The Effect of Burial Depth on Removal of Seeds of *Phytolacca americana*

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Abstract - Although burial is known to have important effects on seed predation in a variety of habitats, the role of burial depth in affecting the removal of seeds in early-successional systems is poorly known. *Phytolacca Americana* (pokeweed) is a model species to examine the role of burial depth in affecting seed removal because it is common in early-successional habitats, studies suggest that seed removal is indicative of seed predation, and seed predation is related to the recruitment of mature plants. To determine how burial depth affects *P. americana* seed removal, 20 seeds of *P. americana* were buried at depths of 0, 1, or 3 cm in early-successional habitats at the Savannah River Site in South Carolina for over 6 weeks. The frequency with which seeds were encountered (as measured by the removal of at least one seed) and the proportion of seeds removed was significantly greater when seeds were on the soil surface (0 cm depth) compared to seeds that were buried 1 cm or 3 cm; there was no difference in encounter or removal between seeds at 1 cm or 3 cm. Our findings suggest that burial may have important consequences for *P. americana* population dynamics, because seed survival depends upon whether or not the seed is buried, and relatively shallow burial can yield large increases in seed survival. Because seed limitation is known to be an important determinant of plant community composition in early-successional systems, our work suggests that burial may play an unappreciated role in the dynamics of these communities by reducing predator-mediated seed limitation.

Introduction

The vertical position of a seed within the soil (burial depth) influences seed dormancy characteristics (Baskin and Baskin 1998) as well as the likelihood that a seedling will successfully emerge from the soil (Baskin and Baskin 1998). Burial depth may also affect the removal of seeds by seed predators because buried seeds are less likely to be detected and removed by seed predators in desert ecosystems (Reichman 1979), coastal dunes (Maron and Simms 1997), grasslands (Hulme 1994, Maron and Simms 1997), temperate forests (Crawley and Long 1995, Hulme and Borelli 1999), and tropical forests (Andresen and Levey 2004). In early-successional species, burial depth is known to reduce germination because many species will only germinate near the soil surface (Baskin and Baskin 1998, Orrock et al. 2006). Moreover, burial depth influences the survival of seeds after germination because larger-seeded species are more likely to successfully emerge

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from deeper in the soil profile (Baskin and Baskin 1998, Grundy et al. 2003). However, despite evidence that the establishment of early-successional plant species is often limited by the number of seeds that survive, germinate, and emerge (seed limitation; Turnbull et al. 2000), little is known about how burial depth affects the predation of seeds in early-successional systems.

Phytolacca americana Linneaus (pokeweed) is a perennial plant whose distribution is largely within eastern North America (Mitich 1994). *Phytolacca* is typically found in early-successional habitats, forest clearings created by disturbance, and other frequently disturbed habitats (McDonnell et al. 1984). Birds and other vertebrates consume the fruits of *P. americana* and subsequently disperse the seeds via defecation (Martin et al. 1951, McDonnell et al. 1984, Mitich 1994). Because of reliance upon vertebrate dispersal and the deterrent effect of *P. americana* fruit pulp on rodent granivores (McDonnell et al. 1984), pre-dispersal seed predation is probably extremely rare. Evidence suggests that post-dispersal seed predation by arthropods, rodents, and birds removes substantial numbers of *P. americana* seeds (Boman and Casper 1995, Hyatt 1998, Orrock et al. 2003, Willson and Whelan 1990) and may affect the size of *P. americana* populations (Orrock et al. 2003). The importance of seed predation in the population dynamics of *Phytolacca americana* and evidence that burial depth also affects seed germination (Orrock et al. 2006) makes *P. americana* a model species for the examination of how burial depth affects seed predation of early-successional plants. In this paper, we examine the role of burial depth in affecting the predation of *P. americana* seeds. Specifically, we examine how burial depth affects the rate at which seed predators encounter seeds, defined as the removal of at least one seed from a particular depth treatment at a site (Hulme 1994, Willson and Whelan 1990), as well as the percentage of seeds removed.

Methods

Study area and design

Mature *P. americana* fruits were collected on July 28, 2003 at the Savannah River Site (SRS), a National Environmental Research Park (NERP) located near Aiken, SC. Fruits contain approximately 10 seeds (Armesto et al. 1983); each seed is 2.5–3 mm in size (Radford et al. 1968). Seeds were removed from ripe fruits by rubbing the fruits against a sieve. Collected seeds were then thoroughly washed and allowed to dry prior to use. Seeds used for seed-removal trials were thus similar to the pulp-free seed predators would encounter in the field after dispersal by frugivores (McDonnell et al. 1984).

Seed-removal trials were conducted at three sites at the SRS, each separated by several kilometers (Fig. 1A). At each site, we used two early-successional patches created in 1999 by clearcutting mature pine forest (> 30 years old; Kilgo and Blake 2005), followed with a prescribed burn (Fig. 1A). Each patch was 1-ha in size and was connected to an identical adjacent patch by a narrow strip of clear-cut habitat (a "corridor"). These patches represent typical early-successional habitat of *P. americana* (Radford et al. 1968), as evidenced by natural recruitment of *P. americana* within this study system (Orrock et al. 2003). Based upon vegetation surveys conducted in the study sites in 2003, vegetation was characterized by *Quercus falcata* Michaux, *Rhus copallina* Linnaeus, *Rubus cuneifolius* Pursh, *Sassafras albidum* Nuttall, *Vaccinium stamineum* Small, and *Vitis* spp. (for additional description of the plant community and survey methodology, see Damschen 2005).

Within each of the six patches (three sites, two patches per site; Fig. 1), seed removal was examined at four locations within a 50- x 50-m square area centered on the patch. Each location was 25 m from the closest patch edge (Fig. 1). Plastic sample cups (approximately 6-cm diameter, 9-cm height, 120-ml volume) were used to hold seeds during seed removal trials. Screw-on lids prevented rain from changing the burial depth of seeds or from washing seeds out of the cups, and 0.5-mm diameter holes drilled in the bottom of each cup provided drainage. Each cup had a 2.5-cm diameter hole drilled into the side to allow seed removal by rodents and invertebrates. Although avian granivores may also exhume seeds, cups were designed to allow us to focus our examination on removal by rodents and invertebrates because of past evidence of their importance in *P. americana* seed removal in the study area (Orrock et al. 2003).

Within each cup, 20 *P. americana* seeds were placed at one of three depths: 0, 1, and 3 cm. These depths were selected because germination of *P. americana* approaches zero as burial depth increases to 3 cm (Orrock et al.

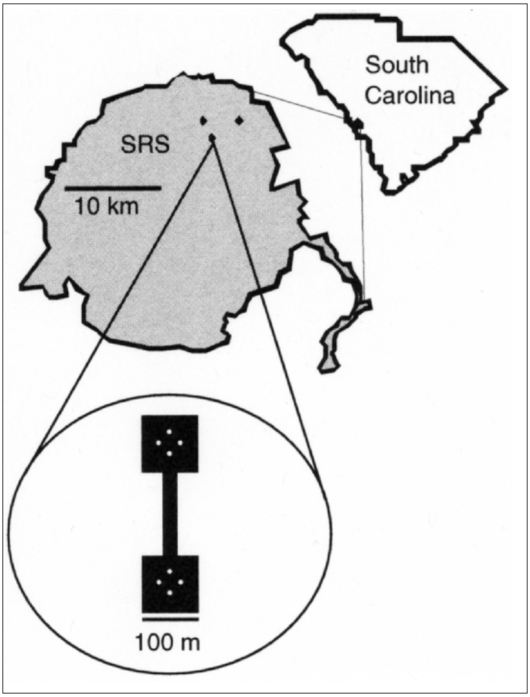

Figure 1. The experimental landscape at the Savannah River Site (SRS) near Aiken, SC, where seeds were collected and burial trials were conducted. At each of three sites, two patches were used. Patches consisted of clearcuts within a matrix of mature pine forest that were connected with a narrow corridor of clearcut habitat as part of another study (see Orrock et al. 2003). Four stations were placed in each patch. Each station had three cups, each containing seeds buried at either 0, 1, or 3 cm below the surface.

2006). Sand, chosen to match the sand-rich soils at the site (Kilgo and Blake 2005), was added to each cup until the surface of the sand was level with the entrance hole. At each location, one cup of each depth treatment was buried so that the lowest point of the entrance hole was flush with the ground, leaving only 4 cm of the cup (the entrance hole and lid) visible above the soil surface. This design resulted in 12 cups per patch, for a total of 72 observations (3 cups per location x 4 four locations per patch x 6 patches).

Cups were placed in the field from May 20–21 until July 4–6, 2004, which exceeds the relatively short duration of many seed-removal studies (< 4 weeks; Hyatt 1998). Because of disturbance by feral pigs at nine of the 24 locations, only locations where all cups were undisturbed were used for analysis (N = 45 observations from 15 locations, at least one location per patch was not disturbed).

Statistical methods

We quantified seed predation using two response variables: the frequency of seed encounter and the proportion of seeds removed. The frequency of seed encounter, defined as the removal of at least one seed from a particular depth treatment at a site (Hulme 1994, Willson and Whelan 1990), was examined using chi-square tests of independence (Quinn and Keough 2002). Due to low frequency of encounter for buried seeds, we used randomization tests with 100,000 randomizations to generate Monte Carlo estimates of significance when expected cell frequencies were less than 5 (Quinn and Keough 2002); Monte Carlo results were qualitatively identical to results using asymptotic chi square. The proportion of seeds removed was examined using one-way analysis of variance (ANOVA). Blocks and patches were treated as random-effects blocks, with burial depth (0, 1, or 3 cm) treated as a fixed effect (Quinn and Keough 2002). All proportions were arcsin squareroot transformed to improve normality prior to analysis. Examination of residuals from ANOVA suggested that they were normally distributed and that variance was homogeneous among groups (Quinn and Keough 2002). All analyses were performed using SAS v. 9.1 (SAS Institute 2004).

Results

Burial significantly reduced both the encounter and removal of *P. americana* seeds by seed predators (Fig. 2). Seeds on the soil surface were more frequently encountered (100%) compared to seeds buried at 1 cm (60%) or 3 cm (53%) depth. The depth of seed burial was not as important as burial itself, as seeds on the surface were more frequently encountered compared to seeds buried at 1 cm and 3 cm (χ^2 = 9.14, d.f. = 1, P < 0.01), while differences among encounter rates for buried seeds were not significant (χ^2 = 0.14, d.f. = 1, P = 0.71). The proportion of seeds removed by seed predators was also greatest for *P. americana* seeds on the soil surface: 83.9, 14.4, and 14.4% for 0, 1, and 3 cm burial depth, respectively (ANOVA, $F_{2,39}$

= 57.83, P < 0.01; Fig. 2). As was found for encounter frequency, there was no difference in the proportion of seeds removed between seeds buried at depths of 1 or 3 cm (linear contrast, $F_{1,39} = 0.01$, P = 0.94; Fig. 2).

Discussion

Although burial is known to reduce seed predation in a variety of plant communities (Andresen and Levey 2004, Crawley and Long 1995, Hulme 1994, Hulme and Borelli 1999, Maron and Simms 1997, Reichman 1979), our study provides the first evidence that burial also affects seed predation in early-successional communities. We show that burial itself, regardless of whether at 1 cm or 3 cm, confers a large reduction in seed predation by rodents and arthropods (Fig. 2). Burial is likely to decrease predation of *P. americana* seeds because it reduces the likelihood that arthropod or rodent seed predators will detect and exhume seeds (Andresen and Levey 2004, Hulme 1994, Hulme and Borelli 1999, Maron and Simms 1997, Reichman 1979). Comparison of our findings for seeds on the soil surface (see Results) with other studies of *P. americana* seed removal from the soil surface (Boman and Casper 1995, Hyatt 1998, Willson and Whelan 1990) suggests that our design accurately captures patterns of seed removal without biasing granivore foraging behavior.

We assume that seeds were not removed by forces other than animal seed predators. Field observations support this assertion, as no sign of seed washout from undisturbed cups was detected during cup collection. Seeds were not "removed" via germination over the course of the experiment, because lids and drainage holes prevented moisture from collecting in cups in sufficient amounts to cue germination; no germinants or seedlings were found in cups when cups were collected. Seeds were also unlikely to be destroyed by fungal pathogens because *P. americana* seeds are resistant to fungal attack (Orrock and Damschen 2005). We also assume that seed removal is indicative of seed predation. This assumption is supported by a study by Orrock et al. (2003) in the same system that showed a strong, negative relationship

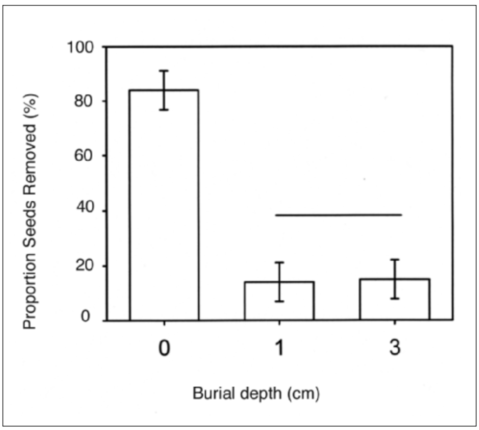

Figure 2. The effect of burial depth (0, 1, or 3 cm) on the proportion of *P. americana* seeds removed from early-successional habitats from May 20–21 to July 4–6, 2004. Error bars represent ± 95% confidence intervals, and horizontal bar indicates means that are not significantly different.

between seed removal and the recruitment of *P. americana* plants: 41% of the variation in the number of *P. americana* in 40 different habitat patches was explained by seed removal. Additionally, the abundance of *P. americana* plants in the 40 patches studied by Orrock et al. (2003) was negatively related to the abundance of the most common rodent granivore at our study sites, *Peromyscus polionotus* old field mouse Wagner (Pearson correlation, r = 0.70, N = 40, P < 0.01; J.L. Orrock, unpubl. data). Moreover, secondary dispersal by ants is unlikely because *Phytolacca americana* seeds do not have eliasomes (Radford et al. 1968).

Considered in light of evidence that seed predation is related to the density of mature *P. americana* (Orrock et al. 2003) and additional evidence that high levels of *P. americana* seed predation occur on or near the soil surface (Boman and Casper 1995, Hyatt 1998, Willson and Whelan 1990), our results suggest that burial may affect seed limitation of *P. americana* by decreasing the likelihood that seeds will be consumed by seed predators. Despite removal of nearly 90% of the seeds presented on the soil surface, our results are likely to be conservative because *P. americana* dispersed in summer would be susceptible to predators for longer periods of time than used in this study (4–6 months compared to the 6 weeks used in this study). The importance of seed predators in affecting *P. americana* over longer time periods is reflected by additional work in this study system: recruitment of *P. americana* did not increase despite the addition of over 10,000 seeds to a patch, and instead, the abundance of *P. americana* in each patch was lower in patches where seed removal was greatest (Orrock et al. 2006).

The immediate benefit of reduced predation conferred by burial may be offset by the effect of burial depth on successful germination. A study by Orrock et al. (2006) has shown that % germination of *P. americana* is 39, 3, and 3% at 0, 1, and 3 cm, respectively (Orrock et al. 2006). Combined with our estimates of seed predation, these data suggest that the proportion of seeds on the soil surface that are likely to recruit to the seedling stage is 0.161 x 0.39 = 0.063, or 6.3%. The proportion of seeds buried at either at 1 or 3 cm that are likely to recruit to the seedling stage is 0.856 x 0.03 = 0.026, or 2.6%. Although a smaller proportion of recruits will come from buried seeds, the reduced mortality of buried seeds yields greater contribution to future *P. americana* recruitment when the total number of seeds is finite, assuming that losses of buried seeds to soil pathogens and birds is negligible (an assumption supported by the resistance of P. *americana* to fungal attack; Orrock and Damschen 2005). These data suggest that, for early successional plants like *P. americana*, the best overall strategy would combine rapid germination on the soil surface (i.e., when predation is greatest), but high levels of dormancy when seeds are buried, because deep seed burial is likely to affect emergence if seedlings die while pushing through the soil (Baskin and Baskin 1998, Grundy et al. 2003). We suggest that a general model of *P. americana* population dynamics is that seeds are stored deep in the soil at depths greater than those from which they will germinate, which are also depths where they are effectively safe from seed predators. *Phytolacca americana* may be particularly well-suited to this strategy because its resistance to fungal pathogens (Orrock and Damschen 2005) would promote persistence in the seedbank. Upon disturbance (e.g., treefall, soil erosion), seeds are moved closer to the soil surface, where germination becomes probable. At this critical stage, when recruitment is either successful or thwarted, subtle differences in burial depth yield large differences in predation.

Within the broader context of early-successional plant communities, seed limitation has been shown to be an important determinant of community composition (Turnbull et al. 2000). As such, burial depth may also play an important role in determining the predation, and subsequent limitation, of seeds of other early-successional plant species. However, because the role of burial in protecting seeds is likely to be a function of the value of seeds to granivores (Hulme 1994) and seed size (Hulme and Borelli 1999), different seeds are likely to reap different levels of protection via burial. Similarly, although burial may provide protection from seed predators, the likelihood of successful germination and emergence is influenced by depth and varies among species (Baskin and Baskin 1998, Grundy et al. 2003). We have shown that burial depth is important for affecting the removal of one early-successional species, *Phytolacca americana*. Future studies manipulating burial depth of a suite of early-successional plant species are needed to provide insight into the role of burial depth in affecting the seed limitation common to early-successional communities (Turnbull et al. 2000).

Acknowledgments

Candice Schneberger and Christy Prenger provided excellent assistance. We thank John Blake, US Forest Service Savannah River, for logistic support. Funding and support was provided by the Department of Energy-Savannah River Operations office through the US Forest Service Savannah River under Interagency Agreement DE-IA09-00SR22188. Funding was also provided by NSF grant DEB-9907365, a Professional Advancement Grant from Iowa State University, and a STAR Fellowship to J.L. Orrock from the US Environmental Protection Agency. Portions of this work were conducted while J.L. Orrock was a Postdoctoral Associate at the National Center for Ecological Analysis and Synthesis, a Center funded by the National Science Foundation (Grant #DEB-0072909), the University of California, and the Santa Barbara campus.

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