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Reconsidering the effects of tannin on seed dispersal by rodents: Evidence from enclosure and field experiments with artificial seeds

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ABSTRACT

The question of how tannin affects feeding and hoarding preferences of rodents still remains poorly understood, in part, because it is difficult to control for other seed traits when considering the sole effect of tannin. Here, we constructed a series of artificial 'seeds' with different tannin levels, made from wheat flour, peanut powder and hydrolysable tannins, to determine the direct effects of tannin on both feeding and hoarding preferences. We first presented 'seeds' to individual rodents of two species (*Tamias sibiricus* and *Apodemus peninsulae*) confined in semi-natural enclosures and then monitored patterns of seed dispersal and consumption by free-ranging animals in a temperate forest in the Xiaoxing'an Mountains, Heilongjiang Province of China. Our results showed that small rodents displayed a significant preference for low-tannin 'seeds' for both consumption and caching in both captive and field experiments. Moreover, our two-year study consistently showed that tannin concentration was significantly and negatively correlated with the number of cached 'seeds' at both the individual and population levels. Seed size, compared with tannin concentrations, appeared to have little effect on dispersal distances and the number of 'seeds' cached. Low-tannin 'seeds' tended to be dispersed greater distances by rodents in the field than those with higher levels of tannin. These results failed to support those of previous reports indicating that acorns containing higher tannins are more likely to be cached by food hoarding animals.

1. Introduction

Many animal species, especially small rodents, play a crucial role in seedling establishment, plant regeneration, and the spatial distribution of plants by scatter-hoarding seeds for later use and subsequently failing to recover a portion of these seeds (Xiao et al., 2004; Li and Zhang, 2007). However, the mutualism between plants and these rodents is a complicated one, as the rodents act as both seed dispersers and seed predators (Hulme, 2002; Vander Wall and Longland, 2004; Yi and Zhang, 2008).

Seed selection for both consumption and subsequent caching by rodents is influenced by a range of seed traits (e.g., seed size, nutritional status, morphological and chemical defensive properties), some that reduce seed predation rates, others that allow the plant to tolerate partial seed consumption, and still others that promote seed dispersal, survival in caches and seedling establishment (Steele et al., 1993; Jansen et al., 2004; Xiao et al., 2006). Although some studies show the integrated effect of seed traits on seed dispersal by mammals and other species (Shimada, 2001a; Jansen et al., 2002; Muňoz and Bonal, 2008), there is much still unknown about the effects of seed characteristics on seed dispersal.

Several experimental studies have helped to clarify how various seed characteristics influence hoarding decisions of rodents, including germinations schedules (Hadj-Chikh et al., 1996; Steele et al., 2006), susceptibility to insect infestation (Steele et al., 1996), seed size (Jansen et al., 2004; Xiao et al., 2004; Zhang et al., 2008) and nutritional value (Shimada, 2001b; Smallwood and Peters, 1986). Tannins, a group of defensive phenolic compounds in plants, are also regarded as yet another important factor influencing seed selection and dispersal by rodents. Small rodents are reported to sometimes avoid high-tannin seeds (Shimada, 2001a) because tannin can disrupt digestive physiology (Chung-MacCoubrey et al., 1997; Downs et al., 2003) and cause loss in body mass, and even death in some rodents (Shimada and Saitoh, 2003; Shimada et al., 2006). However, several studies collectively suggest that tannin levels in acorns affect feeding and hoarding preferences of rodents, and that rodents often selectively cache acorns that are higher in tannins (the 'high tannin hypothesis') (Smallwood and Peters, 1986; Steele et al., 1993; Xiao et al., 2006, 2008; but see Smallwood et al., 2001). However, these secondary compounds often covary with other seed traits making interpretation of such effects

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difficult. Wang and Chen (2008) overcame some of this problem by creating artificial seeds with varying tannin concentrations and concluded that scatter-hoarding rodents often selectively cache seeds with higher tannins. However, even under such experimental conditions, caching decisions vary with season (see Smallwood and Peters, 1986), seed size (Wang and Chen, 2009), foraging conditions (Wang and Chen, 2008; Xiao et al., 2008), seed crops (Xiao et al., 2006) and even consumer body composition (Jiao et al., 2011).

In this study, we produced a series of artificial 'seeds' with different tannin levels to determine how tannin concentration affects seed fates and rodent caching decisions. Our goal was to test the effects of tannins and its interaction with seed size in the semi-natural enclosures and field experiments over two consecutive years.

2. Materials and methods

2.1. Study site

The study was conducted in late September 2009 and 2010 in the Dongfanghong Forestry Center (average elevation 750 m, located at 46°50′–46°59′ N, 128°57′–129°17′ E) in the Dailing district, Yichun city, Heilongjiang Province, northeastern China. The climate of the experimental site is dominated by northern temperate zonal monsoons with long, severe winters and short summers. The annual average air temperature is 1.4 °C with a maximum of 37 °C and minimum of −40 °C. Annual precipitation averages 660 mm, 80% of which falls in the short growing season from May to September. The study site is located in a mixed secondary broad-leaved and coniferous forest. In the experimental site, common tree species include *Betula platyphlla*, *Juglans mandshurica*, *Quercus mongolica*, *Pinus koraiensis*, *Fraxinus mandshurica*, *Phellodendron amurese*, *Acer mono* and *Tilia amurensis*; beneath the tree canopy, common shrubs are *Corylus mandshurica*, *C. heterophylla*, *Fructus schisandrae* and *Acanthopanax senticosus*. *Apodemus peninsulae*, *Clethrionomys rufocanus*, and *Tamias sibiricus* are the main seed consumers in the experimental areas. *A. peninsulae*, a nocturnal species, frequently scatter hoards seeds, while another nocturnal species *C. rufocanus* only larder hoards seeds (XF Yi unpublished data). Siberian chipmunks, *T. sibiricus*, are diurnal rodent species and mainly scatter hoard seeds of several local tree species (Yi et al., 2011a,b).

2.2. Tannin content of seeds

We first measured variation of tannin contents of seeds from several large-seeded tree species in the study area. We collected seeds from rodent-dispersed species in the study area (*J. mandshurica*, *Q. mongolica*, *P. koraiensis*, *C. mandshurica*, and *C. heterophylla*) and sent samples to the Cereal Quality Supervision and Testing Center, Ministry of Agriculture, China (No.12, Southern Zhongguancun Road, Haidian District, Beijing) for tannin analyses. Seed coats were removed and a sample of several seeds was combined, dried and ground. Hydrolysable tannins of 3–5 g kernels (dry mass) were measured by using the Folin–Denis method for each seed species (Mueller-Harvey, 2001). This colorimetric method is based on the capability of tannins to reduce the Folin–Denis reagent, in a buffer of borax-sodium hydroxide, into a bluish compound that is easily quantified via a spectrophotometric analysis (Taira, 1996). Seeds of all five tree species were regularly consumed and dispersed by rodents in the study area (Yi and Zhang, 2008; Yi et al., 2008, 2011a, b; Yi and Yang, 2011). Tannin concentration of the five seed species ranged from 0 to 12%, which helped guide our design of artificial seeds. Hydrolysable tannin was used in this study because it is considered the primary group of defense compounds in seeds (Smallwood and Peters, 1986; Bergvall et al., 2006) and similar in its effect to that of condensed tannin (Wang and Chen, 2008, 2009).

2.3. Artificial seeds

After numerous laboratory trials, in which we sought to ensure hardness and consistency of artificial food items that were similar to that of nut cotyledons (e.g., acorns), we settled on a mixture of wheat flour and peanut powder (7:3 in mass) to make artificial 'seeds' (hereafter 'seed (s)'). By adding different amounts of hydrolysable tannin (analytical grade tannic acid obtained from Tianjin Chemical Reagent Company, China), we created tannin concentrations of 0, 2, 5, 10, 12 and 15%, thereby simulating the variation in tannin concentrations in natural seeds found at the study area. To further explore if rodent abundance and seed size affect seed selection and caching, we created 'seeds' of different sizes (diameter, 0.5 and 1.5 cm) and released them in the field in two consecutive years of different rodent abundances (see below). We also presented these 'seeds' to small rodents caged in the semi-natural enclosures, to determine individual feeding and caching responses to tannin concentrations. We attached a 10 cm-long thin steel thread and a white plastic tag (2.5 cm × 3.5 cm) to each 'seed'. Each tag was numbered individually to make each 'seed' easy to relocate and identify. When rodents disperse and scatter hoard the 'seeds' in the soil or under tree leaves, the tags remain attached to the buried 'seeds' and are visible above the ground surface and easily observed (Yi and Zhang, 2008; Yi et al., 2011a). Previous studies indicate that this tagging procedure has a negligible effect on seed caching and recovery by small rodents (Xiao et al., 2006).

2.4. Seed caching in the semi-natural enclosures

We conducted experiments in separate semi-natural enclosures (10 m × 10 m) previously established in the area (Yi et al., 2011b). The enclosures were built using bricks about 2.5 m above ground and 0.5 m below the soil surface. The walls of the enclosures were smoothed to prevent escape of small rodents. To prevent predators from entering the enclosures from outside, the top of enclosures were covered with plastic nets. An artificial nest area was constructed of bricks ($H \times W \times L$ = 20 cm × 15 cm × 30 cm) in one corner of the enclosure and provisioned with a water bowl to provide shelter and a water source. A seed station of 1 m^2 was established at the center of each enclosure. We conducted captive experiments with *T. sibiricus* and *A. peninsulae* previously shown to be the primary scatterhoarders in this study area (Yi and Zhang, 2008; Yi and Yang, 2011). One individual animal was released in each enclosure allowing them to acclimate to the enclosure environments for two days prior to the experiments. Only one animal was tested at a time in each enclosure. Ten tagged 'seeds' of each tannin level (0, 2, 5, 10, 12 and 15%) were supplied to each individual of *T. sibiricus* at 0700 hours in the morning. Seed fates were checked in the afternoon (1700 hours). Similarly, ten tagged 'seeds' of each tannin level were provided to each *A. peninsulae* at 1700 hours and checked the next morning (0800 hours). Seed fates in the enclosures were defined as: intact in situ (IS), eaten in situ (EIS); eaten after removal (EAR), intact after removal (on surface) (IAR), cached after removal (CAR). Each animal was returned to the lab after its experimental trial and then to the field at the site of capture following the conclusion of experiments. In 2009, only small 'seeds' (diameter = 0.5 cm) were presented to small rodent species (8 adult *T. sibiricus*, 4♀ and 4♂, body mass: 83.01 ± 6.25 g and 8 adult *A. peninsulae*, 4♀ and 4♂, body mass: 33.45 ± 3.14 g). In 2010, we tested the effects of tannin on 'seed' caching using small (diameter = 0.5 cm) and large (diameter = 1.5 cm) 'seeds' separately. 10 adult *T. sibiricus* (5♀ and 5♂, body mass: 81.01 ± 4.80 g) and 8 adult