EFFECTS OF INHIBITORS OF ELECTRON TRANSPORT ON THE INDUCTION OF PHOTOSENSITIVITY IN LACTUCA SATIVA VARIETY MESA SEEDS

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EFFECTS OF INHIBITORS OF ELECTRON TRANSPORT ON THE INDUCTION OF PHOTOSENSITIVITY IN LACTUCA SATIVA VARIETY MESA 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

Lillian Bradley Nanney

December 1975

ABSTRACT

Lactuca sativa L. variety Mesa seeds were irradiated with far-red and red light to determine if photosensitivity could be induced in these dark germinating seeds. The effects of various inhibitors of electron transport and inhibitors plus ATP on the induction of photosensitivity were observed. A study was made of the interrelationship between protein synthesis and the induction of photosensitivity.

Results indicate that a far-red, red photosensitivity was induced in the Mesa lettuce seeds. Gibberellic acid and thiourea reversed the effects of far-red light and stimulated germination. At certain concentrations DCMU, hydroxylamine, phenazine methanosulfate, and dinitrophenol caused a reversal of the induction of photosensitivity. However, when seeds were irradiated with far-red light, DCMU, hydroxylamine or phenazine methanosulfate plus 10 μM ATP, there was a reinstatement of induced photosensitivity. The results of ^{14}C-leucine incorporation into protein did not indicate that the various inhibitors of electron transport act to influence protein synthesis. The data from these experiments seem to indicate that there is an electron transport system that acts to influence germination in the Mesa variety of lettuce seeds.

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To the Graduate Council:

I am submitting herewith a Thesis written by Lillian Bradley Nanney entitled "Effects of Inhibitors of Electron Transport on the Induction of Photosensitivity in Lactuca Sativa Variety Mesa 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.

Major Professor

We have read this thesis and recommend its acceptance:

Second Committee Member

Third Committee Member

Accepted for the Council:

Dean of the Graduate School

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CHAPTER I

INTRODUCTION

Many seeds such as Lactuca sativa variety Grand Rapids (Borthwick et a., 1954) and Nicotiana tabacum (Boucher, 1956) require light in order to germinate. Lactuca sativa variety New York and variety Mesa, however, are not dependent upon light and germinate quite well in darkness. Experimentation by Borthwick et al., (1952) indicated that germination in Grand Rapids lettuce seeds could be controlled by red and far-red light. Studies by Mills (1971) demonstrated that a far-red, red photosensitivity could be induced in the dark germinating variety of New York lettuce seeds.

Various inhibitors such as DCMU (3, 4-dichlorophenyl)-1, 1-dimethylurea) and hydroxylamine are known to block the flow of electrons in the electron transport system of photosystem II of photosynthesis (Katoh, 1972; Cheniae and Martin, 1971). Phenazine methanosulfate is an inhibitor of electron flow in photosystem I (Papageorgion, 1975). Yet another inhibitor, 2, 4-dinitrophenol, is commonly used to inhibit the electron transport system in oxidative phosphorylation (Schneider and Stimson, 1972).

This study was undertaken to: 1) determine if a far-red, red photosensitivity could be induced in the dark germinating variety of

Lactuca sativa variety Mesa; 2) investigate the response of the photo-induced Mesa seeds to some stimulators of germination, gibberellic acid and thiourea; 3) study the effects of various inhibitors of electron transport on the induction of photosensitivity in the Mesa lettuce seeds; 4) observe the interrelationship of various inhibitors of electrons and also the inhibitors combined with adenosine triphosphate on protein synthesis and the induction of photosensitivity in the Mesa lettuce seeds.

CHAPTER II

REVIEW OF LITERATURE

Certain varieties of seeds such as Lactuca sativa variety Grand Rapids (Borthwick et al., 1954), Lepidium virginicum (Toole, 1955), Rheum rhaponticum, Nicotiana tabacum, Agrostis alba (Boucher, 1956), and Arabidopsis thaliana (Shropshire et al., 1961), require light for germination. Others such as Lactuca sativa variety New York and various Italian varieties (Mancinelli and Borthwick, 1964) have no light requirement and germinate quite well in darkness.

Borthwick and associates (1952) found that the red region of the spectrum between 590 and 680 nanometers (nm) was the most effective wavelength for the promotion of germination in Grand Rapids lettuce seeds. Longer wavelengths between 700 and 800 nm, commonly called far-red, inhibited germination. The red (R), far-red (Fr) reversible system which controls germination in Grand Rapids lettuce seeds has been described for many other seeds (Borthwick et al., 1964; Downs, 1964; Chon and Briggs, 1966). Mancinelli (1966) demonstrated that exposure of dark germinating tomato seeds to prolonged periods of far-red light induced a red, far-red photoreversible system controlling germination. Toole (1956) found the photoreaction in light requiring seeds to be controlled by two forms of a blue chromoprotein.

The term photochrome was later proposed for this substance (Butler et al., 1960).

Phytochrome exists in at least two different forms (Hendricks and Borthwick, 1965). The biologically inactive form phytochrome P_R has an absorption maximum near 660 nm. The unstable and biologically active form of phytochrome P_{FR} has an absorption maximum near 730 nm. After irradiation by red light, P_R is transformed into P_{FR}. The opposite reaction occurs after exposure to far-red light (Rollin, 1966). Other investigations have indicated that there may be some phytochrome intermediates (Spruit et al., 1975; Grill and Vince, 1966). P_{FR} is unstable and in darkness undergoes a reversion to P_R. This spontaneous destruction of P_{FR} to P_R is influenced by temperature and inhibitors (Rollin, 1966).

Phytochrome has been detected, extracted and purified (Butler et al., 1959) and many of its physical properties determined (Butler) et al., 1963). It is known to mediate anthocyanin synthesis (Schneider and Stimson, 1972). However, the mechanism or mechanisms by which phytochrome induced various photomorphogenic responses has not been fully elucidated. Hendricks (1967) proposed that phytochrome controls growth responses by altering cell permeability. Mohr (1966) suggested that P_{FR} may be involved in the activation of 'potentially active' genes. Rollin (1966) theorized that P_{FR} is the active form which acts as an enzyme. Additional studies by Kendrick and Spruit (1974) postulated

that there is a continual production of P_{FR} during the imbibition stage of germination. Supplemental studies suggested that in dried seeds a certain proportion of P_{FR} is present and acts from the start of imbibition.

Recent investigators have examined the High Energy Response (HER) that controls germination. High Energy Reactions are believed to arise through the maintenance of a low level of P_{FR} over a prolonged time (Hartman, 1966; Schneider and Stimson, 1972). The term HER is perhaps an inaccurate one because the initiation of germination depends on the duration of light rather than the intensity (Rollin, 1966). Responses that are regulated by phytochrome exhibit photoreversibility. Photoresponses that exhibit evidence of a high energy reaction also have a phytochrome reversibility (Schneider and Stimson, 1972). Hartman (1966) found that under high energy conditions P_{FR} will be destroyed. Rollin (1966) supported this same conclusion when he suggested that periods of far-red irradiation are necessary to transform the molecules of P_{FR} (already present from the start of imbibition) into P_R. Furthermore he proposed that in dark germinating seeds the light requirement is not by-passed but the required P_{FR} is present in the seed. However, when all the evidence is reviewed there is still some uncertainty. Grill and Vince (1966) concluded that at least two photochemical reactions are involved in the response to red and far-red light; the first leading to the formation of substrates used in the second

reaction. Thus, they have used their studies of anthocyanin synthesis to link the red, far-red photoreversible system to the high energy response.

In 1960, Kahn reported that gibberellic acid could substitute for red light in promoting germination in seeds that do not germinate in darkness because of far-red inhibition. In every situation examined, it was found that gibberellic acid could replace red light. Studies correlating gibberellic acid to phytochrome were conducted by Toole et al., (1961). They investigated the mode of action of gibberellic acid on the germination of light requiring seeds such as Grand Rapids lettuce seeds and Lepidum virginicum. They established that gibberellic acid allowed the germination process to by-pass the light requirement. They also noted that the inhibitory action of far-red light was influenced by the concentration of gibberellic acid. Gibberellic acid has been used in other studies as a stimulator of germination (Poljakoff-Mayber et al., 1958; Haber and Luippold, 1960; Ikuma and Thimann, 1960; Burdett, 1972; Black et al., 1974). Ikuma and Thimann reported that far-red light of short duration does not inhibit germination of seeds that have been treated with gibberellic acid. They further speculated that gibberellic acid acted to initiate one of the chemical reactions which normally result from the light reaction, so that the end product is the same as that produced by the light. Different studies by Burdett (1972) noted that far-red light suspends the action of gibberellic acid,

but does not prevent seeds from responding to gibberellic acid when they are placed in the dark. He proposed that gibberellic acid has an effect on permeability. Gibberellic acid studies to date are best described in a paper by Black et al. (1974) when they stated, "the method by which the action of phytochrome is translated into seed germination is still unknown. The interaction between gibberellic acid and phytochrome also remains unsolved."

The chemical thiourea has been used to stimulate germination (Poljakoff-Mayber et al., 1958; Haber and Luippold, 1960; Hendricks and Taylorson, 1975). Its mode of action has not been as thoroughly investigated as that of gibberellic acid, but there is agreement that thiourea can break dormancy in lettuce seeds. Haber and Luippold (1960) announced that thiourea inhibited mitotic acitvity yet it still stimulated germination.

In recent years many investigators have employed the herbicide DCMU in studies involving photosynthesis. In 1958 Bishop probed into the effects of DCMU and noted that it was a powerful photoreductant. Since this time his work has been confirmed by Kimmura and Katoh (1972). Additional studies by these men were designed to discover the exact site where DCMU interfered with electron transport in photosynthesis. It was determined that DCMU blocked electron transport on the reducing side of photosystem II. DCMU was thought to block the transfer of electrons between Q and the adjacent pool of endogenous

electron acceptors in photosystem II (Yamashita and Horio, 1968). This was supported by the work of Katoh (1972). Thus, photosynthesis has been closely studied by the use of artificial electron donor systems (Vandermuelen et al., 1972; Shneyour et al., 1970; Sargent and Taylor, 1972; Vernon et al., 1971; Arnon et al., 1971). Investigators decide that electron transport has been blocked by measuring the amount of ATP that is formed (Yamashita and Horio, 1968; Stranger and Appleby, 1972).

Another chemical used to inhibit photosynthetic electron transport is hydroxylamine. This chemical inhibits a site on the oxidant side to photosystem II, and thus serves as an electron donor (Katoh et al., 1970; Cheniae and Martin, 1971). This role of hydroxylamine was investigated by measuring the oxygen evolving capacity in the presence of hydroxylamine. This inhibitor attacks a site different from DCMU, and it also does not inhibit photosystem I mediated electron transport in any way (Ort and Izawa, 1973). The role of hydroxylamine in Grand Rapids lettuce seeds and pigweed Amaranthus albus was recently investigated by Hendrick and Taylorson (1974, 1975). They reported that hydroxylamine did promote germination by inhibiting the formation of catalase enzymes which thereby trigger a different electron transport system.

Yet another inhibitor which is used in electron transport studies is phenazine methanosulfate. It is thought to be a synthetic cofactor

which supports very rapid phosphorylation (Jagendorf and Avron, 1958). It may act as an intermediate electron carrier across some gap in electron transport. It is known to stimulate cyclic photophosphorylation in photosystem I (Papageorgion, 1975). This inhibitor functions to cause respiratory inhibition by causing a change in the ATP/ADP balance (Sargent and Taylor, 1972). Furthermore in plant mitochondria, phenazine methanosulfate can serve as a redox mediator. Its presence can cause a marked stimulation of ATPase and thus phenazine methanosulfate can effectively short-circuit reversed electron transport (Lambowitz et al., 1974).

Dinitrophenol is known to inhibit oxidative phosphorylation (Schneider and Stimson, 1971, 1972). This uncoupling of mitochondrial phosphorylation serves to block ATP synthesis (Lado, 1973). In addition dinitrophenol is known to reduce anthocyanin synthesis in turnip seedlings (Schneider and Stimson, 1971). Subsequent studies have reported that P_{FR} action and photoreversibility are maintained in the presence of low amounts of dinitrophenol (Schneider and Stimson, 1972).

Recent investigations have examined the effects of adenosine triphosphate on seed germination and protein synthesis. The data reported by Ching and Ching (1972) indicate that the ATP level is directly correlated with growth, organogensis, and morphogenesis in seed development. Studies by Bewley and Gowdz (1975) utilizing dried mosses indicated that protein synthesis was not mediated directly

through ATP availability. However, in 1974 there were conflicting studies by Obendorf and Marcus. They suggested that ATP could be the limiting factor in polyribosome formation and therefore be a significant factor in the regulation of embryo germination. They noted that the rapid increase in ATP during imbibition was consistent with the energy requirement for polyribosome and protein synthesis during early germination.

CHAPTER III

MATERIALS AND METHODS

Seed Germination

Seeds of Lactuca sativa variety Mesa were purchased from Joseph Harris Seed Company, Rochester, New York, and used throughout the study. All seeds were first imbibed in distilled water in darkness for 1.5 hours prior to any light treatments. At this point seeds in lots of approximately 100 were sown in 5 centimeter (cm.) petri dishes. Each dish contained a Whatman No. 1 filter paper which was saturated with 1.5 milliliters (ml.) of an appropriate experimental solution. The method was similar to that of Black and Richardson (1965).

After exposure to 24 hours of far-red light, seeds were taken to a darkroom. For the rinsed seeds, seeds were rinsed 3 times and 1 ml of the appropriate solution was added for the germination period. For germination all seeds were placed in light-proof petri dish sterilization cans and allowed to germinate at 25° Celcius for 48 hours in darkness. Emergence of the radicle was used as the criterion for germination. All germination percentages that follow represent the mean germination based on a minimum of two different runs of triplicate dishes.

The light source used in these experiments consisted of a 150 watt reflector flood operating at 120 volts. The lamp was attached by a metal clamp to a ring stand and placed 78 cm above the seeds. Far-red light was obtained by passing light through a Carolina Biological Supply (CBS) No. 750 plastic filter. An 8 cm solution of water was positioned between the light source and the filter to reduce heat effects. Red light was obtained by passing light through a CBS filter No. 650. A solution 8 cm in depth containing a 1 percent copper sulfate solution was positioned between the light source and the filter to absorb far-red light. The darkroom was equipped with one 20 watt fluorescent lamp wrapped in 10 layers of green cellophane and 10 layers of blue cellophane. This lamp was used when seeds were manipulated in the absence of red or white light.

Labeled Precursor Incorporation into Seeds

Seeds in lots of 100 milligrams (mg) were placed in solutions of the various inhibitors and inhibitors with adenosine triphosphate. All seeds were exposed to 1 microcurie of ^{14}C-leucine and 0.1 ml of 10^{-1} millimole (mM) of penicillin. This radioactive label was exposed to the seeds during the entire 24 hours of far-red irradiation. The extraction method employed for lettuce seed protein was modified from Mans and Novelli (1966) as follows. Seeds were placed in a Gooch crucible attached to a suction flask and rinsed with distilled water to remove any precursor adhering to the seed coat. The tissue was ground in a

mortar and pestle chilled on ice. The mortar and pestle contained 2.5 ml of 10 mM Tris Buffer at pH 7.8 containing 0.2 mg per ml of l-leucine.

Soluble label precursor pool within the tissue was estimated by placing 0.1 ml of homogenate into a test tube containing 3 ml of 10% trichloroacetic acid (TCA). The solutions were chilled on ice for one hour and then centrifuged for 10 minutes. At this point 0.1 ml of the supernatent was pipetted onto glass fiber papers and dried for 15 minutes under an infra-red lamp. To determine the soluble precursor pool the fiber paper was transfered to a liquid scintillation vial containing 10 to 15 ml of scintillation fluid (4 grams of 2, 5-diphenyloxazole and 50 mg of 1, 4-bis-2(5-phenyloxazolebenzene per liter of toluene) and counted in a Nuclear Chicago Unilux II liquid scintillation counter for 10 minutes.

The protein homogenate in lots of 0.1 ml was pipetted onto glass fiber papers and dried under an infra-red lamp. To precipitate protein, the papers were submerged in ice cold 5% TCA for 15 minutes and rinsed twice with cold 5% TCA and 100 ppm l-Leucine. The papers were then incubated in 5% TCA for 10 minutes at 90°C. This was followed by another cold 5% TCA treatment. Ethanol was added and warmed 3 minutes at 70°C. The papers were subsequently treated for 3 minutes in ethanol/ether/chloroform (2:2:1). After decanting the liquid, the papers were washed in acetone and dried overnight. The

papers were placed in vials containing scintillation fluid and counted in a liquid scintillation counter for 10 minutes.

A colorimetric assay for protein was accomplished using the method of Schacterle (1973). Alkaline copper reagent in the amount of 1 ml was added to both 1 ml aliquots of the remaining homogenate and various concentrations of bovine albumen. These mixtures were allowed to stand for 10 minutes. At this time 4 ml portions of phenol reagent were pipetted into the mixtures. These solutions were incubated in a water bath for 5 minutes at 55°C. To estimate colorimetrically the total seed protein the experimental solutions were placed in a Gilford Spectrophotometer and read at 650 nm. These values were compared with the readings from the similarly treated bovine protein standard.

The chemicals used in these tests were obtained from the following sources: hydroxylamine monohydrochloride and adenosine-5-triphosphate from Sigma Chemical Company, St. Louis, Missouri; 2,4-dinitrophenol from Matheson, Coleman, and Bell; phenazine methanosulfate from Nutritional Biochemical Corporation, Cleveland, Ohio; diuron 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) from E. I. duPont de Nemours and Company, Inc., Wilmington, Delaware; ^{14}C-leucine from International Chemical and Nuclear Corporation, Irvine, California.

CHAPTER IV

RESULTS

Effects of Far-Red Light Upon Germination of Mesa Variety Lettuce Seeds

Seeds were exposed to 24 hours of continuous far-red light to determine if this treatment could induce a photosensitivity in these seeds. Normally the Mesa variety of seeds will exhibit nearly 100% germination when placed in darkness in water for 48 hours. The data in Table I indicate that exposure to 24 hours of continuous far-red irradiation does indeed induce a photosensitivity in the Mesa variety. Whether seeds are rinsed or non-rinsed before germination, all germination percentages remained below 40%.

Experiments were designed to see if the classical photoreversibility tests for Grand Rapids lettuce seeds could be established for the photoinduced Mesa variety. Mesa seeds were exposed to 24 hours of far-red light. This light treatment was followed by exposure to 5 minutes of red light and seeds were then allowed to germinate 48 hours in the dark. The results (Figure 1) indicate that red light stimulates germination in the photoinduced seeds, and thus reverses the inhibitory effects of far-red light. When seeds were exposed to 24 hours of far-red light, 5 minutes of red, and a final 5 minutes of far-red light, there was a low germinating response. In seeds that were

TABLE I

INDUCATION OF PHOTOSENSITIVITY IN MESA VARIETY OF LACTUCA SATIVA

LIGHT TREATMENT	CHEMICAL	% GERMINATION
Darkness	water	98.5 ±1 *
Darkness	phosphate buffer	98 ±1
24 hours-Fr	water	10 ±5
24 hours-Fr	water, rinsed	30 ±6
24 hours-Fr	phosphate buffer	20 ±4
24 hours-Fr	phosphate buffer rinsed	35 ±4

* Represents the mean germination percentages and standard error.

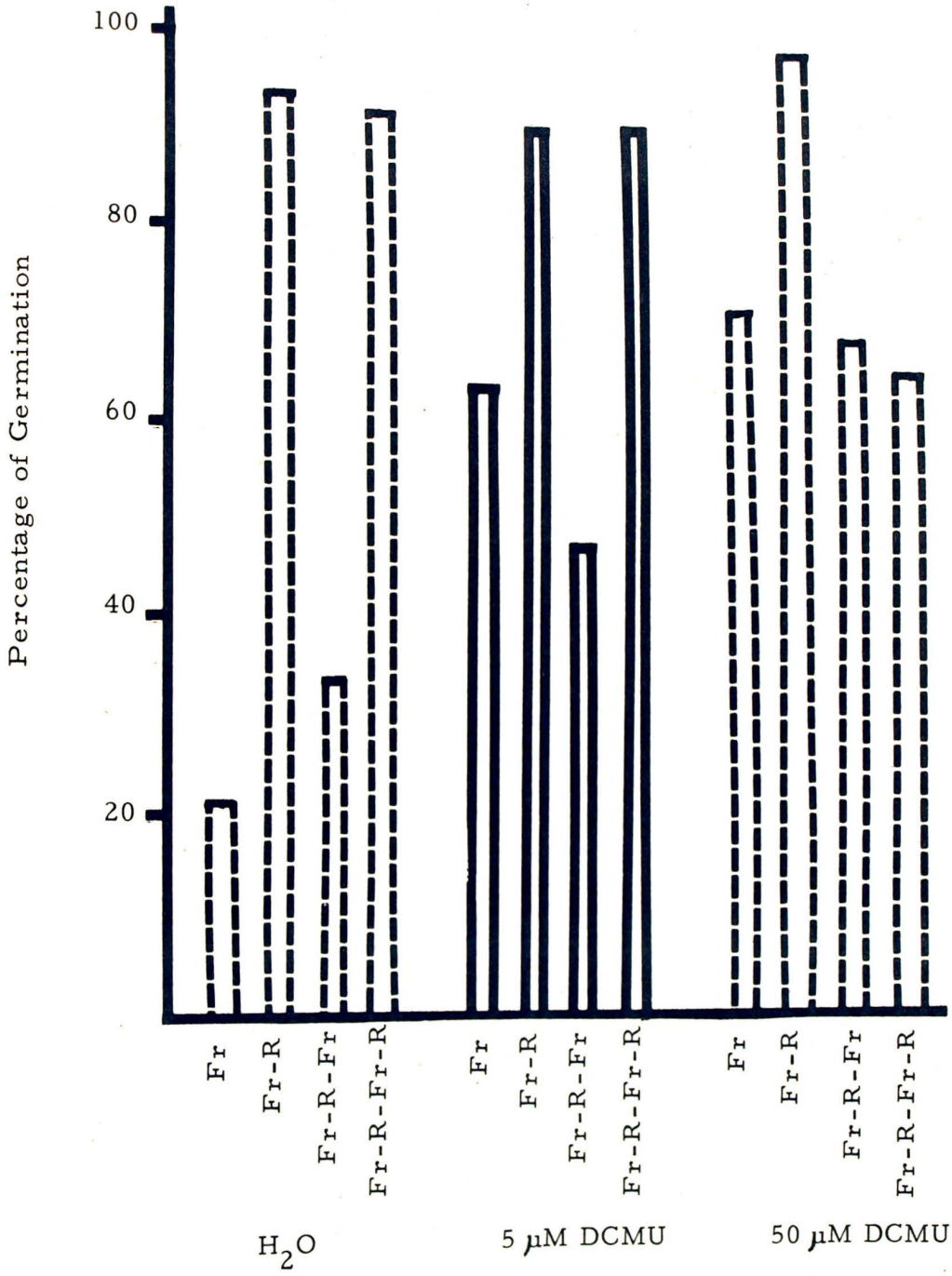


Figure 1. Effects of DCMU concentrations of photoreversibility in non-rinsed seeds of Lactuca sativa variety Mesa

Seeds were exposed to 24 hours of continuous far-red light and then followed by 5 minute light treatments.

exposed to 24 hours of far-red, 5 minutes of red, 5 minutes of far-red, and a final 5 minutes of red, the germination responses were very high. Consequently, the final germination response of the photoinduced Mesa variety of lettuce seeds depends upon the final light exposure prior to the 48 hours of dark incubation.

Effects of Gibberellic Acid and Thiourea on the Induction of Photosensitivity in Mesa Lettuce Seeds

Gibberellic acid (Kahn, 1960, 1966; Ikuma and Thimann, 1960; White et al., 1972; Burdett, 1972; Vidaver and Hsiang-Hsiao, 1974) and thiourea (Haber and Luippold, 1960) have been used to stimulate germination in several varieties of seeds. The following experiments were conducted to investigate the induced photodormancy in Mesa lettuce seeds. When seeds were irradiated for 24 hours in far-red light with distilled water, a photodormancy was induced in the seeds. When gibberellic acid was added for the 48 hour period of germination, the hormone could slightly overcome the induced photodormancy (Figure 2). The results of varying concentrations of thiourea upon the induced photosensitivity are also presented in Figure 2. Thiourea at concentrations ranging from 10 to 100 mM could overcome the induced photosensitivity in the Mesa variety of seeds.

Effects of DCMU on the Mesa Lettuce Seeds

DCMU is a well known inhibitor of electron transport in photosystem II (Bishop, 1958; Shneyour et al., 1970; Katoh, 1972; Shone

and Wood, 1974). To investigate the effects of an inhibitor of electron transport in photosystem II on the induction of photosensitivity, seeds were exposed to 24 hours of far-red light in the presence of DCMU. The results are indicated in Figure 3. Seeds that were rinsed and allowed to germinate in water displayed a reversal of the induction of photosensitivity. In seeds that were not rinsed before germination the reversal of the induced photosensitivity was evident at concentrations between 5 and 10 μM of DCMU.

Photoreversibility experiments with DCMU were conducted and the results are shown in Figure 1. When seeds were exposed to 24 hours of far-red light in the presence of 5 μM of DCMU, there was a reversal of the induced photosensitivity. When seeds were treated with 24 hours of far-red light and this was followed by 5 minutes of red light, germination was stimulated. This indicated that DCMU does not interfere with the response of Mesa seeds to red light. When photo-induced seeds are treated with far-red light and this was followed by red and then far-red light, there is again a slight reversal of the induced photosensitivity. The effects of the far-red light can be completely reversed by another 5 minutes of red light. When photoreversibility tests were conducted in the presence of 50 μM of DCMU, photoreversibility is not as sharply delineated (Figure 1).

The next studies with DCMU investigated the combined effects of DCMU and ATP. Previous experiments indicated that 50 μM DCMU

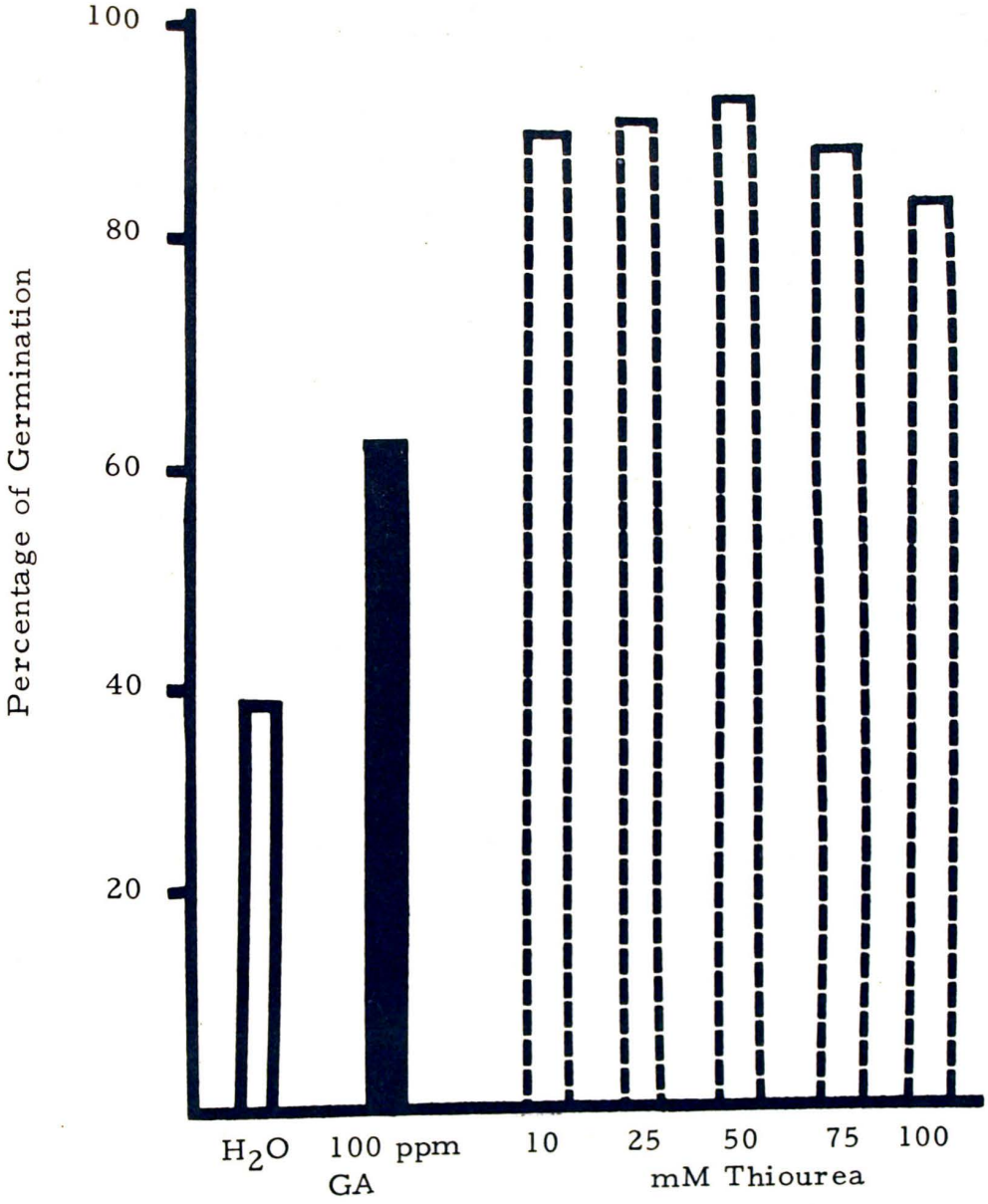


Figure 2. Effects of Gibberellic acid or Thiourea treatments on induced photosensitivity in Lactuca sativa variety Mesa.

Seeds were exposed to 24 hours of continuous far-red irradiation in the presence of distilled water. Seeds were rinsed and GA or Thiourea was added at the beginning of 48 hours of germination in darkness.

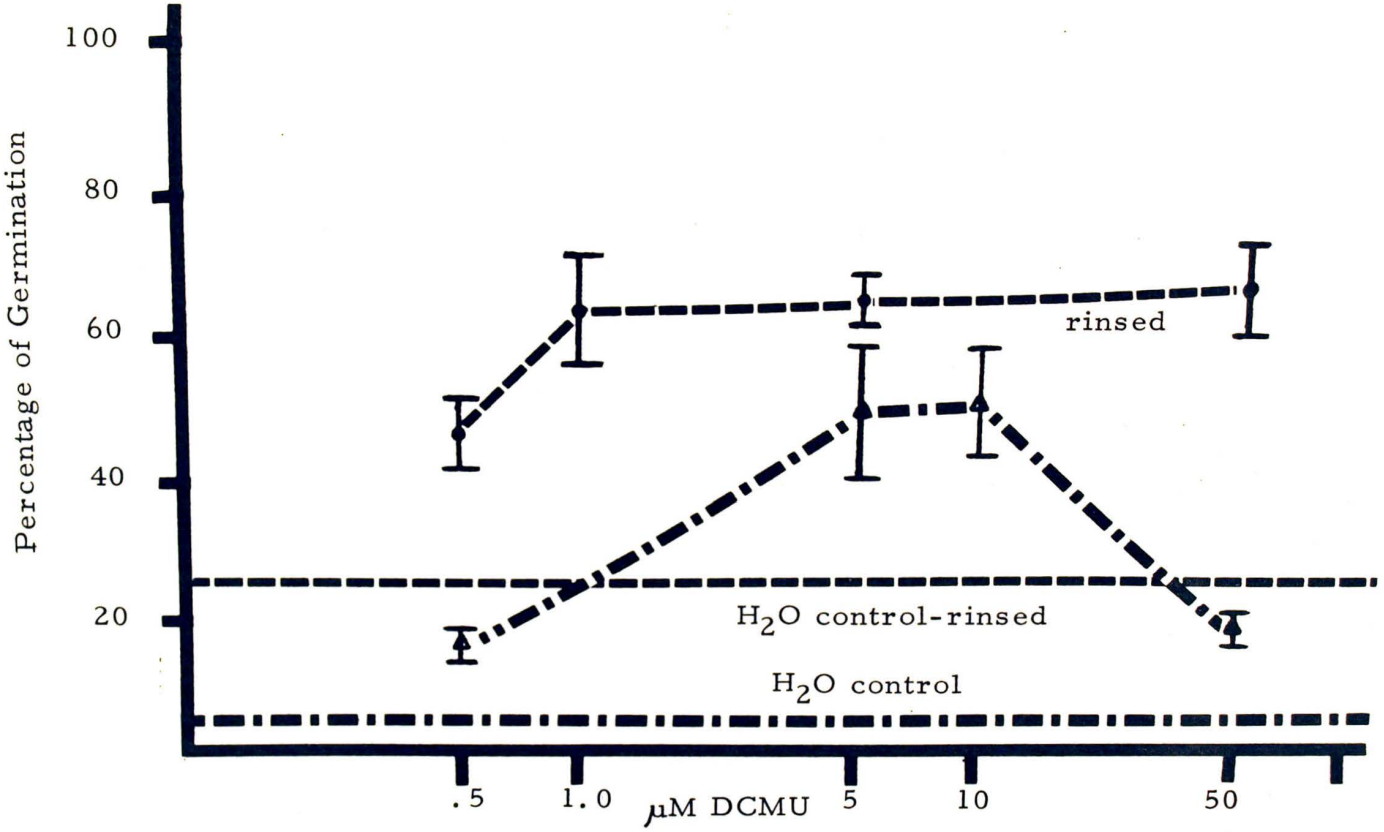


Figure 3. Effects of DCMU on the Induced Photosensitivity in Lactuca sativa variety Mesa.

All seeds were exposed to 24 hours of far-red light in the presence of DCMU. Some seeds were rinsed prior to the 48 hours of germination in darkness and others were not rinsed before germination.

present in the seeds during the 24 hours of far-red light could cause a reversal of the induced photosensitivity (Figure 3). Therefore, in the following experiment seeds were irradiated for 24 hours in far-red light in the presence of 50 μM DCMU and ATP concentrations ranging from 0.01 to 10 mM. The ATP was in a 0.01 M phosphate buffer at a pH of 6.4. The results of these experiments are shown in Figure 4. When ATP was present in a concentration ranging from 0.01 to 5 mM, there was a reversal of the induced photosensitivity that was very similar to the treatment with DCMU alone. However, when 10 mM of ATP was present there was a reinstatement of the induced photodormancy only in those seeds germinated in the presence of 10 mM ATP.

Effects of Hydroxylamine on the Induction of Photosensitivity in Mesa Lettuce Seeds

Hydroxylamine has been used as an inhibitor of Photosystem II (Katoh et al., 1970; Ort and Izawa, 1973; Hendricks and Taylorson, 1974). Seeds were exposed to 24 hours of far-red light in the presence of hydroxylamine ranging from 1 to 100 mM. The results (Figure 5) indicate that the concentration of 1 and 10 mM can cause a reversal of the induced photosensitivity in the Mesa variety of seeds. However, at concentrations ranging from 25 to 100 mM of hydroxylamine the germination percentages reveal a severe inhibition of germination. In seeds that were allowed to germinate in hydroxylamine in darkness for 48 hours without any kind of light treatments, it was found that

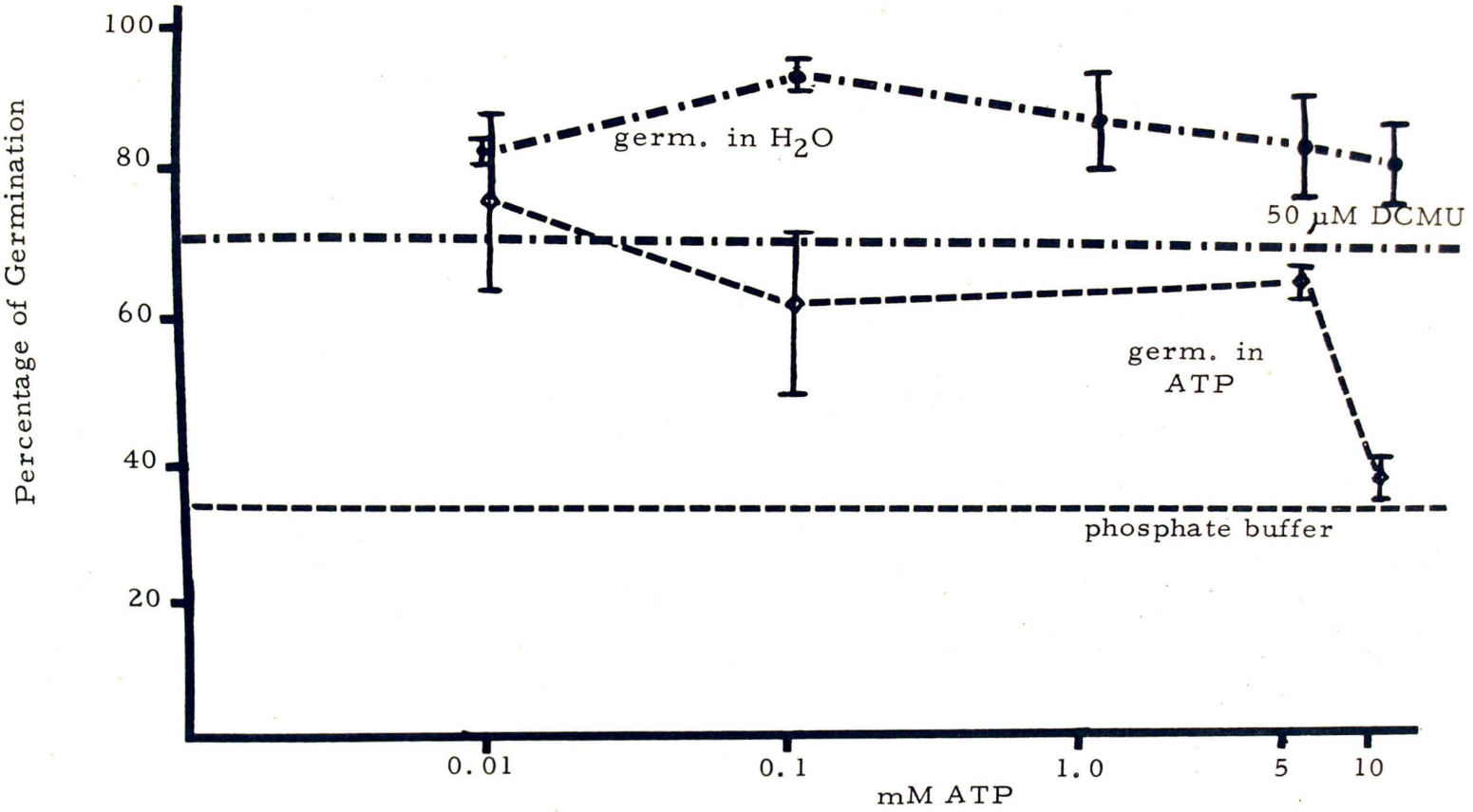


Figure 4. Effects of 24 hours of far-red light, 50 μM DCMU, and various concentrations of ATP on the germination of Lactuca sativa variety Mesa.

All seeds were exposed to 24 hours of far-red light in the presence of DCMU and ATP. All seeds were rinsed and water was added to some for the germination period and fresh ATP solution was added to other seeds for the germination period. All vertical bars represent the standard error of the mean.

25-100 mM was inhibitory to normal germination in the Mesa variety of seeds (Table II).

Seeds were also tested with various concentrations of hydroxylamine and ATP. In this experiment the seeds were irradiated for 24 hours in far-red light in the presence of 1.0 mM of NH_2OH and ATP. A 1.0 mM concentration of hydroxylamine alone is sufficient to cause a reversal of the induction of photosensitivity (Figure 6). When 0.1 mM of ATP is also added during the treatment, there is still a characteristic reversal of the induced photosensitivity by hydroxylamine. However, between the concentrations of 1.0 and 10 mM there is a tendency to reinstate the induction of photosensitivity.

Effects of Phenazine Methanosulfate on the Induction of Photosensitivity in Mesa Seeds

Phenazine methanosulfate has been used as an inhibitor of photosystem I (Lambowitz et al., 1974). To examine the effects of an inhibitor of electron transport in photosystem I, this chemical was added during the 24 hours of continuous far-red light treatment. At concentrations between 1.0 and 100 μM, it is evident that phenazine methanosulfate can cause a reversal of the induced photosensitivity in the Mesa lettuce seeds (Figure 7).

In the next experiment seeds were placed in 10 μM of the inhibitor and also concentrations of ATP ranging from 1-10 mM. A reinstatement of the induced photosensitivity in the Mesa seeds was

TABLE II

EFFECT OF CHEMICALS ON DARK GERMINATED SEEDS OF LACTUCA SATIVA VARIETY MESA

CHEMICAL TREATMENT	PERCENTAGE OF GERMINATION
water	98.5 ± 1 *
phosphate buffer	98 ± 1
10 mM ATP	99 ± 1
1.0 mM ATP	99 ± 1
50 μM DCMU	73 ± 9
1.0 μM DCMU	95 ± 3
10 mM NH_2OH	91 ± 5
100 mM NH_2OH	0
0.1 μM PMS	94 ± 3
100 μM PMS	0
100 μM DNP	79 ± 8
1000 μM DNP	0

* The mean germination percentages and the standard error.

All seeds were imbibed 1.5 hours in water, placed in petri dishes in 1.5 ml of solution and germinated in darkness for 48 hours.

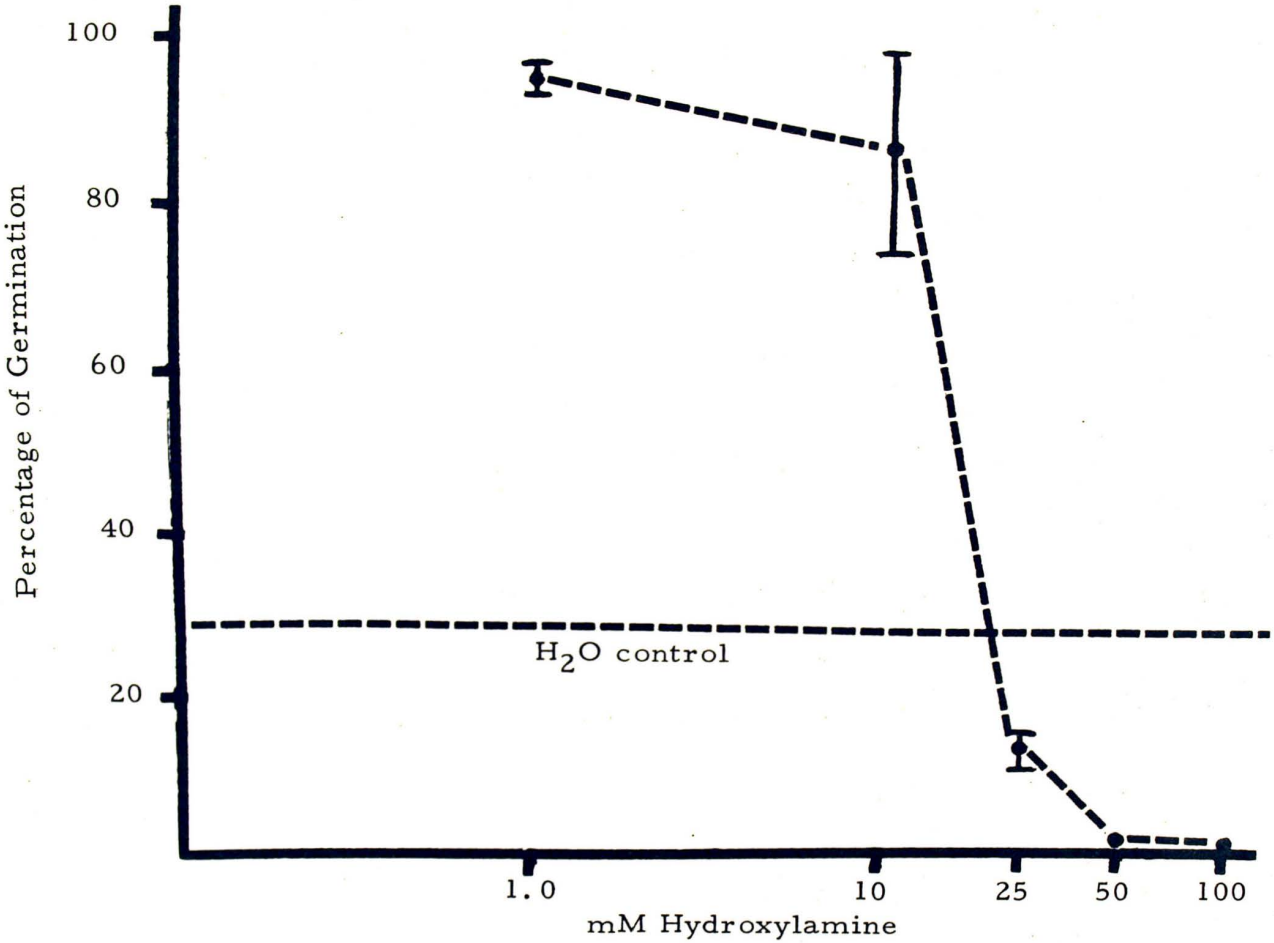


Figure 5. Combined Effects of 24 hours of far-red light and concentrations of hydroxylamine on the germination of seeds of Lactuca sativa variety Mesa.

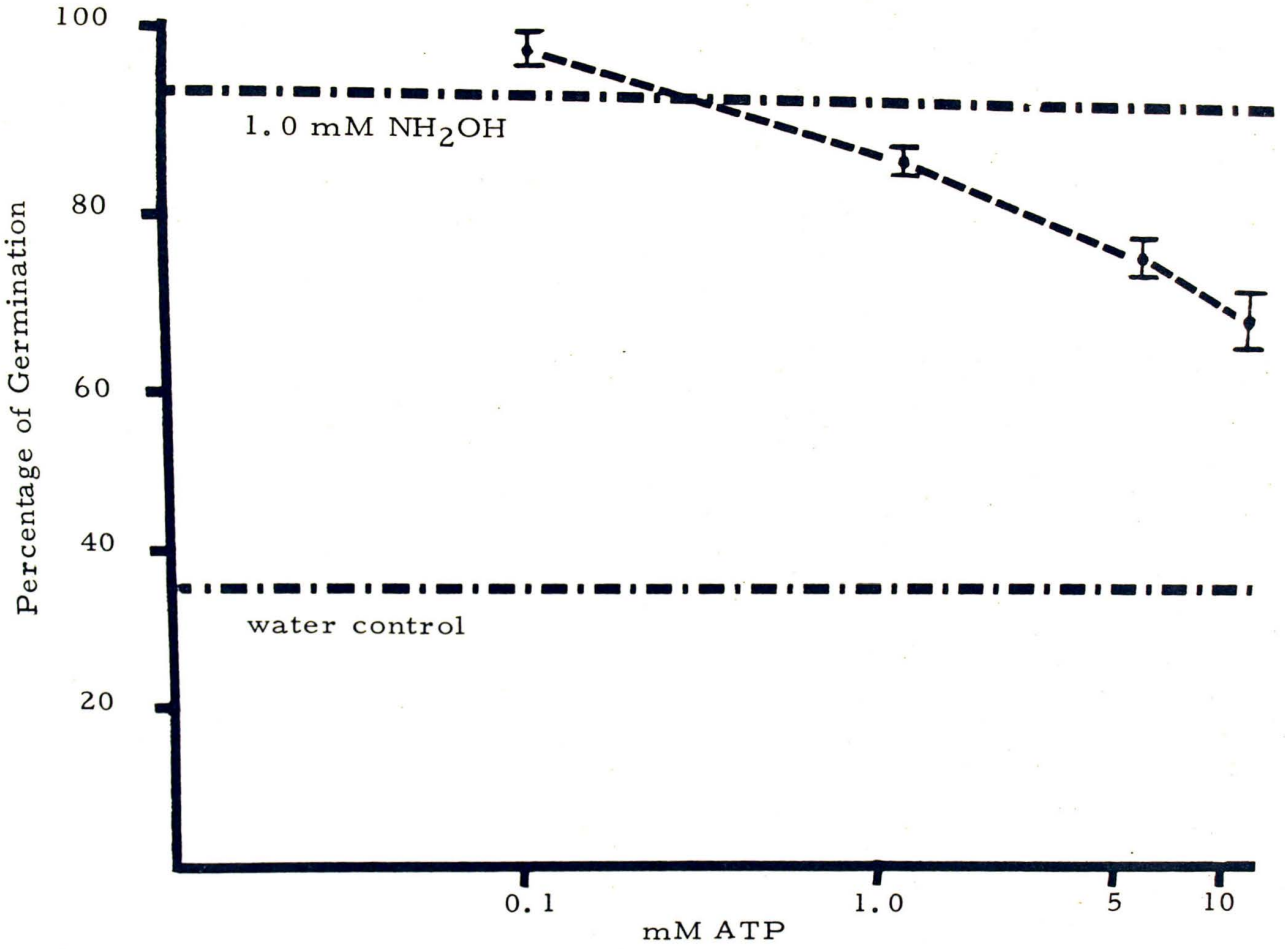


Figure 6. Effects of 24 hours of far-red light and 1 mM hydroxylamine and various concentrations of ATP on the germination of Lactuca sativa variety Mesa.

observed in those seeds germinating in the presence of 10 mM ATP (Figure 8).

Effects of Dinitrophenol on the Induction of Photosensitivity in the Mesa Lettuce Seeds

Dinitrophenol has been used in several studies as an inhibitor of oxidative phosphorylation (Schneider and Stimson, 1971, 1972). To investigate the effects of dinitrophenol on the induced photodormancy in the Mesa variety of lettuce seeds, this inhibitor was added during the 24 hours irradiation with far-red light. In seeds that were not rinsed before the germination period, it was found that concentrations of dinitrophenol ranging from 0.1-1000 mM did not influence the induction of photosensitivity (Figure 9). However, in seeds that were rinsed before germination and concentrations of dinitrophenol ranging from 1.0-100 μM, there was a reversal of the induced photodormancy. A 1000 μM concentration of dinitrophenol completely inhibited any germination response in both rinsed and non-rinsed seeds.

Photoreversibility studies were conducted in the presence of dinitrophenol. When non-rinsed seeds were treated with 1 μM of dinitrophenol for 24 hours of far-red light followed by 5 minutes of red light, it was found that this inhibitor did not interfere with the classical photoreversibility response in lettuce seeds (Figure 10). When this light treatment was followed by a final 5 minutes of far-red light there was an inhibition of germination. If all light treatments were followed

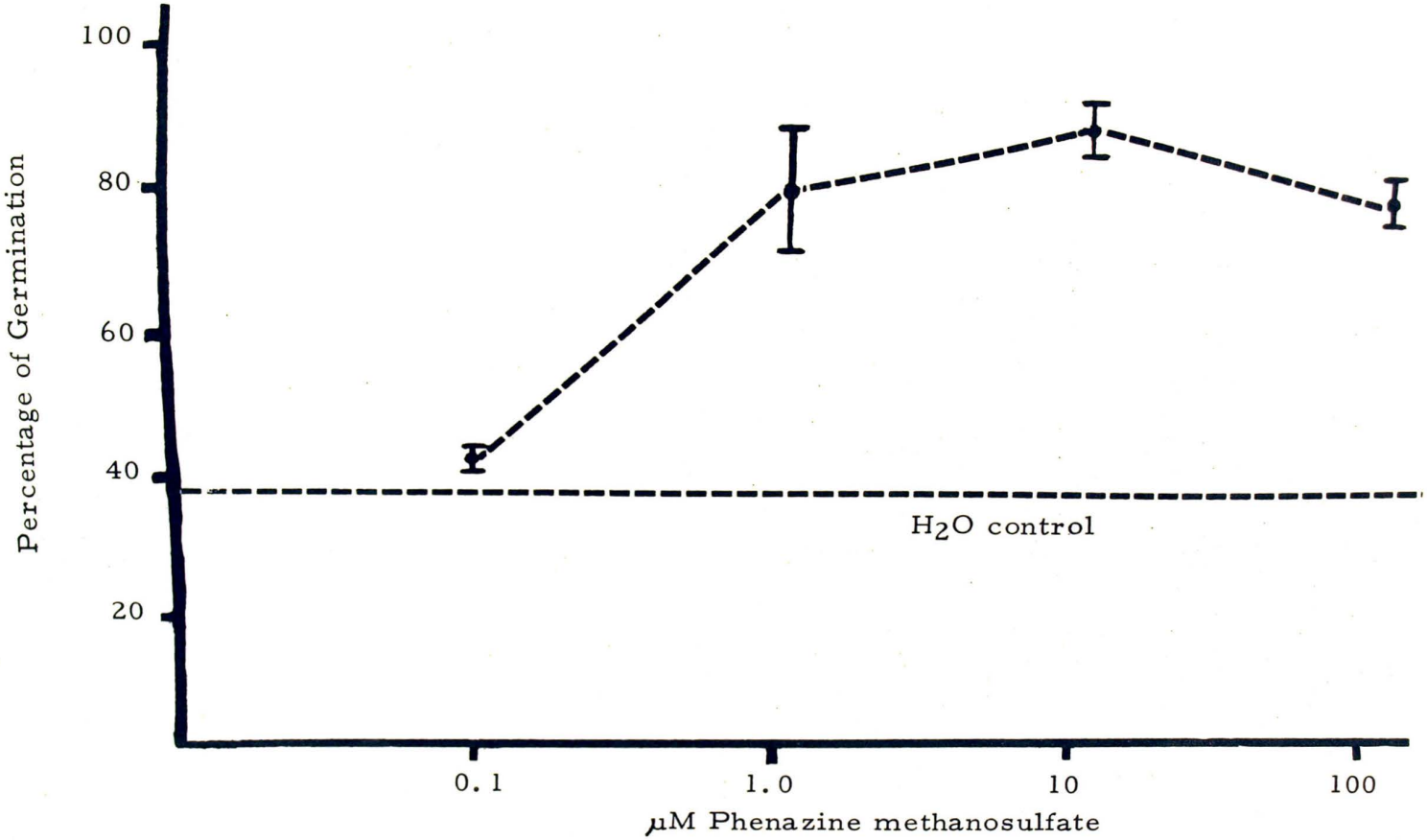


Figure 7. Effects of 24 hours of far-red light and concentrations of phenazine methanosulfate on germination of Lactuca sativa variety Mesa.

Seeds were exposed to 24 hours of far-red irradiation in the presence of PMS. Seeds were rinsed and allowed to germinate in 48 hours of darkness.

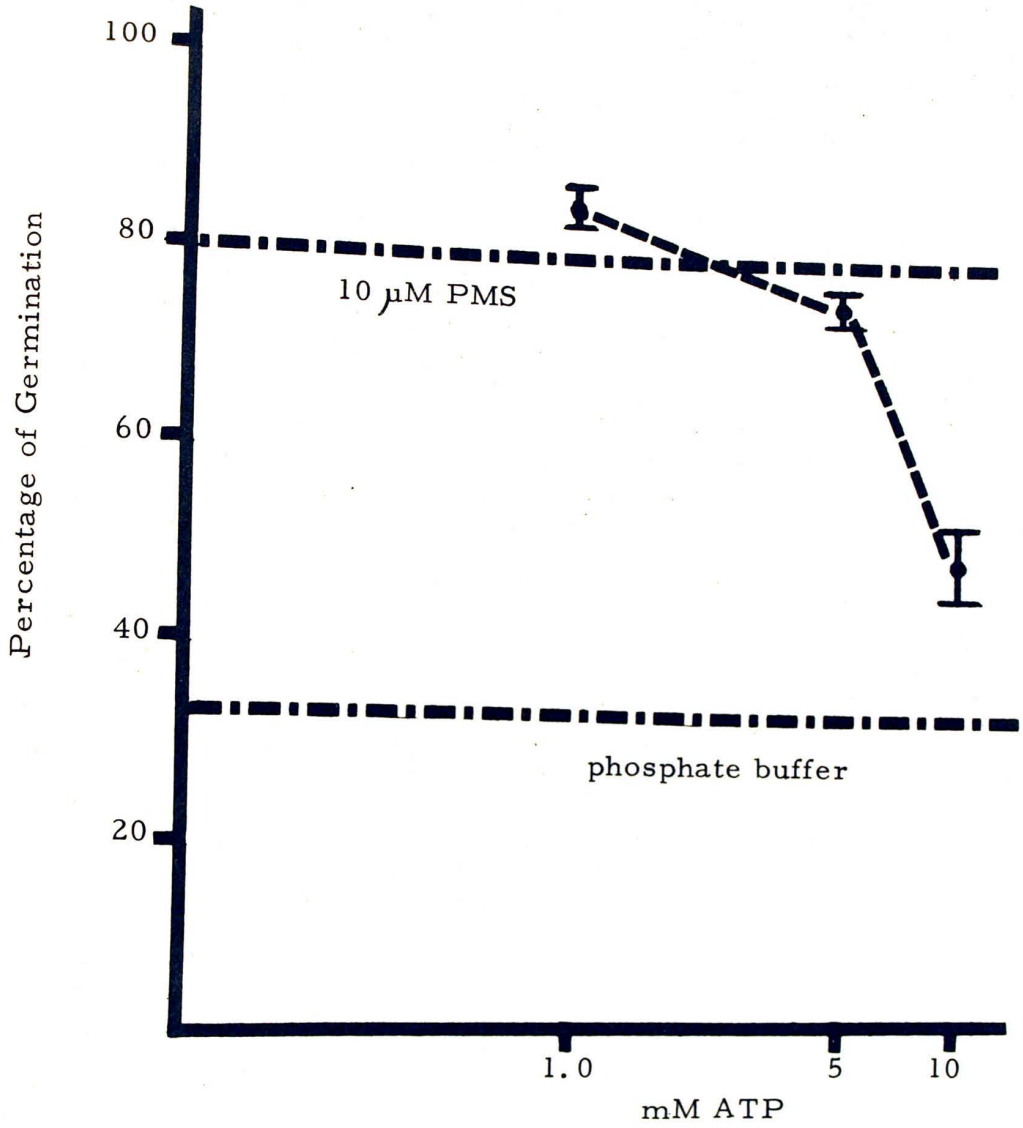


Figure 8. Effects of 24 hours of far-red irradiation, 10 μM phenazine methanosulfate, and ATP on germination in Lactuca sativa variety Mesa.

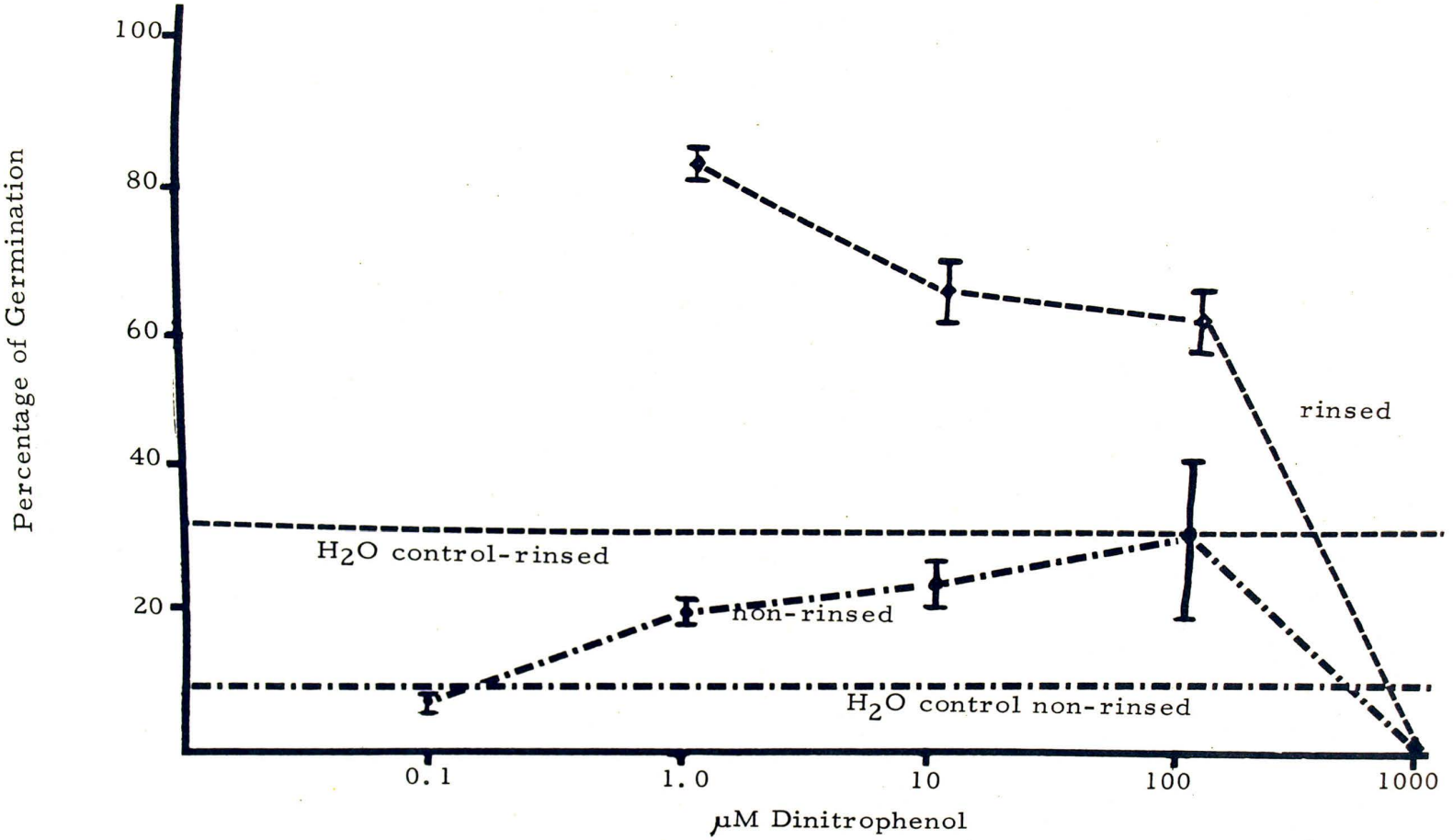


Figure 9. Effects of 24 hours of far-red irradiation in the presence of dinitrophenol on the germination in Lactuca sativa variety Mesa.

by an additional 5 more minutes of red light, germination responses were reversed again. Photoreversibility experiments were conducted using 1000 μM of dinitrophenol. The seeds were not rinsed in these testings. It was found that all germination responses were quite low regardless of the light treatment. Separate testings were then conducted with non-irradiated seeds to determine the effects of 1000 μM of dinitrophenol on seeds not having any light treatments. The results (Table II) indicate that 1000 μM of dinitrophenol is inhibitory to seeds that are totally dark grown and thus not irradiated.

Further studies were conducted to determine the combined effects of dinitrophenol and ATP on the induction of photosensitivity in the Mesa lettuce seeds. Results indicate that seeds irradiated with dinitrophenol and ATP concentrations between 0.1 and 5 mM had a significantly higher germination percentage than seeds irradiated solely with dinitrophenol (Figure 11). At the 10 mM level of ATP which had caused a reinstatement of the induction of photosensitivity in previous treatments with other inhibitors, there was not a significant reinstatement with ATP and dinitrophenol.

Experiments were conducted to investigate more thoroughly the effects of ATP singularly on the Mesa variety of seeds. Results indicated that a range of 0.01 to 5 mM ATP did not significantly influence the induction of photosensitivity (Figure 12). When additional studies with non-irradiated seeds were carried out, it was found that

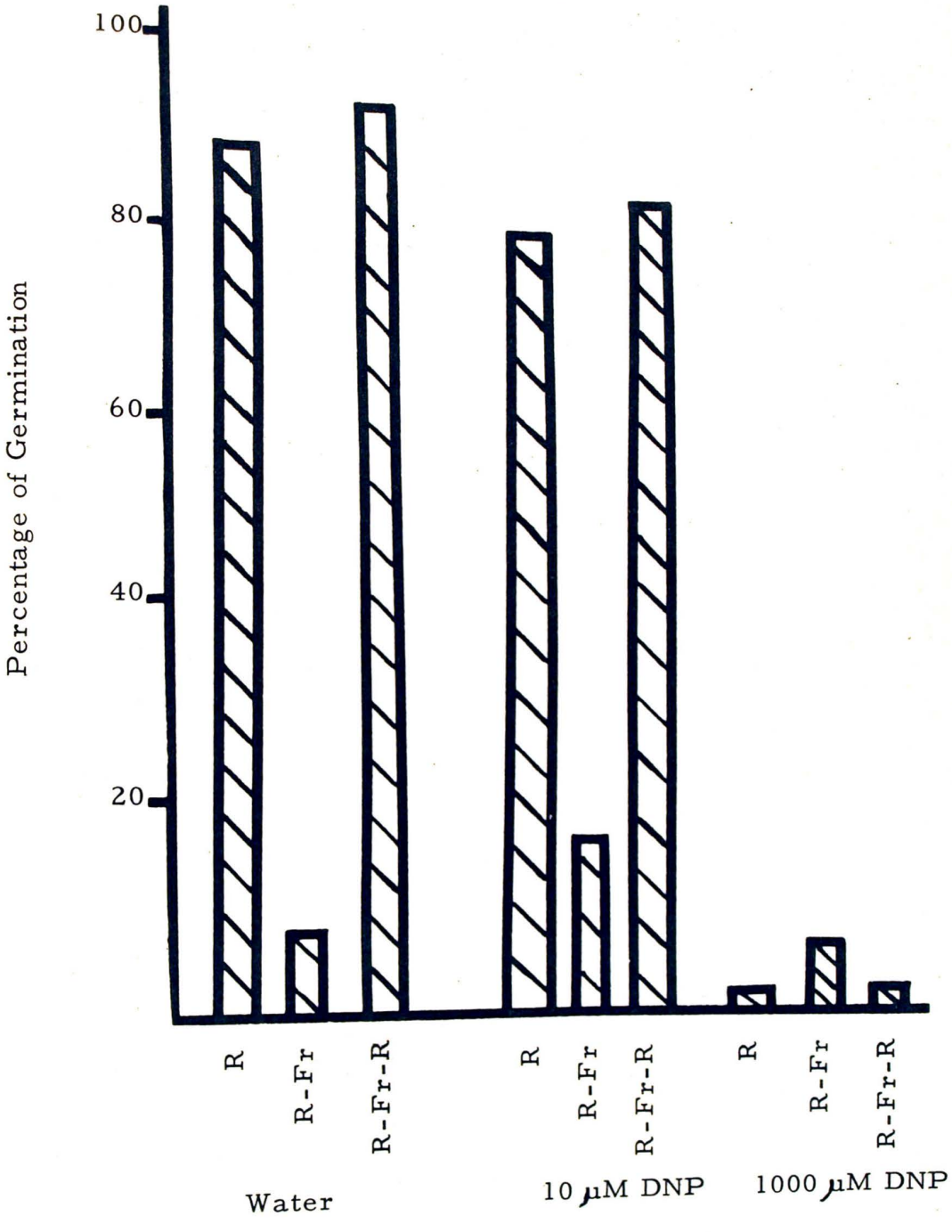


Figure 10. Effects of dinitrophenol on photoreversibility in non-rinsed seeds of Lactuca sativa variety Mesa.

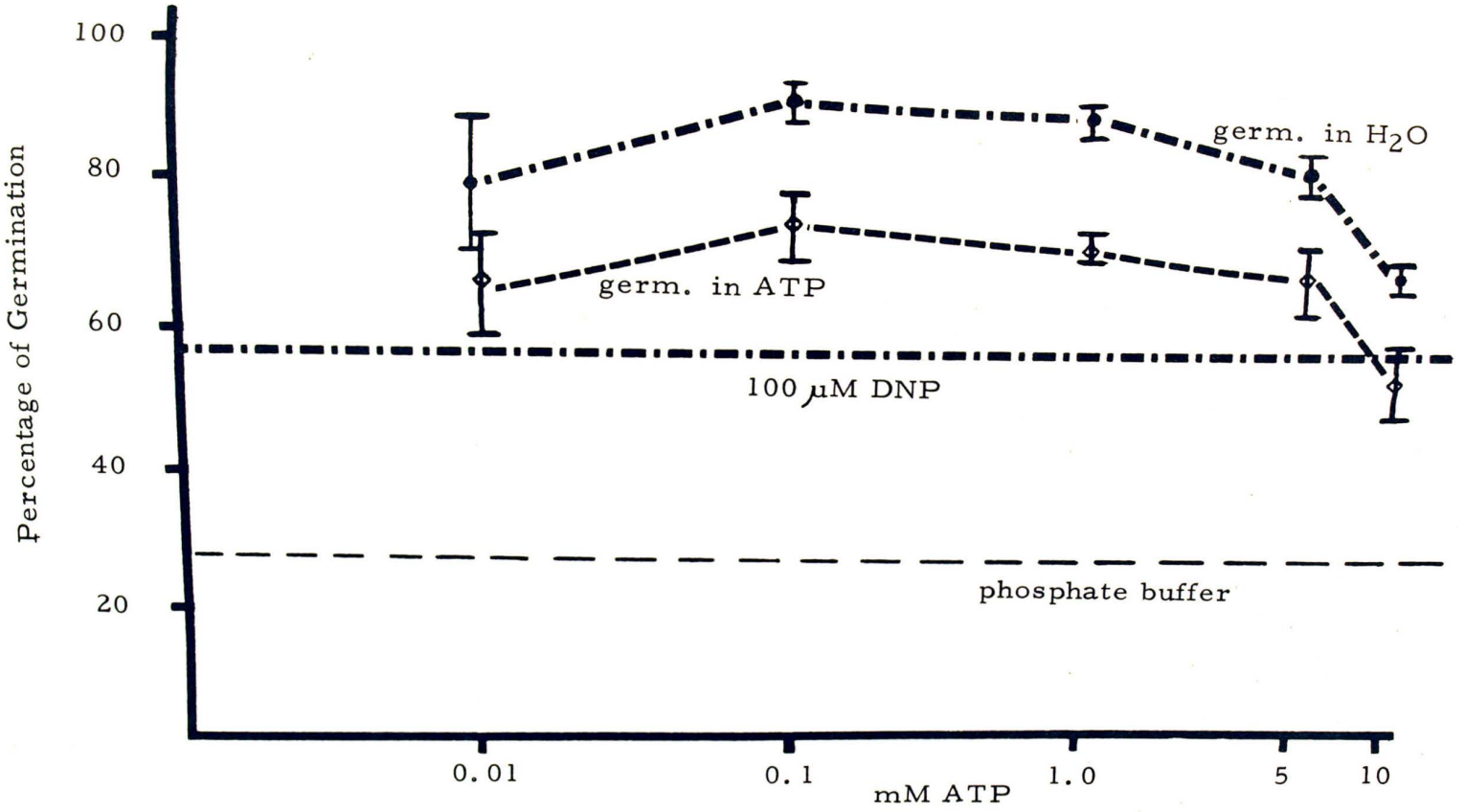


Figure 11. Effects of 24 hours of far-red irradiation, 100 μM dinitrophenol, and ATP variation on germination of Lactuca sativa variety Mesa.

Seeds were exposed to 24 hours of continuous far-red light in the presence of DNP and ATP. All seeds were rinsed. Some were allowed to germinate in H_2O and fresh ATP was added to others.

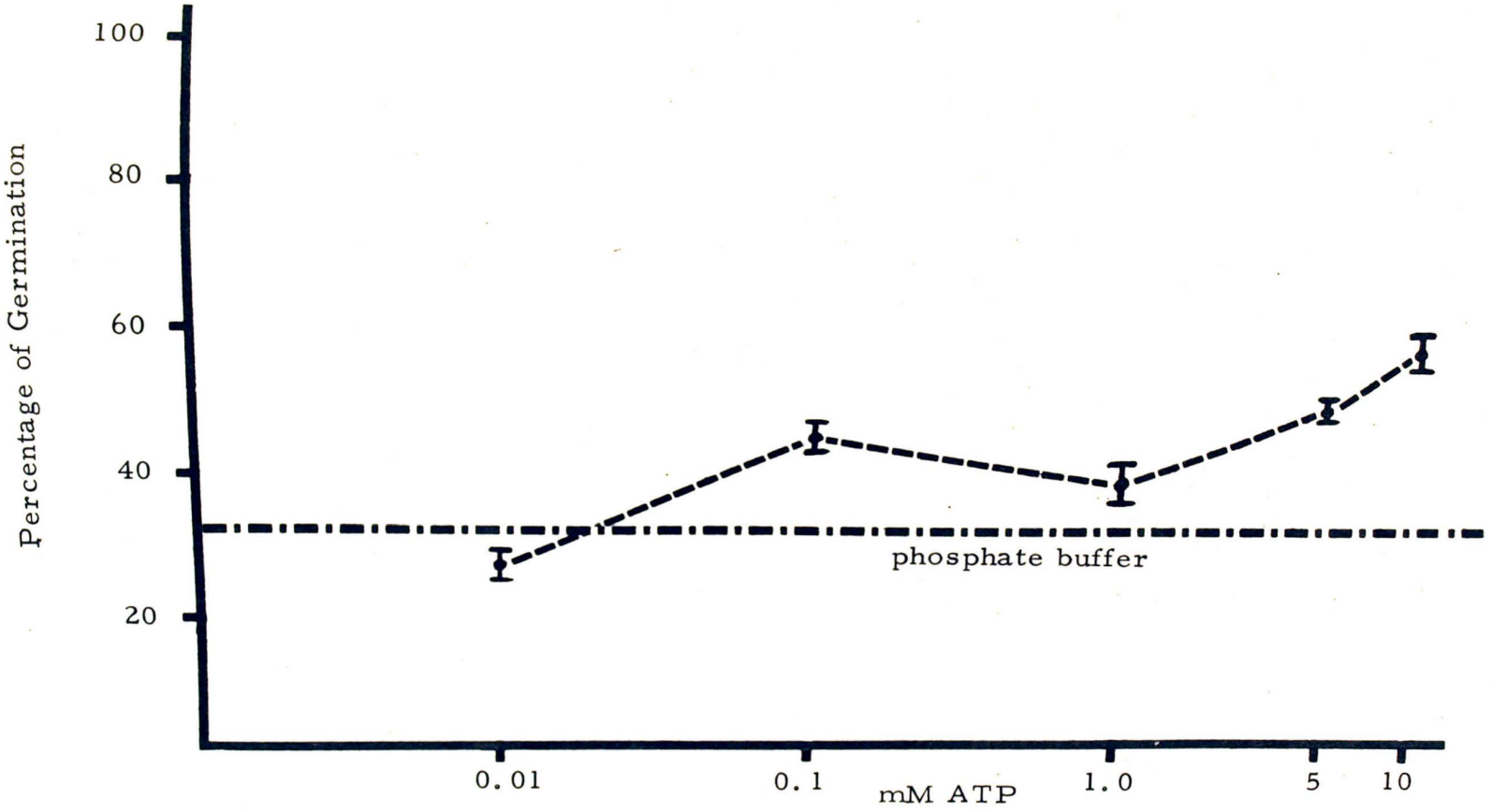


Figure 12. Effects of 24 hours of far-red irradiation and ATP on the germination of Lactuca sativa variety Mesa.

Seeds were exposed to 24 hours of continuous far-red light in the presence of ATP. All seeds were rinsed before the 48 hours of dark germination.

concentrations of 1.0-10 mM of ATP did not influence the germination percentages in the normally dark-germinating Mesa lettuce seeds (Table II).

Effects of Inhibitors and Inhibitors with ATP on Protein Synthesis in Mesa Lettuce Seeds

Seeds were exposed to 24 hours of far-red light in the presence of the various experimental solutions and ^{14}C-leucine. The results are shown in Table III. It was found that none of the various experimental solutions significantly influenced the ^{14}C-leucine incorporation into protein in the Mesa lettuce seeds.

TABLE III

^{14}C-LEUCINE INCORPORATION INTO PROTEIN IN MESA LETTUCE SEEDS IRRADIATED FOR 24 HOURS IN FAR-RED LIGHT

TREATMENT	^{14}C-LEUCINE COUNTS PER MINUTE INCORPORATION INTO PROTEIN	MILLIGRAMS OF PROTEIN IN SEEDS
Buffer	198	3.1
DCMU	180	3.0
DCMU + ATP	199	3.15
DNP	173	3.45
DNP + ATP	191	3.10
NH_2OH	166	3.0
NH_2OH + ATP	170	3.0
PMS	177	3.15
PMS + ATP	193	3.0
ATP	169	3.0

Seeds were exposed to 24 hours of far-red light in the presence of the various experimental solutions and 1 microcurie of ^{14}C-leucine and 0.1 ml of 10 mM penicillin. Seeds were treated in lots of 100 mg.

CHAPTER V

DISCUSSION

The data in Table I indicate that a photosensitivity can be induced in the seeds of Lactuca sativa variety Mesa. When seeds that germinate maximally in darkness are instead treated with 24 hours of continuous far-red irradiation, germination is severely inhibited. This same photosensitivity has been induced in Lactuca sativa variety New York (Mills, 1971). The data in Figure 1 indicate that a far-red, red photoreversibility response was induced in the Mesa lettuce seeds. If the 24 hours of continuous far-red irradiation is followed by 5 minutes of red irradiation, the percentage of germination is similar to that of the dark control seeds. When seeds are irradiated for 24 hours in the presence of far-red light followed by 5 minutes of red light, and a final 5 minutes of far-red light, germination percentages are very low. This photoreversibility phenomena agrees with data obtained in earlier experiments. Borthwick et al. (1954) found far-red light to inhibit germination and red light to promote germination. They also noted that red light could reverse far-red inhibition, and the germination was determined by the final light exposure. Schneider and Stimson (1972) and Rollin (1966) noted that responses which are regulated by phytochrome exhibit photoreversibility and these experimentors also

mentioned that photoresponses which show evidence of a 'High Energy Reaction' also have phytochrome reversibility. Thus in the Mesa lettuce seeds, it seems that the photoreversibility response is controlled by the pigment phytochrome.

When induced photosensitivity was further investigated in the Mesa lettuce seeds (Figure 2), it was shown that both gibberellic acid and thiourea could overcome the induced photodormancy. This data is supported by the work of Kahn (1960). He reported that gibberellic acid could substitute for red light in promoting germination in seeds that do not germinate in darkness because of far-red inhibition. Many other investigators (Poljakoff-Mayber, 1958; Haber and Luippold, 1960; Ikuma and Thimann, 1960; Black et al., 1971) have attempted to determine the mode of action which relates phytochrome to gibberellic acid, but the relationship remains unsolved. The data (Figure 2) indicate that thiourea can also overcome the induced photosensitivity. These findings are also in agreement with the work of other investigators (Pojakoff-Mayber, 1958; Haber and Luippold, 1960; Hendricks and Taylorson, 1975).

Several studies were conducted to investigate the effects of inhibitors of electron transport on the induction of photosensitivity. Studies with DCMU indicate that this inhibitor does influence the induction of photosensitivity (Figures 1, 2, 3). DCMU in 5 μM concentrations does not interfere with the response of the seeds to red light. However,

at concentrations of 50 μM DCMU, the response of the seeds to red light becomes less clearly defined. The response of Mesa lettuce seeds to continuous far-red light in the presence of DCMU reveals a reversal of the induction of photosensitivity (Figure 3). DCMU is a well-known inhibitor of electron transport in photosystem II of photosynthesis (Sargent and Taylor, 1972; Arnon et al., 1971). It acts as an artificial electron donor in the electron transport system of photosynthesis. Thus, it would seem possible that DCMU might also act as an artificial donor in some yet unknown electron transport system that activates germination. Another possibility has been suggested by Hendrick and Taylorson (1975). They suggested that hydrogen peroxide works in the electron transport system of the pentose pathway of glucose use and thereby arrests dormancy in seeds. It might be that DCMU or other inhibitors could function in this electron transport system. These ideas have yet to be thoroughly investigated.

In photosystem II the action of DCMU ultimately blocks the formation of ATP. Many investigators have determined that electron flow has been blocked by measuring the amount of ATP formed. In studies on the induced photosensitivity in the Mesa seeds additional ATP was added to the seeds during the treatment with far-red light and DCMU. It was noticed that 10 mM ATP is successful in reinstating the induction of photosensitivity in these lettuce seeds.

Another inhibitor hydroxylamine was used and it also caused

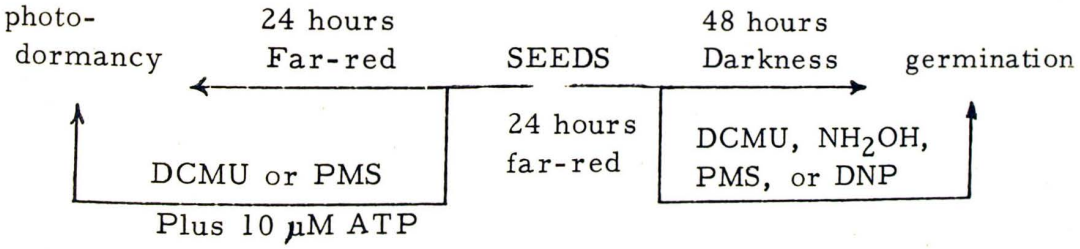
a reversal of the induction of photosensitivity. This inhibitor is known to serve as an electron donor in photosystem II of photosynthesis (Ort and Izawa, 1973). Other investigators have reported that it promotes germination in some seeds by inhibiting the formation of catalase enzymes which trigger an electron transport system (Hendricks and Taylorson, 1974, 1975). Thus, it would seem likely that hydroxylamine is not only an inhibitor of electron transport in photosystem II, but does indeed function in other electron transport systems. When seeds were exposed to 24 hours of far-red light, hydroxylamine, and ATP, it was noted that 10 μM ATP was not capable of reinstating the induction of photodormancy.

The inhibitor of electron transport in photosystem I of photosynthesis, phenazine methanosulfate was used to investigate the induction of photosensitivity. The data indicate that phenazine methanosulfate can cause a reversal of the induction of photodormancy (Figure 7). It would seem likely that this inhibitor can also influence germination by functioning in an electron transport system. In studies with 24 hours of far-red light, phenazine methanosulfate, and 10 μM ATP, the ATP caused a reinstatement of the induction of photosensitivity.

The experiments with dinitrophenol, an inhibitor of oxidative phosphorylation, indicate that it too can cause a reversal of the induction of photosensitivity in the Mesa lettuce seeds (Figure 9). Photo-

reversibility tests with dinitrophenol using 10 μM indicate that the photoreversibility response can still function. However, studies using 1000 μM indicate a severe inhibition of germination and no photoreversibility. These findings are supported by Schneider and Stimson (1972) who reported that phytochrome (Far-red) and photoreversibility were maintained in the presence of low amounts of dinitrophenol. In studies with 24 hours of far-red light, dinitrophenol, and ATP, it was generally noted that ATP reinforced the reversal of the induction of photosensitivity (Figure 11). It would seem likely that the mode of action of dinitrophenol differs from that of DCMU, hydroxylamine and phenazine methanosulfate.

The following model has been constructed to summarize the response of seeds of Lactuca sativa variety Mesa.



The investigation conducted on protein synthesis did not reveal that any of these inhibitors or inhibitors with ATP has a significant effect on the ^{14}C-leucine incorporation into protein in lettuce seeds.

CHAPTER VI

SUMMARY

This study was undertaken to 1) determine if a far-red photosensitivity could be induced in the dark germinating variety of Lactuca sativa variety Mesa; 2) investigate the responses of the photoinduced Mesa seeds to stimulators of germination such as gibberellic acid and thiourea; 3) study the effects of various inhibitors of electron transport on the induction of photosensitivity in the Mesa lettuce seeds; 4) observe the interrelationship of various inhibitors of electron transport and inhibitors plus adenosine triphosphate on protein synthesis and the induction of photosensitivity in the Mesa lettuce seeds.

The data indicate that a far-red photosensitivity can be induced in the dark germinating variety of Lactuca sativa variety Mesa. Gibberellic acid and thiourea can reverse the induced photosensitivity in these seeds. It was noted that various inhibitors of electron transport such as DCMU, hydroxylamine, phenazine methanosulfate, and dinitrophenol could cause a reversal of the induction of photosensitivity in the seeds. The presence of ATP in seeds treated with DCMU or phenazine methanosulfate allowed a reinstatement of the induction of photodormancy by continuous exposure to far-red light. However, it was also illustrated that dinitrophenol plus ATP can stimulate germination. These

findings could indicate that an electron transport system is involved in the regulation of germination in the Mesa lettuce seeds. The data from these studies also indicates that these inhibitors of electron transport do not appreciably effect protein synthesis in the Mesa lettuce seeds.

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