

Seed longevity and dormancy state in a disturbance-dependent forest herb, *Ageratina altissima*

Mame E. Redwood¹, Glenn R. Matlack^{1*} and Cynthia D. Huebner²

¹Environmental and Plant Biology, Porter Hall, Ohio University, Athens, Ohio 45701, USA; ²Northern Research Station, USDA Forest Service, Morgantown, West Virginia, USA

(Received 2 October 2015; accepted after revision 9 February 2016; first published online 10 March 2016)

Abstract

Does seed dormancy allow disturbance-oriented forest herbs such as *Ageratina altissima* to persist in heterogeneous natural communities? To document seed longevity and dormancy state, *Ageratina* seeds were buried in nylon mesh bags in second-growth forest stands in south-eastern Ohio, USA. Bags were recovered at 2-month intervals, and seeds were tested for viability and germinability in the lab. Live seed numbers declined rapidly, with seed banks exhausted in an estimated 33–37 months. Seeds showed a strong dormancy polymorphism, with 71–84% of live seeds germinable between March and July, the season of natural seedling emergence. At other dates, most seeds appeared to be in a state of induced dormancy, allowing little (0–21%) germination. The slope aspect of the burial site, an important factor controlling above-ground vegetation, had no effect on seed longevity or dormancy condition. Dormancy in *Ageratina* appears to be adapted to allow opportunistic germination in late spring–early summer, but to prevent germination in less benign periods. Seed longevity is shorter than the natural frequency of gaps in mesophytic forest. We conclude that a long-term dispersal/dormancy trade-off is probably an oversimplification of the spatial ecology of this species. The primary function of dormancy appears to be short-term tracking of seasonal variation.

Keywords: *Ageratina*, bet-hedging, dispersal, environmental heterogeneity, *Eupatorium*, germination, induced dormancy

Introduction

Seed dormancy and dispersal have often been considered alternative mechanisms allowing sedentary

organisms to persist in heterogeneous, dynamic landscapes (Venable and Brown, 1988). The guild of gap-dependent forest herbs allows a test of this hypothesis. Whereas most temperate-zone forest herb species show little seed dormancy and only occasionally appear in the soil seed bank (Matlack and Good, 1990; Hyatt and Casper, 2000; Leckie *et al.*, 2000), a subset of forest herbs does appear in seed banks. These species are generally recognizable by their low tolerance of shade, small seeds, effective dispersal and opportunistic use of forest gaps (Jankowska-Blaszczuk and Grubb, 1997; Hyatt and Casper, 2000). Canopy gaps are isolated and ephemeral microhabitats, placing severe limitations on the life histories of light-demanding species (Reader and Bricker, 1992). A light-requiring forest herb may benefit from long-range seed dispersal, allowing new gaps to be colonized as existing gaps close by succession (Gadgil, 1971). Alternatively, dormancy in the soil may allow populations to persist between successive gaps at a single site, accomplishing dispersal in time rather than space (Hyatt and Casper, 2000).

Seed dormancy may serve to increase reproductive success of a species in an uncertain environment by distributing emergence of seedlings over several growing seasons, thereby buffering the failure of reproduction or germination in occasional poor years (Rees, 1993; McPeck and Kalisz, 1998). Dormancy will be favoured when the risk of mortality as a seedling exceeds the risk of mortality of seeds in the soil. Mature seeds can germinate quickly in response to environmental cues (enforced dormancy; Harper, 1977) or may occupy a deeper state of dormancy in which they are insensitive to environmental cues until exposed to a specific trigger condition (induced dormancy). Both dormancy types may occur within a single cohort of seeds, which can be considered a way of spreading risk in an unpredictable environment analogous to spatial dispersal ('bet-hedging' *sensu* Venable and Brown, 1988).

In this paper we examine seed longevity and seasonal changes in dormancy state in the perennial forest herb *Ageratina altissima* to test the complementarity of

* Correspondence
Email: Matlack@ohio.edu

dormancy and dispersal. This species prefers isolated disturbed sites but dispersal ability appears to be limited. If seed dormancy is an evolutionary alternative to dispersal between treefall gaps, then we would expect *A. altissima* to have long-term persistence in the soil and a low proportion of germinable seeds.

Ageratina altissima (L.) King and H. Rob. var. *altissima* (Asteraceae; formerly *Eupatorium rugosum*, hereafter '*Ageratina*') is a short-lived perennial herb native to deciduous forests of eastern North America, and common in forests of the upper Midwest. *Ageratina* prefers partially shaded moist or mesic microhabitats, typically occurring in forest edges, natural treefall gaps and burned areas (Clewell and Wooton, 1971; Nuzzo *et al.*, 1996; Landenberger and Ostergren, 2002).

The species blooms in late summer and early autumn, potentially producing thousands of seeds per plant in well-lit sites. Seeds have the achene-pappus morphology characteristic of the Asteraceae, suggesting wind dispersal. However, a rapid rate of descent in free air, low release height and a preference for forest sites (which have little air movement near the ground) imply that the dispersal range is limited (Matlack, 1987). Seeds are released in mid-late autumn. At maturity they are in a state of induced dormancy which can be broken by high temperatures, decreasing the likelihood of autumn or winter germination (Lau and Robinson, 2010). Most germination occurs in early-mid spring (Walck *et al.*, 1997). If germination has not occurred, seeds may re-enter the induced dormancy state (Donohue, 2005). *Ageratina* has been observed in forest seed banks (Leckie *et al.*, 2000; C.D.H., pers. obs.), although it's not clear whether the seeds were in long-term dormancy or recently produced.

Materials and methods

Seed longevity and dormancy condition were examined experimentally by burying seeds in forest sites and recovering them at regular intervals, following the method of Sarukhán (1974). To obtain large numbers of uniformly healthy seeds, we used commercially available material from outside our region. Seeds were produced in a common garden in southern Minnesota in autumn 2009 from parent genotypes collected in a single wild population in central Illinois, USA (Prairie Moon, Winona, Minnesota, USA). They were buried at study sites in south-eastern Ohio, USA, in the following year. Although the experimental seeds were probably genetically distinct from populations in the study area, south-eastern Ohio is similar to central Illinois in rainfall, temperature, day length and natural vegetation types. The weedy (sometimes invasive) character of the species suggests it is not sensitive to minor geographic differences. Thus, seed origin is

unlikely to have affected dormancy behaviour substantially.

Seeds were stitched into 10 × 10 cm nylon mesh bags, which were placed in deciduous forest sites near Athens, Ohio, in July 2010 (i.e. after the period of natural seedling emergence). One hundred seeds were included in each of 120 bags. Bags were placed on the soil surface under a thin litter layer to mimic natural seed deposition. To test for environmental control of dormancy, seed bags were placed on both north- and south-facing slopes. Above-ground vegetation differs strongly between north- and south-facing slopes in the study area, reflecting variation in moisture, temperature and day length (Olivero and Hix, 1998). Thus, slope aspect can be considered a conservative test for environmental control of dormancy. Bags were recovered at 2-month intervals for 24 months. The complete design included 5 replicate sites × 2 slope aspects × 12 recovery dates = 120 bags, with 100 seeds in each bag.

Recovered seeds were tested for germinability on moist filter paper in the laboratory. Seeds were tested at room temperature (18–21 and °C) under fluorescent lighting (c. 12/12 h photoperiod) for 2 weeks, after which germination had ceased in all samples. Seeds that did not germinate were tested for viability by visually inspecting the embryo for firmness and a yellow or green colour. Although a tetrazolium test is the preferred method of assessing viability (Huebner, 2011), such tests were not used here because seeds screened for germinability had already imbibed water and did not readily absorb the tetrazolium solution. The visual and chemical methods agreed strongly in trials on subsets of seeds, so visual examination was considered an acceptable measure of viability.

Differences between recovery dates are assumed to represent changes that would occur in a single sample through time (a 'chronosequence', *sensu* Pickett, 1989). The number of living seeds (germinable and non-germinable) plotted across recovery dates can be interpreted as a survivorship curve. However, because sampling was destructive and each sampling date represents a separate group of seeds, we do not know the fate of individual seeds. Uncertainty about dates of mortality leads to intractable problems of right-censoring (Crawley, 2007), so conventional survival analysis could not be used. Instead, we compared the number of surviving seeds using a general linear model set up as an analysis of covariance (package GLM), with aspect as a categorical variable and recovery date (number of months in the soil) as a covariate. Recovery date was log-transformed to improve normality.

The analysis was repeated with germinable seeds, defined as the proportion of all living seeds germinating at each recovery date; an arcsin square-root transformation was applied as appropriate to proportional data. Normality and homogeneity of variance were

assessed with Shapiro–Wilk and Fligner–Killen tests. Finally, longevity in the seed bank was estimated empirically by regressing surviving seed numbers on recovery date and extrapolating to the time in months when seed number reached zero. All procedures were conducted in the R statistical environment (R Core Team, 2013).

Results

Seed viability declined rapidly, with only 39–50% of seeds surviving to spring of the second growing season (May 2012; Fig. 1), and no seeds surviving to the July sampling date. The best model predicting seed survival included only date ($F_{\text{date}} = 75.81$ with 94 df, $P < 0.0001$, $R^2_{\text{adjusted}} = 0.441$). The Shapiro–Wilk and Fligner–Killen statistics were not significant ($P > 0.05$), indicating that residuals were normally distributed and variance was homogeneous among recovery dates. Slope aspect did not significantly influence seed longevity. Regression estimated a maximum survival of 33.5 months on south-facing slopes ($R^2 = 0.46$) and 36.5 months on north-facing slopes ($R^2 = 0.57$).

The proportion of germinable seeds varied seasonally, with high germinability corresponding to periods of natural seedling emergence (Fig. 1). *Ageratina* showed a maximum of 71 and 84% germinability in March 2011 and 2012 on north-facing slopes, but only 0–21% germinability between September and November (both years; and see January 2011). The transformed date term was significant in explaining *Ageratina* germinability ($F_{\text{date}} = 21.6$ with 92 df, $P < 0.0001$) but had little predictive power ($R^2_{\text{adjusted}} = 0.18$), consistent with the episodic pattern of germination. Again, the slope-aspect term was not significant.

Discussion

Ageratina seed longevity was short relative to estimates for the co-occurring *Rubus allegheniensis* and *Phytolacca americana*, perennial forest herbs which are similarly gap-dependent (Hyatt and Casper, 2000). Consistent with short seed longevity and severe mortality in the soil (Rees, 1993), high proportions of *Ageratina* seeds were germinable at some sample dates. A period of dormancy in the soil potentially allows *Ageratina* to colonize short-lived forest microsites if such sites occur frequently enough, substituting dispersal in time for dispersal in space. Populations appear to be vulnerable, however, because our projected longevity is substantially less than gap return-times estimated for deciduous forests of eastern North America (estimated return interval 20–30 years at herb level; based on Runkle, 1985; Barden, 1989).

It is possible that the buried-seed method underestimated the longevity of seeds (Van Mourik *et al.*, 2005). However, *Ageratina* would have difficulty surviving between canopy gaps even if seed longevity was considerably longer than reported here, so it appears that dormancy is not providing long-term dispersal between gaps. We infer that reports of abundance in the soil seed bank (Leckie *et al.*, 2000) and rapid germination following disturbance (Nuzzo *et al.*, 1996) reflect frequent reproduction rather than longevity in the soil. Consistent reproduction and reliable vegetative survival may explain *Ageratina*'s persistence in forest sites better than long-term soil dormancy (e.g. Carey and Watkinson, 1993). *Ageratina* perennates vegetatively as a rhizome, giving populations a degree of stability independent of seed dormancy (although it's not clear whether vegetative longevity is sufficient to survive between canopy gaps). Thus, any analysis of life history and environmental heterogeneity in forest herbs should include aspects of vegetative growth and longevity in addition to seed traits.

Dormancy also provides a short-term benefit, potentially allowing a fine-grained response to climatic variation between and within years (Honek *et al.*, 1999; Gibson *et al.*, 2002). *Ageratina* showed little autumn and winter germination, consistent with a stratification requirement demonstrated in the laboratory (Walck *et al.*, 1997; Lau and Robinson, 2010), thus avoiding weather unsuitable for seedling establishment. In spring and early summer, the period of natural germination, a large portion of seeds switched to a germinable state, potentially allowing opportunistic establishment in response to short-term environmental cues. The small fraction remaining in the induced state would provide a hedge against seedling failure in unsuitable years, allowing the possibility of survival to a third growing season (Pake and Venable, 1996; Gibson *et al.*, 2002). Ungerminated seeds returned to an induced state in mid summer, allowing *Ageratina* to avoid autumn germination and greatly increasing the number of induced seeds. We infer that opportunities for germination in the autumn are not reliable predictors of seedling survival (Densmore, 1979; Walck *et al.*, 1997). Return to the induced state is presumably cued by environmental variation, e.g. high summer temperatures (Allen and Meyer, 1998; Roman *et al.*, 2000). Laboratory trials have shown the proportion germinating to be dependent upon light, temperature and thermoperiod (Walck *et al.*, 1997), consistent with our interpretation of climatic control in natural populations.

In conclusion, a strict dispersal/dormancy trade-off is probably an oversimplification of the spatial ecology of this species. With relatively small seeds, weak spatial dispersal and short soil longevity, *Ageratina* has few of the seed traits that theoretically compensate

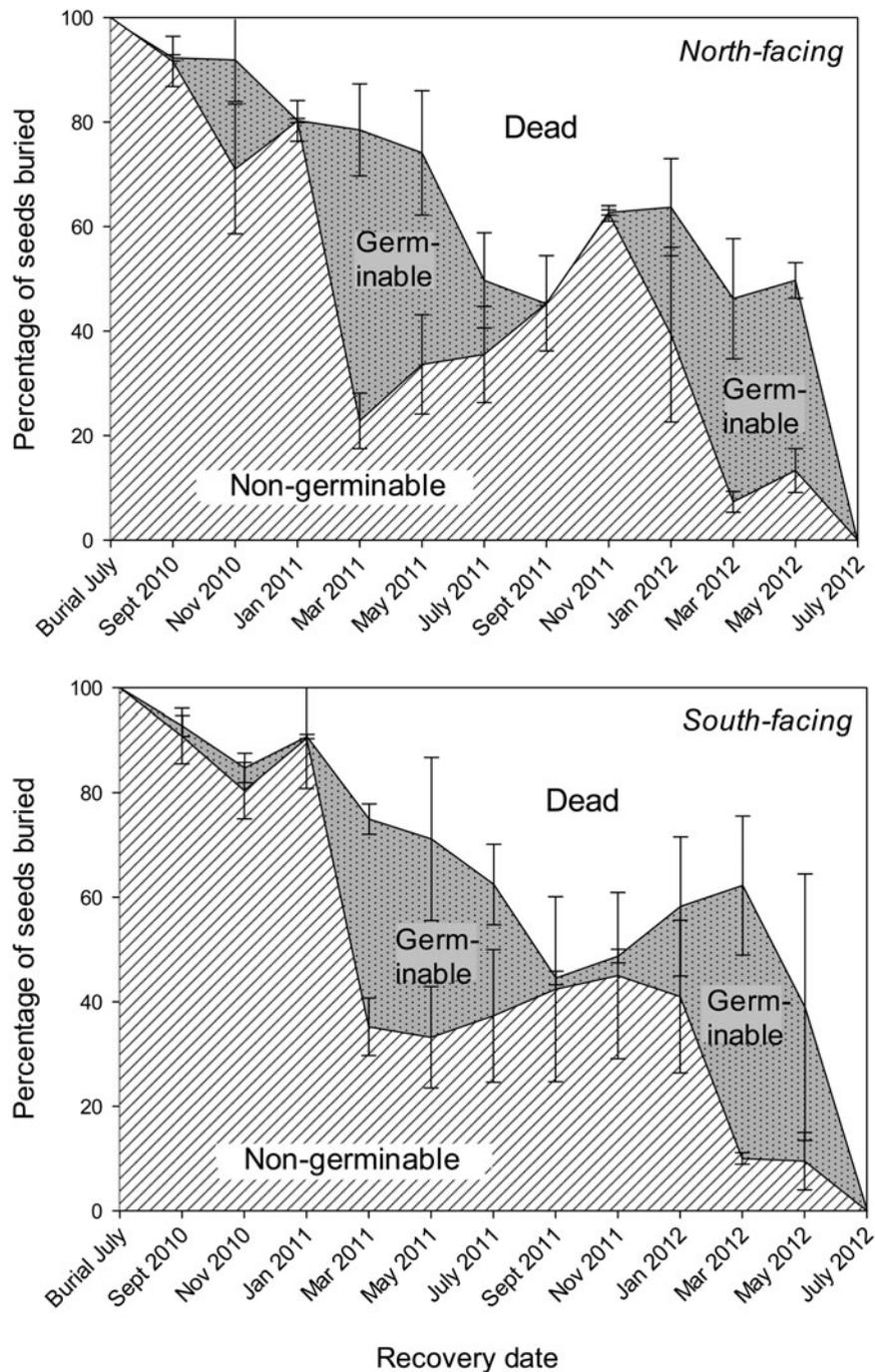


Figure 1. Seed viability and dormancy condition of *Ageratina altissima* at ten sites in south-eastern Ohio, USA, as a proportion of the total number of seeds buried. North- and south-facing slopes. The 'Dead' section of the graph (white) represents the proportion of seeds that disappeared, presumably decayed. The 'Germinable' (shaded) area indicates seeds recovered from buried bags, which subsequently germinated in laboratory trials. 'Non-germinable' seeds (diagonal hatching) appeared to be viable but did not germinate in the laboratory. Means and standard errors are plotted. Natural seedling emergence occurs in March and April.

for spatial and temporal heterogeneity (Venable and Brown, 1988). The large germinable fraction and seasonal variation described here imply that seed dormancy in *Ageratina* is an adaptation to short-term variation in climate rather than long-term patterns of site disturbance.

Acknowledgements

We are grateful to Carol and Jerry Baskin, who made helpful suggestions at early stages of this work. Marion Holmes assisted in the field work. Two anonymous reviewers made helpful comments. We

thank the landowners, public and private, who allowed us to use their woodlands.

Financial support

This work was supported under Joint Venture Agreement 10-JV-11242303-014 from the USDA Forest Service.

Conflicts of interest

None.

References

- Allen, P.S. and Meyer, S.E. (1998) Ecological aspects of seed dormancy loss. *Seed Science Research* **8**, 183–191.
- Barden, L.S. (1989) Repeatability in forest gap research: studies in the Great Smoky Mountains. *Ecology* **70**, 558–559.
- Carey, P.D. and Watkinson, A.R. (1993) The dispersal and fates of seeds of the winter annual grass *Vulpia ciliata*. *Journal of Ecology* **81**, 759–767.
- Clewell, A.F. and Wooten, J.W. (1971) A revision of *Ageratina* (Compositae: Eupatorieae) from Eastern North America. *Brittonia* **23**, 123–143.
- Crawley, M.J. (2007) *The R Book*. New York, John Wiley & Sons.
- Densmore, R.E. (1979) Aspects of seed ecology of woody plants of the Alaskan taiga and tundra. Dissertation, Duke University, Durham, North Carolina.
- Donohue, K. (2005) Seeds and seasons: interpreting germination timing in the field. *Seed Science Research* **15**, 175–187.
- Gadgil, M. (1971) Dispersal: population consequences and evolution. *Ecology* **52**, 253–261.
- Gibson, D.J., Spyreas, G. and Benedict, J. (2002) Life history of *Microstegium vimineum* (Poaceae), an invasive grass in southern Illinois. *Journal of the Torrey Botanical Society* **129**, 207–219.
- Harper, J.L. (1977) *Population biology of plants*. New York, Academic Press.
- Honek, A., Martinkova, Z. and Jarosik, V. (1999) Annual cycles of germinability and differences between primary and secondary dormancy in buried seeds of *Echinochloa crus-galli*. *Weed Research* **39**, 69–79.
- Huebner, C.D. (2011) Seed mass, viability, and germination of Japanese stiltgrass (*Microstegium vimineum*) under variable light and moisture conditions. *Invasive Plant Science and Management* **4**, 274–283.
- Hyatt, L.A. and Casper, B.B. (2000) Seed bank formation during early secondary succession in a temperate deciduous forest. *Journal of Ecology* **88**, 516–527.
- Jankowska-Blaszczuk, M. and Grubb, P.J. (1997) Soil seed banks in primary and secondary forest in Bialowieza, Poland. *Seed Science Research* **7**, 281–292.
- Landenberger, R.E. and Ostergren, D.A. (2002) *Eupatorium rugosum* (Asteraceae) flowering as an indicator of edge effect from clearcutting in mixed-mesophytic forest. *Forest Ecology and Management* **155**, 55–68.
- Lau, J.M. and Robinson, D.L. (2010) Phenotypic selection for seed dormancy in white snakeroot (*Eupatorium rugosum*). *Weed Biology and Management* **10**, 241–248.
- Leckie, S., Vellend, M., Bell, G., Waterway, M.J. and Lechowicz, M.J. (2000) The seed bank in an old-growth, temperate deciduous forest. *Canadian Journal of Botany* **78**, 181–192.
- Matlack, G.R. (1987). Diaspore size, shape, and fall behavior in wind dispersed plant species. *American Journal of Botany* **74**, 1150–1160.
- Matlack, G.R. and Good, R.E. (1990) Spatial heterogeneity in the soil seed bank of a mature coastal plain forest. *Bulletin of the Torrey Botanical Club* **117**, 143–152.
- McPeck, M.A. and Kalisz, S. (1998) The joint evolution of dispersal and dormancy in metapopulations. *Archive fur Hydrobiologie* **52**, 33–51.
- Nuzzo, V.A., McClain, W. and Strole, T. (1996). Fire impact on groundlayer flora in a sand forest 1990–1994. *American Midland Naturalist* **136**, 207–221.
- Olivero, A.M. and Hix, D.M. (1998) Influence of aspect and stand age on ground flora of southeastern Ohio forest ecosystems. *Plant Ecology* **139**, 177–187.
- Pake, C.E. and Venable, D.L. (1996) Seedbanks in desert annuals: implications for persistence and coexistence in variable environments. *Ecology* **77**, 1427–1435.
- Pickett, S.T. (1989) Space-for-time substitution as an alternative to long-term studies. pp. 110–135 in Likens, G.E. (Ed.) *Long-term studies in ecology: Approaches and alternatives*. New York, Springer-Verlag.
- R Core Team (2013) *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. Available at <http://www.R-project.org/> (accessed 19 February 2016).
- Reader, R.J. and Bricker, B.D. (1992) Response of five deciduous forest herbs to partial canopy removal and patch size. *American Midland Naturalist* **127**, 149–157.
- Rees, M. (1993) Tradeoffs among dispersal strategies in British plants. *Nature* **366**, 150–152.
- Roman, E.S., Murphy, S.D. and Swanton, C.J. (2000) Simulation of *Chenopodium album* seedling emergence. *Weed Science* **48**, 217–224.
- Runkle, J.R. (1985) Disturbance regimes in temperate forests. pp. 17–33 in Pickett, S.T.A.; White, P.S. (Eds) *The ecology of natural disturbance and patch dynamics*. New York, Academic Press.
- Sarukhán, J. (1974) Studies on plant demography – *Ranunculus repens* L., *R. bulbosus* L., and *R. acris* L. *Journal of Ecology* **52**, 151–177.
- Van Mourik, T.A., Stomph, T.D. and Murdoch, A.J. (2005) Why high seed densities within buried mesh bags may overestimate depletion rates of soil seed banks. *Journal of Applied Ecology* **42**, 299–305.
- Venable, D.L. and Brown, J.S. (1988) The selective interactions of dispersal, dormancy, and seed size as adaptations for reducing risk in variable environments. *American Naturalist* **131**, 360–384.
- Walck, J.L., Baskin, C.C. and Baskin, J.M. (1997) Comparative achene germination requirements of the rockhouse endemic *Ageratina luciae-brauniae* and its widespread close relative *A. altissima* (Asteraceae). *American Midland Naturalist* **137**, 1–12.