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The effects of some environmental factors on growth and control of northern nutgrass.

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THE EFFECTS OF SOME ENVIRONMENTAL FACTORS ON GROWTH AND CONTROL OF NORTHERN NUTGRASS

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BY

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THE EFFECTS OF SOME ENVIRONMENTAL FACTORS ON GROWTH AND CONTROL OF NORTHERN NUTGRASS

INTRODUCTION

Northern nutgrass (Cyperus esculentus L.) is considered to be one of the most serious perennial weeds of the Northeast. It is most troublesome in those fields planted to row crops such as corn, potatoes and vegetables, where in addition to crop losses through competition for nutrients, it renders harvest very difficult and often impossible. When once established, this weed is extremely difficult to eradicate. The earliest suggested control methods were taking the land out of production and disking continually for two or more years. While this treatment was effective in allowing the culture of crops for a year or so, the recovery of nutgrass excapees and their uncanny development during this short period ultimately rendered the fields useless for further culture of most row crops. Recent studies by Durfee (15) indicate that certain chemicals offer promise for this weed. These chemicals, however, are usually specific for a certain crop, and as yet a general control procedure has not been developed.

An ever increasing amount of work is being conducted with this weed to develop methods of control. Studies relating to growth rate of the plant, effects of environment on germination, and means of herbicide translocation within the plant are being conducted and will undoubtedly add to our basic knowledge of the plant. This increased familiarity with the plant may well increase the chances of an effective eradication measure being found.

REVIEW OF LITERATURE

Bailey (4) reports that Cyperus esculentus is common throughout North America. Although its common name, nutgrass, would indicate membership to the family Gramineae, it actually belongs to the sedges, classified as family Cyperaceae. Nutgrass is a stout leafy plant, developing up to three feet tall, bearing many oblong underground tubers which are generally accepted as the main means of reproduction. Seeds are also produced in a large terminal umbel which has long broad involucral leaves. Peduncles, of varying lengths, end in a spike, to the rachis of which are attached the spikelets in a sessile and squarrose manner. The achenes are oblong, obtuse and three angled.

It was first believed that the primary method of reproduction was by development of the tubers and that increase from seed was unimportant. Recent work by Durfee (15), and others has shown that the seed may be solely responsible for establishing new stands of this pest.

Inhibitor

It has been reported that some crop plants, when grown in the presence of certain weeds, do not perform as well as those in areas where the weed is absent. Kommedahl, Kotheimer, and Bernardini (26) discovered that alfalfa, flax, wheat, oats, and barley were affected adversely when grown in soil that was infested with quackgrass. The results of these experiments revealed a decrease in yield when water extracts of the comminuted rhizomes were added to the growing media. Germinating wheat grains produced roots but no plumules when a leachate from the quackgrass rhizomes was added to the soil.

Ohman, Lambert, and Kommedahl (31) reported on the effect of quackgrass residues on alfalfa. Growth of alfalfa was reduced where dried and

ground quackgrass rhizomes were added to the soil. Addition of ammonium nitrate resulted in less inhibition. Further investigations by Olman and Kommedahl (30) determined the relative toxicity of extracts from the vegetative organs of quackgrass to alfalfa. Hot water extracts of the roots, stems, rhizomes, and leaves reduced the seedling lengths of alfalfa by 65 to 80 per cent. The germination was reduced from 5 to 15 per cent. The extracts from the leaves and rhizomes were passed through activated carbon and found to be dissimilar. All of the toxicity was removed from the rhizome extracts while only part of it was removed from the leaf extracts, as measured by a bioassay with alfalfa.

Tourneau and Heggeness (39) characterized, to some extent, the inhibitors present in leafy spurge foliage and quackgrass rhizomes. A more generalized study by Tourneau, Failes, and Heggeness (38) showed that neither the pH nor osmotic pressure of the extracts is responsible for the inhibition. All of their inhibitors were present after autoclaving and were water soluble. In some cases, the inhibitory fraction was still present after ether extraction.

Tumbleson (40) worked with an inhibitor found in the tubers of yellow nutgrass where water extracts from the tubers inhibited germination of seeds of alfalfa, red clover, peas, soybeans, barley and oats. Extensive work revealed many of the chemical characteristics of this compound, but it was not identified.

Growth Studies

Land owners and research workers have recognized that the reproductive potential of nutgrass is tremendous. Such cultural practices as cultivation and harrowing disseminate the tubers in a field, and thereby aid the process

of infestation. Until recently, the rate of infestation under natural conditions was not known.

Tumbleson and Kommedahl (41, 42) have published data on the reproductive potential of nutgrass. They found that seven to nine tubers were produced per plant, each one terminating a rhizome. Each tuber in turn will produce up to seven shoots. These form many rhizomes, up to 40 cm. long, which end in new shoots or tubers. The majority of the tubers are produced in the upper six in. of the soil. Tubers planted 12 in. deep will, however, germinate and produce new plants.

Tubers were measured, and their diameter ranged from three to eleven mm., with a mean of seven mm. The average weight per tuber was 209 mg. The growth rate was determined by planting a single tuber in the field and recording the data. After one year, a single tuber produced 1900 plants and 6900 tubers in an area seven ft. in diameter and ten in. deep.

The type of soil determined to some extent the yield of tubers. In peat, they counted 823 tubers per square ft. to a depth of 18 in. Computing the yield per acre from this, the result shows 8.3 tons of tubers were produced. The yield was less on sandy loam and least in sand.

Light Studies

It has been known for a long time that the seed of some species require a certain amount of light before they will germinate. Toole, et. al. (35) reported that the germination of Lepidium virginicum, L. campestre, Sesymbrium officinale, Fragaria virginana, Verbascum thapsus, Nicotiana tabacum, and Brassica juncea was enhanced by exposure to red light and diurnal alteration of temperature. Toole, et.al. (36) reported that the seed of Pinus virginiana was also sensitive to light. Germination could be

promoted either by red light or imbibition of the seed at 5°C. This action was immediate and repeatedly reversible.

Typha latifolia L. will not germinate without light, and is sensitive to small differences at low intensity. Sifton (34) demonstrated that when the seed vials were wrapped in blue cellophane, the germination was reduced. Infra red light also acted as an inhibitor. He postulated that the inhibiting effect of the blue and green was due to their transmission of the infra red while shutting out the stimulating red. The swelling of the aleurone grains was more rapid and vigorous in the white or yellow light than in the blue or dark.

Jones and Bailey (25) worked with seeds of Laminum amplexicaule L. Far red radiation (7300 to 8700A) obtained from an incandescent lamp filtered through two layers of red and two layers of blue cellophane, inhibited seed germination. Red light offset this effect. They indicated that light sensitive seeds generally showed a greater sensitivity to one or more parts of the spectrum.

The most commonly studied light sensitive seed is lettuce. As early as 1934, Flint (17) observed that red, orange, and yellow light promoted seed germination, while violet, blue, and green inhibited it. He also changed non light sensitive seed into sensitive by exposing them to blue light.

The method for obtaining the various colors employed was through the use of colored cellophane. Miller (29) obtained red light by filtering the radiation from a standard daylight fluorescent tube through two sheets of DuPont red cellophane. This was exposed 205 cm. from the tube. Hagen, Borthwick, and Hendrick's (21) source of red radiation was a white fluo-

rescent tube with a cover of red cellophane that gave 75 per cent transmission above 6000A and less than 0.1 per cent transmission below 5600A.

Flint and McAlister (18) conducted a more detailed study and found regions of inhibition at 4400A, 4800A, and 7600A. He concluded that the effects obtained with colored cellophanes should not be interpretated without an analysis of the spectral transmission curve. Borthwick, et.al. (5) continued this type of work, with the action spectra for promotion and inhibition measured in detail for wave lengths greater than 4000A. The maximum sensitivity for promotion of germination was 6400-6700A (Red) and for inhibition, it was 7200-7500A. The blue portion of the spectrum was not as important as the red.

Amitrol and Autoradiographing

The use of amitrol (3-amino-1,2,4-triazole) as a herbicide has increased steadily in popularity and is now one of the more important herbicides. Yamaguchi and Crafts (44), Leonard (27), and others, have determined that this chemical has the characteristic of being readily mobile in the plant, making it useful in the control of perennial weeds, where a compound must be translocated to all parts of the plant in order to be effective.

The technique for studying the areas of translocation has been greatly simplified by the use of autoradiograms. The first biological application of autoradiograms occurred in 1904. Gradual progress in the development of this technique continued until World War II, when the resulting interest in fisson products and nuclear physics led to a vastly improved methodology.

Boyd (6) defines autoradiography as the production of a two or three dimensional image on a photographic film or plate by radioactive radiation. In translocation studies, the image appears as a blackened area on the film,

produced by low energy beta particles. Comar (8) indicated that five to ten million beta particles per square cm. must strike the photographic emulsion to produce the image. In general, the greater the concentration of isotope in the tissue, the more intense the image will be.

Gross or survey autoradiography is obtained when the entire plant containing the radioisotope is placed in contact with the photographic emulsion. This is exposed and developed to reveal the paths of translocation and distribution of the tracer within the plant.

Inselberg (24) has conducted work in the Horticultural field using radioactive phosphorous and potassium. His technique was slightly different than that adapted for studies using carbon. With the advent of C^{14}-labeled compounds, research on absorption and translocation of herbicides has greatly increased in scope. Carbon 14 has been incorporated into the molecule of several herbicides, including 2,4-D (2,4-Dichlorophenoxyacetic acid), 2,4,5-T (2,4,5-Trichlorophenoxyacetic acid), dalapon (2,2-Dichloropropionic acid), maleic hydrazide (1,2-Dihydropyridazine-3,6-Dione), and amitrol.

Crafts (10) reported on the absorption and translocation of 2,4-D by wild morning-glory. The amount of translocation was found to be a function of time, being directly proportional to the duration of the treatment. The physiological maturity of the plant was also important in affecting the extent and direction of herbicide movement. From the cotyledons, the movement was into the roots, while from the middle leaves, it was in both directions. There was no translocation out of the tip leaves. The treating of preblossoming plants resulted in only a downward movement, while there was no translocation in blossoming plants.

Saidak (33) determined with a direct counting procedure the effect of dalapon on yellow nutsedge. After leaf absorption, there was negligible basipetal movement of C^{14}, but there was excellent distribution after root absorption. There was very little C^{14} accumulation in the parent tuber. Further investigations revealed only a slight breakdown of dalapon occurred in the plant.

Crafts and Yamaguchi (13) compared the uptake and distribution of labeled herbicides by Zebrina and Tradescantia. Within four days after treatment, amitrol had accumulated in the growing tips. Translocation was in the xylem; movement being predominantly acropetal. The amount of movement was not dependent upon rapid food transport, so that late season treatments were effective.

Leonard and Weaver (28) studied the absorption and translocation of amitrol in the shoots of Tokay grape. Amitrol was readily absorbed by the leaves, but translocation was only apical when the flowers were in the pre-bloom stage. The bulk of translocation was from source to sink, but amitrol was not recoverable from the plant after three days of treatment.

Herrett and Linck (22) indicated that the plant species may have had some effect on the fate of amitrol after it was absorbed. A dilute solution of the chemical penetrated the leaf at a constant and rapid rate and could be detected in the thistle plant 20 days after application; the chemical was metabolized at a more rapid rate in field bindweed, however, for after a similar period, its presence could not be detected by the chromatographic technique.

Studies by Crafts (11) on the comparative mobility of labeled herbicides showed that amitrol moves fairly freely throughout the plant. He

believed that the chemical enters the phloem and moves in the assimilate stream and into the roots where it accumulates in their tips, due to the active assimilate utilization. This point was also studied earlier by Crafts, Currier and Drever (12).

Freed and Montgomery (19) studied the influence of surfactants on the absorption of amitrol by bean plants. They determined that the reduction of surface tension, by the use of specific surfactants, was an important factor in enhancing absorption and translocation. Dybing and Currier (16) found that chemicals enter the leaf stomata in bulk, rather than moving through the cuticle and walls of the guard cells. All of the surfactants tested increased stomatal penetration.

Yamaguchi and Crafts (43) have summarized the methods involved in the autoradiographic study of absorption and translocation of C^{14}-labeled herbicides. Their experience with various plants, including Cyperus rotundus, indicated that between 0.5 and 1.0 microcurie, with an activity of 0.5 to 1.0 millicurie per millimole, would give adequate results for translocation studies. There are several methods of handling the specimens after the treatment period, but each has its weak point. Crafts (9) considered the process used in drying the plants for autoradiography very important. Toth and Romney (37) compared the dry press, dry ice, freeze-dry, and propylene glycol methods, and determined that the method of preparation did not affect distribution or the interpretation of results.

Anderson (2) worked with the absorption and translocation of amitrol in Cyperus rotundus L. His treatment consisted of 20 μl of one μc activity in a 8450 ppm amitrol solution. A series of treatments involving various times showed that accumulation in meristematic tissue, such as the

root tip and sprouts, increases with the passage of time. From this he concluded that amitrol moves in the plant with the photosynthetic food stream. The translocation was much faster than penetration, and this latter characteristic was considered to be the limiting factor in the process involving the distribution of the chemical from the surface of the leaf into the plant system.

Donnalley and Rahn (14) traced the translocation of amitrol, atrazine (2-chloro-4,ethylamino-6,isopropyl-amino-S-triazine), dalapon, and EPTC (N,N-di-n-propylthiolcarbamate) in Cyperus esculentus L. Amitrol was applied to the foliage and autoradiograms later revealed the presence of amitrol in the tubers. Later work showed that the viability of such treated tubers was reduced significantly. Of the various chemicals used, only amitrol showed any basipetal movement following a foliar application.

Studies have been conducted to determine the mode of action of amitrol in the plant, but the results indicate that it is rapidly metabolized to other compounds. Carter and Naylor (7) obtained 13 different compounds produced from the applied C^{14}-labeled amitrol. The principle substance, Compound "1", must have a phytotoxic action, since amitrol could not be recovered from the meristem where symptoms are usually noted. Various methods described by Aldrich and McLane (1), Green and Feinstein (20), Racussen (32), and Herrett and Linck (23) have been used to detect amitrol in plant tissue.

MATERIALS AND METHODS

Inhibitor

Mature nutgrass tubers were gathered from the field and stored at room temperature until used. Plants grown in the greenhouse produced tubers that matured during the winter, and these were also used in the following experiments. The tubers were comminuted in a Waring Blender with water, and this mixture was then filtered. Twelve ml. of the resulting extract was used to moisten the germination blotters. Water was used as a moistening agent for the check treatment.

In the bioassay tests, oats were used as the indicator crop. Four replications, of twenty-five seeds each, were used for each treatment. They were germinated in alternating environments of 8 hours of light at 35°C and 16 hours of darkness at 20°C. After ten days, data were taken on the stem length, root length, and number of roots per seed.

Growth Studies

The experiment was started on July 14, 1960 when seed were placed in a 20°-35°C germinator. On July 29, 1960, after the seeds had germinated and the seedlings were three-quarters of an inch tall, they were removed from the germinator. These were planted individually in a field of Scarborough very fine sandy loam of moderate fertility in 40 plots, each four feet square. The pH of the soil was almost 5.8.

On August 9, 1960, an additional 80 seedlings were planted in a similar manner. Twenty plots were seeded directly at the rate of 20 seeds per plot. All plots were watered lightly and a "Hot-Cap" was placed over each "hill" to prevent damage from wind and rain.

Light Studies

Tests were conducted in the University of Massachusetts Seed Laboratory to determine the effect of light quality and quantity on the germination of nutgrass seed. Four hundred seeds from each of four separate lots, harvested at different stages of plant maturity, were subjected to each test in the experiment.

The seed from all treatments was alternated between 8 hours under the appropriate light treatments at 30°C and 16 hours of darkness at 20°C. The amount of water used to moisten the germination blotters was kept constant.

Various qualities of light were obtained by the use of DuPont colored cellophane. A glass enclosed germinator was used, and the light source was suspended 22 in. above it. The colored cellophane was placed over the glass top and the light allowed to pass through it.

Light quantity was provided with Sylvania F42T12/D Daylight Instant Start forty-two in. fluorescent tubes. Three different intensities were obtained for each color treatment by the use of two, four, and eight tubes. The intensity for each treatment and number of bulbs was then measured in foot candles at the site of seed germination with the aid of a photo electric exposure meter.

The treatments with light included the following colors: red, pink, light yellow, dark yellow, light green, dark green, orchid, light blue, dark blue, red plus dark blue, and clear. The intensity of the light transmitted through these cellophanes varied greatly with the color. The check treatment received 100 per cent of the emitted light.

The spectral energy distribution curve of the lamps, provided by the Sylvania Company, was used to define the conditions under which germination

occurred. This was presented in graphic form and it showed the amount of energy given off for each wave length of the visible spectrum. The spectrum was divided into units of 200 A, and the area per unit under the curve measured with a planimeter. By determining the total area beneath the curve, the per cent of the area occupied by the 200 A unit was calculated, giving the per cent of total light emitted.

The DuPont Company provided data on the spectral characteristics of each of the cellophanes used. This data gave the per cent of light transmission through the cellophane at the various wave lengths. By multiplying the per cent of the total light emitted by the per cent of light transmitted through the cellophane, the per cent of the emitted light that was transmitted to the seed was found. This was then converted to a one hundred per cent basis. The data for each treatment is listed in Tables 1 through 11.

The number of foot candles of each wave band transmitted to the seed was calculated from a knowledge of the total number of foot candles exposed to the seed, as measured by the light meter, and the per cent of transmitted light for each 200 A wave band. This is shown in Figures 1 to 11 for each of the color treatments and light intensities. Each graph also illustrates the results of the effect of the light quantity factor. The separate curves, A, B, and C, represent the number of tubes used for each treatment, eight, four, and two tubes respectively. The total intensity in foot candles is listed for each curve. The per cent germination shown is the mean of the four seed lots tested at each light setting.

TABLE I

Light Quality and Light Quantity Distribution

of DuPont Light Green Cellophane

Wave Length (Ang-(stroms)	Plani-Meter Reading	% Of Total Light Emitted	% Of Light Transmitted Through Cellophane	% Of Emitted That Trans-mitted To Seed	% Of Trans-mitted Light
29-3100	.002	.13	68	.0884	.1276
31-3300	.004	.26	66	.1716	.2477
33-3500	.007	.456	64	.2918	.4213
35-3700	.012	.781	58	.4530	.6540
37-3900	.023	1.498	39	.5842	.8434
39-4100	.041	2.671	18	.4803	.6941
41-4300	.074	4.82	17	.8194	1.1829
43-4500	.115	7.491	36	2.6968	3.8932
45-4700	.132	8.599	60	5.1594	7.4482
47-4900	.132	8.599	79	6.7932	9.8068
49-5100	.123	8.013	86	6.8912	9.9483
51-5300	.112	7.296	86	6.2746	9.0582
53-5500	.128	8.338	84	7.0039	10.1110
55-5700	.151	9.837	81	7.9680	11.5028
57-5900	.161	10.488	77	8.0758	11.6584
59-6100	.141	9.185	74	6.7969	9.8122
61-6300	.091	5.929	74	4.3875	6.3339
63-6500	.047	3.061	76	2.3264	3.3585
65-6700	.024	1.563	76	1.1879	1.7149
67-6900	.013	.846	83	.7022	1.0137
69-7100	.002	.13	90	.1170	.1689
Total	1.535	99.991		69.2700	100.0000

TABLE II

Light Quality and Light Quantity Distribution

of DuPont Dark Yellow Cellophane

Wave Length (Angstroms)	Photometer Reading	% Of Total Light Emitted	% Of Light Transmitted Through Cellophane	% Of Emitted That Transmitted To Seed	% Of Transmitted Light
29-3100	.002	.13	40	.0624	.1265
31-3300	.004	.26	45	.1170	.2372
33-3500	.007	.456	35	.1596	.3235
35-3700	.012	.781	4	.0312	.0632
37-3900	.023	1.498	-	-	-
39-4100	.041	2.671	-	-	-
41-4300	.074	4.82	-	-	-
43-4500	.115	7.491	-	-	-
45-4700	.132	8.599	-	-	-
47-4900	.132	8.599	-	-	-
49-5100	.123	8.013	27	2.1635	4.3854
51-5300	.112	7.296	62	4.5235	9.1692
53-5500	.128	8.338	73	6.0867	12.3378
55-5700	.151	9.837	78	7.6729	15.5551
57-5900	.161	10.488	88	9.2294	18.7081
59-6100	.141	9.185	92	8.4502	17.1287
61-6300	.091	5.929	94	5.5733	11.2972
63-6500	.047	3.061	94	2.8773	5.8323
65-6700	.024	1.563	94	1.4692	2.9781
67-6900	.013	.846	94	.7952	1.6119
69-7100	.002	.13	94	.1222	.2477
Total	1.535	99.991		49.3336	100.0000

TABLE III

Light Quality and Light Quantity Distribution

of DuPont Pink Cellophane

Wave Length (Ang-(stroms)	Plani-Meter Reading	% Of Total Light Emitted	% Of Light Transmitted Through Cellophane	% Of Emitted That Trans-mitted To Seed	% Of Trans-mitted Light
29-3100	.002	.13	77	.1001	.1186
31-3300	.004	.26	84	.2184	.2587
33-3500	.007	.456	87	.3967	.4699
35-3700	.012	.781	86	.6717	.7956
37-3900	.023	1.498	85	1.2733	1.5082
39-4100	.041	2.671	84	2.2436	2.6574
41-4300	.074	4.82	86	4.1452	4.9098
43-4500	.115	7.491	88	6.5921	7.8080
45-4700	.132	8.599	87	7.4811	8.8610
47-4900	.132	8.599	83	7.1372	8.4536
49-5100	.123	8.013	77	6.1700	7.3080
51-5300	.112	7.296	73	5.3261	6.3085
53-5500	.128	8.338	74	6.1701	7.3082
55-5700	.151	9.837	78	7.6729	9.0881
57-5900	.161	10.488	89	9.3343	11.0559
59-6100	.141	9.185	93	8.5421	10.1177
61-6300	.091	5.929	95	5.6326	6.6715
63-6500	.047	3.061	95	2.9080	3.4444
65-6700	.024	1.563	95	1.4849	1.7588
67-6900	.013	.846	95	.8037	.9519
69-7100	.002	.13	95	.1235	.1463
Total	1.535	99.991		84.4276	100.0000

TABLE IV

Light Quality and Light Quantity Distribution

of DuPont Clear Cellophane

Wave Length (Ang-(stroms)	Plani-Meter Reading	% Of Total Light Emitted	% Of Light Transmitted Through Cellophane	% Of Emitted That Trans-mitted To Seed	% Of Trans-mitted Light
29-3100	.002	.13	85	.1105	.1177
31-3300	.004	.26	89	.2314	.2465
33-3500	.007	.456	91	.4150	.4420
35-3700	.012	.781	92	.7185	.7653
37-3900	.023	1.498	92	1.3782	1.4680
39-4100	.041	2.671	93	2.4840	2.6459
41-4300	.074	4.82	94	4.5308	4.8261
43-4500	.115	7.491	94	7.0415	7.5005
45-4700	.132	8.599	94	8.0831	8.6099
47-4900	.132	8.599	94	8.0831	8.6099
49-5100	.123	8.013	94	7.5322	8.0232
51-5300	.112	7.296	94	6.8582	7.3052
53-5500	.128	8.338	94	7.8377	8.3486
55-5700	.151	9.837	94	9.2468	9.8495
57-5900	.161	10.488	94	9.8587	10.5013
59-6100	.141	9.185	94	8.6339	9.1967
61-6300	.091	5.929	94	5.5733	5.9366
63-6500	.047	3.061	94	2.8773	3.0648
65-6700	.024	1.563	94	1.4692	1.5650
67-6900	.013	.846	94	.7952	.8470
69-7100	.002	.13	94	.1222	.1302
Total	1.535	99.991		93.8808	100.0000

TABLE V

Light Quality and Light Quantity Distribution

of DuPont Orchid Cellophane

Wave Length (Ang-(stroms)	Plani-Meter Reading	% Of Total Light Emitted	% Of Light Transmitted Through Cellophane	% Of Emitted That Trans-mitted To Seed	% Of Trans-mitted Light
29-3100	.002	.13	81	.1053	.1278
31-3300	.004	.26	84	.2184	.2646
33-3500	.007	.456	87	.3967	.4806
35-3700	.012	.781	88	.6873	.8326
37-3900	.023	1.498	89	1.3332	1.6152
39-4100	.041	2.671	90	2.4039	2.9124
41-4300	.074	4.82	91	4.3862	5.3141
43-4500	.115	7.491	91	6.8168	8.2588
45-4700	.132	8.599	90	7.7391	9.3762
47-4900	.132	8.599	88	7.5671	9.1679
49-5100	.123	8.013	83	6.6508	8.0577
51-5300	.112	7.296	79	5.7638	6.9831
53-5500	.128	8.338	75	6.2535	7.5764
55-5700	.151	9.837	72	7.0826	8.5809
57-5900	.161	10.488	75	7.8660	9.5299
59-6100	.141	9.185	77	7.0725	8.5686
61-6300	.091	5.929	85	5.0397	6.1058
63-6500	.047	3.061	91	2.7855	3.3748
65-6700	.024	1.563	93	1.4536	1.7611
67-6900	.013	.846	94	.7952	.9634
69-7100	.002	.13	94	.1222	.1481
Total	1.535	99.991		82.5394	100.0000

TABLE VI

Light Quality and Light Quantity Distribution

of the Check Treatment

Wave Length (Ang-(stroms)	Plani-Meter Reading	% Of Total Light Emitted	% Of Light Transmitted to the Seed	% Of Emitted That Trans-mitted To Seed
29-3100	.002	.13	100	.13
31-3300	.004	.26	100	.26
33-3500	.007	.456	100	.456
35-3700	.012	.781	100	.781
37-3900	.023	1.498	100	1.498
39-4100	.041	2.671	100	2.671
41-4300	.074	4.82	100	4.82
43-4500	.115	7.491	100	7.491
45-4700	.132	8.599	100	8.599
47-4900	.132	8.599	100	8.599
49-5100	.123	8.013	100	8.013
51-5300	.112	7.296	100	7.296
53-5500	.128	8.338	100	8.338
55-5700	.151	9.837	100	9.837
57-5900	.161	10.488	100	10.488
59-6100	.141	9.185	100	9.185
61-6300	.091	5.929	100	5.929
63-6500	.047	3.061	100	3.061
65-6700	.024	1.563	100	1.563
67-6900	.013	.846	100	.846
69-7100	.002	.13	100	.13
Total	1.535	99.991		99.991

TABLE VII

Light Quality and Light Quantity Distribution

of DuPont Light Yellow Cellophane

Wave Length (Ang-(stroms)	Plani-Meter Reading	% Of Total Light Emitted	% Of Light Transmitted Through Cellophane	% Of Emitted That Trans-mitted To Seed	% Of Trans-mitted Light
29-3100	.002	.13	74	.0962	.1515
31-3300	.004	.26	72	.1872	.2947
33-3500	.007	.456	67	.3055	.4810
35-3700	.012	.781	52	.4061	.6394
37-3900	.023	1.498	24	.3595	.5660
39-4100	.041	2.671	-	-	-
41-4300	.074	4.82	-	-	-
43-4500	.115	7.491	-	-	-
45-4700	.132	8.599	8	.6879	1.0830
47-4900	.132	8.599	36	3.0956	4.8738
49-5100	.123	8.013	71	5.6892	8.9572
51-5300	.112	7.296	87	6.3475	9.9936
53-5500	.128	8.338	93	7.7543	12.2085
55-5700	.151	9.837	94	9.2468	14.5584
57-5900	.161	10.488	94	9.8587	15.5217
59-6100	.141	9.185	94	8.6339	13.5934
61-6300	.091	5.929	94	5.5733	8.7747
63-6500	.047	3.061	94	2.8773	4.5301
65-6700	.024	1.563	94	1.4692	2.3131
67-6900	.013	.846	95	.8037	1.2654
69-7100	.002	.13	95	.1235	.1944
Total	1.535	99.991		63.5154	100.0000

TABLE VIII

Light Quality and Light Quantity Distribution

of DuPont Light Blue Cellophane

Wave Length (Ang-(stroms)	Plani-Meter Reading	% Of Total Light Emitted	% Of Light Transmitted Through Cellophane	% Of Emitted That Trans-mitted To Seed	% Of Trans-mitted Light
29-3100	.002	.13	71	.0923	.1343
31-3300	.004	.26	67	.1742	.2535
33-3500	.007	.456	70	.3192	.4646
35-3700	.012	.781	82	.6404	.9320
37-3900	.023	1.498	85	1.2733	1.8532
39-4100	.041	2.671	85	2.2704	3.3044
41-4300	.074	4.82	85	4.0970	5.9628
43-4500	.115	7.491	86	6.4423	9.3762
45-4700	.132	8.599	86	7.3951	10.7629
47-4900	.132	8.599	85	7.3092	10.6379
49-5100	.123	8.013	82	6.5707	9.5631
51-5300	.112	7.296	78	5.6909	8.2826
53-5500	.128	8.338	71	5.9199	8.6159
55-5700	.151	9.837	61	6.0006	8.7334
57-5900	.161	10.488	53	5.5586	8.0901
59-6100	.141	9.185	42	3.8577	5.6145
61-6300	.091	5.929	42	2.4902	3.6243
63-6500	.047	3.061	43	1.3162	1.9156
65-6700	.024	1.563	44	.6877	1.0009
67-6900	.013	.846	59	.4991	.7264
69-7100	.002	.13	80	.1040	.1514
Total	1.535	99.991		68.7090	100.0000

TABLE IX

Light Quality and Light Quantity Distribution

of DuPont Red Cellophane

Wave Length (Ang-(stroms)	Plani-Meter Reading	% Of Total Light Emitted	% Of Light Transmitted Through Cellophane	% Of Emitted That Trans-mitted To Seed	% Of Trans-mitted Light
29-3100	.002	.13	-	-	-
31-3300	.004	.26	-	-	-
33-3500	.007	.456	-	-	-
35-3700	.012	.781	-	-	-
37-3900	.023	1.498	-	-	-
39-4100	.041	2.671	-	-	-
41-4300	.074	4.82	-	-	-
43-4500	.115	7.491	-	-	-
45-4700	.132	8.599	-	-	-
47-4900	.132	8.599	-	-	-
49-5100	.123	8.013	-	-	-
51-5300	.112	7.296	-	-	-
53-5500	.128	8.338	-	-	-
55-5700	.151	9.837	-	-	-
57-5900	.161	10.488	1	.1048	.6267
59-6100	.141	9.185	66	6.0621	36.2211
61-6300	.091	5.929	90	5.3361	31.8832
63-6500	.047	3.061	93	2.8467	17.0090
65-6700	.024	1.563	94	1.4692	8.7785
67-6900	.013	.846	94	.7952	4.7513
69-7100	.002	.13	94	.1222	.7301
Total	1.535	99.991		16.7364	100.0000

TABLE X

Light Quality and Light Quantity Distribution

of DuPont Dark Green Cellophane

Wave Length (Angstroms)	Planck-Meter Reading	% Of Total Light Emitted	% Of Light Transmitted Through Cellophane	% Of Emitted That Transmitted To Seed	% Of Transmitted Light
29-3100	.002	.13	4	.0052	.0338
31-3300	.004	.26	-	-	-
33-3500	.007	.456	-	-	-
35-3700	.012	.781	-	-	-
37-3900	.023	1.498	-	-	-
39-4100	.041	2.671	-	-	-
41-4300	.074	4.82	-	-	-
43-4500	.115	7.491	-	-	-
45-4700	.152	8.909	-	-	-
47-4900	.152	8.909	27	2.3217	15.1000
49-5100	.123	8.013	50	4.0065	26.0629
51-5300	.112	7.296	50	3.6480	23.7303
53-5500	.108	8.938	40	3.3352	21.6960
55-5700	.151	9.837	20	1.9674	12.7903
57-5900	.161	10.488	-	-	-
59-6100	.141	9.185	-	-	-
61-6300	.091	5.929	-	-	-
63-6500	.047	3.061	-	-	-
65-6700	.034	1.563	-	-	-
67-6900	.013	.846	1	.0169	.1099
69-7100	.007	.13	53	.0715	.4631
Total	1.535	99.991		15.3724	100.0000

TABLE XI

Light Quality and Light Quantity Distribution

of DuPont Dark Blue Cellophane

Wave Length (Ang-(stroms)	Plani-Meter Reading	% Of Total Light Emitted	% Of Light Transmitted Through Cellophane	% Of Emitted That Trans-mitted To Seed	% Of Trans-mitted Light
29-3100	.002	.13	36	.0468	.1432
31-3300	.004	.26	9	.0234	.0716
33-3500	.007	.456	9	.0410	.1255
35-3700	.012	.781	57	.4452	1.3624
37-3900	.023	1.498	71	1.0636	3.2549
39-4100	.041	2.671	70	1.8697	5.7218
41-4300	.074	4.82	70	3.3740	10.3253
43-4500	.115	7.491	72	5.3935	16.5055
45-4700	.132	8.599	70	6.0193	18.4206
47-4900	.132	8.599	66	5.6753	17.3679
49-5100	.123	8.013	54	4.3270	13.2417
51-5300	.112	7.296	59	2.8454	8.7077
53-5500	.128	8.338	18	1.5008	4.5928
55-5700	.151	9.837	-	-	-
57-5900	.161	10.488	-	-	-
59-6100	.141	9.185	-	-	-
61-6300	.091	5.929	-	-	-
63-6500	.047	3.061	-	-	-
65-6700	.024	1.563	-	-	-
67-6900	.013	.846	-	-	-
69-7100	.002	.13	40	.0520	.1591
Total	1.535	99.991		32.6770	100.0000

Figure 1. The Distribution of Light Quality and Light Quantity from Light Green and Its Effect on Germination.

Curve	Intensity	% Germination
A	160 Foot Candles	67
B	78 " "	59
C	36 " "	67

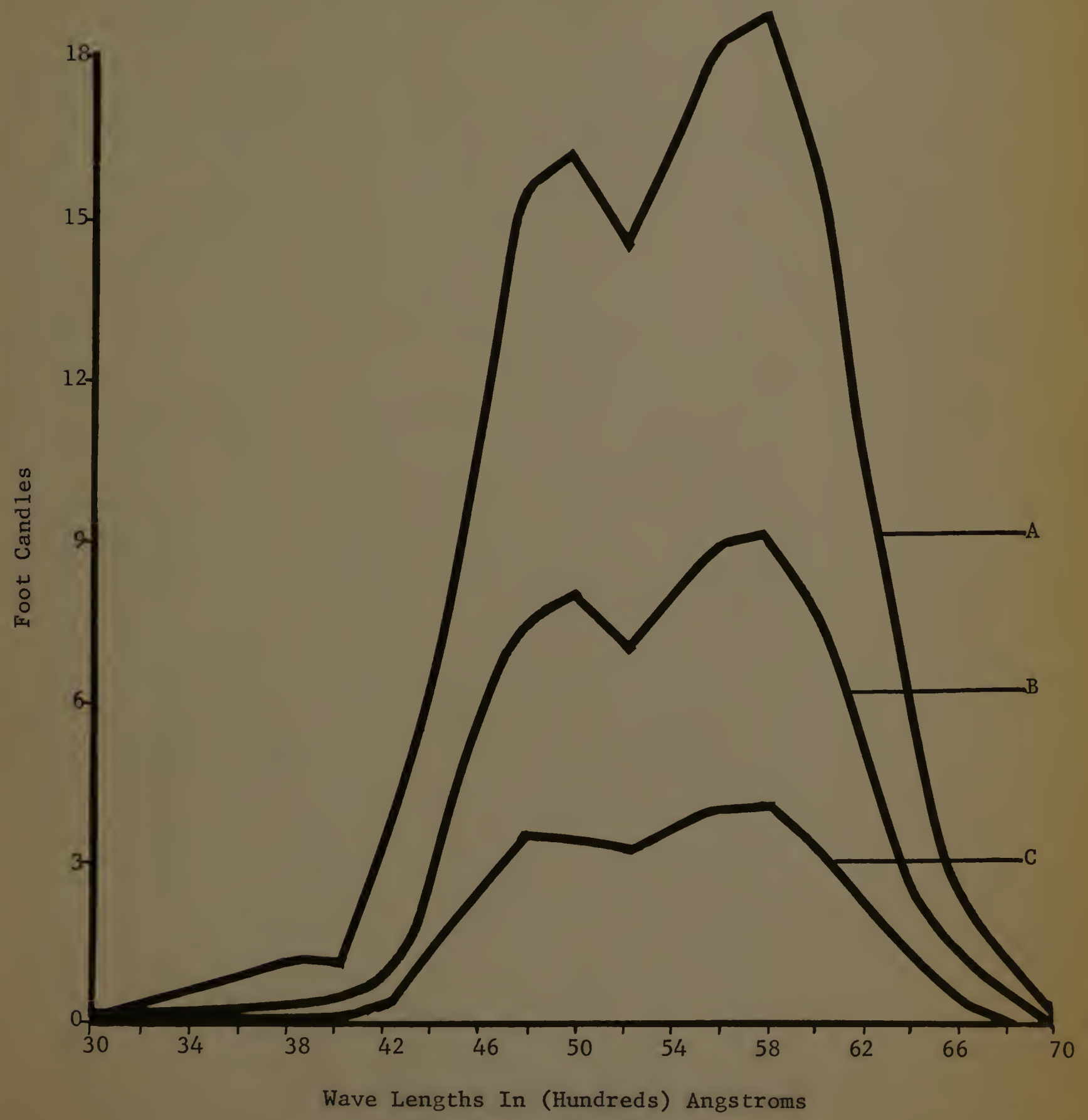


Figure 2. The Distribution of Light Quality and Light Quantity from Dark Yellow and Its Effect on Germination.

Curve	Intensity	% Germination
A	140 Foot Candles	65
B	76 " "	59
C	35 " "	64

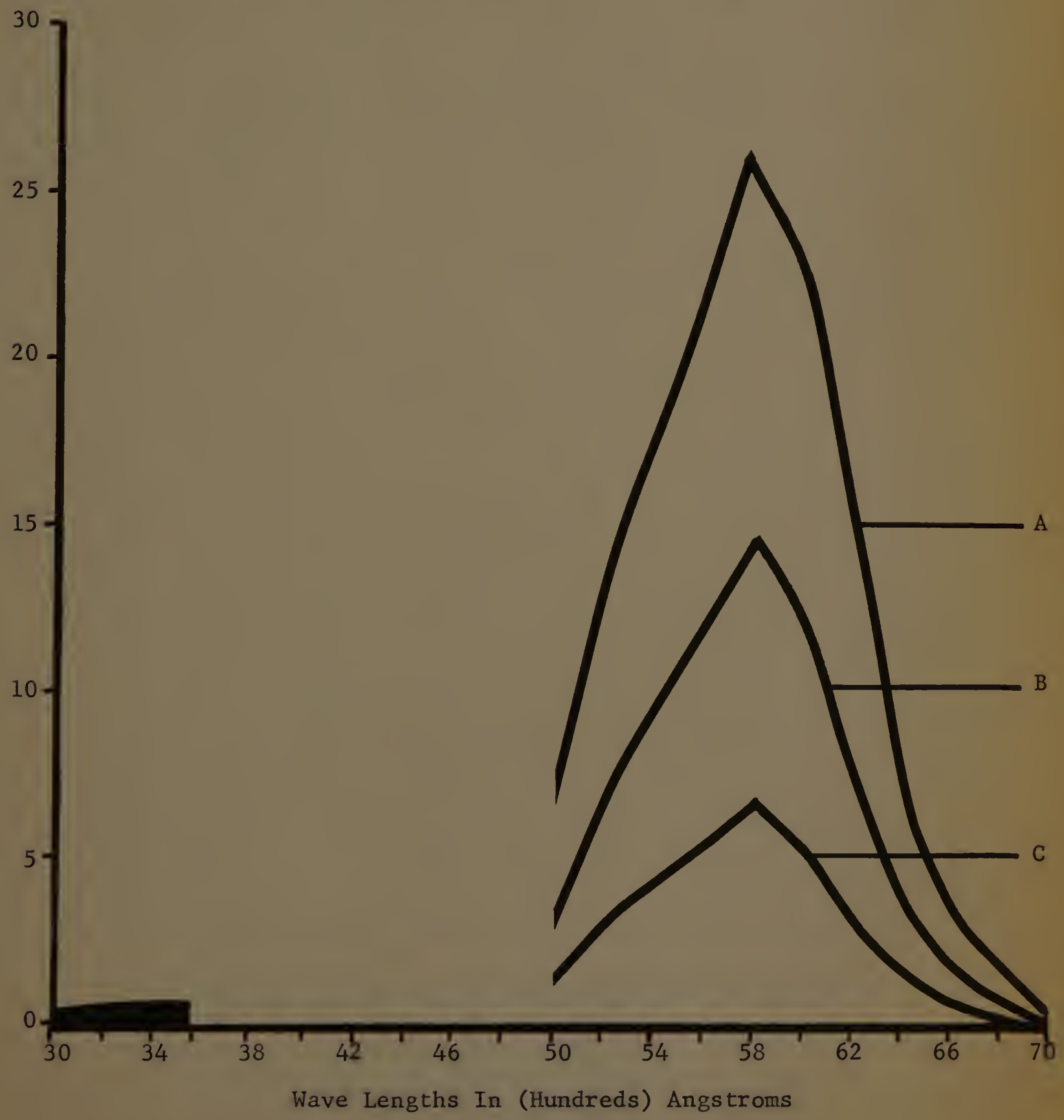


Figure 3. The Distribution of Light Quality and Light Quantity from Pink and Its Effect on Germination.

Curve	Intensity	% Germination
A	185 Foot Candles	61
B	110 " "	65
C	48 " "	61

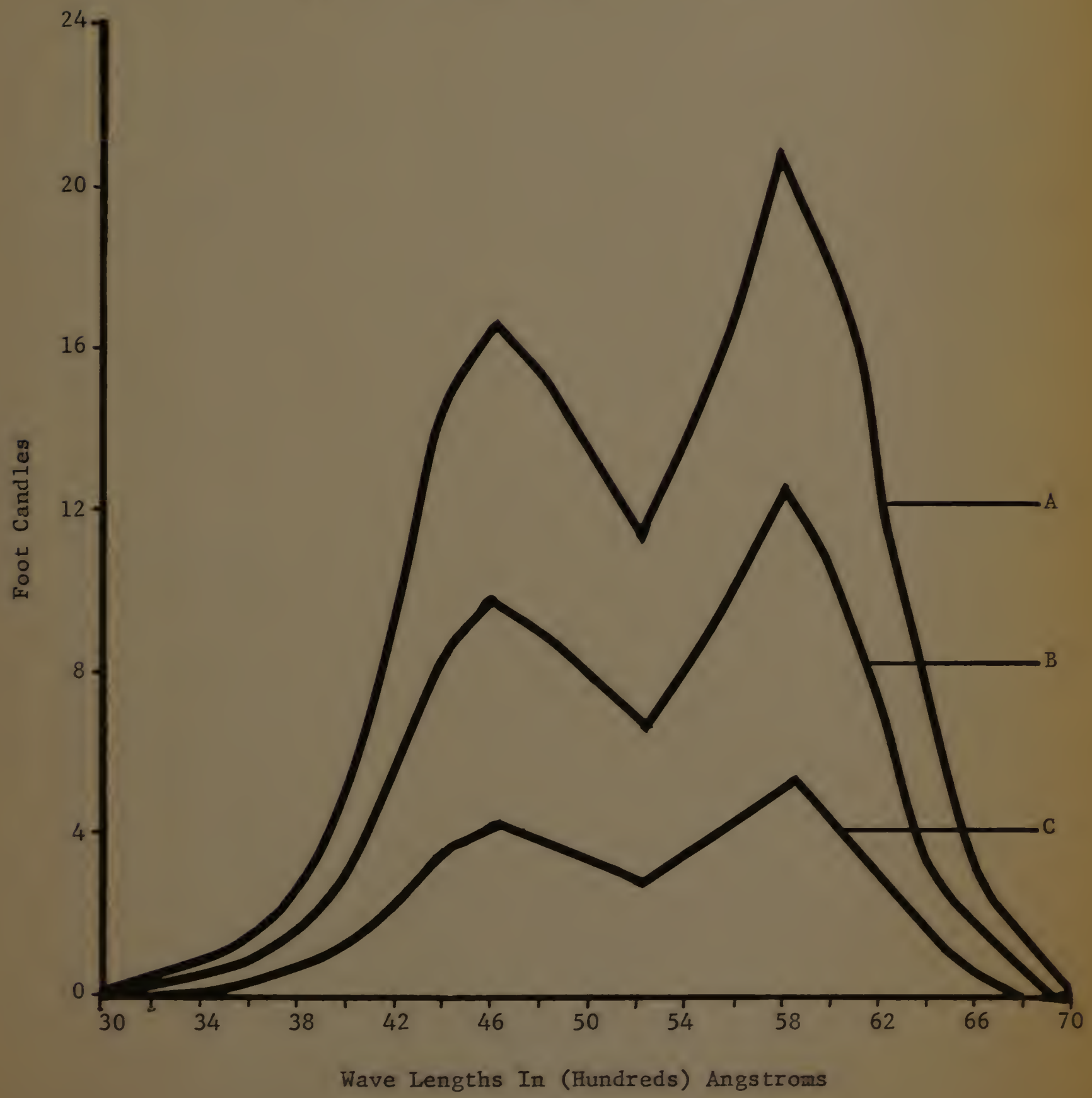


Figure 4. The Distribution of Light Quality and Light Quantity from Clear and Its Effect on Germination.

Curve	Intensity	% Germination
A	270 Foot Candles	65
B	160 " "	57
C	74 " "	65

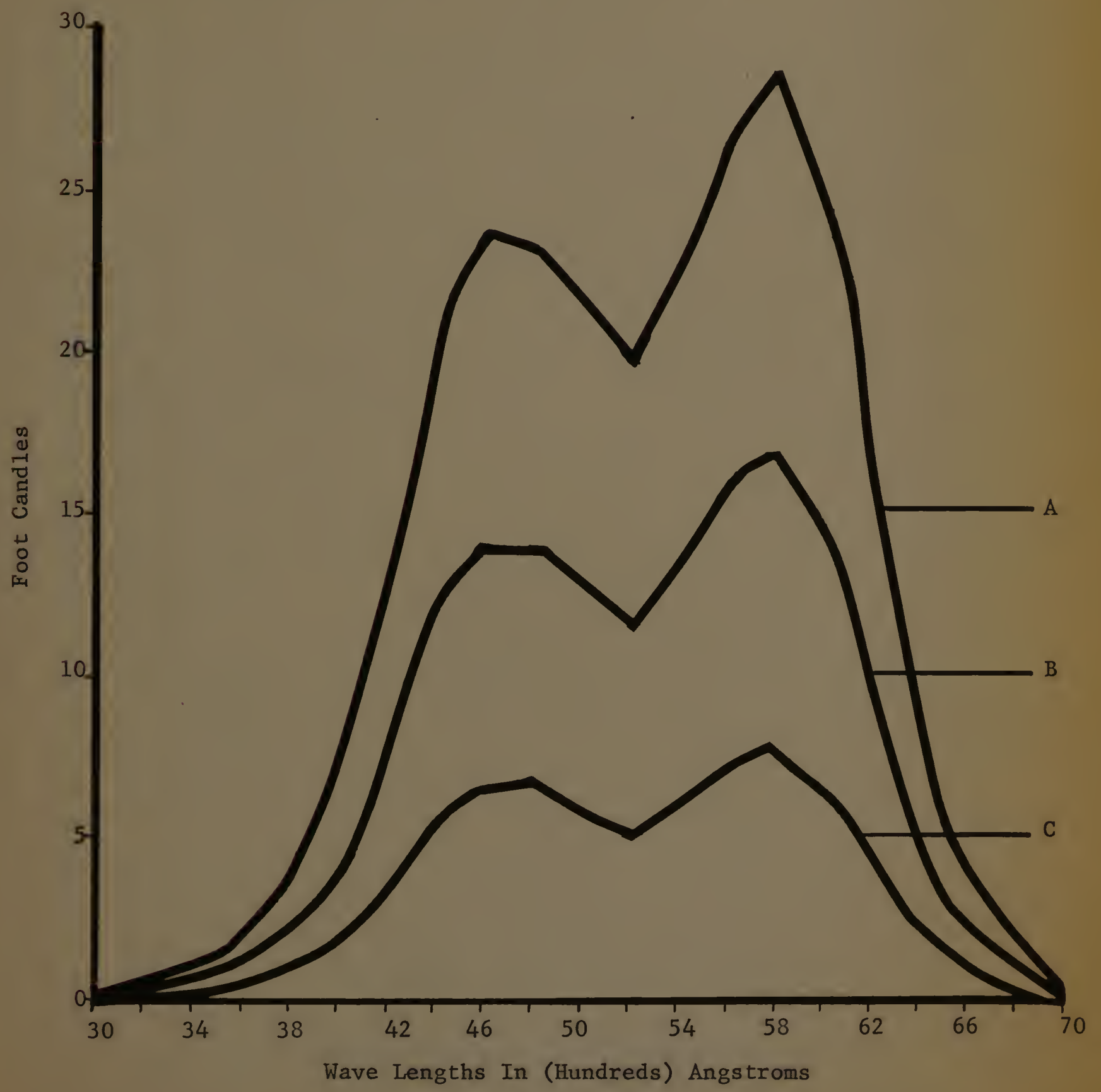


Figure 5. The Distribution of Light Quality and Light Quantity from Orchid and Its Effect on Germination.

Curve	Intensity	% Germination
A	170 Foot Candles	67
B	110 " "	57
C	45 " "	61

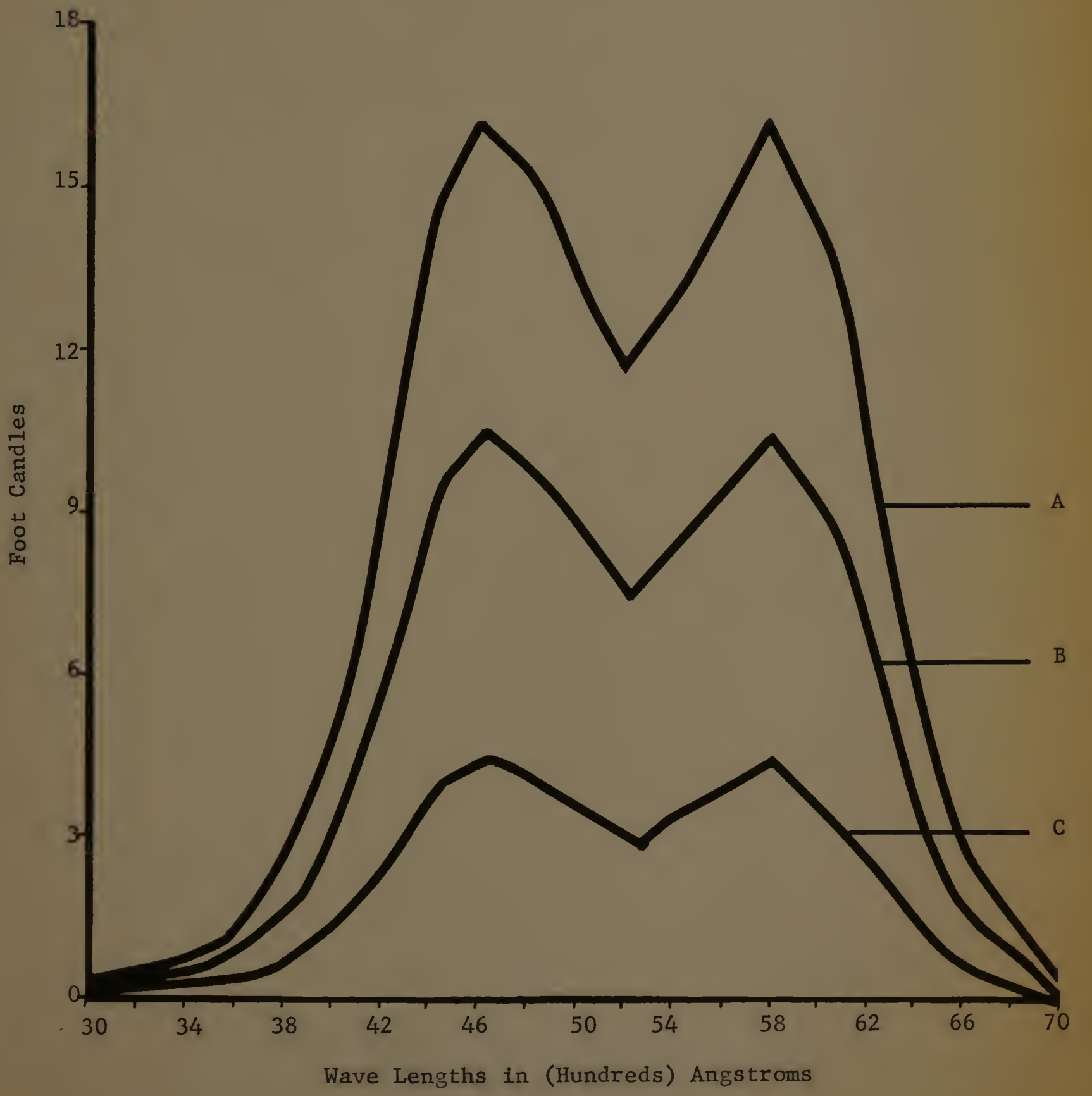


Figure 6. The Distribution of Light Quality and Light Quantity from Check and Its Effect on Germination.

Curve	Intensity	% Germination
A	280 Foot Candles	61
B	140 " "	65
C	65 " "	59

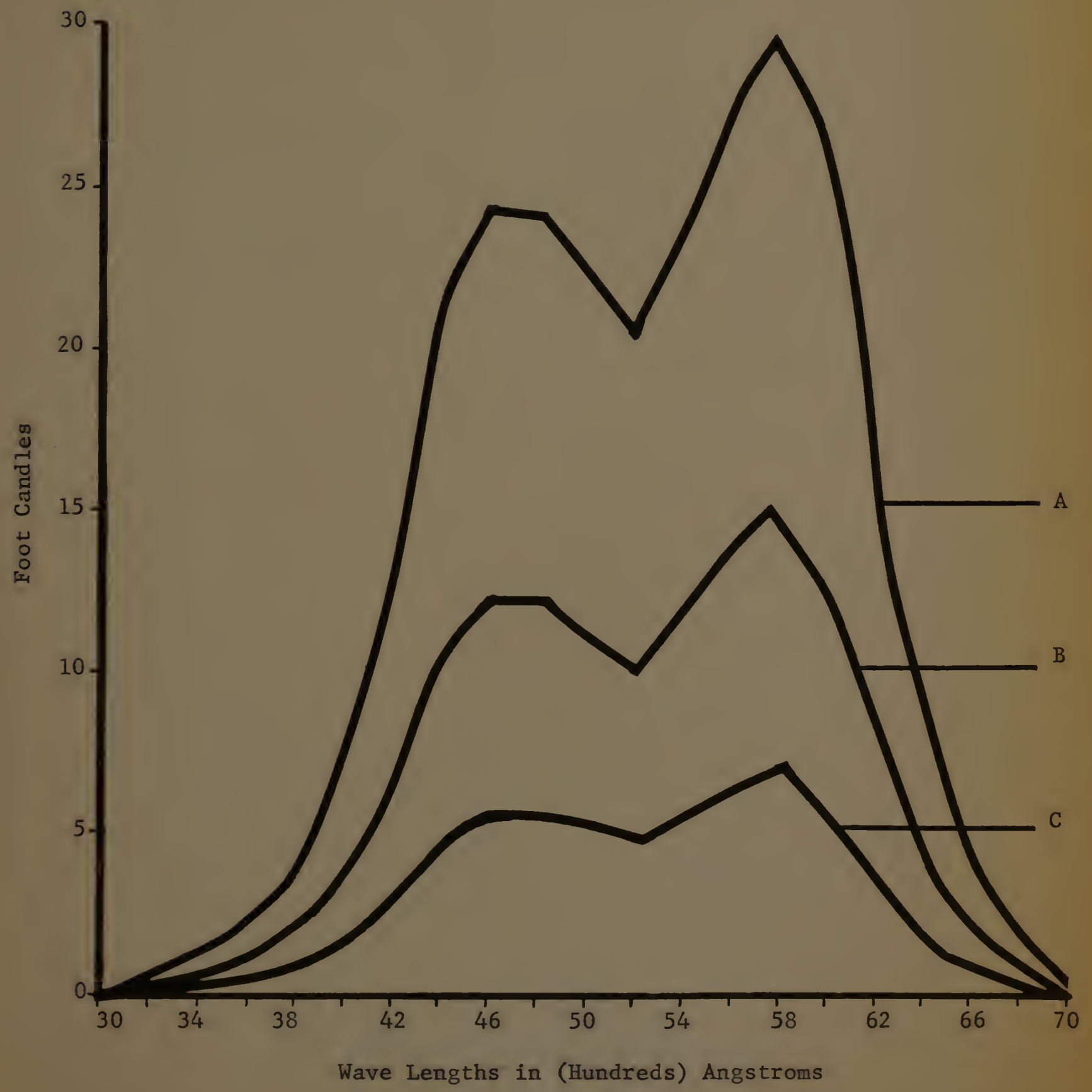


Figure 7. The Distribution of Light Quality and Light Quantity from Light Yellow and Its Effect on Germination.

Curve	Intensity	% Germination
A	170 Foot Candles	64
B	110 " "	55
C	45 " "	63

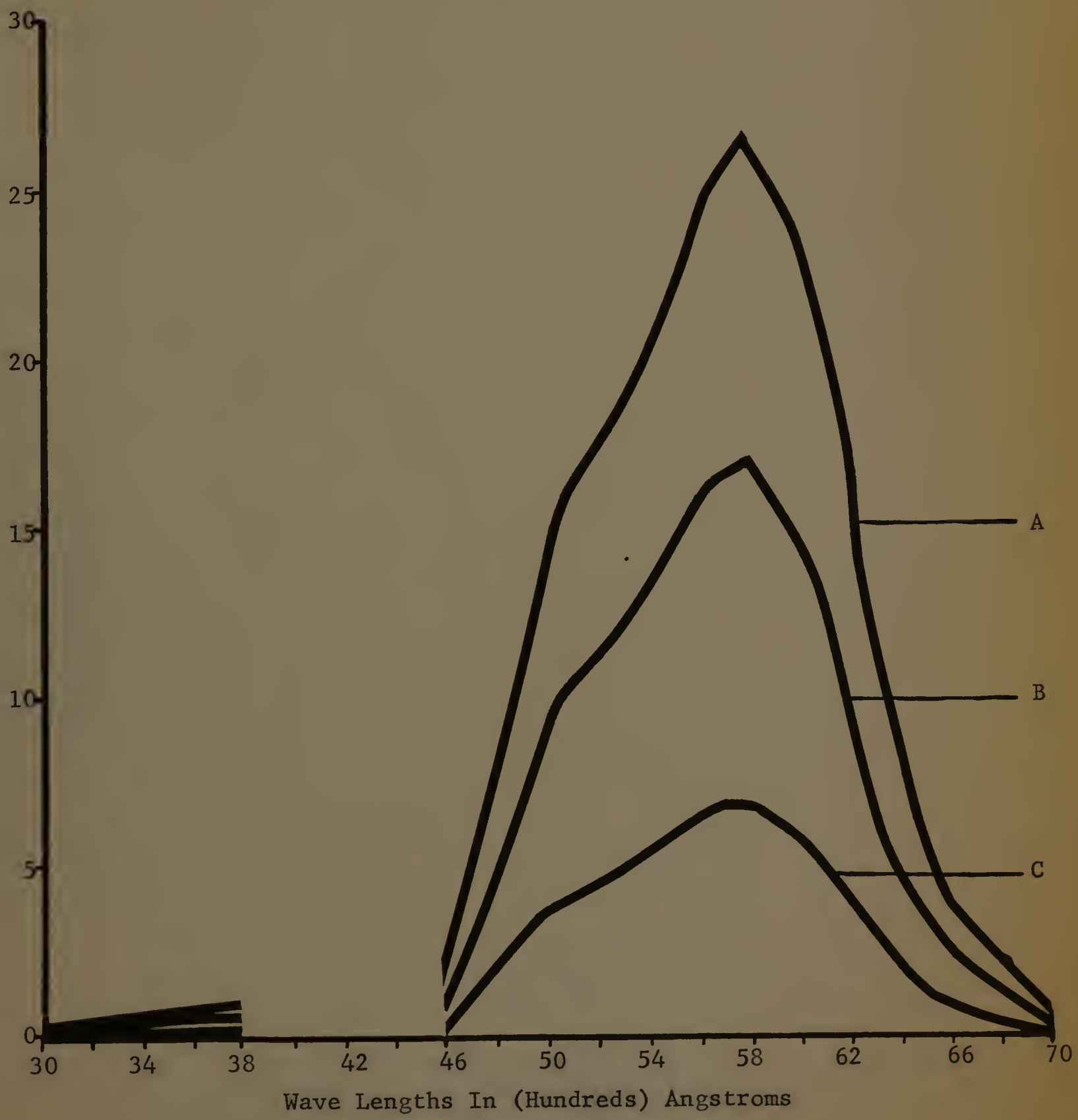


Figure 8. The Distribution of Light Quality and Light Quantity from Light Blue and Its Effect on Germination.

Curve	Intensity	% Germination
A	130 Foot Candles	64
B	54 " "	53
C	26 " "	62

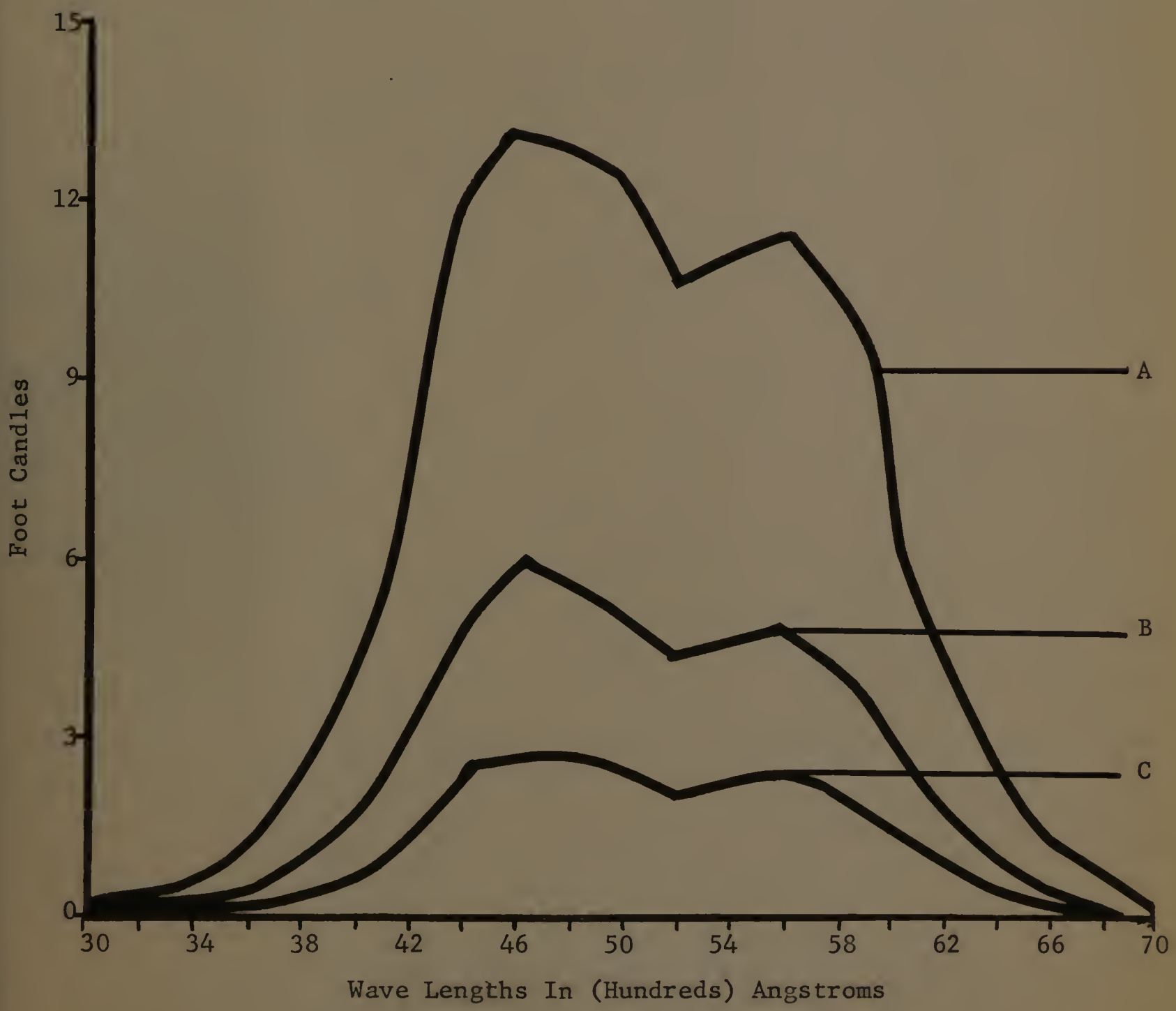


Figure 9. The Distribution of Light Quality and Light Quantity from Red and Its Effect on Germination.

Curve	Intensity	% Germination
A	41 Foot Candles	57
B	18 " "	59
C	10 " "	62

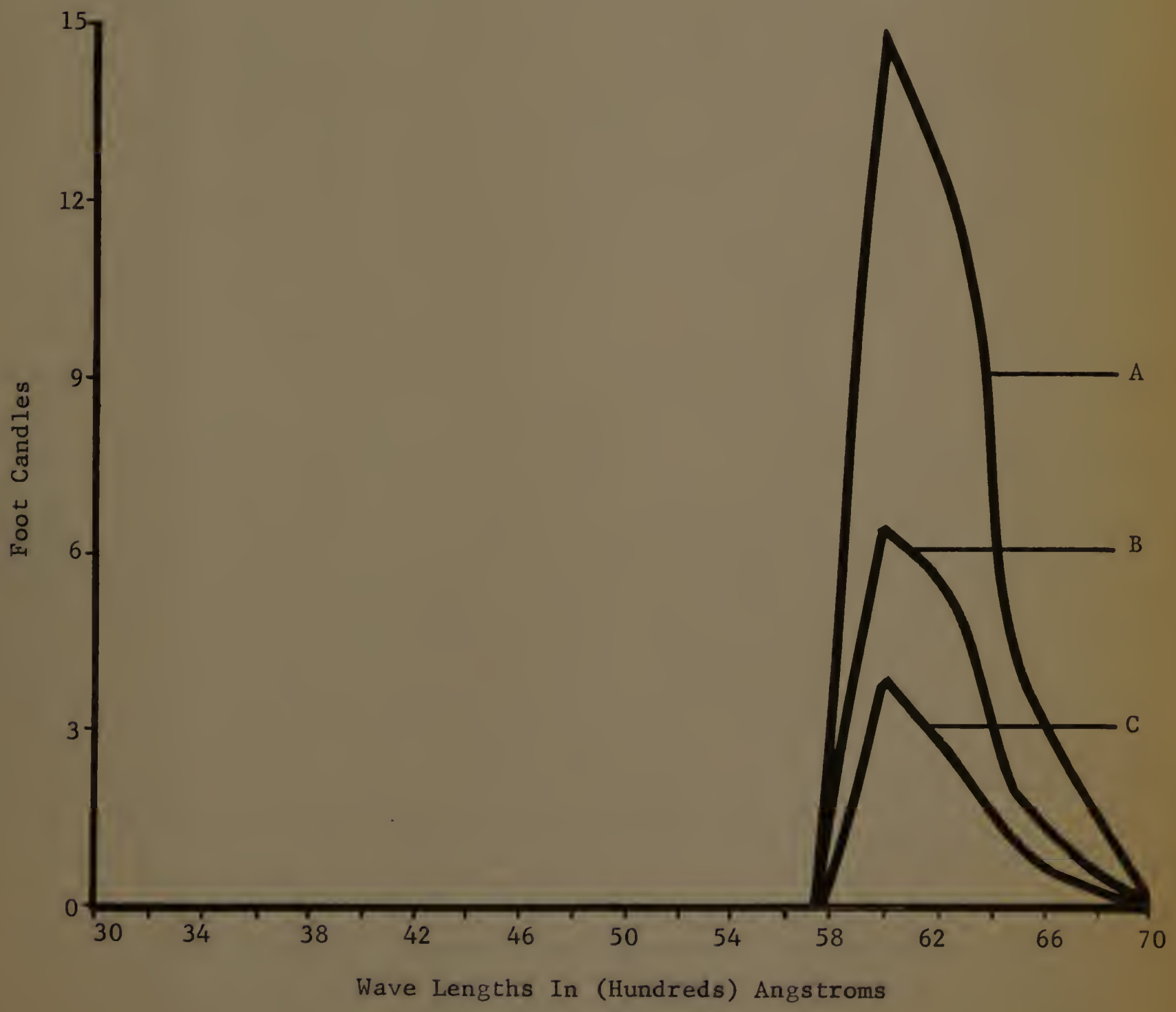


Figure 10. The Distribution of Light Quality and Light Quantity from Dark Green and Its Effect on Germination.

Curve	Intensity	% Germination
A	40 Foot Candles	54
B	19 " "	57
C	9 " "	53

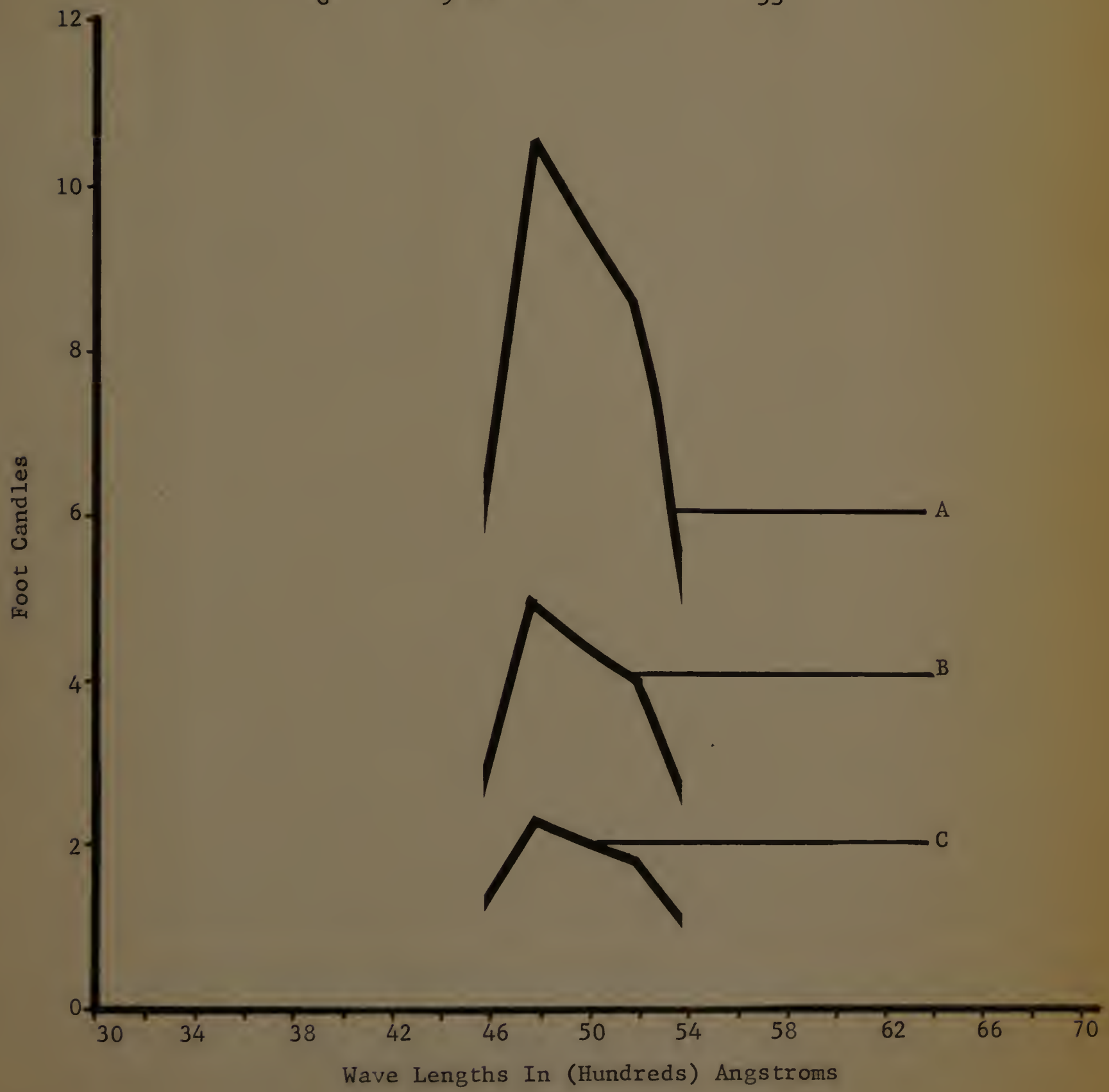
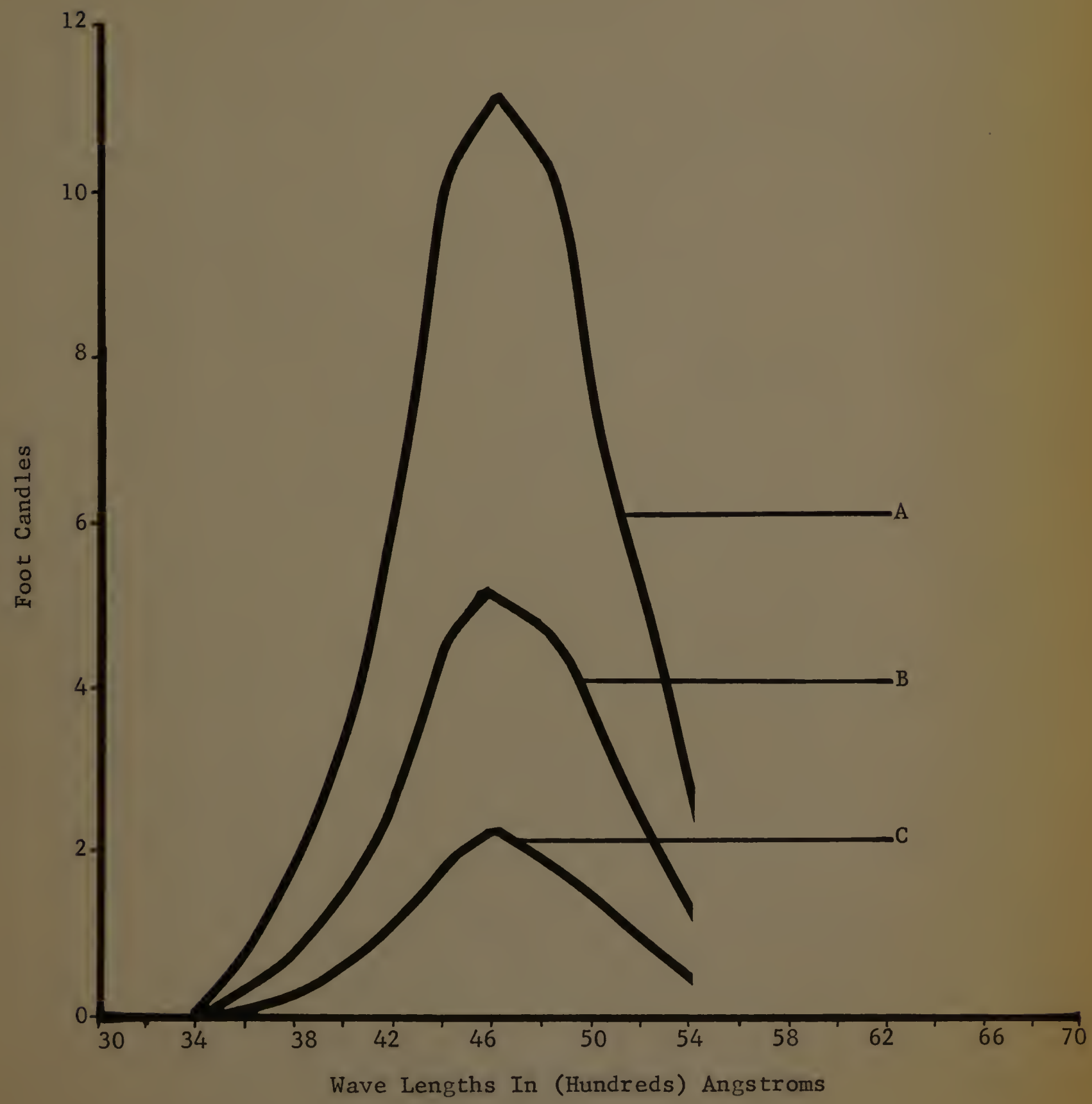


Figure 11. The Distribution of Light Quality and Light Quantity from Dark Blue and Its Effect on Germination.

Curve	Intensity	% Germination
A	60 Foot Candles	38
B	28 " "	39
C	12 " "	37



Amitrol and Autoradiographing

Plants of northern nutgrass were grown in the greenhouse and treated with C^{14}-labeled amitrol; the tagged atom was on the 5-position of the triazole ring. The specific activity of this chemical was 0.95 μc per millimole. A 4500 ppm stock solution, containing 50 μc, was prepared by adding distilled water to the sample.

Each treatment consisted of a 10 μl droplet, with an activity of 0.5 μc, applied to the plant. Since the penetration of amitrol is enhanced by the use of a surfactant, a 0.1 per cent Triton B-1956 spreader solution was added to each droplet at the rate of five μl per treatment. It was necessary to confine the droplet with a lanolin ring because of the decrease in surface tension. The activity was similar for all treatments, but the site of application and the duration of the treatment was varied. Plants at different stages of physiological maturity were treated and comparisons made.

At the end of the various treatment periods, the lanolin was removed with absorbent tissue. The aerial portion of the plant was harvested, sectioned, placed between blotters, bound in a plant press and dried in a forced draft oven at 80°-90°C. Sectioning of the plants into nine in. lengths was necessary in order to accommodate the available equipment and to obtain specimens that were flat when dried. Immediately following each cut, the ends were immersed in melted paraffin to seal them and prevent leakage of plant sap out of the tissue during drying. The plants were mounted on white cardboard using Sanford Ink Company's Grippit paper cement, and then enclosed in an acetate film.

Autoradiograms were made in the following manner: The mount was placed face up in a lead lined Kodak X-ray exposure holder and the Kodak no-screen X-ray medical film placed over it. The emulsion side of the film was facing the mount. The light proof holder was then closed and stored in the dark for 28 days. After the exposure period, the film was developed in Kodak liquid X-ray developer and replenisher for five minutes. It was then transferred to a water bath for one minute to stop the reaction and rinse the film. From this bath, it was placed in a solution of Kodak liquid X-ray fixer and replenisher for ten minutes. After the fixing operation, the film was rinsed in water for thirty minutes and then allowed to dry. Throughout the processing, aggitation of the solutions or movement of the submerged film was provided to insure even development of the film. All operations involving the film was conducted in complete darkness.

In field studies, single applications of amitrol were applied to a dense stand of northern nutgrass at two different stages of maturity, to determine possible "seedicidal" effects. The first application was made on August 17, when the inflorescences were about 50 per cent mature, the second on August 28, when the inflorescences were about 75 per cent mature.

Three rates of the chemical were applied on each date; 2, 4, and 8 pounds of active material were used in 100 gallons of water per acre. Seed was harvested from the plots on September 1 and 11. The seed was air dried at room temperature, hand threshed, and cleaned in an air blower.

Germination tests were conducted in the Seed Laboratory at the University of Massachusetts in the manner prescribed by the Association of Official Seed Analysts (3). All treatments were replicated four times

with one hundred seeds in each replication. Using a procedure described by Durfee (15), the seeds were placed on moist blotters for 16 hours of darkness at 20°C and alternated with 8 hours of light at 35°C. All seeds were subjected to a twenty-one day germination period.

RESULTS

Inhibitor

In the first experiment, eight grams of the tubers were ground in 50 ml. of water. Oat germination was not affected, for all treatments gave almost one hundred per cent germination.

From the results shown in Table 12, it can be seen, however, that there is a highly significant difference in stem length between the check and those treated with the tuber extract. A significant difference among the root lengths of the various treatments was present at the five per cent level. The various treatments had no influence on the number of developing roots.

A second experiment was conducted in which a more concentrated extract was used. Twenty-four grams of the 1961 tubers were ground in 50 ml. of water and 12 ml. of the extract applied to each blotter. A second treatment involved the same amount, except that the extract was boiled. A third treatment was made up of tubers harvested the previous year.

The results of this experiment in Table 13 show that there is a highly significant difference among the treatments when stem development is considered. Greatest growth resulted in the check, which was significantly better than the treatment using the extract from the 1960 tubers, which in turn resulted in better growth than did the other two treatments.

TABLE XII

Bioassay Determination of an Inhibitory Substance in Nutgrass Tubers

A. Mean stem length per oat seed (CM).

Rep.	Check	8 gr./50 ml. Fall Tubers	Winter Tubers
1	7.12	5.72	4.70
2	8.52	3.48	3.68
3	8.26	4.5	3.76
4	8.94	2.61	3.98
Total	32.84	16.31	16.12

L.S.D. .05 = 1.87
.01 = 2.83

B. Mean total root length per oat seed (CM).

1	19.0	16.64	15.44
2	19.56	13.48	15.28
3	17.16	15.84	16.82
4	19.64	14.86	19.58
Total	75.36	60.82	67.12

L.S.D. .05 = 2.77
.01 = N.S.

C. Mean number of roots per oat seed.

1	3.48	3.52	3.0
2	3.48	3.28	2.92
3	3.2	3.32	3.44
4	3.2	3.36	3.44
Total	13.36	13.48	12.80

L.S.D. .05 = N.S.
.01 = N.S.

TABLE XIII

Bioassay Determination of an Inhibitory Substance in Nutgrass Tubers

A. Mean stem length per oat seed (CM).

Rep.	1961 Tubers Check	1961 Tubers 24 Gr./50 Ml.	1961 Tubers 24 Gr./50 Ml. (Boiled)	1960 Tubers 24 Gr./50 Ml.
1	8.36	3.47	4.32	5.01
2	9.4	4.81	3.85	4.12
3	6.67	3.48	3.79	7.05
4	8.31	3.94	4.77	4.78
Total	32.74	15.70	16.73	20.96

L.S.D. .05 = 1.70
.01 = 2.45

B. Mean total root length per oat seed (CM).

1	22.25	9.86	12.06	12.48
2	23.97	11.45	13.50	13.33
3	21.15	8.94	12.50	17.57
4	21.55	10.09	13.98	11.39
Total	88.92	40.34	52.04	54.77

L.S.D. .05 = 2.76
.01 = 3.97

C. Mean number of roots per oat seed.

1	3.2	3.36	3.73	3.42
2	3.76	3.17	3.57	3.43
3	3.21	3.35	3.59	3.25
4	3.26	3.22	3.48	3.29
Total	13.43	13.10	14.37	13.39

L.S.D. .05 = N.S.
.01 = N.S.

In a comparison of root development, the roots in the check were significantly longer than from any of the lots treated with extracts. The unboiled 1961 extract resulted in poorest growth, while the boiled 1961 and the 1960 extracts showed similar results. There were no significant differences in the number of roots produced.

Growth Studies

Referring to the July 29, 1960 experiment, records were taken from June 13, 1961 until the end of the growing season. Plant counts per plot were taken from 6/13 to 7/13, at which time the counting was terminated because of the rapid spread and density of the stand. The mean value of the 36 plots for the plot increase in number of new plants per day is given in Table 14. The values among the plots were extremely variable. The most rapidly growing plot multiplied at the rate of 3.7, 16.0, 23.4, and 13.0 plants per day during the respective weeks.

Data taken on the rate of growth, which was roughly in the form of a circle, is expressed as the diameter between the two most widely separated plants in the plot, and was recorded from 6/13 to 8/10. Measurements were terminated at that time because most of the plots had grown together, forming a dense stand. The mean calculated spread, in in., per plot per day is shown in Table 14. One plot expanded at the rate of 0.28, 1.0, 0.42, 1.28, 0.14, 1.28, 0.57, and 4.28 in. per day during the respective weeks.

Inflorescences were counted from 7/6 to 8/10, after which they became toonumerous to count. The number per plot varied greatly, ranging from 0 to 89. If this latter figure is expanded to an acre basis, there would have been 242,303 inflorescences produced per acre. As the inflorescences matured, seed was collected from each plot until the end of

TABLE XIV

Growth Rate of Nutgrass Planted

on July 29, 1960

(Values are the means of 36 plots)

Date	Plant Increase Per Day	Spread in in. Per Day	Number Inflorescences Per 36 Plots
6/13/61	-	-	-
6/20/61	4.2	.26	-
6/26/61	6.3	.46	-
7/6/61	8.6	1.15	12
7/13/61	6.9	.53	35
7/19/61	-	.35	73
7/26/61	-	1.11	142
8/4/61	-	.83	274
8/10/61	-	1.98	606

TABLE XV

Reproductive Potential In Seed Yield and Germination from the July 29, 1960 Planting

	Yield	Wt./100 Seed	No. Produced	% Germ.	No. Viable
Total (33 Plots)	211.813 Gr.	.5798 Gr.	1205674	-	561823
Mean " "	6.419	.0176	36535	46.4	17025
High Plot	16.476	.0209	90033	65.75	46141
Low Plot	1.443	.0150	9402	29.25	4136

the growing season. The seed was air dried, hand threshed, and cleaned in an air blower with only the heaviest seed being saved. The yield from each plot was weighed and a germination test conducted.

From the 40 original plots, there were seven that failed to produce seed. Production from the remaining plots was very variable, ranging from 1.443 gr. to 16.476 gr. Data illustrating the results of this phase of the experiment are shown in Table 15. One hundred seeds were counted from each plot and weighed. From the known weight of the total yield and the known weight per 100 seeds, the number of seeds produced per plot could be calculated. This ranged from a low of 9,402 to a high of 90,033. Considering the latter plot on an acre basis, 44,855.9 grams containing 245,114,780 seeds would have been produced.

The per cent germination was then determined for each plot, the mean of which was 46.4 per cent. By multiplying the per cent germination by the number of seeds produced per plot, the number of viable seeds per plot could be determined. This varied between 4,136 and 46,141, or on an acre basis, between 11,260,260 and 125,618,872.

It was noted at a different location, however, that seeds were not produced even though growth of the umbel and flowering occurred. This area was composed of a very dense stand of nutgrass that had not been disturbed for several years. This would indicate that the majority of the seed is produced by young vigorously growing plants.

Counts from the 8/9/60 planting were taken at weekly intervals from 6/12 to 7/26/61. Table 16 summarizes the results obtained. The mean plant increase per plot per day has been calculated for both the seed planting and the transplants. Each value given for the seed planting is the mean

of 19 countings, and for the transplants, there were 63 countings. If both the seed plots and transplant plots are considered together, the mean daily plant increase per plot would be 2.09, 2.64, 2.85, 1.84, 1.19, and 1.43 for the respective weeks.

Measurements on the rate of plot spread were taken from 6/12 to 8/24. The mean spread per plot per day for both the seeded plots and transplanted plots is given in Table 16. The plot with the greatest spread expanded from 12 in. to 72 in. Considering the mean daily spread for the seeded plots and transplanted plots together, gave values of 0.18, 0.15, 0.18, 0.27, 0.40, 0.78, 1.08, 0.76, 0.94, and 0.82 in. during the respective weeks.

Inflorescences started to appear during the week of 7/26, and increased in number as time passed. Counts were taken from 7/26 to 8/24, and the results are shown in Table 16. The weekly increase for both groups was 24, 73, 210, and 335. The number per plot was variable, ranging from 0 to 71.

Light Studies

Analyses of the data demonstrated highly significant differences among the seed lots, substantiating the results of previous experiments. A significant, though slight, difference in germination existed when the number of bulbs was varied. This would indicate that the intensity of light played some role in the germination process. This small difference, however, was not totally indicative of the results because germination was not affected when seed were subjected to a wider range in foot candles than those produced from merely a change in the number of bulbs per se. There was a highly significant difference among the treatments as demonstrated by Duncan's Multiple Range Test in Table 17.

TABLE XVI

Growth Rate of Nutgrass Planted

on August 9, 1960

Date	Plant Increase Per Day		Spread in in. Per Day		No. Inflorescences Per Group	
	Values are the means of					
	19 Seed Plots	63 Transplant Plots	19 Seed Plots	63 Transplant Plots	Seed	Transplant
6/19/61	2.48	1.97	.05	.22	-	-
6/26/61	3.95	2.26	.23	.13	-	-
7/5/61	3.48	2.66	.16	.19	-	-
7/12/61	1.22	2.02	.26	.27	-	-
7/19/61	1.26	1.17	.47	.38	-	-
7/26/61	.92	1.59	.61	.83	-	4
8/4/61	-	-	1.23	1.02	-	28
8/10/61	-	-	.67	.79	14	87
8/17/61	-	-	.83	.97	44	267
8/24/61	-	-	-	-	91	555

TABLE XVII

The Use of Duncan's Multiple Range Test to Determine the Effect of Light Quality on the Germination of Northern Nutgrass

Values Are the Means of 48 Observations

Treatments	Ranked Means	5%	1%
Light Green	64.56	a	a
Dark Yellow	62.81	a	a
Pink	62.54	a	a
Clear	62.50	a	a
Orchid	61.79	a	a
Check	61.62	a	a
Yellow	60.33	a	a
Light Blue	59.56	a	a
Red	59.50	a	a
Green	54.77	a	a
Red and Blue	38.75	b	b
Blue	38.27	b	b

Where all the wave bands were present, 2900-7100 A, no significant difference in germination occurred. These treatments included the check, light green, pink, clear, orchid, and light blue. Blocking out wave lengths between 3700-4900 A, or the violet and blue range, but providing all the others, had no effect on germination. This was achieved with use of the dark yellow cellophane. Light yellow cellophane blocked out the 3900-4500 A bands, or violet, and this had no affect on germination. Blocking out the 2900-5700 A bands, or the middle ultraviolet, near ultraviolet, violet, blue, green, and yellow, thus providing only orange and red, did not affect germination. There was no significant difference between the following treatments; light green, dark yellow, pink, clear, orchid, check, yellow, light blue, and red. This group was, however, significantly better than the dark green, red plus blue, and dark blue. The treatments of yellow, light blue, red, and dark green could be grouped together. These resulted in significantly poorer germination than the light green, dark yellow, pink, clear, orchid, and check treatments, and were significantly better than the red plus blue and dark blue illumination. With the green treatment, the 3100-4700 A and 5700-6700 A wave lengths were blocked, thus providing only green and very little red light. Here germination was significantly lower than the check. Providing wave lengths of 2900-5900 A, thus blocking the orange and red waves, reduced germination significantly below all other treatments.

Light intensity, as measured in foot candles, does not apparently affect germination. When all the wave lengths were present, a range of 10 ft-c to 270 ft-c had no influence on germination. It can also be said that intensity had no affect even in treatments whose germination was reduced by some other causes.

The three varying factors were tested to ascertain the presence of any interactions among them. Analyses of the possible interactions: treatments X bulbs, treatments X seed source, and bulbs X seed source, gave no indication of an association. The triple interaction of treatments X bulbs X seed source was used as the error for determining the F values. The remaining 432 degrees of freedom were used to determine the accuracy of the laboratory technique.

Amitrol and Autoradiographing

In the 3-amino-1,2,4 triazole-5-C^{14} studies, the chemical was applied to either the bract leaf, basal leaf, peduncle, or culm. The latter treatment was one in. below the umbel or six in. below the umbel. Plants were treated at several different stages of umbel maturity; these being initiation of umbel development, dehiscence of the anthers, quarter mature, half mature, three-quarters mature, and mature. The treatment time varied from two hours to thirty days.

Considering the treatments to the basal leaf, it was found that translocation was primarily in an acropetal direction. A treatment period of two hours on a half mature plant had this type of movement, but only within the leaf margins. There was also a slight trace basipetally for a distance of eight cm. A six and ten hour treatment at umbel initiation gave results which were very similar to the two hour treatment. One-quarter and three-quarter mature plants treated for six hours resulted in the same acropetal type movement, but it was accompanied by a basipetal movement to a distance of 14 cm. A one-half mature plant treated for ten hours had a more extensive basipetal movement, extending for 37 cm. In all of the above cases, the activity was confined to the treated leaf.

Treatments applied to the bract leaf resulted in translocation, first to the leaf tip, followed by basipetal movement. This was the typical response from plants which were treated at all stages of maturity. A treatment of two hours to a half mature umbel responded with a basipetal translocation of 12 cm. plus the acropetal movement. A six hour treatment to an umbel that was just forming, revealed activity in other bract leaves; the chemical moving basipetally to the umbel and then acropetally toward the leaf tip. A similar treatment of ten hours differed only in that the extent of basipetal movement was not quite as extensive. The treating of a quarter mature, half mature, and three-quarters mature umbel for six, ten, and six hours respectively, revealed only acropetal movement.

An experiment to determine the effect of a surfactant was performed also. Two similar umbels were treated for 24 hours, one with the surfactant, a 5 μl sample of a 0.1 per cent Triton B-1956 solution, and one without. In both cases, there was acropetal movement to the leaf tip. Where Triton was used, the translocation was basipetally to other bract leaves, peduncles, rachises, spikes, and culm. Without the Triton, there was no activity appearing in other bract leaves. This would indicate that the movement was enhanced by the surfactant, and it was used in all the following treatments unless otherwise indicated.

A three day treatment to a three-quarter mature umbel, (Figure 12), showed translocation to the spikes, with slight accumulation in all connecting tissue. There was no activity below the umbel. A seven day treatment yielded greater accumulation in the umbel parts and considerable movement below the umbel. There was slight accumulation in the tip of

the basal leaves, involving distances of 80 and 83 cm. from the point of application.

The treating of a one-quarter mature plant for thirty days, (Figure 13), resulted in translocation throughout the plant. Seeds taken from the inflorescence also demonstrated radioactivity. This indicates that the chemical was translocated basipetally to the umbel and then acropetally to the seed.

Applications to the culms, six in. below the umbel, gave erratic results. A six hour treatment to a plant initiating the umbel, resulted in a downward movement of 12 cm. where it entered a basal leaf and traveled 65 cm. to its tip. There was a slight trace visible for 10 cm. above the point of application. The same treatment for ten hours resulted in only a trace moving acropetally. Treatments giving similar results were: six hour treatment of a one-quarter mature plant, two hour treatment of a half mature plant, and a ten hour treatment of a half mature plant. Treating a three-quarter mature plant for six hours resulted in no downward movement. There was, however, translocation into the umbel where movement within the peripheral tissue of the leaf blades allowed the chemical to reach the leaf tip, 80 cm. from the point of application. Activity was also present in the peduncles, rachises and spikes. A half mature plant treated for thirty days contained activity from its seed to the base of the plant. The greatest distance involved was 68 cm.

A series of plants were then treated on the culms one in. below the umbel. Treatments of six and ten hours to a forming umbel gave similar

results, with translocation into all the bract leaves and very little movement toward the basal leaves, as shown by Figure 14. By extending the period to two days, (Figure 15), activity also appeared in the peduncles and in a basipetal direction from the point of application for 22 cm. Treating a plant at the time of anthesis, for 24 hours, a one-quarter mature plant for six hours, a half mature plant for two hours, and a half mature plant for ten hours, gave similar results. Translocation was to the tip of all the bract leaves and into several peduncles. A half mature plant treated for thirty days showed totally systemic translocation.

A three-quarter mature umbel treated for three and seven, (Figures 16 & 17), days gave similar results except the image from the seven day treatment was of greater extent and intensity. Translocation was generally acropetally to all bract leaves, peduncles, rachises and spikes. A treatment for six hours gave essentially similar results.

A mature umbel treated for two and seven days had widespread activity in the umbel. Traces of activity from the latter treatment appeared in the basal leaves.

In the last experiment, the 10 μl droplet was divided into three parts and each portion applied to a separate peduncle, midway between the rachis and peduncle base. Translocation was entirely acropetally to the rachises and spikelets when a three-quarter mature plant was treated for three days. A mature plant treated in the same manner had a slight accumulation in the lower peduncles. Extending the time to seven days allowed for movement into individual seed and for slight accumulation in other bract and basal leaves as shown in Figure 18.

Figure 19 shows the radioactivity in the individual seeds. Each row of seed was taken from a different umbel. The treatments are as follows, in ascending order: Application to a bract leaf of a one-quarter mature umbel for thirty days, the seed coming from the umbel shown in Figure 13; Application to the culm of a half mature umbel, one inch below the umbel for thirty days; The third row received the same treatment as the second, except it was applied six in. below the umbel; The fourth row received a seven day treatment to the peduncles of a mature umbel, the seeds coming from the umbel shown in Figure 18. The last row came from a mature umbel that was treated for seven days. The application was to the culm, one in. below the umbel. These treatments show definitely that the radioactive carbon is translocated to the seed.

Field grown plants which were treated on August 17 exhibited a marked change in their appearance by August 28. In the plot receiving two pounds per acre, the nutgrass had developed a slight chlorosis; plants in the four pound plot were darker yellow, and those in the eight pound plot were nearly light brown.

On September 1 and 11, the plants that were sprayed on August 28 had about the same color as those in the check plots but those that had been sprayed on August 17 were still chlorotic. It was noticed that the plants sprayed on August 17 had fewer seeds per inflorescence and plants treated with eight pounds of the chemical produced only 25 per cent of the seed yield that was harvested from the two pound treatment.

Analyses of the data are presented in Table 18. It is readily apparent that amitrol sprays had a deleterious and significant effect on seed germination. When the treatments are compared, it is seen that seed

Figure 12. Autoradiogram of a 3/4 mature umbel. Ten μl., containing 0.5 μc., of a 4500 ppm amitrol solution was placed 17 cm. from the base of the bract leaf. The treatment time was three days.

Figure 13. Autoradiogram of an umbel treated when it was 1/4 mature for 30 days. The treatment was the same as in Figure 12. The images at the side of the umbel were made from shattered seed.

Figure 14. Autoradiogram of a forming umbel treated on the culm 1 in. below the umbel for 10 hours. The break in the main leaf was caused by several rachises and spikes overlapping the leaf.

Figure 15. Autoradiogram of a plant similar to Figure 14, except the treatment time was two days. The amount of translocation was greater in this treatment.

Figure 16. Autoradiogram of a 3/4 mature umbel treated 1 in. below the umbel for three days. There was no basipetal movement.

Figure 17. Autoradiogram of a plant similar to Figure 16, except it was treated for 7 days. Although the pattern of translocation was the same, except for a slight movement basipetally, the image is of greater intensity.

Figure 18. Autoradiogram of a mature umbel. The 10 μl. treatment was applied to 3 peduncles for seven days. There was slight movement basipetally, extending to the lower leaves.

Figure 19. Autoradiogram of seed taken from treated umbels. In ascending order the treatments are: to a bract leaf of a 1/4 mature umbel for 30 days, to the culm 1 in. below a 1/2 mature umbel for 30 days, to the culm 6 in. below a 1/2 mature umbel for 30 days, to the peduncles of a mature umbel for 7 days, to the culm 1 in. below a mature umbel for 7 days.

from the check plots germinated significantly greater than those from any of the other treatments. A lineal effect exists among the rates of application, with the higher rates of the chemical resulting in poorest germination. Seed from plots treated on August 17 with eight pounds of amitrol germinated only 25-30 per cent as well as those from the check plots. When comparing the time of application, it can be seen that seed from the August 17 treatments germinated only twenty per cent as well as those that were treated on August 28. This difference is highly significant. In these tests, the time of harvest had no influence on the rate of germination.

TABLE XVIII

The Effect of Amitrol Sprays and Time of Harvest on Germination of Nutgrass Seed

Treatment	Time Of Treatment	9/1 Harvest Mean Per Cent Germination (Angles)	9/11 Harvest Mean Per Cent Germination (Angles)
Check		44.56	52.44
2 Lb./Acre	8/17	32.10	33.81
4 Lb./Acre	8/17	30.78	26.14
8 Lb./Acre	8/17	14.25	14.31
Total Minus Check		77.13	74.26
2 Lb./Acre	8/28	34.73	45.57
4 Lb./Acre	8/28	35.94	37.17
8 Lb./Acre	8/28	31.15	35.03
Total Minus Check		101.82	117.77
	Total	223.51	244.47

L.S.D. (.05) = 12.13

L.S.D. (.01) = 19.02

DISCUSSION

Inhibitor

The presence of an inhibitor in the nutgrass tuber seems to be established definitely, but its identity is not known at present. This compound affects adversely the stem and root development of a bioassay crop such as oats, but does not affect the number of roots formed. There was apparently no difference in the effect of the inhibitor extracted from the fall or winter tubers. Considering root development, however, the inhibition resulting from winter tubers was significantly less than that from the fall tubers. The reason for this is not apparent. The experiment was conducted only once, and therefore, the results are not conclusive. The small difference could be due to a variation among samples.

In the second experiment involving stem length, it might be possible that there is a different amount of the inhibitor produced from one year to the next, or that the inhibitor decomposes with age of the tuber. An indication of this premise is shown by the fact that growth of oat seedlings was not inhibited as much by the 1960 tubers as by the 1961 crop. A similar situation exists in the root development. There was a significant difference here between samples of the extract that were boiled and unboiled. Boiling of the extract reduced significantly the inhibiting effect of the compound. This may give some clue as to the nature of the inhibiting factor.

Amitrol and Autoradiographing

Results of the amitrol studies add further evidence to the fact that amitrol is readily translocated in the plant. The degree of translocation

is dependent upon many variables. Among these are the formulation of the chemical and its physical properties, the environmental conditions in which the plant is growing, the characteristics of the plant as to thickness of cuticle and number of stomates, the physiological stage of plant maturity, the point of chemical application, etc.

Workers have reported that amitrol is translocated in the transpiration stream, thus moving mainly in the phloem. Results of this work demonstrate that movement is in an acropetal direction before any is carried down the phloem. This was especially noticable in treating the culms one in. below the umbel. Activity was usually widespread in the umbel before movement was noted toward the roots. The chemical was apparently absorbed and dissolved in the solution of the large xylem tubes, allowing for a portion of the radioactive chemical to enter each of the xylem branches that feed the various umbel parts.

The results seemed to indicate that the translocation was throughout the aerial portion of the plant before there was any movement into the roots. Basipetal movement, that reached the base of the treated bract leaves, reverted to an acropetal direction, transporting activity into other bract leaves and peduncles. This occurred before any appreciable movement below the umbel. From these results, one can get an even better idea of its mobility. As the chemical is moving down the phloem and reaches the vicinity of a xylem tube entering another leaf or peduncle, it moves out of the assimilate stream and into the xylem system. The reason for the change in transporting systems is not known.

Although the treatment of peduncles resulted in radioactivity appearing in individual seeds, it did not give a systemic type of translocation; for the chemical was confined to the treated segment.

With its potential types of translocation, complete plant coverage during spraying is not of paramount importance in assuring its maximum activity. A single droplet striking the base of a leaf, will in effect, treat the whole leaf. The most effective point of application appears to be just below the umbel where one droplet will treat the leaves and the multitude of seeds produced. Probably the least effective spot would be centered on the basal leaves or the lower portion of the culm. The experimental results showed that translocation was poorest at the latter site, possibly because the cells making up this region are used for support instead of absorption.

The physiological maturity of the plant did not affect the direction of translocation to any great extent, with all types of treatments moving primarily acropetally. The slight differences that did occur were probably caused by individual plant variability. Thus, a late application will control the new seedlings as well as the mature plants in a stand. The timing of an application may be dependent on the type of crop grown.

As might be expected, accumulation of the chemical was a function of time. This was evidenced by the fact that when two similar plants were treated for different lengths of time, the more intense image was produced by the plant having the longer treatment time.

The toxic effects observed in the field sprayings were caused possibly by the greater concentration of the chemical used and the higher rates, and thus more complete coverage. The applications were 2, 4, and 8 pounds active material per acre in 100 gallons of water, or 4800 ppm, 9400 ppm, and 19,100 ppm respectively. The laboratory treatment was composed of a

4500 ppm concentration, with a single 10 μl. droplet applied. In addition to being more concentrated, the field treatment also contained a greater volume of the solution. These combined factors led to the production of the toxicity symptoms.

The fact that seed from the treated plots showed a significant decrease in germination again indicates that the amitrol is translocated internally into the seed where it affects essential processes. The extent of the damage depends not only on the rate of application, but also on the stage of maturity at which it is applied. Seed from the treatments on two dates of application gave the same pattern of results when treatments were varied. The decrease in germination as the rate is increased could be expected. An increase in concentration in the same volume of water would mean that, if the rate of translocation was the same, more active chemical would be entering the seed per unit of time.

The early application was most effective in reducing production and germination of the seed leading one to speculate that a juvenile plant is more susceptible to the effects of the chemical. In this developmental stage it is metabolically active, allowing for the chemical to be drawn up the peduncles and into the seed. The seeds of an umbel do not reach maturity at the same time, and there are gradations of maturity within an umbel at any one time. Thus the older seeds, which are not as active, would not take up as much of the chemical and, therefore, would not be affected as much. Flowers and recently fertilized embryos might be killed. The survival of those in the intermediate stages would depend on the amount of the chemical absorbed.

If the maturity of the seed can be considered in a series of steps, the decrease in yield and germination might possibly be explained on the basis of the following hypothesis. A two pound application may disrupt the proper functioning of existing flowers and those that have completed pollination, enter the immature seeds and affect their germination at maturity, and not be able to affect the more mature seeds. A four pound application may kill those between flower opening and several days past pollination and decrease germination from this stage to almost maturity. An application of eight pounds would kill all stages between flower formation and seed of more advanced stage of maturity than the four pound application. The remainder, except those that were mature at the time of spraying, would have their germination decreased. In this way, the production of new seed is stopped, a greater percentage of the existing immature seed is killed, and more of the intermediate stages of seed maturity have their germination reduced.

The stage of maturity at which the chemical is applied is important because while the plants are in a more immature state, the chemical is more effective. At this stage, there are a greater number of flowers and immature seeds which are more susceptible. Thus the effect, if considered in stages of seed maturity, is pushed upward, killing and affecting a greater percentage of the total number of flowers produced. The effect of a later application, when more of the seeds are mature, will be lower on the scale of maturity. The yield is not affected because all the seed have set and are approaching maturity. In this way, only the later ones that develop are affected by the spray. This might explain why the per cent germination was not affected to such a great extent in the late application as in the early.

SUMMARY

Several areas of research were investigated to ascertain the characteristics of the nutgrass plant. The presence of a growth inhibitor was shown to exist in the nutgrass tubers. A bioassay, using oats as an indicator crop, revealed that a water extract from the tubers reduced significantly the elongation of the stem and roots. This effect was reduced partially by boiling the extract.

A series of growth studies to determine the reproductive potential of the nutgrass was initiated. Single seedlings, 3/4 in. in height, were transplanted in the field and data were taken on the daily increase in the number of plants, the daily rate of spread, and the number of inflorescences produced. Seed was collected from each plot and germination trials conducted. Seed weights and the number produced were also determined. Within a period of one growing season, a single seedling multiplied into a stand of plants that produced 90,033 seeds, having a germination of 65.75 per cent, or 46,141 viable seeds.

The effects of light quantity and quality on germination were also investigated. Germination was not affected where light from the orange and red region of the spectrum was provided. The elimination of this spectral region, with the use of filters, however, reduced germination significantly.

The absorption and translocation of 3-amino 1,2,4 triazole in the nutgrass was also investigated. C^{14}-labeled amitrol was applied to several sites on plants of different physiological maturity for varying lengths of time. The extent of translocation, determined by the auto-

radiographic technique, was primarily in an acropetal direction. Radioactivity was absorbed and translocated from the point of application to individual seed of the umbel.

Field studies revealed that foliar applications of amino triazole had a deleterious and significant effect on seed germination. An early application, when the umbels were immature, was more effective in reducing germination than a later application. A lineal effect exists between the rate of application with the higher rate giving the poorest germination.

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