GERMINATION PATTERNS SHOWN BY THE LIGHT-SENSITIVE SEED OF *ANAGALLIS ARVENSIS*

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Summary

At 25°C germination of seed of *Anagallis arvensis* subsp. *foemina* (Mill.) Schinz & Thell. is promoted by red and inhibited by far red radiation with maximum efficiencies in the vicinity of 670 and 750 mμ respectively.

Dormancy, the effect of duration of dark incubation before exposure to red light, and low temperature (12°C) dark germination were studied for a number of samples. Wide differences between samples were apparent. The possibility of a relationship between the germination behaviour of the seeds and either their genetic make-up or the environment in which they were grown is discussed. The only clear-cut relation observed was between dormancy and temperature at which the seeds were formed.

I. Introduction

The work reported here shows that the seeds of *Anagallis arvensis* belong to the class whose germination is stimulated by red and inhibited by far red radiation. Several detailed studies of the germination of such seeds have been made (Evenari 1952; Borthwick *et al.* 1954; Kadman-Zahavi 1960; to mention a few). The purpose of the present work is not so much to add an account of germination of *Anagallis* to those already in existence for other species, but to bring to notice the differences in germination requirements which exist between samples of seed of this species. To date, no more than passing references have been made in the literature to differences of this type.

In the present work, some aspects of germination were followed in a number of seed samples during their passage out of dormancy, and in a larger number of samples in the relatively stable non-dormant state. The observations are interpreted in terms of blocks in the sequence of metabolic steps culminating in germination. An attempt is then made to trace the differences between samples to factors operating during formation of the seeds. These factors are divided into the complex of the seeds' genetic endowment and the complex of the environment.

II. General Methods

(*a*) *Seed Samples*

Seed of *Anagallis arvensis* subsp. *foemina* (Mill.) Schinz & Thell., a blue-flowered variety, was used in all experiments. Seed was originally collected from a single plant in the field and all subsequent generations of seed have been derived from it as depicted in Table 1. With one exception, samples used were obtained from glasshouse-grown plants. Growing periods and, where known, glasshouse temperatures are recorded in the table; 25°C/20°C, for example, means 25°C for 8 hr during

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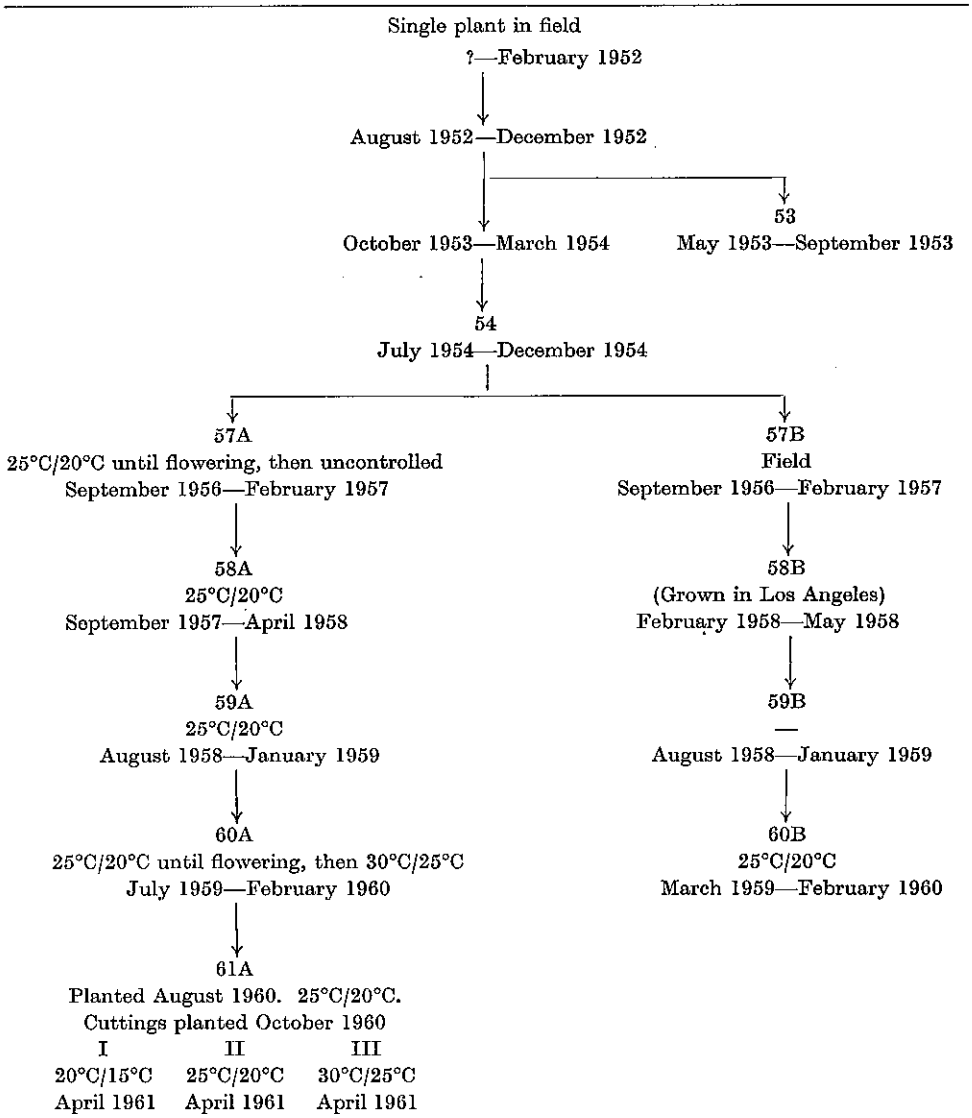
the day and 20°C for the rest of the time. Where no temperature is given, temperature was uncontrolled except for provision of heating to prevent excessively low temperatures occurring. No artificial lighting was used. *Anagallis* is a long-day annual plant,

TABLE 1

ORIGIN OF SEED SAMPLES OF ANAGALLIS ARVENSIS



hence plantings were made in winter or early spring and seed harvested the following summer. Cross pollination within one lot of plants could take place, but that between lots was prevented. Seed constituting any one sample was derived from several plants. Although flowering and capsule formation takes place over a period of many

weeks, maturation of capsules is condensed into a much shorter period. Seeds were harvested as soon as capsules were dry and brittle, then stored in screw-top jars at laboratory temperatures. They were sorted by hand to exclude shrivelled or otherwise abnormal seeds before being used in germination experiments.

Seed samples are named by the year in which they were harvested, e.g. sample 60 was harvested in 1960. When more than one sample was harvested in a given year, the samples are distinguished by the letter A or B according to their parentage.

Samples 61AI, 61AII, and 61AIII were obtained from one plant grown until it was large enough to provide about 30 cuttings, which after rooting were potted and divided between three glasshouses maintained at different temperatures.

(*b*) *Experimental Procedures*

For all except a few of the earliest experiments, four lots of approximately 100 seeds constituted a treatment. The seeds were scattered on filter paper in petri dishes. The filter paper was laid over cotton-wool saturated with distilled water. For dark incubation, the dishes were stacked in light-proof tins. Both dark incubation and irradiation were at 25°C, except in the group of experiments involving low temperature dark incubation (usually 12°C). Any necessary manipulations were done in complete darkness.

Red light was obtained by passing the light from warm white fluorescent tubes through red "Perspex" (I.C.I., Red 400) providing about 360 erg/cm^2/sec in the plane of the seeds for short exposures, or through three layers of red "Cellophane" (Dupont, Red) for continuous exposure (approx. 20 erg/cm^2/sec). Far red radiation, providing about 50 and 500 erg/cm^2/sec between 700 and 900 mμ for continuous and short exposures respectively, was obtained by filtering the light from incandescent lamps through red "Perspex" and two layers of blue "Cellophane" (Dupont, Dark Blue). Narrow wavebands with half-band widths of 20 mμ or less were obtained by passing incandescent light through interference filters (Schott & Co., Mainz).

Results are expressed as mean percentage germination. The criterion for germination was protrusion of the radicle. In the experiments involving short exposures to red light, those seeds which germinated without the intervention of red light were recognized by the greater length of their radicles and were excluded from the calculations. The results for experiments involving continuous exposure include those seeds which would have germinated in darkness, but their inclusion makes little difference to the value obtained because the level of dark germination at the temperature used was low, except in one sample (see Table 5). For experiments designed to compare the efficiencies of several wavebands, results for percentage promotion or inhibition of germination were transformed to probits and plotted against log relative energy (Borthwick *et al.* 1954). From the straight-line relationship so obtained, the relative energy for 50% promotion or inhibition at each wavelength was calculated.

In this paper, germination is said to be complete when all viable seeds had germinated. Tests other than those described indicated that some samples contained a proportion of non-viable seeds. In such cases complete germination is thus less than 100%. By maximum germination is meant the highest germination obtainable

after saturation of the photosystem; it is less than complete when a sample is dormant or has received inappropriate dark incubation. A dormant sample is here defined as one which is incapable of germinating completely at 25°C in continuous red light, or with short, saturating, red light given after optimum dark incubation.

III. Methods and Results

In general, the experiments were designed so that each investigated one, or only a few aspects of germination behaviour. All aspects were not investigated on all samples.

(*a*) *Evidence for Participation of the Phytochrome System*

When the opposing effects of red and far red radiation were to be observed, seeds were exposed, after appropriate dark incubation (see Section III(*d*)), to red alone or to red immediately followed by far red in the sequences depicted in Table 2. The

Table 2

Germination of Anagallis seeds exposed to short saturating irradiation

Sample 53, 10 days dark incubation at 25°C. FR, far red

Radiation	Mean Germination (%)
Red	78·4
Red–FR	4·4
Red–FR–Red	78·3
Red–FR–Red–FR	3·5
Red–FR–Red–FR–Red	78·8
Red–FR–Red–FR–Red–FR	1·8
Dark	0·4

amount of each kind of radiation was more than enough for maximum promotion or inhibition of germination. Red elicited complete germination (this sample contained 80% viable seeds). When red was followed immediately by far red, subsequent germination was brought to a very low level. When a series of red and far red exposures alternated, the germination obtained depended only on the nature of the last irradiation.

The red and far red filters used in the preceding experiment transmitted broad bands of the spectrum. In order to characterize the system further, the relative amounts of energy in several narrow wavelength bands required for 50% promotion or inhibition were measured. When it was desired to promote germination seeds were taken from darkness, exposed to the appropriate wavelengths, and returned to darkness. When germination was to be inhibited, seeds were first exposed to red light to promote germination then immediately exposed to the inhibitory wavelengths before being returned to darkness. At each wavelength a range of energies was

obtained by varying time or intensity or both, having previously established that the reciprocity law held over the range of irradiances used.

The most efficient wavelengths were 670 mμ for promotion, and 748 mμ for inhibition (Table 3).

TABLE 3

RELATIVE ENERGY AT SEVERAL WAVELENGTHS REQUIRED FOR 50% PROMOTION OR INHIBITION OF GERMINATION

Sample 58A, 2 days dark incubation at 25°C

Wavelength (mμ)	Relative Energy	
	Promotion ($1 \cdot 0 = 2 \cdot 7 \times 10^1$ erg/cm^2)	Inhibition ($1 \cdot 0 = 1 \cdot 8 \times 10^4$ erg/cm^2)
586	10·2	—
611	3·3	—
671	1·0	—
703	8·6	1·6
748	—	1·0
760	—	1·6

(*b*) *Germination under Continuous Red Light*

Once the period of post-harvest dormancy was past, seeds which were exposed to red light at 25°C from the time they were set out germinated completely. For all

TABLE 4

DURATION OF DORMANCY AS INDICATED BY GERMINATION AT 25°C UNDER CONTINUOUS RED LIGHT

Sample	Age (weeks)	Germination (%)	Sample	Age (weeks)	Germination (%)
58A	12	98	60B	2	63
				6	90
58B	7	87		23	90
	9	95		43	96
59A	3	22	61AI	3	2
	29	99		10	6
				50	22
59B	5	99			
			61AII	5	53
60A	2	95		12	92
	6	98		52	96
			61AIII	5	99

non-dormant samples for which information is available germination was usually more than 90% by day 2, and was almost complete by day 3 or 4.

The duration of post-harvest dormancy varied greatly from sample to sample. Values in Table 4 show a range from less than 2 weeks for sample 60A to more than a year for sample 61AI.* While seed was dormant, not only was germination incomplete, but the rate was lower (Fig. 1).

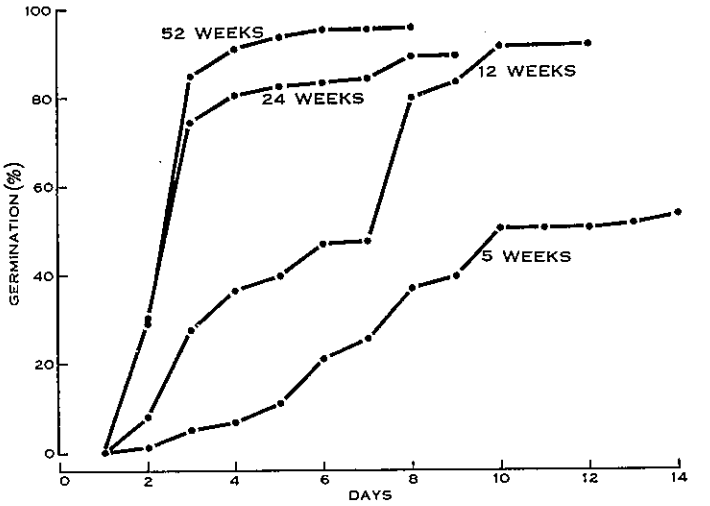


Fig. 1.—Germination of a sample of *Anagallis* seed under continuous red light at four post-harvest ages. Sample 61AII, 25°C.

(c) *Germination in Darkness*

Seeds were held in darkness for 5 or more days at 25°C, or for 14–17 days at 12°C, before counting the number of germinated seeds.

TABLE 5

GERMINATION OF NON-DORMANT ANAGALLIS SEEDS IN DARKNESS

Sample	Mean Germination (%)		Sample	Mean Germination (%)	
	25°C	12°C*		25°C	12°C*
53	0·4	—	59A	2·1	54(10°C)
54	0·0	1·5(10°C)	59B	2·1	100(10°C)
57A	1·4	53	60A	1·3	52
57B	2·1	41	60B	0·8	44(13°C)
58A	1·2	16	61AIII	0·8	56
58B	26†	87			

* Except where indicated. † 0% at 30°C.

At 25°C germination in complete darkness was low (Table 5). In all cases listed in this table the seeds were capable of germinating completely in continuous red

* The viability of this sample is at least 80% as indicated by germination procedures not discussed in this paper.

light. Sample 58B is the only one with appreciable dark germination. Its dark germination rose from harvest until a steady level of about 25% was reached approximately 6 months after harvest, i.e. about 4 months after complete germination could be induced by red light.

Dark germination was usually increased by holding the seeds at temperatures lower than 25°C (Table 5). It varied from a level which was little different from that at 25°C (sample 54) to 100% (sample 59B), with most of the samples in the middle of the range. Samples grown under the same temperature regime (25°C/20°C), but in different years, did not all behave similarly.

While a sample was still dormant, low temperature dark germination was lower than in the same sample when non-dormant, and did not exceed the values then obtainable with continuous red light at 25°C.

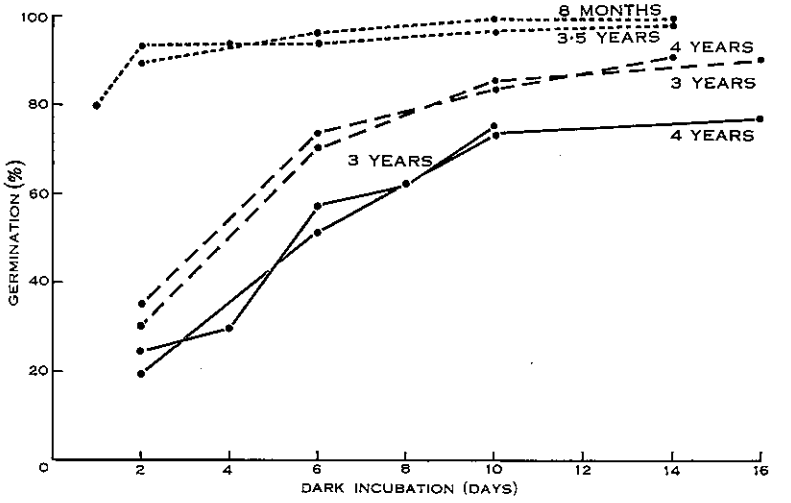


Fig. 2.—Promotion of germination in non-dormant samples of *Anagallis* seed by a short red irradiation given after increasing periods of dark incubation. ——— sample 53; — — — sample 54; · · · · · sample 58A.

Dark germination, both at 25°C and at low temperature, was completely inhibited by continuous exposure to far red radiation, but not by brief exposure. The effect of continuous and short exposure to far red is further illustrated by the following experiment. Seeds were exposed to red light for 24 hr at 25°C. Then, while there was still no visible sign of germination they were transferred to (1) dark, (2) 15 min far red, then dark, (3) continuous far red. Two days later 98% of those in (1) and (2) were germinated normally, while in (3) only 42% had germinated and only one-quarter of these had normally elongated radicles.

(*d*) *Effect of Duration of Dark Incubation*

As stated earlier, non-dormant *Anagallis* seeds germinate completely when exposed to a brief but saturating red irradiation, but only if they have previously been held imbibed in darkness for some time.

Details of the time relationship were investigated by placing batches of seeds in darkness at intervals, and at the appropriate time exposing all to saturating red light. Immediately after exposure the seeds were returned to darkness for 3 or 4 days before scoring for germination.

From the knowledge that water uptake is accomplished in 2–3 hr, and by analogy with results obtained by Borthwick *et al.* (1954) for lettuce, an optimum dark period of several hours was expected. Experiments using sample 53 showed,

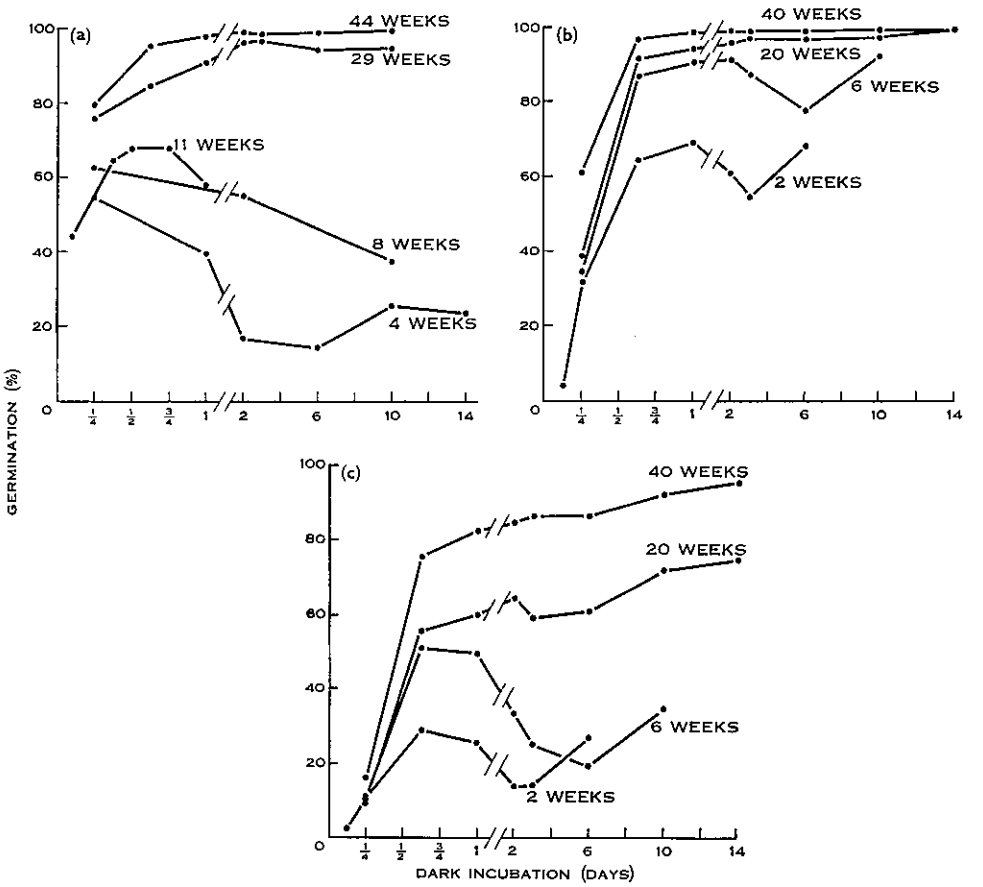


Fig. 3.—Promotion of germination by a short red irradiation given after various periods of dark incubation at several stages during passage out of dormancy: (*a*) sample 59A; (*b*) sample 60A; (*c*) sample 60B.

however, that this expectation was not realized. Ten days or more were required to elicit complete germination in this sample, which at that time contained about 80% viable seed (Fig. 2). Sample 54 showed a closely similar requirement for dark incubation, but with a viability of about 90% (Fig. 2). Both these samples were 3 years old when first tested. They were re-tested several times during the following year and always showed the same dark incubation requirement.

Sample 58A was first tested 8 months after harvest. Although germination after 2 days dark incubation was significantly less ($P < 0{\cdot}01$) than that for longer times, the difference was slight compared with those found for the previous samples (Fig. 2). The difference between this sample on the one hand and 53 and 54 on the other does not appear to be a matter of age because this pattern of incubation requirement was still evident when the seed was 4 years old and viability was decreasing.

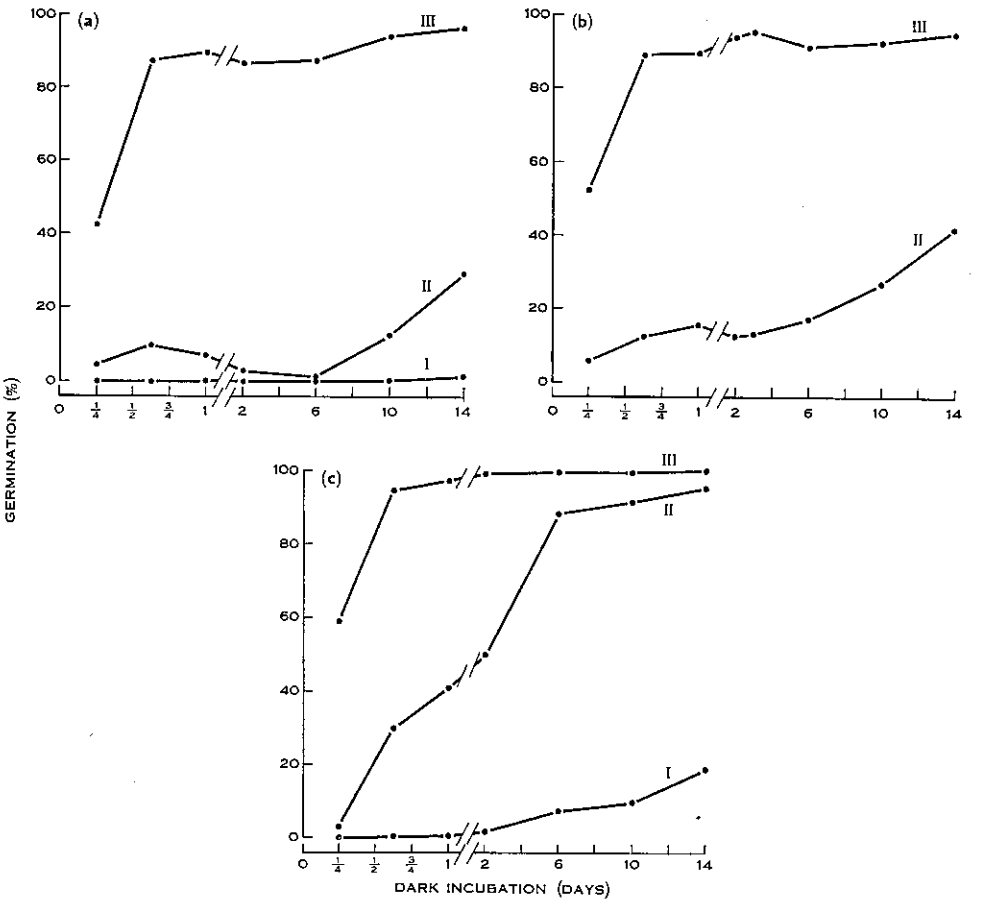


Fig. 4.—Germination of clonally derived seed samples promoted by a short red irradiation given after various periods of dark incubation. Sample 61A. Seed produced at: 20°C/15°C (I); 25°C/20°C (II); 30°C/25°C (III). Post-harvest ages: 4 weeks (*a*); 28 weeks (*b*); 49 weeks (*c*).

The three samples above were not dormant at the time they were tested. Data for three samples (59A, 60A, and 60B), which were partially dormant when first tested, are given in Figure 3. The general picture is the same for all three samples. Germination was low soon after harvest, but increased with the passage of time. While the samples were still incapable of germinating completely, the highest germination was obtained when the seeds were held imbibed for only a short period (15–24 hr). For samples 60A and 60B there was a suggestion of a second rise in germination at the

longest imbibition period. When the samples became capable of substantially complete germination, periods of 1–14 days were equally effective. That is, their reaction to dark incubation became similar to that of sample 58A (see Fig. 2).

The level and duration of dormancy, measured by inability to germinate completely after a single exposure, varied between the three samples. For samples 60A and 60B, while the seeds were still dormant, continuous red light was capable of eliciting a higher germination than the best germination resulting after a short exposure (compare data of Fig. 3 and Table 4). It is thus possible for a sample to be classed as dormant on the basis of its reaction to a short exposure, and non-dormant on that of its reaction to continuous exposure.

Widely different behaviours were exhibited by the genetically identical samples 61AI, 61AII, 61AIII (Fig. 4). Sample 61AIII was almost completely germinable 4 weeks after harvest with a dark incubation requirement similar to that of the samples just considered. Sample 61AII was much more dormant and, unlike other seeds for which information is available, it germinated best after long dark incubation while it was still dormant. At the most recent test, 1 year after harvest it was scarcely dormant and its dark requirement was similar to that for samples 53 and 54. Sample 61AI was very dormant; 1 year after harvest only about 20% germinated with either short or continuous exposure. This sample too germinated better after a long dark period.

IV. Discussion

(*a*) *Participation of the Phytochrome System*

Reversibility of the effects of red and far red radiation, with maximum efficiencies in the vicinity of 660 and 730 mμ respectively, provides reliable evidence for the participation of phytochrome in a process (Borthwick *et al.* 1954). The results of Section III(*a*) indicate that light-induced germination of *Anagallis* seed is mediated by this system. The absolute amount of far red energy required for inhibition of *Anagallis* seed germination is of the same order of magnitude as that required for the control of germination, flowering, and other phytochrome-dependent phenomena (Hendricks and Borthwick 1955). The amount of red energy required by *Anagallis* is, however, only 10^{-3}–10^{-4} of that required for these other phytochrome-dependent processes. The close similarity in amounts of energy required for a wide range of processes is considered to be evidence that pigment conversion by radiant energy is a basic requirement in all such processes. The apparent ability of *Anagallis* seed to achieve pigment conversion in one direction with an abnormally small amount of energy is therefore unexpected.

(*b*) *Dark Germination*

With the exception of sample 58B, germination at 25°C is completely dependent on the participation of phytochrome. At lower temperatures, however, where some germination takes place in darkness, a series of reactions by-passing the photo-reaction evidently occur. A similar effect of low temperature has been observed in other species of light-sensitive seeds (Evenari 1952; Toole *et al.* 1955; Kadman-Zahavi 1960).

The relatively high dark germination at 25°C of sample 58B did not reach a steady level until several months after complete germination could be induced by red light. There was thus a loss of requirement for red light by a fraction of the seed population during the early post-harvest stage. Absence of dark germination at 30°C in this sample seems to indicate that its dark germination differs in degree, but not in kind from that of the other samples. The situation in the *Anagallis* seed samples could be analogous to that found by Evenari (1952) for several varieties of lettuce seed.

Far red radiation can apparently inhibit germination at a later stage than the initial photoreaction discussed above. Evidence for this is provided by the action of continuous far red radiation in inhibiting germination and interfering with radicle elongation at a stage when brief far red irradiation was unable to reverse the germination-promoting effect of red light. Inhibition of dark germination, both at 25 and 12°C, by continuous far red radiation is probably due to the same action.

(c) *Impediments to Germination*

Incomplete germination of a sample under given conditions implies the existence in some individuals of the sample of a block in the sequence of reactions required for germination. When the condition is that of exposure to brief saturating red light, the samples investigated fall into four groups on the basis of the dark incubation required to elicit maximum germination:

1. Dormant: (*a*) requiring a short dark period and for which a long dark period is inhibitory;
   (*b*) requiring long dark incubation.
2. Non-dormant: (*a*) for which short or long dark periods are equally effective;
   (*b*) requiring long dark incubation.

This grouping is consistent with the existence of blocks, but provides no evidence for their nature or position in the reaction chain. They may occur before, at, or after the photoreaction. For convenience they are considered to be due either to deficiency, but not complete absence, of a substrate, or to the presence of an inhibitor. Three blocks are postulated:

A: that present in all dormant samples (groups 1(*a*) and 1(*b*)) and overcome as the seeds pass from the dormant to non-dormant state. The degree of blocking varies from one sample to another. Where continuous red light allows higher germination than does a short exposure, the former apparently prevents dark reversion of phytochrome and so permits enough of a limiting process to occur to allow germination.

B: that introduced by incubating imbibed seeds in darkness for several days. In addition to possessing block *A*, most samples of dormant seed (group 1(*a*)) acquire this block, whereas non-dormant samples do not.

C: that overcome by incubating imbibed seeds in darkness for several days. This block is present in two samples of dormant seed (group 1(*b*)—61AI and 61AII) and in several samples of non-dormant seed (group 2(*b*)). A slow process, either of inhibitor removal or substrate synthesis, is evidently necessary before these samples can germinate completely after brief red

irradiation. The rapid germination of group 2(*b*) seeds in continuous red light is at first sight unexpected, but the explanation is probably similar to that already advanced to account for the germination of dormant seeds in continuous red light.

Seeds of group 2(*a*) do not possess any blocks. With the passage of time the dormant samples of group 1(*a*) became members of group 2(*a*) and one sample in group 1(*b*) has become a member of 2(*b*). These changes would involve disappearance of block *A* and, in addition, for the transition from group 1(*b*) to 2(*b*), of block *B*.

Sufficient samples have not been examined to justify a conclusion that seeds of groups 2(*a*) and 2(*b*) always originate as seeds of groups 1(*a*) and 1(*b*), respectively, as observed here. There could be types of behaviour other than those shown by the samples examined.

(*d*) *Effect of Internal and External Factors on the Germination Pattern*

The foregoing discussion emphasizes the differences which exist between seed samples of a single strain of *Anagallis* and the changes which occur within individual samples as they age. These differences must owe their existence to differences between samples in genetic constitution and in the environment in which the seeds were formed and matured. There is no reason to believe that storage conditions acted differentially on the various samples, or that any slight differences in the stage at which the seeds were harvested were of any significance. In the pre-harvest environment, temperature was the only factor which was controlled and therefore differed in a known manner. Nothing is known about differences in genetic constitution. For all except the clonally derived samples 61AI, 61AII, and 61AIII both genetic and environmental factors would have been operating to produce the observed germination pattern.

(i) *Dormancy.*—Since samples 61AI, 61AII, and 61AIII are genetically identical, the great differences in their dormancy status must be related to environmental differences. The obvious difference between the three environments is in temperature, increased dormancy being associated with lower temperatures.

A similar relation between dormancy and temperature during seed formation is shown by several other samples, in particular by 59A and 59B and by 60A and 60B. The plants bearing samples 60A and 60B were all maintained at 25°C/20°C until flowering, when those bearing 60A were transferred to 30°C/25°C. The seed formed on them was less dormant. There are also differences in dormancy which are not obviously to be related to temperature. The four samples coming from both the A and B lines formed at 25°C/20°C in successive years show quantitative differences in dormancy. There are sure to be genetic differences between these samples, and, in addition, factors of the environment other than temperature such as day length (a result of different growing periods) and insolation would have varied from year to year.

(ii) *Dark Incubation Requirements.*—Comparisons between the requirements of the three genetically identical 61A samples are restricted because of prolonged dormancy of one member. The only known examples of dormant seed for which long

dark incubation is necessary occur here (61AI and 61AII—group 1(*b*)). In the non-dormant state, one member belongs to each of the two groups above—61AIII to group 2(*a*) and 61AII to 2(*b*)—and the third, 61AI, is as yet unassigned. Samples falling into group 2(*a*) occur in all except the earliest generations (several examples in both the A and B lines, in addition to those mentioned here, are known). In addition to sample 61AII, group 2(*b*) is represented by two samples in the earliest generations. All samples grown at 25°C/20°C, except 61AII, fall into group 2(*a*). The anomalous behaviour of this sample is presumably the result of some fortuitous combination of genetic and environmental factors.

(iii) *Dark Germination.*—Since values for low temperature dark germination of samples 61A cannot yet be obtained, effects of environment on this feature unconfounded with genetic differences cannot be observed. The samples grown at 25°C/20°C in different years show a range of values for low temperature dark germination. The negligible low temperature dark germination of sample 54 has not been repeated in later generations in either the A or B line. Only the B line has members with a low temperature dark germination of $> 80\%$ and only the A line has a member with $< 20\%$; both lines have several members in the middle of the range.

The reason for the relatively high dark germination of sample 58B at 25°C is unknown, but is probably due to some unrecognized difference in the location in which it was grown (Los Angeles).

Ample evidence for differences between samples of a single strain of *Anagallis* has been presented. There is no evidence for a divergence of the A and B lines. Neither low temperature dark germination nor dark incubation period required for maximum response to red light can be seen to fall into a pattern related either to the position of a sample relative to other samples or to a single facet of the environment. The differences observed are consistent with those to be expected from the method of breeding used in which each generation of seeds arose from a very small fraction of the seed population of the preceding generation. Dormancy, however, is definitely related to a factor of the environment, namely temperature.

V. Acknowledgments

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VI. References

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