PHOTOREVERSIBILITY OF FLOWER INITIATION[1]

R. J. DOWNS

HORTICULTURAL CROPS RESEARCH BRANCH, PLANT INDUSTRY STATION,
BELTSVILLE, MARYLAND

Action-spectrum studies have shown that such diverse light-controlled plant responses as flower initiation (1, 17, 18), internode elongation (2, 8, 19), leaf expansion (8, 19), and seed germination (4, 23) are regulated most effectively by red radiation near 6500 Å. Borthwick et al (4) recently demonstrated that the red reaction inducing the germination of light-sensitive lettuce seed could be reversed. Their studies show that far-red near 7350 Å is the region of maximum effectiveness in reversing the red reaction and thereby inhibiting seed germination. Thus, seed irradiated with red are put into a germinating condition that is nullified or reversed if a far-red irradiation immediately follows the red. This duodirectional reaction was shown to be repeatedly reversible and to be independent of temperature. This same reversible photoreaction was also established for the control of flower initiation of Xanthium (3), cuticle coloration of tomato fruits (20) and photomorphogenic effects such as leaf expansion (8, 12), hypocotyl elongation (8) and the disappearance of the plumular hook of dark-grown beans (26).

The purpose of the studies reported herein was to investigate some of the details of this photoreversible reaction as it applies to the control of flower initiation.

METHODS AND RESULTS

PRELIMINARY STUDIES: Preliminary experiments were conducted to test the occurrence of the reaction in several kinds of photoperiodically sensitive plants. By use of the established technique of interrupting the dark period, two short-day plants, soybean (*Glycine Max* (L.) Merr. var. Biloxi) and pigweed (*Amaranthus caudatus* L.), and two long-day plants, barley (*Hordeum vulgare* L. var. Wintex) and *Hyoscyamus niger* L., were tested for evidence of the far-red action. A brief irradiation near the middle of an inductive dark period of 13 hours for soybean and 12 hours for pigweed prevented initiation of flowers, whereas a similar interruption near the middle of a non-inductive dark period of 12.5 hours for barley and 12 hours for Hyoscyamus induced flowering. In each case a far-red irradiation immediately following the dark-period interruption with red reversed the effect of the red light (table I).

PLANT MATERIALS: Studies into the details of the photoreversible reaction controlling flower initiation were conducted principally with the short-day plants cocklebur, *Xanthium pensylvanicum* Wallr. (*X. saccharatum* Wallr.) and Biloxi soybean.

There is considerable confusion arising from personal variations in the choice of the specific name for this species of Xanthium. Wallroth (24) described *X. pensylvanicum* and *X. saccharatum* as distinct species and published his descriptions in the same journal on the same date. According to Section 10 of the International Rules of Nomenclature (6), when two such species are combined, the older name must be retained. If neither name is the older, then the choice remains with the person making the combination, provided both names are valid. S. F. Blake, basing his decision on the work of Widder (25), declared the valid name to be *X. saccharatum* (18). Further in-

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TABLE I

EFFECT OF DAILY INTERRUPTIONS OF THE DARK PERIOD WITH RED AND FAR-RED RADIATION ON THE FLOWER INITIATION OF TWO LONG-DAY AND TWO SHORT-DAY PLANTS

PLANT	TREATMENT	PLANTS PER LOT	PLANTS FLOWERING PER LOT	MEAN STAGE OF FLORAL DEVELOPMENT *	TOTAL FLOWERS	MEAN STEM LENGTH *
		no.	*no.*		*no.*	*mm*
Hyoscyamus	Control	4	0	...	...	0.0
	Red	8	8	...	...	18.5
	Red + far-red	8	3	...	...	4.6
Wintex barley	Control	12	12	2.3	...	3.2
	Red	16	16	6.7	...	19.4
	Red + far-red	18	18	3.2	...	3.9
Amaranthus	Control	10	10	...	...	...
	Red	10	1	...	...	...
	Red + far-red	12	12	...	...	...
Biloxi soybean	Control	4	4	...	18	...
	Red	4	0	...	0	...
	Red + far-red	4	4	...	10	...

* Floral-development and stem-length data were taken according to the procedures described in references (1) and (17).

vestigation has shown that Millspaugh and Sherff (14) had made an earlier combination in favor of *X. pensylvanicum*. Thus, *X. pensylvanicum* is established as the valid specific name and *X. saccharatum* must become a synonym.

EVALUATION OF RESPONSE: Although investigations of photoperiodic responses have generally been reported in terms of the presence or absence of flower primordia, proper evaluation of the results reported here demanded a graduated measure. It was obvious from previous reports (9, 15) and from preliminary dissections that the development of the inflorescence of cocklebur was not an all or none affair and that the amount of floral development in a given time depended upon the level of the initial stimulus that initiated it. Thus, it was decided to divide the development of the inflorescence into several easily identifiable stages.

Other investigators (7, 11, 13) have assigned numerical values to arbitrarily selected stages of development of the cocklebur inflorescence, but unfortunately their techniques cannot be used because the descriptions of their stages are inadequate. Recently, however, Salisbury (21) adequately described stages of development based on the linearity of development with time. The experiments reported herein were finished before Salisbury reported his work; the stages of development, while not identical with his, differ only in minor detail.

Eight stages of development of the staminate inflorescence were chosen as indicators of the response of cocklebur to photoperiodic treatment. These stages were designated 0 to 7, 0 indicating a vegetative plant and 1 through 7 plants in which increasing inflorescence development is signified by increasing numerical value. Stage 1 is the earliest stage at which differences in the terminal meristems of vegetative and reproductive plants can be recognized. The terminal meristem of the reproductive plant is slightly higher and more dome-shaped than that of the vegetative one. This development probably represents the beginning of the receptacle of the inflorescence. In stage 2 the receptacle is enlarged, constricted at the base and definitely dome- or egg-shaped, with primordia of floral bracts at the base. When the inflorescence primordium reaches stage 3, the smooth, rounded primordia of the individual flowers are visible on the lower half of the receptacle and each flower is subtended by a bract. The acropetal succession of bracts and flower primordia continues until at stage 4 the flowers are visible more than half way up the receptacle, but not covering the top. At stage 5 the flower primordia completely cover the receptacle and the lobes of the five-parted corolla on the lowermost flowers are visibly differentiated. At stage 6 corolla lobes are differentiated in all flower primordia except those at the very top of the receptacle, and at stage 7 all flower primordia have clearly differentiated corolla lobes and the primordia of the five stamens are present in the lowermost flowers. The response of cocklebur to experimental treatments was evaluated on the basis of these stages of development.

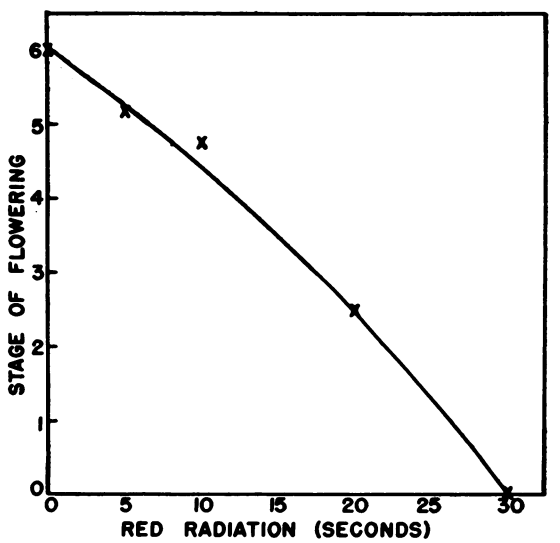
The response of Biloxi soybean, however, was evaluated in terms of the number of nodes bearing flower primordia, a technique already established (5, 18) as a measure of the amount of stimulation afforded by photoinductive treatments. In this system of evaluation reference is made only to flower primordia initiated on the main axis.

EXPERIMENTAL METHODS: Burs of *Xanthium* were planted in pots of soil and placed immediately on the greenhouse bench where the resultant plants received normal daylight plus a 3-hour period of light near the middle of the dark period. This dark-period interruption was made with about 30-fc illumination from 100-watt incandescent-filament lamps. When the vegetative plants produced in this way had 4 or 5 fully expanded leaves, they were taken to a controlled-environment room (16), where all leaves below the two most recently expanded were removed. The plants were then placed on inductive cycles of 12 hours of light and 12 hours of darkness at a temperature of 20° C. Three such inductive cycles were used and the plants were returned to the long-day conditions of the greenhouse. They were dissected 10 days after beginning of treatment. Interruptions were made near the middle of each inductive dark period with radiation obtained through two layers of red cellophane from 18 standard cool white, slimline fluorescent lamps (20). The unfiltered bank of lamps provided an illuminance of 1000 fc at plant level. A variable-energy experiment (fig 1) showed that 30 seconds at a distance of 1 meter from this source was adequate to inhibit the flower initiation potentially induced by the 12 hours of darkness. This was equivalent to about 10^4 ergs cm^{-2} sec^{-1} at 6500 Å, the region of maximum effectiveness. However, in order to be assured that the flowering stimulus was completely removed, an exposure of 2 minutes was adopted as the standard interruption irradiance.

Biloxi soybeans were grown in a like manner, but treatment was begun when the third compound leaf had fully expanded. All leaves below the third compound leaf were removed and the plants were placed on cycles of 10 hours of light and 14 hours of dark, at a temperature of 20° C. After 9 such cycles the plants were returned to long-day conditions until the time of dissection, 15 days after the beginning of treatment. Tests showed that the 2-minute interruption standardized for cocklebur also was adequate to prevent floral initiation in soybean.

REVERSIBILITY: The far-red region of the spectrum, near 7350 Å, was satisfactorily isolated from sources of relatively high far-red emittance such as the sun (8000 fc) or incandescent-filament lamps (800 fc) by a filter of two layers of 300-gauge red and two layers of 300-gauge dark-blue cellophane (20). Using the experimental methods just described, a comparison of the efficiencies of the two sources in supplying far-red for reversing the effect of a red interruption is given in table II. When the sun was

used as the far-red source in a variable-energy experiment, flowering of cocklebur plants previously inhibited by the 2-minute red interruption of the dark period was repromoted to about two-thirds that of the control by a 10-second exposure to far-red (fig 2). When exposures of 1 or 2 minutes were given, the repromotion of flowering approximated that of the controls, but as exposure times were increased further the amount of repromotion was slightly less.



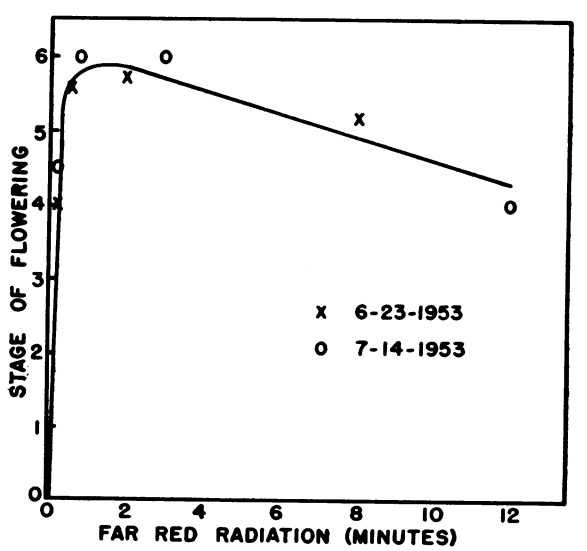


FIG. 1 (*top*). Flowering response of cocklebur plants exposed to various amounts of red energy near the middle of a 12-hr dark period.

FIG. 2 (*bottom*). Repromotion of flowering of cocklebur plants by various far-red energies immediately after a saturating exposure to red radiation.

TABLE II

COMPARISON OF THE EFFECTIVENESS OF THE SUN AND INCANDESCENT-FILAMENT LAMPS AS SOURCES OF FAR-RED RADIATION GIVEN AS DAILY DARK-PERIOD INTERRUPTIONS FOR THE REPROMOTION OF FLOWER INITIATION OF COCKLEBUR

TTEATMENT	STAGE OF DEVELOPMENT OF INFLORESCENCES *	
	SUN	FLOOD LAMPS, 3 × 300 W.
Dark control	6, 6, 6, 6, 6, 6	6, 6, 6, 6, 6, 6
Red	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0
Red + far-red	5, 4, 3, 4, 5, 4	4, 3, 4, 3, 3, 3
	5, 3, 4, 5, 5, 5	2, 4, 5, 3, 3, 4

* Each value represents a single plant.

Biloxi soybeans seemed to be less sensitive to far-red than cocklebur and the sensitivity seemed to vary with the vigor and quality of the plants. The low light intensities of the winter months resulted in soybeans with elongated hypocotyls and small leaves. Far-red repromotion of flowering in these plants was poor, even with exposures of 8 to 16 minues. However, the stocky, larger leaved plants grown under the moderate temperatures and higher light intensity of the spring months were vigorously repromoted by as little as a 2-minute exposure to far-red.

It was shown with light-sensitive lettuce seeds (4) that several successive cycles of red and far-red resulted in promotion of germination when the cycles ended with a red irradiation and in inhibition of germination when the treatment cycles terminated with an exposure to far-red. The reaction controlling flower initiation is also repeatedly reversible (table III). With both cocklebur and soybean, the plants remained vegetative when the last irradiation was

TABLE III

EFFECT OF DAILY INTERRUPTIONS OF THE DARK PERIOD WITH SEVERAL CONSECUTIVE IRRADIATIONS WITH RED AND FAR-RED IN SEQUENCE ON FLOWER INITIATION OF COCKLEBUR AND SOYBEAN

TREATMENT	MEAN STAGE OF FLORAL DEVELOPMENT IN COCKLEBUR *	MEAN NO. OF FLOWERING NODES IN BILOXI SOYBEAN **
Dark control	6.0	4.0
R	0.0	0.0
R, FR	5.6	1.6
R, FR, R	0.0	0.0
R, FR, R, FR	4.2	1.0
R, FR, R, FR, R	0.0	...
R, FR, R, FR, R, FR	2.4	0.6
R, FR, R, FR, R, FR, R	0.0	0.0
R, FR, R, FR, R, FR, R, FR	0.6	0.0

* Two min far-red from the sun. Values are for lots of 5 plants.

** Eight min of far-red from three 300-watt internal reflector flood lamps. Values are for lots of 4 plants.

with red, but when far-red was given last the effects of the previous treatments were removed and flower initiation was repromoted. It was noted, however, that the degree of repromotion became less with each red–far-red cycle until finally after several such cycles repromotion could no longer be effected.

TIME INTERVAL BETWEEN RED AND FAR-RED: The loss of far-red repromotion of flowering after several cycles of red alternating with far-red suggested that the passage of time might be the limiting factor since the total experimental time was 25 minutes for cocklebur and 45 minutes for soybean. To test this hypothesis there was set up an experiment in which dark periods of various durations were inserted between the red and the far-red irradiations. Near the middle of the 12-hour dark period, plants were irradiated with red for the usual 2-minute period and then returned to the dark. After various durations of darkness the plants were irradiated with far-red for 3

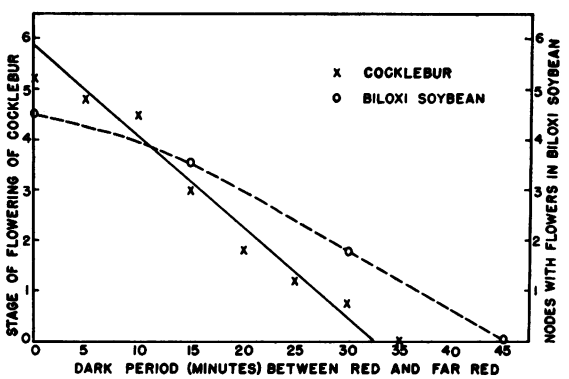


FIG. 3. Effect of various durations of darkness between the red and the far-red irradiation on the promotion of flowering of cocklebur and Biloxi soybean by far-red.

minutes. It was found that as the duration of darkness between the red and far-red irradiations was increased, the amount of far-red repromotion of flowering in both cocklebur and soybean decreased and finally failed (fig 3). The complete loss of far-red repromotion generally occurred after an intervening dark period of about 30 minutes for cocklebur and of about 45 minutes for soybean. A more precise measure of the critical time lapse between the red and the far-red irradiations could not be made for either soybean or cocklebur, because the time necessary for loss of repromotion varied directly with the potential stage of flowering as evidenced by the controls.

As would be expected from a photochemical reaction, the temperature at which the irradiations are made had no measurable effect upon the results of irradiation with either red or far-red (4). It seemed reasonable to assume, however, that temperature would affect whatever was taking place during the interval between the red and the far-red irradiation. Table IV shows the results of studies with cocklebur

TABLE IV

LOSS OF FAR-RED EFFECTIVENESS FOR REPROMOTION OF FLOWERING OF COCKLEBUR AND BILOXI SOYBEAN AS INFLUENCED BY DURATION AND TEMPERATURE OF DARK PERIOD INTERVENING BETWEEN SATURATING IRRADIATIONS WITH RED AND FAR-RED GIVEN NEAR THE MIDDLE OF 12-HOUR DARK PERIODS

DARK INTERVAL (MIN)	MEAN STAGE OF FLORAL DEVELOPMENT IN COCKLEBUR *		MEAN NO. OF FLOWERING NODES IN BILOXI SOYBEAN *	
	20° C	5° C	20° C	5° C
0	6.5	5.5 **	2.6	2.6 **
20	3.8	5.7	...	...
30	1.8	4.0	0.6	1.8
40	0.5	4.5	0.0	1.2
50	...	...	...	1.6
60	0.0	2.8	0.0	...
90	...	...	0.0	0.6
Dark control	7.0	7.0 **	5.0	5.0 †
Red control	0.0	...	0.0	...

* Temperatures of 20° C maintained during photoperiods and dark periods except during the dark interval between the red and far-red irradiations and as otherwise indicated. Values are means for lots of 4 cocklebur and 5 soybean plants.
** Held at 5° C for 60 min.
† Held at 5° C for 90 min.

and soybeans in which the temperature during the interval was varied. When the temperature was lowered to 5° C, the rate of loss of far-red repromotion of flower initiation became extremely slow in contrast to the rather rapid loss at 20° C.

DISCUSSION

There are several hypotheses that might be advanced to explain why flower primordia are initiated when far-red irradiation is given immediately after red, but not when a 30 to 45-minute time interval elapses between the red and the far-red irradiations. Any conclusions must agree with the experimental evidence obtained from studies of other plant responses controlled by the same photoreaction (4, 8, 20, 23). One of the more obvious of these hypotheses is that the far-red-absorbing form of the pigment might undergo thermal decomposition. However, all the results except those of the flowering response indicate that it takes about 8 hours between the red and the far-red irradiations for a 50 % loss of far-red reversibility. This solution, therefore, seems improbable since in the other plant responses the availability of the far-red-absorbing form of the pigment does not seem to be the limiting factor. Since the pigment seems to be present and operating, then some other factor must be involved.

Another possibility would be that the far-red-absorbing form is unstable and reverts to the stable red-absorbing form in a short time. However, previous studies on the photoperiodic control of cocklebur (18, 22) and soybean (18) present evidence that at the end of the light period the pigment is in the

far-red-absorbing form and that a period of about 3 hours is necessary for the dark thermal conversion to the red-absorbing form. Since this is about six times the period necessary for the complete loss of the far-red-repromotive effect, this hypothesis is also improbable.

The third hypothesis that suggests itself is that since the far-red-absorbing form of the pigment is the biologically active form (10), it starts a series of temperature-dependent events that in 30 to 45 minutes lead to a condition inhibitory to flowering. This seems to be the only proposal that agrees with the available experimental evidence. Generally the far-red irradiation does not completely repromote flowering even when given immediately after the red (table II). The difference between the flowering stage of the control plants and that of the far-red-repromoted plants is proportional to the duration of time lapsing between the two irradiations, reaching a maximum difference after about 30 minutes when the far-red treatment completely fails to repromote flowering (fig 3).

It seems reasonable to conclude that while the pigment is in the biologically-active, far-red-absorbing form there is a slow build-up of a condition inhibitory to flower initiation. Conceivably this inhibitory condition could be due to the formation or destruction of an inhibitor, or to the production or destruction in the presence of the far-red-absorbing form of the pigment of a substance that directly or indirectly promotes flowering.

Summary

The reversible photoreaction previously shown to control the germination of light-sensitive seeds and the flowering of cocklebur was extended to two short-day plants, Biloxi soybean and pigweed, and two long-day ones, Wintex barley and *Hyoscyamus niger*.

Studies into the details of this photoreaction were made with plants of cocklebur and Biloxi soybean. Measurement of the treatment stimulus on Xanthium was based on 8 stages of the development of the staminate inflorescence and on soybean on the number of nodes bearing flower primordia.

The action of the red energy was reversible by far-red several times during a brief period near the middle of the inductive dark period. However, the degree of repromotion by far-red decreased with each successive red–far-red cycle until finally no repromotion could be attained. Long exposures to far-red were less effective than exposures of 2 to 5 minutes. Far-red repromotion of flowering was reduced as the duration of darkness between the red and the far-red irradiations was increased. Finally, when 30 to 45 minutes of darkness intervened between the two kinds of radiation, no repromotion could be attained. The effect of this intervening period of darkness was nullified by reducing the temperature during that period.

It is suggested that the far-red-absorbing form of the pigment is the biologically active form and causes a slow build-up of a condition inhibitory to flowering.

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EXOGENOUS SUBSTRATE UTILIZATION AND FERMENTATION BY THE POLLEN OF PINUS PONDEROSA [1]

HENRY HELLMERS [2] AND LEONARD MACHLIS
DEPARTMENT OF BOTANY, UNIVERSITY OF CALIFORNIA, BERKELEY, CALIFORNIA

The pollen of *Pinus ponderosa* lives under circumstances suggesting that it should be capable of absorbing and metabolizing externally supplied carbohydrate and that it should be a facultative anaerobe. The data submitted in this paper indicate that the implied capacities are in fact characteristic of the growing pollen.

The question of the use of exogenous carbohydrate arises because the time between pollination and fertilization in pine is unusually long, approximately one year. The pollen (fig 1), shed in the spring, is carried by air to the micropyle of an ovule. The exudation and subsequent resorption of a drop of liquid pulls the grain through the micropyle to the surface of the nucellus where the grain develops a pollen tube directed into the nucellar tissue. Growth ceases over the winter but resumes again the following spring when the pollen tube completes its passage through the nucellus into the neck of an archegonium where it ruptures, releasing the stalk cell and the tube and sperm nuclei (1, 3, 4, 9, 13). During this year of life the pollen grain develops a relatively long tube and presumably respires actively except for the cold winter months. On water-agar at 25° C the developing pollen becomes moribund after 10 days. In situ it must survive much longer and would appear to require exogenous food reserves, presumably from the nucellus.

The second question, that of a capacity for anaerobiosis, arises because of the location of the pollen in the nucellus. After pollination the megasporophylls contract to form a tightly closed cone. In addition, the cells on the lower rim of the micropyle grow until the opening is completely closed (5). Thus, the pollen is doubly encased in a relatively massive structure with no obvious access to air.

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[2] Present address: California Range and Experiment Station, U. S. Department of Agriculture, Kerckhoff Laboratory of Biology, California Institute of Technology, Pasadena, California.

MATERIALS AND METHODS

COLLECTION, STORAGE, AND HANDLING OF POLLEN: In May 1948 nonshedding catkins were removed from a tree bearing shedding catkins to insure the collection of ripe pollen. The catkins were placed in a single layer on screens through which passed filtered air at 28° C and 45 to 50 % relative humidity. Four to 6 days later the pollen and chaff in the traps under the screens were passed through a 60-mesh screen. The clean pollen was stored at 0° C in sealed jars containing open test tubes of sulfuric acid to maintain a relative humidity of 30 % (6, 7, 22). This pollen was used between May and September 1949. Over this period, the percentage germination decreased 2 % from an initial 90 %. Pollen was also collected from other trees in 1948 and again in 1949. The behavior of the pollen from the several collections was similar to that of the single collection used in the experiments reported in this paper.

Samples of pollen were removed from the jars in the refrigerator room with glass divers. Divers containing 100 to 200 mg of pollen were brought to room temperature in desiccators, weighed, emptied, and reweighed. Dry weights refer to the pollen as described.

CONTAMINATION: Pollen, collected as described, is contaminated with bacteria. All efforts to obtain bacteria-free pollen failed. Attempts were made by: (a) washing pollen in sterilizing agents including sterile water, boric acid, merthiolate, ethyl alcohol, mercuric chloride, streptomycin, "Chlorox," bromine water, "Roccal" (alkyl-dimethyl-benzyl ammonium chloride); (b) germinating the pollen on agar that had been washed with a sterilizing agent so as to leave a thin film on the surface of the agar; and (c) germinating the pollen directly in solutions of sterilizing agents of various strengths. Agents effective in killing bacteria also damaged or killed the pollen. Attempts were also made to control contamination during the process of pollen collection. Unopened