NOTE / NOTE

The effect of gut passage by two species of avian frugivore on seeds of pokeweed, *Phytolacca americana*

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Abstract: Although avian frugivores are known to be important dispersers of seeds of pokeweed, *Phytolacca americana* L., there are no studies that rigorously examine the effect of gut passage through avian frugivores on *P. americana* seeds. I examined how passage through avian frugivores affected the proportion of *P. americana* seeds germinating, the rate of germination (average number of days required for all seeds to germinate), and the total number of viable seeds. Field-collected fruits were either cleaned of pulp (control seeds), fed to northern Mockingbirds (*Mimus polyglottos*), or fed to Brown Thrashers (*Toxostoma rufum*). The proportion of seeds germinating after passage through avian frugivores was greater than control seeds (0.88 vs. 0.67, respectively), but did not differ between Mockingbirds or Brown Thrashers. However, seeds consumed by Mockingbirds germinated significantly faster on average (4.2 d) compared with seeds consumed by Brown Thrashers (4.6 d). Consumption by either species led to faster germination than control seeds (5.5 d). The total number of viable seeds did not differ among seeds consumed by avian frugivores or control seeds. These results suggest that avian frugivores do not change the viability of *P. americana* seeds. Rather, avian frugivores shifted the timing of germination, such that more seeds germinate more quickly after passage through frugivores. The adaptive implications of accelerated germination following passage through frugivores are briefly discussed.

Key words: dispersal, frugivore, germination, viability.

Résumé : Bien qu’on sache que les oiseaux frugivores dispersent de façon importante les graines du raisin d’Amérique, le *Phytolacca americana* L., il n’existe pas d’étude ayant examiné rigoureusement les effets du passage dans l’intestin d’oiseaux frugivores sur les graines du *P. americana*. L’auteur a étudié comment le passage via les oiseaux frugivores affecte la proportion des graines qui germent, le taux de germination (nombre moyen de jours requis pour que toutes les graines germent), et le nombre total de graines viables. Les fruits récoltés sur le terrain ont été dépouillés de leur pulpe (graines témoins), et offert à des moqueurs polyglottes (*Mimus polyglottos*) ou à des moqueurs roux (*Toxostoma rufum*). La proportion des graines ayant germé après un passage dans ces oiseaux frugivores est plus élevée que chez les graines témoins (0,88 vs. 0,67, respectivement), mais ne diffère pas entre le moqueur polyglotte et le moqueur roux. Cependant, les graines consommées par le moqueur polyglotte germent en moyenne significativement plus vite (4,2 jours) que celles consommées par le moqueur roux (4,6 jours). La consommation par une des espèces d’oiseaux se traduit par une germination plus rapide que les témoins (5,5 jours). Le nombre total de grains viables ne diffère pas entre les graines consommées par les oiseaux frugivores et les graines témoins. Ces résultats suggèrent que les oiseaux frugivore ne changent pas la viabilité des graines du *P. americana*. Plutôt, les oiseaux frugivores modifient le temps de germination, de sortes qu’un plus grand nombre de graines germent plus rapidement, après le passage dans les oiseaux frugivores. L’auteur discute les implications d’une accélération de la germination, suite au passage dans des oiseaux frugivores.

Mots clés : dispersion, frugivore, germination, viabilité.

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Introduction

Recent reviews of over 350 experiments suggest that seeds passed through the guts of avian frugivores often exhibit increased overall germination relative to nonconsumed seeds, although gut passage may also reduce germination (Travaset 1998; Travaset and Verdú 2002; Verdú and Travaset 2004). Although less frequently examined, the rate of germination is also often increased (Travaset and Verdú 2002). However, as noted in a recent review of the effect of gut passage on nearly 200 species, the viability of consumed and nonconsumed seeds is rarely measured (Travaset 1998). As such, it is frequently unknown whether consumption by frugivores affects dormancy, viability, or both.

Pokeweed, *Phytolacca americana* L., is consumed by a variety of animals, including birds (Martin et al. 1951; McDonnell et al. 1984), mammals (Martin et al. 1951), and reptiles (Braun and Brooks 1987). Despite the importance of avian frugivores in the dispersal of pokeweed (McDonnell et al. 1984), a rigorous examination of whether passage through birds affects the viability and germination characteristics of *P. americana* seeds has not been conducted. I examined how gut passage by two species of avian frugivore affects the germination and viability of *P. americana*. Four questions were of interest. (1) Does gut passage affect the proportion of *P. americana* seeds that germinate? (2) Does gut passage affect the rate of germination? (3) Does gut passage affect overall seed viability? (4) Does the effect of gut passage differ between two common avian frugivores, Northern Mockingbirds (*Mimus polyglottos*) and Brown Thrashers (*Toxostoma rufum*)?

Materials and methods

Seed collection and treatment

On July 28, 2003, ripe fruits were collected from naturally occurring *P. americana* plants at the Savannah River Site, a National Environmental Research Park near Aiken, South Carolina. Because germination characteristics can vary among fruits collected from the same plants (Armesto et al. 1983), half of the fruits were randomly removed along the length of each raceme as controls, whereas the other half of the fruits were fed to Mockingbirds and Brown Thrashers housed at a wildlife rehabilitation facility. This collection method ensured that seeds were collected from the same plant population and were randomly mixed and sampled prior to feeding to frugivores, two procedures suggested for more robust examination of the effect of gut passage on seeds (Travaset and Verdú 2002).

Seeds were extracted from the control fruits and thoroughly washed to remove all traces of pulp and liquid. Seeds defecated from birds were collected the next morning and washed clean of fecal material. These three groups of seeds (control seeds, Mockingbird-consumed seeds, and Thasher-consumed seeds) were placed in separate bags constructed of fiberglass window screening. On November 20, 2003, bags were buried together at the Savannah River Site approximately 8 cm deep to allow seeds to stratify over winter in a manner similar to naturally dispersed seeds. Seeds were exhumed on 28 March 2004 and stored in a dry container at room temperature until germination trials were initiated.

On 25 May 2004, samples of 15 seeds each were counted into 60-mm plastic Petri dishes containing a cotton cosmetic pad wetted with distilled water. Cosmetic pads were used because preliminary experiments suggested that cosmetic pads had superior moisture-holding ability compared with that of filter paper. Twenty samples were counted for control, Mockingbird, and Brown Thrasher seeds, yielding a total of 60 Petri dishes (20 samples for each of three treatments) and a total of 900 seeds. Dishes were randomly arranged along a single level within a Percival PGC-15.5 growth chamber (approximate light output 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ from cool white florescent and incandescent bulbs) set to 14 h day : 10 h night photoperiod, with day and night temperatures of 34 and 27 °C, respectively. This regime was chosen as a compromise between the settings used in two other studies of *P. americana* germination (Farmer and Hall 1970; Edwards et al. 1988). Seeds were checked daily and distilled water added if necessary. Seeds were considered to have germinated when the radicle was >1 mm in length (Farmer and Hall 1970) and were then removed from the dish. The experiment was terminated on 17 June 2004, in accordance with the duration of other *P. americana* germination studies (Farmer and Hall 1970; Edwards et al. 1988).

At the conclusion of the germination study, remaining ungerminated seeds were collected and subjected to a tetrazolium assay to determine if seeds contained viable embryos. Ungerminated seeds were pooled into two groups for viability analysis: seeds consumed by avian frugivores and control seeds. Pooling was necessary because few seeds consumed by avian frugivores had not germinated by the conclusion of the germination trial and was justified since the proportion of seeds germinating did not differ between seeds consumed by the two bird species (see Results). The tetrazolium assay was performed at the Iowa State University Seed Laboratory using standardized methods (Grabe 1970). Embryos exhibiting any metabolic activity were considered viable.

Statistical methods

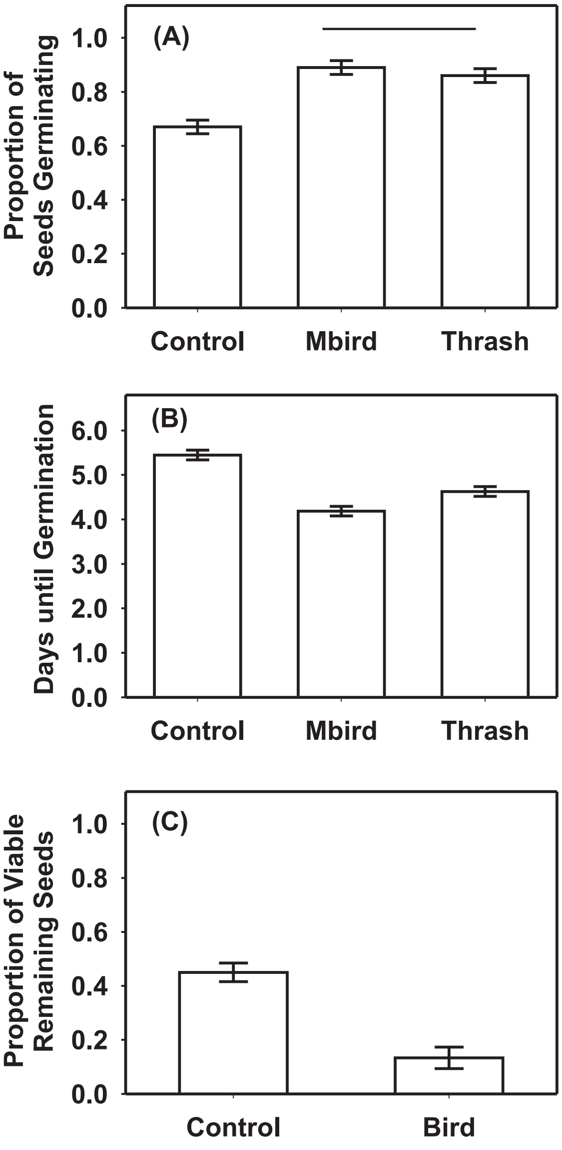
I used a one-way analysis of variance (ANOVA) to compare the proportion of seeds germinating among control, Mockingbird, and Brown Thrasher treatments. Similarly, I used a one-way ANOVA to determine if germination rate differed because of treatment. Germination rate was defined as the mean number of days required for a seed to germinate in each sample (e.g., if half of the seeds in a sample germinated in 2 d and the other half germinated in 3 d, the mean number of days required for that sample was 2.5). Although not presented, identical results were obtained if other measures of germination rate were used, such as the number of days required for the first seed in a sample to germinate or the number of days it took for at least half of the total number of seeds to germinate (Travaset 1998). Following significant ANOVAs, I used Tukey's least significant difference procedure to control type 1 error rates during post-hoc comparison of means (Zar 1996). Examination of residuals suggested that assumptions of ANOVA were met (although nonparametric Wilcoxon tests yielded identical results). The proportion of ungerminated seeds that were viable was compared using a *t* test (Zar 1996). Because ungerminated samples were pooled for viability analysis, testing for differences in the total number of viable seeds (germinants and dormant seeds) between control and bird-consumed seeds was done using a linear contrast performed with Program CONTRAST (Sauer and Williams 1989). The use of CONTRAST allowed a controlled comparison of the four estimates (germinants of control and bird-consumed seeds, and the viability of ungerminated control and bird-consumed seeds) grouped by treatment. Specifically, coefficients for the linear contrast would be 1, –1, 1, –1 among estimates grouped as above. All other analyses were conducted using SAS® version 8.1 (SAS Institute Inc. 2000). Means ± 1 SE are presented.

Results and discussion

Passage through the gut of Mockingbirds or Brown Thrashers significantly increased the proportion of *P. americana* seeds that germinated ($F_{[2,57]} = 22.09$, $p < 0.01$; Fig. 1), which is consistent with recent reviews (Travaset 1998; Travaset and Verdú 2002; Verdú and Travaset 2004). The increase in the proportion of seeds that germinated did not differ among Mockingbirds or Brown Thrashers (Fig. 1A). Gut passage by avian frugivores also increased the rate of germination ($F_{[2,57]} = 34.01$, $p < 0.01$; Fig. 1B). Germination rate was highest when seeds passed through Mockingbirds, lower when seeds passed through Brown Thrashers, and lowest for control seeds (Fig. 1B). As measured by the tetrazolium assay, viability of ungerminated seeds was greater for control seeds than for bird-consumed seeds ($t = 6.03$, df = 12, $p < 0.01$; Fig. 1C). As such, passage through avian frugivores increased the proportion of seeds germinating and the rate of germination but did not affect the total number of viable seeds ($\chi^2 = 0.02$, 1 df, $p = 0.90$). On average, 14.46 of 15 seeds (96.4%) in each bird-consumed sample were viable and 14.55 of 15 seeds (97%) in each control sample were viable.

Considered in light of the reliance of *P. americana* on bird dispersal (McDonnell et al. 1984; Martin et al. 1951), is it possible that shifts in germination following consumption (Fig. 1) have adaptive significance? A common hypothesis is that frugivore-induced changes in germination increase the overall variance in germination patterns, which is thought to be adaptive in unpredictable environments (Harper 1977). Because *P. americana* inhabits disturbed, early successional habitats (Armesto et al. 1983; McDonnell et al. 1984), the location of suitable microsites may be highly unpredictable in time and space.

Fig. 1. The effect of passage through Eastern Mockingbirds or Brown Thrashers on seeds of pokeweed, *Phytolacca americana*. (A) The mean proportion of seeds germinating in control, Mockingbird (Mbird), and Brown Thrasher (Thrash) treatments. (B) The average number of days elapsing before germination. (C) The mean proportion of viable seeds remaining after germination trials (i.e., seeds that did not germinate during the germination trial). To obtain sufficient samples, seeds consumed by either Mockingbirds or Brown Thrashers were combined into a single group (Bird). The horizontal bar in Fig. 1A indicates group means that are not statistically significant ($p = 0.68$ after Tukey's LSD correction); all other comparisons are significantly different (all $p < 0.02$ after Tukey's LSD correction). Error bars represent ± 1 SE.



The increased proportion of germinating seeds and increased rates of germination may be adaptive if seeds are more susceptible to consumption by granivores after dispersal by frugivores. Evidence suggests that intact *P. americana* fruits are not palatable to deer mice (*Peromyscus maniculatus*) but that seeds are readily consumed once free of the fruit (McDonnell et al. 1984). Field studies of post-dispersal seed predation of *P. americana* also support the importance of granivores on the abundance of *P. americana* seed and mature plants (Orrock et al. 2003). Rapid germination following consumption may lower the risk of granivory by reducing the amount of time a seed is available for consumption by granivores. Differences in germination of just a few days may have considerable significance: Duncan et al. (2002) found that less than 1% of *P. americana* seed piles were discovered by granivores on the first day of deployment, but more than 35% were discovered by the fourth day. Because bird-dispersed seeds may accumulate beneath perching sites (McClanahan and Wolfe 1993), rapid germination may be particularly advantageous because high-density patches of seeds may be more quickly discovered by granivores (Hulme 1998).

Accelerated germination after passage through frugivores may provide *P. americana* seeds with a competitive advantage over seeds that do not germinate as rapidly because the outcome of seedling competition may often depend upon which seeds germinate first (e.g., Loiselle 1990). In the case of bird-dispersed species like *P. americana*, accelerated germination may be particularly important because birds often consume more than one species of seed (Loiselle 1990) and seeds may be defecated in high-density groups (McClanahan and Wolfe 1993). Competition among seedlings in these groups may be intense (Loiselle 1990), and accelerated germination may provide a competitive advantage under these conditions. The potential importance of accelerated germination can be illustrated using ancillary data from *P. americana* seedlings grown in potting soil (unpublished data). Based on the relationship between above-ground seedling height and the number of days of growth (linear regression through the origin, $R^2 = 0.98$, $F_{[1,8]} = 458.93$, $p < 0.001$; oldest seedling was 22 days old), seedlings from seeds consumed by Mockingbirds would be 2.46 ± 0.12 mm taller than control seedlings by the time control seeds germinated. Brown Thrasher-consumed seedlings would be 1.60 ± 0.08 mm taller than control seedlings and 0.86 ± 0.04 mm shorter than Mockingbird-consumed seedlings.

This work suggests that passage through frugivores can affect the proportion of seeds germinating and the rate of seed germination of *P. americana*, supporting general trends exhibited by a variety of plant species in recent reviews (Travaset 1998; Travaset and Verdú 2002; Verdú and Travaset 2004). Although overall viability was not affected by gut passage, differences in the number of seeds germinating and the rate of germination may serve to exploit unpredictable habitats, reduce losses to granivores, and increase success in competition. Ultimately, the ecological relevance of differences in germination depends upon the alternatives: *P. americana* seeds that remain within fruits are unlikely to germinate (Edwards et al. 1988). As such, for plants such as *P. americana*, where most seeds pass through frugivores (McDonnell et al. 1984), control (unconsumed) seeds are more likely the rare situation (Samuels and Levey, unpublished data). To fully evaluate the ecological relevance of frugivore-mediated shifts in germination, future work may need to consider the potentially different ecological conditions experienced by seeds consumed by frugivores compared with those experienced by seeds that rely upon time to free them from the fruit.

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