

Ability of chestnut oak to tolerate acorn pruning by rodents

The role of the cotyledonary petiole

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Abstract Acorns of many white oak species germinate soon after autumn seed fall, a characteristic widely interpreted as a general adaptation to escape predation by small rodents. However, the mechanism by which early, rapid germination allows escape and/or tolerance of seed damage remains unclear. Here we reported how specific germination traits of chestnut oak (*Quercus montana*) acorns, and those of other white oak species, allow successful escape from acorn pruning by rodents. During germination, chestnut oak acorns develop elongated cotyledonary petioles, which extend beyond the distal end of the acorn (1–2 cm) to the point at which the epicotyl and radicle diverge. However, granivorous rodents often prune the taproots above or below the plumule when eating or caching these germinated acorns in autumn. Hence, we hypothesized elongation of cotyledonary petioles allows chestnut oaks to escape acorn pruning by rodents. We simulated pruning by rodents by cutting the taproot at different stages of germination (radicle length) to evaluate the

regeneration capacity of four resulting seedling remnants following taproot pruning: acorns with the plumule (remnant I), acorns without the plumule (remnant II), and pruned taproots with (remnant III) or without the plumule (remnant IV). Our results showed that remnant I germinated into seedlings regardless of the length of the taproot previously pruned and removed. Remnant III successfully germinated and survived provided that taproots were ≥ 6 cm in length, whereas remnant IV was unable to produce seedlings. Remnant II only developed adventitious roots near the severed ends of the cotyledonary petioles. Field experiments also showed that pruned taproots with the plumule successfully regenerated into seedlings. We suggest that the elongated cotyledonary petioles, typical of most white oak species in North America, represent a key adaptation that allows frequent escape from rodent damage and predation. The ability of pruned taproots to produce seedlings suggests a far greater resilience of white oaks to seed predation than previously anticipated.

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Introduction

For many trees that bear large-sized seeds, small rodents play important roles as both seed dispersers, as a result of caching activities (Vander Wall 2001), and seed predators, by consuming and killing seeds or seedlings (Crawley 2000; Kitajima and Fenner 2000; Hulme and Benkman 2002). Many seed consumers exhibit close ecological and evolutionary relationships with the plant seeds on which they feed, often responding very specifically to certain seed traits, which in turn influences the probability of seed dispersal and caching, and hence seed survival and

establishment (Steele et al. 2001a, b; Vander Wall 1990, 2001, 2010; Jansen et al. 2006; Cao et al. 2011; Yi et al. 2012).

Seed storability (i.e., seed perishability) has proven to be a particularly important factor for hoarding animals when selecting seeds for long-term caching (Reichman 1988; Gendron and Reichman 1995), including those that scatter hoard seeds and typically facilitate seed dispersal (Hadj-Chikh et al. 1996; Chang et al. 2009). The long evolutionary relationship between seeds and the rodents that both consume and disperse seeds has resulted in a variety of adaptations of rodents to avoid seed perishability or cache losses (Elliott 1978; Fox 1982; McEuen and Steele 2005; Steele et al. 2001a, 2007). Numerous recent studies, for example, have revealed a variety of behavioral responses of rodents (e.g., embryo excision, pericarp removal) for maximizing returns from scatter hoarded seeds (Vander Wall 1990; Jansen et al. 2006; Xiao et al. 2009, 2010; Cao et al. 2011; Yi et al. 2012).

Seed perishability and its influence on scatter hoarding decisions have been studied extensively in the oak (*Quercus*) dispersal system, where it is clear that perishability due to germination schedules significantly influences caching decisions (Hadj-Chikh et al. 1996), seed survival (Moore et al. 2007), and possibly even the distribution of many oak species (Steele et al. 2007). The lack of dormancy in white oak species (section *Quercus*), for example, results in the selective consumption of these acorns by rodents over those in the red oak group (section *Lobatae*), which typically exhibit a dormancy period (Steele et al. 2001a,b). However, acorns of most white oak species germinate in autumn soon after seed fall or even while still attached to the tree (Fox 1982), which results in the rapid transfer of nutritional reserves from the cotyledons into an inedible, robust taproot (Lewis 1911; Fox 1974; Barnett 1977; Vander Wall 1990; Hadj-Chikh et al. 1996; Jansen et al. 2006; Steele et al. 2001a; Xiao et al. 2010). This early germination of white oak species is thus widely considered a general adaptation to escape seed consumption by seed predators, especially rodents (Barnett 1977; Fox 1982; Vander Wall 1990; Steele and Smallwood 2001). In response, various rodent species in several oak biomes of North America, Mexico, and China appear to retard or completely arrest germination of acorns by either pruning the radicles (Yang et al. 2012; Yi et al. 2012) or removing the entire embryo (Fox 1982; Steele et al. 2001b; Steele 2008; Xiao et al. 2009, 2010), resulting in an ability to further exploit early germinating white oaks. Although one study demonstrates how several oak species that produce multiple-seeded (embryos) within single acorn may overcome embryo removal by tree squirrels (McEuen and Steele 2005), we know little about whether white oak species can successfully escape taproot pruning and similar damage by small rodents (but see Yi et al. 2012). Here, we

demonstrate that the chestnut oak *Quercus montana* (Sands and Abrams 2009) and several other white oak species exhibit a germination morphology distinct from that of many red oak species, which allows the seedling to sustain early radicle pruning (i.e., cotyledon and plumule removal) by rodents.

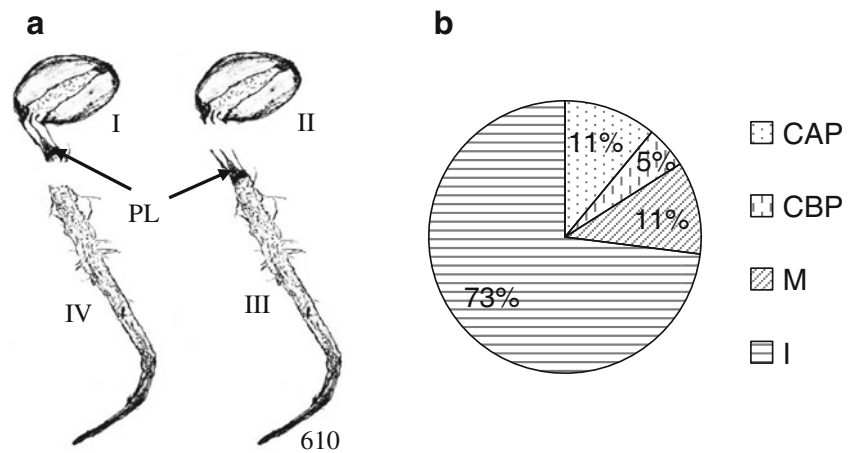
During germination, cotyledonary petioles (Lewis 1911) of acorns of the white oak species *Q. virginiana* elongate and extend well beyond the distal end of the acorn to the cotyledonary node (the point at which the epicotyl and radicle appear to separate) resulting in a considerable distance (1–3 cm) between the distal end of the acorn and the developing epicotyl (see also Lewis 1911) (Fig. 1a). The length of the cotyledonary petiole of white oak species (e.g., *Q. alba* and *Q. montana*) usually reaches its maximum when the radicle is more than 2 cm long (Yi, personal observation). These extended cotyledonary petioles push the developing radicle (taproot) into the soil (Fig. 1b), thus allowing it to rapidly anchor itself prior to the onset of winter. In the spring, the epicotyl then begins to grow from the plumule (embryonic stage of the epicotyl) at the interface (cotyledonary node) of the cotyledonary petiole and the radicle. Rodents frequently harvest and cache germinated chestnut oak acorns by severing the cotyledons from the anchored taproots using their incisors (Yi, personal observation; Fig. 1b,c). Because rodents (eastern chipmunks [*Tamias striatus*] and eastern gray squirrels [*Sciurus carolinensis*]) consistently prune (i.e., sever the cotyledons with incisors) the cotyledons from taproots, either above or below the plumule, which protrudes at the cotyledonary node, they generate four types of remnants: pruned acorns (cotyledons) with the plumule (remnant I) or pruned cotyledons without the plumule (remnant II) and pruned taproots with (remnant III) or without the plumule (remnant IV) (Fig. 2a).



a Anatomy of germinating acorns of *Quercus montana* **b** Remnant I with adventitious roots **c** Remnant II and III

Fig. 1 Germinated chestnut oak acorns and evidence of acorn pruning in the field. Shown are **a** germinated acorn with cotyledon (CL), cotyledonary petiole (CP), plumule (PL, embryonic stage of the epicotyl), taproot (TP), and pericarp (P); **b** germinated acorn pruned below the plumule by rodents (note the pruned acorn re-sprouted and produced adventitious roots); **c** germinated acorn pruned above the plumule (note the pruned taproot containing the plumule)

Fig. 2 Simulated acorn pruning and rodent pruning of acorns in the field. Shown are: **a** artificially pruned acorns, below (left) and above (right) the plumule; I pruned acorns with plumule, II pruned cotyledons without the plumule, III pruned taproots with the plumule, IV pruned taproots without the plumule; **b** proportions of acorns pruned above (CAP) and below (CBP) the plumule, missing acorns (M), and intact acorns (I) on five transects in May 2011



We hypothesize (1) that both pruned cotyledons (remnants I) and pruned taproots (remnant III) will regenerate and produce seedlings because they still contain the plumule, (2) that elongated cotyledonary petiole is the specific structure that allows chestnut oak acorns to still regenerate and establish following pruning, and (3) that cotyledonary petioles should be consistently longer in early germinating white oak species than in those of red oaks.

Materials and methods

Study area

Field surveys were conducted in 2010, on a 60-ha, middle-aged (50–70 years), second-growth stand of oak, hickory (*Carya* spp.), and maple (*Acer* spp.), 4 km south of Mountaintop, Pennsylvania, USA (41°05'N, 75°55'W) during a year with a large mast crop of chestnut oak acorns. Based on a 12-year mast survey in three nearby study sites in central and northeastern PA (Steele, unpublished data), we documented that the oak mast crop in 2010 was the second largest acorn crop in 12 years (2000–2011).

Field surveys of pruned and non-pruned acorns

Experimental field monitoring of acorn pruning

Preliminary observations in autumn 2010 also indicated intense rodent pruning of germinating chestnut oak acorns during the period of seed fall. Consequently, we tagged and monitored germinating acorns to follow their fates. In late November 2010, we marked five 60-m transects each separated by a minimum distance of approximately 20 m. All transects were set out in the forest site described above in an area where the canopy was dominated by chestnut oak. On each transect, we used flags to tag the nearest germinating

chestnut oak acorn with a well-developed, anchored taproot (>10 cm) every 2–3 m along the length of each transect ($n=20$ acorns on each transect). In May 2011, we located the tagged acorns and grouped each into one of four categories: intact in situ, pruned above the plumule, pruned below the plumule, and missing.

Collections of intact germinating acorns

We also randomly collected intact germinating seedlings of varying taproot lengths (i.e., 2 (0–2.0), 4 (2.1–4.0), 6 (4.1–6.0), 8 (6.1–8.0), 12 (8.1–11.9) and 16 cm (14.1–16.0, $n=20$ of each taproot length), and a sample of sound (non-infested, undamaged), but non-germinating acorns ($n=20$) from the same locations in the field in November of 2010. In May 2011, we collected another sample of acorns with developing taproots ($n=20$), 5 months after initial germination. Acorns were then dried in a drying oven at 80 °C and their masses compared to estimate the proportion of energy reserves (mass) transported to seedlings during different stages of germination. For these comparisons, we collected acorns of similar dimensions, to control for the variation in acorn sizes that typically occur between individual trees.

Field observations of pruning by squirrels

To further estimate the frequency of taproot pruning, we provided free-ranging eastern gray squirrels (*S. carolinensis*) with 160 germinated acorns with long taproots (5–10 cm). We presented squirrels with eight replicate piles of 20 germinated acorns at independent locations on the campus of Wilkes University (41°14'N, 75°53'W), and recorded if and how taproots were pruned by these squirrels and whether acorns were eaten by squirrels. Wilkes University is an urban campus with numerous gray squirrels but no other mammalian seed predators. We observed some squirrels feeding at these piles but allowed piles to remain on the ground for 10 h. to ensure that they were encountered

by gray squirrels. In all cases, gray squirrels were found to prune the taproots before they dispersed the remaining acorn cotyledons from the feeding station. The pruned taproots were then collected and we determined whether each was pruned above or below the plumule.

Assessment of germination and seedling performance

Laboratory germination of pruned cotyledons and taproots

We conducted laboratory experiments to evaluate the germination success of acorns, cotyledons and taproots. In early December 2010, when taproots of chestnut oak acorns approached their maximum autumn length just before the onset of heavy ground frost, we randomly selected approximately 400 germinated acorns of *Q. montana* from under the canopy of >10 potential parent trees. We carefully excavated the taproots of these germinating acorns, returned them to the lab, and temporarily stored them in humid conditions at 4 °C for subsequent germination experiments. In January, we first determined the frequency distribution of these randomly collected acorns with different taproot lengths and then sorted these germinated acorns into separate samples ($n=40$) for each of six categories based on taproot length (i.e., 2 (0–2.0), 4 (2.1–4.0), 6 (4.1–6.0), 8 (6.1–8.0), 12 (8.1–11.9) and 16 cm (14.1–16.0)). These categories represented the general distribution of taproot sizes observed in field and more importantly, allowed adequate samples of taproot lengths, which were necessary to determine the critical size at which pruned taproots may not survive.

To simulate the pruning of germinating acorns by rodents, we cut the taproots from each acorn either above or below the plumule (enclosed at the base of the cotyledonary petiole), generating four types of remnants: pruned acorns with plumule (remnant I) or pruned cotyledons without the plumule (remnant II) and pruned taproots with (remnant III) or without the plumule (remnant IV) (Fig. 2a). Thus, we generated a sample of 20 of each remnant type for each of the six taproot categories (480 total remnants). We planted these 480 remnants and followed their growth and survival. We planted a single remnant of each remnant type of the six taproot length classes into a single plant container (18 cm diameter × 17 cm high) and replicated this with 20 containers. All remnants were planted in a composite growing medium (3:1 mixture of Pro-Mix® potting medium: commercial top soil). This approach allowed us to control for any container effects across treatments (taproot lengths). This procedure was repeated for each of the four remnant types (80 containers). Plant containers were kept in the laboratory at room temperature and regularly watered. All containers were randomly moved every 2 weeks to eliminate any further possibility of a

position effect in the experiment. Rates of epicotyl emergence were measured at several intervals after planting until the experiment was completed.

Seedling performance

Following germination, we followed seedling growth to compare performance measures. For remnant III, seedling height and leaf number were measured 52 days after planting. This period ensured adequate time for seedling growth, which takes approximately 5 weeks for normal oak seedlings to reach about two to three tiers of leaves (Yi, Steele personal observations). For remnant I, seedling height, leaf number, root number, root length, fresh mass of epicotyls and root, and dry mass of the remaining cotyledon were measured 52 days after planting. Final survival rates of seedlings developing from remnants I and III were also recorded accordingly. At the same time, we reported the number of remnants II and IV that were still viable (alive). We also compared the dry masses of a random subsample of 20 sound acorns prior to germination, with an equal random sample of germinating acorns from each of the six categories of taproot length.

Field germination of pruned taproots

In early December 2010, we conducted a field experiment to determine if pruned taproots could still produce seedlings under natural conditions. We prepared ten additional transects at the Mountaintop site and on each transect tagged 20 germinating acorns with well-anchored taproots. Each acorn was spaced 2–3 m along the transect. On five transects, we severed taproots above the plumule, and on the remaining five transects, we severed taproots below the plumule. We then revisited these tagged remnants in May and August 2011 to determine the proportion of seedling establishment.

A survey of cotyledonary petioles in red and white oak species

To test the hypothesis that the cotyledonary petioles are substantially larger in early germinating white oak species (WO, subgenus, *Quercus*) than in red oak species (RO, subgenus, *Lobatae*), we measured the length of the cotyledonary petioles in samples (ranging from six to 51 seedlings) of seven WO and nine RO species from North America. Six of these species (three RO and three WO) were native to the USA; the others were native to Mexico. Seedlings of *Q. montana* were germinated acorns from the field without developing epicotyls, whereas all others were laboratory-grown seedlings with well-developed epicotyls, grown either in the laboratory at Wilkes University (*Q. alba*, *Q. rubra*, *Q. velutina*, *Q. palustris*, and *Q. macrocarpa*) or

in a field greenhouse at Benemerita Universidad de Puebla, in Puebla Mexico (all other species; M.A. Steele. unpublished data). All laboratory and greenhouse grown seedlings were measured prior to 6 weeks of growth. Because the length of cotyledonary petioles does not appear to change much after leaf emergence, any variation in conditions across these germination trials (maximized for oaks in each of the two regions) should not contribute to the size of these cotyledonary petioles.

Statistics and analysis

Analyses were performed using the program JMP 9.0.0 (SAS Institute Inc., Cary, NC, USA). The distribution of each variable was examined prior to analysis. When the data deviated significantly from a normal distribution (Shapiro–Wilk W test for goodness-of-fit, $P < 0.05$), they were \log_{10} -transformed to meet the assumptions of analysis of variance (ANOVA). In the case of Poisson distributed data, a generalized linear model (GLM) was used.

The effect of taproot length category (growth stage) on cotyledon dry mass, seedling height, leaf number, root length, epicotyl mass, and root mass was analyzed using ANOVA. Bartlett's test for unequal variances was used to check for heteroscedasticity among groups prior to each test. Variances were consistently homogeneous (all $P > 0.10$) except in one case for which we used Welch's test, which does not assume variances are equal. For several variables (cotyledon dry mass [for remnants I and II], seedling height and leaf number [for acorns with the plumule], root number, epicotyl mass and root mass), pot effects were modeled as a random variable with the response variable of interest fixed, and analyzed with mixed-model (Model III) ANOVAs using restricted maximum likelihood (REML) for parameter estimation. Pot effects were generally small or close to zero. For estimation, we bounded the variance components to be greater than zero. This assumes that any small non-positive estimates of variance components (which we found using unbounded estimation) are effectively zero. Our results were unaffected by choice of bounded or unbounded variance component estimation.

Data on root number were Poisson distributed. To test the effect of taproot length category on root number we used GLM with Poisson distribution and Log link function (log-linear model). Because GLM does not handle random effects, we modeled pot effects as fixed in this analysis.

Cumulative germination rates (i.e., on the final day of the experiment) for the different taproot length categories were initially analyzed using a chi-square test for independence. However, germination was generally very high (close to 100 %) regardless of taproot length category and the chi-square assumption of no more than 20 % of cells with less

than five observations was violated. Therefore, we do not present a statistical analysis of these data.

Finally, differences in cotyledonary petiole lengths between red oak species and white oak species were tested with a two-way ANOVA with species means as the response and group (red or white), region (USA or Mexico), and their interaction as fixed effects.

All statistical tests were considered significant at $P < 0.05$. All data are presented in graphical or tabular form without transformation. The following variables were \log_{10} -transformed prior to statistical analysis: cotyledon dry mass for Remnant I and Remnant II only; seedling height for taproots only; epicotyl mass and root mass. Remaining variables were not transformed.

Results

Our initial observations revealed that 73 % of the 100 germinated acorns tagged in the field remained intact on the transects in early May 2011, reflecting a strong satiation effect from the 2010 mast crop. Overall, 11 % of acorns were pruned by rodents above the plumule, 5 % were pruned below the plumule, and 11 % were missing (Fig. 2b). Similarly, when germinated acorns with long radicles were presented to free-ranging gray squirrels, >90 % ($n=144$) of taproots were pruned above the plumule. Squirrels then dispersed, ate or cached the pruned acorns; however, the final fates of these pruned acorns were not followed. These observations show that small rodents regularly prune the cotyledons above the plumule when eating or caching germinated chestnut oak acorns. Field surveys in August 2011 showed that, among these 100 control acorns, 23 seedlings with intact acorns survived; 4 seedlings without an acorn survived; 15 seedlings with acorns and 12 seedlings without acorns died; and 46 tagged germinated acorns were removed (i.e., neither seedlings, acorns, nor taproots could be relocated).

The frequency distribution of field-collected, germinated acorns was right-skewed towards those with longer taproots. The proportions of germinating acorns with taproot lengths of 2, 4, 6, 8, 12 and 16 cm were 10.02 %, 10.76 %, 17.35 %, 26.41 %, 20.78 %, and 14.67 %, respectively ($n=409$). These data suggest that a large proportion of acorns have already produced long taproots by early winter. Interestingly though, the biomass reserves (i.e., dry mass) in cotyledons of these germinated acorns did not differ significantly from those of non-germinated acorns, regardless of the stage of autumn germination (i.e., taproot length) (one-way ANOVA, $F_{7, 152} = 1.283$, $P = 0.2622$) (Fig. 3). This suggests that a relatively small proportion of energy reserves are actually transported into taproots during autumn germination, at least in this region of the chestnut oak's range.

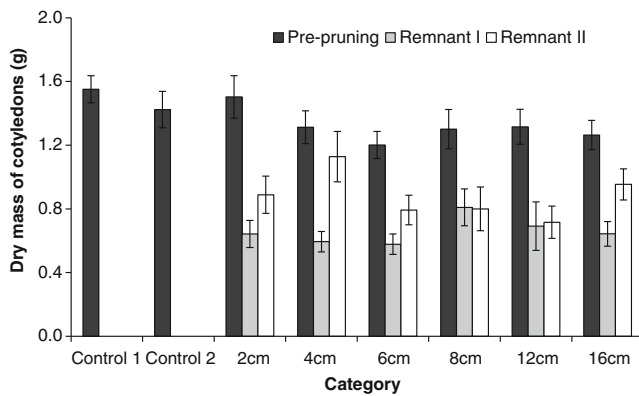


Fig. 3 Variation in dry mass of acorn cotyledons of field-collected acorns at different stages of development and with artificial pruning. Shown are dry mass of: sound, non-germinated acorns collected in autumn 2010 (Control-1); germinated acorns with taproots collected in May 2011 (Control-2); field-collected, germinated acorns with varying taproot sizes (2–16 cm) at the time of artificial pruning (Pre-pruning); artificially pruned acorns (with the plumule); following germination experiments (Remnant I); and artificially pruned acorns (without the plumule) following germination experiments (Remnant II). Data are expressed as mean \pm SE

In laboratory germination experiments, remnant I (acorns with the plumule) developed into seedlings regardless of the lengths of taproots pruned (Fig. 4a), and showed higher survival rates than the pruned taproots with the plumule (Yi et al. 2012). However, there were no significant differences among all measures of seedling performance in remnant I acorns at different stages of germination for seedling height (mixed-model ANOVA, $F_{5, 105}=0.209$, $P=0.958$, % total variance from pots=0; Fig. 5a), leaf number (mixed-model ANOVA, $F_{5, 105}=0.574$, $P=0.720$, % total variance from pots=0; Fig. 5b), root number (GLM, whole-model $\chi^2_{14,43}=11.635$, $P=0.636$; Fig. 6a), root length (one-way ANOVA, $F_{5, 52}=0.712$, $P=0.617$; Fig. 6a), epicotyl mass (mixed-model ANOVA, $F_{5, 51}=0.849$, $P=0.522$, % total variance from pots=0; Fig. 6b), and root mass (mixed-model ANOVA, $F_{5, 43.45}=0.824$, $P=0.539$, % total variance from pots = 18.70; Fig. 6b). Interestingly, remnant I consistently produced multiple seminal roots instead of a single taproot, although this did not appear to influence subsequent seedling performance, which presumably benefits seedling establishment and growth following pruning. There was a clear decrease in dry mass of these pruned acorns compared to controls (Fig. 3), suggesting the role of cotyledonary reserves in supporting seedling establishment after pruning, but no difference in dry mass for the different taproot length categories (mixed-model ANOVA, $F_{5, 45}=0.772$, $P=0.575$, % total variance from pots = 12.50; Fig. 3).

Remnant III successfully regenerated a seedling regardless of its length (Fig. 4b). Moreover, these taproot-generated seedlings survived and successfully established provided the taproots were ≥ 6 cm in length at the time of

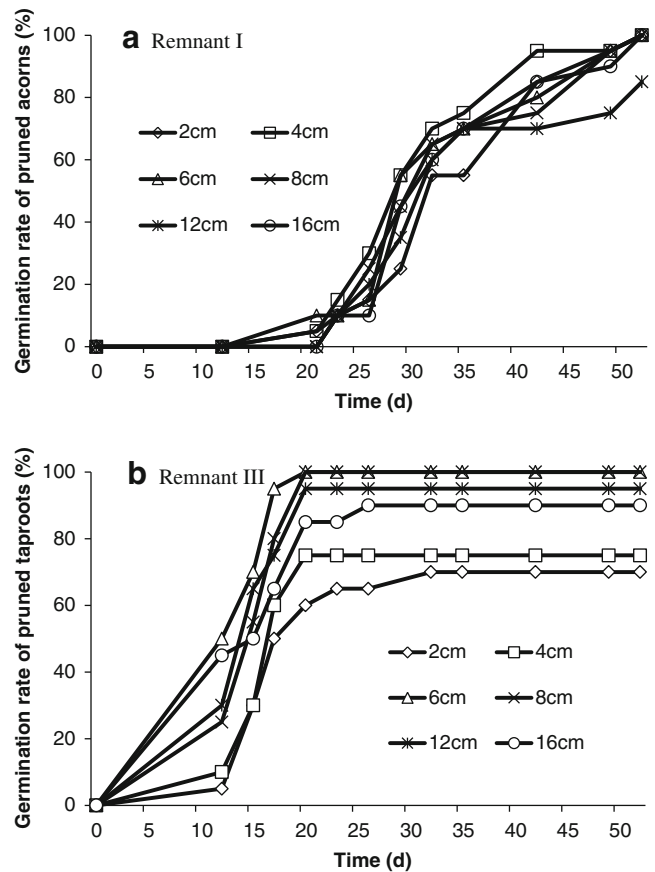


Fig. 4 Seedling emergence from artificially pruned acorns and taproots. Shown are: **a** pattern of seedling emergence from remnant I for each of the six categories of taproot size; and **b** pattern of seedling emergence from remnant III for each of the six categories of taproot size

pruning (Yi et al. 2012). No difference was found in the cumulative germination rates of taproots with different lengths ($\chi^2=9.434$, $df=5$, $P=0.093$). Although taproots less than 4 cm did elongate the epicotyl, none of these seedlings survived to the end of the experiments. Neither seedling height (one-way ANOVA, $F_{3, 49}=0.494$, $P=0.688$; Fig. 5a) or leaf number (one-way ANOVA, $F_{3, 49}=0.540$, $P=0.657$; Fig. 5b) was significantly different among the different taproot length categories of remnant III. Across taproot length categories for which seedlings survived and established (i.e., 6–16 cm), seedlings regenerated from remnant III exhibited lower performance than those from remnant I in terms of seedling height (Welch's test, $t_{1, 121.03}=1.899$, $P=0.060$) and leaf number (Welch's test, $t_{1, 123.89}=1.950$, $P=0.053$) (Fig. 5).

Remnant II remained alive for more than 4 months in the laboratory, producing multiple adventitious roots near the severed ends of the cotyledonary petioles regardless of the length of taproots removed, further indicating that even plumule removal cannot cut off the transfer of nutrients from cotyledons. In addition, we observed a significant

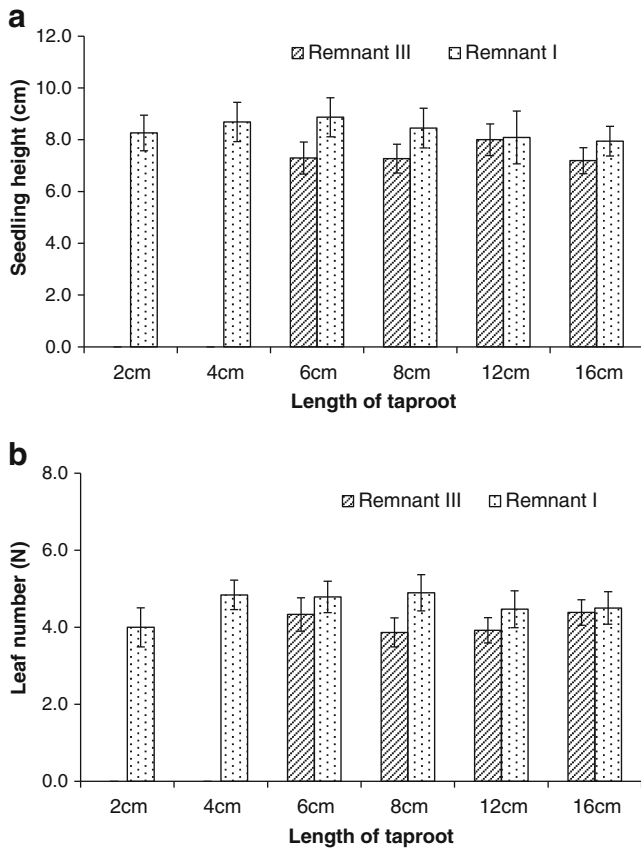


Fig. 5 Comparisons of performance in terms of seedling height (a) and leaf number (b) for seedlings grown in the laboratory from remnants I and III. Data were expressed as mean ± SE. All measurements were taken at the end of the experiment (day 52). Seedlings that developed from pruned taproots ≤4 cm all died prior to day 52

decline in dry mass of the cotyledons of remnant II compared with control acorns, most likely indicating significant nutrient transfer from cotyledons into multiple adventitious roots (Fig. 4e), but no difference among taproot length categories (mixed-model ANOVA, $F_{5, 45}=1.395$, $P=0.244$, % total variance from pots = 6.56; Fig. 3). Remnant IV only produced fine lateral roots.

Overall, there were no substantial pot effects in that they contributed little variance to the response variables. The exception was root mass where variation among pots accounted for 18.7 % of the total variance.

Field surveys in May 2011 showed that 51 % ($n=100$) of the tagged taproots with the plumule successfully germinated into seedlings on the five transects (Fig. 7). Moreover, we found 13 seedlings that developed from these taproots (with the plumule) survived on the transects in August 2011. None of the taproots without the plumule germinated and produced seedlings. We found that some tagged taproots with or without the plumule were excavated and partially eaten by rodents, possibly voles (*Myodes gapperi*).

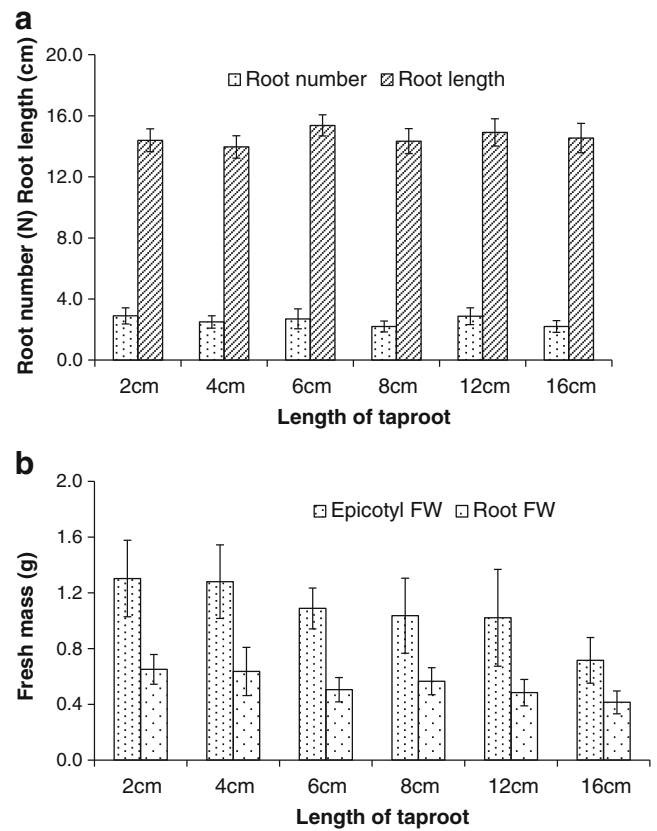


Fig. 6 Seedling performance of remnant I following artificial removal of taproots of different lengths. Shown are the root length and number of roots from pruned acorns with the plumule (a), and the fresh, above- and below-ground mass of seedlings from pruned acorns with the plumule (b). Data are expressed as mean ± SE. FW fresh weight

Cotyledonary petioles of the seven white oak species were consistently larger than those of the seven red oak species (Table 1). However, in two red oak species from northeastern deciduous forests (*Q. rubra* and *Q. velutina*),

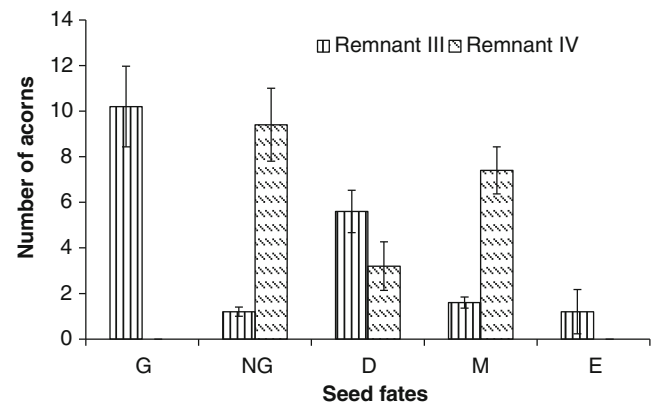


Fig. 7 Fates of 100 artificially pruned taproots on five transects in May 2011. Shown are the fates of remnants III and IV; fates include germinating (G) non-germinating (NG), dead (D), missing (M), and partially eaten by voles or other rodents (E). Data are expressed as mean ± SE

this structure was well within the range of that of white oaks. Cotyledonary petioles of white oaks from central Mexico were 3-fold the size of the homologous structure in red oaks from the same region, although in many cases it appeared that the cotyledonary petiole in these red oaks was virtually non-existent (Table 1). Two-way ANOVA revealed a significant interaction between group (red oak vs. white oak) and region (USA vs. Mexico) (Table 2), consistent with large differences in the length of cotyledonary petioles between red oaks and white oaks in Mexico, but no difference in the USA (Table 2).

Discussion

Although rodents selectively consume acorns of white oak (WO) species over those of red oaks (RO), which they selectively disperse and cache (Steele et al. 2007), results of the present study suggest that rapid regeneration from taproots, following acorn pruning by rodents, provides WO species with an alternative ability for tolerating damage by rodents. Previous authors have argued that autumn germination of white oak species results in the rapid transfer of

Table 1 Length of cotyledonary petioles (distance between the apical end of the acorn and the point at which radicle and epicotyl diverge) of several white oak (Section, *Quercus*) and red oak (Section, *Lobatae*) species

	Section	Species	(n)	Length (mm) mean ± SE
Northeastern deciduous forests	<i>Quercus</i>	<i>Q. alba</i>	39	12.77±0.53
		<i>Q. montana</i>	25	12.68±0.47
		<i>Q. macrocarpa</i>	39	9.84±0.33
	<i>Lobatae</i>	<i>Q. rubra</i>	31	14.25±0.78
		<i>Q. velutina</i>	20	11.57±0.73
<i>Q. palustris</i>		30	7.07±0.50	
Mexico	<i>Quercus</i>	<i>Q. glaucooides</i>	8	18.3±2.4
		<i>Q. laeta</i>	6	27.0±2.3
		<i>Q. microphylla</i>	51	21.6±0.2
		<i>Q. peduncularis</i>	6	17.0±2.4
		<i>Q. crassipes</i>	8	6.2±0.9
	<i>Lobatae</i>	<i>Q. crassifolia</i>	9	7.0±0.6
		<i>Q. dysophylla</i>	9	2.3±0.6
		<i>Q. mexicana</i>	17	3.5±0.4
		<i>Q. scytophylla</i>	5	2.6±1.1
		<i>Q. conspersa</i>	19	2.9±0.7

Sample sizes are number of seedlings measured. Seedlings of *Q. montana* are from germinated acorns without developing epicotyls, randomly collected in the field. All other seedlings had produced epicotyls. Seedlings of *Q. alba*, *Q. rubra*, *Q. velutina*, *Q. palustris*, and *Q. macrocarpa* are from germinating acorns in laboratory experiments at Wilkes University. All other results are for seedlings from greenhouse experiments conducted in Central Mexico

Table 2 Two-way ANOVA of the length of cotyledonary petioles in red oak and white oak species (Group) from the USA and Mexico (Region) (Model $F_{3, 11}=15.239$, $P=0.0003$)

Source	df	Type III SS	F ratio	P>F
Group	1	0.504	18.871	0.0012
Region	1	0.032	1.195	0.298
Group × Region	1	0.398	14.896	0.0027
Error	11	0.294		
Corrected total	14	1.514		

nutritional reserves from acorn cotyledons into the taproot, which allows white oak species to escape predation by reducing the acorn's attractiveness to small rodents and jays (Lewis 1911; Barnett 1977; Fox 1982; Vander Wall 1990). While perhaps true, our results also show strong evidence that there is no significant difference in cotyledon biomass before and after germination of *Q. montana* acorns, and that rodents regularly remove these cotyledons following autumn germination. Moreover, we found that the pruned taproots (containing a plumule) produce normal seedlings both in the laboratory and field.

We show that taproots of a white oak species can survive following acorn (cotyledon) removal by small rodents (see also Yi et al. 2012). The only other report of successful establishment from rodent-pruned taproots was recently observed for a tropical species (*Pittosporopsis kerrii*) by Cao et al. (2011). Although pruned taproots (with the plumule) showed high shoot survival, suggesting an ability to escape predation by small rodents following cotyledon removal, the highest germination rates were exhibited by pruned cotyledons which retained the plumule. Furthermore, the seedlings produced from the cotyledons with a plumule showed higher performance (e.g., seedling height) than those produced by taproots.

Cotyledonary petioles appear to show three key functions that collectively increase the chances of escape from rodent predation: (1) movement of the plumule outside the acorn, which may be a direct response to embryo excision by tree squirrels, (2) rapid extension (pushing) of the taproot into the soil, and (3) significant separation of the radicle and epicotyl from the acorn, which allows potential seedling survival following pruning of the cotyledon. This conclusion is further supported by our observations that cotyledonary petioles are far more common and better developed in most early germinating WO species than in RO species, at least in some regions where taproot pruning is common (Tables 1 and 2).

Why two species of red oaks in northeastern deciduous forests also show a well-developed cotyledonary petiole is not clear. However, it is interesting to note that whereas a cotyledonary petiole is produced in *Q. rubra*, the plumule

remains in the acorn until the taproot reaches >8 cm in length (Yi et al. 2012). This retention of the plumule means that *Q. rubra* acorns retain the ability to germinate regardless of how many times they are cached and re-cached by small mammals, which appears to be common (>25 % of initial caches) in eastern deciduous forests of North America (A. Bartlow, unpublished data). This delay in plumule emergence may be an important mechanism during the pruning and caching of these acorns in the spring. Red oak species (*Q. rubra* and *Q. velutina*) also experience acorn removal by animals in spring after they break dormancy (Bossema 1979; Yi, personal observation), therefore, elongation of cotyledonary petioles may also help seedlings of red oak species avoid damage by post-seedling acorn predation. However, regardless of the specific function of cotyledonary petioles, our observations that pruned taproots with the plumule can successfully germinate and establish provide strong evidence for how rapid germination of chestnut oak acorns may allow for seedling establishment following rodent damage.

Based on these findings, we argue that dispersal and establishment of chestnut oak, and WO species in general, may be more complicated than previously considered. Past studies suggest that the higher perishability (due to early germination schedules) of WO species, compared with that of RO species, results in selective dispersal and caching of RO species (Steele et al. 2001a), selective consumption of WO acorns over RO acorns in the autumn (Steele et al. 2001a, 2007) and embryo excision of early germinating WO species by several genera of squirrels (*Sciurus*, *Sciurotamias*, *Caliosciurus*, *Dremomys*) in North America and China (Fox 1982; Steele et al. 2001b; Steele 2008; Xiao et al. 2009, 2010; Xiao and Zhang 2012). Moreover, this differential pattern of dispersal and predation of RO and WO species is predicted to alter patterns of seedling establishment, with WOs establishing closer to parent sources and ROs showing a greater pattern of dispersion (Moore et al. 2007; but see Steele et al. 2007 for evidence of greater dispersion of seedlings of some WO species). Results of the present study suggest, however, that germinating chestnut oak acorns that first establish near the parent tree may be pruned and thus have the potential to be moved following initial germination.

Finally, our results support a growing body of evidence to suggest that the acorn is equally important for dispersal and thwarting seed predation as it is for supporting the developing seedling. Although previous authors have advanced the notion that white oak cotyledons allow rapid transfer of nutritional reserves into an inedible robust taproot (Fox 1982; Hadj-Chikh et al. 1996; Steele et al. 2001a; Jansen et al. 2006; Xiao et al. 2010), we report that the dry mass of acorns of non-germinated acorns did not differ significantly from those following autumn germination, regardless of the length of the taproots (even 5 months after germination).

Moreover, we show that pruned taproots with an intact plumule can successfully germinate, and pruned acorns (cotyledons) can still serve as a food source for some rodents. Elsewhere, it is shown that both WO and RO species can germinate and establish after sustaining considerable damage by insects (e.g., *Curculio* spp.), rodents, and birds (Steele et al. 1993; Yi and Zhang 2008; Yi and Yang, 2010; Perea et al. 2011). In addition, tannin and lipid gradients in the several acorn species may direct partial acorn predation away from the embryo (Steele et al. 1993; Steele, unpublished data) and enhance seedling establishment of these partially damaged seeds. These results, coupled with those suggesting that larger acorn size enhances caching and dispersal of acorns by rodents (Xiao et al. 2004; Moore et al. 2007; Steele 2008), suggest that the acorn may serve more as a fruit to attract potential seed dispersers and to sustain damage by seed predators than it does for directly supporting the young seedling. Such an assertion is supported by recent experimental evidence that several oak species can sustain simulated insect and rodent damage and even acorn removal at early stages in seedling growth (S. Agosta et al., unpublished data).

We conclude that the ability of chestnut oak to sustain significant early acorn pruning and still produce viable seedlings shows a previously underappreciated resilience to rodent (and jay) predation which, in many years, is assumed to contribute to complete failure of regeneration. It also suggests why the dispersal syndrome of white oak species differs considerably from that of red oak species. We suggest that additional research focus on the differential response of red and white oak species to damage by insects, rodents, and birds, as well as investigate this adaptation in other white oak species in North America and other biomes throughout the world.

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