Differential effects of acorn burial and litter cover on Quercus rubra recruitment at the limit of its range in eastern North America

Daniel García, María-José Bañuelos, and Gilles Houle

Abstract: Primary predators or dispersers such as birds and rodents cache acorns of northern red oak (Quercus rubra L.). A proportion of these acorns are not retrieved, and thus, animals may favour oak regeneration by placing acorns in microsites suitable for recruitment. We experimentally investigated the effects of acorn burial and litter cover on red oak recruitment at two sites at the northern limit of the species’ range in North America. Laboratory experiments also tested the effects of acorn burial and litter cover on desiccation and germinability and the influence of soil moisture on germination. Burial and litter protected acorns against predation by deer in the field. Germination was promoted by burial both in field and laboratory experiments. Germination was proportional to acorn water content and to soil moisture. Seedling emergence in the field was enhanced by burial but reduced by litter cover. Acorns buried but uncovered by litter had the highest probability of recruiting a seedling. A potential effect of seed predators or dispersers on red oak regeneration and expansion is suggested, as acorn caching by birds and rodents may actually enhance population recruitment, despite high mortality through acorn consumption.

Key words: acorn burial, litter, microsite effects, recruitment, northern red oak, southern Quebec.

Introduction

Many oak species in North America depend on acorn-caching animals (primary predators such as birds and rodents) for seed dispersal (Vander Wall 1990; Farmer 1997 and references therein). Besides its role in the transport of acorns from parental plants, caching by animals is assumed to enhance oak regeneration for two reasons (Vander Wall 1990; Johnson et al. 1997): first, by providing escape from secondary predators, e.g., deer; and second, by placing them in physiologically suitable sites for both acorn germination and seedling establishment. Thus, the effectiveness of primary predators as dispersers depends on the trade-off between acorn retrieval by these or other predators and the suitability of the site where acorns are delivered (Schupp 1993; Kollmann and Schill 1996). Yet, information on how caching activity by dispersers modifies microsite conditions for oak recruitment in the field is scarce (but see Kollmann and Schill 1996; Fuchs et al. 2000). This is especially lacking for sites such as the limits of the species’ ranges where...
regeneration is mainly constrained by abiotic factors, and thus, the positive effect of dispersers might be more evident, favoring not only population recruitment but also species’ expansion (Griffin 1971; Vander Wall 1990; Clark et al. 1998).

In this paper, we experimentally analyze microsite requirements for seedling recruitment of northern red oak (*Quercus rubra* L.) in the southern part of Quebec to understand how caching may influence the species’ regeneration at the northern limit of its range in eastern North America. We considered two microsite features, burial and litter cover, both of which may differ dramatically between cached and uncached acorns after primary dispersal (on soil surface, under parental trees, and covered by litter; Barnett 1977; Crow 1988; Kollmann and Schill 1996; Johnson et al. 1997). We hypothesized that burial and litter might affect oak establishment by influencing humidity in the nearby environment around the acorns, which may be relevant to recruitment since acorns lose their viability under dessication (Watt 1919; Shaw 1968; Griffin 1971). Moreover, we studied the effect of caching on different regeneration stages (e.g., post-dispersal survival, germination, and emergence) to evaluate the importance of each stage on final recruitment as well as how different stages might be differentially affected by the same microsite conditions (Schupp 1995; Kollmann and Schill 1996). More specifically, we addressed the following questions:

1. Do burial and litter cover affect acorn germination, seedling emergence, and recruitment in the field?
2. Is germination influenced by acorn water status under burial or litter cover conditions?
3. Does soil moisture level affect germination?

**Materials and methods**

**Study sites**

Two sites were used for the field experiments: the first one was on Île-aux-Grues (47°02'N, 70°33'W), an island in the Montmagny archipelago in the St. Lawrence River, Quebec, and the other was in the Réserve Léon-Provancher (46°18'N, 72°31'W) on the south shore of the St. Lawrence River near Trois-Rivières, Que. Both sites are characterized by mixed hardwood stands in which red oak is associated with sugar maple (*Acer saccharum* Marsh.), yellow birch (*Betula alleghaniensis* Brit.), American beech (*Fagus grandifolia* Ehrh.), and red maple (*Acer rubrum* L.). These forests are typical of the middle St. Lawrence section of the Great Lakes – St. Lawrence Forest Region of Rowe (1972). Both stands represent red oak populations at the northern limit of the species’ distribution (Sander 1990). Potential hoarding animals such as red squirrels (*Tamiasciurus hudsonicus* L.) and blue jays (*Cyanocitta cristata* L.), and evidence of acorn caching were found at both sites. In fact, most first-year oak seedlings located in the stands apparently emerged from caches (D. García, data not shown). Mean annual temperature at nearby weather stations is ca. 5°C, and annual precipitation totals ca. 1000 mm of which ca. 24% falls as snow (Atmospheric Environmental Service 1981). The frost-free season is ca. 150 days (Atmospheric Environmental Service 1982).

**Field experiment: effect of acorn burial and litter cover on acorn germination, seedling emergence, and recruitment**

The experiment was simultaneously set up at both study sites in fall 2000. At each site, we collected acorns during the peak time of dispersal from at least six different trees. Acorns were tested for soundness by water floatation and removal of those defective or infested by weevil larvae (Dey and Buchanan 1995). At each site, 800 sound acorns were then placed in the field in groups of 10, each group homogeneously distributed on a surface of 20 × 20 cm and covered by a wire cage of 25 × 25 × 5 cm (1-cm mesh). Acorns were placed with the micropyle horizontal, their longitudinal axis being equally oriented. Treatments were established in a completely randomized block design, in which each of 20 blocks had a group of acorns assigned to one of four combinations of two factors (burial and litter cover): (i) buried and covered by litter, where acorns were buried in the soil at 1-cm depth above the acorn and covered by litter (B'L'); (ii) buried and uncovered by litter (B'L'); (iii) unburied and covered by litter (B'L'); and (iv) unburied and uncovered by litter (B'L'). Blocks were separated by 2 m and arranged in four transects of five blocks per transect. Litter was taken directly from the surroundings and was composed of a mixture of leaves freshly fallen from different species, mainly red oak, sugar maple, yellow birch, and beech. Litter cover was homogeneously distributed within the cage, representing a cover of three to five layers of leaves (the number of leaf layers at the study site was 2.80 ± 0.11 (mean ± SE)). Litter that fell on the cages during the fall was systematically removed until snowfall to make sure that the only litter covering the acorns was that added experimentally. Plots were surveyed in late spring 2001 by removing the cage and carefully searching for the acorns, checking for germination (acorn split, radicle protrusion), seedling emergence (epicotyl development on germinated acorns), and recruitment (seedlings from sown acorns).

During the experiment, some cages at Réserve Léon-Provancher evidenced attack and acorn predation by white-tailed deer (*Odocoileus virginianus* Zimmermann). Thus, we recorded the treatment of those cages damaged by deer and collected the data on germination, emergence, and recruitment for the surviving acorns (number of acorns: B'L', 180; B'L', 197; B'L', 156; B'L', 19). Deer were not present at Île-aux-Grues, and no cage removal or acorn predation was detected at this site.

**Laboratory experiment 1: effect of burial and litter on acorn germination**

In November 2000, one hundred sound acorns from Île-aux-Grues were individually weighed and placed in 250-mL plastic pots filled with 150 g of sterilized sand, which were then watered to saturation level (ca. 19% moisture content) by adding 35 mL of distilled water per pot. Two factors considered were acorn burial and litter cover, with 25 pots assigned to each of the four combinations of burial and litter as above. Buried acorns were placed in the sand with the micropyle horizontal at 1-cm depth above the acorn. Litter was applied by covering the sand surface with three to five layers of leaves collected from Île-aux-Grues. Acorn weights did not differ between treatments at the be-
Acorn water loss during stratification was followed on 10 additional acorns in the combination no burial – no litter, following the procedure described above. We assumed that acorn water loss in the remaining combinations should be lower or similar to those of no burial – no litter, since this combination represents the lowest level of acorn protection against desiccation. These control acorns were individually weighed every 2 weeks, and dry weight was determined at the end of the stratification period. Acorn water content was then calculated for each date, showing a progressive decrease and passing the threshold value for acorn viability (20% water content; Watt 1919; Dey and Buchanan 1995) after 60 days stratification.

Laboratory experiment 2: effect of decreasing soil moisture levels on acorn germination

In November 2000, one hundred sound acorns from Île-aux-Grues were individually weighed and placed in 250-mL plastic pots filled with 150 g of sterilized dry sand. Acorns were partially buried (half of the acorn) with the micropyle horizontal. Four groups of 25 pots were assigned to each one of four treatments representing a decreasing gradient of soil moisture from saturation: 19, 14, 6, and 3%. These values were established by adding, respectively, 35, 25, 10 and 5 mL of distilled water to the pots. No differences in acorn weight were found between treatments at the beginning of the experiment (F3,96 = 0.07, p = 0.97, one-way ANOVA). The initial acorn moisture content was 50.55 ± 0.56% (calculated from a subsample of 10 acorns from the initial pool, by oven-drying at 80°C for 24 h). All pots were sealed with plastic wrap and vaseline, to preclude soil water loss, and stratified in darkness in a chamber at 4°C, for 3 months. After that, acorn germination (acorn split, radicle protrusion) was checked and temperature regime was changed to 25:15°C (day-night) with a 16 h light:8h dark photoperiod, and pots were watered to saturation level (35 mL). After 1.5 months, germination was checked again, and dry weight was determined for all acorns.

Laboratory experiment 1: effect of burial and litter cover on acorn germination, seedling emergence, and seedling recruitment

Germination percentages were 80.5% at Île-aux-Grues and 94.9% at Réserve Léon-Provancher. Burial significantly affected germination probability (Tables 1 and 2), being higher for buried acorns. Total emergence for germinated acorns was 31.7% at Île-aux-Grues and 48.8% at Réserve Léon-Provancher. Emergence was significantly higher when acorns were buried but uncovered by litter (Tables 1 and 2). The percentage of initially sown acorns recruiting seedlings was 25.9% at Île-aux-Grues and 46.4% at Réserve Léon-Provancher. Recruitment was positively affected by burial but negatively affected by litter at both sites, with no interaction between factors (Table 2). As a result, the highest probability to recruit a seedling appeared for acorns buried and uncovered by litter (Tables 1 and 2). A significant block effect was detected at both study sites for most analysed variables (Table 2).

Deer attacked 36.3% of acorn cages at Réserve Léon-Provancher. Cages with acorns unburied and uncovered by litter had the highest probability of attack (Fig. 1). Both burial (χ² = 19.4, p < 0.0001) and litter cover (χ² = 6.5, p = 0.011) significantly reduced the probability of attack by deer. The interaction between these factors was not significant (B × L, p = 0.10; NLM).

Laboratory experiment 1: effect of burial and litter on acorn germination

Total acorn germination was 27.0%, mostly occurring during the stratification period. Acorns buried and covered by litter showed the highest germination percentage (Table 3). Both burial and litter had significant and independent effect on germination (burial: Wald χ² = 14.2, p < 0.0001; litter: Wald χ² = 5.9, p < 0.05; df = 1; B × L: Wald χ² = 1.5, p > 0.05, df = 1), while initial acorn weight did not affect germination (Wald χ² = 2.7, p > 0.05, df = 1; NLM).

Acorn moisture content after stratification was significantly higher for acorns buried and covered by litter than for the other treatments (Table 3). Burial and litter were significant factors on acorn water content (burial: F1,95 = 196.1, p < 0.0001; df = 1; litter: F1,95 = 89.0, p < 0.0001; df = 1), but litter effect was only evident for buried acorns (B × L: F1,95 = 21.9, p < 0.0001; df = 1; NLM).

Statistical analysis

Multivariate contingency models (nominal logistic model (NLM) procedure of JMP statistical package; SAS Institute Inc. 2001) were used to analyse simultaneously the effects of (i) burial, litter, their interaction, and block on germination, emergence, and recruitment for individual acorns in the field experiments; (ii) burial, litter, and their interaction on predator attack for individual cages at Réserve Léon-Provancher; (iii) burial, litter, their interaction, and initial seed weight on germination for individual acorns in the laboratory experiment 1; and (iv) soil moisture level and initial seed weight on germination for individual acorns in the laboratory experiment 2. Pairwise comparisons between treatments were made by partial χ².
Table 1. Percentage of *Quercus rubra* germination, emergence (seedlings from germinated acorns) and recruitment (seedlings from sown acorns) at two sites, in relation to burial and litter cover.

<table>
<thead>
<tr>
<th></th>
<th>B+</th>
<th>B-</th>
<th>L+</th>
<th>L-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germination</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Île-aux-Grues</td>
<td>86.50a</td>
<td>92.46a</td>
<td>70.50b</td>
<td>72.50b</td>
</tr>
<tr>
<td>Réserve Léon-Provancher</td>
<td>96.67a</td>
<td>97.46a</td>
<td>89.74b</td>
<td>94.74ab</td>
</tr>
<tr>
<td>Emergence</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Île-aux-Grues</td>
<td>32.37a</td>
<td>70.65b</td>
<td>2.13c</td>
<td>10.34d</td>
</tr>
<tr>
<td>Réserve Léon-Provancher</td>
<td>56.32a</td>
<td>75.52b</td>
<td>7.86c</td>
<td>11.11c</td>
</tr>
<tr>
<td>Recruitment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Île-aux-Grues</td>
<td>28.00a</td>
<td>66.83b</td>
<td>1.50c</td>
<td>7.50d</td>
</tr>
<tr>
<td>Réserve Léon-Provancher</td>
<td>54.44a</td>
<td>73.60b</td>
<td>7.05c</td>
<td>10.53c</td>
</tr>
</tbody>
</table>

Note: Values with different letters are significantly different (*p* < 0.05) after partial *χ*². B⁺, buried; B⁻, unburied; L⁺, covered by litter; L⁻, uncovered by litter.

**Discussion**

Results from both field and laboratory experiments show that red oak recruitment is affected by acorn burial and by the presence of a litter cover. However, the effect of these two factors differs depending on the regeneration stage. Our field experiment suggests a significant role for deer as acorn predators (see also Griffin 1971; Barnett 1977; Vander Wall 1990; Dey and Parker 1996) and shows that burial and litter cover protected acorns against secondary predators, perhaps by decreasing the encounter probability (Ovington and McRae 1960; Kikuzawa 1988; Myster and Pickett 1993; Kollmann and Schill 1996). Thus, acorns benefit from burial and litter by escaping post-dispersal secondary predators.

For those acorns surviving predation, both field and laboratory data showed that burial also promotes germination. Related to this, our laboratory experiments also showed that a reduction in acorn water content is associated with a decrease in germination ability. On the other hand, germination was strongly prevented at low soil water contents. These re-
García et al.

Fig. 2. Percentage of *Quercus rubra* germination in relation to soil water content in the laboratory experiment 2. Bars with different letters are significantly different ($p < 0.05$) after partial $\chi^2$.

results support the idea that the positive effect of acorn burial depends on the provision of a moist environment preventing seed desiccation. By holding the environment at a higher humidity level and by permitting good seed-soil contact, burial enhances acorn moisture and, therefore, contributes to maintaining acorn viability through the dormancy period (Watt 1919; Shaw 1968; Griffin 1971; Nyandiga and McPherson 1992; Kollmann and Schill 1996; Germaine and McPherson 1998). Conversely, no litter effect on germination was observed by our field data. It is possible that litter presence in our study site did not impose differences in substrate humidity strong enough to affect germination. This was indirectly suggested by our laboratory experiment, where, under conditions of poor watering and strong desiccation, acorns covered by a protective layer of leaves suffered lower water losses and had a higher percent germination (see also Barrett 1931; Shaw 1968; Sander 1990; Dey and Parker 1996). In addition, the existence of snow cover during the entire winter might modify the role of litter in the field by altering the humidity gradients at ground surface (see also Shimano and Masuzawa 1998).

Emergence was positively affected by burial at both study sites, as shown for other *Quercus* species (Barrett 1931; Tripathi and Khan 1990; Kollmann and Schill 1996; Fuchs et al. 2000). Lower emergence from unburied acorns was apparently caused by drying out and freezing of the growing radicle (Watt 1919; Ovington and McRae 1960; Kollmann and Schill 1996; Germaine and McPherson 1998, 1999). Conversely, emergence was consistently reduced by litter cover, for both buried and unburied acorns. This reduction in emergence may be due to allelopathic effects on radicle growth (Farmer 1997; and references therein), physical barrier and anomalous epicotyl development leading to withering (Barrett 1931), or damage by litter animals or pathogens (Barrett 1931; Facelli and Pickett 1991).

Seedling recruitment in the field was mainly affected by processes acting between germination and emergence, as this regeneration stage decreased the recruitment probability more than the germination stage itself. More importantly, the cumulative effects of burial and litter on germination and emergence determined a strong microsite effect on red oak recruitment in the field, with acorns buried but uncovered by litter having the highest recruitment probability. This forest-microsite specificity contrasts with the common assumption of low site sensitivity for larger-seeded trees like oaks (Seiwa and Kikuzawa 1996; Farmer 1997). On the other hand, considering germination and emergence separately as stages leading to recruitment enabled us to determine a differential effect of burial and litter depending on the stage. In fact, litter may be beneficial to seeds by reducing predation and increasing germination under water stress conditions but detrimental to seedlings by inhibiting emergence. This can be interpreted as a seed-seedling conflict determining the final outcome of recruitment, as safe sites for seeds are not necessarily safe sites for emerging seedlings (Lamont et al. 1993; Schupp 1995; see also Kollmann and Schill 1996 for *Quercus petraea*; Welter and McPherson 1999 for *Quercus emoryi*).

Our results suggest a potential beneficial effect of seed dispersers on red oak recruitment, by determining where acorns are delivered. In this sense, dispersers such as blue jays are known to selectively cache acorns in patches such as regeneration forest, forest gaps, and forest edges (Harrison and Werner 1984; Johnson and Adkisson 1985; Johnson et al. 1997). Caching in these open environments would match red oak requirements for germination and, more importantly, emergence (i.e., burial and no litter cover) and might be considered as “directed dispersal”, since seeds are dispersed nonrandomly into more suitable patches (Vander Wall 1990; Johnson et al. 1997; Wenny 2001). In addition, this effect of directed dispersal may be even more significant at the northern limit of the species’ range, where germination and emergence are more constrained by abiotic factors like frost and desiccation (Griffin 1971; Barnett 1977; Crow 1988; Germaine and McPherson 1999). By demonstrating a microsite effect on red oak recruitment, our study provides empirical support to the role of animal dispersers in the northwards expansion of red oak in North America (Johnson and Webb 1989; Vander Wall 1990). This role would be related both to the long dispersal distance provided by animals (Johnson and Webb 1989; Clark et al. 1998) and to the quality of caches as microsites where acorns escape abiotic (desiccation, frost) as well as biotic (seed predators, litter) mortality factors.

Acknowledgements

We thank Dominique Lapointe and Éric Lavoie for field work assistance. Regino Zamora and J. Ramón Obeso provided some logistical support. Dr. P.G. Krannitz and two anonymous referees reviewed the manuscript, providing helpful suggestions. We also acknowledge a postdoctoral fellowship from Ministerio de Educación y Cultura and a contract from Programa Ramón y Cajal, Ministerio de Ciencia y Tecnología, to D.G., a predoctoral grant from Fundación para el Fomento en Asturias de la Investigación Científica y la Tecnología to M.J.B., and a research grant from Natural Sciences and Engineering Research Council of Canada to G.H.
References


