

## SEED MASS, SEEDLING EMERGENCE, AND ENVIRONMENTAL FACTORS IN SEVEN RAIN FOREST *PSYCHOTRIA* (RUBIACEAE)

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**Abstract.** We examined the effects of seed mass on performance between the time of seed dispersal and emergence within seven sympatric rain forest woody species of *Psychotria* in two contrasting natural habitats: gaps and shaded forest. We determined the effects of seed mass on emergence (the total proportion of emerged seedlings) and on the speed of emergence (the time necessary to reach 50% total emerged seedlings) in both greenhouse and field conditions, and we observed the effects of seed mass on the risk of removal by animals in the field. The effects of seed mass on performance were specific to species and habitat. For example, in two species, seed mass had a positive effect on emergence, while another showed a negative effect; for most of the species, effects on performance restricted to only one habitat (shaded forest or gap). Similarly, the results for effects of seed mass on the risk of removal were species- and habitat-specific. In the greenhouse, seed mass did not affect either emergence or the speed of emergence under either high or low light conditions. Our results suggest that: (i) seed mass did not have a general effect on emergence success and (ii) the effects of seed mass on seedling emergence are driven by external ecological factors more than by intrinsic effects of seed mass. Seed-removing animals appear to be an important ecological agent operating on intraspecific seed mass variation.

**Key words:** germination; neotropical rain forest; *Psychotria*; *Rubiaceae*; seed mass; seed mass variation; seed size; seed size variation; seedling emergence.

### INTRODUCTION

Offspring size plays a key role in the establishment of the juvenile phase of an organism's life cycle. In plants, seed mass can influence the probability of seedling establishment by affecting the distance to which seeds disperse, the likelihood that they will reach suitable habitats for germination, and the probability of early survival (Westoby et al. 1992). Several studies have observed that within populations large seed mass confers an advantage during at least one stage of the life cycle, principally under conditions where resources are scarce (Harper 1977, Silvertown 1989). Seedlings emerging from larger seeds often survive longer than those from smaller seeds under adverse conditions such as high defoliation (Careaga 1989), low light (Howe et al. 1985), low soil moisture (Manga and Yadav 1995; but see Hendrix and Nielsen [1991]), and nutrient limitation (Allsopp and Stock 1995). In addition, in comparison to seedlings emerging from relatively small seeds, seedlings from larger seeds have been observed to have a higher probability of survival and emergence from beneath the forest litter layer (Gross 1984, Tripathi and Khan 1990), and to emerge from greater depths in the soil (Wulff 1986, Yanful and Maun 1996).

In contrast to these advantages of relatively large seed mass, several studies have detected ecological factors that can lead to the opposite trend, particularly during the phase when seeds remain in the soil. Vertebrate predation has been observed to generate a disadvantage to large seeds, presumably because large seeds represent a richer energy resource or are more apparent to vertebrates than small seeds (Hulme 1993, Van der Wall 1994). Invertebrates such as bruchid beetles may also select against larger seeds, which are preferred over smaller seeds as oviposition sites (Howe et al. 1985, Moegenburg 1996).

The direct and indirect effects of seed mass on seed coat thickness, seed coat permeability, and the surface: volume ratio may also influence processes occurring at the soil–seed interface, which may in turn determine the speed and probability of successful germination or the persistence of a dormant seed bank (Thompson et al. 1977, 1993, Leck 1989, Chambers 1995). For example, seed coat permeability may influence the rate of rehydration necessary for germination, and consequently the proportion of seeds that germinate and the speed with which they do so (Harper 1977).

Ultimately, for any given species, the effect of seed mass on plant performance may depend both on the intrinsic effects of seed mass on seed–seedling behavior and on its interactions with other ecological factors. In natural habitats, the selective pressures influencing

Manuscript received 9 September 1997; revised and accepted 18 June 1998; final version received 10 August 1998.

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the evolution of seed mass are likely to differ among species and habitats (Foster 1986). Few studies, however, have explored the function of seed mass under field conditions in a variety of habitats (see Stanton 1984, 1985, Howe et al. 1985, Wulff 1986, Schupp 1995).

One approach toward a general understanding of the ecology and evolution of seed mass is to combine intraspecific studies of the ecological effects of seed mass variation with a comparative study of closely related species that vary widely in their seed masses. This allows the investigation of the function of seed mass across a very wide phenotypic range, as expressed by taxa that share similar life histories, ecological roles, and phylogenetic history. In this study, we focus on the effect of seed mass on seed behavior very early in the life cycle, a phase that has not been examined in detail for many species. While many studies have evaluated the effects of seed mass on performance through the period of seedling establishment, few focus on the influence of seed mass on the probability of successful emergence itself (e.g., Gross 1984, Tripathi and Khan 1990).

We conducted a two-year study of the effects of seed mass on seedling emergence, speed of emergence, and the probability of seed removal by animals in seven closely related, ecologically similar, and sympatric lowland rain forest woody species in the genus *Psychotria* (Rubiaceae). In this paper, we address some questions that aim to detect general, as well as species- and habitat-specific influences of seed mass on seedling emergence.

1) Within species, does seed mass affect the probability of seedling emergence, the speed of emergence, or the probability of removal by animals within species? If so, are these effects similar in rain forest gaps and under shaded forest?

2) Does the intraspecific relationship between seed mass and seed/seedling behavior differ among species? If so, does the qualitative relationship between seed mass and seed/seedling behavior depend on the habitat or on the mean seed mass of a taxon?

## METHODS

### *Species and study site*

Experimental work was conducted from December 1992 through August 1993 in the tropical rain forest located at Los Tuxtlas Tropical Biology Field Station, Veracruz, Mexico. The study system included seven tree species of the genus *Psychotria* (Rubiaceae): *P. limonensis* K. Krause, *P. graciliflora* Benth., *P. chagrensis* Standley, *P. papantlensis* (Oersted) Hemsley, *P. flava* Oersted ex Standley, *P. faxlucens* Lorence and Dwyer, and *P. simiarum* Standley. According to Steyermark (1974), at least two subgenera can be recognized within *Psychotria*, including the subgenera *Psychotria* and *Heteropsychotria*. The first five species list-

ed above are found in the subgenus *Psychotria*; *P. faxlucens* is in the subgenus *Heteropsychotria*, and *P. simiarum* belongs to a group of taxa that has yet to be named (Taylor and Lorence 1992; D. Lorence, *personal communication*). In all species, flowers have whitish tubular corollas and are typically heterostylous. The fruits are fleshy two-seeded drupes, whose shape varies among species between ovoid and spherical. All species are primarily dispersed by birds and produce fruit primarily between November and December, with considerable overlap between species (G. Ibarra-Manriquez and S. Sinaca, *personal communication*).

All seven species are shrubs or small trees (1.5–12 m in height) and are important structural components of the forest understory (Bongers et al. 1988). Our field observations indicate that the adults of these species inhabit a variety of forest habitats that differ with respect to the size of openings, or light gaps, in the forest canopy. Classifying this variety into relatively discrete categories, it is possible to recognize species that are common in shaded sites (*P. faxlucens* and *P. simiarum*) and species that are restricted to gaps >300 m<sup>2</sup> in area (*P. limonensis*). Some species (*P. graciliflora*, *P. papantlensis*, and *P. chagrensis*) are found in intermediate habitats, while *P. flava* occupies habitats ranging from intermediate habitats to shaded sites (Table 1).

This group of species exhibits high variation in fresh seed mass, both within and among species. Within species, seeds range in mass by a factor of two to four, and exhibit coefficients of variation between 20 and 33% (see Table 1). Among species, mean seed mass varies by two orders of magnitude, although there is a high degree of overlap between the seed mass distributions of different species. These species do not form persistent seed banks as seeds germinate in <5 mo.

### *Field experiment*

*Seed collection.*—During the peak fruiting period of each species, fruits from  $\geq 20$  individuals were collected. The fruits were stored fresh for 5–7 d until the number of fruits collected was sufficient for the planned experiment. The seeds were removed from the fruit and exposed to air until their surface was dry. Seeds were individually weighed to the nearest milligram using a digital balance. From the range of seed masses exhibited by each species, four seed mass classes were then identified for each species, with each class including one-fourth of the absolute range of seed masses. The seed mass ranges identified for each of the seven species were estimated from a total of 600–943 seeds.

Seeds for the field experiment were obtained from 20 individuals per species (the same individuals from which seed mass was determined) and were treated as in the paragraph above. Seeds were collected by random sampling among individuals to achieve a sample size of 420 seeds per seed mass class per species (total  $N = 11\,760$  seeds). When dry, seeds were stored in-

TABLE 1. Seed properties of seven *Psychotria* species present at Los Tuxtlas, Mexico. Sample sizes (*N*) refer to the total number of seeds weighed.

Species	Fresh mean seed mass (g)	<i>N</i>	Coefficient of variation	Range of variation (g)	Relative abundance		
					Shaded forest	Border of gap	Center of gap and forest border
<i>P. faxlucens</i>	0.380	943	0.26	0.028–0.634	VC	C	
<i>P. flava</i>	0.230	661	0.33	0.020–0.412	VC	C	C
<i>P. simiarum</i>	0.042	880	0.23	0.015–0.068	VC	C	R
<i>P. papantlensis</i>	0.037	792	0.29	0.010–0.100	R	VC	R
<i>P. chagrensis</i>	0.023	800	0.20	0.012–0.038	R	VC	C
<i>P. graciliflora</i>	0.011	600	0.25	0.004–0.019	C	VC	R
<i>P. limonensis</i>	0.007	799	0.26	0.003–0.015		C	VC

Notes: The last three columns give the relative abundances of mature individuals of each species in each of three rain forest habitats, based on a 5-ha survey: VC, very common species; C, common; R, rare; empty cells indicate absence from a particular habitat (M. Martinez-Ramos, unpublished data, and H. Paz, unpublished data).

dividually in plastic bags for 1–2 d before introducing them to experimental treatments, as follows.

**Experimental design.**—At Los Tuxtlas reserve, we selected three forest sites where a pair of plots representing a treefall gap and a shaded forest were present. Gaps were required to be 1 yr old and to include an area of ~290–320 m<sup>2</sup> of projected open canopy (sensu Brokaw 1982). Shaded forest patches were required to include an area of 320 m<sup>2</sup>, to be located below a uniformly closed canopy, and to be a distance of 40–80 m from the gap with which it was paired. Each of the three pairs of plots were on a slope of <10° and were located in a 500 × 300 m area with the same north-facing orientation.

Within each plot, eight treatments were established per species: four seed mass classes × two levels of exclusion of potential seed predators. In the “animal-exclusion” treatment, seeds were protected from vertebrates and invertebrates by enclosing each one within a small mesh cylinder with a lid (described in detail below); in the “open” treatment, seeds were placed in a mesh cylinder without a lid. Using a random sampling procedure, 70 seeds of each seed mass class were selected from each species and assigned at random to one of the two exclusion treatments. Consequently, within each of the six plots, each species was represented by a total of 280 seeds (35 seeds × 4 seed mass classes × 2 exclusion treatments).

With the aim of reducing possible effects due to environmental heterogeneity within plots, each plot was divided into a grid of 35 (1.8 × 1.0 m) quadrats, and a single seed representing each of the species and eight treatments was assigned at random to each quadrat. Thus, each quadrat included a seed from each size class, exclusion treatment, and species. In total, we sowed 56 seeds per quadrat, 1960 seeds per plot, and 11 760 seeds in the entire experiment. Within each quadrat, seeds were separated by 15–20 cm. To ensure that seeds remained in the same position on the soil, and in order to follow the fate of each seed, each seed was put on the soil surface within the margins of an aluminum

mesh cylinder fixed to the soil. These cylinders were made of mesh with 4-mm<sup>2</sup> apertures (facilitating the free movement of water and soil particles), and were 3 cm in diameter and 4 cm in height. The cylinders were buried in the soil to a depth of 2 cm, leaving 2-cm walls exposed above the soil surface. To create the “animal-exclusion” treatment, cylinders were capped with nylon mesh with 4-mm<sup>2</sup> apertures. The “open” treatment cylinders were not capped, and the seeds within them were accessible to animals. Preliminary trials showed that cylinders were effective in retaining the seeds in their original position, in spite of strong storm rains, and the open cylinders allowed animals to remove *Psychotria* seeds from the soil surface (H. Paz, unpublished data).

To avoid any contamination of the treatments with *Psychotria* seeds that might be held in the seed bank, the 5-cm column of soil originally contained in each cylinder was replaced with soil free of *Psychotria* seeds. During the germination period of each species, experimental seeds were monitored every 4 d and the number of emerging seedlings was recorded as those seedlings with hypocotyl visible above soil surface. The census was continued until seedling emergence ceased for four 4-d census periods. After that time, censuses were conducted at increasing time intervals. Plants were censused after each of four 16-d periods, followed by four 56- to 64-d periods, unless a seedling was observed to emerge, in which case the censuses were conducted again at 4-d intervals until four intervals passed without emergence. After 1 yr, we recorded the total proportion of emerged seedlings (hereafter referred to as “emergence”) for each species, exclusion treatment (open vs. animal exclusion), and habitat.

In this study, the animal exclusion treatments were only used to derive a response variable: the “risk of seed removal.” This variable estimates the reduction in the probability of emergence due to seed removal by animals as the difference in emergence between the animal-exclusion and the open treatments. Animal exclusion treatment per se was not used as a factor in our

statistical analyses. To ensure that there were no abiotic treatment effects causing this difference, we conducted a greenhouse experiment (described in *Methods: Greenhouse experiment*).

Finally, to estimate the amount of time that seeds of each species, size class, and habitat remained dormant on the soil surface in the absence of animal disturbance, we determined the number of days that passed until 50% of seeds in the animal-exclusion treatment had successfully germinated (hereafter referred to as "speed of emergence"). This variable is of interest because a delay in emergence could provide an increased opportunity for pre-emergence seed predation.

In sum, seeds from seven species of *Psychotria* were sown within three pairs of gap-shaded forest plots. For each species, four seed mass classes and two animal-exclosure treatments were represented within each plot. Seeds were monitored until no newly emerging seedlings were observed. For each species, we considered emergence, time to emergence, and risk of seed removal as response variables, and habitat and mean seed mass as explanatory class variables.

#### *Greenhouse experiment*

An experiment was conducted in a greenhouse (a metallic frame covered with transparent nylon mesh with 4-mm<sup>2</sup> apertures) located outside the forest, with three objectives: (i) to explore the potential abiotic effects of the animal-exclosure treatment on seedling emergence (e.g., due to the lower light level and higher humidity created by the nylon mesh cap), (ii) to explore the effects of seed mass on seedling emergence in contrasting light conditions, and (iii) to determine whether there is an intrinsic effect of seed mass on seed behavior in the absence of ecological factors (e.g., animal interference, pathogens, and litter accumulation) that are restricted to the field habitats.

Seeds of each of three species (*P. limonensis*, *P. papantlensis*, and *P. flava*), representing the smallest and the largest of the four size classes identified for the field experiment, were processed following the same protocol used in the field experiment. Seeds were sown under two light treatments: "low" and "high." Within the greenhouse, these treatments were created within rectangular metallic frames (height  $\times$  width  $\times$  length = 1.5 m  $\times$  1.5 m  $\times$  2 m) covered with mesh that permitted the flow of air and the entry of rain (these frames are referred to below as "main plot" units). For the high light treatment, one single layer of green mesh (with 9-mm<sup>2</sup> apertures) was used; for the low light treatment, three layers of mesh were used: one layer of black mesh (with 4-mm<sup>2</sup> apertures) and two layers of green mesh (with 9-mm<sup>2</sup> apertures). To control for spatial variation in light exposure observed in the greenhouse, three pairs of frames (each pair including one low-light and one high-light treatment) were established, blocking the light treatments. The three pairs

(blocks) were oriented along the main axis of light variation within the greenhouse.

During four cloudless days, the following physical variables were recorded hourly within each frame: active photosynthetic light (photosynthetic photon flux density [PPFD], measured in moles per square meter per second), using quantum sensors and a LI-COR data logger (Model LI-1000, LI-COR, Lincoln, Nebraska); the ratio of red/far red light (R/FR), using a radiometer with filters for 660 and 700 nm (SKYE Model SKR-100, Skye Instruments, Landrindod Wells, UK); and minimum ( $T_{\min}$ ) and maximum ( $T_{\max}$ ) air temperatures, using a bulb thermometer. The light variables were estimated by integrating the instantaneous measures taken every 5 min to derive a mean ( $\pm 1$  SE) value per hour, while the R/FR ratio was measured hourly at each of three fixed points inside each frame. Pooling all frames, the mean daily values were: PPFD =  $12.0 \pm 2.54$  mol·m<sup>-2</sup>·s<sup>-1</sup>; R/FR =  $0.32 \pm 0.02$ ;  $T_{\min}$  =  $22.9 \pm 1.0^\circ\text{C}$ ;  $T_{\max}$  =  $26.7 \pm 0.3^\circ\text{C}$ , for the low light treatment, and PPFD =  $108.8 \pm 7.17$  mol·m<sup>-2</sup>·s<sup>-1</sup>; R/FR =  $1.23 \pm 0.05$ ;  $T_{\min}$  =  $22.9 \pm 1.8^\circ\text{C}$ ;  $T_{\max}$  =  $33.0 \pm 0.8^\circ\text{C}$  for the high light treatment. These values coincided with the range of values reported in the understory of shaded and natural gaps forest sites at Los Tuxtlas (Vazquez-Yanes et al. 1990).

For each species, four treatments (two seed mass classes [small vs. large seeds] and two exclosure treatments [the same treatments as used in the field experiment]) were represented within each frame (main plot). Exclosure treatments were established using the same procedures described for the field experiment. The experimental unit corresponding to each of the four combinations of seed mass class and exclosure treatments was a plastic 40  $\times$  20 cm tray containing a 7-cm layer of forest soil free of *Psychotria*. Within each tray, 20 seeds representing one of the four treatments were placed on the soil surface. Each frame contained 12 trays, one replicate of each of the four treatments per tree species. The positions of the trays within each frame were assigned at random.

During the germination period of each species, the number of emerging seedlings was recorded every 4 d.

#### *Statistical analyses*

*Greenhouse experiment.*—To detect the effects of seed mass, animal exclosures, and light environment on emergence and on speed of emergence, we performed a split-plot ANOVA for each species. To avoid pseudoreplication in these analyses, we used the proportion of emergence per tray and the mean speed of emergence per tray as the response variables. The main plot factor was the light environment, which was spatially arranged in three pairs of high light and low light treatments, hereafter called blocks. Seed mass class and the exclosure treatment were fixed factors split within each light environment (commonly called subplot factors). The mean square of the block  $\times$  light environ-

ment interaction term was used as the error term to evaluate the statistical significance of the light environment effect (plot factor), while the residual error of the model was used to test the remaining terms (subplot factors), as indicated in Milliken and Johnson (1984). For *P. papantlensis*, due to a failure to record the time to emergence in the low light treatment, data were analyzed only for the high light treatment, using a randomized block model. In this case, we tested only for significant effects of seed mass class, exclusion treatment and their interactions. To compare means we used a Tukey hsd test, following proper use of standard errors for a split plot design, according to Milliken and Johnson (1984).

*Field experiment.*—We performed ANCOVA to detect effects of seed mass within species on emergence, risk of seed removal, and speed of emergence. In addition, we aimed to detect the potential sensitivity of such effects to habitat. In this model, the mean seed mass of each seed mass class was considered as a continuous and linear variable, and the habitat as a discrete factor. Because seed mass was a fixed factor split within each plot representing each habitat, and because habitats were paired in three sites, to test for a habitat effect we used the mean square of the site  $\times$  habitat interaction term, while to test the rest of the model we used the residual error, as indicated by Milliken and Johnson (1984). For most of species the relationships between seed mass and the response variables were clearly linear. Only for emergence in *P. simiarum* did we include seed mass as a quadratic term in the model. For those cases where the ANCOVA detected any significant seed mass effect, we performed for each habitat an a posteriori linear regression to examine the relationship between the response variable and mean seed mass. To control for the site effect, we considered site as a block in these regression analyses. As a conservative approach, we considered only those relationships that were statistically significant in the separate regression analyses to be truly biologically significant.

For some species and response variables a high heterogeneity of variances between habitats prevented the use of ANCOVA. This was the case for *P. papantlensis* for emergence, for *P. simiarum* and *P. flava* for the risk of seed removal, and for *P. flava* for the speed of emergence. Heterogeneity of variances persisted even after several transformations of the response variables. In all such cases, we performed separate linear regressions between the response variables and seed mass for each habitat, as indicated above. Among all these regressions, within each species, we detected a significant seed mass effect in only one of the two habitats. Therefore, it was not necessary to test for slope differences between habitats.

To approach normality and to reduce the heterogeneity of variances, emergence values were arcsin(square-root)-transformed. All statistical analyses

were conducted using the GLM procedure in SAS version 6.08 (1994).

## RESULTS

### *Greenhouse experiment*

Under controlled greenhouse conditions, total seedling emergence varied between 73% (*Psychotria flava*) and 93% (*P. papantlensis*). Seedling emergence occurred 3–5 mo after seeds were sown. After 7 mo, at which time the experiment was terminated, none of the seeds that remained ungerminated were viable according to a tetrazolium test.

Our animal-exclosure units did not impose any abiotic factors that influenced seed behavior. In the greenhouse, in both light treatments, there was no statistically significant effect of the animal-exclosure treatment on either emergence or on the speed of emergence (Fig. 1).

We detected only a minor effect of light environment on seed behavior, and this effect was specific to both trait and species. ANOVAs detected no significant effect of the light environment on emergence in any species (Figs. 1 and 2). There was, however, a significant effect of the light treatment on the speed of emergence in *P. limonensis* ( $F_{1,2} = 454.4$ ,  $P < 0.01$ ). In this species, seeds in the low light treatment emerged faster than those in high light (Figs. 1 and 2). Finally, there was no effect of seed mass class on either emergence or the speed of emergence in any species, nor was there a significant light environment  $\times$  seed mass interaction affecting either of these variables (Fig. 2).

### *Field experiment*

*Seedling emergence.*—Seed mass influenced seed behavior in the field, at least in some species. The amount of variance in emergence explained by seed mass varied from 0 to 64% among species in the gaps, and from 0 to 68% among species in the shaded forest. ANCOVA detected a significant seed mass effect on emergence in three species: *P. limonensis*, *P. simiarum*, and *P. flava* (Table 2a), while linear regressions detected seed mass effects in *P. papantlensis* (Fig. 3). The direction of the relationship between seed mass and the total proportion of seedling emergence differed among these species (Fig. 3). In two species (*P. limonensis* and *P. papantlensis*), the proportion of seedling emergence increased linearly with seed mass (Fig. 3). In contrast, in *P. flava*, seedling emergence diminished linearly with seed mass. In *P. simiarum*, both trends occurred, generating a maximum of emergence for seeds of intermediate sizes.

Within species, we found no evidence that the direction of the seed mass effects on emergence differed consistently between habitats. In *P. limonensis* and *P. simiarum* the behavior of smaller and larger seeds was similar in both habitats, according to ANCOVAs and a posteriori linear regressions (Table 2a, Fig. 3). For

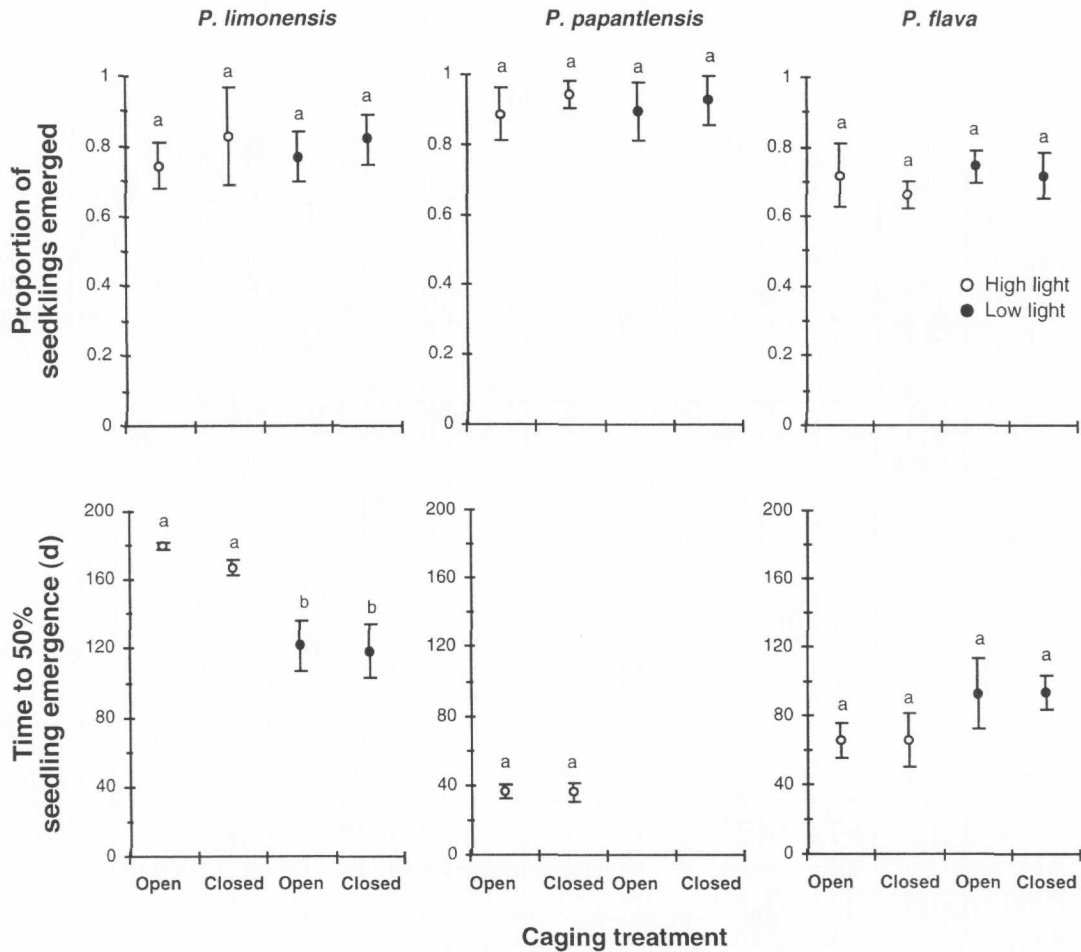


FIG. 1. Effect of animal exclusion units on seed behavior of three *Psychotria* species under greenhouse conditions at Los Tuxtlas, Mexico. Mean values ( $\pm 1$  SE, vertical bars) are shown for seedling emergence and speed of emergence. Values are indicated for each caging treatment ("open" = no exclusion; "closed" = animals excluded), represented in each light environment (high light vs. low light) treatment. Dots not sharing the same letter are significantly different ( $P < 0.05$ , Tukey hsd test).

the other two species, we detected significant mass effects only in one habitat. In *P. papantlensis* we found a positive relationship between emergence and seed mass, but only in shaded forest. In *P. flava*, although ANCOVA did not detect a significant interaction between seed mass and habitat, a posteriori regressions indicated a significant negative relationship that was restricted to gaps (Table 2a, Fig 3).

The amount of variance in emergence explained by seed mass depended on both the mean seed mass of a species and on the habitat in which it was observed. In species with relatively small seeds, seed mass explained a higher proportion of variance in shaded forest than in gaps (*P. limonensis* and *P. papantlensis*, Fig. 3). In contrast, in relatively large-seeded species, seed mass explained a similar or even higher proportion of variance in the gaps than in the shaded forest (*P. simiarum* and *P. flava*, respectively, Fig. 3).

*Effects of animal exclusion on seedling emer-*

*gence.*—In most species and habitats, emergence was higher when animals did not have access to seeds; *P. chagrensis* and *P. simiarum* were the only species in which the mean risk of seed removal (in gaps only) did not differ from zero. This indicates that, in most of these species, seeds deposited on the soil surface were frequently either removed, eaten, or otherwise destroyed by animals.

In *P. papantlensis*, *P. simiarum*, and *P. flava*, seed mass had a significant effect on the risk of seed removal in one of the habitats (Table 2b, Fig. 4). The direction of the seed mass effect on the risk of removal varied among species, even exhibiting opposite trends among taxa. In *P. papantlensis*, larger seeds had a lower risk of removal than smaller ones. In contrast, for *P. simiarum* and *P. flava*, larger seeds had a higher risk of removal than smaller ones (Fig. 4). These seed mass effects were not associated with habitat type. For example, *P. flava* exhibited significant seed mass effects

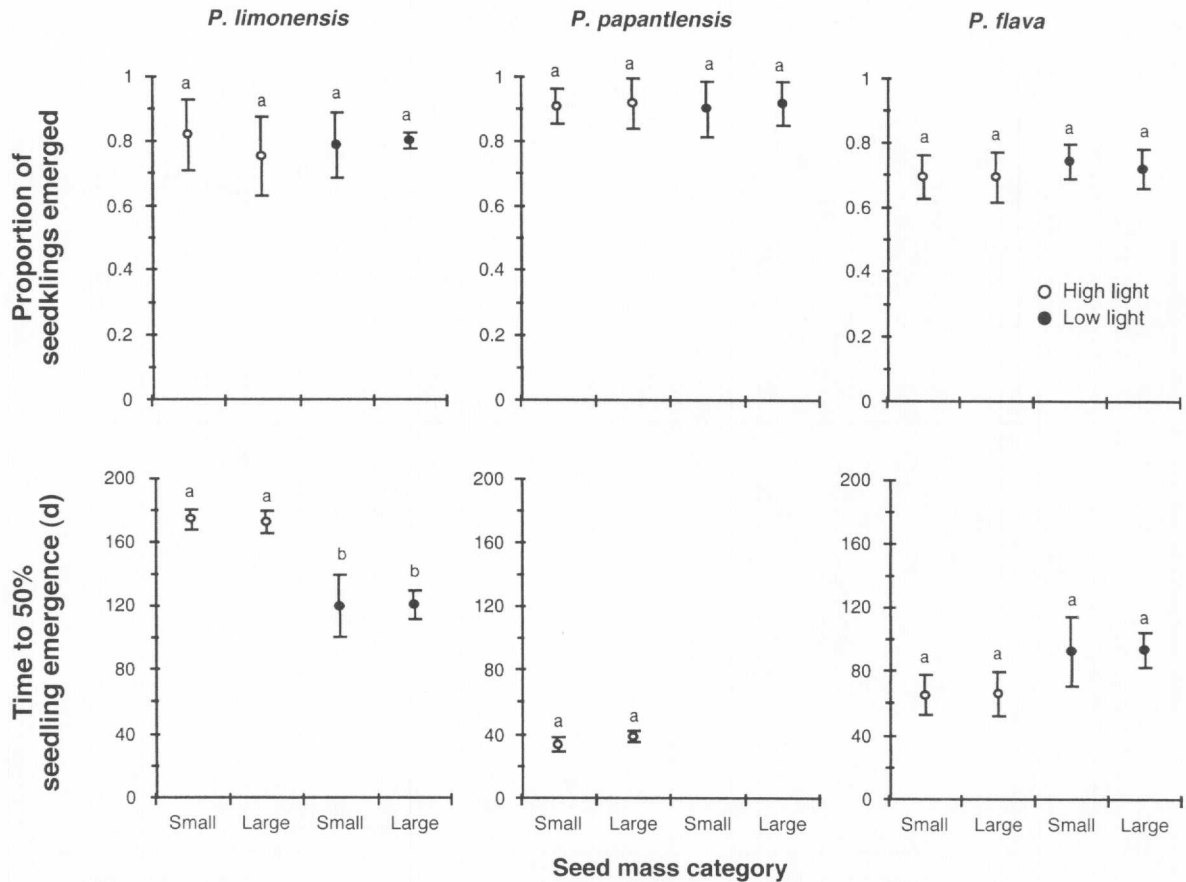


FIG. 2. Effect of seed mass and light environment on seedling emergence and speed of emergence in three *Psychotria* species under greenhouse conditions at Los Tuxtlas, Mexico. Mean and two standard errors are shown (vertical bars). Small and large seed mass classes are, respectively: 0.005 and 0.011 g for *P. limonensis*, 0.029 and 0.047 g for *P. papantlensis*, and 0.163 and 0.298 g for *P. flava*. Dots not sharing the same letter are significantly different ( $P < 0.05$ , Tukey hsd test).

only in gaps, while *P. papantlensis* and *P. simiarum* exhibited seed mass effects only in shaded forest (Table 2b, Fig. 4).

**Speed of seedling emergence.**—The effect of seed mass on the speed of emergence was specific to each species. Seed mass affected this trait only in *P. papantlensis* and *P. flava* (Table 2c, Fig. 5). In *P. papantlensis* larger seeds emerged faster than smaller seeds while in *P. flava* larger seeds took longer to emerge. These effects were restricted to gaps (Fig. 5).

#### DISCUSSION

##### *Effects of seed mass on emergence*

The absence of seed mass effects on emergence in the greenhouse suggests that the effects of seed mass on seedling emergence observed in the field experiment were due to interactions between seed mass and external ecological factors, and not the result of intrinsic effects of seed mass on viability or germination behavior.

In the field, we did not detect a general effect of seed mass on the probability of seedling emergence in all

seven species studied. Only in four species did seed mass have a strong effect on this trait (Fig. 3). The absence of seed mass effects in the other species is not the statistical result of a lack of seed mass variation. We found that each of these species exhibits as much variation in seed mass as at least one species with significant seed mass effects (Table 1).

In this study, we did not find support for the frequent observation that large seeds have an advantage over small ones during the seed to seedling transition (e.g., Gross 1984, Tripathi and Khan 1990). This was so even for those species in which seed mass appeared to have an important effect on emergence. For example, in two species, in the shaded forest, larger seeds had a higher probability of emergence than small ones. In another species, however, small seeds had a relatively high probability of emergence (in gaps), and in yet another species, seeds of intermediate mass had the highest emergence probability. It is possible that differences among species in the relationship between seed mass and emergence are associated with interspecific differences in the interaction between mean seed mass

TABLE 2. Summary of ANCOVAs evaluating the effects of habitat and seed mass on seedling emergence in seven *Psychotria* species in the field.

Species	Habitat			Seed mass			Seed mass × habitat		
	MS	$F_{1,2}$	$P$	MS	$F_{1,16}$	$P$	MS	$F_{1,16}$	$P$
a) Proportion of seedlings emerging									
<i>P. limonensis</i>	0.016	0.481	0.558	0.094	21.33	<b>0.001</b>	0.007	1.600	0.225
<i>P. graciliflora</i>	0.013	0.186	0.708	0.004	0.349	0.563	0.001	0.081	0.782
<i>P. chagrensis</i>	0.028	2.037	0.289	0.004	0.366	0.554	0.016	1.394	0.255
<i>P. simiarum</i>	0.014	2.333	0.267	0.024	1.991	0.181	0.013	1.028	0.328
<i>P. flava</i>	0.003	0.086	0.797	0.152	12.39	<b>0.004</b> †	0.008	0.688	0.420‡
<i>P. faxlucens</i>	0.019	0.427	0.581	0.083	13.16	<b>0.003</b>	0.007	1.116	0.307
				0.000	0.081	0.780	0.001	0.102	0.754
b) Risk of seed removal									
<i>P. limonensis</i>	0.002	0.209	0.689	0.010	1.072	0.316	0.000	0.033	0.859
<i>P. graciliflora</i>	0.022	0.319	0.628	0.014	0.835	0.375	0.021	1.305	0.271
<i>P. chagrensis</i>	0.042	840	<b>0.001</b>	0.001	0.054	0.819	0.060	4.056	0.060
<i>P. papantlensis</i>	0.014	35.00	<b>0.024</b>	0.030	4.545	<b>0.047</b>	0.012	1.812	0.198
<i>P. faxlucens</i>	0.005	0.666	0.501	0.008	0.701	0.415	0.001	0.119	0.735
c) Time to seedling emergence									
<i>P. limonensis</i>	3.429	0.006	0.950	299.2	2.354	0.145	0.002	0.000	0.997
<i>P. graciliflora</i>	45.67	0.615	0.516	3.333	0.023	0.881	37.41	0.261	0.617
<i>P. chagrensis</i>	54.62	0.906	0.442	0.149	0.003	0.954	83.21	1.910	0.186
<i>P. papantlensis</i>	0.004	0.000	0.993	105.8	5.516	<b>0.032</b>	1.715	0.089	0.769
<i>P. simiarum</i>	17.48	4.197	0.177	22.53	3.502	0.080	0.533	0.083	0.778
<i>P. faxlucens</i>	98.59	0.510	0.550	5.574	0.138	0.715	48.14	1.192	0.291

Notes: For emergence in *P. simiarum*, we used a quadratic model resulting in two coefficients associated with seed mass and the seed mass × habitat interactions. Terms in the model were tested using proper errors (see *Methods: Statistical analysis*). ANCOVAs were not performed for some species and variables (see *Methods: Statistical analysis* and Figs. 3–5 for alternative procedures).

† Seed mass × seed mass  $P$  value.

‡ Seed mass × seed mass × habitat  $P$  value.

and habitat. Positive associations between seed mass and emergence were restricted to small-seeded species, while negative or convex associations were restricted to medium and large-seeded species. Furthermore, where there were positive associations, seed mass tended to explain a higher proportion of variance in emergence in the shaded habitat than in gaps, while where there were negative associations the opposite trend was observed. This suggests that ecological factors in the shaded forest provide a disadvantage to relatively small seeds, particularly within small-seeded species, and that ecological factors in gaps provide a disadvantage to relatively large seeds, particularly within large-seeded species. These trends may help to explain why small-seeded species of *Psychotria* tend to be more abundant in gaps. A firm conclusion, however, would require the study of more species and the observation of the effects of seed mass on the demographic fate of individuals throughout the life cycle. From an evolutionary perspective, the direction of selection on seed mass depends on the combined effects of seed mass on all fitness components. Large-seeded species must have experienced direct (or correlated) selection favoring large seeds relative to their close relatives in the past, but our data suggest that large seeds are not necessarily advantageous during all phases of the life cycle.

#### Ecological factors operating on seed mass

*Light environment.*—Results of the greenhouse experiment indicated that seed mass did not affect emer-

gence given variation only in the level of light. This suggests that the habitat-specific effects of seed mass emergence observed in the field were the result of interactions between seed mass and other ecological factors that differ between the habitats.

*Animal seed removers.*—Within some species and habitats, seed mass affected the risk of removal by animals, which in turn influenced the relative success of seeds of different mass in making the transition from seed to seedling. Our results do not support the idea that larger seeds, as a rule, have a higher risk of removal (e.g., Harper et al. 1970, Fenner 1983, Facelli and Pickett 1991). We found one case in which the risk of seed removal within species was actually higher for smaller than for larger seeds while in other cases larger seeds were at greater risk (Fig. 4). Such risks of removal, however, were restricted either to the shaded forest or to the gaps. The frequency of these habitats change across space and time due to forest patch dynamics (Martinez-Ramos and Alvarez-Buylla 1988). Therefore, the net effect of seed removers as a potential selective factor operating on intraspecific seed mass variation may depend on the frequencies of each habitat type (Martinez-Ramos et al. 1988).

The observed differences among species, regarding the direction with which seed removal risk changed with seed mass, might be due to distinct groups of animals removing *Psychotria* seeds in different seed mass ranges. Three observations provide support for this idea.



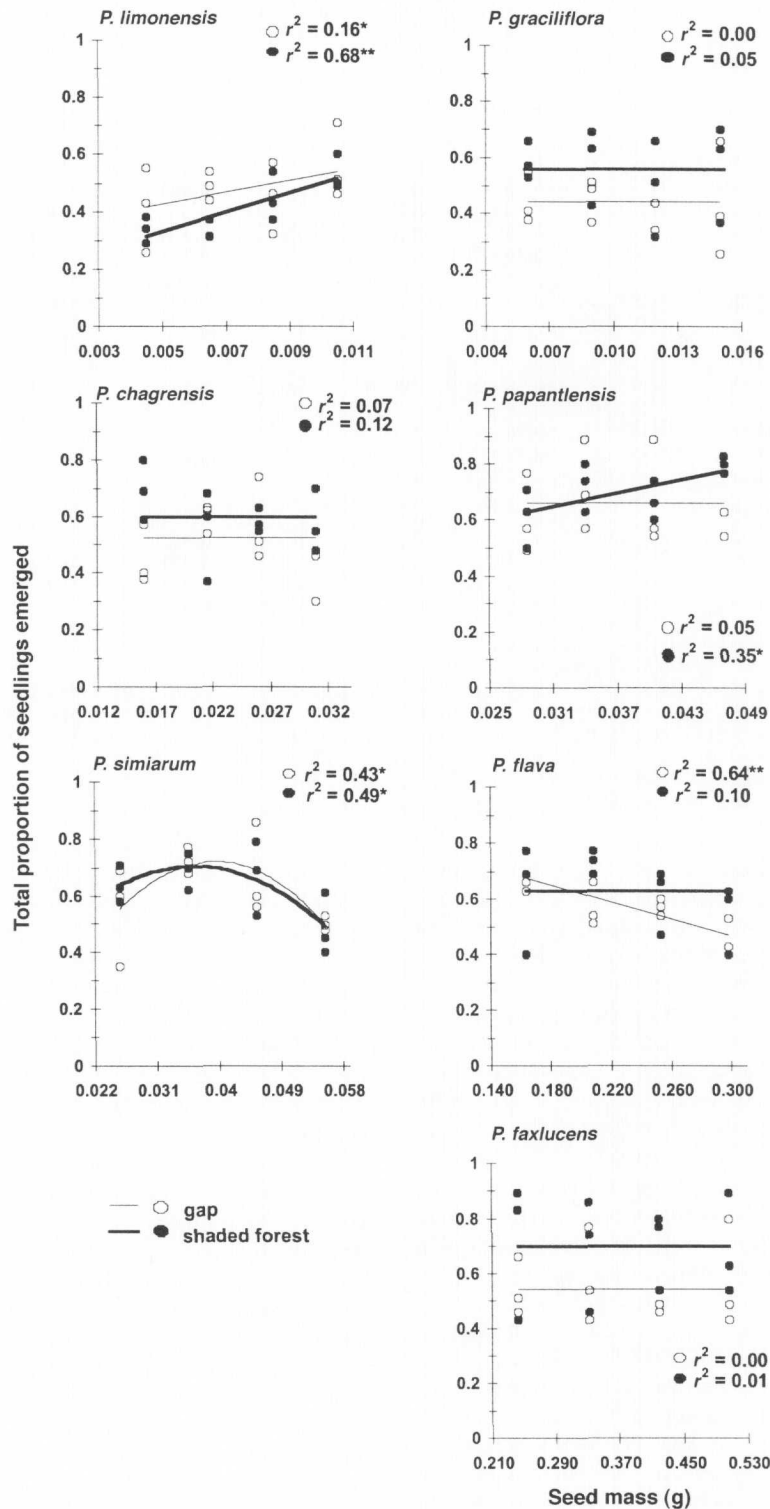


FIG. 3. Relationship between seed mass and seedling emergence in seven species of *Psychotria* under experimental field conditions at Los Tuxtlas, Mexico. Species appear in order of increasing mean seed mass from the left at the top to the right at the bottom. Each dot represents the emergence proportion for all seeds of a given seed mass class monitored in a particular gap or shaded forest site. The  $r^2$  values of regressions are given for each habitat; significant values are indicated: \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ . In the case of nonsignificant regressions, horizontal lines indicate the mean value of seedling emergence across the entire seed mass range.

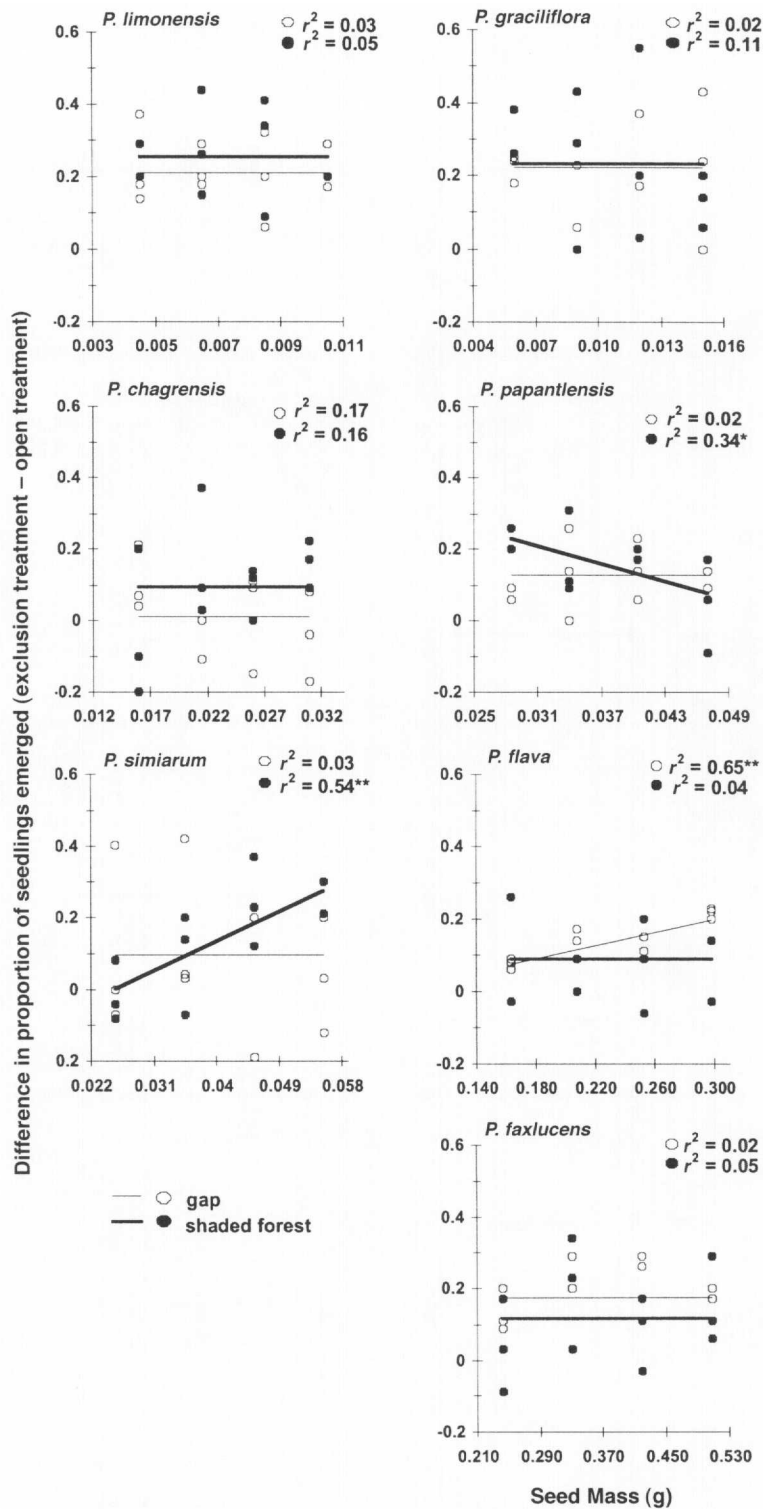


FIG. 4. Effect of seed mass on the risk of seed removal for seven species of *Psychotria* under experimental field conditions at Los Tuxtlas, Mexico. Each dot represents the risk of seedling removal among all seeds of a given seed mass class monitored in a particular gap or shaded forest site. In the case of nonsignificant regressions, horizontal lines indicate the mean value of the risk of seed removal across the entire seed mass range. Other specifications are as in Fig. 3.

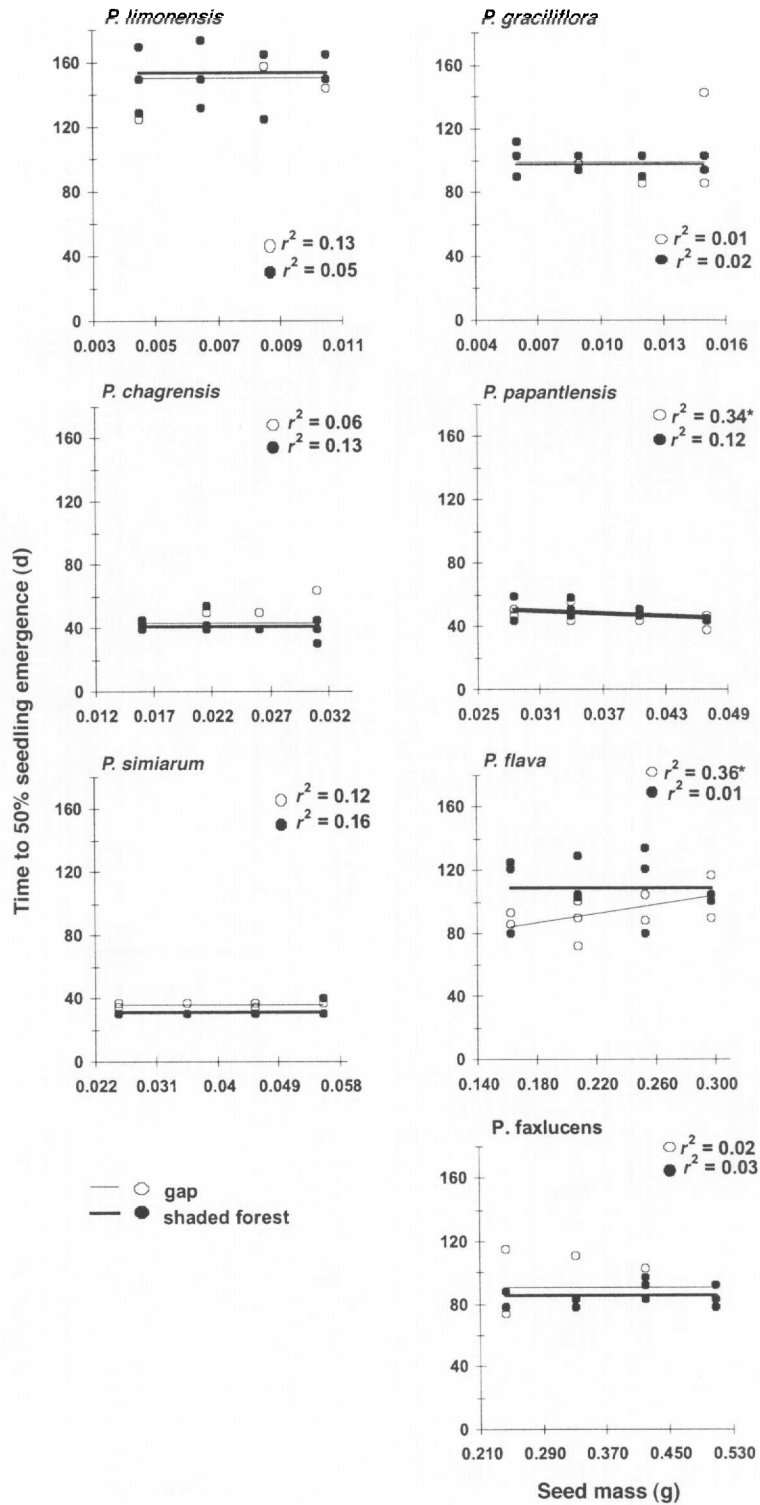


FIG. 5. Effect of seed mass on speed of seedling emergence, for seven species of *Psychotria* under experimental field conditions at Los Tuxtlas, Mexico. Each dot represents the time (in days) to achieve 50% seedling emergence among all seeds of a given seed mass class monitored in a particular gap or shaded forest site. In the case of nonsignificant regressions, horizontal lines indicate the mean time to 50% emergence across the entire seed mass range. Other specifications are as in Fig. 3.

First, our field observations indicate that different groups of animals attack different *Psychotria* species: vertebrates are attracted to the species with relatively large seeds (*P. simiarum*, *P. flava*, and *P. faxlucens*), while ants attack the relatively small-seeded species (*P. limonensis* and *P. graciliflora*). Second, the significant effects of seed mass on the risk of removal are not random with respect to the mean seed mass of a species. We found no effects of seed mass on the risk of removal within the small-seeded species *P. limonensis* and *P. graciliflora*, whereas seed mass explained a high degree of variance in the risk of removal in the medium- to large-seeded species. Third, the direction of seed mass effects on the risk of removal was positive in the relatively large-seeded species, while negative in the medium-seeded species. These observations suggest that vertebrates preferentially remove larger seeds, but only in those species with large seeds, and that ants do not discriminate among seed sizes within the small-seeded species. In contrast, the relatively high levels of removal of smaller seeds in the medium-seeded species does not appear to be easily explained by the activity of any particular group of animals, such as ants or rodents. Selective removal by vertebrates on the basis of intra-specific seed size variation has been observed before (Hulme 1993), but there is little information about seed size preferences in ants (but see Kaspari [1996]).

The length of time that seeds were exposed to animals did not explain the association between seed mass and the risk of removal, except in one species. In *P. flava* we observed significant effects of seed mass on both the number of days to 50% emergence (Fig. 5) and on the risk of removal (Fig. 4); larger seeds exhibited higher values for both variables. In this species the higher risk of removal for larger seeds could have been due to a higher attractiveness to animals or to the longer time that larger seeds remained exposed to animals.

*Effects of seed mass on speed of emergence.*—Our results in both the greenhouse and the field indicated that seed mass did not have a consistent effect on the speed of seedling emergence. Only under field conditions did we find significant relationships between these variables and those cases were restricted to two species in gaps. Moreover, the relationship was opposite in the two species (Fig. 5). These observations suggest that there is no common mechanism that accounts for the relationship between seed mass and speed of emergence in the studied species, such as changes in surface: volume ratio or in seed coat permeability with seed size. Contradictory evidence for the effects of seed size on speed of emergence has been found in other studies (Stanton 1985, Marshall 1986, Stamp 1990, Tripathi and Khan 1990, Hendrix and Trapp 1992, Zhang and Maun 1996).

#### CONCLUSIONS

Our results indicate that, within species, seed mass does not have a general effect on seed or seedling be-

havior that was shared by all seven *Psychotria* species investigated. Seed mass did not affect the risk of seed removal, the speed of emergence, or the probability of seedling emergence for all species, and the significant relationships found varied depending on the habitat and species. The probability of seedling emergence was more sensitive to seed mass than the risk of seed removal or the speed of seedling emergence.

The variation that we observed among species in the relationship between seed mass and emergence was related to the species mean seed mass in a manner that suggests two hypotheses. First, ecological factors acting on seed mass may operate in opposite directions in different taxa, depending on their mean seed mass. Second, the nature and magnitude of such factors may be habitat specific. However, since significant seed mass effects were found only in four of the seven species studied, the potential contribution of such habitat-specific factors to the evolutionary and phenotypic seed mass differentiation in our *Psychotria* system seems to be globally weak. It is possible that the seed mass variation we observed may have evolved due to selection operating more strongly on postemergence life stages than on the seed to seedling transition itself. Additional studies on a wide variety of taxa that differ in seed mass would be necessary to corroborate these possibilities.

The effects of seed mass on seedling emergence detected in the field appear to be the result of external ecological factors, and not due simply to intrinsic effects of seed mass. Seed-removing animals appear to be an important factor acting on seed mass. The activities of seed-removers or seed predators, however, could not fully explain the seed-mass-dependent trends of seedling emergence that we observed, suggesting that additional ecological factors not examined here also contribute to these trends.

#### ACKNOWLEDGMENTS

This work was conducted in partial fulfillment of the requirements for the Ph.D. of Horacio Paz, a Ph.D. candidate at the Universidad Nacional Autonoma de Mexico (UNAM). The field work was financed by PADEP-UACPyP-CCH, UNAM. Data were analyzed and the text written during H. Paz's 18-mo stay at the University of California, Santa Barbara, with economic support provided by the DGIA-UNAM. H. Paz is especially grateful to Santiago Sinaca Colin, Braulio Gomez Chagala, and Lorenzo Chagala, for their invaluable collaboration in the planning and development of this field work, as well as to the people working at Los Tuxtlas Tropical Biological Station for the support provided. We thank Allan Stewart-Oaten for statistical advice.

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