973) reported that the active form of phytochrome slowly reverts back to the inactive P_r form in the dark if the water content of the seed is too low for germination. They noted that this dark reversion does not occur in dry seeds. Ikuma and Thimann (1959) found that phytochrome was located in the hypocotyl end of the seed.

Evenari et al. (1957) reported that blue light in the region of 350 nm. to 590 nm. also caused an increase in the germination of Grand Rapids lettuce seeds. A narrow band within this region, from 410 nm. to 455 nm., depresses germination (Wareing and Black, 1958; Gwenn and Scheibe, 1972).

Dark storage causes a gradual loss of responsiveness to red light (Black and Richardson, 1965). This condition, known as skotodormancy, can be prevented with

chloramphenicol and actinomycin D (Black and Richardson 1967). Certain germination promoting chemicals given in conjunction with red light can overcome skotodormancy (Speer et al., 1974).

Bewley et al. (1968) reported that phytochrome (P_{fr}) was active within five minutes of irradiation with red light. Such a quick response lends support to Burdett's (1972a) suggestion that phytochrome exerts its control at the level of membrane permeability.

Hsiao and Vidaver (1971) reported that imbibed seeds irradiated with red or far red light could be dried and re-imbibed without changing the form of the phytochrome (P_r or P_{fr}). They concluded that phytochrome mediation of germination consisted of two phases: (1) induction, or activation of the phytochrome and (2) expression. Loercher (1974) reported that the phytochrome could be activated in seeds with a water content of 15%. Seeds will not germinate with a water content this low. Later imbibition causes the seeds to respond to the last light treatment. These results are in agreement with others (Hsiao and Vidaver, 1970, 1973; Berrie et al. 1974).

Kahn et al. (1957) reported that gibberellic acid could replace red light in promoting dark germination of Grand Rapids variety lettuce seeds. The gibberellic acid-induced germination is not reversed by far red light (Kahn, 1960b). Many workers have since confirmed the promotion of germination by gibberellic acid (Kahn, 1960b; Toole

and Cathey, 1961; and Jarvis et al., 1968). Germination is also induced by kinetin (Sankla and Sankla, 1968; Reynolds and Thompson 1971, 1973). Ethylene promotes germination (Burdett and Vidaver, 1971) and man be responsible for the expression of the red light effect (Burdett, 1972b, 1972c). Coumarin and abscisic acid depress germination (Berrie et al., 1968; Bewley and Fountain, 1972).

The effect of various inhibitors of protein and nucleic acid synthesis on germination has been the object of much research. Poljakoff-Mayber (1953) and Kefford et al. (1965) have reported that thiourea increases dark germination of Grand Rapids lettuce seeds. Black and Richardson (1965, 1967) reported that germination is promoted by chloramphenicol and actinomycin D. Germination may require actinomycin D-resistant RNA synthesis (Khan, 1967).

Bewley and Black (1972) reported that germination was inhibited by cyclohexamide. The dwarfing compounds chlormequat, B995, and phosphon D depress germination (Berrie and Robertson, 1973).

Certain physical alterations have been shown to alter dark germination of Grand Rapids lettuce seeds. Heating seeds to 97^{C} induces a dormancy which can be overcome by gibberellic acid and ethylene, (Burdett and Vidaver, 1971). Cold treatment (0^{o}C) enhances germination at 30^{o} (Roth-Bejerano, 1966). Reynolds (1973) reported that Grand Rapids lettuce seeds have a sharp upper temperature maximum and there is a great variability in germination of seeds incubated

at the transition zone. Kahn (1960a) reported that germination could be suppressed by placing the seeds in an osmoticum. Puncturing the seeds increased the germination of seeds in 0.18 M mannitol solution in darkness (Kahn 1960a). Ikuma and Thimann (1960) found that injection of gibberellic acid into the seeds with a fine needle lowered the concentration of gibberellic acid needed to induce dark germination. Speer (1973) reported that punching removes penetration barriers to the entrance of chemicals. Speer (1974) noted that punching increased the dark germination of seeds incubated in distilled water. Removal of the endosperm eliminates the light requirement (Scheibe and Lang 1965). The light requirement is restored if the naked embryos are placed in 0.3 M mannitol (Nabors and Lang, 1971a). Deuteron irradiation of the seed coat and endosperm also promoted dark germination. The endosperm and the osmoticum act as alternate external physical restraints (Nabors and Lang, 1971a). Irradiation with red light causes an increased turgor pressure to be generated in the seed to overcome these restraints (Nabors and Lang 1971b).

Seeds can overcome the endosperm in two ways: (1) the mechanical force of the growing embryo and (2) chemican weakening of the endosperm (Palvista and Haber 1970).

Fountain and Bewley (1973) found that protein synthesis occurs early in the imbibition phase of lettuce seeds. Black and Richardson (1967) reported that dormant seeds synthesize protein at the same rate as germinating seeds. Net

protein synthesis is not altered by either red light or gibberellic acid (Rai and Laloraya, 1965; Klein et al., 1971; Bewley and Black, 1972). The activity of several enzymes has been found to be altered even though no change in net protein synthesis can be detected (Fountain and Bewley 1973). There are several growth promoters that influence enzyme activity in lettuce seeds. Gibberellic acid promotes α-amylase activity (Galsky and Lippincott, 1969; and Amen 1968). Thiourea decreases catalase activity and increases peroxidase activity (Poljakoff-Mayber 1953). Red light increases the activity of pectinase, cellulase (Amen, 1968) and peroxidase (Poljakoff-Mayber 1953). Red light also causes polysome formation (Mitchell and Villars, 1972). Exposure of seeds in a 0.5 M mannitol solution to red light causes extensive degradation of protein in the radicle (Nabors et al., 1974). Poljakoff-Mayber et al. (1957) found that two acidic growth inhibitors present in dry seeds both disappear after imbibition. Shain and Mayer (1968) reported that the activity of a trypsin-like enzyme increased during germination. The activity of NADH-cytochrome C reductase activity increases rapidly early in germination (Eldan and Mayer, 1972). Haber and Tolbert (1959) and Poljakoff-Mayber (1955) also reported the tricarboxylic acid cycle to be functioning during the earliest phase of germination. Park and Chen (1974) found that digestion of the endosperm and transfer of the nutrients to the embryo occurs during germination.

The rate of respiration of dormant seeds is only 20% less than that of nondormant seeds (Chen et al., 1970). Respiration is stimulated by red light and depressed by far red light (Evenari et al., 1955; Leopold and Guernsey, 1954). The increase in the activity of enzymes associated with germination may be the result of activation of pre-existing enzymes (Eldan and Mayer 1972).

Mayer and Poljakoff-Mayber (1962) noted a correlation between nuclear RNA and germination, but thiourea promotion of germination did not directly interfere with RNA metabolism. Frankland et al. (1971) reported a higher rate of RNA synthesis in illuminated seeds. RNA inhibitors prevent light and gibberellic acid promoted germination (Khan 1967). Red light causes a rapid increase in extractable cytokins (Staden et al., 1972). Gibberellic acid and red light convert cytokinins to their nucleosides which promote germination (Staden 1973).

CHAPTER III

MATERIALS AND METHODS

Germination

Lactuca sativa, variety Grand Rapids, lettuce seeds purchased from Joseph Harris Company, Moreton Farm, Rochester, New York were used throughout this study. They were stored in a moisture-proof container at 4°C. Seeds were chosen at random and placed in 5 centimeter (cm.) petri dishes containing one layer of Matheson Type D filter paper. Initially approximately 40 seeds were used per dish. This was increased to 100 seeds per dish. They were soaked in 1.5 milliliters (ml.) of distilled water for one hour before being irradiated with 5 minutes of far red light to inhibit germination. The light source was a 150 watt reflector flood operated at 120 volts. It was attached to a ring stand 72 cm. above the seeds. An 8 cm. water screen was placed under the lamp to reduce the heat from the lamp. The seeds were irradiated in a light-proof box equipped with a Carolina Biological Supply (CBS) N. 750 far red filter between the seeds and the light source. The seeds were stored in light-proof petri dish canisters. The seeds were initially germinated at two different temperatures, 20° and 25°, to determine the optimum temperature conditions.

All subsequent treatments were carried out at 20°. The far red irradiated seeds were stored in the dark for 0, 2, 4, 6, 8, or 10 days to induce varying degrees of skotodormancy before experimental treatments. After the appropriate length of dark storage the seeds were treated with gibberellic acid, 0.5 millimolar (0.5mM), thiourea, 50mM, 5 minutes irradiation with red light, or distilled water. The treatments were applied by transferring the seeds tc 5 cm. dishes containing one layer of filter paper and 1.5 ml. of the appropriate solution. The seeds to be treated with red light were transferred to a dish containing distilled water. The seeds were irradiated in a light-proof box equipped with a CBS No. 650 red filter between the seeds and the light source. An 8 cm. solution of 1% copper sulfate was placed directly below the lamp. The seeds were allowed to germinate in the dark for 48 hours after treatment. Germination was determined by visible protrusion of the radicle tip past the seed coat as determined under a 10X microscope.

The punched seeds were pierced in the midsection through the cotyledons with a #0 insect pin to break the seeds' penetration barriers (Speer, 1973). The seeds were punched before imbibition.

Evacuated seeds were placed in a desicator attached to a vacuum pump immediately after treatment. Twenty pounds of vacuum pressure was applied for 3 minutes to facilitate the entrance of the chemicals through the penetration barriers.

All dishes were run in triplicate. All experiments were run in duplicate; the vacuum-treatment was run three times.

Appearance of Radioactive Leucine in the Soluble Precursor Pool and Incorporation into Protein

Gibberellic acid, thiourea, red light, and distilled water were applied to unpunched skotodormant seeds to determine the effect on protein synthesis. The seeds were tested after 96 hours and 144 hours of dark storage.

For each treatment 0.5 grams of seeds were soaked for one hour in a sterile 10 cm. petri dish containing one layer of Matheson Type D filter paper and 6 ml. of distilled water. The seeds were irradiated with far red light for 5 minutes and stored in the dark in light-proof petri dish canisters at 20°. After the appropriate length of dark storage, the seeds were transferred to a sterile 10 cm. petri dish containing one layer of Matheson Type D filter paper and the appropriate solution. All germinated seeds were removed. The seeds to be treated with red light were transferred to a dish containing distilled water and irradiated with red light for 5 minutes. All solutions contained 10^{-4} Molar (M) penicillin and streptomycin to inhibit bacterial growth. Immediately after application of the experimental treatments, two microcuries of ^{3}H-L-leucine or leucine-^{14}C was added to each dish. The seeds were incubated in the radioactive amino acid for the first twelve hours of germination.

The seeds were rinsed with distilled water. All germinated seeds were removed. The ungerminated seeds were ground in a chilled mortar and pestle containing 5.0 ml. of 0.01 M tris buffer pH 7.5 containing 0.2 mg. leucine per ml. The homogenate was centrifuged at 1640 Xg for 10 minutes to remove the cellular debris. The supernatant was poured into a polypropylene test tube. The debris was washed in 5 ml. of buffer and recentrifuged. The supernatant was added to the first supernatant and the volume was determined. Six 0.2 ml. aliquots were placed on one inch squares of Type A glass fiber filter paper and dried under a 250 watt reflector infra-red heat lamp operating at 120 volts. Three of the paper squares were used as a measure of soluble precursor pool and received no further treatment.

The incorporation of ^{3}H-leucine and leucine-^{14}C was assayed by a modification of the procedure of Mans and Novelli (1960). The three remaining papers were submerged in a beaker of ice cold 5% Trichloroacetic acid (TCA) and dept on ice for 15 minutes. The cold TCA was decanted and the filter papers were rinsed two times with cold 5% TCA containing leucine. The papers were covered with 5% TCA and heated to 90^{o} for 10 minutes. The TCA was decanted and cold 5% TCA was added and the beaker was placed on ice for 10 minutes before decanting. The papers were covered with 95% ethanol and warmed to 65^{o} for 3 minutes. The ethanol was decanted and the papers were covered with a mixture of

ethanol/ether/chloroform (2: 2: 1) and warmed to 57° for 3 minutes. This was decanted and the papers were washed with acetone and dried under the infra-red lamp. Both the treated and the untreated filter paper squares were placed in scintillation vials. Each vial contained 18 ml. of scintillation solution (4 grams of 2, 5-diphenyl-oxazolyl and 50 mg. of 1, 4-bis-2 (5-phenyloxazyl) benzene/liter of toluene). The radioactivity in the vials was counted in a Nuclear Chicago Unilux III liquid scintillation counter for 10 minutes.

A 0.2 ml. aliquot of the homogenate was dissolved in 1 ml. of 3 N sodium hydroxide and further diluted with 2 ml. of distilled water. The protein of each sample was determined by the method of Lowry et al. (1951).

CHAPTER IV

RESULTS

Determination of Optimum Temperature for Breaking Skotodormancy in Grand Rapids Lettuce Seeds Treated with Red Light, Gibberellic Acid, and Thiourea

Different lots of seeds have different optimum temperatures for germination (Reynolds, 1973; Khan, 1968). Seeds were tested at 20° and 25° to determine the optimum temperature of this particular lot. Experimental treatments were applied after 0, 2, 4, 6, 8, and 10 days dark storage. Germination was scored after a 48 hour dark germination period. As indicated in Tables I through IV, seeds grown at 20° yielded higher germination counts for all treatments including the water control. In all subsequent tests, seeds were incubated at 20° to avoid confusing the inhibiting effect of higher temperature with those of skotodormancy.

Effects of Red Light, Gibberellic Acid, and Thiourea on the Prevention of Skotodormancy in Unpunched Seeds

Red light treatment (figure 1) caused very high germination for day 0 but, thereafter, the percent germination fell rapidly. After 6 or more days of dark storage, germination was less than 7%.

TABLE I

DETERMINATION OF OPTIMUM TEMPERATURE FOR BREAKING SKOTODORMANCY IN GRAND RAPIDS LETTUCE SEEDS INCUBATED IN WATER

Incubation Temperature	Days of Dark Storage Percent Germination					
	0	2	4	6	8	10
20°	7.8	8.0	6.9	0.4	0.4	0.4
25°	8.6	2.1	2.0	0.5	0.4	1.4

Seeds were imbibed in distilled water for 1 hour before being irradiated with far red light for 5 minutes. After the appropriate length of dark storage all germinated seeds were removed and the seeds were allowed to germinate in water for 48 hours. Six replicates of approximately 40 seeds each were employed for each incubation temperature at each time interval.

TABLE II

DETERMINATION OF OPTIMUM TEMPERATURE FOR BREAKING SKOTODORMANCY IN GRAND RAPIDS LETTUCE SEEDS IRRADIATED WITH RED LIGHT

Incubation Temperature	Days of Dark Storage Percent Germination					
	0	2	4	6	8	10
20°	96.3	73.5	64.7	6.5	1.5	3.6
25°	54.8	42.6	7.1	2.0	0.0	1.2

The treatment was the same as described in Table I except that the seeds were irradiated with red light for five minutes at the beginning of the 48 hour germination period.

TABLE III

DETERMINATION OF OPTIMUM TEMPERATURE FOR BREAKING SKOTODORMANCY IN GRAND RAPIDS LETTUCE SEEDS INCUBATED IN GIBBERELLIC ACID

Incubation Temperature	Days of Dark Storage Percent Germination					
	0	2	4	6	8	10
20°	98.8	99.4	93.8	41.1	48.3	19.2
25°	97.9	94.2	82.3	27.0	37.2	47.3

The treatment was the same as described in Table I except that the seeds were incubated in 0.5 mM gibberellic acid during the 48 hour germination period.

TABLE IV

DETERMINATION OF OPTIMUM TEMPERATURE FOR BREAKING SKOTODORMANCY IN GRAND RAPIDS LETTUCE SEEDS INCUBATED IN THIOUREA

Incubation Temperature	Days of Dark Storage / Percent Germination					
	0	2	4	6	8	10
20°	55.1	72.6	77.7	30.0	25.2	7.0
25°	89.0	67.7	23.7	19.1	2.6	2.0

The treatment was the same as in Table I except that the seeds were incubated in 50 mM thiourea during the 48 hour germination period.

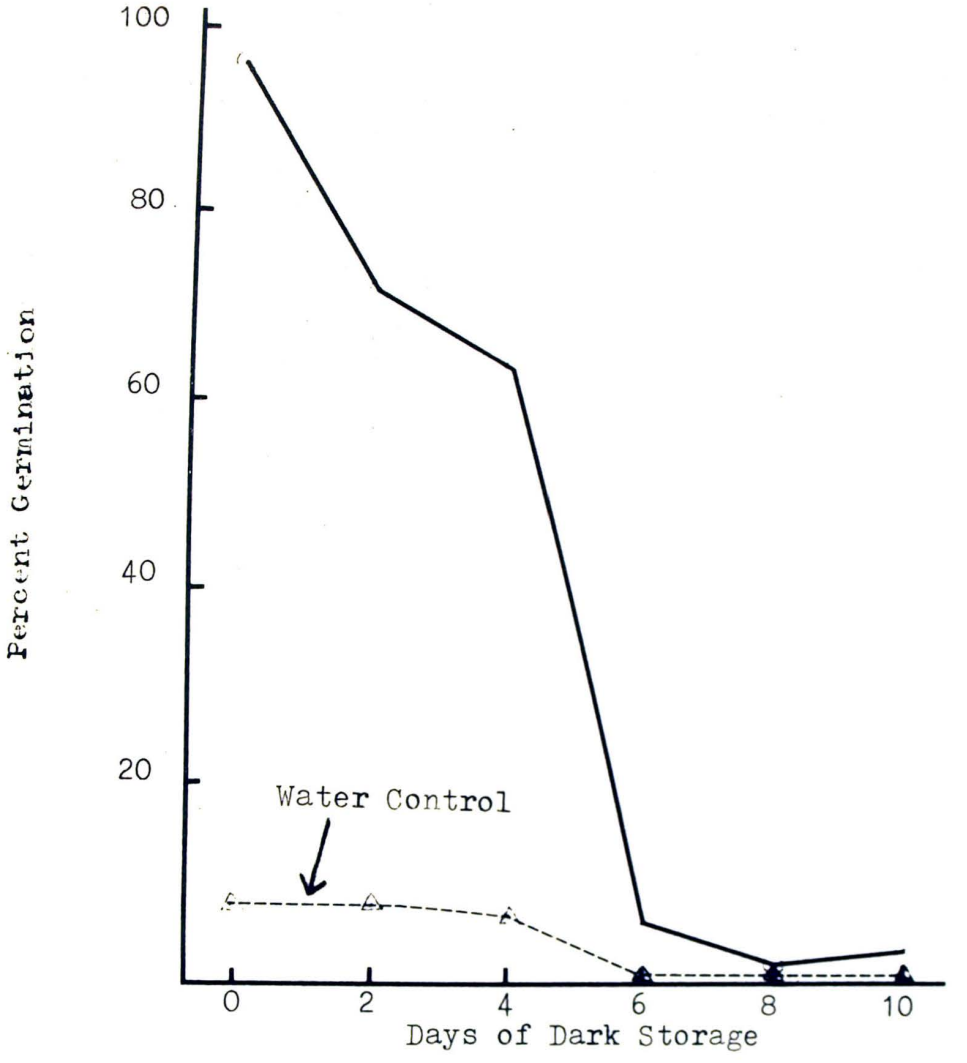

FIGURE 1. EFFECTS OF RED LIGHT ON SKOTODORMANCY IN GRAND RAPIDS LETTUCE SEEDS

Seeds were imbibed in distilled water for 1 hour before being irradiated with far red light for 5 minutes. After the appropriate length of dark storage all germinated seeds were removed and the seeds were irradiated with red light and allowed to germinate in water for 48 hours.

Seeds treated with gibberellic acid (figure 2) gave better than 90% germination for up to 4 days of dark storage. After 6 or more days of dark storage the percent germination fell rapidly. After 10 days, the percent germination was still higher than the water control.

Thiourea-treated seeds (figure 3) yielded 55% germination for 0 days of dark storage. It then rose to 77% after 4 days before falling to 7% after 10 days.

The water control germinated 7 to 8% for up to 4 days of dark storage. On the 6th day, the germination response was less than 1% and remained there through the 10th day.

The germination percentage decreased markedly for all treatments including the water control between the 4th and 6th day of dark storage.

The Effects of Red Light, Gibberellic Acid, and Thiourea on the Prevention of Skotodormancy of Punched Seeds

Speer (1973, 1974) reported that punching seeds with a #0 insect pin removes penetration barrier to the entrance of chemicals. Punching was tested to determine its effect on skotodormancy.

Germination of punched seeds irradiated with red light (figure 4) was not seriously inhibited by dark storage. Seeds thus treated exhibited a 90% germination even after 8 days dark storage. After 10 days in the dark, the seeds germinated at a level of 72%.

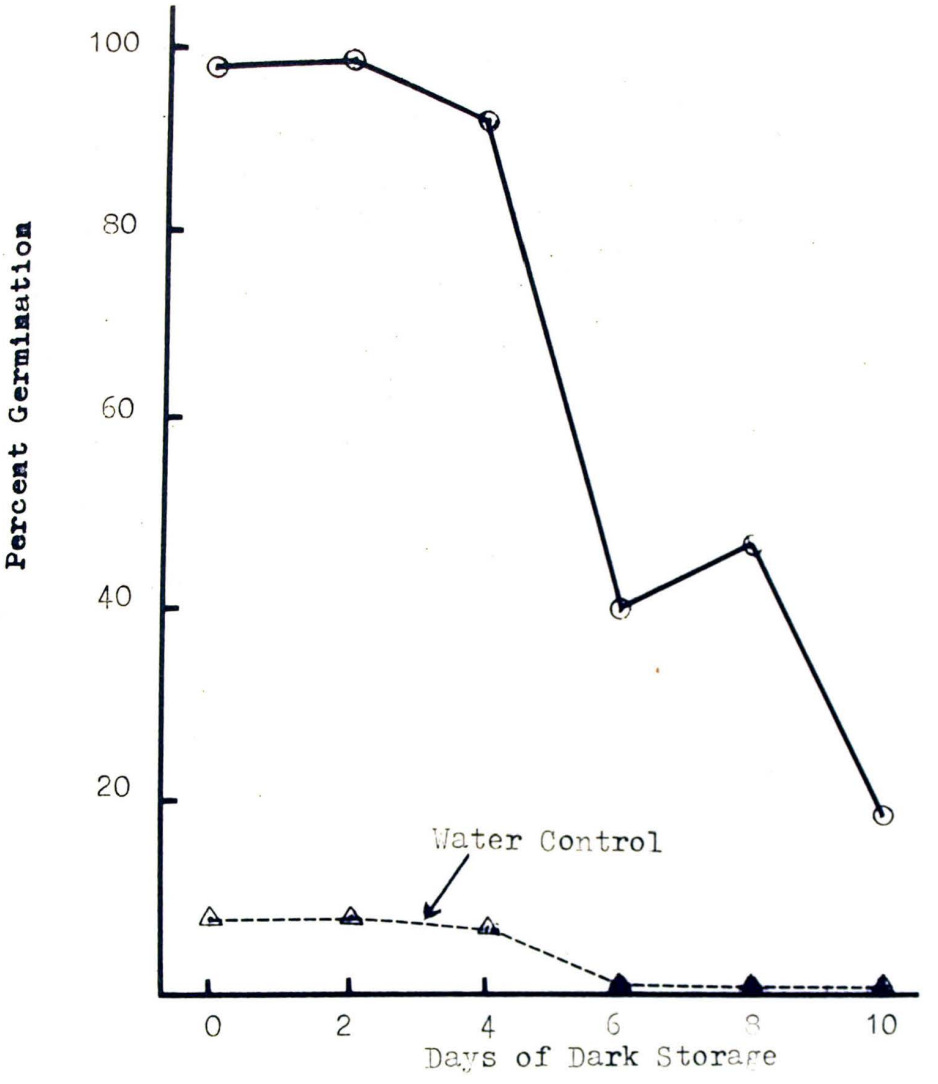

FIGURE 2. EFFECTS OF GIBBERELLIC ACID ON SKOTODORMANCY IN GRAND RAPIDS LETTUCE SEEDS

The treatment was as described in figure 1 except that the seeds were incubated in .5 mM gibberellic acid during the germination period rather than being irradiated with red light and incubated in water.

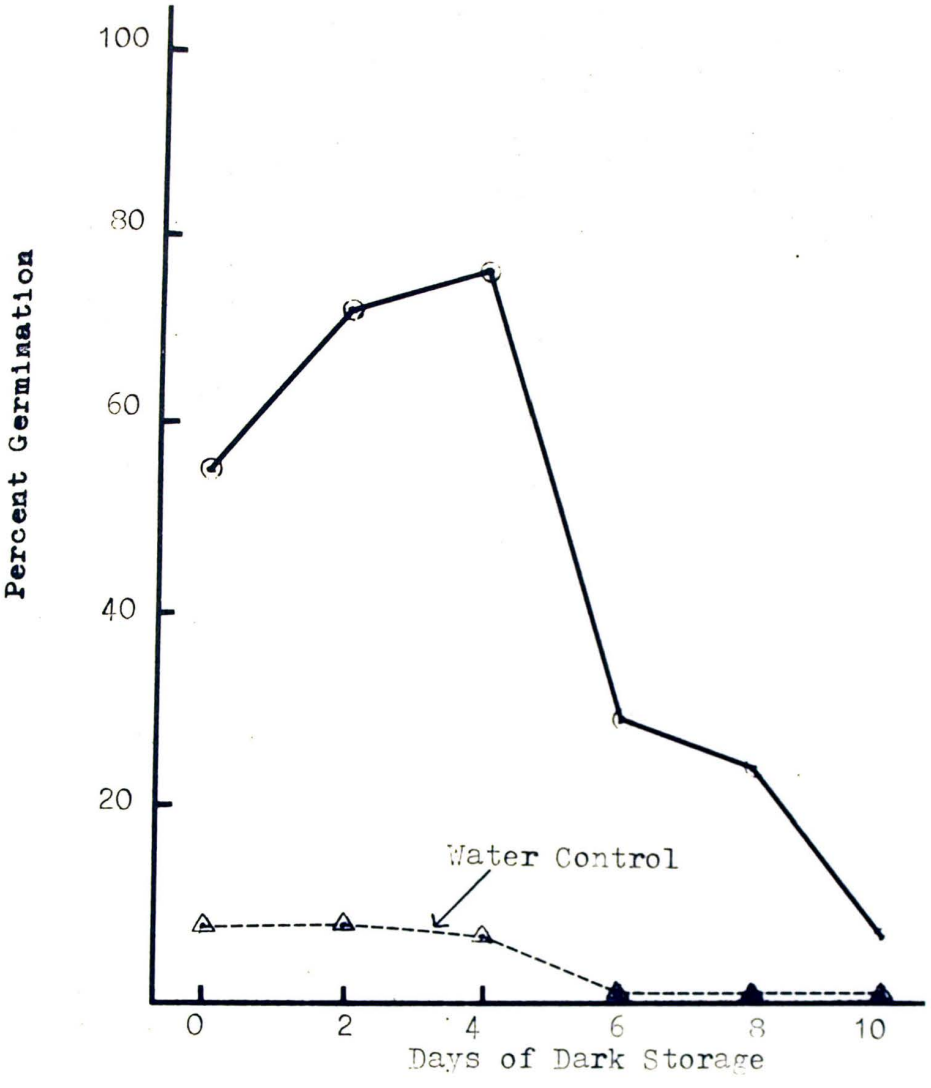

FIGURE 3. EFFECTS OF THIOUREA ON SKOTODORMANCY IN GRAND RAPIDS LETTUCE SEEDS

The treatment was as described in figure 1 except that the seeds were incubated in 50mM thiourea during the germination period rather than being irradiated with red light and incubated in water.

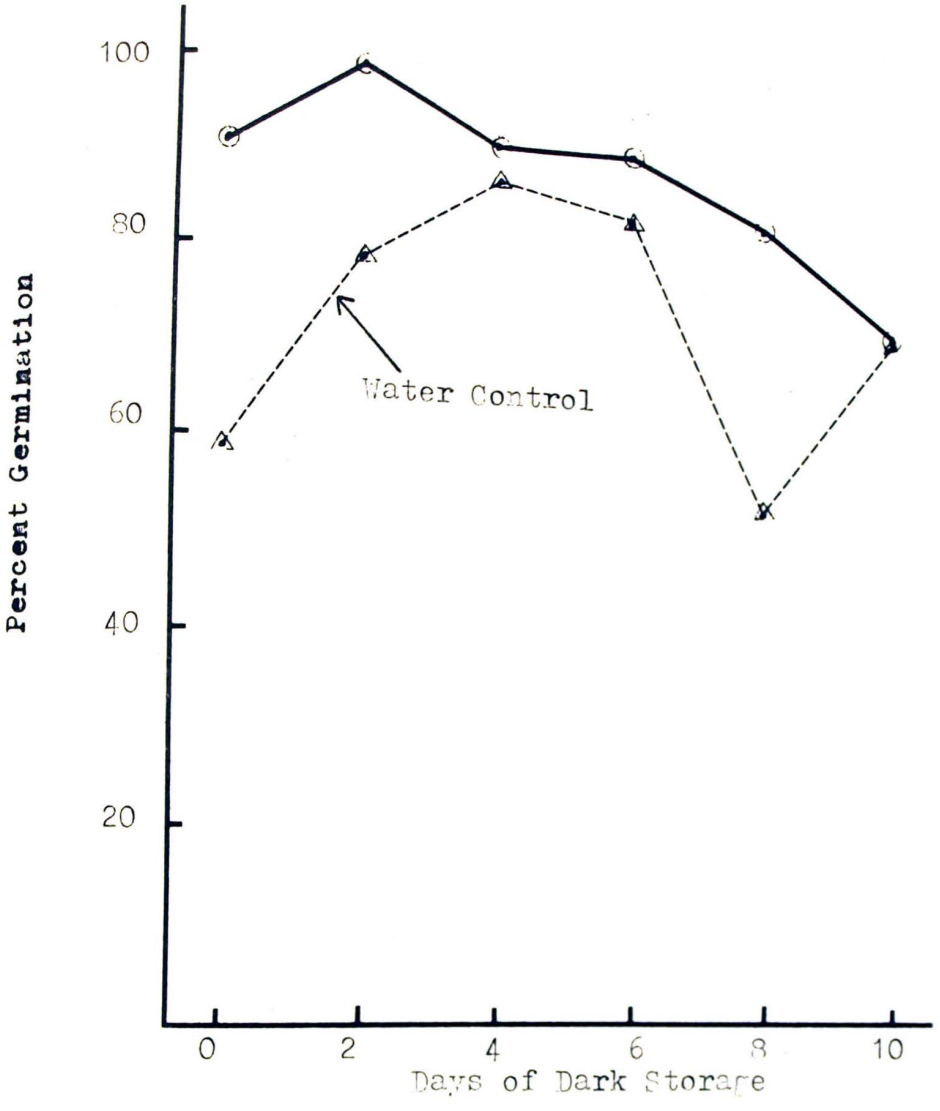

FIGURE 4. EFFECTS OF RED LIGHT ON SYNCODORMANCY IN PUNCHED GRAND RAPIDS LETTUCE SEEDS

Seeds were punched in the midsection with a #0 insect pin before imbibition and treated as described in figure 1.

Dark storage also did not have much effect on punched seeds treated with gibberellic acid (figure 5). Germination was above 90% after 6 days dark storage. After 10 days in the dark, the germination was still approximately 85%.

Punched seeds incubated in thiourea (figure 6) overcame the inhibitory effects of dark storage, but not as well as the other experimental treatments. Germination was above 90% after 4 days in the dark. Germination declined after 6 or more days to 55% on the 10th day.

In all experimental treatments, punching the seeds was very effective in negating the inhibitory effect of extended dark storage, especially after 6 or more days. The most obvious effect was on the punched water control (figure 4). Punched seeds incubated in water germinated at least 650% higher than unpunched water controls for every time period tested (figure 1 and 4). Punching very effectively prevented skotodormancy for at least 10 days.

The effects of punching and of the various growth promoters seem to be additive. Punching alone increased dark germination as did each of the experimental treatment. Treatment of the punched seeds with one of the growth promoters resulted in higher germination than either punching alone or any of the growth promoters alone, especially after 6 or more days.

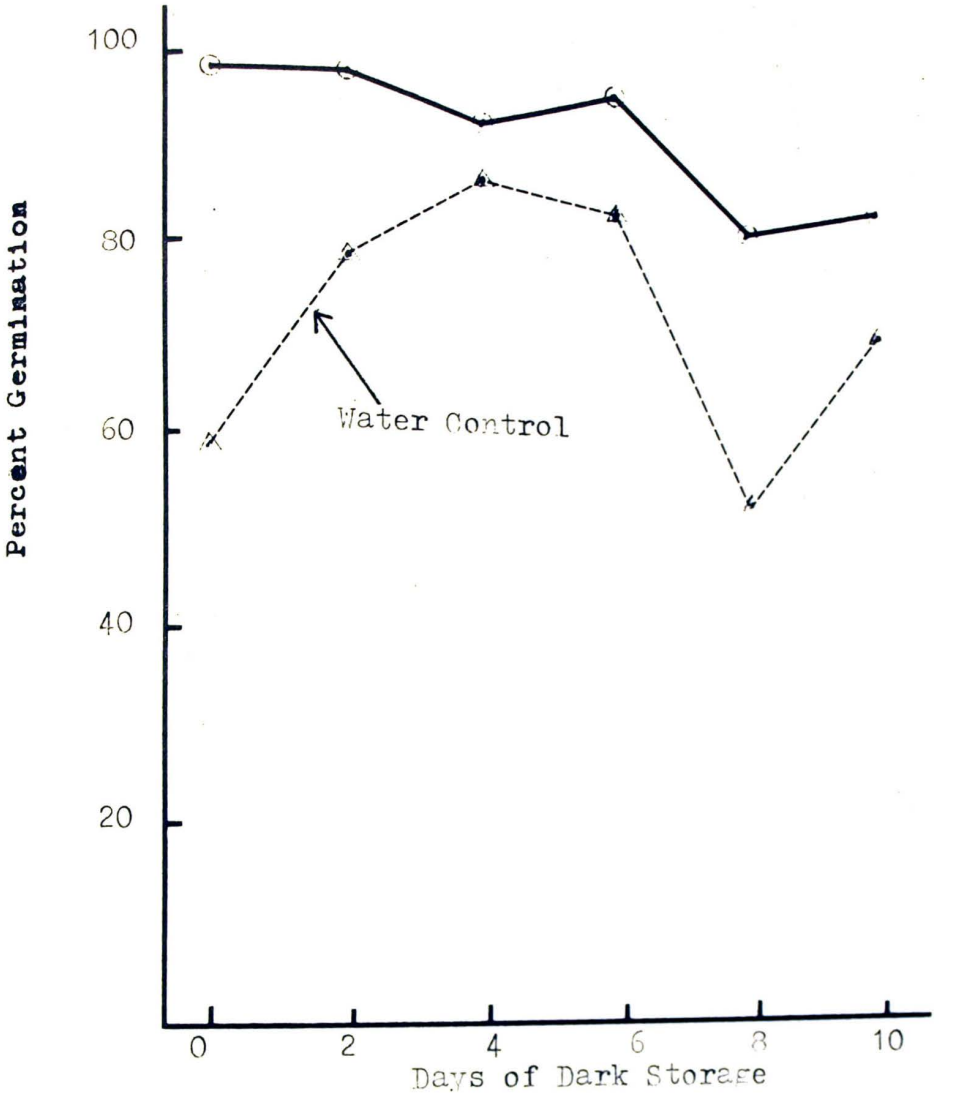

FIGURE 5. EFFECTS OF GIBBERELLIC ACID ON SKOTODORMANCY IN PUNCHED GRAND RAPIDS LETTUCE SEEDS

The seeds were punched in the midsection with a #0 insect pin before imbibition. The remaining treatment was as described in figure 1 except that the seeds were incubated in .4 mM gibberellic acid during the germination period rather than being irradiated with red light and incubated in water.

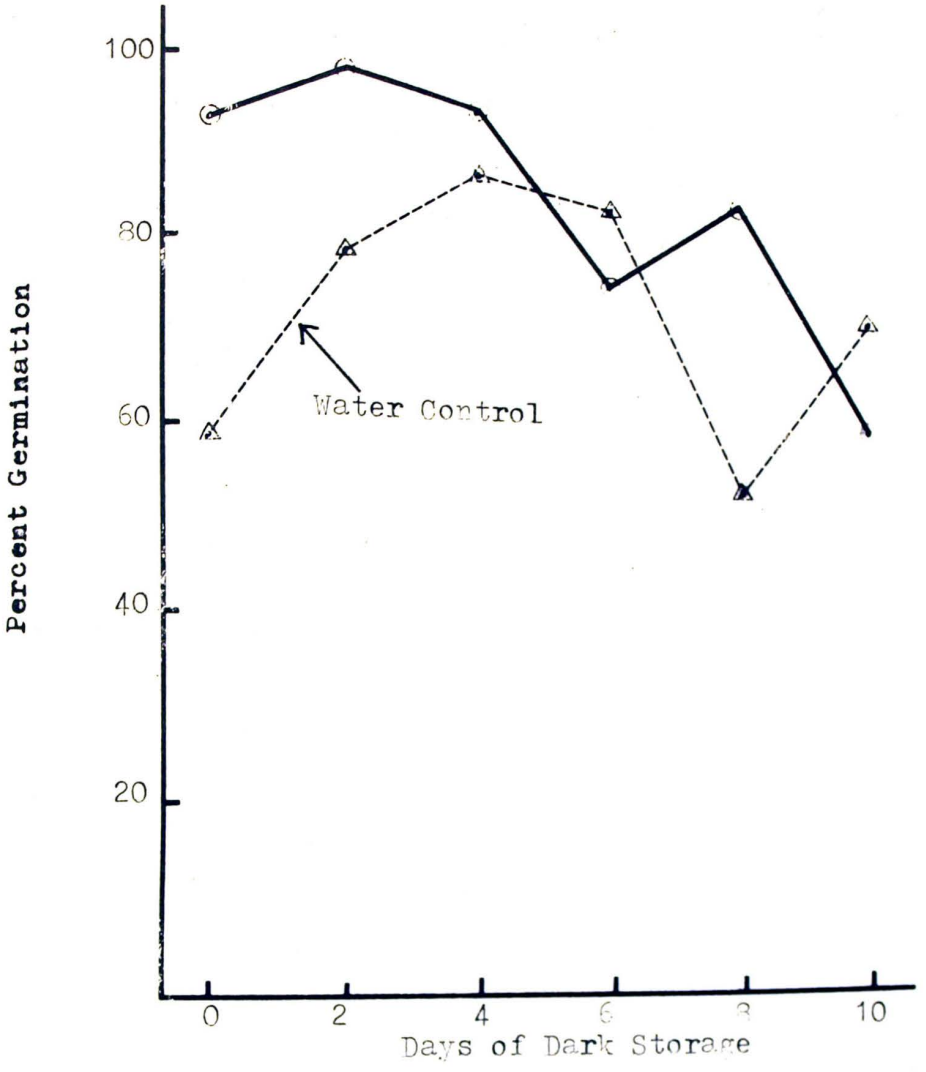

FIGURE 6. EFFECTS OF THIOUREA ON SKOTODORMANCY IN PUNCTURED GRAND RAPIDS LETTUCE SEEDS

The seeds were punched in the midsection with a #0 insect pin before imbibition. The remaining treatment was as described in figure 1 except that the seeds were incubated in 50 mM thiourea during the germination period rather than being irradiated with red light and incubated in water.

It should be noted that many of the punched seeds germinated during the dark storage periods before the experimental treatments could be applied. These germinated seeds were removed at the end of each time interval before the application of experimental treatment and subsequent 48 hour germination period. As a result, the number of seeds scored for germination for the longer time intervals was considerably less than for the shorter ones. Because of the smaller numbers involved, the germination percentages obtained from the latter portion of the experiment are not as reliable as those from the first part.

The Effects of Red Light, Gibberellic Acid, and Thiourea on the Prevention of Skotodormancy in Vacuum-treated Seeds

Since punching seeds effectively prevented skotodormancy, vacuum-treatment of seeds was tested. If the beneficial effect of punching was the breaking of penetration barrier to the entrance of the chemicals (Speer 1973, 1974), vacuum-treatment should also increase germination. At each time interval a 20 lb. vacuum was applied to the seeds for three minutes after the appropriate experimental treatments had been administered.

Vacuum-treatment of seeds irradiated with red light (figure 7) did not produce the same effects as punching. The germination response more closely resembled that of the

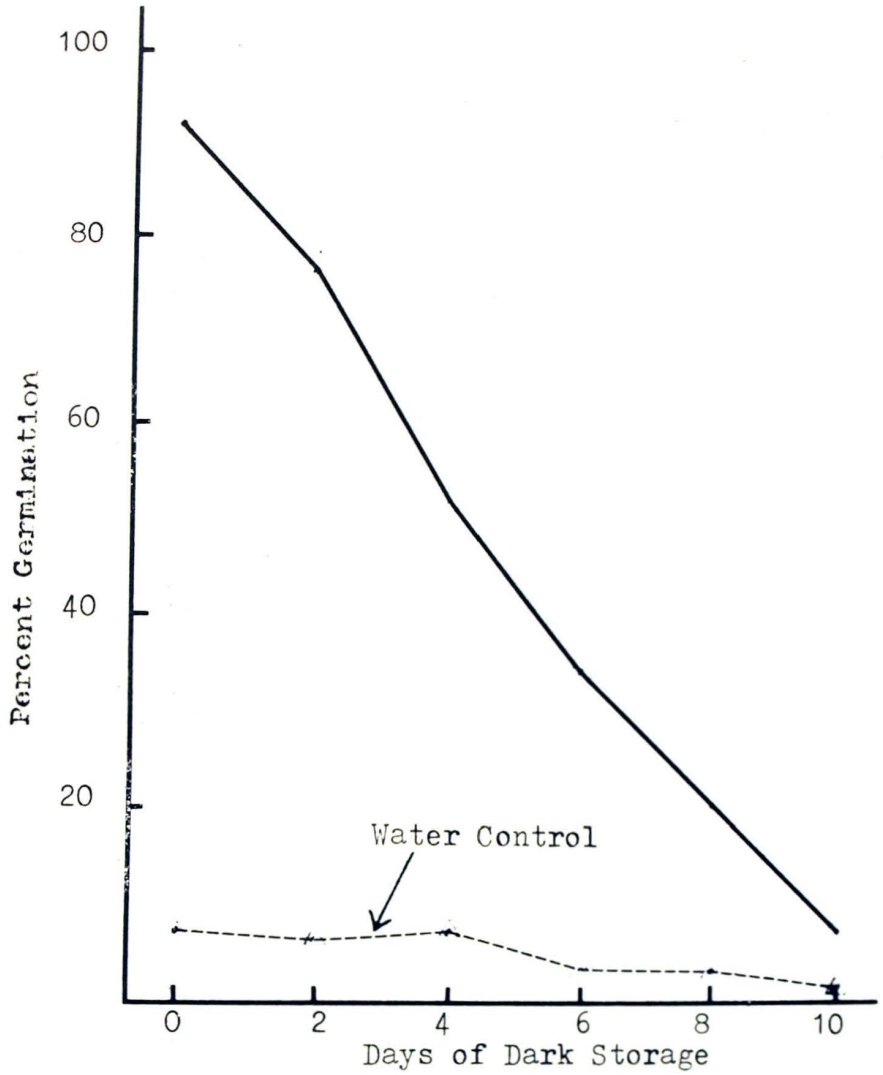

FIGURE 7. EFFECTS OF VACUUM-TREATMENT ON SKOTODORMANCY IN RED LIGHT-IRRADIATED GRAND RAPIDS LETTUCE SEEDS

Treatment was as described in figure 1 except red light irradiation was followed by 20 lbs. vacuum-treatment for 3 minutes.

unpunched seeds irradiated with red light. Germination decreased linearly from 92% on day 0 to 7% on day 10. The germination for days 6 and 8 was somewhat higher for the vacuum-treated seeds than for the unpunched. The observed difference may be associated with the smaller number of unpunched seeds scored. The average number of seeds scored at each time interval for each experimental treatment was 750 for vacuum-treated seeds as compared to 250 for the unpunched seeds.

Evacuated gibberellic acid-treated seeds (figure 8) gave a germination response very similar to that of the unpunched seeds. The observed variations were attributed to the smaller population of unpunched seeds.

Evacuated thiourea-treated seeds (figure 9) were able to overcome longer periods of dark storage than the unpunched thiourea-treated seeds (figure 3). Germination increased slightly from 45% on day 0 to 57% on day 8. Germination was sharply depressed on day 10. Only 13% of the seeds germinated.

The germination response for the evacuated water control was almost identical to the unpunched.

Vacuum-treatment did not duplicate effects of punching. Except with thiourea, it has very little effect on germination. This was most obvious in the water control where more than 50% of the punched seeds germinated every day tested, and the unpunched and evacuated never germinated higher than 8%.

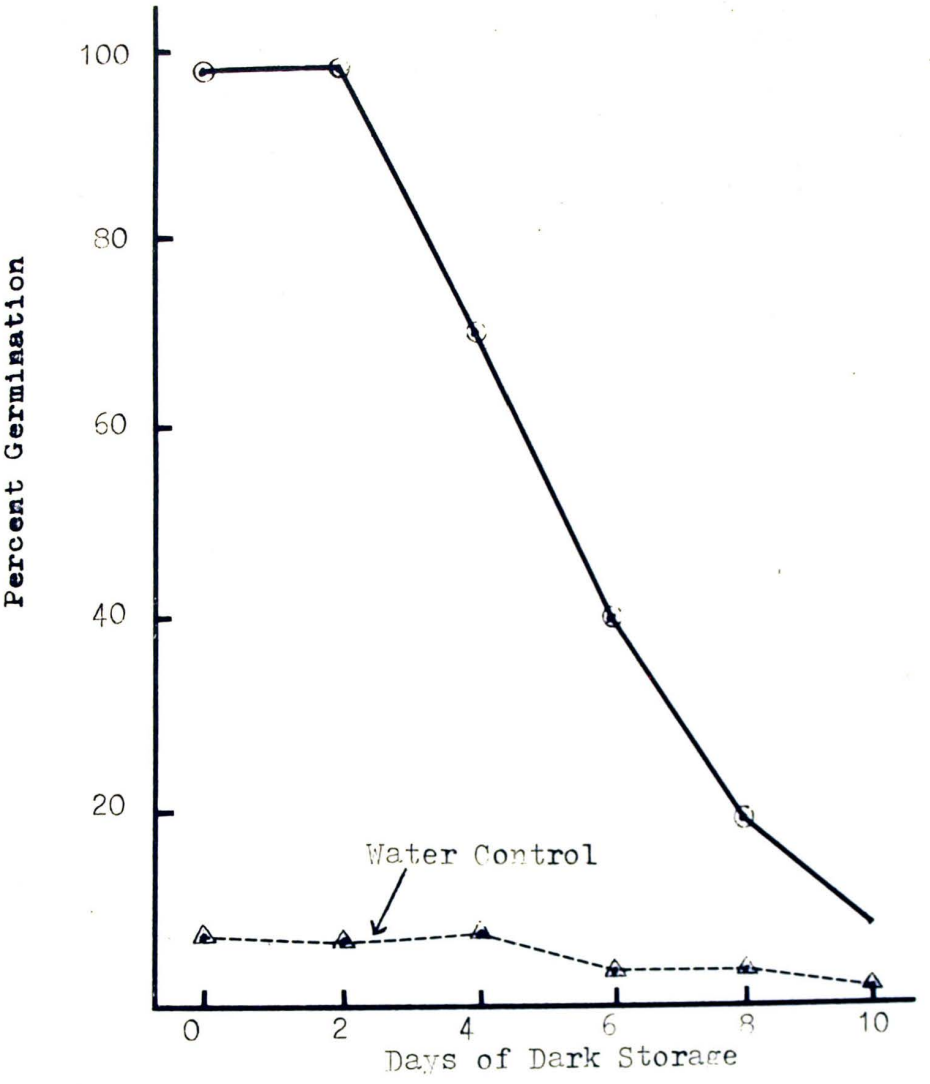

FIGURE 3. EFFECTS OF VACUUM-TREATMENT ON SKOTODORMANCY IN GIBBERELLIC ACID-TREATED GRAND RAPIDS LETTUCE SEEDS

The treatment was as described in figure 1 except that the seeds were incubated in .5 ml gibberellic acid during the germination period rather than being irradiated with red light and incubated in water. A 20 lb. vacuum was applied for 3 minutes at the beginning of the germination period.

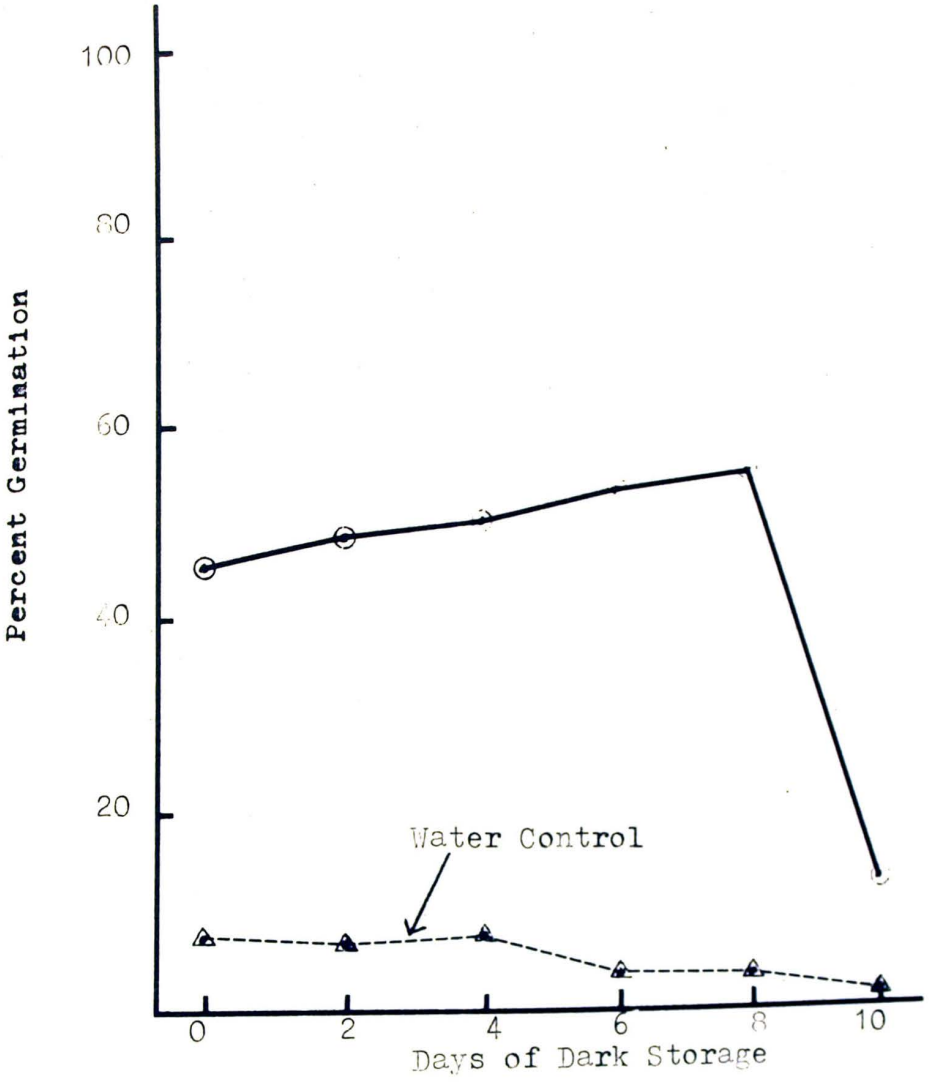

FIGURE 9. EFFECTS OF VACUUM-TREATMENT ON SKOTODORMANCY IN THIOUREA-TREATED GRAND RAPIDS LETTUCE SEEDS

The treatment was as described in figure 1 except that the seeds were incubated in 50 mM thiourea during the germination period rather than being irradiated with red light and incubated in water . A 20 lb. vacuum was applied for 3 minutes at the beginning of the germination period.

Effect of Red Light, Gibberellic Acid, and Thiourea on Leucine-^{14}C Permeability and Protein Synthesis in Skotodormant Seeds

The onset of skotodormancy in unpunched seeds was most dramatic between day 4 and day 6 of dark storage. These two time intervals were selected to determine whether protein synthesis was involved in inhibiting germination. Unpunched seeds were treated with red light, gibberellic acid, thiourea or water on day 4 and day 6. ^{3}H-Leucine or leucine-^{14}C was added and the seeds were allowed to incubate for 12 hours before extraction.

Experiments involving ^{3}H-leucine showed no significant incorporation into protein. The counts per minute for all experimental treatments were very near background counts.

Incorporation into protein was detectable using leucine-^{14}C. Table V illustrates the effects of the experimental treatments given on day 4 and day 6 of dark storage on subsequent permeability of the seeds to leucine-^{14}C. Red light irradiation on day 4 and day 6 had little effect on the permeability to leucine-^{14}C. This is consistent with the findings of Klein *et al.* (1971). Seeds treated with gibberellic acid and thiourea on day 4 contained slightly higher amounts of leucine-^{14}C. Thiourea caused a small reduction in uptake of label on day 6 and gibberellic acid had no effect on day 6. The 6 day water control contained more leucine-^{14}C than the 4 day water control.

TABLE V

PERMEABILITY TO LEUCINE-^{14}C OF GRAND RAPIDS LETTUCE SEEDS TREATED WITH RED LIGHT, GIBBERELLIC ACID, AND THIOUREA AFTER 4 OR 6 DAYS DARK STORAGE

Treatment		Soluble Leucine Pool Percent of Water Control
Days Dark Storage	12 Hour Dark Germination	
4	H_2O	100*
4	Red Light	95
4	GA	125
4	Thiourea	127
6	H_2O	100**
6	Red Light	89
6	GA	90
6	Thiourea	81

* 95,222 cpm of soluble leucine-^{14}C per 0.5 gram seeds in 4 day water control

** 134,746 cpm of soluble leucine-^{14}C per 0.5 gram seeds in 6 day water control

0.5 gram of seeds was employed for the indicated time for each treatment. Dark storage was preceded by irradiation with far red light for 5 minutes.

Bewley and Black (1972) found that gibberellic acid did not affect net protein synthesis in dark germinated lettuce seeds. Table VI illustrates that gibberellic acid also does not affect net protein synthesis in seeds stored in extended darkness. Klein (1971) reported that red light did not affect the incorporation of leucine-^{14}C into protein in dark germinated seeds. Table VI illustrates that this is also true after extended dark storage. Treatment with thiourea causes a 50% increase in the incorporation of leucine-^{14}C into protein on day 4 and a small decrease on day 6.

The decrease in germination between the 4th and 6th day of dark storage was apparently not due to net protein synthesis. There is no significant difference between incorporation of leucine-^{14}C into protein on day 4 and day 6 of dark storage (Table VI) for any of the treatments except thiourea.

TABLE VI

INCORPORATION OF LEUCINE-^{14}C INTO PROTEIN IN GRAND RAPIDS LETTUCE SEEDS TREATED WITH RED LIGHT, GIBBERELLIC ACID, OR THIOUREA AFTER 4 OR 6 DAYS DARK STORAGE

Treatment		
Days of Dark Storage	12 Hours Dark Germination	% Water Control
4	H_2O	100*
4	Red Light	105
4	GA	116
4	Thiourea	151
6	H_2O	100**
6	Red Light	97
6	GA	106
6	Thiourea	77

* 402 cpm of leucine-^{14}C per mg. protein in 4 day water control

** 539 cpm of leucine-^{14}C per mg. protein in 6 day water control

0.5 gram of seeds was employed for the indicated time for each treatment. Dark storage was preceded by irradiation with far red light for 5 minutes.

CHAPTER V

DISCUSSION OF RESULTS

Many workers have noted that Grand Rapids lettuce seeds irradiated with far red light and stored in the dark progressively lose their responsiveness to germination promoters (Black and Richardson, 1965, 1967; Speer et al., 1974a; Vidaver and Hsiao, 1974). This condition is known as skotodormancy.

Gibberellic acid, red light, and thiourea were able to overcome the inhibitory effects of skotodormancy after as much as 4 days dark storage (figures 1, 2, and 3). More than 4 days dark storage caused a rapid loss of responsiveness to the experimental treatments. The water control also exhibited an additional depression in the already low germination rate between the 4th and 6th day of dark storage. These depressions in germination ability were interpreted as the onset of skotodormancy.

Speer et al. (1974) reported that red light given in conjunction with either gibberellic acid or thiourea could overcome even 10 days dark storage of fully imbibed Grand Rapids lettuce seeds. He suggested that the loss of effectiveness of gibberellic acid and thiourea given alone

after extended dark storage might be due to a change in the permeability of the seed coats. Speer (1973) reported that punching seeds with a #0 insect pin removes penetration barriers to the entrance of chemicals. He also reported that punching reduced the far red light inhibition of germination. Punching was tested as a means of preventing skotodormancy.

Punching the seeds greatly increased the germination rate. Punched seeds incubated in only distilled water germinated better than 50% after every period of dark storage tested. Red light, gibberellic acid, and thiourea increased the germination rate of punched seeds. The reduction of germination observed between the 4th and 6th day with intact seeds was prevented by punching.

The fact that the punched water control seeds had germinated so well indicated that the effect of punching on skotodormancy was not due primarily to the removal of penetration barriers.

In an effort to determine how punching elicited its response, vacuum-treatment was tested as an alternate method of overcoming penetration barriers. A twenty pound vacuum was applied after experimental treatment to facilitate the entrance of chemicals. If skotodormancy involved a decreasing permeability of the endosperm, vacuum-treatment should allow an increased response to chemical treatments after extended dark storage. However, vacuum-treatment did not duplicate the effects of punching (figures 7, 8, and 9). It did

cause thiourea to be effective after longer periods of dark storage (figure 9). The results of the other experimental treatments were very similar to those obtained with intact seeds. The most convincing data, though, was provided by the water controls. Germination of the punched water controls was at least 650% higher than the water control for either the intact or the vacuum-treated seeds at every time interval tested.

These results indicate that skotodormancy is not the result of a decrease in endosperm permeability. The increased germination brought about by punching may have been due to the damage to the mechanical restriction of the endosperm. Kahn (1960a) reported that removal of the endosperm allowed full germination even after far red light. Germination could be completely inhibited by placing the naked embryos in a mannitol solution with a high osmotic pressure. He found that the germination rate could be manipulated by changing the molarity of the solution the embryos were incubated in. The osmotic pressure substituted for the mechanical restraint of the endosperm. Nabors and Lang (1971a) reported that the force necessary for the radicle to break through the seed coats was equal to the osmotic pressure of 0.16 to 0.38 M mannitol. Red light could overcome the osmotic restraint and induce germination. Scheibe and Lang (1965) reported that gibberellic acid could also overcome the osmotic restraint. Palvista and Habor (1970)

reported that partial destruction of the endosperm increased germination. Klein and Preiss (1958) reported that deuteron irradiation of the seed coat and endosperm also promoted dark germination. It is clear that removing or damaging the endosperm can increase the seeds' germination ability. The increased germination caused by punching was probably due to damaging the physical restraints to growth rather than increasing the endosperm's permeability to chemicals.

Experiments involving leucine-^{14}C further demonstrated that permeability does not decrease during skotodormancy. The effect of skotodormancy in unpunched seeds was most dramatic between the 4th and 6th day of dark storage. These two time intervals were selected for experiments involving leucine-^{14}C. After 4 or 6 days of dark storage, seeds were treated with either red light, gibberellic acid, thiourea, or water and allowed to incubate in leucine-^{14}C for 12 hours. Table V indicates that the leucine-^{14}C pools for all experimental treatments were higher for day 6 than for day 4. Permeability was somewhat increased rather than decreased by extended dark storage.

Net protein synthesis (Table VI) does not appear to be affected by skotodormancy. The slight increase in leucine-^{14}C incorporated into protein in the seeds stored for 6 days can be attributed to the higher leucine-^{14}C soluble pool. Red light and gibberellic acid did not appreciably alter net protein synthesis. This is in agreement with Klein *et al.* (1971) and Bewley and Black (1972).

Thiourea caused an increase in protein synthesis on day 4 and a small decrease on day 6.

Even though extended dark storage does not appear to involve a change in net protein synthesis, it may involve synthesis of specific enzymes which this procedure is not sensitive enough to detect. Changes in the activity of several enzymes have been reported to occur during dark germination although no change in net protein synthesis was discernible (Eldan and Mayer, 1972; Amen, 1968; Poljakoff-Mayber, 1958; Poljakoff-Mayber, 1953). Nabors et al. (1974) demonstrated that red light-promoted germination involved chemical activity. Combining the mechanical restriction of the endosperm with the osmotic force of 0.5 M mannitol solution allowed the effects of red light to build up. The result was extensive degradation of protein in the cortical cells of the radicle. Park and Chen (1974) reported that digestion of the embryo and transfer of nutrients from the endosperm to the embryo occurred during germination. Although net protein synthesis does not appear to be altered before visible germination, it is apparent that enzymatic activity is associated with germination ability.

Shain and Mayer (1965 and 1968) suggested that increased enzyme activity may not be the result of de novo synthesis. The enzymes may be pre-existing and need only be activated to induce germination or dormancy.

That skotodormancy did not appreciably alter net protein synthesis does not rule out the possibility that skotodormancy is under enzymatic control. Polyacrylamide gel electrophoretic separation of lettuce seed proteins extracted after different lengths of dark storage could probably reveal more the relationship of enzymes to germination ability.

CHAPTER VI

SUMMARY

The objective of this study was to determine the following: (1) if red light, gibberellic acid, thiourea, or punching could overcome the effects of skotodormancy in Grand Rapids variety lettuce seeds, (2) if skotodormancy involves a decrease in the permeability of the endosperm, and (3) if skotodormancy or any of the experimental treatments involved a change in net protein synthesis.

Red light, gibberellic acid, and thiourea overcame skotodormancy after as much as 4 days of dark storage. After 6 or more days of dark storage these treatments rapidly lost their effect. Punching effectively overcame skotodormancy after every length of dark storage tested. Red light, gibberellic acid, and thiourea each increased the level of germination when applied to punched seeds.

The onset of skotodormancy is not accompanied by a decrease in endosperm permeability. Vacuum infiltration of the seeds with the experimental treatments had no effect on germination. Experiments involving leucine-^{14}C indicated that endosperm permeability actually increased during skotodormancy.

Skotodormancy was not found to be related to net protein synthesis. Red light and gibberellic acid also had no effect on net protein synthesis. Thiourea increased protein synthesis on day 4 and caused a small decrease on day 6.

LITERATURE CITED

Amen, Ralph D. 1968. A model of seed dormancy. Bot. Review. 34: 1-31.

Berrie, A. M. M., W. Parker, B. A. Knights, and M. R. Hendrie. 1968. Studies on lettuce seed germination. I. Coumarin induced dormancy. Phytochemistry. 7: 567-573.

_____, J. Patterson and H. R. West. 1974. Water content and the responsivity of lettuce seeds to light. Physiol. Plant. 31: 90-96.

_____, and James Robertson. 1973. Growth retardants and the germination of light sensitive lettuce seeds. Physiol. Plant. 28: 278-783.

Bewley, J. D. and M. Black. 1972. Protein synthesis during gibberellin-induced germination of lettuce seeds. Can. J. Bot. 50: 53-59.

_____, and David W. Fountain. 1972. A distinction between the actions of abscisic acid, gibberellic acid and cytokinins in light-sensitive lettuce seed. Planta. 102: 368-371.

_____, M. Negbi, and M. Black. 1968. Immediate phytochrome action in lettuce seeds and its interaction with gibberellins and other germination promoters. Planta. 78: 351-357.

Black, M. 1969. Light-controlled germination of seeds, p. 193-197. In dormancy and survival. Cambridge, England.

_____, and M. Richardson. 1965. Promotion of germination in light-requiring seed by chloramphenicol. Nature. 208: 1114-1115.

_____. 1967. Germination of lettuce induced by inhibitors of protein synthesis. Planta. 73: 344-356.

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.

Boucher, R. V. 1956. Effects of light on various processes: plants, p. 459. In William Spector ed., handbook of biological data. Philadelphia, Pennsylvania.

Burdett, A. N. 1972a. Two effects of prolonged far red light on the response of lettuce seeds to exogenous gibberellin. Plant Physiol. 69: 531-534.

_____. 1972b. Antagonistic effects of high and low temperature pretreatments on the germination and pregermination of ethylene synthesis of lettuce seeds. Plant Physiol. 50: 201-204.

_____. 1972c. Ethylene synthesis in lettuce seeds: Its physiological significance. Plant Physiol. 50: 719-722.

_____, and W. Vidaver, 1971. Synergistic action of ethylene with gibberellin or red light in germinating lettuce seeds. Plant Physiol. 48: 656-657.

Butler, W. L., K. H. Norris, H. W. Siegelman, and S. B. Hendricks. 1959. Detection, assay, and preliminary purification of the pigment controlling photoresponsive development of plants. Proc. Nat. Acad. Sci. 45: 1703-1708.

Chen, Shepley, S. C. and J. E. Varner. 1970. Respiration and protein synthesis in dormant and after-ripened seeds of Avena fatua (wild oat). Plant Physiol. 46: 108-112.

Eldan, M. and A. M. Mayer. 1972. Evidence for the activation of NADH-cytochrome C reductase during germination of lettuce. Physiol. Plant. 26: 67-72.

Evenari, M., G. Neumann, and S. Klein. 1955. The influence of red and infra-red light on the respiration of photoblastic seeds. Physiol. Plant. 8: 33-47.

_____, _____, and G. Stein. 1957. Action of blue light on the germination of seeds. Nature, London. 180: 609-610.

Fountain, D. W., and J. D. Bewley. 1973. Polyribosome formation and protein synthesis in imbibed but dormant lettuce seeds. Plant Physiol. 52: 604-607.

Frankland, B., B. C. Jarvis, and J. H. Cherry. 1971. RNA synthesis and the germination of light-sensitive lettuce seeds. Planta. 97: 39-49.

Galsky, A. G., and J. A. Lippincott. 1969. Promotion and inhibition of α-amylase production in barley endosperm by cyclic 3',5' adenosine monophosphate and adenosine diphosphate. Plant Cell Physiol. 10: 607-620.

Gwynn, D. and Joseph Scheibe. 1972. An action spectrum in the blue for inhibition of germination of lettuce seed. Planta. 106: 247-257.

Haber, A. H., and N. E. Tolbert. 1959. Metabolism of C-14-Bicarbonate p^{32}, or s^{35}-sulfate by lettuce seed during germination. Plant Physiology. 34: 376-380.

Hendricks, S. B., and H. A. Borthwick. 1967. The function of phytochrome in regulation of plant growth. Proc. Nat. Acad. Sci. 58: 2125-2130.

_____, E. H. Toole, V. K. Toole, and H. A. Borthwick. 1959. Photocontrol of plant development by the simultaneous excitations of two interconvertable pigments. III. Control of seed germination and axis elongation. Bot. Gaz. 121: 1.

Hsiao, A. I-Hsiung, and W. Vidaver. 1970. Seed water content in relation to phytochrome-mediated germination of lettuce seeds (Lactuca sativa L. Var. Grand Rapids). Can. J. Bot. 49: 111-115.

_____. 1971. Water content and phytochrome induced potential germination responses in lettuce seeds. Plant Physiol. 47: 186-188.

_____. 1973. Dark reversion of phytochrome in lettuce seeds stored in a water-saturated atmosphere. Plant Physiol. 51: 459-463.

Ikuma, H. and K. V. Thimann. 1959. Photosensitive site in lettuce seeds. Science. 130: 568-569.

_____. 1960. Action of gibberellic acid on lettuce seed germination. Plant Physiol. 35: 557-566.

Jarvis, B. C., B. Frankland, and J. H. Cherry. 1968. Increased nucleic-acid synthesis in relation to the breaking of dormancy of hazel seed by gibberellic acid. Planta. 83: 257-266.

Kahn, A. 1960a. An analysis of "dark-osmotic inhibition" of germination of lettuce seeds. Plant Physiol. 35: 1-7.

_____. 1960b. Promotion of lettuce seed germination by gibberellin. Plant Physiol. 35: 333-339.

_____, James A. Gross, and D. E. Smith. 1957. Effect of gibberellin on germination lettuce seed. Science. 125: 645-646.

Kefford, N. P., J. A. Zwar, and M. Bruce. 1965. Enahncement of lettuce seed germination by some urea derivatives. Plants. 67: 103-106.

Khan, A. A. 1967. Dependence of lettuce seed germination on actinomycin D-resistant RNA synthesis. Phys. Plant. 20: 1039-1044.

_____. 1968. Inhibition of gibberellic acid-induced germination by abscisic acid and reversal by cytokinins. Plant Physiol. 43: 1463-1465.

Klein, S., M. Negbi, A. Witztum, and L. Rothberg. 1971. The role of the endosperm in uptake and distribution of exogenous leucine in germinating lettuce seeds. New Phytol. 70: 143-147.

_____, and J. W. Preiss. 1958. Depth control deuteron irradiation of Lactuca sativa seeds. I. Effects on germination and growth. Plant Physiol. 33: 3-21.

Leopold, A. C., and F. S. Guernsey. 1954. Respiratory responses to red and infra-red light. Phys. Plant. 7: 30-40.

Loercher, L. 1974. Persistence of red light induction in lettuce seeds to varrying hydration. Plant Physiol. 53: 503-506.

Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randell. 1951. Protein measurement with folin phenol reagent. Jour. Biol. Chem. 193: 265-275.

Mans, R. J. and G. D. Novelli. 1960. A convenient, rapid, and sensitive method for measuring the incorporation of radioactive amino acids into protein. Biochem. Res. Comm. 3: 540-547.

Mayer, A. M. and A. Poljakoff-Mayber. 1962. Quantitative changes in nucleic acids during germination of lettuce seeds. Phys. Plant. 15: 283-292.

_____. 1963. The germination of seeds. The Macmillan Co. N. Y. p. 37-195.

Mitchell, R. C., and T. A. Villars. 1972. Polysome formation in light-controlled dormancy. Plant Physiol. 50: 671-674.

Nabors, M. W., Paul Kugrens, and Cleon Ross. 1974. Photodormant lettuce seeds: Phytochrome-induced protein and lipid degradation. Planta. 117: 361-365.

Nabors, M. W., and Lang, A. 1971a. The growth physics and water relations of red-light induced germination in lettuce seeds. Part I. Planta. 101: 1-25.

Nabors, M. W., and Lang, A. 1971b. The growth physics and water relations of red-light induced germination in lettuce seeds. Part 2. Planta. 101: 26-42.

Palvista, A. D., and A. H. Habor. 1970. Embryo expansion without protrusion in lettuce seeds. Plant Phys. 45: 636-637.

Park, Won-mok, and Shepley S. Chen. 1974. Patterns of food utilization by the germinating lettuce seeds. Plant Physiol. 53: 64-66.

Poljakoff-Mayber, A. 1953. Peroxidase activity in germinating lettuce seeds. Enzymology. 16: 122.

_____. 1955. Oxidative activity of particles prepared from lettuce seedlings. J. Exptl. Bot. 6: 313-320.

Poljakoff-Mayber, A., Goldschmidt-Blumenthol, Shulamith, and M. Evenari. 1957. The growth substances content of germinating lettuce seeds. Physiol. Plant. 10: 14-19.

Rai, V. K., and M. M. Laloraya. 1965. Correlative studies on plant growth and metabolism. I. Changes in protein soluble nitrogen accompanying gibberellin-induced growth in lettuce seedlings. Plant Phys. 40: 437-441.

Reynolds, T. 1973. A temperature-dependent source of variability in estimates of germination behavior of lettuce fruits. Planta. 113: 327-332.

Reynolds, T., and P. A. Thompson. 1971. Characterisation of the high temperature inhibition of germination of lettuce (Lactuca sativa). Physiol. Plant. 24: 544-547.

Reynolds, T. and P. A. Thompson. 1973. Effects of kinetin, gibberellins and (±) abscisic acid on the germination of lettuce (Lactuca sativa). Physiol. Plant. 28: 516-522.

Roth-Bejerano, N., D. Koller, and M. Negbi. 1966. Mediation of phytochrome in the inductive action of low temperature on dark germination of lettuce seed at supra-optimal temperature. Plant Phys. 41: 962-964.

Sankhla, A. and D. Sankhla. 1968. Reversal of (±) abscisin II induced inhibition of lettuce seed germination and seedling growth by kinetin. Physiol. Plant. 21: 190-195.

Scheibe, J. And A. Lang. 1965. Lettuce seed germination: Evidence for a reversible light-induce increase in growth potential and for phytochrome mediation of the low temperature effect. Plant Phys. 40: 485-492.

Shain, Y. and A. M. Mayer. 1965. Proteolytic enzymes and endogenous trypsin inhibitor in germinating lettuce seeds. Physiol. Plant. 18: 853-859.

_____. 1968. Activation of enzymes during germination-trypsin-like enzyme in lettuce. Phytochemistry. 7: 1491-1498.

Speer, H. L. 1973. The effect of arsenate and other inhibitors on early events during the germination of lettuce seeds (Lactuca sativa L.). Plant Physiol. 52: 142-146.

_____. 1974. Some aspects of the function of the endosperm during the germination of lettuce seeds. Can. J. Bot. 52: 1117-1121.

_____, A. I. Hsiao, and W. Vidaver. 1974. Effects of germination-promoting substances given in conjunction with red light or the photochrome-mediated germination of dormant lettuce seeds (Lactuca sativa L.). Plant Physiol. 54: 852-854.

Staden, J. V. 1973. Changes in indogenous cytokinins of lettuce seed during germination. Physiol. Plant. 28: 222-227.

_____, and P. F. Wareing. 1972. The effect of light on endogencus cytokinin levels in seeds of Rumex obtrusifolius. Planta. 104: 126-133.

Toole, E. H., H. A. Borthwick, S. B. Hendricks, and V. K. Toole. 1955. Photocontrol of *Lepidium* seed germination. Plant Physiol. 30: 15-21.

Toole, V. K. and H. M. Cathey. 1961. Responses to gibberellin of light-requiring seeds of lettuce and *Lepidium virginicum*. Plant Phys. 36: 663-671.

Vidaver, W. and A. I. Hsiao. 1974. Actions of gibberellic acid and phytochrome on lettuce seed germination. Plant Physiol. 53: 266-268.

Wareing, P. F. and M. Black. 1957. Sensitivity of light-inhibited seeds to certain spectral regions. Nature, London. 180: 395.

_____. 1958. Similar effects of blue and infra-red radiation on light sensitive seeds. Nature, London. 181: 1420-1421.