

Are We Misinterpreting Seed Predation in Palms?

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ABSTRACT

The inadvertent inclusion of weevil-infested seeds when evaluating seed predation by vertebrates, and particularly rodents, may lead to an overestimation of predation rates, thereby confusing the roles of rodents and invertebrates as ecological filters. A study of weevils, rodents and *Syagrus romanzoffiana* palm seeds indicates the usefulness of X-rays to improve evaluation of invertebrate seed predation.

Abstract in Portuguese is available at <http://www.blackwell-synergy.com/loi/btp>.

Key words: Brazil; pre-dispersal seed predation; *Revena rubiginosa*; seed fate; seed removal; *Syagrus romanzoffiana*; weevil; X-rays.

CURRENT METHODS USED TO EVALUATE WEEVIL PALM SEED PREDATION, which are mostly based on the observation of larval perforations (Forget *et al.* 1994, Harms & Dalling 2000, Gálvez & Jansen 2007), may fail in detecting cryptic larvae. In consequence, weevil seed predation may be underestimated, and rodent seed predation overestimated. This problem may result in misleading conclusions about the impact of rodents and invertebrates acting as biological filters on palm demography, a classical issue in the ecology of tropical forests. The use of X-rays may provide a more reliable evaluation of invertebrate seed predation, as already shown for acorns (Steele *et al.* 1996, Dixon *et al.* 1997).

Palms in particular are heavily affected by post-dispersal seed predation and have frequently been used to investigate the consequences of seed predation for plant recruitment (Hoch & Adler 1997, Silva & Tabarelli 2001, Wyatt & Silman 2004, Fleury & Galetti 2006, Salm 2006 and references therein). Given that rodents also manipulate palm endocarps to eat the larvae enclosed within them, ecologists must be careful to accurately evaluate pre- or post-dispersal seed predation by weevils, and other invertebrates, in order to avoid counting a previously insect-predated seed as one eaten by rodents. Indeed, the perception and preference of rodents by infested or sound palm endocarps (Forget *et al.* 1994, Silvius 2002, Gálvez & Jansen 2007), as well as acorns (Steele *et al.* 1996), has been the subject of an ongoing debate in ecological studies. Furthermore, predation rates by invertebrates and vertebrates may also be confused in the case of many tropical plant species with seeds enclosed in stony endocarps, which make it difficult to identify predated seeds with cryptic weevil infestation.

In this study, we evaluated: (1) if the observation of larval perforation as means of seed predation evaluation of palm seeds un-

derestimates the role of weevils as biological filters; (2) if the use of X-rays can provide a more precise evaluation of invertebrate seed predation by identifying cryptic weevils in palm seeds; and (3) if the identification of cryptic weevils in seeds is important to avoid overestimating seed predation by rodents in ecological studies.

METHODS

We selected as our model for this study the single-stemmed palm *Syagrus romanzoffiana* Cham. (Arecaceae), which is an emergent species widely distributed in different vegetation types of South America, where it typically attains 10–20 m in height (Giombini *et al.* 2009). Its seeds are highly susceptible to pre-dispersal seed predation by the curculionid beetle *Revena rubiginosa* Boheman (Coleoptera:Curculionidae), which is probably a specialist seed predator of this palm (Alves-Costa & Knogge 2005), and to post-dispersal seed predation by rodents (Fleury & Galetti 2006). Seed infestation by *R. rubiginosa* begins while fruits are still unripe, and continues until the fruits are fully ripe, when the larvae leave the endocarp to penetrate into the soil where they complete their development into pupae (Freitas *et al.* 1999, Alves-Costa & Knogge 2005). *Syagrus romanzoffiana* seeds are borne within a stony endocarp (11.7 mm diam × 18.5 mm length) which encloses a single seed.

Syagrus romanzoffiana seeds were harvested in natural populations from restinga forest (RF), seasonally dry forest (SDF) and cerrado (C) located in natural reserves in the state of São Paulo, SE Brazil. RF and SDF are contrasting forest types from the Atlantic forest biome, while C is part of Cerrado biome. The study areas are large and well-preserved forest remnants, harboring both small- and medium-sized rodents that predate *S. romanzoffiana* seeds (e.g., *Pecari tajacu*, *Tayassu pecari*, *Sciurus ingrami*, *Coendou prehensilis* and *Agouti paca* – Fleury & Galetti 2006, Galetti *et al.* 2009).

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Syagrus romanzoffiana seed harvesting was carried out at the beginning of the fruiting season. A total of 14 bunches bearing ripe fruits were collected from each tree in each study site, with a minimum distance of 50 m between mother palms. After seed cleaning in a pulp extraction machine, seeds were kept shaded for 7 d to allow further larvae exit from the endocarps. In order to test the first and the second hypotheses, all seeds with *R. rubiginosa* exit holes were manually separated and counted to estimate the number of predated seeds per bunch. Nonperforated endocarps were not necessarily unpredated, because larvae might still be inside the endocarp. Thus, predation of nonperforated seeds was evaluated using a Faxitron X-ray machine – model MX-20 (Faxitron X-Ray Corporation, Lincolnshire, Illinois, U.S.A.). Seeds were exposed for 300 s with radiation intensity of 20 kV. To obtain the X-ray image, an acrylic plate containing 108 compartments (one seed per compartment) was placed over a radiographic film (Kodak MIN-R 2000 (Kodak, Rochester, New York, U.S.A.), 18 × 24 cm) at 40 cm from the radiation source. The radiographic films were processed in automatic Hope X-Ray equipment – model 319 Micro-Max (Hope X-Ray, Warminster, Pennsylvania, U.S.A.). The position of each seed was previously determined in the acrylic plate and consequently in the X-ray image, which allowed us to identify and separate predated seeds in the sample by observing the interior of their endocarps (Fig. 1). All nonperforated seeds present in each bunch were evaluated using X-rays.

In order to test the third hypothesis, discrimination by rodents between sound seeds and seeds with a cryptic *R. rubiginosa* weevil was evaluated. Fourteen infested seeds with *R. rubiginosa* larvae (without exit hole) and 14 sound seeds (one per mother palm) were distributed in individual 20 × 20 cm plots (sound seed plots and infested seed plots), 2 m distant from each other. The seeds were buried in the soil to half of their diameter, leaving the other half exposed. This amount of seeds used in this experiment is similar to the amount found in feces of carnivorous and ungulate mammals that disperse *S. romanzoffiana* seeds naturally (Galetti *et al.* 2001). Ten experimental blocks (one sound seed plot and one infested seed

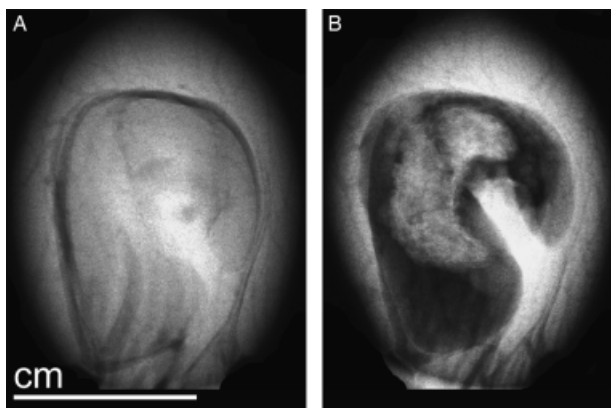


FIGURE 1. Sound *Syagrus romanzoffiana* Cham. (Arecaceae) endocarp (A), in which the cavity is filled by the endosperm, and (B) a predated seed, showing a *Revena rubiginosa* Boheman (Coleoptera:Curculionidae) larva inside the stony endocarp, from which all endosperm has been consumed.

plot per block) were set up along two different trails for each forest type (five blocks per trail). In each trail, the experimental blocks were separated by 100 m. Considering that the main *S. romanzoffiana* seed dispersers are ungulate mammals that feed on fruit pulp and that clean seeds are predominantly predated by rodents (Galetti *et al.* 2001, Fleury & Galetti 2006), endocarps removed from the experimental site were considered to have been predated. Seedlings started to emerge in the plots 5 mo after the experiment was established. At this time all remaining seeds were counted, and endocarps with rodents' tooth marks were considered predated along with those that had been removed.

Rodent discrimination between sound and infested seeds by *R. rubiginosa* was analyzed using a generalized linear mixed models (GLMM) in R, fitting the dependent variable (seed fate) to a binomial, and including treatment as fixed factor and forest type and trail nested into forest type as random factors. In these kinds of analyses, significance is obtained only for the fixed factor. The significance of random factors was determined by comparing models with and without those factors (Pinheiro & Bates 2000).

RESULTS AND DISCUSSION

The evaluation of invertebrate seed predation by simply observing larval perforations with the naked eye leads to underestimation of *S. romanzoffiana* seed predation by weevils. The pattern of underestimation of weevil seed predation was consistently observed. In the three forest types where seeds were harvested in our study, *R. rubiginosa* weevils predated approximately 30 percent more seeds than

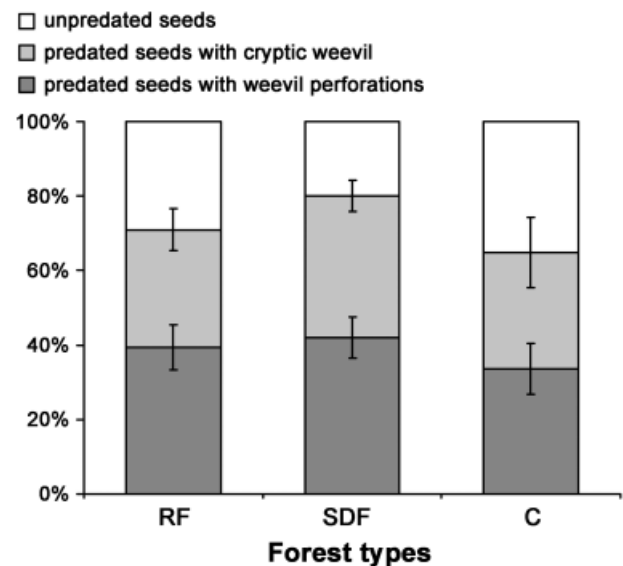


FIGURE 2. Percentages of unpredated seeds, predated seeds by the weevil *Revena rubiginosa* Boheman (Coleoptera:Curculionidae) with larvae perforations, and predated seeds with cryptic weevil for the palm *Syagrus romanzoffiana* Cham. (Arecaceae) in the tropical forest types restinga forest (RF), seasonally dry forest (SDF) and cerradão (C). Bars represent the mean \pm SE of seeds obtained from 14 individuals in each forest type.

indicated by the naked eye evaluation (Fig. 2), as shown by X-ray analysis. Rodents did not discriminate between sound and infested endocarps (GLMM: $\chi^2 < 0.0001$; $P = 0.999$). As a result, if only predated seeds with perforations were removed, and both sound and infested seeds with cryptic weevils were used, seed predation by rodents would have been reduced in RF from 65 percent to 30.5 percent, in SDF from 31 percent to 12.9 percent and in C from 23 percent to 12.2 percent. Because the level of seed predation by weevils was found to be an underestimate in all three forest types, and rodents did not detect the difference between sound and weevil-infested endocarps in any habitat (Fig. 2), there is strong evidence that our findings are noteworthy, and merit experimental corroboration in other tropical biomes.

Consequently, the use of X-rays may improve evaluation of invertebrate seed predation, not only for palms but also for other taxa with stony endocarp. The X-ray method has several advantages over traditional methods. It allows a faster evaluation, because a group of several seeds can be evaluated at the same time and it is not necessary to break open each endocarp to evaluate its interior, as is often done (e.g., Freitas *et al.* 1999). Moreover, it is a nondestructive method, which allows the use of proven predated or unpredated seeds in further experiments, like the one carried out in the present work. Other studies have used immersion in water to evaluate invertebrate palm seed predation, but for *S. romanzoffiana* predated seeds with cryptic weevil this method was not effective.

Other studies have also used X-rays to detect pre-dispersal seed predation by chalcid wasps (Chung & Waller 1986, Fabre *et al.* 2004), and acorn predation by weevils (Steele *et al.* 1996, Dixon *et al.* 1997). This is the first attempt we are aware of, however, to apply this method to separate the ecological roles played by weevils and rodents as palm seed predators. As a result, we suggest that previous studies on palm seed predation may have underestimated palm seed predation by weevils by unwittingly using infested endocarps in field experiments aiming at estimating palm seed predation by rodents. In other words, in some previous studies, the removal or handling of palm seeds enclosing cryptic weevils by rodents may have been counted as seed predation, rather than just 'grubivory' (Silvius 2002).

The nondiscrimination or preference by rodents between sound and infested *S. romanzoffiana* endocarps underlines the importance of accurate evaluation of weevil seed predation. The value of tooth marks for distinguishing among vertebrate seed predators is also limited because such marks are not easy to differentiate among species.

We propose that the use of X-rays allows an accurate evaluation of both pre-dispersal seed predation by weevils and post-dispersal seed predation by rodents, avoiding in many cases misleading conclusions about their importance for plant demography.

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