# ORIGINAL ARTICLE

Xianfeng Yi · Zhishu Xiao · Zhibin Zhang

# Seed dispersal of Korean pine *Pinus koraiensis* labeled by two different tags in a northern temperate forest, northeast China

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Abstract Two types of tagging methods, i.e., a  $1 \times 3$ -cm tin tag attached to seed with a 10- to 12-cm metal wire (total weight 0.32 g) and a  $2 \times 4$ -cm white plastic tag fastened to seed with an identical metal wire (total weight 0.57 g) were used to study their effects on seed dispersal of Korean pine by small rodents. A total of 600 seeds were released to assess four main points: (1) difference in seed survival rates, (2) difference in caching behaviors of small rodents, (3) difference in dispersal distances, and (4) proportion of seed missing. The results demonstrated that seed removal for wire-plastic-tagged seeds was faster than that for wire-tin-tagged seeds. There was no apparent difference in the proportion of seeds eaten in situ (42% and 52% for wire-plastic-tagged seeds and wire-tin-tagged seeds, respectively). We found 41% and 1% of seeds were moved and hoarded for wireplastic-tagged seeds and wire-tin-tagged seeds, respectively. However, 2.33% and 14% of seeds were missing, and their ultimate fates were not known for wire-plastictagged seeds and wire-tin-tagged seeds, respectively. We found the wire-plastic-tagged seeds easier to track than the wire-tin-tagged seeds due to the fact that the white plastic tags were more salient than the tin tags in field environments. The average dispersal distances were  $4.11 \pm 2.40$  m and  $3.01 \pm 2.06$  m for wire-plastic-tagged seeds and wire-tin-tagged seeds, respectively, and showed great difference. Despite most being eaten in situ or after removal, 41% of seeds were cached for wireplastic-tagged seeds, much more than for wire-tin-tagged seeds. A total of 71 primary caches (123 seeds) were

X. Yi · Z. Xiao · Z. Zhang (⊠) State Key Laboratory of Integrated Management of Pest Insects and Rodents in Agriculture, Institute of Zoology, The Chinese Academy of Sciences, Beisihuanxilu 25, Haidian District, Beijing 100080, China E-mail: zhangzb@ioz.ac.cn

*Present address*: X. Yi College of Agriculture, Henan University of Science and Technology, Luoyang 471003, China found for wire-plastic-tagged seeds, with the average and maximum cache sizes being 1.73 and 6, respectively. However, only three caches were found, and cache size was equal to one for wire-tin-tagged seeds. The above data suggests there is some uncertainty in different tagging methods to used track seed fates. Despite their effectiveness in helping to trace seed dispersal or movement by seed-dispersing rodents, different tagging methods—including size, color, and mass—need to be fully understand in enclosure experiments.

**Keywords** Korean pine · Seed dispersal · Seed fate · Tagging methods · Xiaoxing'an mountain

### Introduction

Many propagules of large-seeded plant species are dispersed by animals (e.g., birds, mammals, ants) that eat or disperse the fruits or seeds of such plants. In most ecosystems, a great number of vertebrate animals are identified as predators or/and dispersers of seeds or fruits of a particular plant species (Forget and Milleron 1991; Forget 1996; Godoy and Jordano 2001; Kaplin and Lambert 2002; Xiao et al. 2006). However, seed dispersal is a very complicated process that includes many steps and links the end of the reproductive cycle of adult plants with the establishment of the offspring of those plants (Wang and Smith 2002). Therefore, to trace the ultimate fate of every dispersed seed or fruit seems to be a major obstacle (Levey and Sargent 2000; Wang and Smith 2002), yet it is of great importance for evaluating reproductive success and seedling establishment of given plant species. Although a great deal of research has recently focused on animal-mediated seed dispersal and provided valuable information, seed-marking techniques, a key step for tracking dispersed seeds, varied greatly according to different researchers and experimental conditions (Vander Wall and Joyner 1998; Li and Zhang 2003; Xiao et al. 2004a, b, 2005; Forget and Wenny 2005).

A variety of marking methods have been applied in seed-dispersal ecology, especially for large-seeded species dispersed by small rodents (Forget and Wenny 2005). Among these methods, radioisotopic markingconsidered to cause no injury to seeds and to be undetectable to small rodents-does not affect the removal and further handling of seeds by animals (Forget and Wenny 2005), is widely used (Jensen 1985; Jensen and Nielsen 1986; Vander Wall 1992, 1993, 1994; Vander Wall and Joyner 1998), and shows high tracking efficiency. Besides radioisotopic marking, thread-marking methods also gained wide popularity (Yasuda et al. 1991, 2000; Wang and Ma 1999; Brewer 2001; Jansen et al. 2002, 2004; Chauvet et al. 2004; Theimer 2001, 2003; Dennis 2003; Zhang and Wang 2001; Hoshizaki and Hulme 2002; Li and Zhang 2003; Xiao et al. 2004a, b, 2005). With the development of new technologies, the use of stable isotope techniques and molecular genetic markers also holds great promise for the study of seed dispersal (Grivet et al. 2005; Hardesty et al. 2006; Pairon et al. 2006). Microsatellites would provide accurate information on individual dispersal events and lead to a better insight into the dispersal process. However, the above techniques have respective shortcomings due to condition limits. For example, radioisotope methods, stable isotope techniques, and molecular genetic markers are relatively expensive and require specific detection equipment (Xiao and Zhang 2003; Forget and Wenny 2005). Due to their unique traits of lower cost, simple disposition, and versatile manipulation (Xiao and Zhang, 2003; Forget and Wenny 2005), thread-marking methods have been popularly accepted and used, especially in China (Li and Zhang 2003; Xiao et al. 2004a, b, 2005).

In recent years, we have developed a new marking method known as wire tagging to track seed fates (Li and Zhang 2003; Xiao et al. 2004a, 2004b, 2005). However, the size and mass of tags are still under final determination. To what extent the tags would not affect seed removal and seed handling by animals and consequently seed survival and germination still remains unanswered (Zhang and Wang 2001; Li and Zhang 2003; Xiao et al. 2006). Despite the fact that most thread-marking studies assume that marking has little or negligible effects on seed fate or animal behavior (Wenny 2000; Forget and Wenny 2005), it is necessary to evaluate the effects of tags on seed removal and dispersal by animals, because we have actually witnessed side effects of wire tagging.

In the present study, we investigated the effects of tags on seed removal and dispersal by animals using two kinds of marking tags: (1) metal-wire tin tagging and (2) metal-wire plastic tagging. The former method has been used for several years, but the latter was just developed in late 2005 (Xiao ZS and Chang G. unpublished data). For metal-wire tin tagging, a  $1 \times 3$ -cm tin tag is attached to each individual seed with a 10- to 12-cm metal wire (Zhang and Wang 2001; Li and Zhang 2003; Xiao et al. 2004a, b, 2005; Zhang et al. 2005), the total mass of tag and wire being 0.32 g (Fig. 1). Compared with metal-

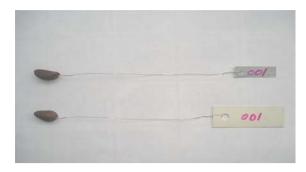


Fig. 1 Two kinds of tags used to track seed fates and dispersal in this study

wire tin tagging, metal-wire plastic tagging is designed to fasten a  $2 \times 4$ -cm white plastic tag to each individual seed with a 10-cm identical metal wire, the total mass of tag and wire being 0.57 g (Fig. 1). This transformation is intended to facilitate seed relocation because the former methods result in a great proportion of seed missing in the field.

Four main points are addressed in this paper: (1) difference in seed survival rates between the two methods, (2) effects of the two methods on the behavior of small rodents as revealed by the proportion of seed eating, moving, and caching, (3) difference in dispersal distance by small rodents using the two methods, and (4) proportion of seed missing.

#### **Materials and methods**

# Study areas

The study was conducted in September 2006 in the Dongfanghong Forestry Center (elevation averaged 750 m, 45°58'N, 129°08'E) in Dailing District, Yichun City, Heilongjiang Province, northeast China. The climate of the experimental site is dominated by the north temperate zonal monsoon with long, severe winters and short, cool summers. The annual average air temperature is 1.4°C, with extremes of a maximum of 37°C and a minimum of -40°C. Average annual precipitation averages 650 mm, 80% of which falls in the short summer growing season from May to September. Vegetation is characterized by secondary broad-leaved and conifer mixed forest. Dominant or common canopy tree species include Quercus mongolica, Pinus koraiensis, Fraxinus mandshurica, Phellodendron amurense, Acer mono, and Tilia amurensis. Beneath the tree species, shrubs such as Corylus mandshurica, C. heterophylla, Fructus schisandrae, and Acanthopanax senticosus dominate.

#### Methods

In the experimental site, 20 individual plots were spaced 15–20 m apart along two transecting lines over 200 m long. When the seeds of the Korean pine were available

in September 2006, we invited local farmers to collect directly from fruiting plants and used water flotation to distinguish between sound and insect-damaged/empty seeds. Sound seeds were randomly selected and labeled using methods reported by Zhang and Wang (2001) and Li and Zhang (2003), with minor modifications. A tiny hole 0.5 mm in diameter was drilled through the husk far from the embryo of each seed without damaging the cotyledon or the embryo. A tin tag or a hard, flexible plastic tag (4 cm  $\times$  2 cm, < 0.5 g) was tied through the hole in each seed using a 10- to 12-cm-thin metal thread. Each tag was consecutively and discriminatively numbered to make each seed easy to relocate and identify. When rodents buried the seeds in the soil or fallen tree leaves, the tags were often left on the surface of ground, making them easy to find.

Thirty wire-plastic-tagged seeds and 30 wire-tin-tagged seeds were respectively placed at each plot. Every day after seed release, we checked the tagged seeds at each plot to investigate seed harvest (eaten in situ, removed, and later seed fate). At the same time, a 10-min random search was undertaken of the area surrounding each plot (radius  $\approx 10$  m) for tagged seeds and fragments removed from each plot by small rodents and to examine the proximate fates of the released seeds: intact in situ, eaten in situ, eaten after removal, intact after removal (on surface), cached after removal (in soil), and missing (may be in burrow or not seen). When a cache was found, the microhabitat around that cache and seed code numbers were carefully recorded, the distance the tagged seeds had traveled was measured, and the cache location was determined using a chopstick that was coded in indelible ink with the number of seeds. The sticks were set 20 cm away from the seed cache. In subsequent visits, we also checked all caches relocated in previous visits until the caches were removed or eaten by the rodents. If a marked cache was removed, the area around the cache (radius <10 m) was randomly searched. The ultimate fates of the hoarded seeds were not determined in this study due to time limitations.

### Small seed-eating mammal identification

In the experimental site, we used wooden snare kill traps baited with one peanut to monitor rodent species and their numbers in the autumn (late September) in 2006. Three transects were selected, and 16 trap stations were set at 5-m intervals along each transects for two consecutive nights. We determined the species and abundance of captured rodents. In late September 2006, two rodent species were trapped in the experimental site: *Apodemus speciosus* (four, body mass range 19–26 g) and *Eutamias sibiricus* (one, body mass 87 g). Besides the above-mentioned small rodents, the Eurasian jay *Garrulus glandarius*, and *Clethrionomys rufocanus* are expected to be other, less important, species participating in large-sized seed dispersal. Although sometimes witnessed, we failed to capture them.

# Data analysis

Independent one-sample test or one-way analysis of variance (ANOVA) was used to test the differences between each variable (time to seed harvest, proportion of seeds eaten in situ, proportion of seeds removed or hoarded, dispersal distance) using the two different tagging methods. Cox regression was used to test whether there was significant difference in seed removal rates between the two kinds of tagging methods.

### Results

Seed removal rates

Seed removal rates of *P. koraiensis* differed significantly between the two seed-tagging methods (Wald = 14.436, df = 1, P = 0.000). Seed half time of *P. koraiensis* were 1 and 3 days for wire-plastic-tagged and wire-tin-tagged seeds, respectively, indicating wire-plastic-tagged seeds were removed faster than wire-tin-tagged seeds (Fig. 2). Despite these differences, 95.7% and 90% seeds had been harvested from the seed stations for wire-plastictagged and wire-tin-tagged seeds, respectively, at the last survey.

## Postdispersal seed fate

For *P. koraiensis*, rodents cached and recached the marked seeds up to three times. We found 42% and 52%

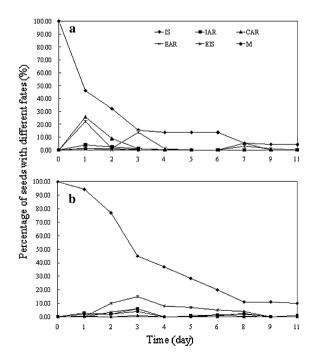


Fig. 2 Seed-survival curve of *Pinus koraiensis* by wire plastic tagging (a) and wire tin tagging (b) after placing at the released location

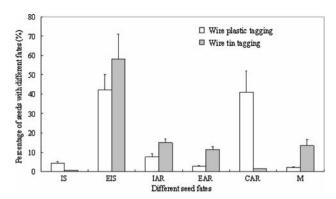


Fig. 3 Seed fates of *Pinus koraiensis* by wire plastic tagging and wire tin tagging after primary dispersal by small rodents

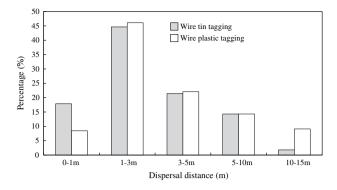
of tagged seeds were eaten in situ by small rodents for wire-plastic-tagged and wire-tin-tagged seeds, respectively. There was no apparent difference in the proportion of seeds eaten in situ. We found 41% and 1% of seeds were moved and hoarded for wire-plastic-tagged and wire-tin-tagged seeds, respectively (Fig. 3). The proportions of cached and recached seeds were greater for wire-plastic-tagged seeds than for wire-tin-tagged seeds. However, 2.33% and 14% of seeds were missing and their ultimate fates unknown for wire-plastic-tagged and wire-tin-tagged seeds, respectively (Fig. 3). The proportion of seeds that we retrieved upon removal from seed stations was marginally higher for wire-plastictagged than wire-tin-tagged seeds (97.67% vs. 86.5%, respectively; F = 4.535, df = 1, P = 0.051). We found wire-plastic-tagged seeds much easier to track than wiretin-tagged seeds due to the fact that the white plastic tags were more salient than the tin tags in field environments.

## Dispersal distances

Figure 4 illustrates the frequency distributions of dispersal distances of wire-plastic-tagged and wire-tin-tagged seeds at primary dispersal from seed-release stations. Many more wire-plastic-tagged seeds were moved further than 10 m than wire-tin-tagged seeds (Fig. 4). The average dispersal distances were  $4.11 \pm 2.40$  m and  $3.01 \pm 2.06$  m, respectively, and showed great difference (F = 4.786, df = 1, P = 0.030). However, the maximum dispersal distances were identical for wire-plastic-tagged and wire-tin-tagged seeds (15 m vs. 14 m, respectively).

### Cache size

At our last survey, the proportion of seeds remaining in release stations was much lower for wire-plastic-tagged (4.33%) than for wire-tin-tagged (10%) seeds. Despite the fact that most were eaten in situ or moved and eaten, 41% of wire-plastic-tagged seeds were cached, far more



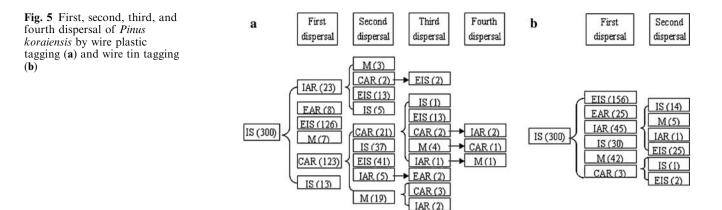
**Fig. 4** Frequency distributions of dispersal distances of *Pinus koraiensis* by wire-plastic-tagged and wire-tin-tagged seeds at primary dispersal from seed-release stations

than wire-tin-tagged seeds. A total of 71 primary caches (123 seeds) were found for wire-plastic-tagged seeds, of which two pine seeds were contained in 29 caches, three were contained in six caches, four were contained in two caches, and six were contained in one cache, with the average and maximum cache sizes being 1.73 and 6, respectively (Fig. 5). However, for wire-tin-tagged seeds, only three caches were found, and each cache size was one (Fig. 5). Small rodents performed larder hoarding for wire-plastic-tagged seeds but not for wire-tin-tagged seeds (Fig. 5). Although seeds were dispersed up to four times (fourth dispersal), 42 caches survived for wire-plastic-tagged seeds at the last survey; however, only one remained for wire-tin-tagged seeds.

# Discussion

Our study indicates that wire plastic tagging and wire tin tagging was highly effective for retrieving individual seeds from their caches due to removal by seed-dispersing rodents. Despite the fact that some tagged seeds were harvested, moved, and hoarded more than two times (up to four times), dispersal patterns were roughly similar to other studies using radioisotope methods (Vander Wall and Joyner 1998; Vander Wall 2002, 2003), thread-marking methods (Forget and Milleron 1991), and microsatellite approaches (Pairon et al. 2006), indicating less influence of tagging on animal behavior (Forget and Wenny 2005).

However, different dispersal patterns were also found between the two tagging methods. Seed removal rates were higher for wire plastic tagging than for wire tin tagging. The striking salience of plastic tags was thought to be responsible for fast removal of seeds by small rodents. Despite the fact that seed markers are assumed to provide cues for cache pilferage by small rodents (Jacobs and Liman 1991; Vander Wall 1991, 2003), a great number of wire-plastic-tagged seeds were hoarded (41%), but this was not the case for wire-tin-tagged seeds (1%). The salience of plastic tags might have served as an accelerator for seed hoarding due to heavier



predation pressure on plastic-tagged seeds. As mentioned in "Materials and methods," plastic tags were larger and much more salient than tin tags in size and color in this study. Among 123 primary cached seeds, 69.92% (86/123) were pilfered or moved several times. Tagging methods seemed to have some effect on the caching behavior of small rodents. However, whether the differences in dispersal patterns of seeds with different tags were independent of types of seed dispersers is unknown. We cannot confirm that the caching of seeds tagged with plastic up to four times was completely due to the striking salience of plastic tags or any particular animal species. These questions remain to be answered.

Furthermore, wire-plastic-tagged seeds were on average dispersed to farther locations than were wiretin-tagged seeds (F = 4.786, df = 1, P = 0.030). This was consistent with the optimal cache spacing models that large seeds were cached further than small ones (Stapanian and Smith 1978; Clarkson et al. 1986), as wire plastic tags were significantly heavier and larger than wire tin tags (t = 43.265, df = 14, P = 0.000). Most important, the rate of missing wire-plastic-tagged seeds (2.33%) was lower than for wire-tin-tagged seeds (14%), which made tracking results more accurate. Both seed-tagging methods showed some shortcomings in estimating postdispersal seed fate, such as the relative longer time the seeds remained at the release locations and potential injury to embryo and cotyledons. These factors expose tagged seeds to predation pressure and fungus infestation for a relative long time, and consequently, surveys of seed removal and survival by seed tagging may ultimately be underestimated (Forget and Wenny 2005).

The above facts suggest that three rodent species, *A. speciosus, C. rufocanus*, and *E. sibiricus*, together with the Eurasian jay *G. glandarius,* are expected to be important species participating in large-sized seed dispersal. Our results indicate that wire plastic tagging has a potential advantage over wire tin tagging and other previous methods used by others, as wire plastic tagging is characterized by faster removal, higher proportion of

cached seeds, lower numbers of seed missing, and longer dispersal distances. Wire plastic tagging seems to be more suitable and applicable than does wire tin tagging in the field and might be a good alternative to the previous wire-tin-tagging method developed by us. However, resistance would increase with the increasing in size and mass of tags during removal, as we witnessed a few tagged seeds abandoned by small rodents due to high resistance of dense grasses and shrubs. However, the larger size and white color of the plastic tags provided a highly obvious clue to potential pilferage, as evidenced in Fig. 5, where some tagged seeds were recached more than three times. The respective effect of size, color, and mass of plastic tags needs to be determined in the future. Certainly, radioisotopes, stable isotopes, and even molecular genetic markers (highly preserved microsatellite sequences) are not perceivable to small rodents and may show a bright future in helping to match dispersed seeds and seedlings with parent plants and easy detection of long-distance dispersal events (Grivet et al. 2005; Jones et al. 2005; Hardesty et al. 2006; Pairon et al. 2006).

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