2023

Disentangling Directed Dispersal: Seed Traits, Shade Tolerance, And Squirrel Caching Decisions

Patrick Lynch
William & Mary - Arts & Sciences, plytheman@gmail.com

Recommended Citation

Lynch, Patrick, "Disentangling Directed Dispersal: Seed Traits, Shade Tolerance, And Squirrel Caching Decisions" (2023). *Dissertations, Theses, and Masters Projects.* William & Mary. Paper 1697552500.
https://dx.doi.org/10.21220/s2-cnsw-qh70

Disentangling Directed Dispersal: Seed Traits, Shade Tolerance, & Squirrel Caching Decisions

Patrick M. Lynch

Lawrence, Massachusetts

Bachelor of Science, University of Massachusetts, 2012

A Thesis presented to the Graduate Faculty of
The College of William & Mary in Candidacy for the Degree of
Master of Science

Department of Biology

College of William & Mary
August, 2023

APPROVAL PAGE

This Thesis is submitted in partial fulfillment of
the requirements for the degree of

Master of Science

Patrick M. Lynch

Approved by the Committee, June 2023

Harmony

Committee Chair
Harmony J. Dalgleish, Associate Professor, Biology
College of William & Mary

Martha A. Case

Martha A. Case, Associate Professor, Biology
College of William & Mary

Matthias Leu, Associate Professor, Biology
College of William & Mary

ABSTRACT

Animal-mediated dispersal of seeds is a crucial step for many trees to relocate propagules to favorable locations for establishment. Scatter-hoarding rodents, such as the Eastern grey squirrel (*Sciurus carolinensis*), choose cache locations in such a way as to balance the value of the seed and effort taken to cache it against the risk of pilferage from conspecifics. Pilferage avoidance strategies observed in squirrels and other rodents have been described by the optimal density model and the habitat structure hypothesis. These mutually non-exclusive models suggest, respectively, that rodents increase the dispersion of seeds proportional to the value of the seeds and utilize the landscape of fear to reduce pilferage. These caching outcomes often increase the likelihood of establishment for the seedling as well. If seeds of higher value, which are placed in more open locations, are also of lower shade tolerance than it would be an example of directed dispersal. We performed two experiments to better understand these dynamics. The first was a field experiment which found support for both the optimal density model and the habitat structure hypothesis in a typical Eastern mixed forest. The second, a greenhouse experiment, established a quantitative measure of shade tolerance and found an inverse relationship between caching value and shade tolerance which suggests directed dispersal in rodent-dispersed hardwoods.

TABLE OF CONTENTS

ACKNOWLEDGMENTS

I would like to thank the numerous people who helped me in preparing this M.S. thesis. Without their hard work, patience, and guidance it would not have been possible.

First, I would like to thank my advisor, Harmony, for her vision and organizational prowess to keep the wheels on this project and guide me in doing good science. I've learned so much from her with regards to planning and executing a project, performing thorough analyses, and communicating in a clear and concise manner.

Next, I would like to thank my committee, Martha and Matthias, for taking the time to offer their opinions, guidance, and support on so many aspects of the project. I'd also like to thank Michael Steele and Ned Fetcher, who freely offered their time and knowledge to collaborate and inform this project in its infancy.

Additionally, I would like to thank all my friends in my cohort as well as the plant ecology lab who have volunteered so much of their time to help in the last few years. From prepping seeds and filling pots with soil to scavenger-hunting tagged seeds and harvesting plants, this thesis would have been impossible without all their help. Even more so, though, without their friendship there's no way I'd have made it these two years!

Lastly, I'd like to thank Kate, my family, and all my friends from back home who have encouraged and supported me throughout. Be home soon!

I would like to dedicate this thesis to my long-gone dog, Bailey, who is probably still the only one who understands me.

LIST OF TABLES

LIST OF FIGURES

Chapter 1

Introduction

Seed dispersal is a critical stage in a plant's life history which allows propagules to escape density dependent mortality as well as colonize suitable sites for establishment such as canopy gaps [21, 74]. In a mature forest with a closed canopy, light is frequently a limiting resource where as little as 1-5% of light will reach the forest floor [45]. Seeds may reach canopy gaps via wind dispersal and air currents (reviewed by Wenny (2001)) or via animals. Globally, up to 80% of temperate species rely on animals to disperse their seeds [44]. The Eastern grey squirrel is one such important disperser of seeds in temperate forests.

Eastern grey squirrels (*Sciurus carolinensis*, henceforth referred to as squirrels) engage in a caching strategy known as scatter-hoarding in which they cache food items individually in a multitude of caches dispersed within their territory [30]. Due to the dispersed nature of these caches it is impossible for squirrels to defend all of their food and, as such, pilferage is frequent [28, 34, 37, 52]. To defend against losses of seeds, scatter-hoarding rodents have adopted strategies described by the optimal density model (ODM) [52, 53] and the habitat structure hypothesis (HSH) [55, 57].

The ODM suggests rodents will invest an amount of time and energy into caching food items which is equal to the value of the food. Because naive pilferers are likely to search for additional food close to where a cache was found, more valuable seeds should be cached further away to minimize the risk of additional losses. The HSH describes a separate insurance strategy utilizing the landscape of fear [27], or gradient of predation risk within an environment. Squirrels cache more valuable seeds in these riskier locations and naive pilferers must trade time spent searching with vigilance for predators. These two strategies are not mutually exclusive and both have support. Rodents are known to cache larger, more valuable seeds further from the point of origin [34, 52, 53] as well as caching them further from canopy edges and in the open [37, 55, 57, 60].

The perceived value of a seed, or caching utility, increases with kernel size but that is only one of many traits which rodents take into consideration. Other traits including perishability, tannin content, or shell thickness (among others) can increase or decrease the utility of a seed [59, 58]. There is growing evidence that squirrels and their caching behavior have exerted pressure on the seed traits of trees which they cache [59]. Further support for coevolution would be evident if the larger seeds, which are more likely to be cached in more open locations, are also of species with lower shade tolerance.

Lichti *et al.* (2020) have found evidence of this coevolution by modeling the relationships between estimated caching utility and shade tolerance for a number of deciduous broad-leaf trees. They found that, as suggested, seeds of increasing caching utility were also of lower shade tolerance. The measure of shade tolerance used in this study, however, was a qualitative scale aggregated from expert opinion in the literature [39]. We wanted to further explore this coevolutionary link but with a more rigorous and quantitative measure of shade tolerance.

Shade tolerance is, by its simplest definition, the minimum light threshold at which a plant is able to survive [62]. Two explanations of shade tolerance have been proposed: the carbon gain hypothesis and the stress tolerance hypothesis (reviewed in Valladares & Niinemets 2008). The carbon gain hypothesis proposes that shade-tolerant plants are able to outcompete light dependent plants in the shade due to greater leaf area and rates of growth. Ensuing research, however, has found that it is actually light-demanding species which exhibit greater leaf area and growth rates in low light [45]. Shade-tolerant plants, on the other hand, favor thicker leaves and stems which are better able to tolerate stresses such as predation or structural damage in the understory [75]. Here, too, seed size can confer advantages.

A general association has been made in both the tropics and temperate forests between shade-tolerant species of more mature forests and larger seed size [4, 11, 19, 46, 73]. Larger seeded trees could gain advantage by producing germinants which are initially of larger size that can better resist stresses, have greater initial energy reserves, or penetrate leaf litter [19, 71, 75]. These observed relationships between seed size and shade tolerance, however, are counter to our proposal and the findings of Lichti *et al.* (2020); possibly because the broader studies to not account for mode of dispersal.

To better understand these dynamics of seed dispersal and shade tolerance we performed two experiments. A field study offering tagged seeds to squirrels in a typical Eastern mixed forest found support for both the optimal density model and the habitat structure hypothesis. In a greenhouse experiment we grew a multitude of species under varying shade treatments. Using growth traits of seedlings we quantified a measure of shade tolerance and supported our proposition that larger seeded species are also of lower shade tolerance.

Chapter 2

Squirrel Caching Decisions

2.1 Background

Animals that cache seeds typically follow one of two strategies: larder-hoarding or scatter-hoarding [70]. The larder-hoarding strategy relies on caching a majority of seeds in a few limited locations which are easier to defend but more susceptible to substantial pilferage if left unguarded [10, 68]. Scatter-hoarding decreases the likelihood of cache pilferage by dispersing seeds over a broad area and thus minimizing consecutive discoveries by naive conspecifics [28, 34, 37, 52]. The decision of where to cache any given seed is predicated by a number of factors including seed value, food availability, conspecifics, the surrounding landscape, and more (Reviewed by Lichti *et al.* (2017)). In general, the density and distribution at which seeds are cached is often determined by a cost-benefit decision made by the cacher, first described in 1978 as the optimal density model (ODM) [52]. Stapanian & Smith (1978) proposed that a lower density of cache placements reduces the likelihood of pilferage but greater dispersal comes at the costs of time and energy to space caches further apart.

Despite the energetic costs of dispersing caches, scatter-hoarding merely slows down pilferage rather than abates it entirely [26] as anywhere from 2-30% of cached seeds are pilfered daily - losses would be catastrophic if not for the fact that the victim of pilferage is concurrently pilfering from others as well [69]. Larder-hoarding red squirrels (*Tamiasciurus hudsonicus*) similarly lost 25% of cones in their midden to pilferage but also stole an equal amount from others [15]. This reciprocal pilferage is not borne of altruism, though. When pilferage rates become too high animals will employ additional strategies to further secure their caches such as Merriam's kangaroo rats which will switch from scatter-hoarding to more easily defensible larder-hoards [47]. Another option is to cache seeds where naive pilfers must trade off searching time and vigilance against predators, effectively limiting the time they can spend searching for food [6, 57]. Proposed as the habitat structure hypothesis (HSH) [55], cache owners may utilize the landscape of fear to reduce pilferage.

The landscape of fear is a general description of how prey animals balance foraging cost against risk of predation within a heterogeneous environment [14, 27]. Foragers spend less time searching in open areas where the threat of predation is high [7, 55, 57, 63]. Similarly, conspecifics pilfer more caches when under the cover of the canopy or near trees [37, 43, 61]. Algerian mice (*Mus spretus*) exploit this gradient of fear: when pilferage rates increase they begin selectively caching in open areas which significantly improves recovery rates by the owner [37]. Grey squirrels have also been shown to maintain a recovery advantage over conspecifics and will protect more valuable seeds by preferentially caching them outside the canopy and further from potential cover [54, 55].

Rodents that cache seeds, whether under the canopy or in the open, rely on visual cues and spatial memory to relocate caches and maintain a recovery advantage [54, 66, 69]. Rodents have a keen sense of smell, however, which is important both for social behaviors as well as foraging [67]. Naive pilferers rely on olefactory cues when searching for caches but these cues are dependent upon soil moisture [56, 65]. Seeds which are in dry soil emit little scent but, when soils are wet, seeds absorb moisture and release oderant molecules [65, 66, 67]. Naive pilferage rates then gain parity with cache owners as their spatial recovery advantage is lost [66, 69].

Previous investigations have begun to show that Eastern gray squirrels *Sciurus carolinensis* and Eurasian red squirrels (*Sciurus vulgaris*) utilize the landscape of fear to preferentially cache seeds of higher value in more open locations [55, 57, 60]. Steele (2014) described this as the habitat structure hypothesis (HSH). These experiments examining cache locations and seed trait preferences, however, have been largely performed in open, park-like settings. Additionally, investigations into the effect of soil moisture on caching and pilferage have largely focused on chipmunks and mice in Western habitats [65, 66] with less work done in Eastern temperate forests.

It was our goal with this research to examine how the environment influences cache placement by offering tagged seeds of low and high utility to squirrels in a forested landscape. We hypothesize that squirrel-mediated cache locations are selected based upon the value of the seed, the risk of pilferage within the environment, and soil moisture. We predict that higher utility seeds will be dispersed A) further distances and to more dispersed caches and B) to more open, riskier locations and drier cache sites.

2.2 Methods

2.2.1 Seed Selection and Preparation

Four species were chosen in 2021 to offer to squirrels, two of high estimated utility (*Juglans nigra* & *Quercus rubra*) and two of low (*Corylus americana* & *Q. alba*). Estimated utilities of species were determined based upon numerous seed traits including, but not limited to: kernel size, shell thickness, and lipid content [59, 58]. In 2022, only *Q. rubra & J. nigra* were offered. *Juglans nigra* and *Quercus spp.* fruits were collected in the areas of Colonial Williamsburg and the campus of William & Mary by trained volunteers between the dates of September 18, 2021 and November 7, 2021. *Corylus americana* seeds were procured from F. W. Shumacher Co. Inc (Sandwhich, MA, USA) in the Fall of 2021. *Juglans nigra* fruits were husked to the endocarp and rinsed and *Quercus spp.* acorns were float tested to provide quality control against weevil damage. All fruits (here-after referred to as seeds) were stored at approx. 35°C in moist peat moss until further processing.

All seeds used in the field study were weighed (Sartorius ED6202S-CW scale) and cataloged. The seed IDs were written on the seeds with the exception of *J. nigra* which were stored in individual bags and labeled. For each species 80 seeds were selected at random and tagged by threading 30 cm of narrow gauge wire through a hole drilled near where the pedicel and/or cap had been. A fluorescent pink tag was then attached to the wire and labeled with the seed's ID. Starting on the third trial (12/3/21) a 30 cm length of flagging tape was added to the wire to aid in recovery.

2.2.2 Site Selection and Offering

Seed fate trials were run at two locations in the William & Mary College Woods, Williamsburg, VA. The two locations Strawberry Light (StrawLight) and Strawberry Dark (StrawDark) were approximately 100m apart from one another, the average territory size of Eastern grey squirrels. The forest composition is a maturing Eastern Coastal Plain Forest with mixed hardwoods and pine with an understory that is heavily affected by deer browse.

Four trials were run in 2021 on November 12 and 19, and December 03 and 10. Trials in 2022 were performed on November 6, 14, and 17, and December 1. In 2021 species selection for each site was randomly selected such that each species was presented at each site only once with the exception of Dec. 10, 2021, where *Q. rubra* was offered a second time at StrawDark in lieu of *C americana* which had not been cached at all in previous trials. Twenty seeds were selected at random with the caveat that all weighed within 20% of the population mean for each respective species. This was done to assure an even distribution of seed sizes across the length of experiment. Each seed station was protected from deer browse by a 1x1 m square of hardware cloth on PVC posts $1/2$ m above the ground. Game cameras (Reconyx Hyperfire HC600) were deployed at randomly chosen offering sites to confirm post-hoc that only squirrels were browsing the seeds. For the 2022 field trials, 25 seeds were offered and each species was presented twice in an alternating pattern at both stations. Because the 2022 trials were also part of another project to assess the influence of tannin content on squirrel decision making, the standard deviation in the mass of seeds offered were minimized for each trial.

Shelled peanuts were presented one to two days prior to the actual seeds to pre-bait squirrels. Peanuts were offered alongside tagged seeds as well to satiate squirrels and encourage caching. After 48 hours of seed presentation technicians searched for seeds within a 30m radius of the station for a combined 60 person-minutes. Seed fates were recorded for those recovered (cached, consumed, not cached, consumed at exclosure, or lost) and seeds which were cached or consumed away from the exclosure were flagged for later micro-habitat characterization.

2.2.3 Environmental Measures

Cache site microhabitats were measured for distance from origin, distance to nearest cover (such as a tree or log), distance to nearest cache, and canopy openess. All distances were calculated by mapping locations with a Hageloff Postex laser system and calculating nearest neighbors in R (Nate Lichti, 2015). Canopy gaps were estimated as total light that was measured via orthophotography and Gap Light Analyzer [1]. Using a tripod to center the camera, Google Streetview (ver.2.17.3) was used to create a hemispherical photograph. Each photo was then cropped and polarized using ImageMagick (ver. 7.1.0) and binarized in ImageJ (ver. 1.53q) with the Hemispherical 2.0 macro [5]. Lastly, canopy openness was measured using Gap Light Analyzer (ver. 2.0). Additionally, in 2022, soil moisture was recorded (Field Scout TDR100) at all new cache sites and each random point for each offering.

The same response measures as at squirrel-chosen cache locations were recorded at a bumber of randomly selected points at each site. In 2021 up to five points per plot were randomly established in a 360°radius and within 30m of the offering location. In 2022 the count of random points was increased to 20 per site.

2.2.4 Analysis

Only seeds which were found within 48 hours of presentation were used in this analysis. To analyze species level differences in response variables either ANOVA & Tukey tests or Mann-Whitney & Dunn tests were performed dependent upon the normality of the residuals. To assess intraspecific effects of seed size linear regressions were performed. Seed masses were log transformed to standardize against the discrepancy in mass between *Quercus spp.* seeds and *J. nigra.* The response of distance cached was also log transformed to correct for non-normal residuals in the model.

Because canopy data from 2021 was inconsistent and taken later in the winter only canopy data from 2022 was used in this analysis. Photos were taken over multiple dates from November 11 to December 13. The site at which the photos were taken (StrawLight or StrawDark) was a significant effect so each site was analyzed independently. Soil moisture results were standardized using z-scores within each date to account for varying ambient moisture levels between dates.

Species	**Cached**, N=172[1]	**Consumed**, N=160[1]	**Ignored**, N=113[1]
2021	***N=52***	***N=147***	***N=56***
C_amer	0 (0%)	48 (80%)	12 (20%)
J_nigra	16 (28%)	5 (8.6%)	37 (64%)
Q_alba	16 (26%)	42 (69%)	3 (4.9%)
Q_rubra	11 (16%)	52 (78%)	4 (6.0%)
Random	9 (100%)	0 (0%)	0 (0%)
2022	***N=120***	***N=13***	***N=57***
J_nigra	28 (34%)	2 (2.4%)	52 (63%)
Q_rubra	52 (76%)	11 (16%)	5 (7.4%)
Random	40 (100%)	0 (0%)	0 (0%)

[1] n (%)

Table 2.1: Seed fates at both Strawberry Dark and Strawberry Light for 2021 and 2022. 'Consumed' included seeds consumed at offering location and which were carried away.

2.3 Results

In total, 42 seeds across three of the four species offered in 2021 were cached, the majority of which were located at Strawberry Dark and Strawberry Light. In 2022 there were 75 seeds cached of the two species offered (Table: 2.1).

2.3.1 Distance from origin

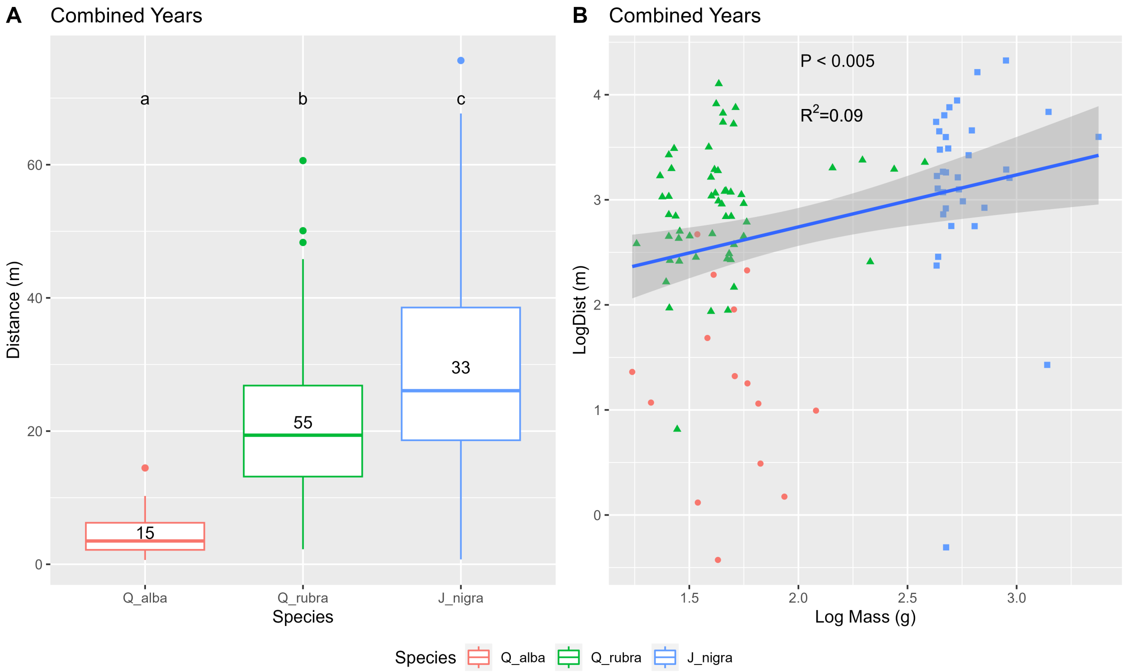

Figure 2.1: A Kruskal-Wallis test ($P<0.005$) (A) of combined 2021 and 2022 data show squirrels cached *J nigra* furthest followed by *Q rubra*, and *Q alba* the shortest distances. A linear regression (B) of log transformed seed mass (g) and distance (m) shows a positive relationship ($\beta=0.5$, $P<0.005$) but per-species regressions revealed no significant relationships suggesting more interspecific differences.

Seeds were cached at shorter or further distances from the origin dependent upon species. *Juglans nigra* was cached the furthest from the origin followed by *Q. rubra* then *Q. alba* (Kruskal-Wallis $P<0.005$; Fig: 2.1A). The same data show that an increase in mass correlated to greater distance to cache ($\beta=0.5$, $P<0.005$; Fig: 2.1B). This relationship didn't hold up on a per-species basis, however.

2.3.2 Cache density

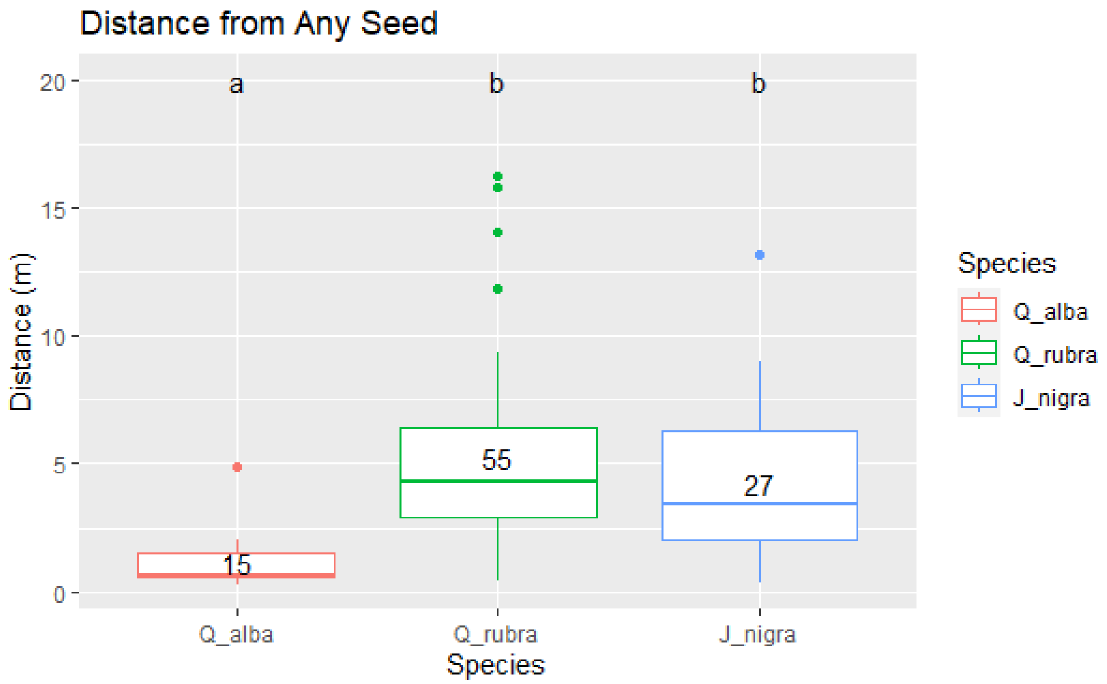

Figure 2.2: Distance in meters to the nearest seed of any type for both years combined (Kruskal-Wallis P<0.005).

Squirrels cached seeds at differing densities dependent upon species with both years combined (Kruskal-Wallis P<0.005; Fig: 2.2). *Quercus alba*, the lowest utility seed, had the most concentrated caching density while both of the high value seeds, *Q. rubra & J. nigra* were more dispersed.

2.3.3 Distance from cover

There were no significant differences in distance from the nearest tree (>4cm DBH) among any of the species or random points when both years were combined (Kruskal-Wallis P=0.29; Fig: 2.3).

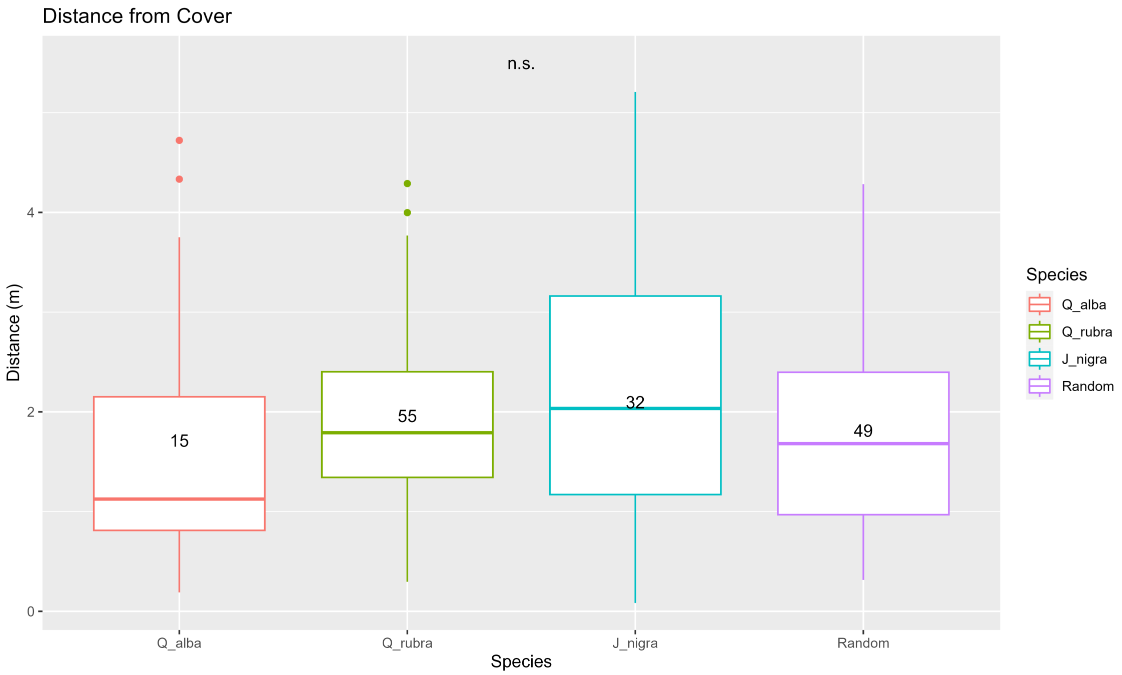

Figure 2.3: Distances to cover (nearest tree >4cm DBH) were not significantly different among any species or random points with both years combined (Kruskal-Wallis P=0.29).

2.3.4 Canopy Cover

The total light of cache sites at Strawberry Light showed significant variation between species (ANOVA P=0.012; Fig: 2.4A). *Quercus rubra* seeds were cached in more open locations than *J. nigra* (Tukey test P=0.045) as well as Random points (Tukey test P=0.021). Total light of *J. nigra* was not different from random points. There was no difference at Strawberry Dark between *Q, rubra* and Random caches (ANOVA P=0.941; Fig: 2.4B). Analyses of each species within each site showed no relationship between seed mass and total light.

2.3.5 Soil Moisture

Soil moisture data of cached seeds and random points show some preference for caching in drier microhabitats (Fig: 2.5). Of the four dates analyzed only one was significantly different than random (*Q. rubra* on 11/16/22; ANOVA P=0.008) but *J. nigra*, on 11/08/22

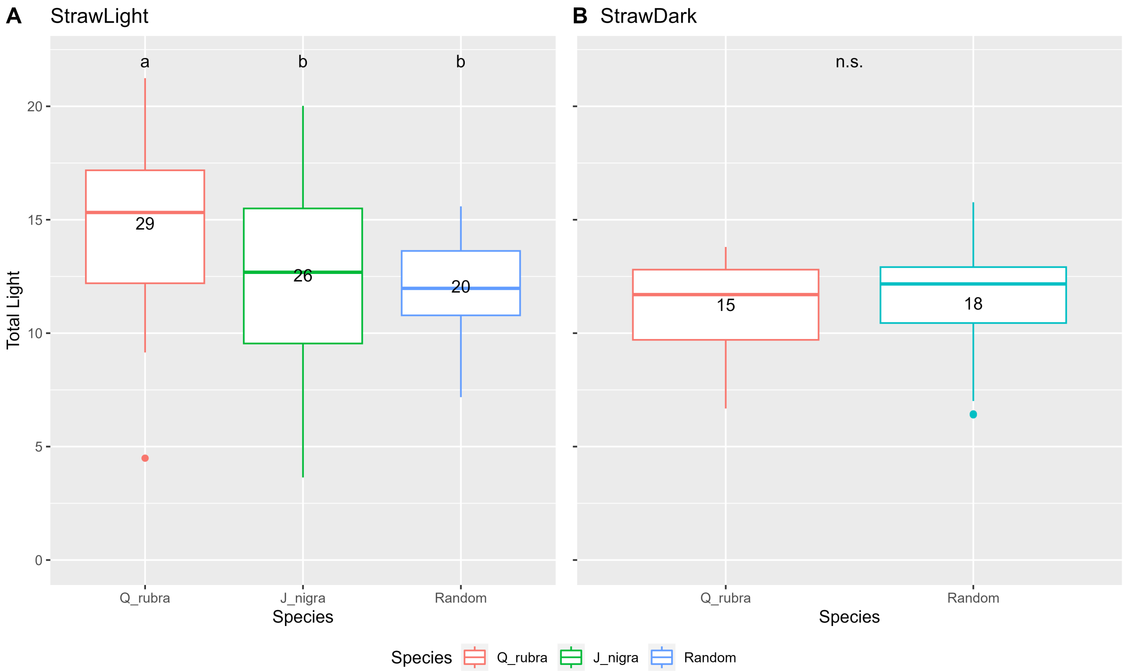

Figure 2.4: Because site showed a significant effect on the light data each location was analyzed separately. At Strawberry Light (A) *Q. rubra* was significantly more open than random (P=0.024) and marginally greater than *J. nigra* as well (P=0.067). There was no significant difference at Strawberry Dark (B). Data from 2022 season only.

was marginally significant as well (ANOVA P=0.065). Both of these trials were at the Strawberry Light site.

2.4 Discussion

We found support for part of the prediction that higher value seeds are cached further away from the point of origin and are more dispersed. Although we saw no response in the distance of caches from cover, we did find that squirrels utilize canopy gaps, as measured by available light, as well as drier microhabitats for cache locations.

Evidence of rodents, and particularly squirrels, caching larger and more valuable seeds at greater distances is well established [18, 23, 42, 55, 59]. Our study supports this as well such that an increase in seed value (*Q. alba* > *Q. rubra* > *J. nigra*) resulted in further caches. It is likely that cache decisions were made in response to seed traits beyond size

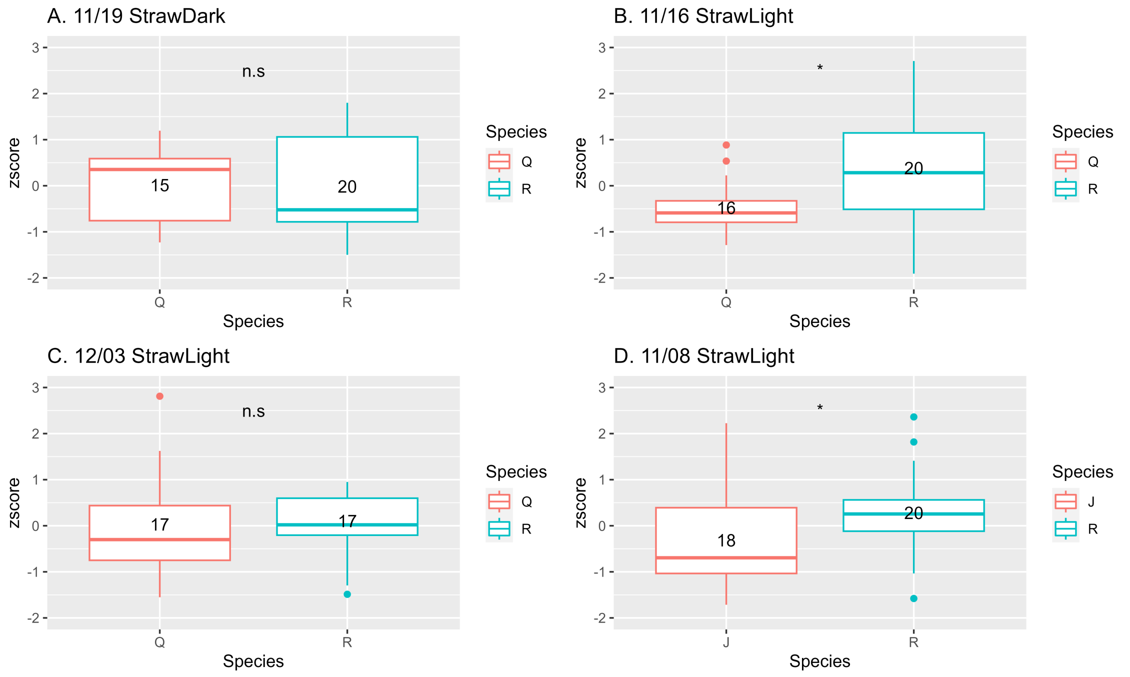

Figure 2.5: Soil moisture of cache locations were significantly lower than random points at one trial: *Q. rubra*, 11/16/22 (B. ANOVA P=0.008) and marginally significant at a second: *J. nigra*, 11/08/22 (D. ANOVA P=0.065). Both trials were at the Strawberry Light site. Moisture data were standardized using z-scores for each day to control for varying ambient moisture.

alone. An increase in the mass of seeds did result in further caches across species but this relationship was not found intra-specifically. Further, *Q. alba* and *Q. rubra* largely overlap in seed mass yet are separated in distance cached suggesting traits beyond seed mass influencing caching outcomes. In this particular example a determining trait between the two species is likely perishability - *Q. alba* germinates in the fall which rapidly decreases the nutritional value for the squirrel, where-as *Q. rubra* can remain in the cache longer without germinating [12].

In addition to increasing the distance carried, the density of caches should also decrease with increasing seed value as described in the optimal density model [52]. This behavior has been observed in fox squirrels (*Sciurus niger*) [52, 53], agoutis (*Dasyprocta spp.*) and acouchies (*Masyprocta ssp.*) [13, 20, 23], and chipmunks (*Tamias amoenus*) [64]. Our results show a similar strategy is employed by Eastern grey squirrels as well: the more valuable *Q. rubra* and *J. nigra* were cached further apart than the low value *Q. alba.* This suggests that squirrels are willing to invest more energy in caching more nutritious seeds further apart but it is worth noting that *Q. rubra & J. nigra* largely overlap, with *Q. rubra* only being slightly more dispersed. If seed size was the only driver of value then we would expect *J. nigra* (avg. mass 16.2 g, s.d.=3.24) to be further dispersed than *Q. rubra* (5.34 g, s.d=1.82).

Jansen *et al.* (2002) raised two caveats in relation to seed size and cache density. The first is that, within the scope of the optimal density model, there may be an upper limit of seed size past which the metabolic costs of carrying a heavy seed a longer distance exceeds the benefits of caching it. This could be a contributing cause to the overlap in dispersion between *J. nigra* and *Q. rubra* in our study. The second caveat is that they found no additive effect of seed mass directly on cache density. Rather, the two-dimensional dispersion was really a result of the one-dimensional distance cached.

Measures of the landscape of fear in our study had mixed results as no effect was found for the distance of caches from cover. One possible cause for this lack of effect is that the distribution of trees in the forest is fairly uniform leaving no appreciable distance to be exploited by squirrels. This is supported by the broad overlap of distances between all cached seeds and the randomly selected points. A second explanation is that the overstory cover is closed enough that the threat of predation from raptors is negligible regardless of distance to a near-by tree.

We found canopy cover to be fairly homogeneous as there were no differences at Strawberry Dark between *Q. rubra* caches and random points nor between *J. nigra* and random at Strawberry Light. Additionally, the random points at both sites were comparable. Despite that homogeneity, squirrels did exploit some gaps in the canopy as they cached *Q. rubra* in more open locations at Strawberry Light and cached *J. nigra* in a greater range of cover than the random points. Anecdotally, there are more downed trees and variation in canopy structure at Strawberry Light than Dark suggesting that, when available, squirrels will utilize this gradient of canopy cover. Our results show the effect of landscape of fear within a forested environment and align with prior studies in more open habitats [37, 55, 60].

Beyond the landscape of fear, an additional cache protection strategy could utilize soil moisture. In two out of four trials squirrels chose cache sites which were drier than random. Soil moisture increases the release of oderants from seeds [65] which naive pilferers then exploit to discover caches [56, 65, 66, 69]. Our findings suggest squirrels choose drier sites to minimize the likelihood of pilferage. The two trials in which squirrels did not choose drier locations also had drier random points than the two where a difference was seen. This suggests that for the two non-significant trials squirrels likely only had dry sites to select from.

Seed traits such as size, perishability, tannin content, and more are important contributors to seed value [30, 59, 58], but they may not be the only factors which determined the outcomes we observed. As an economic model seeds are stored in locations which balance the energy costs of caching with the nutritional value of the seed. While the aforementioned seed traits are fairly static, the benefit is also relative to the dynamics of the habitat the squirrel is in. For example: the presence of conspecific 'spies' can alter behavior towards caching further or making false caches and raise the metabolic costs [30, 56]. Seasonality and food availability can affect the perceived value of a seed as well [30, 53]. Anecdotally, 2022 was a mast year with high acorn availability and we observed greater rates of caching than 2021 when food was more scarce. Measuring seed fall, conspecific populations, and even predator population will likely offer more explanatory power in temporal and geographic differences.

2.5 Conclusion

Caching of food is a behavior that is critical for meeting the metabolic needs of squirrels at future dates (reviewed by Lichti *et al.* (2017)). Faced with conspecific pressure of pilferage, rodents have adopted myriad strategies to protect their investments as described by the optimal density model (ODM) [52, 53] and the habitat structure hypothesis (HSH) [55]. Our research found support for both with Eastern grey squirrels in a typical Eastern broadleaf forest. Supporting the ODM we observed squirrels preferentially cached higher value seeds at greater distances and dispersion. Squirrels also utilized the landscape around them by caching seeds in more open areas and in drier soil when available. To build upon this work future studies should investigate traits beyond seed size, monitor more environmental factors and offer seeds in more heterogeneous environments.

We observed that size likely was not the only trait driving caching outcomes. Measuring additional traits such as tannin or lipid content (see Sundaram *et al.* 2018) in relation to cache location could build upon the results we've found. Further measures of environmental cues such as masting or predator populations could also offer finer resolution to caching behavior. Lastly, previous studies on the landscape of fear have generally been in park-like settings with stark boundaries at the canopy edge [55, 60]. Our study was more representative of forests but may have had too homogeneous a canopy to see strong effects of habitat structure on caching decisions. A more heterogeneous forest with larger canopy gaps likely would offer a better compromise between the two extremes of park and forest.

Chapter 3

Shade Tolerance

3.1 Background

Trees can broadly be categorized by their ability to survive in low light environments along a gradient from shade-tolerant to light-demanding [62]. Previous explanations of a shade tolerance mechanism, known as the carbon gain hypothesis, proposed that shade tolerant plants are more efficient at light capture and growth in the shade [17]. This hypothesis states that shade tolerant plants should be larger, grow faster, and have greater leaf area or photosynthetic capacity to capitalize on limited light in a mature forest. Support for this hypothesis is lacking, though. Light demanding plants, rather than shade tolerators, have been shown to have greater relative growth rate (RGR, rate in increase in biomass/total biomass of plant) and greater leaf area ratio (LAR, surface area of leaves/total biomass of plant) in both high and low light environments [16, 24, 72]. Shade tolerant plants, on the other hand, frequently exhibit higher leaf mass area (LMA, leaf mass/leaf area) which indicates greater leaf thickness but also reduced photosynthetic capability [24, 72, 73].

In contrast to the carbon gain hypothesis the stress-tolerance hypothesis states that shade tolerant plants prioritize thicker leaves, stems, and a strategy of allocating carbon toward defense and storage rather than immediate growth [16, 24]. These plants are better able to resist and recover from predation and mechanical damage in the understory

[8, 24, 38]. For shade tolerant species, thicker leaves provide greater defense against predation, thicker stems can protect from mechanical damage, and allocation of carbon towards carbohydrate reserves allow for recovery from predation and damage [25, 38].

The shade tolerance of a species is often reflected in its seed size and dispersal habit. While there are exceptions, light demanding species are typified by a higher quantity of smaller, wind dispersed seeds which have longer residence in the seed bank [4, 74]. Shade tolerators, though, often invest more resources into fewer, larger fruits and seeds and rely on animals to disperse seeds away from the parent tree [19, 32]. Rodents, such as the Eastern grey squirrel (*Sciurus carolinensis*), are essential in this dispersal process [31].

Grey squirrels engage in a caching behavior known as scatter hoarding where they cache singular seeds in many dispersed locations across the environment [31]. Because squirrels cannot defend all of their caches at all times it is likely that they utilize the landscape of fear - the variation of predation risk in a heterogeneous environment [27, 63] - to insure more valuable seeds against pilferage from naive conspecifics. Higher value seeds have been shown to be carried further and cached in more open, riskier areas [55, 57, 60]. The value, or caching utility, of these seeds are built upon an aggregate of seed traits such as kernel size, shell thickness, lipid content, and more [59, 58].

There is increasing evidence of coevolution between these seed traits and the caching behavior of squirrels [59, 29]. To support of this, it would be expected that larger seeds which are cached in more open areas would also be of species with lower shade tolerances. This, however, is counter to the prevailing literature which suggests that larger seeds are more shade tolerant than smaller. In both tropical and temperate forests large seeded angiosperms outperform smaller seeded trees in the understory and in small gaps [46, 11, 19, 35]. Large seeded species produce seedlings which are initially larger, able to reserve more nutrients to survive in shade, and are also better able to penetrate deep leaf litter [49, 50, 36]. These studies, however, frequently include a wide range of trees of various successional status and seed dispersion methods.

Within the scope of rodent-dispersed tree species we hypothesize that squirrels select for higher utility seeds which has driven the evolution of seed and seedling traits. Support for this mechanic has already been modeled [29] but the measure of shade tolerance used was compiled by qualitative expert opinion from the literature. Using a greenhouse experiment we intend to build upon Lichti *et al.* (2020) and A) quantify a scale of shade tolerance from seedling growth traits and B) test if species with larger, higher utility seeds are of lower shade tolerance.

3.2 Methods

3.2.1 Seed Preparation and Planting

Twenty-two species of hard seeded trees were initially chosen for this study with an average of one hundred and twenty seeds prepared for each. Of these, nine species had sufficient germinants to use in our experiment (Table3.1). The 5 point scale of expert shade tolerance used was compiled from literature by Niinemets & Valladares (2006) and is based on Baker (1949) such that 1 is the lowest tolerance and 5 the greatest. Caching utility was estimated by Lichti, *et al.* (2020).

Seeds were stratified as per the instructions provided by Sheffield's Seed Co. All seeds were soaked in water for 24-96 hours dependent upon species. Seeds were subsequently weighed then packed with damp peatmoss in plastic bags and returned to refrigeration for the recommended amount of days (min: 30, max: 120). Seeds were planted between the dates of May 24-28, 2022, in Jolly Gardener Pro-Line C25 soil (Oldcastle APG, Atlanta, GA, USA) in either 7.62x25.4 cm or 6.35x25.4 cm tree pots manufactured by Stuewe & Sons (Tangent, OR, USA). Seeds were allowed to germinate in approx. 15% of full sun in the William & Mary greenhouse, watered daily, and monitored for emergence. Seedlings were entered into shade treatments on a per species basis when three conditions were satisfied: 1) the daily tally of newly emerged seedling began declining considerably; 2) there were, at minimum, enough seedlings to harvest 5 plants per treatment for two harvests (a minimum of 30); and 3) the majority of seedlings had at least one completely opened leaf. The average

age of seedlings when entered into treatments was 17.6 days.

3.2.2 Greenhouse Design

Three shade treatments were used in this experiment: full sun, part shade (25%), and full shade (2%). Shade boxes were constructed of layered screening on PVC frames and light transmission was measured with a LiCor LI250A light meter and LI791 sensor. Seed mass was considered during assignment such that small, medium, and large seeds for each species were equally distributed to the three treatments and seedlings were watered daily.

The experiment followed a block within a block design such that three of the six available benches in the greenhouse were used with each of the three treatments on a bench. Up to 16 plants were assigned to five trays randomly placed within six potential locations within each treatment. Every two weeks new table/treatment locations were randomly assigned within the greenhouse and trays were randomly relocated to the appropriate new positions. Individual plant positions within each tray were randomly shuffled as well. Greenhouse relocations were only performed after dark to prevent shade-treatment plants from being exposed to sunlight.

Abbr.	Species	Common Name	Scar. (h)	Strat. (d)	Utility	Shade Tolerance
CACO	*Carya cordiformis*	Bitternut hickory	96	90	2.51	2.07
CAGL	*Carya glabra*	Pignut hickory	96	120	2.93	2.69
CAIL	*Carya illinioensis*	Hardy pecan	96	90	UNK	UNK
CAOV	*Carya ovata*	Shagbark hickory	96	120	2.54	3.4
CATO	***Carya tomentosa***	Mockernut hickory	96	150	3.11	2.2
CADE	*Castanea dentata*	American chestnut	24	60	1.74	3.06
CAMO	***Castanea mollissima***	Chinese chestnut	24	60	2.89	1.67
COAM	***Corylis americana***	American hazlenut	24	180	1.58	3.50
FAGR	*Fagus grandifolia*	American Beech	24	90	1.35	4.75
JUCI	***Juglans cinerea***	Butternut walnut	14	120	3.73	1.88
JUNI	***Juglans nigra***	Black walnut	24	120	4.18	1.93
JURE	***Juglans regia***	English walnut	24	120	3.24	2.27
QUAL	*Quercus alba*	White oak	24	60	1.89	2.85
QUBI	***Quercus bicolor***	Swampwhite oak	24	60	2.38	2.98
QUCO	*Quercus coccinea*	Scarlet oak	24	60	2.11	2.07
QUFA	***Quercus falcata***	Southern red oak	24	90	UNK	UNK
QUMA	*Quercus macrocarpa*	Bur oak	24	60	2.05	2.71
QUMI	*Quercus michauxii*	Swampchestnut oak	24	60	3.00	UNK
QUMU	*Quercus muehlenbergii*	Chinkapin oak	24	60	2.00	2.22
QUPA	*Quercus palustris*	Pin oak	24	60	1.95	2.49
QURU	*Quercus rubra*	Nothern red oak	24	60	4.65	2.75
QUVE	***Quercus velutina***	Black oak	24	60	1.93	2.72

Table 3.1: Twenty-two species were planted for use in this study, of those there were enough germinants to include from nine species (bold). Hours of scarification (Scar.) in water and days of stratification (Strat.) in peat moss were obtained from Sheffield's Seed Co. Caching utility was estimated by Sundaram, *et al.* (2015,2018) and shade tolerances (1-5; lowest to highest tolerance) were obtained from Niinemets & Valladares (2006).

3.2.3 Harvests

At a minimum each species was harvested twice: the first just before seedlings entered treatments and once at 14 weeks of growth - the length of the experiment. If there were sufficient seedlings to allow more harvests, however, a harvest was performed at 3 weeks and 7 weeks as well. At each harvest 3 to 7 plants were randomly selected from each treatment and destructively harvested. When possible an even proportion of small, medium, and large seeded germinants from each treatment were selected to control for variation in seed size. Leaves (without the petioles) were removed and placed on a light table, pressed between glass panes, and photographed for leaf area measurement (Cannon 600d DS126311). The stem and roots were then removed from the pot and soil was removed from the roots by hand and using running water, taking care to preserve fine root material. The petioles were separated from the stem, the stem and roots were separated at the root collar, and all plant material was placed into paper bags and subsequently into drying ovens. Harvested plants were dried for a minimum of 48 hours at 60°C and then each portion of the plant, leaves, stem, petioles, and roots, were weighed on a Sartorius ED1245 scale. Leaf area was measured using the ImageJ software. Using these weights and leaf areas we calculated the following morphological traits: leaf mass fraction (LMF), stem mass fraction (SMF), root mass fraction (RMF), leaf area ratio (LAR), and leaf mass area (LMA) (Table: 3.2).

Trait	Name	Units	Equation
LMF	Leaf Mass Fraction	g g^{-1}	L_W/M_D
SMF	Stem Mass Fraction	g g^{-1}	S_W/M_D
RMF	Root Mass Fraction	g g^{-1}	R_W/M_D
LMA	Leaf Mass Area	g m^{-2}	L_W/L_A
LAR	Leaf Area Ratio	m^2 g^{-1}	L_A/M_D

Table 3.2: Seedling traits measured in this study. Variables used are as follows: M_D dry mass of entire plant (g), L_W dry mass of leaves (g) , S_W dry mass of stems (g), R_W dry mass of roots (g), L_A surface area of leaves (m^2)

3.2.4 Analysis

Statistical analysis was performed on plants from the final harvest (14 weeks) as it offered the largest sample sizes across species and treatments. A minimum of 4 plants per species/treatment was required resulting in: nine species in full shade, eight in full sun, and three in the full shade with an average sample size of 9, 9, and 7 plants in each treatment, respectively (Table 3.4). To quantify shade tolerance we performed a principal component analysis (prcomp function, R package stats v. 4.22) of the response traits followed by Spearman rank correlations (corr.test function, R package stats v. 4.2.2) of the first two PC axes and expert opinion shade tolerance. Lastly, we regressed the shade tolerance axis with both estimated caching utility and seed mass (lm function, R package stats v. 4.2.2).

Harvest n(wks):	1 (0)	2 (3)			3 (7)			4 (14)		
Treatment:	PreTreat	Sun	PartShade	FullShade	Sun	PartShade	FullShade	Sun	PartShade	FullShade
CAMO	6	5	6	4	6	4	-	7	10	-
CATO	11	6	5	6	6	6	5	8	7	7
COAM	9	6	6	4	6	5	5	5	6	-
JUCI	8	5	6	-	6	6	-	10	12	-
JUNI	10	-	-	-	6	4	-	-	6	-
JURE	6	-	-	-	-	-	-	9	8	-
QUBI	5	-	-	-	-	-	-	10	10	6
QUFA	5	-	-	-	-	-	-	7	8	-
QUVE	6	9	9	6	6	5	4	12	14	7

Table 3.4: Harvested plants at each harvest number (weeks) from each treatment. Required sample size for a harvest to be included in the analysis was n≥4. Only harvest 4 was used in this analysis as it offered the greatest sample size of species across the three treatments.

3.3 Results

A PCA of all light treatments combined and limited to the species in all three treatments (CATO, QUBI, & QUVE) explained 95.8% of variance in the first two principal components (Fig: 3.1A). The first PC axis accounted for 73.7%, of the variance and is associated with biomass allocation: root mass fraction (RMF, 0.47) was positively correlated while both stem mass fraction (SMF, -0.51) and leaf mass fraction (LMF, -0.51) were negatively correlated. Plants in the full sun treatment were associated with high RMF while SMF and LMF were associated with plants in the part shade and full shade treatments. The second component explained 22.1% of the variance and was associated with the leaf production traits leaf mass area (LMA, 0.93) and leaf area ratio (LAR, -0.26). In PC2 there is segregation of sun treatments towards higher LMA and part shade and full shade with higher LAR. We found similar results when we used data from only the part shade and full sun treatments which increased the sample size to the eight species present in both treatments (Fig: 3.1B). This second PCA explained 85.8% of variation across the first two components. The first axis was again related to carbon allocation (RMF, 0.31; SMF, -0.59; LMF, -0.58) and the second with leaf production (LMA, 0.79; LAR, -0.47).

A PCA of each individual treatment at harvest 4 revealed similar patterns (Fig: 3.2). In all three treatments PC1 (49%-75.2% variance explained) was driven by the above and below-ground biomass split of RMF and LMF/SMF. The second axis of all three PCAs explained 22.3%-31.3% of variation and showed strong loadings of LMA from 0.74 to 0.89 and LAR from -0.29 to -.63. To examine the relationship between shade tolerance and morphological traits, we used the 20% shade treatment as it had the greatest sample size of species (n=9) and explained the most variation on the second component (31.3%).

Neither rank correlations of the biomass allocation axis (PC1) (Fig: 3.3A) nor the leaf production axis (PC2) (Fig: 3.3B) were significantly related to expert opinion shade tolerance (P=0.121, R^2=-0.57; P=0.581, R^2=0.22, respectively). The rank correlation with the leaf production axis, however, showed COAM to be a high leverage point. Despite being the most shade tolerant in expert opinion it occupied traitspace along the shade tolerance axis with light demanding species (high LAR). The removal of this outlier improved the leaf axis correlation to be significant (P=0.046, R^2=0.74) supporting our use of this axis as our measure of shade tolerance.

Linear regressions to examine the relationship of shade tolerance to both seed mass and cache utility showed a negative relationship such that both increased utility and seedmass were associated with lower shade tolerance (Fig:3.4). Initially, with all species included, only seed mass was significant (P<0.005,R^2=0.14)(Fig:3.4B). Removal of COAM, however, resulted in a significant relationship for caching utility as well which also explained the most variance (P<0.005, R^2=0.83) (Fig:3.4A).

3.4 Discussion

Using principle component analysis we found a relationship between the growth metrics of leaf mass area (LMA) and leaf area ratio (LAR) and estimated shade tolerance consistent with the stress tolerance hypothesis. Plants of higher estimated shade tolerance exhibited higher leaf mass areas where-as lower ranked plants favored greater leaf area ratio. The ranking of plants along this second PC axis largely aligned with previous qualitative measures of shade tolerance as described by expert opinion in the literature [39] thus supporting our use of it as a quantitative measure of shade tolerance. Lastly, we found a negative relationship between this measure of shade tolerance and both caching utility and seed size which supports our hypothesis. While most species were well-aligned in this relationship, *Corylus americana* (COAM) was a consistent outlier.

Principal component analysis of plants in all treatments showed a strong division of above- and below-ground biomass allocation (LMF/SMF and RMF, respectively) along the first axis. This biomass allocation is likely driven driven more by optimal partitioning in response to local growth conditions and resource availability - when light is limited plants shift growth to stems and leaves to increase light capture [9, 16, 41, 45]. This partitioning of growth to below- or above-ground biomass is likely more plastic within a species than distinctive between them [48]. Our results are similar to Chmura, *et al.* (2017) such that plants in shaded treatments allocated more growth towards stems and leaves where-as those in full sun could afford more growth in roots. Lastly, we found no correlation of this biomass allocation axis with established shade tolerance.

In contrast, we found a separation of shade tolerant and light demanding species along the second components of our PCAs (Fig: 3.2). To support the carbon gain hypothesis of shade tolerance we would expect to see an association of shade tolerators with greater LAR [17, 62]. However, we observed association of light demanding with greater LAR and shade tolerant plants with higher LMA which is supports by the shade tolerance hypothesis [24, 45, 62]. We found further support for this with Spearman rank correlations of the second (stress tolerance) axis and expert opinion shade tolerances.

We predicted that species with seeds of higher caching utility would also exhibit lower shade tolerance. Both seed size and caching utility were negatively related with shade tolerance when using the stress tolerance axis (PC2). This supports our hypothesis that squirrel caching preferences have selected for certain traits, such as seed size, in hard-seeded trees. This builds upon previous work which has shown likely diffuse coevolution of seed traits and squirrel caching preferences. Caching responses such as time to cache and distance to cache were influenced by family-level related traits such as lipid content and shell thickness as well as more evolutionarily labile traits such as kernel mass [59]. Not only do these traits and cache responses increase the likelihood of a seed escaping predation [59], but increased seed utility can lead to caches being placed in more open areas [55]. Our results show that these larger, higher value seeds are also of lower shade tolerance which suggests directed dispersal and coevolution.

Our results draw contrary conclusions, however, from the conventional literature which suggests that shade tolerance increases with seed size. Significant relationships between angiosperm seed size and tolerance have been shown in tropical [11, 24] as well as temperate species [19, 73]. These studies, though, include species which are either pioneer/early successional or are wind dispersed. Pioneer and early successional trees favor smaller seeds than those of mature forests [2, 11, 33] and seed size increases with dispersal syndrome from wind to bird to mammal [22, 11]. Within our own study it is likely we saw this same discrepancy exemplified by *C. americana*. COAM was the lowest utility seed grown and, although it is ranked as highly shade tolerant [40, 51], it responded similarly to the least shade tolerant species in both our rank correlations with expert opinion and our regressions with utility and size. In field studies performed in conjunction with this project we observed that COAM was of such low utility to squirrels that they didn't cache any, they simply ate the seeds or ignored them entirely (see Chapter 2).

3.5 Conclusion

We present these findings as novel evidence towards the coevolution of trees and the animals which cache them. From this experiment we've expanded upon qualitative measures of shade tolerance and quantified a measure based upon the morphological traits of LMA and LAR, a dichotomy supported by the stress tolerance hypothesis. Using this scale we've shown that species of lower shade tolerance produce seeds of higher utility to squirrels. This finding is supportive both of a shared evolutionary history and directed dispersal. *Corylus americana*, however, was an outlier within the species we tested. It is likely that *C. americana* is of such low caching value to squirrels that evolutionarily it has not responded to the same behavioral pressures as the other species we examined. Expanding the selection of species grown to include more tree species, particularly those not cached by squirrels, could further explore this discrepancy in the relationship of seed size and shade tolerance. Lastly, seed size is not the sole driver of caching utility. Further quantification of traits such as tannin or lipid content of seeds could further refine the estimation of seed value and provide finer resolution to the work begun here.

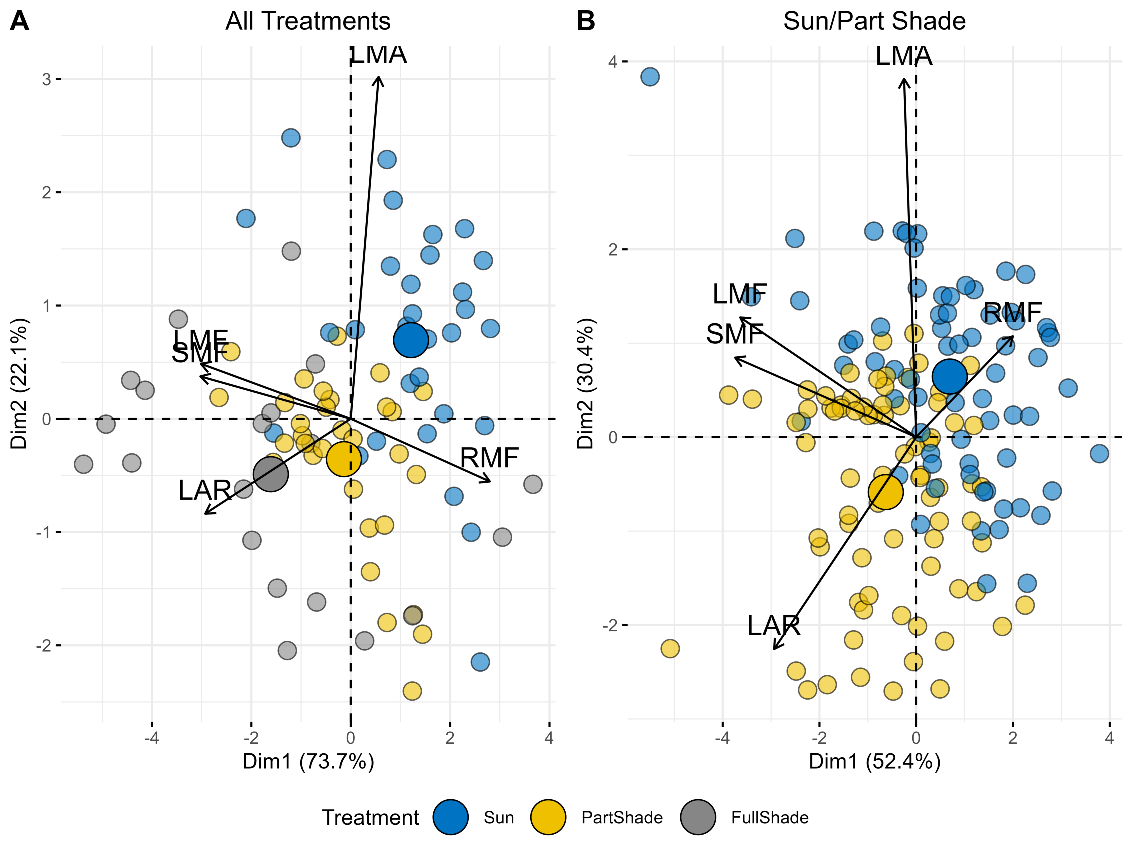

Figure 3.1: Principle component analysis of all treatments combined (A) and the sun and part shade treatments (B) categorized by shade treatment. Both are limited to the species only present in all (A: n=3, B: n=8, see Table 3.4). PC1 describes a division in above- and below-ground biomass allocation (biomass allocation axis) while PC2 shows a split of leaf thickness (LMA) and relative amount of leaves (LAR) (stress tolerance axis). In both analyses, sun treatments aggregated towards RMF and LMA while shade treatments associated with above-ground biomass and LAR.

Figure 3.2: Principle component analyses show similar loadings and distribution of shade tolerance among treatments. PC1 consistently describes above and below-ground biomass allocation and PC2 is driven by opposing leaf traits of LMA and LAR. Categorization by the rounded expert opinion shade tolerance shows higher shade tolerance towards LMA and light dependence towards LAR. Of note: the highest estimated shade tolerance (4) is *C. americana* (COAM) shifts on the second PC axis towards high LAR in the Part Shade (20% light) treatment.

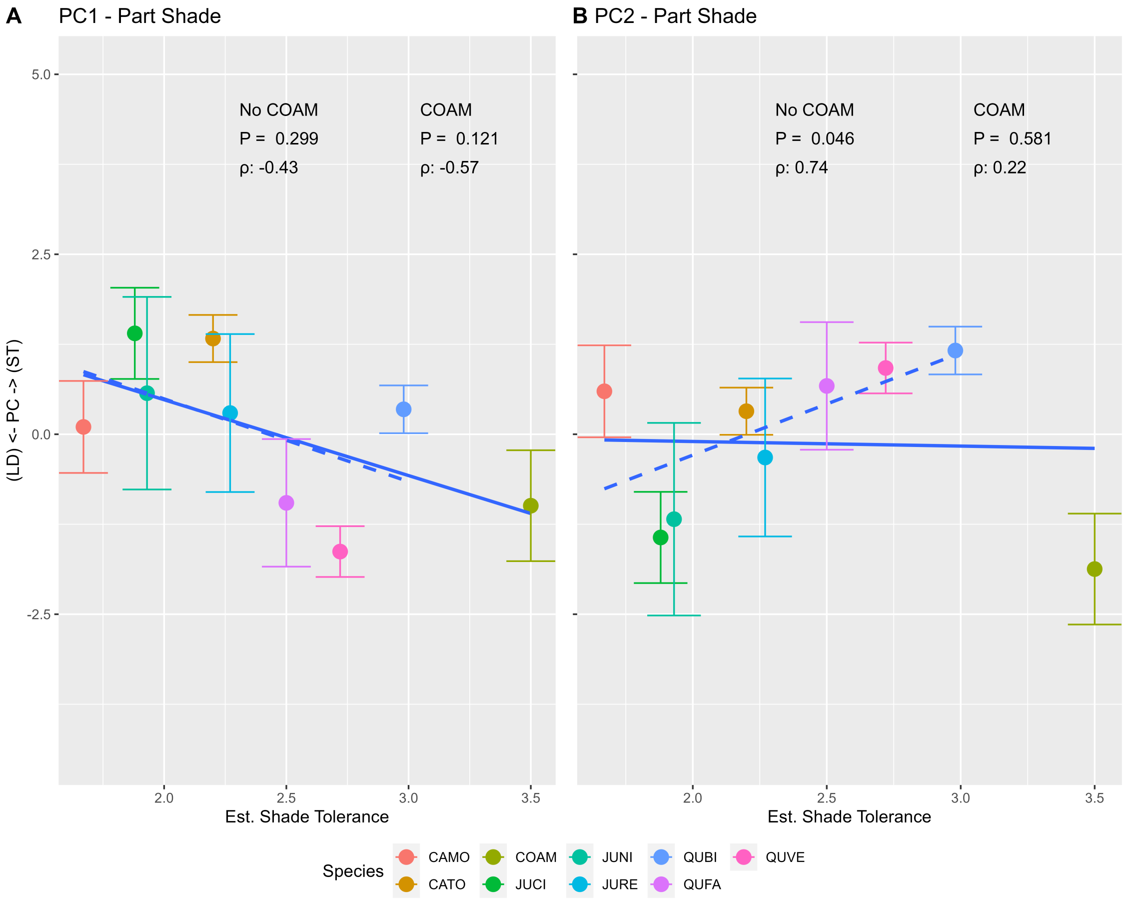

Figure 3.3: Spearman rank correlations of expert opinion shade tolerance correlates with the second PC axis. Correlations were performed on the first two axes of the part shade (20% light) PCA. Statistics are shown for correlations both including (solid line) and excluding (dashed) the high leverage species C. americana (COAM). Only PC2 (LMA/LAR axis) showed a significant high correlation with the removal of COAM.

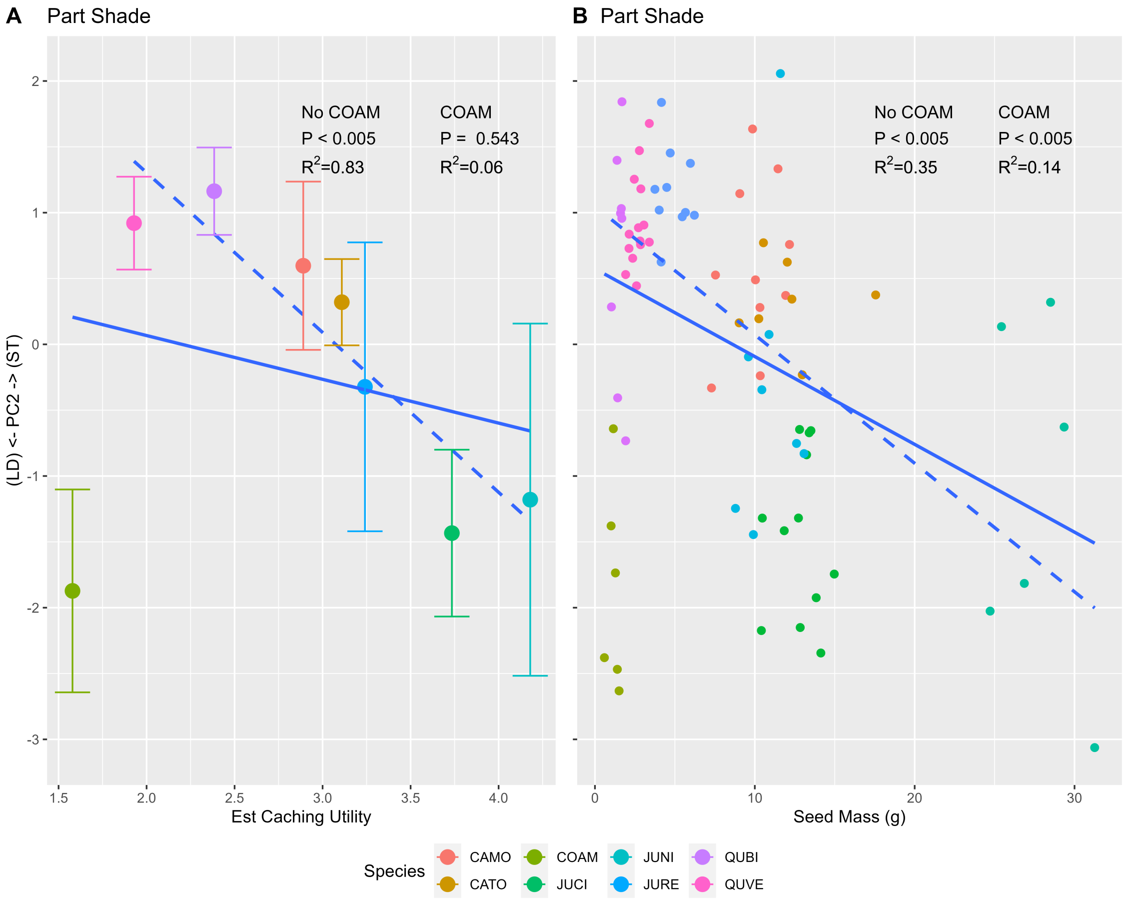

Figure 3.4: Linear regressions of both estimated caching utility and seed mass show negative relationships with shade tolerance. Shade tolerance is here estimated by the second PC axis (LMA/LAR) of the part shade treatment (20% light). Statistics are shown for regressions both including (solid line) and excluding (dashed) the high leverage species *C. americana* (COAM).

Chapter 4

Conclusion

With this work we've found evidence for both the optimal density model (ODM) and the habitat structure hypothesis (HSH) in a typical Eastern mixed forest. In accordance with the ODM Squirrels cached higher value seeds further away and in more dispersed locations. Although we did not find support for for the HSH with regards to the distances of caches from routes of escape (i.e.: nearest tree) we did find evidence that squirrels utilized heterogeneity in the canopy as well as soil moisture when choosing cache locations. It is likely that over-all homogeneity of the environment contributed to the mixed results in the in the use of the landscape.

Our shade tolerance experiment provided a quantitative measure of tolerance which is supported by the stress tolerance hypothesis. We found that shade tolerant species favored greater leaf mass area while light demanding trees exhibited higher leaf area ratio. Using this measure of shade tolerance we found inverse relationships between seed size & caching utility and shade tolerance. Our scale of tolerance and relationship to utility largely aligned with previous expert estimated shade tolerance with the notable exception of *Corylus americana*. *Corylus americana* had the highest expert opinion tolerance to shade in our study but behaved much like a light demanding species in the shade treatment. We propose this exceptional behavior is because *C. americana* is not readily cached by squirrels and likely responds to different selective pressure in the environment than the other species tested here.

Bibliography

[1] AZ Andis Arietta. Estimation of forest canopy structure and understory light using spherical panorama images from smartphone photography. *Forestry*, 95(1):38–48, 2022.

[2] Carol K Augspurger. Seedling survival of tropical tree species: interactions of dispersal distance, light-gaps, and pathogens. *Ecology*, 65(6):1705–1712, 1984.

[3] Frederick S Baker. A revised tolerance table. *Journal of Forestry*, 47(3):179–181, 1949.

[4] F. A. Bazzaz. The physiological ecology of plant succession. *Annual Review of Ecology and Systematics*, 10:351–371, 1979.

[5] Philip Beckschäfer. Hemispherical_2.0 – batch processing hemispherical and canopy photographs with imagej – user manual. 01 2015.

[6] Joel S Brown. Vigilance, patch use and habitat selection: foraging under predation risk. *Evolutionary ecology research*, 1(1):49–71, 1999.

[7] Joel S Brown and Burt P Kotler. Hazardous duty pay and the foraging cost of predation. *Ecology letters*, 7(10):999–1014, 2004.

[8] Charles D Canham, Richard K Kobe, Erika F Latty, and Robin L Chazdon. Interspecific and intraspecific variation in tree seedling survival: effects of allocation to roots versus carbohydrate reserves. *Oecologia*, pages 1–11, 1999.

[9] Daniel J Chmura, J Modrzyński, P Chmielarz, and Mark G Tjoelker. Plasticity in seedling morphology, biomass allocation and physiology among ten temperate tree species in response to shade is related to shade tolerance and not leaf habit. *Plant Biology*, 19(2):172–182, 2017.

[10] Joanna M Dally, Nicola S Clayton, and Nathan J Emery. The behaviour and evolution of cache protection and pilferage. *Animal Behaviour*, 72(1):13–23, 2006.

[11] Susan Foster and Charles H Janson. The relationship between seed size and establishment conditions in tropical woody plants. *Ecology*, 66(3):773–780, 1985.

[12] J. F. Fox. Adaptation of gray squirrel behavior to autumn germination by white oak acorns. *Evolution*, 36(4):800–809, 1982.

[13] Dumas Galvez, Bart Kranstauber, Roland W Kays, and Patrick A Jansen. Scatter hoarding by the central american agouti: a test of optimal cache spacing theory. *Animal Behaviour*, 78(6):1327–1333, 2009.

[14] Kaitlyn M Gaynor, Joel S Brown, Arthur D Middleton, Mary E Power, and Justin S Brashares. Landscapes of fear: spatial patterns of risk perception and response. *Trends in ecology & evolution*, 34(4):355–368, 2019.

[15] Fritz Gerhardt. Food pilfering in larder-hoarding red squirrels (tamiasciurus hudsonicus). *Journal of Mammalogy*, 86(1):108–114, 2005.

[16] Marian J Giertych, Piotr Karolewski, and Jacek Oleksyn. Carbon allocation in seedlings of deciduous tree species depends on their shade tolerance. *Acta Physiologiae Plantarum*, 37:1–15, 2015.

[17] Thomas J Givnish. Adaptation to sun and shade: a whole-plant perspective. *Functional Plant Biology*, 15(2):63–92, 1988.

[18] José M. Gómez, Carolina Puerta-Piñero, and Eugene W. Schupp. Effectiveness of rodents as local seed dispersers of holm oaks. *Oecologia*, 155(3):529–537, 2008.

[19] Nina Hewitt. Seed size and shade-tolerance: a comparative analysis of north american temperate trees. *Oecologia*, 114(3):432–440, 1998.

[20] Ben T Hirsch, Roland Kays, Verónica E Pereira, and Patrick A Jansen. Directed seed dispersal towards areas with low conspecific tree density by a scatter-hoarding rodent. *Ecology Letters*, 15(12):1423–1429, 2012.

[21] Henry F Howe and Judith Smallwood. Ecology of seed dispersal. *Annual review of ecology and systematics*, 13(1):201–228, 1982.

[22] Lesley Hughes, Michael Dunlop, Kristine French, Michelle R Leishman, Barbara Rice, Louise Rodgerson, and Mark Westoby. Predicting dispersal spectra: a minimal set of hypotheses based on plant attributes. *Journal of Ecology*, pages 933–950, 1994.

[23] Patrick A Jansen, Martijn Bartholomeus, Frans Bongers, Jelmer A Elzinga, Jan den Ouden, and Sipke E Van Wieren. 14 the role of seed size in dispersal by a scatter-hoarding rodent. *Seed dispersal and frugivory: ecology, evolution, and conservation*, page 209, 2002.

[24] Kaoru Kitajima. Relative importance of photosynthetic traits and allocation patterns as correlates of seedling shade tolerance of 13 tropical trees. *Oecologia*, 98:419–428, 1994.

[25] Richard K Kobe. Carbohydrate allocation to storage as a basis of interspecific variation in sapling survivorship and growth. *Oikos*, pages 226–233, 1997.

[26] Bill Kraus. A test of the optimal-density model for seed scatterhoarding. *Ecology*, 64(3):608–610, 1983.

[27] John W Laundré, Lucina Hernández, and Kelly B Altendorf. Wolves, elk, and bison: reestablishing the" landscape of fear" in yellowstone national park, usa. *Canadian Journal of Zoology*, 79(8):1401–1409, 2001.

[28] Lisa A Leaver, Lucy Hopewell, Christine Caldwell, and Lesley Mallarky. Audience effects on food caching in grey squirrels (sciurus carolinensis): evidence for pilferage avoidance strategies. *Animal cognition*, 10(1):23–27, 2007.

[29] Nathanael I. Lichti, Harmony J. Dalgleish, and Michael A. Steele. Interactions among shade, caching behavior, and predation risk may drive seed trait evolution in scatter-hoarded plants. *Diversity*, 12(11), 2020.

[30] Nathanael I. Lichti, Michael A. Steele, and Robert K. Swihart. Seed fate and decision-making processes in scatter-hoarding rodents. *Biological Reviews*, 92(1):474–504, 2017.

[31] Nathanael I Lichti, Michael A Steele, Hao Zhang, and Robert K Swihart. Mast species composition alters seed fate in north american rodent-dispersed hardwoods. *Ecology*, 95(7):1746–1758, 2014.

[32] Jiajia Liu, David A Coomes, Guang Hu, Jinliang Liu, Jingjing Yu, Yangqing Luo, and Mingjian Yu. Larger fragments have more late-successional species of woody plants than smaller fragments after 50 years of secondary succession. *Journal of Ecology*, 107(2):582–594, 2019.

[33] Christopher H Lusk and David I Warton. Global meta-analysis shows that relationships of leaf mass per area with species shade tolerance depend on leaf habit and ontogeny. *New Phytologist*, 176(4):764–774, 2007.

[34] Lucinda H Male and Tom V Smulders. Hyperdispersed cache distributions reduce pilferage: a field study. *Animal Behaviour*, 73(4):717–726, 2007.

[35] Daniel J Metcalfe, Peter J Grubb, and IM Turner. The ecology of very small-seeded shade-tolerant trees and shrubs in lowland rain forest in singapore. *Plant Ecology*, 134(2):131–149, 1998.

[36] Jane Molofsky and Carol K Augspurger. The effect of leaf litter on early seedling establishment in a tropical forest. *Ecology*, 73(1):68–77, 1992.

[37] Alberto Muñoz and Raúl Bonal. Linking seed dispersal to cache protection strategies. *Journal of Ecology*, 99(4):1016–1025, 2011.

[38] Jonathan A Myers and Kaoru Kitajima. Carbohydrate storage enhances seedling shade and stress tolerance in a neotropical forest. *Journal of Ecology*, pages 383–395, 2007.

[39] Ülo Niinemets. The controversy over traits conferring shade-tolerance in trees: ontogenetic changes revisited. *Journal of ecology*, 94(2):464–470, 2006.

[40] Ülo Niinemets and Fernando Valladares. Tolerance to shade, drought, and waterlogging of temperate northern hemisphere trees and shrubs. *Ecological monographs*, 76(4):521–547, 2006.

[41] Horacio Paz and Miguel MartÍnez-Ramos. Seed mass and seedling performance within eight species of psychotria (rubiaceae). *Ecology*, 84(2):439–450, 2003.

[42] Ramón Perea, Alfonso San Miguel, and Luis Gil. Acorn dispersal by rodents: The importance of re-dispersal and distance to shelter. *Basic and Applied Ecology*, 12(5):432–439, 2011.

[43] Ignacio M Pérez-Ramos, Itziar R Urbieta, Teodoro Maranón, Miguel A Zavala, and Richard K Kobe. Seed removal in two coexisting oak species: ecological consequences of seed size, plant cover and seed-drop timing. *Oikos*, 117(9):1386–1396, 2008.

[44] Mario B Pesendorfer, T Scott Sillett, Walter D Koenig, and Scott A Morrison. Scatter-hoarding corvids as seed dispersers for oaks and pines: a review of a widely distributed mutualism and its utility to habitat restoration. *The Condor: Ornithological Applications*, 118(2):215–237, 2016.

[45] Hendrik Poorter, Ülo Niinemets, Nikolaos Ntagkas, Alrun Siebenkäs, Maarit Mäenpää, Shizue Matsubara, and ThijsL. Pons. A meta-analysis of plant responses to light intensity for 70 traits ranging from molecules to whole plant performance. *New Phytologist*, 223(3):1073–1105, 2019.

[46] Lourens Poorter and Simmoné A Rose. Light-dependent changes in the relationship between seed mass and seedling traits: a meta-analysis for rain forest tree species. *Oecologia*, 142(3):378–387, 2005.

[47] Stephanie D Preston and Lucia F Jacobs. Conspecific pilferage but not presence affects merriam's kangaroo rat cache strategy. *Behavioral Ecology*, 12(5):517–523, 2001.

[48] Giacomo Puglielli, Lauri Laanisto, Hendrik Poorter, and Ülo Niinemets. Global patterns of biomass allocation in woody species with different tolerances of shade and drought: evidence for multiple strategies. *New Phytologist*, 229(1):308–322, 2021.

[49] SA Rose and Lourens Poorter. The importance of seed mass for early regeneration in tropical forest: a review. *Long-term changes in tropical tree diversity: Studies from the Guiana Shield, Africa, Borneo and Melanesia*, pages 19–35, 2003.

[50] Tharman Saverimuttu and Mark Westoby. Seedling longevity under deep shade in relation to seed size. *Journal of Ecology*, pages 681–689, 1996.

[51] Michael J Schuster, Peter D Wragg, and Peter B Reich. Phenological niche overlap between invasive buckthorn (rhamnus cathartica) and native woody species. *Forest Ecology and Management*, 498:119568, 2021.

[52] Martin A Stapanian and Christopher C Smith. A model for seed scatterhoarding: coevolution of fox squirrels and black walnuts. *Ecology*, 59(5):884–896, 1978.

[53] Martin A Stapanian and Christopher C Smith. Density-dependent survival of scatterhoarded nuts: An experimental appoach. *Ecology*, 65(5):1387–1396, 1984.

[54] Michael A Steele, Melissa Bugdal, Amy Yuan, Andrew Bartlow, Jarrod Buzalewski, Nathan Lichti, and Robert Swihart. Cache placement, pilfering, and a recovery advantage in a seed-dispersing rodent: could predation of scatter hoarders contribute to seedling establishment? *Acta Oecologica*, 37(6):554–560, 2011.

[55] Michael A. Steele, Thomas A. Contreras, Leila Z. Hadj-Chikh, Salvatore J. Agosta, Peter D. Smallwood, and Chioma N. Tomlinson. Do scatter hoarders trade off increased predation risks for lower rates of cache pilferage? *Behavioral Ecology*, 25(1):206–215, 12 2013.

[56] Michael A Steele, Sylvia L Halkin, Peter D Smallwood, Thomas J McKenna, Katerina Mitsopoulos, and Matthew Beam. Cache protection strategies of a scatter-hoarding rodent: do tree squirrels engage in behavioural deception? *Animal Behaviour*, 75(2):705–714, 2008.

[57] Michael A. Steele, Ghislain ROMPRÉ, Jeffrey A. STRATFORD, Hongmao ZHANG, Matthew SUCHOCKI, and Shealyn MARINO. Scatterhoarding rodents favor higher predation risks for cache sites: The potential for predators to influence the seed dispersal process. *Integrative Zoology*, 10(3):257–266, 2015.

[58] Mekala Sundaram, Nathanael I Lichti, Nicole J Olynk Widmar, and Robert K Swihart. Eastern gray squirrels are consistent shoppers of seed traits: insights from discrete choice experiments. *Integrative Zoology*, 13(3):280–296, 2018.

[59] Mekala Sundaram, Janna R. Willoughby, Nathanael I. Lichti, Michael A. Steele, and Robert K. Swihart. Segregating the effects of seed traits and common ancestry of hardwood trees on eastern gray squirrel foraging decisions. *PLOS ONE*, 10(6):1–16, 06 2015.

[60] Yu Takahata, Kenta Uchida, Tatsuki Shimamoto, and Nobuyuki Kutsukake. Keeping treasure safe: Eurasian red squirrels cache valuable food far from the food source with low canopy cover. *Behaviour*, 160(3-4):201–216, 2023.

[61] Noriko Tamura, Yuko Hashimoto, and Fumio Hayashi. Optimal distances for squirrels to transport and hoard walnuts. *Animal behaviour*, 58(3):635–642, 1999.

[62] Fernando Valladares and Ülo Niinemets. Shade tolerance, a key plant feature of complex nature and consequences. *Annual Review of Ecology Evolution and Systematics*, 39:237–257, 12 2008.

[63] Marius van der Merwe and Joel S. Brown. Mapping the Landscape of Fear of the Cape Ground Squirrel (Xerus inauris). *Journal of Mammalogy*, 89(5):1162–1169, 10 2008.

[64] Stephen B Vander Wall. The effects of seed value on the caching behavior of yellow pine chipmunks. *Oikos*, pages 533–537, 1995.

[65] Stephen B Vander Wall. Foraging success of granivorous rodents: effects of variation in seed and soil water on olfaction. *Ecology*, 79(1):233–241, 1998.

[66] Stephen B Vander Wall. The influence of environmental conditions on cache recovery and cache pilferage by yellow pine chipmunks (tamias amoenus) and deer mice (peromyscus maniculatus). *Behavioral ecology*, 11(5):544–549, 2000.

[67] Stephen B Vander Wall, Maurie J Beck, Jennifer S Briggs, Julie K Roth, Ted C Thayer, Jennifer L Hollander, and Jennifer M Armstrong. Interspecific variation in the olfactory abilities of granivorous rodents. *Journal of Mammalogy*, 84(2):487–496, 2003.

[68] Stephen B Vander Wall, Elaine CH Hager, and Kellie M Kuhn. Pilfering of stored seeds and the relative costs of scatter-hoarding versus larder-hoarding in yellow pine chipmunks. *Western North American Naturalist*, pages 248–257, 2005.

[69] Stephen B Vander Wall and Stephen H Jenkins. Reciprocal pilferage and the evolution of food-hoarding behavior. *Behavioral Ecology*, 14(5):656–667, 2003.

[70] Stephen B. Vander Wall, Kellie M. Kuhn, and Maurie J. Beck. Seed removal, seed predation, and secondary dispersal. *Ecology*, 86(3):801–806, 2005.

[71] Michael B Walters and Peter B Reich. Are shade tolerance, survival, and growth linked? low light and nitrogen effects on hardwood seedlings. *Ecology*, 77(3):841–853, 1996.

[72] Michael B Walters and Peter B Reich. Low-light carbon balance and shade tolerance in the seedlings of woody plants: do winter deciduous and broad-leaved evergreen species differ? *The New Phytologist*, 143(1):143–154, 1999.

[73] Michael B Walters and Peter B Reich. Seed size, nitrogen supply, and growth rate affect tree seedling survival in deep shade. *Ecology*, 81(7):1887–1901, 2000.

[74] Dan Wenny. Advantages of seed dispersal: A re-evaluation of directed dispersal. *Evol. Ecol. Res*, 3, 01 2001.

[75] Mark Westoby, Enrique Jurado, and Michelle Leishman. Comparative evolutionary ecology of seed size. *Trends in Ecology & Evolution*, 7(11):368–372, 1992.