

# Differences in seed germination responses may promote coexistence of four sympatric *Piper* species

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## Summary

1. Species-specific responses to the range of microsites resulting from canopy gap formation may contribute to coexistence in tropical forests. We investigated the effects of four factors affected by canopy gap formation (red : far-red light, soil nitrate concentrations, soil temperature fluctuations and soil water potential) on the germination response of four pioneer *Piper* species (*P. dilatatum*, *P. hispidum*, *P. marginatum* and *P. peltatum*) that are typically found in canopy gaps and clearings.

2. All four *Piper* species required light for maximum germination. However, the ratio of red : far-red light (R : FR) resulting in maximum germination varied between species. *Piper peltatum* will germinate in simulated understorey light conditions; *P. dilatatum* and *P. hispidum* require conditions typically found in small to medium gaps; while *P. marginatum* requires the conditions appropriate to large gaps.

3. Only *P. marginatum* was affected by nitrate concentration: elevated concentrations increased the germination rate. This suggests that this species could detect canopy gaps using a combination of high R : FR and elevated soil nitrate concentrations.

4. The germination rate of *P. marginatum* was least sensitive to low water potentials and high daytime temperatures characteristic of large gaps. *Piper peltatum* was most sensitive to these treatments, while *P. dilatatum* and *P. hispidum* were intermediate in response.

5. A principal components analysis of the ratios of germination in understorey to large gap conditions, for four variables, generated a significant axis that explained 88.5% of the variance in germination response between species. Differential species distribution along this axis, based on species-specific responses, may allow germination to occur in the most suitable microsite for onward growth of the seedling and contribute to species coexistence by reducing interspecific competition.

*Key-words:* Niche differentiation, nitrate, red : far-red ratio, temperature, water potential.

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## Introduction

Many explanations have been proposed to account for the coexistence of tree species in species-rich tropical forests. Some of these explanations have focused on niche differences that are expressed during the regeneration phase (Grubb 1977), and range from preferences for different establishment substrates (Lusk 1995) to differential responses of seedlings to light gradients resulting from the formation of canopy gaps (Denslow 1980; Kobe 1999).

Among tropical forest trees and shrubs, a functional group, labelled 'pioneers', has been identified (Swaine

& Whitmore 1988) that consists of fast-growing, gap-demanding species which typically produce many relatively small seeds. Within this group, species growing together can be associated with particular gap sizes and locations within gaps (Brokaw 1987). This requirement for different gap sizes may arise from a trade-off between seedling growth rates and mortality (Dalling, Lovelock & Hubbell 1999).

The transition from seed to seedling is a high-risk period in the life cycle of most plants (Harper 1977). Consequently, mechanisms that minimize the risk to this transition will be under strong selection pressure (Meyer, Allen & Beckstead 1997), and natural selection should favour seed germination patterns that increase the probability of successful seedling establishment.

Thus species can be expected to undergo selection to germinate in conditions that are likely to be most suitable for their onward growth. This has been demonstrated in many studies (for example, Cruden 1974; Evans & Etherington 1990; Meyer *et al.* 1997) dealing with either inter- or intraspecific variation in the germination response of seeds collected from different parts of an environmental gradient. These studies found that when a species is restricted in distribution to a particular part of an environmental gradient, its seed germination characteristics are likely to be adapted to that particular set of conditions. Hence differential patterns of germination, rather than differences in seedling growth, may be partly responsible for the observed partitioning of species between different sizes of canopy gap.

Although seedling studies have commonly focused on the light environment within canopy gaps (Augspurger 1984; Kobe 1999), gap creation leads to a gradient of changing microclimate across each gap that depends on gap size (Brown 1993). In addition to greater irradiance reaching the forest floor, the light quality is altered, with an increase in red to far-red wavelengths (Vázquez-Yanes *et al.* 1990). More irradiance leads to warmer soil during the day and greater drying of the soil surface (Ashton 1992). Furthermore, after gap creation the decomposition of plant debris and the death of fine roots causes a flush of plant-available soil nutrients, particularly nitrate (Denslow, Ellison & Sanford 1998). Fluctuating soil temperatures (Thompson & Grime 1983) and increased soil nitrate concentrations (Hilton 1984) stimulate germination in a wide variety of temperate weed species. Therefore these factors may also enhance the germination of gap-demanding tropical trees.

Previous seedling studies have investigated the potential importance of partitioning of the light environment for coexistence, but none has investigated whether differential germination responses contribute to niche-based coexistence among tropical trees. Although it is well known that some small-seeded pioneer tree seeds detect a canopy gap from changes in spectral composition or soil temperature fluctuations (Vázquez-Yanes 1974; Vázquez-Yanes & Smith 1982), few studies have compared interspecific differences in germination requirements under standardized experimental conditions. Studies that have been conducted have focused on the effect of differences in light quality on germination. For example, Orozco-Segovia & Vázquez-Yanes (1989) investigated the germination of four *Piper* species in relation to light quality, and reported interspecific differences that agreed with their observed gap size preferences in the field. Wide diurnal temperature fluctuations trigger the germination of some pioneer species such as *Ochroma lagopus* (Vázquez-Yanes 1974), but it is not known how soil temperature affects pioneer species that do not require temperature fluctuations for germination, or whether species differ in their tolerance of large fluctuations in

temperature. Similarly, the effect of water availability on germination is not known for pioneer species, although species that require large gaps might be expected to be more tolerant of dry conditions for germination than species typical of small gaps, because of the greater potential for soil drying in the surface layers in large gaps (Ashton 1992).

*Piper* is a pan-tropical genus of herbs, climbers and shrubs containing over 1000 species (Burger 1972). Four pioneer *Piper* species co-occur at our study site in Central Panamá (Croat 1978). This genus has been used frequently as a convenient model system for eco-physiological studies (Field & Vázquez-Yanes 1993), and it enables us to test for differences in germination response to environmental conditions that are likely to result from adaptation rather than from phylogenetic differences. In this paper we test the hypotheses that (a) four co-occurring *Piper* species, which all require canopy gaps as adult plants, possess differential germination responses to four factors that are affected by canopy gap creation: the spectral composition of irradiance; nitrate concentration; water availability; and soil temperature, and (b) the four species will respond in a consistent and predictable manner to these factors in accordance with their patterns of variation across canopy gaps of different sizes. We interpret the responses in relation to potential species coexistence mechanisms.

## Materials and methods

### STUDY SITE AND SPECIES

Ripe fruits of four *Piper* (Piperaceae) species (Table 1) were collected between March and July 1999 from semideciduous tropical rain forest on Barro Colorado Island (BCI) (9°10' N 79°50' W), Republic of Panamá. These species all occupy well-lit microsites as adult plants (Croat 1978; Table 1). Rainfall on BCI averages 2600 mm per year, with a pronounced dry season between January and April. The climate and vegetation of BCI are described in greater detail elsewhere (Leigh *et al.* 1982).

Seeds were cleaned by mashing the fruits in water and decanting off the fruit pulp. Seeds were then air-dried in the dark prior to being air-freighted to Aberdeen, UK, where they were stored at room temperature in the dark until the start of germination experiments, in September 1999. Initial germination of the four species was assessed by germinating seeds in a growth cabinet (Fi-totron 600H, Fison Environmental Equipment, Loughborough, UK) at 26 °C and a ratio of red : far-red light (R : FR) of 2.0 (12 h day/12 h night). Seeds were sown on the surface of 1% agar in water in 50 mm Petri dishes. Germination of *P. hispidum*, *P. marginatum* and *P. peltatum* was between 75 and 90%. However, germination of *P. dilatatum* was only 60%; a cut test on the non-germinated seeds revealed that they were non-viable rather than dormant.

**Table 1.** Characteristics of the four *Piper* species

Species	Growth form	Seed dry weight (mg)	Fruiting period*	Typical habitat
<i>Piper dilatatum</i> L.C. Rich	Shrub	0.15	Late wet to early dry season	Clearings/open areas*
<i>Piper hispidum</i> SW.	Shrub	0.12	Wet season	Open areas/clearings*†
<i>Piper marginatum</i> Jacq.	Shrub	0.15	Late wet to early dry season	Clearings/open frequently disturbed sites*‡
<i>Piper peltatum</i> L.	Herb	0.04	All year	Clearings/along trails*

\*From Croat (1978).

†From Orozco-Segovia & Vázquez-Yanes (1989).

‡From Fleming (1985).

#### LIGHT QUALITY AND SOIL TEMPERATURE DETERMINATIONS

During March 1999, two experimental canopy gaps, each consisting of approximately  $10 \times 10$  m of cleared ground but with different sizes of canopy opening, were created in secondary forest on the Bohio peninsula adjacent to BCI. Hemispherical photographs were taken during June 1999, in the gap centres and at a nearby understorey site 1 m above the ground, using Kodak tri-X pan ASA-400 black-and-white film, a Nikkor 8 mm hemispherical lens and a Nikon FM2 camera (Nikon, Melville, NY, USA). Canopy openness was estimated by analysing the photographs using WINPHOT version 4 (ter Steege 1994). Subsequently, in June 1999 photosynthetically active radiation (PAR) and R : FR were measured in the gap centres and at an adjacent undisturbed understorey site using sensors (SKP 215 and SKR 110, Skye Instruments, Llandrindod Wells, UK) attached to a datalogger (Datahog, Skye Instruments). To enable PAR at each site to be determined as a percentage of full sunlight, an additional PAR sensor was located at an open site outside the forest and approximately 800 m from the paired/gap understorey sites. Concurrent to these measurements, soil temperature at a depth of 1 cm was measured using bead thermistors attached to dataloggers (Datahog, Skye Instruments). Thermistors were inserted horizontally into the soil and 20 cm of cable adjacent to the probe buried to prevent heat transfer along the cable. Measurements were made every 10 s and integrated over a period of 10 min for 16 days.

#### GERMINATION IN RELATION TO LIGHT QUALITY AND NITRATE

Coated polyester filters (Lee Filters, London) were used to achieve a R : FR of 0.08 (filters 322 and 121), 0.15 (filters 121 and 122), 0.21 (filter 322), 0.47 (filter 122) and 1.37 (filter 088), and irradiance in the range  $1.5\text{--}3.1 \mu\text{mol m}^{-2} \text{s}^{-1}$ . Three replicates of 25 seeds per species per treatment were sown on the surface of 1% agar in water in 50 mm Petri dishes. Each of the dishes was placed in an open-topped aluminium foil container ( $19 \times 19 \times 3$  cm) which was then either covered with a coated polyester filter or, in the case of the dark

treatment, aluminium foil. Irradiance was measured using PAR and R : FR sensors (SKP 215 and SKR 110, respectively; Skye Instruments). All treatments were arranged at random in a growth chamber (as above) at a constant  $26^\circ\text{C}$  and 12 h day length.

Germination in response to potassium nitrate at concentrations ranging from  $10^{-2}$  to  $10^{-7}$  M was tested by adding the appropriate concentration of potassium nitrate to molten agar prior to pouring it into the Petri dishes. Petri dishes were then placed in aluminium foil dishes and seeds were germinated either in the dark or in the light at a R : FR of 1.37. In addition, seeds were germinated in the presence of potassium nitrate ( $10^{-3}$  M) in the dark and at R : FR of 0.08, 0.47 and 1.37. In all experiments, germinated (defined as radicle emergence) seeds were removed under a green light with a R : FR of 0.01 and total irradiance of  $0.9 \mu\text{mol m}^{-2} \text{s}^{-1}$  every 2 days for 50 days.

#### GERMINATION IN RELATION TO TEMPERATURE

A two-way temperature gradient plate (Model GRD1, Grant Instruments, Cambridge, UK) was used to obtain a range of diurnally fluctuating temperatures based on a night temperature of  $25^\circ\text{C}$  and a 4 h daytime temperature between 25 and  $43^\circ\text{C}$  ( $25/25$ ,  $25/29$ ,  $25/33$ ,  $25/39$  and  $25/43^\circ\text{C}$ ). These treatments were designed to simulate soil surface temperature conditions in different sized canopy gaps on BCI. During the 4 h simulated day, seeds were exposed to an irradiance of  $200 \mu\text{mol m}^{-2} \text{s}^{-1}$  at a R : FR of 1.8. Because of space constraints on the temperature gradient plate, two replicates of 25 seeds each were used per species per treatment. The actual temperatures seeds experienced on the plate were determined by placing five bead thermistors connected to a datalogger (as above) on the surface of the plate, in the germination cells used during the experiment, and recording every 10 min for 3 days.

#### GERMINATION IN RELATION TO WATER POTENTIAL

Seeds were subjected to water potentials of approximately 0,  $-250$ ,  $-500$  and  $-1000$  kPa by placing three

replicates of 25 seeds each on the surface of a wick connected to a reservoir of polyethylene glycol 8000 (PEG) at a concentration appropriate to the intended treatment (Michel & Kaufmann 1973). The PEG solution flowed over the wick, maintaining the seeds at a constant water potential (Crabtree 2001). Subsamples of the wicks were removed after the experiment had been running for 20 days, and their actual water potential determined using an SC-10A Richards Thermocouple Psychrometer (Decagon Devices, Pullman, USA). Measured potentials were always more negative, but within 30% of their intended values. The germination system was housed in transparent polyethylene boxes and placed in the growth chambers described above. Irradiance inside the transparent boxes averaged  $5 \mu\text{mol m}^{-2} \text{s}^{-1}$ , the R : FR was approximately 2.0 during the 12 h day, and the temperature was 26 °C. The seeds were inspected for germination every 2 days for 45 days.

#### STATISTICAL ANALYSIS

Final percentage germination was plotted against R : FR for each species. Sigmoidal curves were fitted to the data using the curve-fitting function in SIGMAPLOT 5. The equation of the curve was used to calculate the lowest R : FR at which maximum germination percentage occurred. Additionally, because of the low germination percentages in some of these treatments, the mean time to germinate (MTG) for each species at each R : FR was calculated using the equation:

$$\text{MTG} = \Sigma(n \times d) / N \quad \text{eqn 1}$$

where  $n$  is the number of seeds germinated between scoring intervals;  $d$  the incubation period in days at that time point, and  $N$  the total number of seeds germinated in the treatment (Tompsett & Pritchard 1998). The effects of light quality and  $10^{-3} \text{ M}$  potassium nitrate on final germination percentage of the study species were examined using two-way ANOVA on arcsine transformed data.

To calculate germination rates, germination progress curves were plotted for each nitrate concentration, alternating temperature regime or water potential. Subsequently, the times taken ( $t$ ) to reach 50% of the final germination percentage at either  $0 \text{ M NO}_3^-$ , a constant temperature of 25 °C, or zero water potential (at 26 °C) were noted, and the reciprocal ( $1/t$ ) was plotted against nitrate concentration, temperature or water potential. One-way ANOVA and *post hoc* Fisher's LSD test were used to test for intraspecific differences in response to nitrate concentration and interspecific differences in germination rates at each temperature regime or water potential. In addition, the base potential for 50% germination (the lowest water potential at which germination can occur) of each species was calculated by extrapolating the least-squares regression line on the plot of  $1/t_{50}$  against water potential to the  $x$ -axis intercept (Bradford 1990; Gummerson 1986).

Each species' response to the gradient of simulated canopy gap size was characterized by expressing its germination percentage (for R : FR) or  $1/t_{50}$  (all other factors) in simulated understorey conditions (R : FR 0.1,  $10^{-6} \text{ M NO}_3^-$ , constant 25 °C and 0 kPa) as a ratio of its value under simulated large-gap conditions (R : FR 0.4,  $10^{-4} \text{ M NO}_3^-$ , daytime temperature 39 °C and  $-1088 \text{ kPa}$ ). The  $4 \times 4$  species  $\times$  treatment matrix was submitted to principal components analysis (PCA) to explore the intercorrelation among species responses to these factors and determine whether germination response to environmental conditions can be reduced to one or more axes of response to inferred canopy gap size.

## Results

### LIGHT QUALITY AND SOIL TEMPERATURE IN CANOPY GAPS

With increasing canopy gap size, there were increases in the amount of irradiance reaching the forest floor, in R : FR and in the magnitude of diurnal temperature fluctuations (Table 2; Fig. 1). In the understorey, soil temperature at 1 cm depth fluctuated by about 1 °C from a night-time temperature of 25 °C. In contrast, in the large canopy gap fluctuations of 10 °C above 25 °C were common, with occasional fluctuations of up to 20 °C (Fig. 1).

### EFFECT OF LIGHT QUALITY AND NITRATE ON GERMINATION

Each of the four study species required light for maximum percentage germination (Fig. 2). At a R : FR slightly higher than that of full sunlight (1.37), percentage germination was significantly higher than in the dark ( $t$ -test,  $P < 0.05$  for each species). While increasing the R : FR resulted in a higher final germination percentage for each species, the four species differed in the minimum R : FR required for maximum germination. *Piper peltatum* reached maximum germination at an R : FR of 0.08 or less. In contrast, *P. dilatatum*,

**Table 2.** Mean ( $\pm$  SE) photosynthetically active radiation relative to full sunlight and red : far-red ratio in an understorey site, gaps of two different sizes and a nearby open site in the Barro Colorado nature Monument, Panamá

Location	Gap size (m <sup>2</sup> )	PAR (%)	Red : far-red ratio
Understorey	0	0.89 (0.02)	0.23 (0.02)
Small gap	67	5.70 (0.02)	0.45 (0.01)
Large gap	320	34.00 (0.01)	0.95 (0.01)
Open site	–	100	1.10 (0.01)

Measurements were made in June 1999 and the means reported for all values recorded between 06.30 and 18.00 on each of 16 consecutive days. It was not possible to quantify gap size for the open site as it was completely open to the sky.

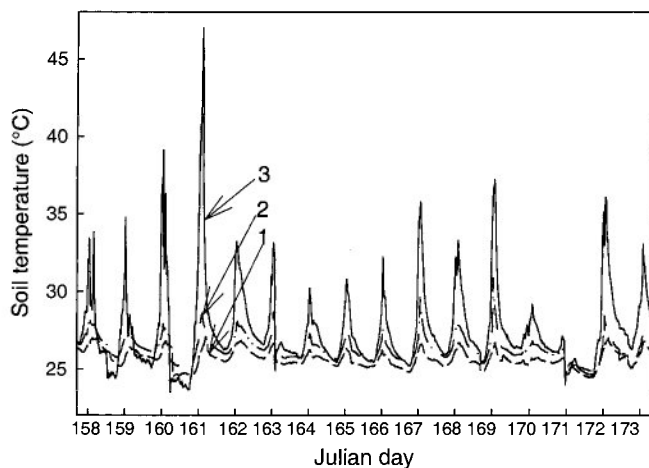


Fig. 1. Effect of canopy openness on soil temperature at a depth of 1 cm (1, understorey; 2, small canopy gap; 3, large canopy gap).

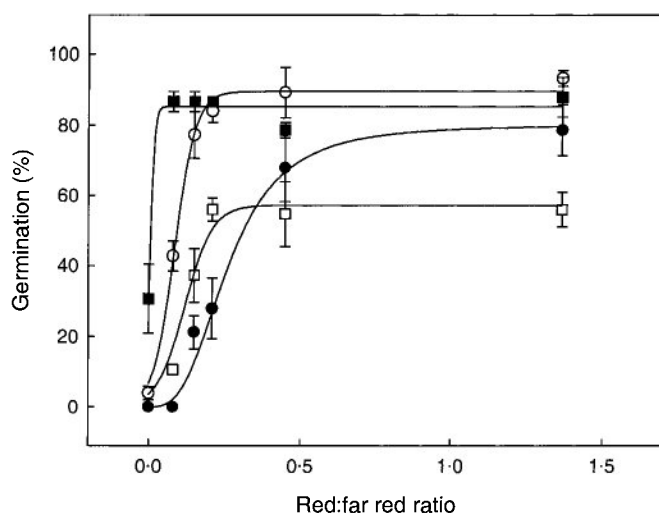


Fig. 2. Effect of R : FR on mean final germination percentage of four *Piper* species (*P. dilatatum*, □; *P. hispidum*, ○; *P. marginatum*, ●; *P. peltatum*, ■). Values for R : FR = 0 represent germination in the dark. Bars  $\pm$  1 SE.

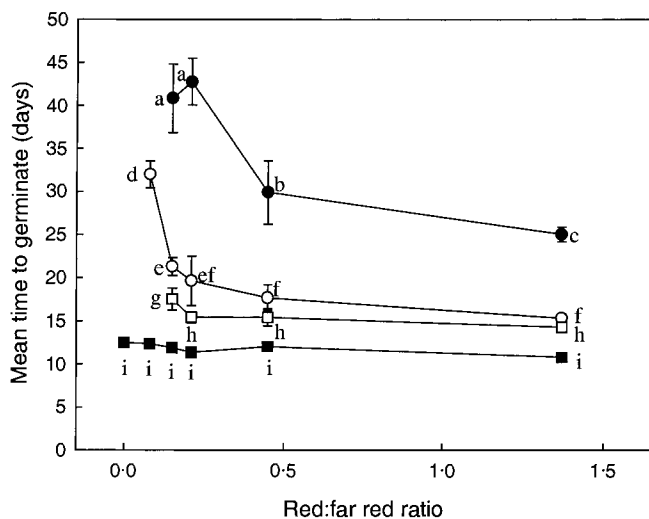


Fig. 3. Mean time to germinate (MTG) in relation to R : FR in four *Piper* species (*P. dilatatum*, □; *P. hispidum*, ○; *P. marginatum*, ●; *P. peltatum*, ■). Different letters indicate significant intraspecific differences ( $P < 0.05$ , Fisher's LSD test) in MTG across the range of R : FR. Bars  $\pm$  1 SE.

*P. hispidum* and *P. marginatum* required ratios of approximately 0.25, 0.30 and 0.64, respectively, for maximum germination (Fig. 2).

Light quality also had a significant effect on the mean time to germinate (Fig. 3). Across the range of light treatments, there was no effect on mean time to germinate for *P. peltatum* (one-way ANOVA,  $F_{5,12} = 2.39$ ,  $P > 0.05$ ). However, for the other three species, the few seeds that did germinate at low values of R : FR took significantly longer to do so than at higher ratios (one-way ANOVA, *P. dilatatum*  $F_{3,4} = 6.57$ ,  $P = 0.05$ ; *P. hispidum*,  $F_{4,10} = 45.4$ ,  $P < 0.001$ ; *P. marginatum*,  $F_{3,4} = 16.5$ ,  $P = 0.01$ ; Fig. 3). In addition, germination of *P. peltatum* was more rapid than in the other three species across all treatments and *P. marginatum* took the longest time to germinate, even at the highest R : FR, 1.37 (one-way ANOVA,  $F_{3,8} = 129.3$ ,  $P < 0.001$ , Fig. 3).

*Piper marginatum* germinated significantly more quickly in the presence of  $10^{-2}$  to  $10^{-4}$  M nitrate than at lower concentrations (one-way ANOVA,  $F_{6,7} = 55.5$ ,  $P < 0.001$ ; Fig. 4). For the other species there were no significant effects of nitrate on germination rate (one-way ANOVA,  $P > 0.05$ ). For *P. marginatum* there was a significant interaction between light and  $10^{-3}$  M nitrate on germination percentage (Fig. 5), with some germination occurring at a low R : FR, and even in the dark, in the presence of nitrate (two-way ANOVA,  $F_{9,27} = 13.8$ ,  $P < 0.001$ ). The other three species showed no significant effect of nitrate on final germination percentage at different ratios of R : FR (one-way ANOVA,  $P > 0.05$ ).

#### EFFECT OF TEMPERATURE

Raising daytime temperatures above a basal, nighttime temperature of 25 °C had no appreciable affect on germination percentage until the daytime temperature reached 43 °C (Fig. 6). A daytime temperature of 39 °C had no effect on percentage germination, although it reduced germination rate for all species. This was most marked in *P. peltatum*, with a 46% reduction in germination rate as the daytime temperature increased from 25 to 39 °C (Fig. 7). For daytime temperatures of up to 34 °C, *P. peltatum* germinated significantly faster than *P. marginatum* (one-way ANOVA,  $P < 0.05$ ), with *P. dilatatum* and *P. hispidum* intermediate in germination rate. At a daytime temperature of 39 °C there were no significant differences in germination rate between the four species (one-way ANOVA,  $F_{3,4} = 0.86$ ,  $P = 0.530$ ).

#### EFFECT OF WATER POTENTIAL

Increasingly negative water potentials reduced germination percentage in all four species (Fig. 8). This was most marked for *P. hispidum*, with germination declining from 95 to 50% as the water potential decreased from 0 to -1088 kPa, whereas the germination percentage of *P. peltatum* was only reduced from 95% at 0 kPa to 82% at -1088 kPa (Fig. 8). The germination rate ( $1/t_{50}$ ) of all four species decreased linearly with decreasing

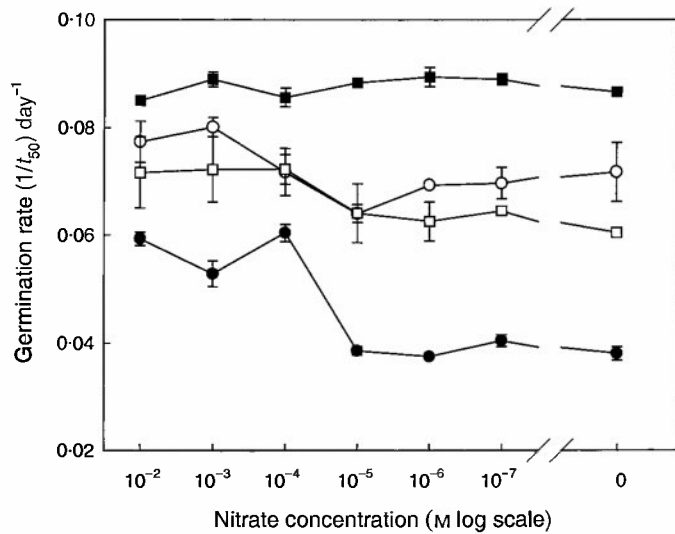


Fig. 4. Effect of potassium nitrate concentration on germination rate ( $1/t_{50}$ ) for four *Piper* species (*P. dilatatum*, □; *P. hispidum*, ○; *P. marginatum*, ●; *P. peltatum*, ■). Bars  $\pm 1$  SE.

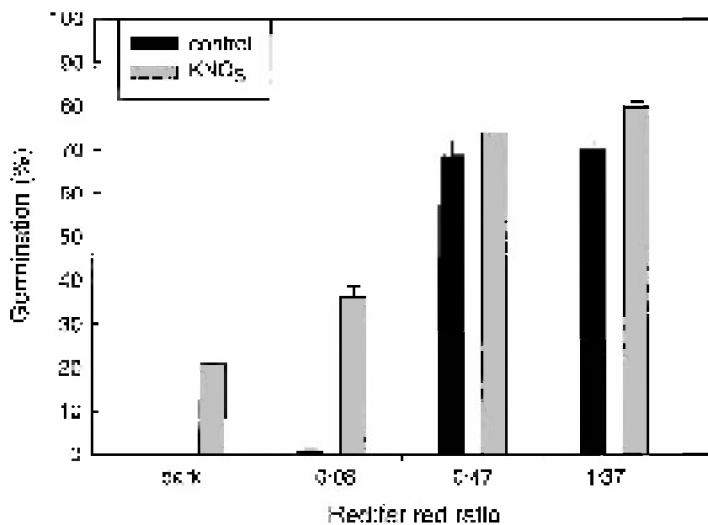


Fig. 5. Effect of potassium nitrate ( $10^{-3}$  M) on final germination percentage of *P. marginatum* at a range of R : FR. Bars  $\pm 1$  SE.

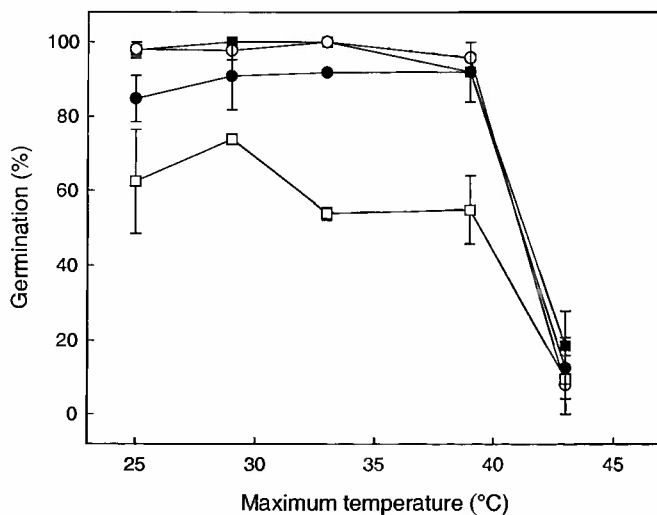


Fig. 6. Effect of maximum daytime temperature on final germination of four *Piper* species (*P. dilatatum*, □; *P. hispidum*, ○; *P. marginatum*, ●; *P. peltatum*, ■). Bars  $\pm 1$  SE.

water potential, as described by the hydrotime equation (Gummerson 1986):

$$1/t_{50} = A + B\Psi_w \quad \text{eqn 2}$$

Values of  $A$  and  $B$  are given in Table 3 as well as the potentials at which germination just fails to occur (the base potential when  $1/t_{50} = 0$ ). The latter values should be treated with caution as they are obtained from extrapolation beyond the range of experimental conditions. At  $-1088$  kPa, there were no significant differences in germination rate between the four species (one-way ANOVA,  $F_{3,12} = 1.12$ ,  $P = 0.383$ ).

#### PRINCIPAL COMPONENT ANALYSIS

The PCA axis I had an eigenvalue of 3.54, and explained 88.5% of the variance in the four germination-response ratios. The four species were arranged along this axis as follows with species' axis I scores: *P. marginatum* ( $-2.684$ ), *P. dilatatum* ( $-0.750$ ), *P. hispidum* ( $0.674$ ) and *P. peltatum* ( $2.502$ ). Most pairwise correlations between the four germination-response ratios were significant despite the low statistical power of these analyses (Table 4). In addition, all four variables were significantly correlated with PCA axis I, but not with axis II.

#### Discussion

##### EFFECT OF LIGHT QUALITY ON GERMINATION

Many small-seeded tropical pioneer species require light for germination (Orozco-Segovia & Vázquez-Yanes 1989; Vázquez-Yanes & Smith 1982). This was the case with all four *Piper* species we studied, and the four (which included *P. hispidum*) studied by Orozco-Segovia & Vázquez-Yanes (1989). Small seeds can emerge only from shallow soil (Bond, Honig & Maze 1999). This means that for small-seeded pioneer species to establish successfully they must germinate in a canopy gap and sufficiently close to the soil surface. As irradiance penetrates only 4–5 mm into soil (Tester & Morris 1987), its detection provides seeds with a means of ensuring germination close to the soil surface and in a location suitable for the onward growth of the seedling.

Although all four *Piper* species required some irradiance for greater than 30% germination, there was a clear sequence from *P. peltatum* to *P. marginatum* in the R : FR required for maximum germination (Fig. 2). The R : FR within intact tropical forests is approximately 0.2, rising to approximately 1.2 in full sunlight (Vázquez-Yanes *et al.* 1990; Table 2). As R : FR is further reduced by a covering of litter (Vázquez-Yanes *et al.* 1990) and will be less in edges of the gap receiving less direct sunlight, the values in Table 2 represent the maxima that seeds would receive in small and large

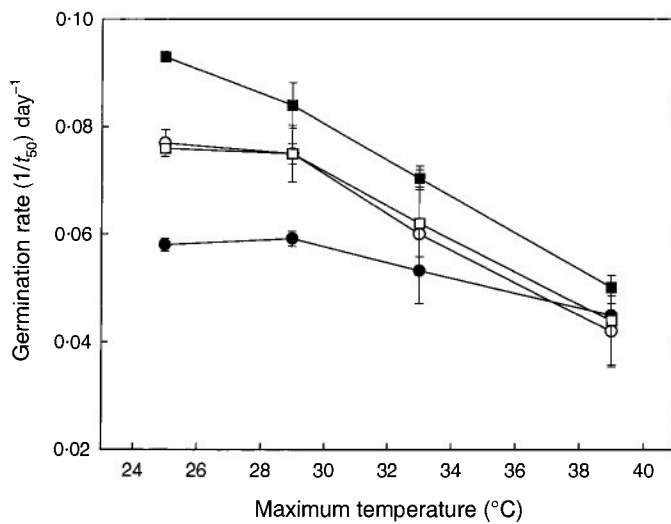


Fig. 7. Effect of maximum daytime temperature on germination rate ( $1/t_{50}$ ); *P. dilatatum*, □; *P. hispidum*, ○; *P. marginatum*, ●; *P. peltatum*, ■. Bars  $\pm 1$  SE.

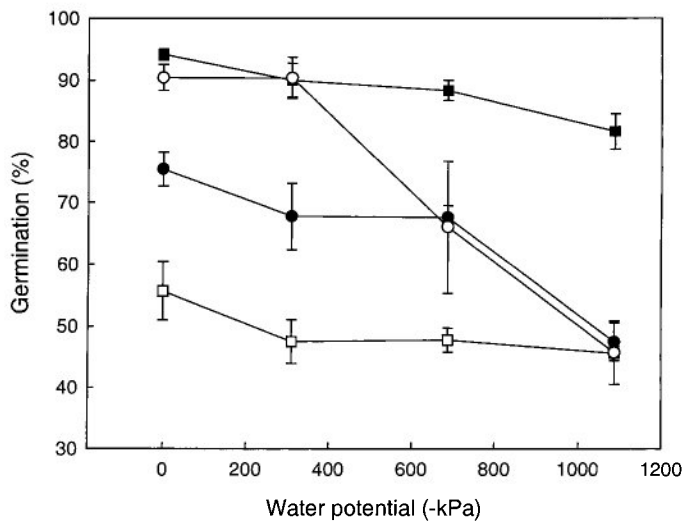


Fig. 8. Final germination percentage after 45 days at 26 °C of four *Piper* species subjected to various water potentials (*P. dilatatum*, □; *P. hispidum*, ○; *P. marginatum*, ●; *P. peltatum*, ■). Bars  $\pm 1$  SE.

Table 4. Pearson's correlation coefficients describing relationships among four species in their understorey: gap germination response ratios for four variables

Parameter	Nitrate	Temperature	Water	PC I†	PC II†
Red: far-red	0.955*	-0.875	-0.983*	-0.984*	0.153
Nitrate		-0.990*	-0.877	0.989*	-0.147
Temperature			0.986*	-0.952*	-0.173
Water				-0.950*	-0.260
PC I					0.000

\* $P < 0.05$ .

†PC I and PC II are the first and second axis of a PCA of the  $4 \times 4$  species  $\times$  variable matrix as described in the text.

gaps. Figure 2 indicates that *P. marginatum* requires substantial canopy gaps for most germination to occur, which is in agreement with Fleming (1985) who found that *P. marginatum* occurs in open, frequently

Table 3. Hydrotime constants describing time to 50% germination ( $t_{50}$ ) in the equation  $1/t_{50} = A + B\Psi_w$ , where  $\Psi_w$  is water potential

Species	A (day <sup>-1</sup> )	B (day <sup>-1</sup> kPa <sup>-1</sup> )	Base potential (kPa)
<i>P. dilatatum</i>	0.0639	$3.689 \times 10^{-5}$	-1732
<i>P. hispidum</i>	0.0722	$4.518 \times 10^{-5}$	-1598
<i>P. marginatum</i>	0.0576	$3.293 \times 10^{-5}$	-1749
<i>P. peltatum</i>	0.0897	$5.863 \times 10^{-5}$	-1530

The base potential is the potential at which  $1/t_{50} = 0$  (as  $1/t_{50}$  tends to infinity).

disturbed sites. In contrast, *P. dilatatum* and *P. hispidum* would require only small to medium gaps. While *P. peltatum* should germinate in the shade of the understorey, and hence also in any size of gap, a covering of litter will further reduce R : FR and prevent germination. This would prevent all the *Piper* species from germinating under litter which would otherwise prevent emergence of the shoots of such small-seeded species, as suggested by Metcalfe & Turner (1998)

While the species responded differently to light quality, each requiring a different R : FR for maximum germination, the few seeds of *P. dilatatum*, *P. hispidum* and *P. marginatum* that did germinate at lower ratios took significantly longer than at higher ratios (Fig. 3). At low R : FR, these species would be at a disadvantage compared to *P. peltatum*. In addition, these species (especially *P. marginatum*) germinated more slowly than *P. peltatum*, even at higher R : FR (Fig. 3), suggesting that *P. peltatum* may have a competitive advantage in all sizes of canopy gap. However, as discussed later, this will be an advantage only if it is more favourable to attempt establishment irrespective of the rainfall regime, as an increasing duration and frequency of surface drying will increase the mortality risk for rapidly germinating species.

#### EFFECT OF NITRATE ON GERMINATION

Nitrate had a significant effect on the germination rate of *P. marginatum*, and a marginal effect on *P. dilatatum* and *P. hispidum*. *P. marginatum* germinated nearly as rapidly as the other species in the presence of nitrate. For intact forest on BCI, Yavitt (2000) reported 11–22 mg NO<sub>3</sub><sup>-</sup> kg<sup>-1</sup> soil. Similarly, at La Selva, Costa Rica, Denslow *et al.* (1998) reported approximately 22 mg NO<sub>3</sub><sup>-</sup> kg<sup>-1</sup> soil for understorey sites. Assuming a soil water content of 45% (typical value from Windsor 1990) and that all the soil nitrate is in solution, this yields a nitrate concentration in the soil solution of between 2.3 and  $4.4 \times 10^{-4}$  M for understorey sites. Denslow *et al.* (1998) reported 44–199 mg NO<sub>3</sub><sup>-</sup> kg<sup>-1</sup> in gaps, which is equivalent to between  $8.7 \times 10^{-4}$  and  $3.9 \times 10^{-3}$  M at a soil water content of 45%. While these concentrations are all sufficient to increase the germination rate of *P. marginatum* (Fig. 4), incorporating

potassium nitrate into the germination medium may provide more nitrate than is realistic, given that nitrate fluxes rapidly in some soils, such as in undisturbed coniferous forests (Stark & Hart 1997). Nonetheless, the concentrations of nitrate that affect the germination rate of *P. marginatum* are similar to those in soils beneath canopy gaps. *Piper marginatum* could therefore detect canopy gaps using a combination of light quality and elevated nitrate concentrations. As the soil nitrate concentrations in gaps increase with gap size (Denslow *et al.* 1998), only species adapted to fairly large sizes of gap would be likely to respond to nitrate as a gap-detection mechanism. *Piper marginatum* required the highest R : FR for maximum germination to occur (Fig. 2), which might explain why it was the only species to respond to nitrate. This is the first suggestion that tropical pioneers could detect gaps using nitrate, although nitrate stimulates the germination of temperate weeds (e.g. *Avena fatua*; Hilton 1984) and has been implicated as a gap-detection mechanism in the chalk grassland herb *Plantago lanceolata* (Pons 1989). However, seeds of the four *Piper* species are dispersed through defecation by bats and birds (Fleming 1985). This may provide seeds with a potential nitrate source, although it is difficult to interpret the observation that only one of the four species responds to nitrate, if faeces contain functionally significant quantities of nitrate.

The effect of nitrate on the germination of *P. marginatum* interacted with light quality; seeds were able to germinate at lower R : FR if nitrate was present (Fig. 5). This interaction could be explained by the influence of nitrate on the sensitivity of phytochrome receptors (Hilhorst 1990). Thus the control of germination in some light-sensitive pioneer species can be more complex than has been suggested (e.g. Orozco-Segovia & Vázquez-Yanes 1989; Vázquez-Yanes & Smith 1982). The omission of nitrate from the germination medium used in experiments on light-sensitive pioneer seeds may therefore limit the ecological significance of previous studies.

#### EFFECT OF TEMPERATURE ON GERMINATION

Diurnally fluctuating temperatures increase the germination percentage in several comparatively large-seeded pioneer species (e.g. *Ochroma lagopus*; Vázquez-Yanes 1974). This response enables them to emerge from soil deeper than irradiance can penetrate. In contrast, the negative effect of high daytime temperatures, typical of large canopy gaps (Fig. 1), on germination (Fig. 6) suggests that all the study species could have difficulty in establishing in the centre of large canopy gaps if they were not buried sufficiently deep, and were not germinating in soil with a moist surface sufficient to avoid lethal temperatures. The amplitude of soil temperature fluctuations decreases with increasing soil depth. The comparative absence of small-seeded species from large gaps may reflect an inherent limitation

of small seed size, namely, that such seeds can emerge from shallow depths (Bond *et al.* 1999) only where the maximum temperature is likely to be lethal. Brokaw (1987) reported gap-size preferences of three pioneer species on BCI. The smallest-seeded species, *Miconia argentea* (seed weight 0.08 mg; Dalling, Hubbell & Silvera 1998), was found most commonly in small gaps, while the larger-seeded species (*Cecropia insignis* and *Trema micrantha*, 0.68 and 3.9 mg, respectively; Dalling *et al.* 1998), which could germinate from deeper soil, were more frequent in larger gaps.

As the maximum daytime temperature increased, the difference in germination rate between the four species reduced. By 39 °C there were no significant differences in germination rate among them (Fig. 7). As the maximum soil temperature decreases with increasing depth, the larger-seeded species (e.g. *P. dilatatum* and *P. marginatum*), which may be able to emerge from greater depth, are more likely to establish in larger gaps than the other species, as the germinating seeds will be less likely to encounter lethal temperatures.

#### EFFECT OF WATER AVAILABILITY ON GERMINATION

Species that grow in dry conditions have seeds able to germinate at more negative water potentials than species from wetter environments (Evans & Etherington 1990). Germination rate also decreases with decreasing water potentials (Gummerson 1986). For our four *Piper* species, germination percentage and germination rate were reduced as water potential decreased (Fig. 8; Table 3). *Piper peltatum* had the fastest germination rate at all potentials except -1088 kPa, and maintained the greatest percentage germination at -1088 kPa. It can be advantageous to have a shorter time to germination because this will increase the probability of establishment in advance of more slowly germinating species after rainfall. However, there is also an associated risk: once a seed has germinated beyond a critical stage it cannot survive and germinate after subsequent desiccation, and the seedling will die unless the root can outpace the rate at which the surface soil layers are drying (Townend *et al.* 1996). Such wetting and drying might be common in gaps during the dry season and at the start of the wet season, and might be more pronounced in larger than in smaller gaps. Consequently, the shorter time to germination in *P. peltatum* can improve its competitiveness in small gaps, but would increase the risk of mortality in large gaps. The seeds of the other three species are three or more times heavier than those *P. peltatum*. Heavier seeds are more likely to be able to emerge from greater depth (Bond *et al.* 1999) and have faster root growth which will favour survival during dry spells.

The germination rate of all four species was the same at -1088 kPa. However, that of *P. marginatum* was proportionately less affected by decreasing potential than the others (smallest *B* in Table 3), suggesting



better adaptation to conditions typical of larger canopy gaps. In addition, *P. marginatum* had a more negative base potential (water potential at which  $1/t_{50} = 0$  day<sup>-1</sup>; Table 3) for germination than the other species, consistent with it being adapted to larger gaps.

#### ECOLOGICAL IMPLICATIONS

The four species separated along only one PCA axis of germination response to the simulated understorey: large gap conditions. The correlations between the four variables used in the PCA (Table 4) and, with the species axis I scores, indicate that species' responses to environmental variables were consistent and predictable. For example, a species able to germinate at low R : FR would be relatively insensitive to nitrate and severely affected by high daytime temperature and low water potentials. This suggests that the four species have germination patterns that are consistent with adaptation to a particular gap size. If so, the axis I score can be considered as a measure of gap size preference.

As a result, the species can be divided into three groups. *Piper marginatum* is likely to germinate only in medium and large gaps, due to its requirement for a high R : FR. Additionally, its response to elevated nitrate concentrations will enable it to germinate almost as rapidly as the other three species in larger gaps. The high daytime soil temperatures and dry soil associated with larger gaps may enable it to germinate as fast as the other three species.

*Piper dilatatum* and *P. hispidum* will germinate only in canopy gaps, although these need not be as large as for *P. marginatum*. *Piper dilatatum* and *P. hispidum* have similar germination responses to R : FR and soil temperature fluctuations. However, percentage seed germination of *P. hispidum* is poor under dry conditions, suggesting that it may perform better in wet sites, while *P. dilatatum* can establish in drier sites.

*Piper peltatum* produces many small seeds all year round. These seeds can germinate rapidly in most sites, provided they are sufficiently close to the soil surface, except in large gaps where they may be killed by the high soil temperatures. The need for *P. peltatum* to germinate near the soil surface, because of its small seeds, may mitigate against either successful establishment or competition with seedlings of the three larger-seeded species in medium-to-large canopy gaps. The fact that this species, unlike the other three, is a herb (Table 1), coupled with its year-round fruiting, prodigious seed production and rapid germination, suggests an opportunism that is based more on increasing the probability of germination in suitable microsites than on seed dormancy until conditions become suitable for germination.

In conclusion, these four *Piper* species show different germination patterns in response to the environmental variation caused by canopy gap formation, and these differences may help explain the coexistence of

these species. The combined responses of these species to more than one environmental cue (or limitation) may enable them to detect different gap sizes and hence to coexist by reducing interspecific competition.

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