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Quantifying Variation in the Seed Scatter-hoarder Relationship

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Quantifying Variation in the Seed Scatter-hoarder Relationship

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ABSTRACT

The relationship between scatter-hoarders and trees varies on a continuum from mutualism to antagonism and can change across time, location, and among species. We examined five tree species across five sites to quantify this variability: red oak (*Quercus rubra*), white oak, (*Quercus alba*), American chestnut (*Castanea dentata*), Chinese chestnut (*Castanea mollissima*), and a hybrid American/Chinese chestnut. In the fall of 2012, 2013, and 2015, we set up field sites across the east coast, including in Virginia, Pennsylvania, Indiana, Massachusetts, and Maine. We tested the potential for mutualism between scatter-hoarders and trees by using a simple model proposed in the literature that compares rates of seedling emergence with and without scatter-hoarders. The model uses the ratio of seed emergence from the surface divided by seed emergence from a cache (*Es/Ec*) as one variable to place seeds on a continuum from mutualism to antagonism. We predicted across different study sites and found that that red oak seeds will have a more mutualistic relationship with scatter-hoarders because of their long dormancy period before germination, white oak seeds will have an antagonistic relationship because they germinate readily, and chestnuts will fall in between. We hypothesized that abiotic conditions surrounding the seed would have a stronger influence on the *Es/Ec* ratio than the provenance of the seed would. We found that different seed provenances caused variation in the *Es/Ec* ratio, but abiotic conditions described more of the variation in the continuum from mutualism to antagonism. We also quantified the effect of temperature at our different study sites to measure the correlation between temperature and emergence. We found that temperature did not predict the high degree of variation in the effect of abiotic conditions on the degree of mutualism.

INTRODUCTION

The relationship between scatter-hoarders (animals that store 1-2 seeds in multiple, shallow caches) and hard-seeded trees varies along a continuum from mutualism to antagonism (Zwolak and Crone 2011, Xiao and Krebs 2015). In a mutualistic relationship, both partners experience a net benefit from the interaction, while in antagonism only one partner benefits. In the scatter-hoarder-tree relationship, scatter-hoarders benefit from having a non-perishable food source throughout the winter months. However, the benefit to trees is not always as clear. While a high number of scatter-hoarders can lead to low seedling recruitment through seed predation, scatter-hoarders can also benefit trees by dispersing seeds through caching and not returning to eat the cache. Such dispersal enables seeds to leave the shadow of the parent tree, preventing density-dependent mortality due to natural enemies such as disease (Connell 1971, Howe and Miriti 2000, Liu *et al.* 2012). Some studies have developed a theoretical framework to attempt to describe the variation in the continuum from mutualism to antagonism (Theimer 2005, Xiao and Krebs 2015). Theimer (2005) describes the scatter-hoarders as "conditional mutualists," whose effect on trees can vary over time and space. He uses the ratio of seeds to scatter-hoarders to predict when scatter-hoarders can act as mutualist with trees. Another model uses the effects of dispersal and burial to understand the variation in the continuum, showing how seed emergence in the presence of scatter-hoarders can sway the continuum (Xiao and Krebs 2015). Because this relationship can fluctuate so readily from mutualism to antagonism, it is difficult to quantify the effects of scatter-hoarders on seed success.

In order to measure the potential for mutualism between scatter-hoarders and trees, Zwolak and Crone (2011) put forward a model to measure this relationship using three variables. The first variable, *Es*, is the rate of emergence of seeds from the surface, i.e., if scatter-hoarders do not cache them. The second variable, *Ec*, is the emergence rate for seeds from a cache. Finally, of seeds that are dispersed by scatter-hoarders, a fraction will be cached and not eaten, and these seeds can then germinate to become seedlings, denoted as *Pc*. When the probability of survival of seeds that are cached and not recovered is higher than the ratio of survival on the surface to survival in a cache, the seed-scatter-hoarder relationship is mutualistic:

$$Es / Ec < Pc.$$

If the ratio of the emergence from the seeds from the surface to the emergence from the seeds in a cache is smaller than the probability of survival in the cache, the relationship is antagonistic (Figure 1).
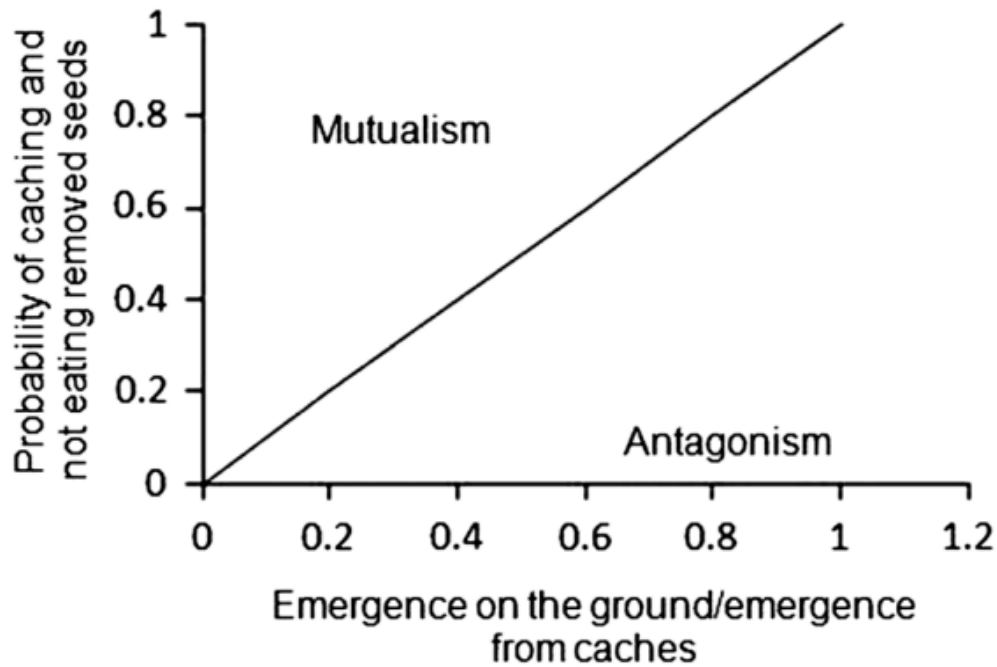
Figure 1: Graphical model of the varying relationship between scatter-hoarders and trees from Zwolak and Crone (2011). The y-axis represents Pc, *which is the proportion of seeds that survive from caches that are never recovered from scatter-hoarders. The x-axis represents* Es/Ec, *which is the emergence rate from the surface divided by the emergence rate from the cache. When the* Es/Ec *ratio is high, i.e. high surface emergence compared to emergence from the cache, the relationship leans towards antagonism because the seeds have higher success without scatter-hoarders. When* Es/Ec *is low, i.e. low surface emergence compared to emergence from a cache, the relationship leans towards mutualism because the seeds are more successful in scatter-hoarder caches.*

Several factors can cause inter-annual variation in both the probability of survival in the cache (*Pc*) and the ratio of the seedlings emerging from surface to seedlings emerging from a cache (*Es/Ec*). For *Pc*, the major source of variation is the ratio of seeds: scatter-hoarders (Theimer 2005). When there is a low seed: scatter-hoarder ratio, for example if few seeds are produced in a year, virtually all of the seeds will be handled by a scatter-hoarder, and the vast majority of seeds will be eaten and *Pc* will have a low value. When there is a high seed to scatter-hoarder ratio, such as in a high seed production year, the scatter-hoarders cache more seeds than they require, which can lead to higher *Pc*. (Garcia and Houle 2002, Zwolak and Crone 2011, Lichti *et al.* 2015). The number of seeds produced in a population of trees is strongly influenced by "masting", which is the synchronous production of seeds by all trees in a population (Sork 1993). The number of seeds produced in a mast varies from year to year, causing the seed: scatter-hoarder ratio to vary substantially.

There are two potential sources of variation in the *Es/Ec* ratio: the provenance, or source, of the seed and the abiotic conditions. The provenance is the place of origin of the seed, and many studies have assessed the differences among seed provenances. Seed provenances may have different responses to abiotic conditions because they are locally adapted to their smaller climate ranges. For example, one study compared growth (height and diameter) among different oak (*Quercus robur* and *Quercus petraea*) provenances and found that Nordic provenances (more northern) grew slower than Danish provenances (more southern) and were more resistant to damage from wind and frost, supporting evidence that northern provenances were locally adapted to harsh abiotic conditions found in the Nordic region (Jensen 2000). Another study comparing European oaks found that German, Polish, and Danish provenances showed more resistance to frost than French, Austrian, and British provenances (Deans and Harvey 1996), implying that northern provenances were more resistant to frost damage. However, another study found that when exposing provenances of three European oak species to temperature and moisture, the differences among provenances was significant, but showed no pattern that could be related to biogeographic climate. In North America, Huang *et al.* (2016) observed 16 provenances of white oak planted in two sites in Indiana and found that the different sites described significantly more of the variation in emergence than provenance did, even though he compared two sites in one state. We predict similar results for our seeds, as we hypothesize that abiotic conditions among sites will cause more variation in the continuum from mutualism to antagonism than provenance will. Because the literature is inconclusive about the effect of provenance, we are interested in comparing different provenances in our oak seeds. If our seed provenances are significantly different from each other, they may have different rates of emergence from the surface and emergence from caches depending on the conditions at their particular location of origin, leading to variation in the *Es/Ec* ratio.

The second source of variation in the Es/Ec ratio includes abiotic conditions such as soil moisture, leaf litter, and temperature. Soil moisture increases seed survival, with higher soil moisture treatments having higher germination rates (Garcia and Houle 2002). Leaf and pine needle litter can also affect seed emergence. Seeds placed on top of pine needle litter are more prone to desiccation (Lopez-Barrera and Gonzalez-Espinosa 2001), potentially causing seeds to dry out by blocking moist soil from reaching the seed. While pine needles may cause desiccation, a thin layer of leaf litter covering the seeds can increase frost resistance (Esteso-Martinez and Gil-Pelegrin 2003). Leaf litter and burial can both induce germination, and studies have shown that burial can increase seed germination in oak trees (Houle 2002) and in pine seeds (Briggs *et al.* 2009). Those studies that focus on the beneficial effects of litter and burial often note that burial can provide insulation for the seed. This insulating factor may include temperature, implying that there may be a temperature difference between seeds under litter and seeds on the surface. One study looked at the effect of temperature on seed germination by exposing *Quercus rugosa* seeds to two different temperature regimes: seeds in one treatment were subjected to warmer temperatures, and seeds in another treatment were subjected to colder temperatures. The study found that seeds in the higher temperature regime had slightly higher germinative energy, but these results were not statistically significant. Germinative energy is the

number of days required to reach 50% germination (Huerta-Paniagua and Rodriguez-Trejo 2010). While this study found no effect of temperature overall, we will instead compare temperature in the cache to temperatures on the surface and see if temperature can predict variation in the *Es/Ec* ratio. There is a gap in the literature directly linking different temperatures to these higher emergence rates from insulation, so we hypothesize that warmer temperatures in burial sites may explain the benefit of being buried.

The goal of this study is to quantify the variation in the seed: scatter-hoarder relationship using the model presented by Zwolak and Crone (2011). Specifically, we focus on two sources of variation in the *Es/Ec* ratio: seed provenance and abiotic conditions. To test these, we set up plots of seeds with predator exclusion cages. We planted seeds in five different sites to expose the seeds to varying environmental conditions, and planted every seed source in Virginia to compare seed provenances. We use seeds from red oak (*Quercus rubra*), white oak (*Quercus alba*), American chestnut (*Castanea dentata*), Chinese chestnut (*Castanea mollissima*), and a hybrid American-Chinese chestnut. We test three hypotheses:

1. Our study species will have differing values for *Es/Ec* that correspond to their seed dormancy requirements. Red oak will have lower *Es/Ec* ratios (higher potential for mutualism) because of its longer dormancy period; white oak will have higher *Es/Ec* ratios (higher potential for antagonism) because of its shorter dormancy period; and the chestnuts will fall somewhere in between because of their intermediate dormancy period.
2. Abiotic conditions will have a stronger effect on *Es/Ec* than the seed provenance. We predict that we will see more variation in *Es/Ec* when comparing across sites and years (differing abiotic conditions), and then when comparing seed provenances within the same site and year.
3. Temperature will explain variation in the *Es/Ec* ratio. We predict that sites with lower temperatures will have lower *Es/Ec* ratios (more potential for mutualism) because these seeds will benefit more from the extra protection and warmth of being cached. We predict that seeds planted in northern sites will have a more mutualistic relationship (lower *Es/Ec*) with scatter-hoarders than those planted in the south.

METHODS

Study Species

The species chosen for this study include red oak (*Quercus rubra*), white oak, (*Quercus alba*), American chestnut (*Castanea dentata*), Chinese chestnut (*Castanea mollissima*), and a hybrid American/Chinese chestnut. Oak trees occupy a large area characterized by eastern deciduous forests, and their range can extend as far south as Texas and as far north as Canada (Bourdeau 1954). They grow slowly and up to about 30 ft tall, with branches that extend upwards and do not form a closed canopy (Bourdeau 1954). Red oaks, characterized by their pointed leaves and round, striped seeds require two growing seasons in order to mature into a seedling (Straub 2016). They require a dormancy period between falling from the parent tree and germination the next spring (Licthi *et al.* 2014). Red oak acorns in eastern deciduous forests typically have higher concentrations of fats and tannins (Smallwood *et al.* 2001), which are bitter chemicals that can have negative physiological effects on granivores (Chung-MacCoubrey 1997). Since red oak seeds have a long dormancy period, granivores prefer to cache red oak seeds because of their low perishability (Smallwood *et al.* 2001).

Rounded leaves and longer, narrower acorns characterize white oaks. White oak trees span from the eastern coast of the United States to as far west as the Mississippi River and as far south as Alabama up to Canada (USDA). Their seeds have lower fat and tannin content, and they germinate in the autumn shortly and readily after falling from their parent tree (Smallwood *et al.* 2001). Squirrels tend to eat white oak seeds immediately because of their perishability and when they do choose to cache them, they often excise the embryos first to prevent germination (Smallwood *et al.* 2001).

Chestnut trees are loved by humans and recognized for their umbrella shape and soft, sweet seeds traditional to American holidays. The natural range of the American chestnut extends from New England to the Mid-Atlantic (Paillet 2002). During the seventeenth century, chestnut pollen was extremely abundant throughout New England during European agricultural practices, but between 1900 and 1925, chestnut populations plummeted. During this time, chestnut blight (*Cryphonectria parasitica*) was introduced to the United States, and American chestnuts have been rapidly declining since the introduction of the blight (Paillet 2002). Early New Englanders relied on chestnuts for a steady supply of timber, and this reliance spurred the chestnut restoration efforts that have been going on ever since (Paillet 2002). American chestnuts can be hybridized with the Chinese chestnut in order to confer blight resistance, creating a F1 hybrid (Jacobs 2007). After three back-crossings with American chestnut (B3F3), and two inter-crossings between selected resistant B3F1 hybrids, a final hybrid is produced that retains the morphological characteristics of American chestnut with the blight resistance of a Chinese chestnut (B3F3) (Jacobs 2007). When presented with chestnut seeds, red oak seeds, and white oak seeds, squirrels prefer to eat white oak seeds, so more chestnuts may be cached when surrounded by white oaks (Lichti *et al.* 2015, Blythe *et al.* 2015).

Study Sites

We conducted experiments over three years in five hardwood forests: Atkinson Grove, Maine; Harvard Forest, Massachusetts; Martell Forest, Indiana; Dorrance Township, Pennsylvania, and College Woods, Virginia (Table 1, Figure 2). Maine has warm summers, with temperatures reaching 26°C in the summer. This climate only lasts for about three months, however, with four to eight months of temperatures as low as -12°C. Similarly, Massachusetts has an equally short summer capped at 26°C, with equally cold winters, bottoming out at -11°C in Barre, MA. The site of Indiana's Martell Forest near West Lafayette has a winter low of -8°C and a summer high of 29°C, but with moderate temperatures from April to October. Pennsylvania has an even milder winter, with a low of -6°C in Williamsport, PA, and a comfortable five to seven months of summer peaking at 30°C. The College of William and Mary's College Woods of Williamsburg, VA experiences minimal winter conditions, with a winter low of -1°C. Similarly to Pennsylvania, the summer there has a high of 30°C.
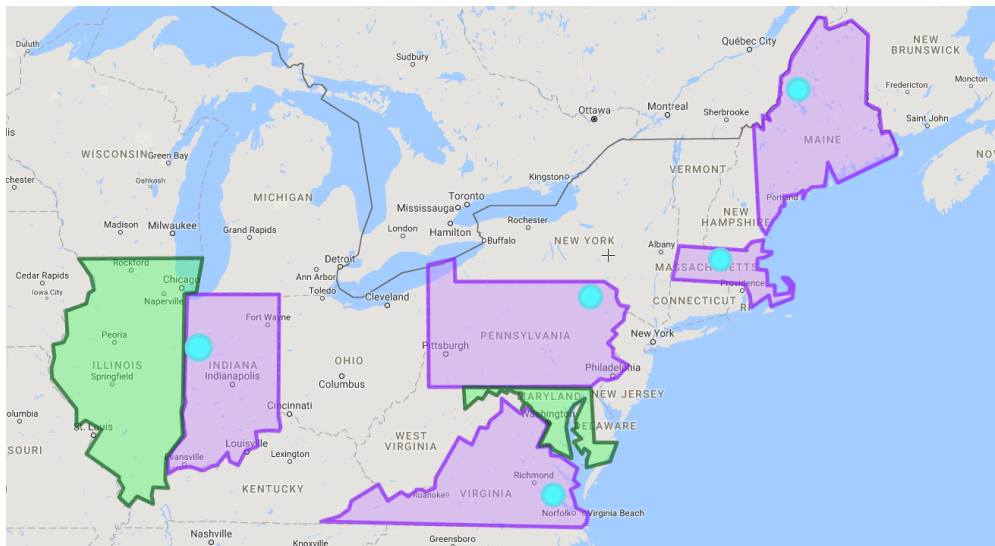
Figure 2: Map of study sites and provenances. Purple states were both sources of seeds and locations of field sites, indicated with blue dots. Green states were exclusively seed sources.

Table 1: Characteristics of the five study sites and years of study.

Site	Coordinates	Mean Annual Temperature	Mean Annual Precipitation	Dominant Canopy	Years as study site
Atkinson Grove, ME	45°9'N, 69°3'W	4.6°C	9.4 cm	*Castanea dentata, Quercus rubra, Abies balsamea*	2013
College Woods, VA	37°16'N, 76°42'W	14.8°C	10.4 cm	*Q. alba, Liriodendron tulipifera, Pinus taeda, Fagus grandifolia, Q. falcata, Acer rubrum, Cornus florida*	2013, 2014, 2015
Harvard Forest, MA	42°31'N, 72°11'W	7.2°C	9.7 cm	*Acer rubrum, Q. rubra, Pinus strobus, Tsuga sieboldii, Picea abies, Pinus resinosa*	2013, 2015
Martell Forest, IN	40°26'N, 86°54'W	11.1°C	7.9 cm	*Q. alba, Carya glabra,* Q. *velutina, Q. rubra,* A. *saccharum*	2013
Dorrance Township, PA	41°13'N, 75°98'W	9.6°C	97.2 cm	*Q. rubra, A. rubrum, Q. alba*	2014, 2015

Seed collection and field methods

We hand-collected seeds from a variety of locations in all the years of study except for 2014 when a mast failure in VA prompted us to purchase red oak seeds from Illinois (Table 2, Figure 2). Upon collection, each seed was visually inspected and float-tested in order to eliminate seeds with weevil infestations. We used the same seed provenance for red oak and white oak seeds in 2013 and 2016, collecting our red and white oak seeds from the same trees in those years. In addition, we had oak seeds from Pennsylvania in 2014 also from the same trees (Table 2). Hybrid chestnuts were donated from three different provenances (Table 2).

Each plot contained a 9x10 unit array where each column of the array contained the seeds of a single species, for a total of 3 rows of each species per cage (30 seeds/species; 90 seeds/cage). In the plot, rows alternated between cached and non-cached seeds, for a total of 10 rows. Buried seeds were cached so that the bottom of the seed was at 2 centimeters below the soil surface. Non-cached seeds remained on the surface of the soil. We pushed the leaf litter aside at each seed site and then replaced the leaf litter immediately after placing the seed. Along with the seeds, each plot had two iButton temperature loggers: one left on the surface and one buried like the seeds. These took temperature readings every 4 hours. Plots were then covered with cages made of hardware cloth that measured 1.2m x 1.2m x 17.8cm and were attached to wire aprons measuring 15.2 cm in width. The cages were secured with landscaping staples in the apron and on the corners of the cages in order to minimize pilferage by small mammals (Barnett 1977).

To investigate the effect of provenance on the *Es/Ec* ratio, we planted several provenances of the different species in Virginia in two different years (Table 2). Each provenance was replicated in 15 plots for a total of 450 seeds per provenance per year. To investigate the effects of abiotic conditions on the *Es/Ec* ratio, we planted a single provenance of each species across 2-4 different sites over three years (Table 2). Each species was replicated in 15 plots/site for a total of 450 seeds per species per site per year.

Table 2: Summary of study design.

Year	Species	Provenances planted in Virginia	Provenance of seeds planted across sites	Sites
2013	Red Oak	Virginia, Pennsylvania, Maine	Virginia	Virginia, Indiana, Massachusetts, Maine
2014		Illinois, Pennsylvania	Illinois	Virginia, Pennsylvania
2016		Virginia	Virginia	Virginia, Pennsylvania, Massachusetts
2013	White Oak	Virginia	Virginia	Virginia, Indiana, Massachusetts, Maine
2014		Illinois, Pennsylvania	Illinois	Virginia, Pennsylvania
2016		Virginia	Virginia	Virginia, Pennsylvania, Massachusetts
2013	Hybrid Chestnut (B3F2)	Maine	Maine	Virginia, Indiana, Massachusetts, Maine
2014		Massachusetts, Maine	Massachusetts	Virginia, Pennsylvania
2016		Maryland	Maryland	Virginia, Pennsylvania, Massachusetts
2013	American Chestnut	Indiana, Maine	NA	NA
2014		Indiana, Virginia	NA	NA
2013	Chinese Chestnut	Virginia	NA	NA

Analysis

For the seed viability study, we computed an average germination percentage for each species in each year. Then we used a chi-squared test to test for differences in germination rate between the two years.

We found high rates of pilferage at some of our cages. We eliminated cages with less than 50% of the seeds of each species remaining in order to reduce the effects of those cages on our averages. After removing plots with high pilferage, we calculated the *Es/Ec* ratios by calculating the percent emergence from the surface and from the cache for each plot. Then we averaged the emergence from the surface and the emergence from the cache of all of the plots at one site or of one provenance.

To recreate Figure 1, we then plotted these ratios against *Pc* values from Lichti *et al.* (2015). He collected these values by placing trays of tagged seeds out for scatter-hoarders to handle. He then tracked the handled seeds that were placed in caches, and put predator enclosures around the cached seeds, and measured the rate of emergence from those caches. He measured these in two different years with red oak, white oak, and American chestnut.

In order to test our temperature hypothesis, we first looked at the pattern of cached and surface temperatures over the months used in our study. We graphed the average temperature in the cache minus the average temperature on the surface at each site in each month. Then we plotted our *Es/Ec* values against average temperature and used a correlation to test the relationship between the two variables. Finally, we graphed surface emergence against average minimum surface temperatures to test the correlation between the coldest average temperatures and surface emergence in each species at each site.

RESULTS

Seed Viability

Across two different years, red oak seeds had similar germination, but white oak and hybrid chestnut seeds differed greatly (Table 3). Red oak viability of seeds from the same trees within the same provenance was high: 99% germination and 100% in 2013 and 2016, respectively ($X_1^2 = 0$, $P = 1$). We found the largest difference in viability in white oak, despite the seeds coming from the same trees within the same provenance, with 99% viability in 2013 (similar to red oak) but only 43% viability in 2016 ($X_1^2 = 67.199$, $P = < 0.001$, Table 3). White oak viability was also much more variable in 2016, with a coefficient of variation (CV) of 0.50 compared to 0.01 in 2013. Hybrid chestnuts had similar viability to red and white oak in 2013 (97%), but had significantly lower viability in 2016 ($X_1^2 = 7.413$, $P = 0.006$, Table 3). Similar to white oak, 2013 CV was lower than 2016: 0.174 and 0.488, respectively (Table 3). Unlike red and white oak, the hybrid chestnuts came from different provenances between years.

Table 3: Comparison of germination between years. Germination is mean ±1 SD. Bold indicates differences between years (P < 0.05)

Species	Year	Germination (%)	CV	Provenance
Red oak	2013	99.1 ± 0.1	0.01	Virginia
Red oak	2016	100.0 ± 0.0	0.00	Virginia
White oak	2013	**99.0 ± 0.1**	0.01	Virginia
White oak	2016	**43.1 ± 0.5**	0.50	Virginia
Hybrid chestnut	2013	**97.0 ± 0.2**	0.17	Maine
Hybrid chestnut	2016	**71.0 ± 0.5**	0.49	Maryland

Placing the species on the continuum from mutualism to antagonism

How does seed provenance affect the **Es/Ec** *ratio?*

All red oak provenances across years fell in the mutualistic range (Figure 3a). These seeds had low surface emergence (12%) and higher cache emergence (26%). Overall, white oak seeds had 24% emergence from the caches and 7% emergence from the surface. In white oak, three of the sources fell in the antagonistic range, and one source was mutualistic. The mutualistic point had a *Es/Ec* ratio of 0.03, whereas the other provenances had a ratio of about 0.40. White oak seeds from Pennsylvania and Illinois in 2013 showed an antagonistic relationship (Figure 3b). Virginia white oak seeds in 2013 had a strongly antagonistic relationship, but in 2015 they had a mutualistic relationship.

Chestnuts overall showed more variation than the oak species (Figure 3c and 3d). Two sources of American chestnuts, Maine and Indiana in 2013, were more mutualistic, but American chestnuts from Virginia in 2013 and Indiana in 2014 were more antagonistic (Figure 3c). The American chestnuts had an average emergence from the cache of 47%, and 5% average emergence from the surface. The Chinese chestnuts, from Virginia, had a strongly mutualistic relationship, and these seeds had absolutely no surface emergence, but and 40% average emergence from the

cache. The hybrid chestnuts had a more antagonistic relationship, with three sources showing antagonism, and only one source, Maryland in 2015, showing a mutualistic relationship. The antagonistic points were sources from Maine in 2013 and 2014, and from Massachusetts in 2014 (Figure 3d). Hybrid chestnuts had an average cache emergence of 39%, and 11% average emergence from the surface.
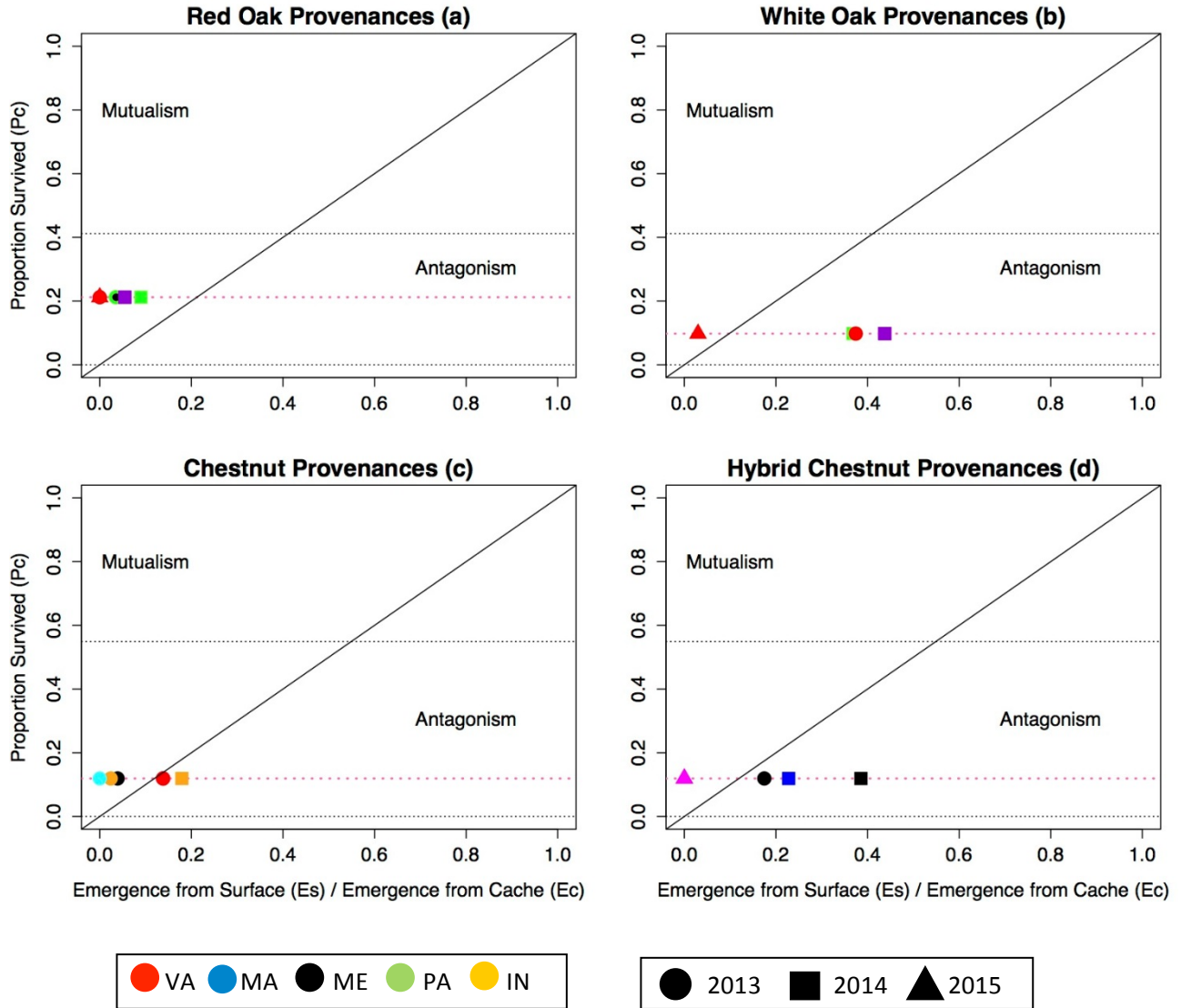

Figure 3: Sources plotted on the continuum from mutualism to antagonism. The Pc *values were taken from Licthi et al. (2015), and we used the maximum, minimum, and average values to create the window of potential survivorship represented by the horizontal lines on the graphs. The top dotted line is the maximum* Pc *value ever observed by Lichti et al. (2015) (0.412 for red oak, 0.407 for white oak, and 0.549 for chestnuts). The bottom dotted line is the minimum* Pc *value ever observed (0 for all three species). The pink dotted line represents the average* Pc *value for that species over 2 years and 2 sites (0.212 for red oak, 0.098 for white oak, and 0.119 for chestnuts). The 1:1 line indicates the separation between mutualism and antagonism.*

How do abiotic conditions affect the Es/Ec ratio?

Comparing across sites and years

We see the highest variation in the ratio in white oak, and the least amount of variation in red oak. Red oak fell more often with thin the mutualistic range (Figure 4a). Only two of the site year combinations (33%) fell in the antagonistic range: Pennsylvania in 2014 and Massachusetts in 2015.

We found the opposite pattern in white oak with 57% of the sites and years falling within the antagonistic range (Figure 4b). Both of the Massachusetts points and Virginia in 2013 and 2014 showed an antagonistic relationship, but Virginia in 2015 and the Maine and Pennsylvania in 2013 were mutualistic (Figure 4b).

Hybrid chestnut had an intermediate relationship with scatter-hoarders, with many of the points lying right on the line between mutualism and antagonism (Figure 4c). In Virginia, the value of *Es/Ec* for 2015 was mutualistic, but in 2013 and 2014 it was antagonistic. In Indiana, Maine, and Massachusetts in 2015, *Es/Ec* was in between mutualism and antagonism. Massachusetts in 2013, the relationship was the most antagonistic (Figure 4c).
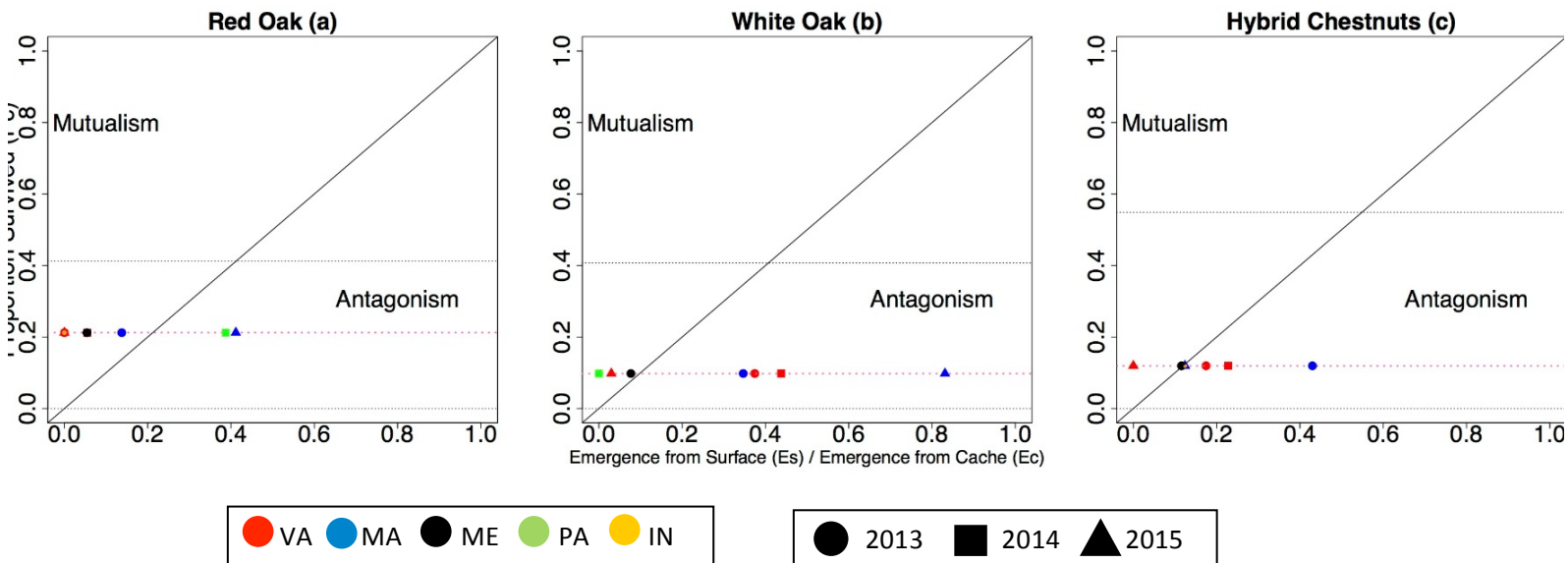

Figure 4: Sites and years plotted on the continuum from mutualism to antagonism. The Pc *values were taken from the literature, and we used the maximum, minimum, and average values to create the window of potential survivorship represented by the horizontal lines on the graphs. The top dotted line is the maximum* Pc *value ever observed by Lichti (2015) (0.412 for red oak, 0.407 for white oak, and 0.549 for chestnuts). The bottom dotted line is the minimum* Pc *value every observed (0 for all three species). The pink dotted line represents the average* Pc *value for that species (0.212 for red oak, 0.098 for white oak, and 0.119 for chestnuts). The 1:1 line indicates the separation between mutualism and antagonism.*

Effects of temperature

Across all sites, caching increased the average temperature in the cold months. From November to February, the average temperatures in a cache were up to 2° C warmer than temperatures on the soil surface at all five sites. In April and May, surface temperatures were on average 1° C warmer than the buried temperature (Figure 5). On average, there was no difference between cache and surface temperature in March.
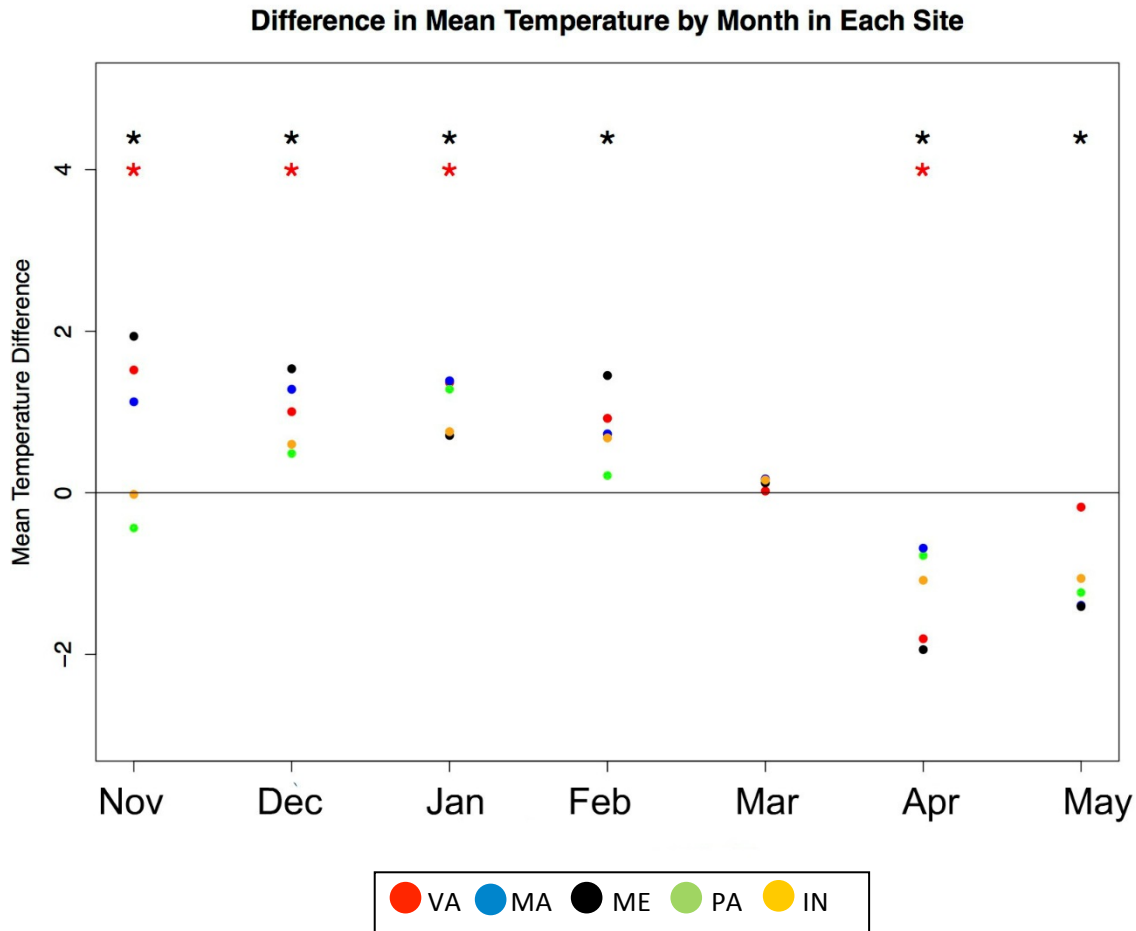

Figure 5: Comparing average temperatures across the year. Each point represents a mean of all cages in that month. In the fall when the seeds are planted, the seeds in the cache are experiencing warmer temperatures than the seeds on the surface. In the spring, seeds on the surface are warmer. The asterisks indicate when a site had significantly different temperatures on the surface than in the cache, which was calculated by using average cache temperature minus average surface temperature.

Despite these consistent effects of caching in the thermal environment experienced by the seeds, we found no correlation between average temperature and the *Es/Ec* ratio in red oak ($P = 0.69$), white oak ($P = 0.54$), or chestnut ($P = 0.70$) (Figure 6). The species did line up in horizontal bars, which aligns with our results from Figure 3: red oak had the lowest *Es/Ec* ratio, white oak had the highest, and chestnut fell in between.
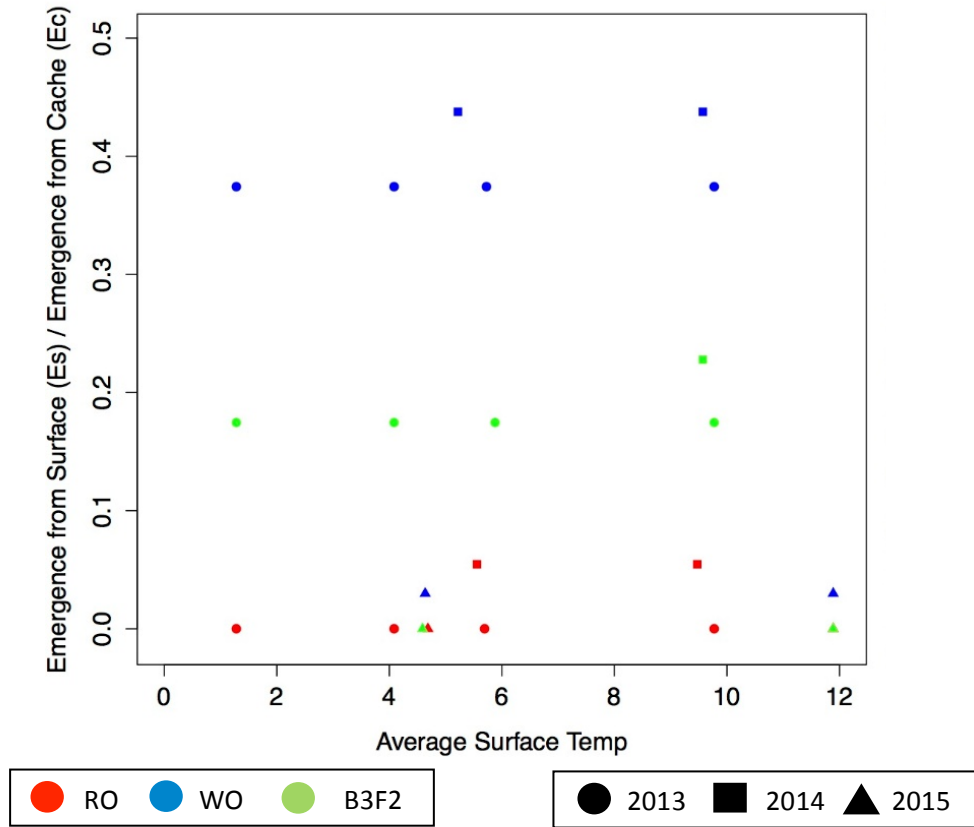

Figure 6: Average temperatures vs. Es/Ec. *Each point represents the average temperature at each site and year.*

We found no correlation between minimum surface temperatures experienced at a cage and surface emergence within that cage in red oak ($P = 0.336$), white oak ($P = 0.193$), or chestnut ($P = 0.754$). However, warmer sites had higher frequency of surface emergence in white oak: 14% of cages had surface emergence in Maine, 31% in Massachusetts, and 54% in Virginia (Figure 7b). We did not find that pattern in red oak, which had very low frequency of surface emergence in Maine (7%), and in Virginia (5%), but higher surface emergence in Massachusetts (35%, Figure 7a).

In chestnut, overall we saw many more cages with surface emergence (Figure 7c). Virginia still had the highest number of cages with surface emergence with 54% of cages having surface emergence, while Massachusetts had 40% and Maine had 38% surface emergence.
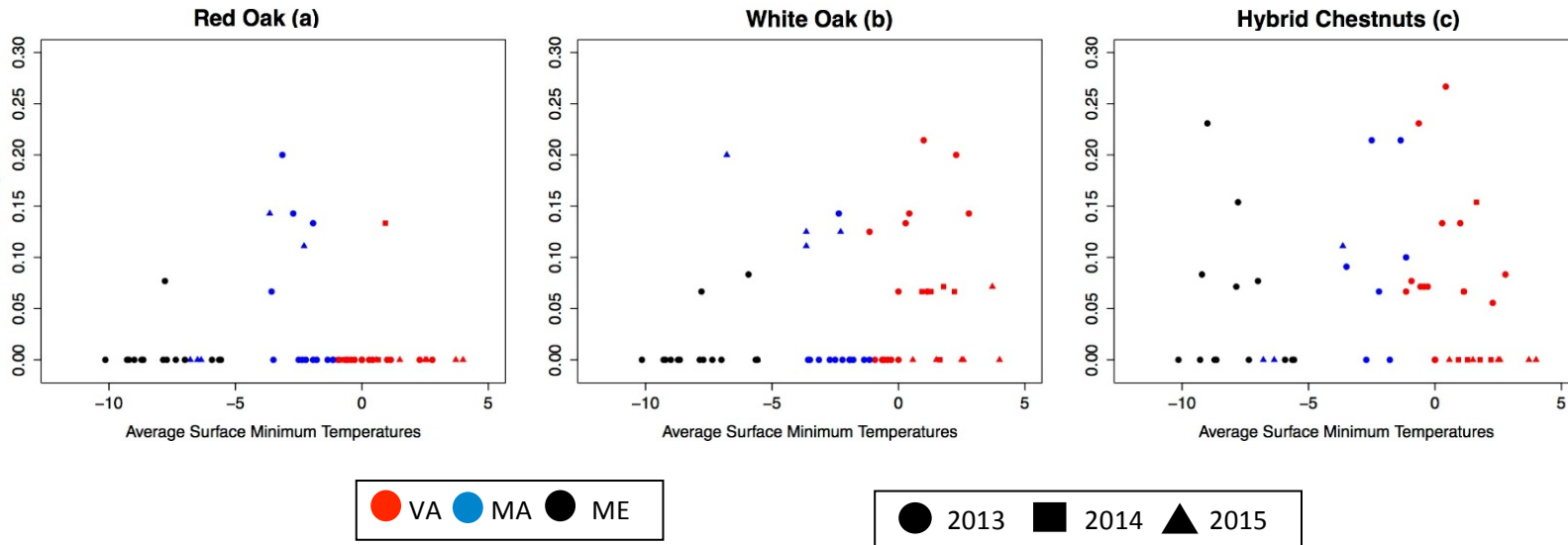

Figure 7: Surface emergence at different minimum temperatures. Temperature was measured in degrees Celsius.

DISCUSSION

We found that red oak seeds have generally lower Es/Ec ratios, plotting them more in the mutualistic range, white oak seeds have higher Es/Ec ratios, plotting them in the antagonistic range, and chestnuts have intermediate ratios. While abiotic conditions had a stronger effect on Es/Ec ratios than provenance, temperature did not explain the high degree of variation caused by abiotic conditions.

We originally hypothesized that red oak would have the lowest Es/Ec value because they have a long dormancy period and could benefit from the extra protection of a cache. We also predicted white oak would have the highest Es/Ec values because the seeds germinate readily and would have a high Es value. We predicted chestnuts would fall in between because of their intermediate dormancy period. In other words, cached red oak seeds would have the greatest cache emergence rates compared to surface seeds, chestnuts an intermediate amount, and white oak the least. Moreover, the lower the Es/Ec value, the more often the seed-granivore relationship sways towards mutualism. We found these hypotheses to be mostly true. In our graphs for provenance and abiotic conditions, the calculated ratios supported our hypotheses. All of the red oak provenances fell in the mutualistic range (Figure 3a), while 67% of red oak sites and years leaned that way as well. The white oak provenances fell in the antagonistic range 75% of the time, and 57% of the sites and years were also in the antagonistic range. It is also noteworthy that the average Pc values follow this hypothesis about how species will fall on the continuum: the highest average Pc value is red oak (0.212), the lowest is white oak (0.098), and in between in chestnuts (0.119), showing that the window for potential mutualism is highest in red oak and lowest in white oak. These data suggest that red oak seeds emerge at consistently higher rates when cached, leading to a more mutualistic relationship with scatter-hoarders. White oak provenances and sites generally fell in the antagonistic range, showing that these seeds emerge at high rates on the surface and do not require caching by scatter-hoarders in order to survive.

While we found more cases of mutualistic relationships in red oak, and more cases of antagonism in white oak, aberrations from the general trend were noticed at some years and sites. Furthermore, we found evidence that certain sites and years had an antagonistic relationship across species. For example, when all three species were planted in Massachusetts in 2015, they all fell within the antagonistic range. In addition, chestnuts and white oaks planted in Massachusetts in 2013 also showed antagonism. These data show that different abiotic conditions in different years play a strong role in whether a species will have a mutualistic or antagonistic relationship. Studies that discuss the differences among years focus on masting; for example, Sork (1993) notes that the number of seeds produced in different mast years impacts scatter-hoarder diets and influences the number of seeds that will establish. Yang and Yi (2012) found data supporting this statement, adding that more seeds are only partially eaten in high mast years, allowing the embryo to survive. They measured the rate of partial consumption by small rodents in a white oak species (*Q. mongolica*) to quantify how partially eaten seeds in varying seed abundances (different mast years) contribute to oak regeneration. We have shown that masting is not the only characteristic that affects seed success between years. In our controlled study we found that abiotic differences alone can determine where seeds will fall on the continuum from mutualism to antagonism.

We expected to see a biogeographical pattern in the *Es/Ec* ratios, with colder sites having more emergence from the cache and therefore smaller *Es/Ec* ratios, as the seeds planted in colder sites may benefit from the extra protection and added warmth of caching. Contrary to our hypotheses, all three species experienced an antagonistic relationship in Massachusetts in 2015, and chestnuts and white oaks planted in Massachusetts in 2013 showed antagonism. These Massachusetts results are surprising, as we expected colder sites to have the highest burial-benefit, leading to a more mutualistic relationship with scatter-hoarders. We found evidence for our hypothesis in Maine, as all species planted there in 2013 fell in the mutualistic range. As we hypothesized for our warmest study site, white oak and chestnut seeds planted in Virginia in 2013 and 2014 showed an antagonistic relationship. Since Virginia is the warmest study site, the white oak and chestnut seeds on the surface may have survived at higher rates, leading to a more antagonistic relationship because they do not rely on caching by scatter-hoarders for survival. We did not see this trend in red oak, however, as these seeds responded positively to being cached in Virginia. So while our study species hypotheses were generally supported, we did not see a consistent biogeographical pattern among study sites.

The large amount of variation of the *Es/Ec* ratios in each species was largely described by differences in abiotic conditions. We saw a much wider range of *Es/Ec* values when graphing different sites (Figure 4) than different provenances (Figure 3). Because we observed more variation when comparing *Es/Ec* across sites and years than we saw when comparing different provenances, we conclude that abiotic conditions caused the seeds to have a more variable relationship with scatter-hoarders than the provenance of the seed. The high degree of variation in Figure 4 supports our hypothesis that abiotic conditions will have a stronger effect on the continuum from mutualism to antagonism than provenance will.

Furthermore, we predicted that temperature would describe the variation we saw in the *Es/Ec* ratios because insulation in the cache and warmer temperatures in more southern sites could induce higher levels of emergence. We observed a consistent pattern across sites in which cache temperatures were warmer in the fall and colder in the spring (Figure 5). Overall we saw that caching seeds increased emergence, so we predicted that there would be some correlation between temperature and emergence across the study sites. However, we found no relationship between average temperature and the *Es/Ec* ratio (Figure 6), and no relationship between our coldest average temperatures and surface emergence (Figure 7), meaning temperature did not predict emergence at different sites.

These results are surprising since many studies that indicated that burial and leaf litter increase survivorship note that insulation by soil and leaf litter may be the mechanism for inducing germination (Garcia and Houle 2002, Briggs and Vander Wall 2004). Garcia and Houle (2002) found that buried red oak seeds and seeds covered by litter had greater soil moisture contents and were significantly less likely to be eaten by deer, suggesting that moisture and protection could be the driving factors in the benefits of burial instead of temperature. While further research is needed to confirm the effects of soil moisture on germination, it would be unlikely that protection from predation is the lone source of different emergence rates from caches and surface, as we saw high variability in the emergence rates without the effect of predators.

Overall, there was less variation in the *Es/Ec* ratio when comparing provenances, especially in red oak and American chestnuts, which had exceptionally small variation (Figure 3a and 3c). White oak and hybrid chestnuts had moderate variation, but sources within a year behaved similarly; e.g., all of the points from 2014 and 2015 in these two species were antagonistic, and in 2013 they were mutualistic (Figure 3b and 3d). Huang *et al.* (2016) found that white oak seeds from different provenances had significantly different heights and diameters after completing a 23-year growth experiment in Indiana, but noticed that seeds planted in two different sites in Indiana experienced more variation in emergence than seeds from different provenances. There are very few provenance studies done in the United States on our other species, red oak and American chestnut. One review on American chestnut restoration noted that testing success rates of different provenances will be important for selecting the best genotypes for tolerance to abiotic factors, which will be helpful for restoration and success of the species in a changing climate (Clark *et al.* 2014). While provenance is still important to understanding emergence patterns from different genotypes, our data shows that the differences among years, explained by varying abiotic conditions, explain some of the differences between provenances that the literature has noted. Inter-annual variation in abiotic conditions could cause differences in growth observed in provenance studies. In order to minimize these differences between years, we collected our oak seeds from the same trees in the two years of this study, and planted in the same place, yet we still saw some variation in the *Es/Ec* ratio, demonstrating strong differences in abiotic conditions present in our provenance study.

In order to quantify where our species fell on the continuum from mutualism to antagonism, we used *Pc* values taken from Lichti *et al.* 2015, plotting our *Es/Ec* ratios against average *Pc* values for each species. If some provenances or sites had had slightly higher- or lower-than-average survivorship from the cache, the relationship could have readily switched from antagonism to mutualism, or vice versa. Some of the points that fell in the antagonistic range for each species may have fallen in the mutualistic range with higher-than-average *Pc* values. For example, hybrid chestnut seeds planted in Virginia in 2013 and 2014 may have been more mutualistic if they had slightly higher survivorship from unrecovered caches (Figure 4c). Alternatively, some points would need such high *Pc* values that falling into the mutualism range is not realistic. For example, the *Pc* value for red oak seeds in Pennsylvania in 2014 would have to be the highest ever observed in order to fall into the mutualism range (Figure 4a). White oak seeds in Virginia and Massachusetts in 2013 could only fall in the mutualistic range if they had maximum survivorship from unrecovered caches (Figure 4b). The extremely variable seed: scatter-hoarder ratio leads to variable *Pc* values, and sometimes our points can readily switch from one side of the continuum to the other in certain years and sites, demonstrating the extreme variability in the relationship seeds have with scatter-hoarders.

Most of the variation observed in the continuum in mutualism to antagonism has been modeled by describing the scatter-hoarder ratio (Theimer 2005, Lichti *et al.* 2015, Xiao and Krebs 2015), but very little research has quantified variation in the *Es/Ec* ratio instead. The Zwolak and Crone (2011) model was one of the first to point out that the *Es/Ec* ratio may contribute to the continuum as much as the seed to scatter-hoarder ratio. Theimer (2005), however, frames the continuum solely on the abundance of seeds and scatter-hoarders, coining the term "conditional mutualism" to describe the varying degree of mutualism. He explained how masting can impact the proportion of seeds that are left on the surface at the end of a season, explaining that in a low

mast year, most of the seeds will be eaten, but in a high mast year, many of the seeds will be left on the surface. He demonstrates through the seed to scatter-hoarder ratio that an intermediate mast is best for seed emergence. Lichti *et al.* (2015) describe the continuum the same way, arguing that the degree of mutualism depends on the success of seeds cached by scatter-hoarders, using data on the probability of surviving from scatter-hoarder caches to calculate the *Pc* values used in our graphs. Xiao and Krebs (2015) provide a model that uses both the effects of scatter-hoarder dispersal and the effects of burial to compare emergence rates. They conclude by predicting that the effects of dispersal will have a greater effect on the continuum than the effects of burial because seeds left un-cached have low rates of survival due to predation, density-dependent death, or desiccation, and therefore handling and dispersal by scatter-hoarders is more important to understanding mutualism than studying burial alone. These studies attempt to model an extremely variable and conditional mutualism between seeds and scatter-hoarders, but they focus on the seed: scatter-hoarder ratio instead of the survival of the seeds in varying abiotic conditions. Our study has built off of these models and shown evidence that the seed: scatter-hoarder ratio alone only describes some of the variation observed in the continuum from mutualism to antagonism.

While the ratio of seeds to scatter-hoarders is absolutely important for quantifying the mutualism between seeds and scatter-hoarders, our data indicates the importance of variable *Es/Ec* ratios as a factor to quantify the continuum. Our results illustrate that the abiotic conditions play a significant and perhaps underappreciated role in shifting the relationship between scatter-hoarders and seeds along the mutualism-antagonism continuum. Understanding the variation in both the seed to scatter-hoarder ratio and the *Es/Ec* ratio is necessary in order to quantify the mutualism between seeds and scatter-hoarders.

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