

Research Paper

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Seed permeability: an essential trait for classifying seed dormancy type

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Abstract

Seed dormancy in plants can have a significant impact on their ecology. Recent work by Rojas-Villa and Quijano-Abril (2023) classified the seed dormancy class in 14 plant species from the Andean forests of Colombia by using germination trials and several microscopy techniques to describe seed anatomy and morphology. The authors conclude that *Cecropia* species have both physical and physiological dormancy (of which they call physiophysical dormancy) based on seed morphology and mean germination times of over 30 days. Here, we present seed permeability and germination data from neotropical pioneer tree species: *Ochroma pyramidale*, *Cecropia longipes*, and *Cecropia insignis*, as well as *Cecropia peltata* (present in Rojas-Villa and Quijano-Abril, 2023), to demonstrate that *Cecropia* species do not exhibit dormancy and also have high levels of seed permeability. We find that the mean germination time for all three *Cecropia* species in our study was less than 30 days. This suggests a need for reporting the conditions in which germination trials take place to allow for comparability among studies and using seed permeability tests to accurately identify the physical dormancy class of seeds. Further, we present data from the literature that suggests that dormancy is not a requirement for seed persistence in the seed bank.

Seed dormancy is an essential trait widely reported in ecological studies. Seed dormancy often influences the ecological dynamics of plant communities by determining germination timing (Baskin and Baskin, 2014). Recent work by Rojas-Villa and Quijano-Abril (2023) used light microscopy and scanning electron microscopy to describe the morphology and anatomy of seeds from 14 plant species in the Andean forests of Colombia, while also determining dormancy class with these techniques and germination trials. The authors found dormancy in 85% of their study species, three of which are in the genera *Cecropia* and were classified as having both physiological and physical dormancy (referred to as physiophysical dormancy). Seeds with physiophysical dormancy have impermeable coats, a fully developed embryo, and a mean germination time (MGT) of over 30 days (Rojas-Villa and Quijano-Abril, 2023). Here, we present seed permeability data measured by fluorescent dye uptake into the seed endosperm and germination trials from a Panamanian lowland population of *Cecropia peltata* (one of the species studied by Rojas-Villa & Quijano-Abril, 2023), as well as *Ochroma pyramidale*, *Cecropia longipes*, and *Cecropia insignis*. Our data do not support the presence of physical dormancy in seeds of *Cecropia*, while *O. pyramidale* does exhibit physical dormancy.

Rojas-Villa and Quijano-Abril (2023) provide extensive anatomical descriptions of the *Cecropia* fruits and seeds included in their study. These descriptions, along with MGT of 30 days or more, were used to determine if seeds exhibited dormancy. Support for their dormancy classification of physiophysical dormancy in *Cecropia* was provided by the presence of a seed coat derived from two integuments, a ~0.14 mm thick membrane, and one to two layers of cuboidal cells with tannins present (Rojas-Villa and Quijano-Abril, 2023). The three *Cecropia* species had MGT varying from 39.35 ± 7.36 days for *C. angustifolia* to 63.73 ± 6 for *C. peltata*, with *C. telealba* averaging 46.21 ± 9.59 days. Although the authors mentioned the inclusion of imbibition tests in their study, no seed permeability data were reported for any of the *Cecropia* species.

There are several ways to test the permeability of seeds. Given that physiophysical dormancy and physical dormancy, by definition, have impermeable seed coats, a test of permeability is needed to determine if a species exhibits these dormancy types. Permeability can be tested using imbibition or conductivity tests, and/or via microscopy by measuring fluorescent dye uptake by seeds (Tieu and Egerton-Warburton, 2000; Chen et al., 2019). Imbibition tests quantify the increase in seed mass when seeds are exposed to water. For impermeable seeds, seed mass should not change after water exposure. However, the imbibition test may have limitations for seeds with small masses, as the change in mass may be difficult to detect for individual seeds. Furthermore, because a single population of seeds may contain intact

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impermeable seeds, and damaged permeable seeds, imbibition tests based on a pooled sample of multiple seeds may be difficult to interpret.

The electrical conductivity test measures the condition of the seed-protecting tissues, where low conductivity suggests there is little electrolyte leaching from the seed interior, and the seeds are likely impermeable or of high quality (Matthews and Powell, 2006). These tests may have similar limitations for small seeds, as the signal-to-noise ratio may be challenging to interpret when electrolyte leaching from small seeds may result in low conductivity rates.

Seed permeability can also be quantified by the extent to which a low molecular weight fluorescent dye penetrates inside of the seed coat (Fig. 1A). Fluorophore seed penetration is often quantified through fluorescence microscopy after 24 to 48 h of exposure. This dye can potentially permeate the seed anywhere, leading to a potential underestimation of permeability if the dye permeates into a location of the seed that is not visible from where the researcher has cut the seed before microscopy processing.

Here, we used Lucifer Yellow (LY) dye to determine seed permeability because LY has a low molecular weight compared to other water-soluble fluorophores, making it especially useful for measuring seed permeability (Tieu and Egerton-Warburton, 2000). Combining data on seed permeability and germination trials on seeds collected on lowland tropical forests in Panama, we suggest that (1) permeability tests should be included when determining physical dormancy, and (2) a need for standardized and/or reported germination trial conditions (e.g., temperature, moisture, and light). In Rojas-Villa and Quijano-Abril (2023), all the *Cecropia* species exhibit physiophysical dormancy and they suggest this may be a characteristic of the genus. We find high levels of seed permeability in all three of the *Cecropia* species

in our study (Fig. 1B), one of which, *C. peltata*, is present in both studies. In comparison, *Ochroma pyramidale*, a species with known physical dormancy (Sautu et al., 2007; Zalamea et al., 2015), shows that only ~50% of seeds are permeable. Using four species with physically dormant seeds (including *O. pyramidale*), Zalamea et al. (2015) showed that fresh seeds (i.e., fully mature seeds that have not been dispersed away from the maternal tree) often consist of two types: one representing a permeable (nondormant) fraction of seeds that is capable of germinating immediately, and a second fraction that is impermeable (dormant) and accounts for the seeds that persist in the soil seed bank.

Further, Rojas-Villa and Quijano-Abril (2023) define species as dormant if the MGT is greater than 30 days. However, several environmental factors can influence MGT for a given species. These factors need to be reported in papers classifying dormancy types on seeds to avoid confounding results. Mean germination time may increase under colder conditions (Sampayo-Maldonado et al., 2019), such as those in Andean montane forests in Rojas-Villa and Quijano-Abril (2023). For example, the time required for germination of 50% of *Cedrela odorata* seeds was 13–42 days longer in temperature treatments of less than 20°C compared to treatments above 20°C (Sampayo-Maldonado et al., 2019). Germination trials (see the methods at the end for details on the environmental conditions of these trials) with *Cecropia* species (and *O. pyramidale* as a physically dormant species as a comparison) in Panama showed the MGT is substantially less than 30 days (*C. insignis* = 18.9 ± 0.6 ; *C. longipes* = 15.6 ± 0.8 , *C. peltata* = 13.6 ± 0.2 , *O. pyramidale* = 10.9 ± 0.7 ; Fig. 2). It is difficult to disentangle whether the differences in MGT between this study and Rojas-Villa and Quijano-Abril (2023), especially for *C. peltata* used in both studies, are from differences in the physical environment of the germination trials or potential differences in

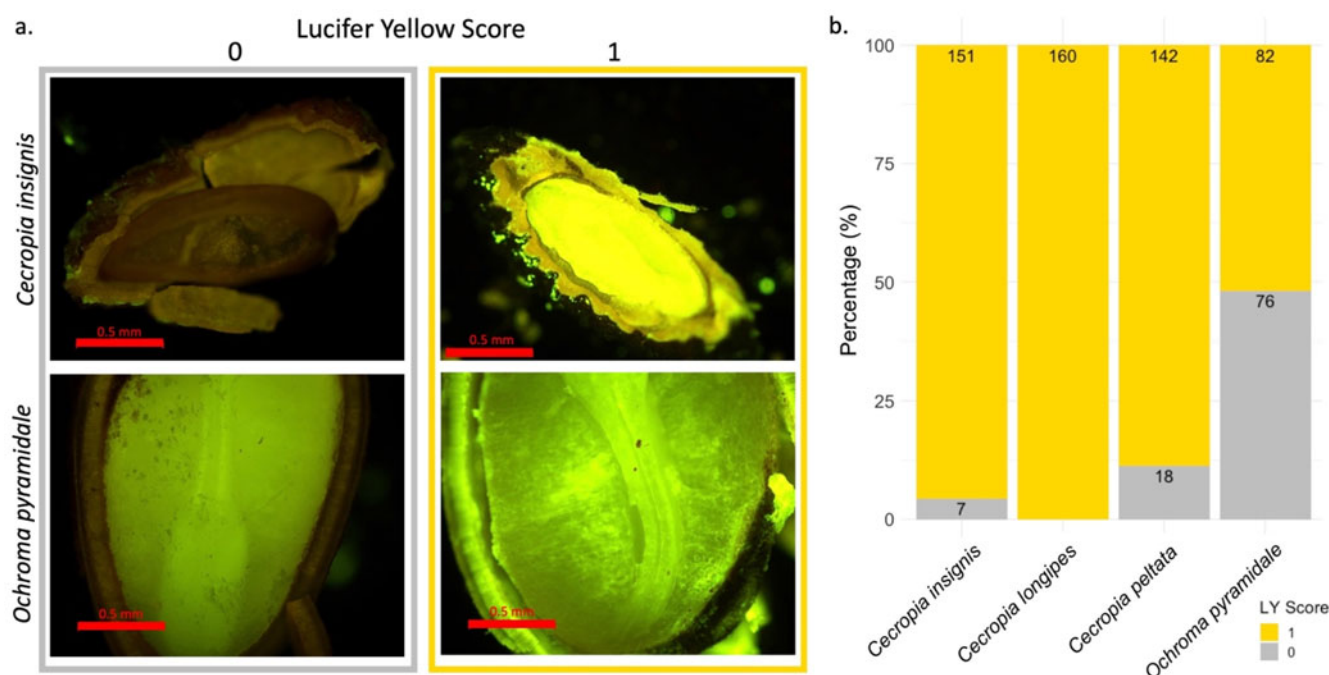


Figure 1. Example images (a) from *C. insignis*, a species with nondormant seeds, and *O. pyramidale*, a species with physically dormant seeds, with seed permeability scores of both 0 and 1. Bar plots (b) showing the percentage of permeable seeds quantified by Lucifer Yellow (LY) uptake from three tree species with nondormant seeds, *C. insignis*, *C. longipes*, *C. peltata*, and one tree species with physically dormant seeds, *O. pyramidale*. Seeds are scored 0 if the seed is classified as impermeable or 1 if the seed is classified as permeable. Numbers at the top of each bar in (b) indicate the sample size for each category.

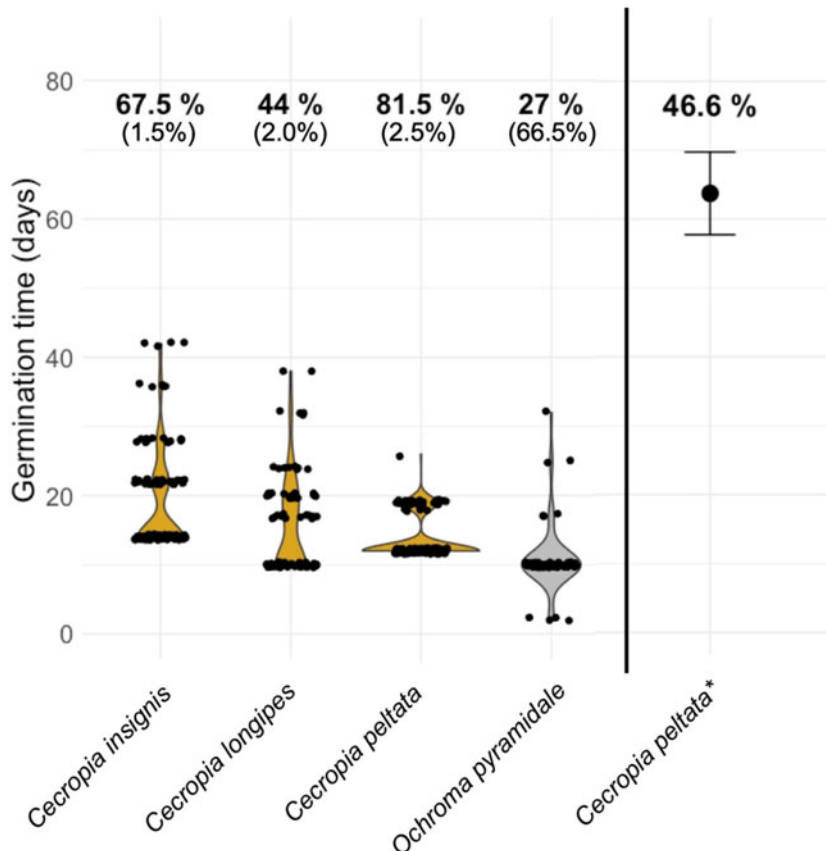


Figure 2. Germination time in days for three tree species with nondormant seeds, *C. insignis*, *C. longipes*, *C. peltata*, shown in gold and one tree species with physically dormant seeds, *O. pyramidale* shown in gray. Mean and standard error of *C. peltata** from Rojas-Villa & Quijano-Abril (2023) are also shown for comparison between studies. Percent of seeds that germinated in germination trials for each species is shown in bold at the top of the figure. Below these percentages, we provide the percentage of seeds that did not germinate but were determined to be viable through tetrazolium staining for the four species in our study.

sub-populations within species. This suggests caution should be taken using MGT as an indicator of physical dormancy without some standardization for comparison between studies and the additional use of permeability tests.

We also find that *O. pyramidale*, a physically dormant species, had the fastest germination time but the lowest percentage of seeds that germinated (27% compared to 44% or higher found in *Cecropia* species; Fig. 2). Further, 66.5% of the seeds in *O. pyramidale* that did not germinate after 6 weeks in the germination trials were determined to be viable using tetrazolium testing compared to the *Cecropia* species where only 1.5–2.5% of the non-germinated seeds were viable. This adds further evidence that there is a subset of seeds in physically dormant species, such as *O. pyramidale*, that do not exhibit dormancy.

Rojas-Villa and Quijano-Abril (2023) comprehensively explain the functional role of physiophysical dormancy in *Cecropia*. They suggested that dormancy allows these seeds to persist in the soil for long periods of time until suitable (gap or high light) conditions arise. However in other work, we found that seeds of *Trema micrantha* and *Zanthoxylum ekmanii*, that have physiologically dormant seeds at the time of dispersal, exhibit seed dormancy alleviation in decades-old viable seeds extracted from the soil seed bank (Zalamea et al., 2023). Never-buried, fresh seeds had a lower proportion of germinated seeds in germination trials, compared to seeds that persisted in the soil for decades, despite both seed types having a similar proportion of viable seeds (determined by germination trials and tetrazolium testing). This suggests that seeds can persist in the soil seed bank even after dormancy begins to be alleviated. In a 30-month long seed burial experiment including the three species of *Cecropia* included in

this study and *Jacaranda copaia* (also a nondormant species; Sautu et al., 2007), we found that the proportion of seeds that can germinate decreased gradually over time (Zalamea et al., 2021). Although the proportion of viable seeds after 30 months of soil incubation was consistently low and varied among localities and species, we were able to confirm that some seeds of three species of *Cecropia* and *J. copaia* persisted in a viable state in the soil without being species that exhibit seed dormancy.

Plant biologists have used morphological and anatomical studies for centuries to characterize and understand the diversity of plant form and function (Haberlandt, 1914). Rojas-Villa and Quijano-Abril's work extensively characterizes anatomical and morphological characteristics of seeds from 14 different species and links these traits to dormancy classes for these species. Contributions like those of Rojas-Villa and Quijano-Abril (2023) are extremely valuable to better understand how seeds of tropical species interact with the physical environment in which they germinate and recruit into as seedlings. In the case of *Cecropia* species, Rojas-Villa and Quijano-Abril's work built upon the pioneering and detailed morphological and anatomical study of Lobova et al. (2003) that used fruits and seeds of *Cecropia* species from French Guiana. Lobova et al. (2003) showed that in *Cecropia* fruits, the pericarp may play a key protective role that is functionally more important than the two thin layers of integuments that form the seed coat. Lobova et al. (2003) hypothesized without any seed permeability measurements that *Cecropia* fruits may not be completely impermeable because the vascular bundle penetrates the pericarp at the pedicel scar; however, they concluded that seed impermeability may have evolved to allow seeds to remain dormant in the soil seed bank. As discussed in

this study and provided empirical evidence in previous contributions (i.e., Zalamea et al., 2021), *Cecropia* seeds do not require dormancy to persist in the soil seed bank. In this contribution, we used seed permeability and germination data collected from four Neotropical pioneer tree species to demonstrate the need for formal testing of permeability when assigning physical dormancy to species. In addition, we also highlight the need to report the environmental conditions in which the germination trials are implemented to make studies comparable and replicable in the future.

Methods

Seeds of four neotropical pioneer species (*Ochroma pyramidale*, *Cecropia longipes*, *Cecropia insignis*, and *Cecropia peltata*) were collected from ripe fruits of at least five different maternal sources per species on the Barro Colorado Nature Monument in Central Panama. Seed collection matched fruit phenology and was done between 2012 and 2014. After seed collection, seeds were pooled among maternal sources and allocated to (1) measurements of seed permeability (160 seeds per species), and (2) germination trials (200 seeds per species).

Seed permeability was measured by qualitatively scoring fluorescent dye uptake into the seed interior. Seeds were incubated in 0.1% (w/v) aqueous solution of LY CH potassium salt (Biotium, Inc., Fremont, California, USA) for 48 h in the dark at room temperature (22°C). After incubation, seeds were manually cut in half and examined using a Nikon Eclipse 600 microscope attached to a XX-V mercury lamp, with a Nikon B-2A fluorescent filter set (450–490 nm excitation/515 nm emission, Nikon Instruments, Inc., Melville, New York, USA). Seed permeability was scored as zero or impermeable (no LY in the endosperm) or one or permeable (LY in the endosperm; for more details, see Zalamea et al., 2015, 2018).

Seed germination was quantified using germination trials. Twenty groups of 10 seeds each per species were placed in a 60 mm Petri dish lined with a paper towel, moistened with ~2 mL of sterile distilled water, and sealed with two layers of Parafilm® (Zalamea et al., 2021). Seeds were incubated in a shade house in Barro Colorado Island in Panama under ~30% sunlight, high red:far-red irradiance (c. 1.4), and ambient temperature (23–30°C). The maximum temperature recorded on the germination bench was c. 38°C. Germination, defined as radicle protrusion, was scored every week over 6 weeks.

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Author contributions. P.-C.Z. and J.W.D. designed the seed permeability and germination trials; P.-C.Z. collected the data; L.A.M. and P.-C.Z. analyzed

the data; L.A.M. drafted the manuscript with contributions from P.-C.Z. All authors contributed substantially to revisions and gave final approval for publication.

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Competing interests. The authors declare none.

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