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Endosperm loss, seed germination, and early seedling growth in large-seeded tropical trees

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ABSTRACT

Endosperm damage was studied in Monteverde, Costa Rica to determine its frequency, extent, and effect on seed germination and early seedling growth. Endosperm damage was found to occur in 72% of seeds from *Chione sylvicola* (Rubiaceae) and 94% of seeds from *Quercus costarricenses* (Fagaceae). The extent of endosperm damage in *Q. costarricenses* was not related to the root and shoot weight of germinated seeds. Experimental endosperm removal was carried out in *Persea americana* and *Cinnamomum paratriplinerve* (both Lauraceae) seeds. The three week time period of the study was inadequate to yield significant *C. paratriplinerve* germination, but seeds with intact endosperm were much less likely to suffer fungal infection. Endosperm removal did not inhibit germination in *P. americana* seeds, even when 80% of the original seed weight had been removed. After 23 days in soil, most seeds were just beginning to produce roots and shoots, the majority of which were still enclosed in endosperm. At this time, *P. americana* seeds with a higher percent of endosperm removed had significantly heavier roots and shoots, suggesting a reduced or even inhibitory role of endosperm in initial seed germination.

RESUMEN

Se estudió el daño al endospermo en Monteverde, Costa Rica, para determinar su frecuencia, grado y efecto en la germinación de las semillas y el crecimiento temprano de las plántulas. Se encontró que el daño al endospermo ocurrió en 72% de las semillas de *Chione sylvicola* (Rubiaceae) y 94% de las semillas de *Quercus costarricenses* (Fagaceae). El grado de daño al endospermo en *Q. costarricenses* no estuvo correlacionado ni con el peso ya sea de las raíces ni el de los brotes de semillas germinados. Se llevó a cabo un experimento de eliminación del endospermo en las semillas de *Persea americana* y *Cinnamomum paratriplinerve* (Familia Lauraceae). Las tres semanas de crecimiento no fueron suficientes para la germinación significativa de *C. paratriplinerve*, pero las semillas con endospermos intactos tuvieron una probabilidad menor de sufrir una infección de hongos. La eliminación del endospermo no inhibió la germinación de las semillas de *P. americana*, aun cuando el 80% del peso original de la semilla había sido removido. La mayoría de las semillas solamente habían empezado a producir raíces y brotes después de 23 días en el suelo y muchas de ellas todavía estaban encerradas en el endospermo. Para entonces, las semillas de *P. americana* con un porcentaje más alto de endospermo eliminado tuvieron raíces y brotes significativamente más pesados. Esto sugiere un papel más reducido o inhibitorio del endospermo en la germinación inicial de la semilla.

INTRODUCTION

Seed endosperm provides nutrition to sustain developing embryos during seed germination and initial growth (Raven and Johnson 1986). Overall, tropical seeds are consistently larger in size, and therefore implicitly higher in nutrient (endosperm) content than temperate species. Despite being relatively large on average, seeds in the tropics cover a size range of ten orders of magnitude, from tiny orchid dust seeds to giant coconuts. A significant relationship exists between growth form and seed size, with canopy trees and lianas having the largest seeds (Leishman et al. 2000). Within a growth form, large seed size may reflect a greater competitive ability. Multiple studies have shown a positive relationship between initial seedling size and seed size, even within species (Dolan, 1984; Moegenburg, 1996; Wulff, 1986; Zhang and Maun, 1991). When a steep light or moisture gradient exists within a few centimeters of the soil surface, even a few extra millimeters of shoot or root length can make a great difference competitively for a seedling (Leishman et al. 2000). As larger seeds produce larger seedlings, larger seed seedlings are able to grow up out of greater depths of soil, where the seed can receive more moisture. Seedlings of larger seeds also emerge from leaf litter more successfully. Larger seeds tend to have a higher percentage of their mass stored as reserve that is gradually deployed to permanent structures. More reserve remains in the seed uncommitted, available for the seed to draw upon in case of undesirable growing conditions or seedling disturbance. Slower germination and growth can put larger seeds at a competitive disadvantage, since less tissue is available for photosynthesis. However, studies have concluded that larger seeds have an advantage in times of drought, intense shade, and mineral nutrient deficiency (Leishman et al. 2000). Dispersal modes are also associated with seed size (Harper et al. 1970, Primack 1987). Larger seeded species are typical of the mammal-dispersal syndrome, (Foster and Janson 1985) but little evidence exists conclusively linking seed size with distance of dispersal (Leishman et al. 2000).

Loss of endosperm to predation or parasitism is widespread in tropical forests due to the length spent on the forest floor (Calvo-Irabién and Islas-Luna 1999; Dalling, Harms, and Aizprúa 1997). Tropical canopy trees, seeds and seedlings commonly suffer severe damage from insects and fungi (Clark and Clark 1991). The role of seed herbivores, or granivores, in selecting for seed size in the tropics has been reported in multiple studies. Harms and Dalling (1997) examined 13 dicotyledon tree species from Barro Colorado Island (BCI), with seeds ranging in weight from 0.2 to 107.6 grams. After each seed produced its first pair of fully-expanded leaves, they clipped the above ground shoot and found that only seeds heavier than five grams were capable of resprouting and producing fully functional seedlings. These results agree with a similar study for seed damage to *Macuna andreana* (Fabaceae), a tropical liana with seeds weighing five to ten grams (Janzen 1976). Further, Ichie et al. (2001) show that germination and seedling growth are accompanied by depleted starch and lipid reserves in *Dryobalanops lanceolata* (Dipterocarpaceae). Once depleted, seedling growth ceases, suggesting that seedling size is a direct result of seed reserves.

Dalling, Harms, and Aizprúa (1997) studied the germination, initial seedling production, and seed and seedling damage tolerance of *Prioria copaifera* (Fabaceae), the largest-seeded tree species on Barro Colorado Island, Panamá. They found that experimental endosperm removal of up to 60% of seed mass did not affect the proportion of seeds germinated, but decreased shoot mass. This supports the portrayal of seed endosperm as a determining factor in seedling growth rather than germination. Janzen (1976) demonstrated that removal of only 1%, 5%, and 10% of *M. andreana* seed weight by drilling holes in the endosperm to mimic insect damage severely reduced seed fitness in the face of artificial granivory. Less seed reserves remained to be utilized to compensate for the herbivore damage.

In the present study I first aimed to confirm the commonness of endosperm damage in the Cloud Forest by examining the frequency of granivory in *Chione sylvicola* (Rubiaceae), a small-seeded member of the coffee family whose seeds are commonly seen along Cloud Forest trails. I next examined the impact of natural and simulated granivory on germination and initial seedling growth for two other species of Cloud Forest trees, *Quercus costarricenses* (Fabaceae) and *Cinnamomum paratriplinerve* (Lauraceae), and along with the commercial avocado, *Persea americana* (Lauraceae). Specifically, I analyze the role of endosperm in seed germination ability and early seedling size. I note the degree of fungal infection in seeds with removed endosperm, and quantify the amount of endosperm missing and development of root and shoot from the two Cloud Forest species. Further, I test for whether root and shoot masses are enhanced by soil nutrients or are accumulated from endosperm stores alone by growing half of the avocado seeds in water rather than soil.

MATERIALS AND METHODS

Field Observations

In mid-November 2005 *C. sylvicola* seeds along a forest trail near the Monteverde Biological Station were examined. The first 100 red *C. sylvicola.* seeds lying exposed on the trail were inspected for granivore damage. These seeds, which were typically around 20 grams in weight, were categorized as damaged if they exhibited exit holes or had portions of the seed removed. While counting the 100 seeds a tally mark was recorded for each counted seed qualifying as damaged in order to determine the percentage of seeds preyed upon.

During the same week 100 *Q. costarricenses* (Fagaceae) seeds (mean weight = 2.26 g, sd = 0.68 g) were collected from the same trail. *Q. costarricenses* drops its seeds, or acorns, synchronously so all the seeds were of relatively the same age (Burger 1975). Almost all seeds were in the early stages of germinating, with a small leafless shoot only a few centimeters in diameter emerging from the center of the seed. To accurately measure seed size only seeds with both halves remaining were gathered. After the seeds were gathered any pieces of shell or debris attached to the seeds were removed and the root and shoots were clipped off. The number of insect exit holes was counted for each seed, and the percent of endosperm removed from the seed was estimated. Each pair of seed halves was weighed, along with their respective roots and shoots. Original seed weights were calculated using the measured seed weights and respective estimates of percent endosperm removed.

Endosperm removal experiments

100 *P. americana* seeds were removed from their fruits. In order for this experiment to yield data, the seeds (at least the ones without endosperm removed) needed to be capable of germinating within the short time span allotted for this study. Out of this concern the seed coat was removed from each seed, as well as a sliver of tissue from both ends, as this has been shown to hasten germination (Eggers 1942). Each seed was weighed after this removal. To determine whether soil nutrients as well as endosperm impacted seedling growth, 50 of the *P. americana* seeds were grown in water and the remaining 50 were designated for soil treatment. Seeds were equally divided between manipulations of 0%, 20%, 40%, 60% and 80% endosperm removal by weight (Table 1). A knife was used to cut away tissue from the seed end distal to the embryo, leaving the actual embryo untouched. The water treatment seeds were each supported within the rim of a water-filled plastic cup by three toothpicks, assuring that at least the embryo-contain bottom two centimeters of the seed was submerged. The soil treatment seeds were planted in large germination trays with .5-1 cm of seed left exposed above the soil, which was a two to one mixture of forest soil and sand. Both treatments were placed indoors along a large westward-facing window. The soil in the germination trays was kept moist and the water cups were kept at a constant level of water.

TABLE 1. *Mean weights of* P. americana *seeds in (a) the water treatment set and (b) the soil treatment set.*

(a) % of endosperm removed	Mean initial weight (g)	Mean after cut weight (g)	(b) % of endosperm removed	Mean initial weight (g)	Mean after cut weight (g)
0	30.895 ± 5.616	30.895 ± 5.616	0	25.012 ± 6.171	25.012 ± 6.171
20	28.650 ± 6.114	23.032 ± 6.270	20	27.538 ± 5.296	21.930 ± 4.056
40	30.075 ± 4.132	18.014 ± 2.433	40	29.902 ± 6.257	17.922 ± 3.776
60	29.826 ± 6.205	12.094 ± 2.583	60	29.331 ± 9.258	11.733 ± 3.642
80	26.009 ± 5.340	5.259 ± 1.053	80	26.449 ± 6.475	5.423 ± 1.351

Fifty intact *C. paratriplinerve* seeds with their seed coats intact were weighed and endosperm was removed from them in the same manner as with the *P. americana* seeds. Ten seeds were left completely intact, and 20%, 40%, 60%, and 80% endosperm removal were each performed on ten seeds (Table 2). All the *C. paratriplinerve* seeds were then planted in germination trays .5 cm deep in two:one forest soil and sand mixture, and placed in the same window as the *P. americana* seeds.

TABLE 2. *Mean weights of* C. paratriplinerve *seeds planted in soil.*

% of endosperm removed	Mean initial seed weight (g)	Mean after cut seed weight (g)
0	0.493 ± 0.077	0.494 ± 0.077
20	0.493 ± 0.060	0.395 ± 0.049
40	0.503 ± 0.057	0.300 ± 0.032
60	0.478 ± 0.077	0.190 ± 0.029
80	0.442 ± 0.067	0.087 ± 0.012

All *P. americana* seeds were removed from their treatments 23 days following planting. The seeds were cut open and the sprouting roots and shoots, none of which had grown longer than a few centimeters, were then removed and weighed separately. Bivariate regression analyses were performed to test for correlations between the percent of endosperm removed and the resulting root and shoot weight. The *C. paratriplinerve* seeds were removed after 22 days in soil and all seeds were examined for shoots or roots. If the seed had germinated the shoot and root were weighed together. Each seed was also inspected for fungal infection.

RESULTS

Field Observations

Endosperm damage is common. Out of the 100 *C. sylvicola* seeds observed, 72 seeds exhibited herbivore damage. This damage was characterized by exit holes or partially missing endosperm. In damaged seeds, the soft tissue on the outside of the seed either had chunks taken out of it, showed small dark round bruises around tiny insect exit holes, was split laterally, or showed any combination of these signs of granivory. Seed damage ranged in severity from only one or two insect exit holes to the removal of over half of the endosperm.

Quercus costarricenses seeds suffer an even higher frequency of damage than *C. sylvicola*. Furthermore, damage in this species is cause by insects both boring holes into the seed endosperm and consuming its contents. Some of the seeds still contained insect larvae within them when examined in the lab. Only six of the 100 *Q. costarricenses* seeds gathered show no signs of endosperm damage. Seeds had an average of 11 ± 13% of their endosperm estimated to be missing (Figure 1a). Ten of the seeds had so little intact tissue remaining that it was impossible to count the number of insect exit holes in the seed. These seeds were excluded from exit hole analyses. With these seeds excluded the number of exit holes in a seed averaged 28 ± 17 (Figure 1b).

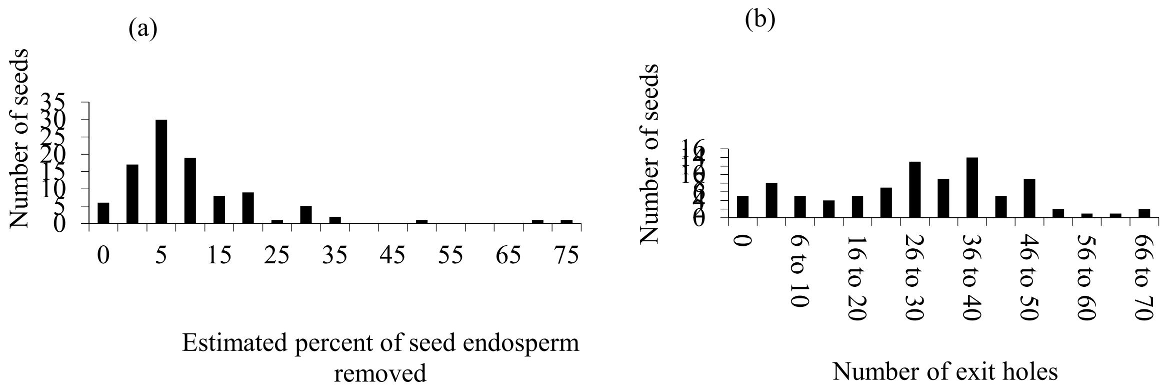

FIGURE 1. Q. costarricenses *seeds with (a) estimated percent of seed endosperm removed (n = 100) and (b) number of exit holes (n = 90).*

Insects do not prey preferentially upon larger *Q. costarricenses* seeds. The number of exit holes in a *Q. costarricenses* seed did not relate to either its measured weight (r^2 = 0.006, P = 0.486, n = 90; Figure 2a) nor its calculated original weight (r^2 = 0.026, P = 0.130, n = 90; Figure 2b). Since insect damage did not vary significantly by seed size, seeds of smaller weight on average suffered a greater proportion of endosperm loss.

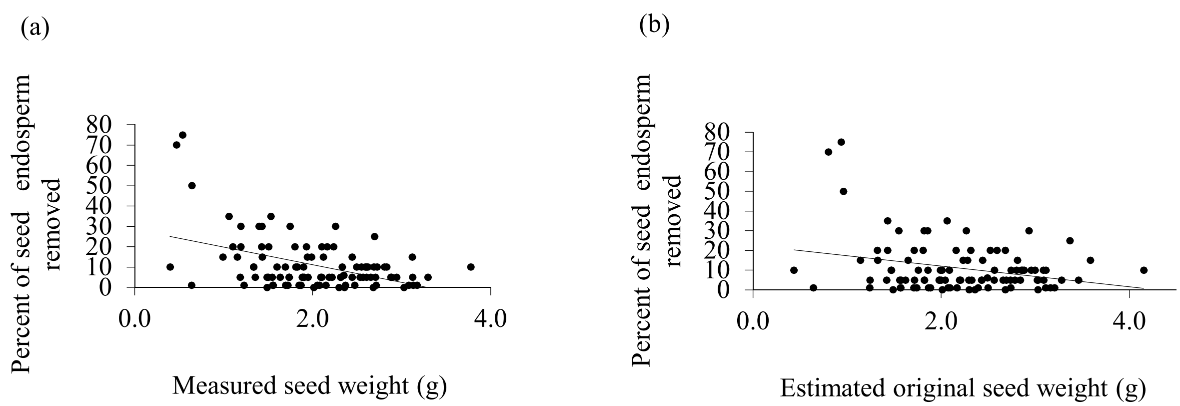

FIGURE 2. *Significant regressions relating the percentage of endosperm removed from* Q. costarricenses *seeds to (a) the measured seed weight (r^2 = 0.21, P < 0.0001, n = 100) and to (b) the calculated estimated original seed weight (r^2 = 0.086, P = .0032, n = 100).*

Insects damaging *Q. costarricenses* endosperm do not regularly damage the seed embryo or seedling. The shoot and root growth of the germinated seeds was not found to be significantly hindered by any amount of endosperm damage. The number of exit holes in a seed neither correlated to its root and shoot weight ($r^2 = 0.0004$, $P = 0.845$, $n = 90$; Figure 3a), nor to its root and shoot weight as a percentage of its calculated original weight ($r^2 = 0.0006$, $P = 0.813$, $n = 90$; Figure 3b).

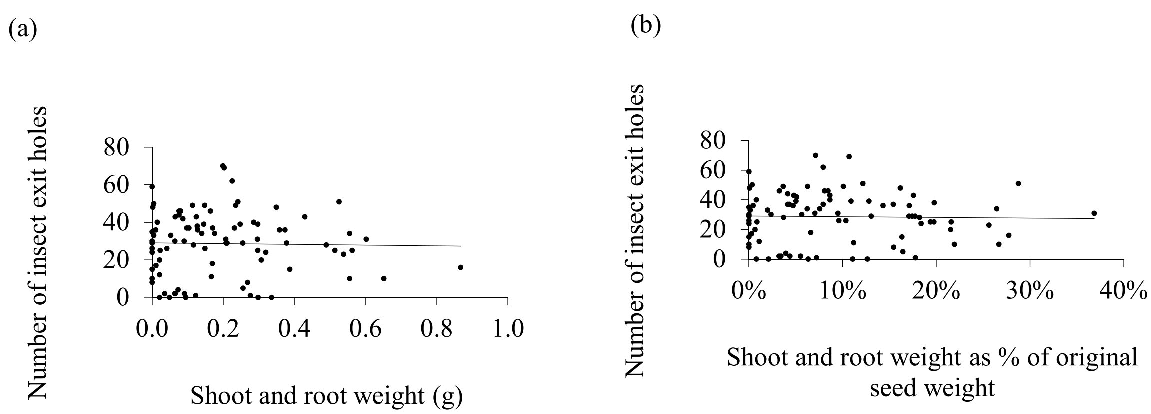

FIGURE 3. *There was no significant correlation between either the number of insect holes removed from a* Q. costarricenses *seed and (a) the weight of its respective root and shoot nor (b) the weight of the root and shoot relative to the original seed weight.*

Endosperm removal experiments

The large avocado seeds did not have inhibited germination even when 80% of their endosperm had been removed. Twenty-three days after planting, 97% of the seeds had germinated. This germination was so subtle, however, that only about a quarter of the shoots had grown taller the top of the seed (mean root and shoot weight of water treatment seeds = 0.170 g, sd = 0.092 g; mean root and shoot weight of soil treatment seeds = 0.142 g, sd = 0.111 g). Most shoots and roots combined were only a centimeter longer than the embryo, and none had begun to visibly deplete the endosperm reserves. Upon the extraction of seedlings from soil treatment seeds it was discovered that the 80% of the endosperm cut from one seed had been inadvertently removed from the wrong end, so that the seed no longer contained an embryo. This seed was excluded from analysis. By examining the contents of each seed when removing seedlings it was confirmed that this error did not occur in any of the other 99 *P. americana* seeds used in the study. Seeds grown in soil differed from those grown in water in that removal of their endosperm increased their root and shoot weights. The water treatment seeds showed no significant correlation between either the percent of endosperm removed and the respective root, shoot, root and shoot combined weight, or root and shoot combined weight as a percentage of the original weight of each seed. The *P. Americana* seeds grown in soil, however, showed a strong positive linear relation was found for each of these relationships except the percent of endosperm removed versus root weight (Fig. 4).

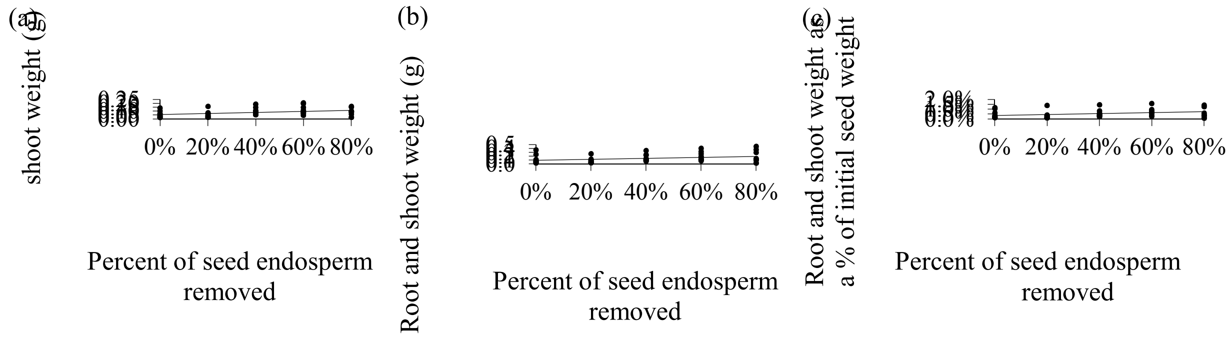

FIGURE 4. *The effect of the percent of endosperm removed on (a) shoot weight (r^2 = 0.131, P = 0.011, n = 49), (b) combined root and shoot weight (r^2 = 0.104, P = 0.024, n = 49), and (c) combined root and shoot weight as a percentage of the initial seed weight (r^2 = 0.084, P = 0.044, n = 49) of* P. americana *seeds grown in soil.*

Only one *C. paratriplinerve* seed had germinated by the end of the growing experiment. This was insufficient data to draw conclusions on the effect of endosperm removal on germination growth. However, a large number of the seeds had developed a fungal infection. The fungus-infested endosperm swelled up above the edges of the seed coat where the seed had been cut, and the endosperm was white and liquefied. All infected seeds were dissected to inspect for germination, and none of their embryos showed any sign of growth; some were so degraded they were barely distinguishable from the rest of the dissolving tissue. Intact seeds showed no infection, but all other categories, even those with as little as 20% removed, had complete or nearly complete infestation (Figure 5). A Chi-squared sample test showed that that the number of seeds infected with the fungus varied significantly between the percent endosperm removed groups ($X^2 = 9.85$, df = 4, $p < 0.05$;).

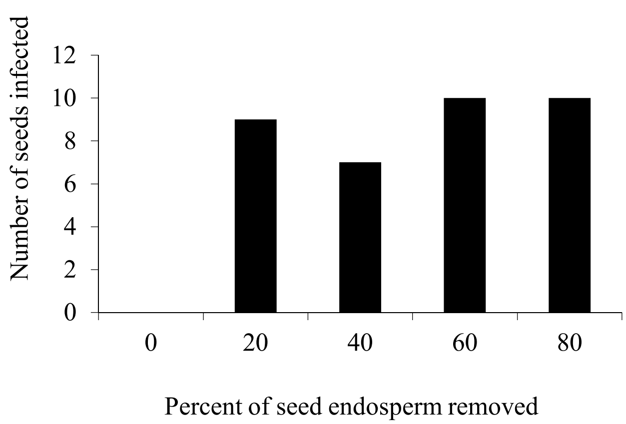

FIGURE 5. Cinnamomum paratriplinerve *seeds with removed endosperm were more susceptible to fungal infection, while those still intact were untouched by the fungus.*

DISCUSSION

Large seeds may be adaptive for many reasons. They are better equipped to respond to abiotic conditions important for tropical trees: shade, seasonal rain, depleted soils (Leishman et al. 2000), as well as the biotic conditions that are stronger in Tropical forests: competition, predation, and parasitism. Further, these conditions are not necessarily mutually exclusive. The current study explores the importance of granivory to germination and subsequent immediate seedling growth.

Seed endosperm damage is common in lowland tropical forests (Calvo-Irabién and Islas-Luna 1999, Clark and Clark 1991, Dalling et al. 1997). Cloud Forest seeds seem to suffer the same high rates of endosperm loss as seeds from lower sites. Of the two seeds studied in the field, both showed high rates of endosperm damage. Predation of *C. sylvicola* seeds was not directly witnessed so its specific granivore is unknown, but insect larvae still remaining in the *Q. costarricenses* allowed the identification of their damager. Larger oak seeds suffered less damage proportionately than smaller oak seeds, as insects seemed to attack both large and small seeds with equal frequency. Larger seeds may also escape fungal infections longer, though here fungal infection resulted just from any endosperm removal. Seed size had no effect on the extent of its insect infestation nor did insect damage reduce root and shoot size. Insect infestation in acorns occurs most frequently on the basal end of the seed, opposite from the embryo, possibly explaining why germination frequencies are not affected by endosperm removal (Steele et al. 1993).

Germination experiments showed that early seedling development is not impacted by endosperm removal, even up to 80% removal. This initially seems to conflict with the *P. copaifera* study by Dalling, Harms, and Aizprúa (1997). However, that study was conducted over a 38 week growing time, after which all the germinated seedlings had a fully developed pair of leaves. Seedlings from avocadoes were smaller than those observed in the oak seeds, and much smaller than those from the *P. copaifera* study, so it is likely due to their small size that only 20% of endosperm remaining was sufficient for their early growth. This successful germination after endosperm damage coincides with the initial results of a study on *Acacia albida* (Mimosaceae) seeds by Hauser (1994). Seeds showing bruchid beetle exit holes initially had a high germination rates. A few months later, though, they all had died. Other studies (Dalling et al. 1997, Ichie et al. 2001) suggest that later seedling development would expose the negative impact of endosperm removal on seedling size, however. It is predicted that though the experimentally endosperm-deficient *P. Americana* seeds had greater initial germination growth, had the experiment been allowed to run longer their roots and shoots would have eventually been surpassed in weight by those of seeds containing more endosperm. This could put plants with more endosperm removed at a competitive disadvantage, as taller seedlings and saplings are more likely to “win” a gap (Brokaw and Busing 2000).

Finally, soil seedlings actually grew larger with more endosperm removed. It is likely that this was due to their earlier germination. Apparently the greater amount of endosperm remaining on the more intact seeds inhibited their germination. The function of endosperm is to fuel growth of a seedling until it is capable of gathering energy from photosynthesis (Ichie et al. 2001); the mechanism of germination may be more significantly fueled by an interaction between the seed embryo and the soil rather than the use of energy reserves in the endosperm. Having less endosperm between the embryo and the soil would have expedited the embryo’s contact with soil compounds and thus may have hastened germination in soil treatment seeds with more endosperm removed. Perhaps avocado seeds grown in water did not show a similar trend in seedling size due to their lack of soil substrate whose proximity could have given seeds with less endosperm around their embryo an advantage. Earlier germination among endosperm-deprived soil treatment seeds may also explain why gap plants have smaller seeds, if fast germination time is more important for them than ultimate seedling size. Large seeds may germinate slower, but their seedlings are larger– which is more important for growth forms such as canopy trees and lianas that have a lot of time on their hands at the seedling level, but once their endosperm is exhausted harvest less energy to draw upon for growth because they receive little light.

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