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PHYTOCHROME CONTROL OF SEED GERMINATION IN THE TROPICAL RAIN FOREST PIONEER TREES *CECROPIA OBTUSIFOLIA* AND *PIPER AURITUM* AND ITS ECOLOGICAL SIGNIFICANCE

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SUMMARY

The tropical forest pioneer trees *Cecropia obtusifolia* and *Piper auritum* germinate and become established in large light gaps of the forest canopy in the rain forest of south-eastern Mexico. Germination of the seeds of both species is under photocontrol and is triggered when the red:far-red ratio (R:FR) of the incident light increases due to a reduction of the green canopy density. Exposure to simulated light canopies retarded and reduced germination. The light environment inside the forest inhibits germination totally. Experiments with alternate R and FR light treatments indicate the need for long periods of exposure to R light for germination, and demonstrate a strong reversibility of the R light stimulation by FR light in both species. This property of the seeds may be related to the detection of light gap size and its differentiation from the normal sunflecks of the forest.

INTRODUCTION

During recent years, ecological research in tropical rain forest areas has shown that naturally-occurring light gaps in the forest canopy play an important rôle in the vegetation dynamics and internal regeneration processes of the forest. The gaps are produced by canopy trees falling, the frequency of such events depending on the occurrence of strong winds and the type and size of the tall trees of each region. Measurements of the frequency of light gap formation in some forests of Central America (Hartshorn, 1978) and south-east Asia (Whitmore, 1978) indicate that the probability of light gap formation in any one place is about one per 100 years in the places studied.

The rain forest tree species can be roughly divided into two groups depending on the type of seeds that they produce, the way those seeds germinate, and the means whereby those seedlings become established (Gómez-Pompa and Vázquez-Yánes, 1974). Apparently, most mature forest tree species produce large seeds, capable of germinating under the unaltered forest canopy to produce umbrophile seedlings. In some species, the seedlings can grow slowly till maturity, but in most tree species the seedlings remain dormant or die unless a light gap is formed in the place where they may grow (Whitmore, 1975).

The other type of tree species, namely the rapidly growing short-lived heliophile pioneers, produces very large amounts of small seeds which remain dormant under

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intact canopies and germinate to become seedlings only when a sizeable light gap is formed. This kind of tree produces very small heliophile seedlings that are incapable of living and growing under canopy shade-light.

The pioneer tree is a very well characterized group of plants (Vázquez-Yánes, 1980a), typical of every tropical rain forest in the world; they are particularly abundant in large light gaps where they replace the mature forest trees acting as a cicatrizal vegetation (Schnell, 1971). Human perturbation of forests has produced a huge increase in pioneer tree abundance.

The purpose of this paper is to explore the nature of the light-induced germination in two pioneer trees from the rain forest of south-eastern Mexico. In previous papers it was demonstrated that *Cecropia obtusifolia* and *Piper auritum* (Vázquez-Yánes, 1979, 1980b) produce light-requiring seeds which remain dormant under the unaltered forest canopy due to the low red:far-red ratio (R:FR) of the shade-light inside the forest. It is well known that the pigment phytochrome participates in the light-regulated germination of seeds, allowing the seeds to detect the spectral composition of the light, triggering the germination when the light conditions become appropriate for the establishment of the plants (Smith, 1973; Frankland, 1976).

Due to the low probability of large light gap formation in the rain forest to which, in natural conditions, both species are restricted, it can be expected that their seeds will possess: (a) a very efficient dormancy mechanism to prevent germination under inappropriate light conditions for establishment, (b) the capacity to germinate quickly when the light environment changes due to the formation of a light gap of a sufficient size and (c) a relatively extended survival capability in the soil.

A series of experiments was designed to assess the survival of the seeds in the soil and in water-imbibed dark storage during a period of 1 year, to test the effects of different wavelengths on germination, to study the effects of natural and simulated canopies of different densities on seed germination, and to investigate the possible role of phytochrome in attuning germination to changes in the natural light environment.

The species

Cecropia obtusifolia Bertol (Moraceae) is a typical pioneer tree, very abundant in light gaps and young secondary vegetation in the lowland tropical rain forest of south-eastern Mexico, central America and northern South America. The trees of this species grow very quickly, up to 3 m per year (Golley *et al.*, 1975) reaching 25 m in height and normally living no more than 30 years. The plants soon reach sexual maturity and continuously produce fleshy infructescences with hundreds of small red seeds which are eaten and dispersed by birds bats and other mammals (Vázques-Yánes, 1979). The trees have an umbrella type of crown with all the leaves exposed to direct sunlight. Synecological studies indicate that this species grows only in large gaps when it is found in mature forest (Knight, 1975).

Piper auritum H.B.K. (Piperaceae) is a small short-lived tree with a similar habitat preference and geographical distribution as *C. obtusifolia*. They are frequently found together in light-gaps and secondary vegetation. This tree reaches about 5 to 8 m in height and normally survives for about 15 years. It also has an umbrella-type crown with heliophile leaves. The plants produce, all the year round, infructescences with thousands of small black seeds, which are eaten by bats and birds.

Materials and Methods

Field Experiments

Seeds of both species employed in the study were collected and the field experiments were performed in the Tropical Biology Station of 'Los Tuxtlas' from the National Autonomous University of Mexico. This reserve is located in the evergreen rain forest area of the coastal lowlands of southern Veracruz State. Its altitude is 160 m, mean temperature 26 °C, and annual rainfall > 4000 mm. More information about this biological reserve has been provided by Lot (1976).

The experiment on the longevity of buried seeds consisted of mixing groups of 200 seeds of each species with rain forest soil previously heated to kill any other seed which may have been present in the soil. Each sample of the mixture, in a small bag of nylon net, was buried 5 cm deep in the mature rain forest soil. A bag was disinterred each month during a year, the soil inside it was spread on a wet surface inside a growing chamber at 25 °C with 12 h photoperiod of white light and the final total germination was recorded.

The experiment on germination in the natural light environment was performed in a recent natural light gap of the forest on the slope of a hill orientated to the east and thus illuminated directly by the sun during the morning. The gap was about 4 m in diameter and the area employed for the experiment was the unearthed root zone of the fallen tree. Each seeded Petri dish was placed in a polyethylene bag to avoid flooding of the dishes during the rains. The dishes were placed in groups of three per species on the soil in a gradient of light from the centre of the light gap (groups 1 and 2) across the periphery of it (groups 3 and 4) into the nearby undisturbed forest of about 25 m in height and several strata of leaves (groups 5, 6 and 7). Germination was recorded during the first 15 days after the dishes were placed in the gap and after a month. Due to the orientation of the gap and the disposition of the dead tree trunk and root, direct sunlight reached the gap only during a few minutes each morning and consequently the Petri dishes located in the open gap were not heated by the sun to more than 27 °C measured by a YSI Telethermometer with surface sensors placed on the agar surface (Yellow Springs. Inst. Co., Nebraska), being illuminated most of the time by scattered sky light only. The visible light energy inside the forest at noon at soil level during a sunny day from early morning till afternoon varied from 0·6 to 17·1 μmol m^{-2} s^{-1}, depending on the place. Measurements were made with a Li-Cor quantum radiometer (LiCor Inc., Lincoln Nebraska).

Laboratory Experiments

Experiments on the light reactions of the seeds were carried out using the same populations of seeds, mostly at the Department of Botany, University of Leicester, U.K. The routine method was to distribute, homogeneously, 100 seeds on 1 % agar in distilled water in plastic Petri dishes. All operations were carried out in a dark room using the green safe light.

The experiment on seed longevity and dark dormancy in dark-imbibed storage was performed (in Mexico) using 24 dishes per species, stored in a dark and humid chamber at 25 °C. Each month two boxes per species were taken out of the dark storage and placed in a growing chamber under fluorescent light at the same temperature. The final percentage germination was recorded 1 month after exposing the seeded dishes to the light.

The experiment on the effects of different wavelengths was performed using

500 W theatre lamp projectors filtered through 'Balzer' narrow band interference filters of wavelengths 397, 452, 493, 544, 599, 660 and 730 nm. Groups of seeded dishes were each exposed to the filtered light, at one of the wavebands, continuously for 6 h, before storing them in a dark room. Germination was recorded after a month of storage.

Germination under simulated natural light spectra was studied using four growth chambers in which the photosynthetically active radiation ($\equiv$ 400 to 700 nm $\equiv$ 40 μmol m^{-2} s^{-1}) was equal, but in which varied amounts of added FR (700 to 800 nm) could be given. In these experiments, the R:FR ratio (defined as the ratio of photon fluence rates in 10 nm bandwidths centred respectively at 660 and 730 nm) was set as follows: chamber 1, 2·3; chamber 2, 0·58; chamber 3, 0·23; chamber 4, 0·2.

R and FR light sources, and the green safe light were used as previously described (Hilton and Smith, 1980). All measurements of spectra and fluence rates for experiments carried out in the laboratory were performed with a Gamma spectroradiometer (Gamma Scientific Corpn., San Diego, California).

RESULTS

The results of the experiments on longevity in the soil are presented in Figure 1. The germination obtained in each buried bag of seeds was very variable for each month, particularly for *C. obtusifolia* but high germination was still obtained for both species in the later samples. The variability may indicate that having the seeds closely together in a bag may produce high mortality if there is a microbial contamination in any of the bags.

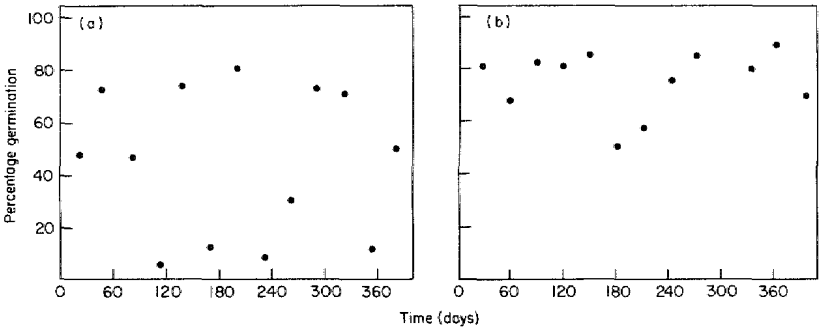

Fig. 1. Germination of seeds buried in rain forest soil as a function of time. Seed samples were recovered from the soil at the times stated and final germination percentage measured after incubation in a white light cabinet at 25 °C with 12 h photoperiod for a period of 1 month. (a) *C. obtusifolia*; (b) *P. auritum*.

The results of longevity and dark dormancy in dark-imbibed storage are presented in Figure 2. The seeds of both species remained dormant in darkness during a whole year, but the germination after exposure to light was high, with no germination at all being obtained in darkness.

The results of the seed germination experiments under simulated canopies are presented in Figure 3. *C. obtusifolia* seeds germinate more slowly under low R:FR but, with sufficient time, most seeds germinated in all the chambers. For *P. auritum*

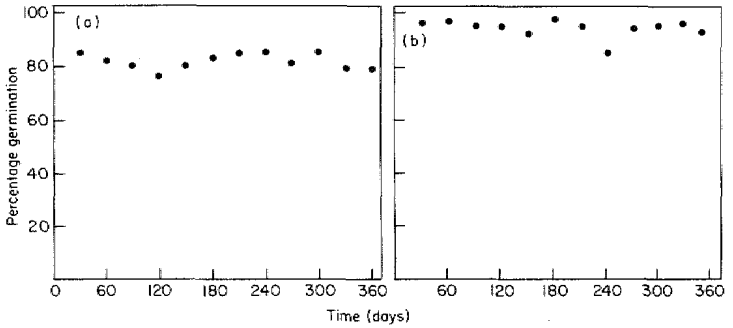

Fig. 2. Germination of seeds stored in total darkness as a function of time. Seed samples stored in the imbibed state in the dark laboratory were transferred at monthly intervals to the white light cabinet (see Fig. 1) and final germination percentage recorded after 1 month. (a) and (b) as for Figure 1.

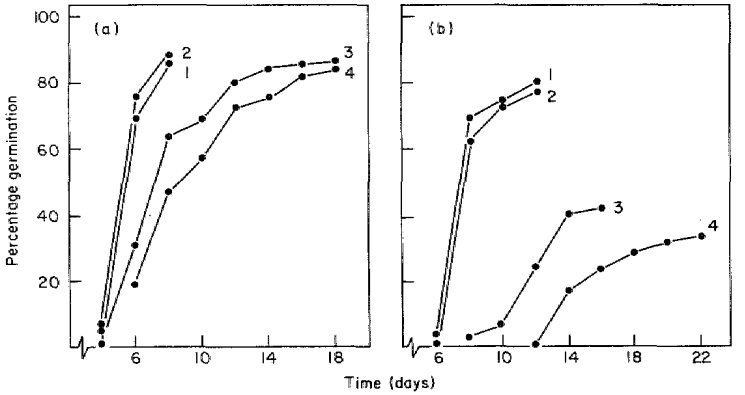

Fig. 3. Time-course of germination under simulated canopy spectra with varying R:FR ratios 1, R:FR = 2·3; 2, R:FR = 0·58; 3, R:FR = 0·23; 4, R:FR = 0·20. (a) and (b) as for Figure 1.

the effect of low R:FR was more drastic and some of the seeds of the samples remained dormant under simulated dense canopies. The fluence rates in the chambers are several times higher than on the forest soil.

The results of the field experiments (Fig. 4) indicate that the seeds germinate freely in the light gap, more slowly in the periphery of it and, after a month, have still not germinated at all inside the forest.

The effects of different wavelengths on germination are presented in Figure 5. Only the R light caused germination, but the time of exposure to the light was not enough to stimulate all the viable seeds.

A single exposure to R light, even of 6 h duration, was not sufficient to induce maximum germination, although within this period an apparent linear relationship between dose (i.e. duration of exposure) and response was evident for *C. obtusifolia* (Fig. 6). No such relationship was obtained with *P. auritum* seeds as single exposures of up to 2 h duration were without effect. Maximum germination required repeated exposure on consecutive days (Fig. 7) but long exposures

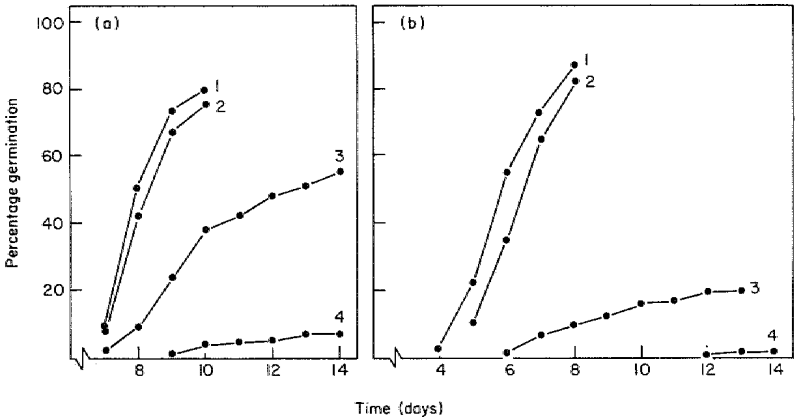

Fig. 4. Time-course of germination in a large natural light gap in the forest. Positions 1 and 2 were in the centre of the light gap, whilst positions 3 and 4 were progressively closer to the periphery. No germination was obtained after 1 month in any of the remaining four positions used in the periphery of the light gap and in the forest itself. (a) and (b) as for Figure 1.

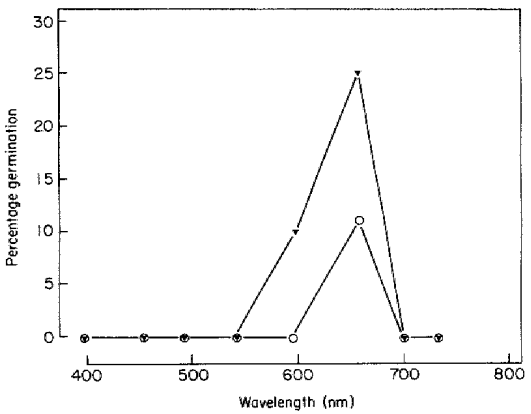

Fig. 5. Wavelength dependence of the effect of light on germination. Seed samples were exposed to 6 h of irradiation with light of various wavelengths, and final percentage germination determined after 1 month in subsequent darkness. (▼) *C. obtusifolia*; (○) *P. auritum*.

(i.e. 2 h) were more effective than short (i.e. 10 min). FR photoreversibility of R-induced germination potential was evident (Table 1). Repeated exposures to R were of no effect if each exposure was immediately followed by FR (treatments 7 and 8, Table 1) or if the FR treatment was delayed by up to 2 h after each R treatment (treatment 8, Table 1). If the FR was given after the last of a series of repeated daily exposures to R, then only partial reversibility was seen (treatments 4, 5 and 6, Table 1). Finally, repeated 10 min exposures to R spaced equally within one 24 h period gave substantial germination, which was completely negated by one 10 min exposure to FR (treatment 9, Table 1).

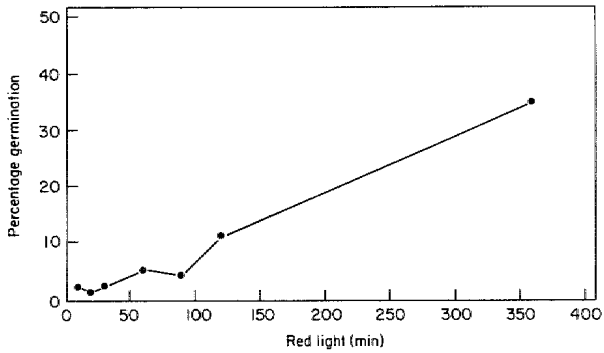

Fig. 6. Relationship between final percentage germination and period of a single exposure to R light (λ_{max} 660 nm) in *C. obtusifolia*.

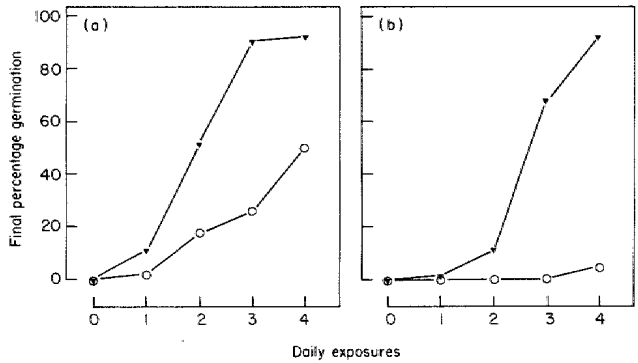

Fig. 7. Final percentage germination (after 1 month) of seed samples given up to four repeated exposures to R light (either 10 or 120 min duration) at 24 h intervals. (a) and (b) as for Figure 1. (▼) 120 min; (○) 10 min.

Discussion

The seeds of *C. obtusifolia* and *P. auritum* can remain dormant in darkness or in the soil for more than a year. This indicates that there is the possibility, for each generation of seeds arriving at the soil, to 'wait' for a light gap to form in the canopy. The change in light quality seems to be the only controlling mechanism acting in natural conditions in relation to light gap formation and it is indeed the most efficient one because it permits germination to be timed precisely with gap formation, when plants of many species may compete for establishment in the new open space.

The germination obtained under simulated canopy light spectra, in comparison with the lack of germination under the forest canopy, may be due either to a lower R:FR value inside the forest, or to the higher fluence rate of visible light in the chambers. There are very few measurements of R:FR in rain forest (Stoutjesdijk, 1972) and they may not be very precise. For the rain forest of Los Tuxtlas, Mujaes (1981, unpublished undergraduate dissertation) using a field spectroradiometer

Table 1. *R and FR photoreversibility of* C. obtusifolia *and* P. auritum *seed germination*

Treatment	*C. obtusifolia*	*P. auritum*
1. Dark	0	0
2. R_{10}	2	0
$R_{10}FR_{10}$	0	0
3. R_{30}	1	0
$R_{30}FR_{30}$	0	0
4. $(R_{10})2$	18	0
$(R_{10})2FR_{10}$	5	0
5. $(R_{10})3$	26	0
$(R_{10})3FR_{10}$	13	0
6. $(R_{10})4$	50	4
$(R_{10})4FR_{10}$	21	1
7. $(R_{120})2$	51	11
$(R_{120}FR_{120})2$	0	0
8. $(R_{120})3$	90	67
$(R_{120}FR_{120})3$	0	0
$(R_{120}D_{120}FR_{120})3$	0	0
9. $R_{10}D_{350}R_{10}D_{350}R_{10}D_{350}R_{10}$	41	0
$R_{10}D_{350}R_{10}D_{350}R_{10}D_{350}R_{10}FR_{10}$	0	0

Seeds imbibed as described in the Materials and Methods were either incubated in darkness (D), given R light (16 μmol m^{-2} s^{-1}), and/or given FR light (32 μmol m^{-2} s^{-1}). The subscript numbers indicate the duration of exposure, in minutes, to the light treatment. The numbers following the parentheses indicate the number of successive days upon which the treatment within the parentheses was applied. Successive treatments were given at 24 h intervals. All seeds were kept in total darkness except for the stated treatments.

(Francois *et al.*, 1975) values of R:FR from 0·3 to less than 0·02 in dense forest parts were obtained.

The long periods of continuous or intermittent R light treatment needed for both species to initiate germination may possibly be related to the ability of the seeds to determine the size of the gap, and to differentiate it from a normal sunfleck reaching the soil of the forest. A small light gap, or sunfleck, will give a short period of unfiltered light exposure to the soil (and therefore a high R:FR) but soon the filtered light (low R:FR) will re-impose dormancy. A large light gap will permit a long period of unfiltered (high R:FR) light exposure on consecutive days with only a much shorter period of filtered (low R:FR) light irradiation during the early morning or afternoon. In the periphery of the gaps, germination takes longer to occur than in the centre of the gap.

The control of germination by phytochrome exerted here does not display reciprocity (Table 1, Fig. 7). Continued, or repeated, photoconversion of Pr to Pfr is necessary for germination of the whole population of viable seeds to be initiated. Such behaviour is normally taken to mean that Pfr reverts to Pr in darkness. If it is assumed that the requirement of prolonged irradiation with a relatively high R:FR confers an ecological advantage in the detection of large light gaps, then an important physiological function for phytochrome dark reversion seems evident. The observations made here on the characteristics of the phytochrome control of germination are essentially preliminary and would repay more detailed investigation.

Acknowledgements

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