Reproductive and offspring trait variation in Northern Red Oak, *Quercus rubra*, at Black Rock Forest

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May 5, 2008

Abstract:

Northern Red Oak, *Quercus rubra* (Fagaceae), is a common and dominant species in the hardwood forest ecosystems in the Eastern United States. At Black Rock Forest in Cornwall, NY, where this species is currently showing limited recruitment, we have initiated a long-term study of reproductive and offspring traits. Over the past two growing seasons reproductive and seed traits were examined with three age classes of the parent red oak trees: 35 years old (young) , 90 years old (middle) and 135 years old (old). Reproductive traits included male flower abundance in spring, mature acorn abundance in fall, and acorn mass, viability, and germination. Seed and seedling traits included individual acorn mass, rate of germination, seedling growth rate, and additional above- and belowground seedling allocation traits.

Flower and acorn production was significantly lower in trees of the young age class, but seed mass in the young age class was significantly larger than the middle and old age classes. When grown in uniform greenhouse conditions, seedlings from acorns produced by trees in the young age were generally smaller than those of the middle and old age classes. This negative correlation between seed mass and seedling growth contradicts the accepted idea that larger seed mass leads to better seedling growth and performance. Soil nutrient composition at the three contiguous study sites showed that the micro site of the 135 age class had twice as much carbon and nitrogen as the micro site of the young and middle age classes. We relate these results to recent studies of oak ecophysiology in these same stands. We anticipate collecting longer-term data to examine how age of the parent tree influences flower and fruit production, seed mass, and seedling quality, and whether factors other than seed mass, such as seed and seedling nitrogen content, also contribute to variation in seed size and seedling quality.

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Introduction:

Understanding the biological components of the carbon cycle and carbon storage is a critical part of comprehending the global climate system. An important part of the carbon cycle is the storage of carbon in terrestrial systems. Better management in forests including reforestation has the potential to sequester significant quantities of carbon (Dixon et al., 1994; Schimel et al., 2001). Recognizing forest dynamics and the changes associated with them are therefore integral to understanding the global climate system.

Forest dynamics have the potential to change by both anthropogenic and natural means. Anthropogenic sources that could affect forests include climate change and the associated increase in CO_2, disease, invasive species, excess nitrogen and population changes in herbivorous animals.

One review (Ceulemans et al., 1999) examined the effect of increased CO_2 in forests concluded that increased atmospheric CO_2 causes an increase in tree biomass most notably in leaf area and also leaf photosynthetic rate. While there is a general understanding of aboveground tree response to increased CO_2, there is a lack of knowledge in the interaction of processes on below-ground tree responses (Ceulemans et al., 1999). One study (Asshoff et al., 2006) looked at the response to elevated CO_2 in mature (100 years or older) temperate forest trees and the results do not support that mature forest trees will acquire biomass faster in a CO_2 enriched environment. While there are inconclusive results of the effect of elevated CO_2 on mature forest trees, seedling biomass accumulation rates will likely increase as seen in multiple enriched CO_2 studies involving a wide range of woody trees including *Picea mariana, Picea sitchensis,* *Citrus aurantium L., Quercus alba, and Betula pendula* (Bigras and Bertrand, 2006; Centritto et al., 1999; Curtis and Wang, 1998; Kimball et al., 2007).

Furthermore, biological invasions, defined as non-native insect, plant or fungal pests, also have the potential to affect forest populations and are probably the most significant environmental threat to the maintenance of natural forest ecosystems in North America (Liebhold et al., 1995). In the southern Appalachian Mountains, the balsam woolly adelgid, brought from Europe almost 100 years ago, has nearly killed every adult Frasier Fir in the Great Smoky Mountains National Park and has spread to other areas (Vitousek et al., 1996). Threats like this have the potential to change forest communities by eliminating a specific tree species or by changing population structures by targeting an age group in a species. Such changes have the potential to fragment intact forests and change population structures, possibly leading to elimination of a certain tree species thus changing the soil chemistry in a forest (Covington, 1981; Ussiri and Johnson, 2007). Soil composition plays a critical role in the growth and survival of plants. Available nitrogen is a limiting nutrient that affects growth and performance on forest trees (Jose et al., 2003). Many plants will respond positively to increased nitrogen if other nutrients are also readily available. A study on annual plants testing seed production on elevated CO_2 and nitrogen found that seed production is limited primarily by nitrogen availability and will be affected by elevated CO_2 only when nitrogen acquisition is increased (Miyagi et al., 2007). Changes in available nitrogen could be anthropogenic, like with the addition of nitrogen fertilizer or increased nitrogen deposition. Through the past 100 years, nitrogen deposition has steadily increased in temperate forest areas (Brimblecombe and Stedman, 1982), affecting the composition of forest soils. Nitrogen can also increase at a micro site with natural means, like animal decay or increased fungal colonization. A review (van der Heijden et al., 2008) of the role of soil microbes in plant diversity and productivity on terrestrial ecosystems explored research that showed soil microbes to have a dramatic effect on forest composition. Soil microbes can have a positive direct effect on forests through fungal symbioses, nitrogen fixation, and have a negative direct effect through pathogens and competition for nutrients (van der Heijden et al., 2008).

In addition to anthropogenic variation, background variation can exist in many forms in a forest. Most notably, there is variation among different tree species of a forest. This variation between tree species can include differences in canopy structure and fungal communities. Within one species, there can also be variation within a population due to micro site habitat (altitude, availability of nutrients, shade etc.), individual genetic variation or the age of the trees. A study (Frey et al., 2007) showed variation among species in density of seedling recruits and also within species variation due to micro site differences. This study examining growth and survival of seedlings of masting trees in a mixed-deciduous forest in southern New England showed that seedling recruitment varied in density within different species, sugar maple, white oak, red oak, white ash and red maple, but also within species in each of the three different topographic positions: ridge, midslope and valley (Frey et al., 2007). Additionally, individual genotype can affect forest variation, for example there is a genetic basis for the large variation in growth rate in the Northern Red Oak (Steiner, 1998).

Forests are continually changing due to natural succession, invasive species and changing climate patterns. Forest composition is partly dependent on the life cycle of forest trees, which could span from decades to centuries. To better understand how the abundance of Northern Red Oak will respond to anthropogenic or natural causes, this study focuses on reproductive and offspring traits. Specifically our study examines how age of the tree affects variation of reproductive traits, examined in the field and offspring traits, evaluated in the lab and greenhouse. Male and female reproductive tissues were both sampled. Northern Red Oak is a monoecious tree, in which both male and female reproductive tissues occur on the same tree. Male reproductive tissues, catkins, are defined as an inflorescence of male flowers. Female reproductive tissues remain in the tree canopy until fertilization, after which it becomes a single-seeded fruit, an acorn, after two growing seasons. To estimate abundance of male and female reproductive tissues, catkins and acorns were sampled on the ground directly below the canopies of individual trees. Individual acorn weight, also a reproductive trait of the mature trees, is a distinct trait from overall abundance. Acorn weight is both a reproductive trait of the parent tree and an offspring trait that potentially influences growth of the seedling.

Our study assesses the quality of offspring by weighing individual acorns and then growing seedlings to assess their quality, because seed mass in other oak species has a positive correlation with growth and survival of seedlings (Bonfil, 1998). A study on *Quercus ilex* found that seed size positively correlated with seedling establishment (Gomez, 2004). Variation in seedling quality and growth was evaluated through individual acorn mass and associated seedling traits including germination time, seedling biomass, specific leaf area and nitrogen content. Variation in these seedling traits is potentially related to variation among parent trees, including not only tree to tree variation, but also among trees of different age classes.

Data collected in the field and greenhouse study is used to test the hypothesis that variation in reproductive and offspring traits in the Northern Red Oak are influenced by variation in tree age class. Alternatively, variation in these traits may reflect variation among trees due to factors other than age. Tree to tree variation and age class variation are not mutually exclusive alternatives and both could affect the traits examined in this study.

Natural History of Northern Red Oak:

The Northern Red Oak, *Quercus rubra*, a hardwood, monoecious species common in the United States, occurs most frequently in a mixed forest with White Pine and Red Maple in northern forests and Yellow Poplar and White Oak in central forest regions (Crow, 1988).

Flowering

The male flowers are borne in catkins that emerge before or at the same time as leaves in April or May and are wind pollinated (Sander, 1990). The female flowers emerge from the axils of the current year's leaves (Sander, 1990). After fertilization in spring, the female flower matures into an acorn over two growing seasons and falls off trees in autumn.

Seed Production

In forests, Northern Red Oak start bearing acorns at around age 25 but do not produce abundantly until around age 50 (Sander, 1990). The Northern Red Oak is a masting species, in which there is a significantly larger than normal acorn crop at irregular intervals, usually every 2-5 years (Sander, 1990). Several hypotheses exist for the occurrence of masting in oaks such as a response to weather conditions and as an antipredator adaptation (Sork et al., 1993). An eight year study of flower and acorn production on three species of oaks concluded that patterns of acorn reproduction for black oak, red oak, and white oak are not simply due to weather events but are also a function of prior reproduction events suggesting masting as an evolved reproductive strategy (Sork et al., 1993). Masting has been suggested to be caused by the number of flowers or the survival of the flowers to fruit (Feret et al., 1982; Sork et al., 1993). The 8-year study's data demonstrated that flowers in the spring and the survival of these flowers have a significant impact on acorn crop size. Furthermore the variation in mature acorn crop size for Northern Red Oak was explained largely by premature abscission of flowers during the final season of development (Sork et al., 1993).

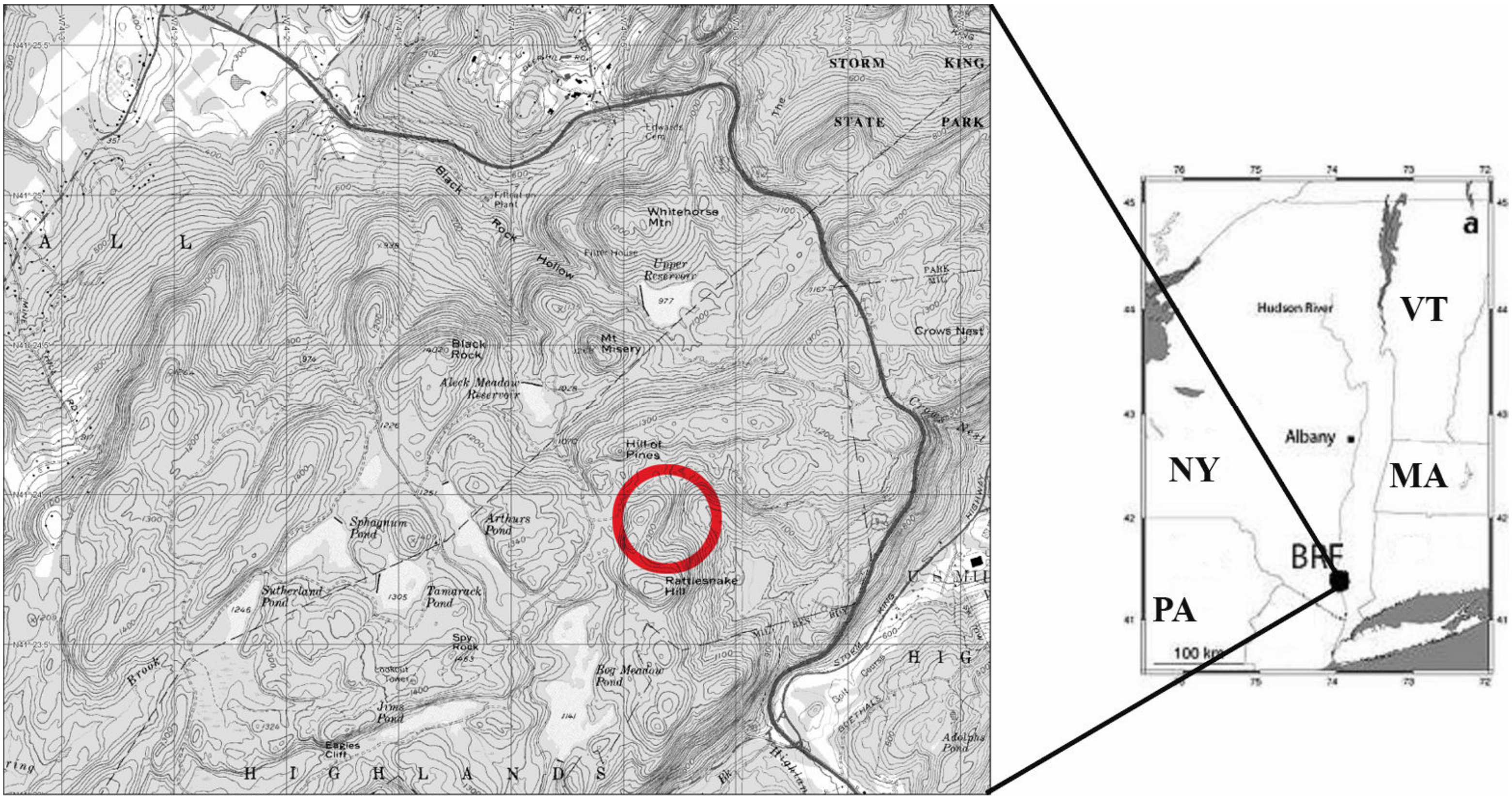
Seed Survival

There is widespread predation of acorns by animals causing few seeds to survive to become seedlings (Crow, 1988; Gomez, 2004). Although organisms such as birds, deer, rodents and insects all have a direct role in acorn survival, the acorn weevil (*Conotrachelus posticactus*) have been known to destroy from 66% to as much as over 90% of a typical acorn crop (Miller and Schlarbaum, 2005; Riccardi et al., 2004; Smith and Scarlett, 1987). Even in a mast year, where there is an unusually large acorn crop, only about one percent of the acorns become available for regenerating Northern Red Oak and as many as 500 or more acorns may be needed to produce a 1-year-old seedling (Sander, 1990).

Methods

Site description

The Black Rock Forest (BRF) is a 1550-ha forest preserve located in Cornwall, New York (41º24´ N, 74º01´ W; Figure 1). It is situated in the Hudson Highlands of New York State and is a mature mixed hardwood forest dominated by oaks and maples. A study looked at the changes in composition in BRF between 1930-2006 showed that the forest canopy remained dominated by red oak and chestnut oak followed by maples and birches. In this period , the forest understory changed with all oak species decreasing , red maple increasing and black birch increasing (Schuster et al., 2008). All three study sites (young, middle, old) are located at approximately at 41º23´58 N, 74º00´45 W.



3-D TopoQuads Copyright © 1999 DeLorme Yarmouth, ME 04096 Source Data: USGS 1000 ft Scale: 1 : 25,000 Detail: 13-0 Datum: WGS84

Figure 1: Map of The Black Rock Forest and its location in New York State. The red circle represents the approximate location of all three study sites (young, middle, old) within The Black Rock Forest.

Sampling

In May of 2006, catkins were collected from three *Quercus rubra* dominated stands at Black Rock Forest, aged 35-, 90-, and 135-years, respectively. The catkins, from 12 trees in each stand, were collected from the forest floor in a standard area of 0.64 m^2 plots. This was achieved by haphazard sampling using a uniform hula hoop where all catkins inside the hula hoop were collected. Two samples were collected at each tree.

From August through October of 2006, acorns from each of the 36 trees were sampled on four separate trips. The acorns were collected from the forest floor in a standard area of 0.64 m^2 plots. Two samples were collected at each tree.

Initial Data Collection

The field samples, consisting of whole acorns, partial acorns, seed caps, and attached material, were weighed for each tree on each sampling date. Samples were then stored in a cold chamber at ~4°C in their collection bags while individual acorns were tested for viability, using the "sink test", over next 3-4 weeks. The "sink test" is a general method to determine acorn viability (Miller and Schlarbaum, 2005). Floating acorns, which were likely rotted consumed and vacated by *Curculio* spp. (acorn weevil), were deemed inviable and discarded while acorns that sank were assumed to be viable. Individual viable acorns were weighed.

Growing Conditions

Having recorded the mass of individual viable acorns, they were placed in flats of 72 (12 x 6) plastic pots filled with moist vermiculite and subjected to cold-moist stratification at ~4°C for roughly 60 days. After cold conditioning, the acorns were placed on greenhouse benches (0.77 m high, 0.93 cm deep) facing east, west, or south, under natural light conditions in Barnard's Ross Greenhouse at ambient temperature (18-23°C daytime, 13-15°C nighttime). The acorns were top-watered with 21°C de-ionized water at every 2 days or as needed to maintain moisture and they were periodically monitored for germination, which was recorded on an approximately bi-weekly basis. In February, all confirmed viable acorns (displaying noticeable seed-coat rupture) were re-potted at a depth of ca. 2.5 cm in standard 21 cm sterilized (5% bleach solution) cones in standard greenhouse potting mix (mix of 70% sterile sphagnum peat, perlite, and vermiculite). A layer with around 4mL of Osmocote 14-14-14 fertilizer was added during re-potting at a depth of ca. 10 cm. Ten 60 x 30 x 18 cm racks were sterilized with 5% bleach solution, with 98 potted cones each. The cones were randomized into blocks with a random number generator to ensure equal distribution of acorns according to tree age, stand age, and collection date across racks, and distributed the racks evenly on greenhouse lab benches in numbered blocks.

After approximately one month of growth, crowding of vigorous seedlings necessitated increased spacing of the cones.

Data Collection and Transformation

There were two different harvests, the early harvest after approximately one month of growth and the late harvest after approximately four months of growth.

The data collected during each harvest were the harvest are harvest time, number of leaves, number of stems, leaf dry weight, stem dry weight, root dry weight and seedling height. Traits that were later acquired are greencover, specific leaf area, percent root mass, percent leaf mass and percent stem mass and total seedling dry weight.

The early harvest was in April, where 366 seedlings were destructively harvested over a span of 3 weeks. The seedlings in the early harvest were placed in a cold chamber of ~10°C to slow seedling growth during the harvest. Aboveground vegetative material was severed from the subterranean material (roots, cotyledons, seed coat). Cones containing soil and subterranean material were placed in a freezer at -10°C for later biomass assessment. Stems and leaves were counted and leaves were scanned on an HP Scanjet 8300, one scan per seedling, and color edited in computer program GIMP2. Edited scans were put in percent-green-cover assessment computer program R to quantify leaf area. The total stem length was measured to allow analysis of resource allocation. The biomass of the leaves and the stems were measured after all material was dried to constant mass at 50°C for 48 hours.

In May, the seedling roots were removed from the freezer and left to thaw at room temperature. The roots were carefully washed to isolate root biomass. First detaching the roots from the acorns and roughly removing the dirt by hand to prepare for washing, the roots were placed onto sieves of different sizes and soaked in a shallow bucket of water. A hose was used to wash off dirt attached to the roots, paying careful attention to fine roots. The roots were placed to dry in the sun for between 2 to 6 hours, labeled and placed in paper envelopes for drying. After being dried to constant mass at 50°C for a minimum of 48 hours, the dried roots were weighed.

The late harvest contained the same procedures as the early harvest. The aboveground and belowground harvesting in the late harvest was throughout the month of June.

Nitrogen:

Six seedlings from each of the three age classes from the early harvest were chosen for nitrogen analysis. The seedlings were chosen from different trees with acorn weight and other offspring traits to be within one standard deviation from the mean. One leaf from each of the 18 seedlings was ground into a fine powder using a ball mill. These samples were weighed on a microbalance, balled in pressed tin caps and sent out for nitrogen analysis at Pennsylvania State University

Soil Analysis

Two soil samples from underneath two trees in each age class were collected in October of 2007. A soil corer attained soil under the leaf litter to approximately 35 cm for each tree. Each age class had a total of four samples from two trees, that was mixed to form one large sample to be analyzed for nitrogen and carbon content at the Cornell Nutrient Analysis Lab.

Data Analysis:

The reproductive trait data were collected from 36 trees within 3 age classes. The traits were analyzed in Statistical Analysis Software (SAS) using a nested ANOVA model that included the effects of age class (young, middle, old) within year (2006, 2007). This model was elaborated into an ANCOVA model that included each tree's canopy volume (as reported by: Carson 2004) as a linear covariate. Individual acorn weight, which was both a reproductive and seedling trait was analyzed differently. This trait was analyzed using a nested ANOVA model with tree number nested within age class and age class nested within year.

For individual acorns, the associated seedling traits were analyzed using a nested ANOVA model with tree number nested within age class, age class nested within harvest time and 10 spatial blocks. Acorn weight and emergence time of seedlings were further put into two ANCOVA models to test as sources of covariation.

Results

Soil nutrient composition for the site at each age class showed a doubling of percent nitrogen and percent carbon in the old age class (Table 1). The C/N ratio was slightly lower in the old age class than the young or middle age class.

Table 1: Soil nutrient composition for the stands of each age class at BRF.

	Percent nitrogen	**Percent carbon**	**Carbon/Nitrogen ratio**
Young	0.28	7.22	26
Middle	0.28	7.38	26
Old	0.59	14.26	24

In the spring of 2006 and 2007, catkins from each of the 36 trees were collected (Table 2). We sampled 7786 and 6066 catkins in 2006 and 2007 respectively. For the young age class, 10% fewer catkins were collected in 2006 than 2007. In the middle and old age classes, there were 16% and 48% more catkins collected respectively in 2006 than 2007. The total number of catkins in 2006 was 22% more than that of 2007.

Table 2: Sum of catkins collected in 2006 and 2007 by age class.

	2006	**2007**	**Percent change from 2006 to 2007**
Young	1481	1629	+9.99
Middle	3577	3013	-15.77
Old	2728	1424	-47.80
Total	7786	6066	-22.09

The average number of catkins collected per tree for each age class was compared for 2006 and 2007 (Figure 2). In 2006, the average number of catkins per tree in the middle and old age class were not statistically different, but the middle age class was significantly higher than the young tree class. In 2007, the average number of catkins collected per tree in the young and old age class were not statistically different, but both were significantly fewer than the middle tree class. For both years, the trend was that the middle age class produced the most number of catkins per tree.

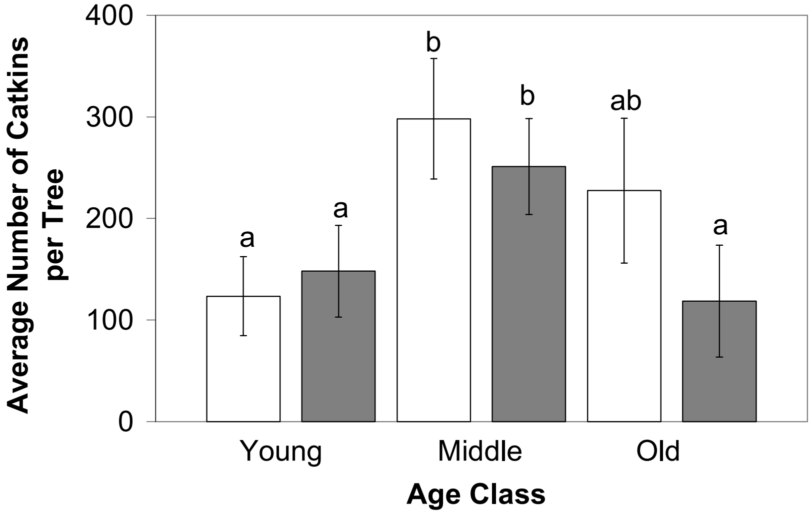


Figure 2: Average number of catkins by age class collected in 2006 and 2007. The unshaded bars represent data from 2006 and the shaded bars represent data from 2007. The error bars represent two standard errors from the mean in each category.

A total of 5017 and 656 acorns were collected at BRF in the fall of 2006 and 2007 respectively (Table 3). The total amount of acorns collected in 2006 was more than 7 times the amount collected in 2007. Among the acorns collected, 50% were mature acorns in 2006, compared to 64% in 2007. Of the mature acorns, 73% were viable in 2006 while 27% were viable in 2007.

Table 3: Totals and percentages for acorns collected in 2006 and 2007.

	2006	2007
Total acorns collected		
Young	970	192
Middle	1487	196
Old	2560	268
Total	5017	656
Total mature acorns		
Young	175	43
Middle	906	149
Old	1428	229
Total	2509	421
Percent mature of total collected		
Young	18	22
Middle	61	76
Old	56	85
Total	50	64
Total viable acorns		
Young	126	18
Middle	637	50
Old	1065	45
Total	1828	113
Percent viable of mature acorns		
Young	72	42
Middle	70	34
Old	75	20
Total	73	27

The adult reproductive traits were tested in nested ANOVA and ANCOVA models and tabulated (Table 4). Age class of the parent tree was highly significant as a source of variation in the number of catkins, the mass of catkins and the proportion of mature acorns. However, age class of the parent tree was not significant in the number of acorns collected. The year the samples were collected, 2006 or 2007, was not a significant source of variation for the number of catkins collected or the mass of the catkins, but was a highly significant source of variation for the number of acorns and the proportion of mature acorns, as 2006 was a mast year. Looking at both age class and year, when paired both are a significant source of variation only for the number of acorns collected. In the analysis of covariance, canopy volume was not a significant source of variation for catkin number, catkin mass, number of acorns and proportion mature of acorns collected.

Table 4: Summary of ANOVA and ANCOVA models to test sources of variation in adult tree reproductive traits. *** represents p-value of <0.001, ** represents p-value of <0.01, * represents p-value of <0.05 and n.s. represents p-value of >0.05. Stand age, year, stand age * year are categorical variables. Canopy volume is a continuous variable.

	ANOVA				ANCOVA
	Age class	Year	Age class * Year		Canopy volume
Catkin Number	***	n.s.	n.s.		n.s.
Catkin Mass	***	n.s.	n.s.		n.s.
Number of Acorns	n.s.	***	**		n.s.
Proportion Mature of Total	**	**	n.s.		n.s.

The mean acorn weight of the mature viable acorns collected from each age class was compared for 2006 and 2007 (Figure 3). The mean acorn weight in 2007 was higher than that of 2006 in all age classes. In the young age class, the mean acorn weight was 12% higher in 2007 than 2006. In the middle age class, the mean acorn weight was 16% higher in 2007 than 2006. In the old age class, the mean acorn weight was 23% higher in 2007 than 2006. Furthermore, in 2006, the mean acorn weight in the young age class was significantly different than that of the old age class. This relationship did not hold true in

2007, as there is no significant difference between any of the age classes in mean acorn weight.

Figure 3: Mean acorn weight of mature viable acorns by age class of the parent tree. The unshaded bars represent data from 2006 and the shaded bars represent data from 2007. The error bars represent two standard errors from the mean in each category.

Seedling traits

Out of the acorns that germinated in 2006, 708 became seedlings with 366 in the early harvest and 343 in the late harvest. Seedlings in the late harvest were about 3 times larger than those of the early harvest (Figure 4). In the early harvest, seedlings of parents from different age classes were not statistically different from one another. In the late harvest, the mean seedling dry weight was 13% larger in the old age class than the young age class, but there was no significant difference between any age classes in the late harvest.

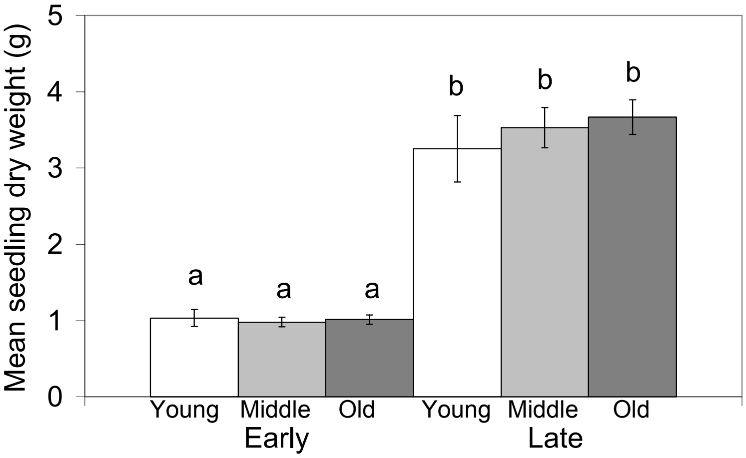


Figure 4: Mean seedling dry weight of above and below ground tissues, separated into harvest time and age class of the parent tree. The error bars represent two standard errors from the mean in each category.

Looking at the variation of the mean seedling dry weight in the late harvest, there was no statistical difference between the aboveground traits in the late harvest, but there was some variation of the mean root dry weight (Figure 5). For the mean root dry weight, there was no statistical difference in the different age classes of the early harvest. However, in the late harvest, there was a significant difference between the young age class and the old age class.

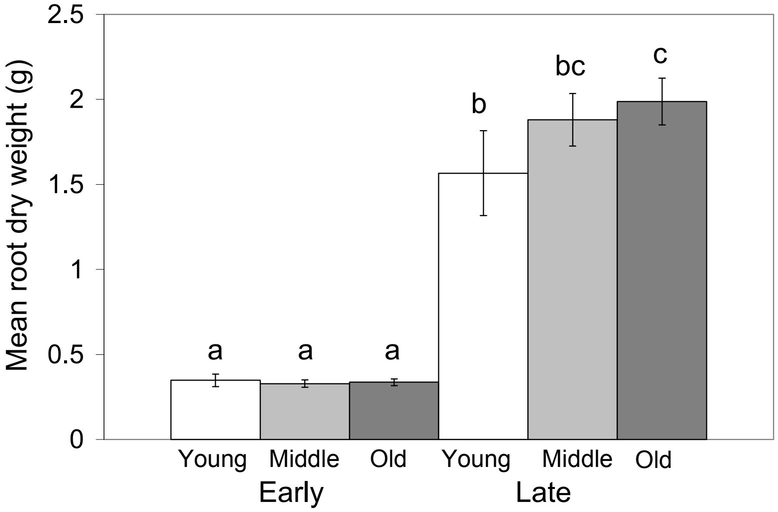


Figure 5: Mean root dry weight, separated into harvest time and age class of the parent tree. The error bars represent two standard errors from the mean in each category.

Sources of variation in all seedling traits were tested in age class of parent tree, tree number and block. Other sources of covariation in the seedling traits were tested in acorn weight, acorn weight and age class, emergence date and emergence date and age class (Table 5). Age class of the parent tree was not a noticeable source of variation for any of the seed and seedling traits. Tree number was a highly significant source of variation in the seed traits, acorn weight and emergence date. But tree number was not a source of variation in the seedling traits except for leaf dry weight. Additionally, block or placement in the greenhouse was a significant source of variation in the emergence date, seedling dry weight, specific leaf area and root dry weight but was not in leaf dry weight and stem dry weight. Acorn weight was a highly significant source of covariation in all of the seedling traits but was not significant in emergence date. Acorn weight paired with age class was not a source of covariation in any of the traits except for specific leaf area. Emergence date was not a source of covariation in the seedling traits except for specific leaf area.

Table 5: Summary of nested ANOVA and ANCOVA models to test sources of variation in seed and seedling traits. *** represents p-value of <0.001, ** represents p-value of <0.01, * represents p-value of <0.05 and n.s. represents p-value of >0.05.

	Nested ANOVA				ANCOVA model		ANCOVA model	
	Age class	Tree number	Block		Acorn weight	Acorn weight * Age class	Emergence date	Emergence date * Age class
Acorn Weight	n.s.	***						
Emergence Date	n.s.	***	*		n.s.	n.s.		
Seedling Total Dry Weight	n.s.	n.s.	***		***	n.s.	n.s.	n.s.
Specific Leaf Area (cm^2/g)	n.s.	n.s.	***		***	*	**	n.s.
Leaf Dry Weight	n.s.	*	n.s.		***	n.s.	n.s.	n.s.
Stem Dry Weight	n.s.	n.s.	n.s.		***	n.s.	n.s.	n.s.
Root Dry Weight	n.s.	n.s.	***		***	n.s.	n.s.	n.s.

Discussion

Data collected in the field and greenhouse study was used to test the hypothesis that variation in reproductive and offspring traits in the Northern Red Oak are influenced by variation in tree age class.

Major patterns in observation show that trees in the middle age class produced a larger amount of catkins in both 2006 and 2007. Acorn rain for catkins in 2006 occurred in 2007 but acorn rain for catkins in 2007 will occur in 2008. We therefore only have data for one complete reproductive cycle and cannot make comparisons between years. It is unlikely that both 2007 and 2008 will be mast acorn years since masting has been suggested as an evolved reproductive strategy related to resource thresholds (Sork et al., 1993) and occurs every 2-5 years (Sander, 1990). Masting has been suggested to be caused by the number of flowers or the survival of the flowers to fruit (Feret et al., 1982; Sork et al., 1993). In our data, age class was a highly significant source of variation in both catkin number and catkin mass (p-value <0.001) (Table 4), suggesting that masting could also be related to tree age.

Even though the amount of catkins collected were 22% higher in 2006 than 2007, there is no baseline for catkin abundance in a non mast year and not enough data exists to make conclusions on how masting is affected by the number of male flowers. However, in analyzing data for one complete reproductive cycle, the middle age class had the highest catkin abundance in 2006 but did not produce the most acorns in 2007. Rather in 2007, total acorns collected from the young and middle age class were practically identical, while that of the old age class was 38% higher. Although we do not have enough data to make conclusions on how catkins affect acorn abundance and therefore masting, our preliminary data does not suggest that catkin abundance directly affects acorn abundance. Our 2006 and 2007 catkin data could serve as a baseline for future sampling and could prove useful in the relationship between catkins and masting.

In 2006, the number of viable acorns was much larger in the old age class than the middle and young age class. The old age class produced more than 8 times the number of viable acorns than the young age class (Table 3), thus supporting that abundant acorn production of Northern Red Oak begins after age 50 (Sander, 1990). Two of the study age classes (middle and old) were older than age 50, while the young age class was only 35 years old.

Year 2006 was a mast year, in which 7 times more acorns were collected than in 2007 (Table 3). The percent of mature acorns was higher in all three age classes in 2007 but the percent of viable acorns was 27% in 2007 compared to 73% in 2006. The phenomenon of masting has been additionally suggested as an antipredation strategy (Sork et al., 1993), in which there is an oversaturation of acorns that exceed the amount predators can consume. If this strategy holds to be accurate, then in 2006 there are a larger percentage of viable acorns because there are too many acorns for predators to consume. But in 2007, a non mast year, acorns are generally scarcer and those that are found by predators are easily consumed leading to many damaged acorns that were nonviable for germination and seedling growth.

Seed mass is both a reproductive trait of the parents and an offspring trait because in oak species seed mass has a positive correlation with germination success and seedling growth (Bonfil, 1998). In 2006, the heavier acorns were produced by the young age class, but these seedlings were generally smaller. Mean seedling dry weight in the late harvest was significantly less in seedlings of the young age class than those of the old age class (Figure 4) suggesting a negative relationship between acorn weight and seedling dry weight. Contrary to the accepted assumption that larger seed mass leads to better quality seedlings, our data for 2006 shows that larger seed mass was not linked to better growth and survival in the seedlings.

In our 2007-2008 study, the acorns collected have not shown similar findings in the relationship of acorn weight and age class. The acorns will be grown into seedlings to test the relation of seedling weight and acorn weight.

Furthermore, emergence date could be a potential source of variation in seedling weight. The seedlings that emerged earlier would have from a day to two weeks extra to grow and therefore seedling size differences would not be due to genetic or parental variation but emergence date. However, when tested, emergence date was not a significant source of variation in seedling weight (Table 5).

Although our experiment found no significant relationship between any aboveground traits and mean seedling dry weight, belowground traits related to much of the variation (Figure 5). There is a general lack of knowledge on belowground tree responses (Ceulemans et al., 1999), suggesting that belowground processes could play a more critical role in forest composition than previously expected. Our study supports further research in belowground traits as they were found to comprise much of the variation in mean seedling dry weight.

Additionally, site differences between the different age stands are evident in the soil as the old age class had about double the amount percent carbon and percent nitrogen than the middle and young age class. Soil composition plays a critical role in the growth and survival of plants. Available nitrogen is a limiting nutrient that affects growth and performance on forest trees (Jose et al., 2003). Many plants will respond positively to increased nitrogen if other nutrients are also readily available. A study (Miyagi et al., 2007) on annual plants testing seed production found that seed production is limited primarily by nitrogen availability. Through the past 100 years, nitrogen deposition has steadily increased in temperate forest areas (Brimblecombe and Stedman, 1982), affecting the composition of forest soils. Differences in soil nitrogen composition at each of the study sites at BRF may have affected the quality of acorns and lead to variations in seedling weight.

For the 2006 acorns, the young tree class produced fewer seeds, the seeds were bigger (Figure 4) yet these seedlings still failed to perform better (Figure 5). This complex relationship shows that many factors may be influencing seedlings in a controlled environment. Unfortunately, seedlings have not been tested in a mature forest ecosystem, where there is predation selection and soil composition differences and such factors may influence their establishment in forests.

In addition to widespread predation of acorns by animals in forests, causing few seeds to survive to become seedlings (Crow, 1988; Gomez, 2004), there is also a lack of seedling establishment. The lack of Northern Red Oak seedling establishment at Black Rock Forest (Schuster, 2007) is not an isolated incident. Unsuccessful oak establishment in the understory of forests is widespread (Crow, 1988; Lorimer et al., 1994; Nowacki and Abrams, 1992) partially due to the poor performance of oaks in shade.

Our study aims to better understand natural variations in reproductive and offspring traits to apply in forest management. Our data display that there is a wide range of variation in reproductive and offspring traits specifically related to tree age, individual variation and micro site differences. However, the specific influence of each of these factors is not known and more research is needed in this area. This study is the first two years of continuous sampling that will span several years to include year to year variation. Understanding the background variation in the reproductive cycle of this important species is crucial in predicting the potential changes in forests due to anthropogenic and natural changes. Since forests has the potential to sequester significant additional quantities of carbon (Dixon et al., 1994; Schimel et al., 2001), recognizing forest dynamics and the changes associated with them are therefore integral to understanding the global climate system.

Conclusion:

Developing forest models and predictions to address problems such as a lack of regeneration could prove to be useful in many forests. Looking at whether reproductive traits vary according to age helps identify how age is a factor associated with reproduction. Research in this area may provide important tools in forest management especially in the current environment of failure in seedling recruitment. In addition, since our data did not show a direct relationship between seed mass and seedling quality, this area needs to be further addressed. Also, future data on catkin abundance and associated acorn crops could shed light on masting events, a key process in the reproductive cycle of the Northern Red Oak.

Acknowledgements:

I thank the Hughes Science Pipeline Project for financial support and the incredibly helpful staff at Black Rock Forest for guidance with field work. Thanks to Dr. Hilary Callahan for mentoring and guiding us through it all and Dr. Martin Stute for advising. Thanks to Dr. Kevin Griffin for the use of the ball mill.

Of course I am extremely grateful to Angelica Patterson who helped every single step of the way, Mei-Ho Lee for offering me much support and my fellow interns who labored beside me and shared equally in all of the work Samantha Roberts and Lu Gomezdelat. Also, thanks to the interns who started the field and lab work, Mia Mattioli, Sarah Tepler and Naomi Wiener. Lastly, thanks to Terry Lin who provided much needed technical assistance.

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