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Seed Mucilage Promotes Dispersal of *Plantago asiatica* Seeds by Facilitating Attachment to Shoes

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Abstract: Understanding the mechanisms underlying seed dispersal is a fundamental issue in plant ecology and vegetation management. Several species demonstrate myxospermy, a phenomenon where the seeds form mucilage after absorbing water. Mucilage is thought to act as a glue, enabling seeds to attach to the external surfaces of dispersing agents. However, there have been no quantitative investigations of the efficacy of this function of seed mucilage. We performed a trampling and walking experiment to investigate the seed dispersal of a perennial herb, Asian plantain (*Plantago asiatica* L.), which forms polysaccharide mucilage upon hydration. Our experiment showed that: (1) after trampling, more seeds of *P. asiatica* attached to shoes in wet conditions (after rainfall), in which seed mucilage was created, than in dry conditions (no rainfall); and (2) after walking for 1000 m, more seeds remained attached to shoes in wet conditions than in dry conditions. Our results indicate that mucilage promotes the adherence of seeds to the surface of vectors. We therefore provide the first empirical evidence that seed mucilage facilitates epizoochory and human-mediated dispersal.

Keywords: seed dispersal; human-mediated dispersal; anthropochory; animal dispersal; epizoochory; mucilage; myxospermy; myxodiaspory; weed; urban ecology

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1. Introduction

The dispersal of fruits or seeds is a major determinant of plant distributions, abundances, and the structure of communities [1–5]. It plays a key role in gene flow within and among populations as well [6,7]. Additionally, seed dispersal is a central issue in vegetation management because it can promote the introduction of new species and weeds [4,8–15]. It is also a major focus of studies on plant–animal interactions [16–26].

Many plants that depend on animal dispersal are transported by specific dispersing agents, while others are be transported by a range of agents, including humans [1,16,27]. Humans are important agents of both intentional (e.g., introducing crops [28]) and unintentional dispersal (e.g., seeds becoming attached to clothing [12]); dispersal by humans is referred to as human-mediated dispersal [4,8,11–13,28–31]. Human-mediated dispersal can also be categorized as methods involving human vectors, such as the attachment of seeds to clothing [1,9,10,12,30–37] or shoes [9,12,29,33,34,38,39], or those involving non-human vectors, such as vehicles [11,12,15,40,41], cargo [8], and livestock [42]. Although the significance of the role of human vectors has long been recognized [1,32,33,38,43–45], there is a paucity of quantitative evidence of dispersal by human vectors (e.g., [2,15,29,36,37]). Furthermore, despite the fact that the necessity of quantification of the dispersal distance is crucial in ecology to determine the distribution of species, there is, to date, little quantitative evidence of dispersal distance in relation to human vectors (e.g., [1,29,37]).

Plants that depend on animal dispersal have evolved various diaspore traits to increase dispersal [1,16,18,26,27,46–52], including polysaccharide mucilage. Diaspores of several species form polysaccharide mucilage after absorbing water, in a phenomenon called myxospermy (in the case of seeds [45,53–58]) or myxodiaspory (in relation to diaspores in general [55,56,59–62]). Mucilage can act as an adhesive in epizoochory (i.e., dispersal by attaching to the external surface of animals) [33,43,45,46,54,56,59,63–72]. However, to the best of our knowledge, there have been no studies that quantitatively analyze the efficacy of seed dispersal by adherence to dispersal agents due the formation of seed mucilage. Therefore, the present study aimed to evaluate whether and how seed mucilage facilitates seed dispersal, including determining whether its efficacy varies with the hydration level of seeds (wet or dry).

Seeds of several species from the genus *Plantago* produce mucilage upon hydration [45,53,56–58,61,62,64,68,70,72–84]. The polysaccharide mucilage of the *Plantago* species is primarily composed of hemicelluloses, such as arabinoxylans, which are responsible for the adherent properties of the mucilage [72,79]. Asian plantain (*P. asiatica* L.) is a perennial rosette herb widely distributed in East Asia [85]. As has been recognized previously [86], the seeds of this particular species also produce mucilage (Figure 1). Empirical data of seed dispersal following attachment to human clothing or vehicles have been reported for *Plantago* spp. (e.g., *P. asiatica* L. [87], *P. coronopus* L. [8], *P. depressa* Willd. [15], *P. lanceolata* L. [8,38,88], and *P. major* L. [38]). However, to date, no studies have quantified the efficacy of seed mucilage that is formed after hydration regarding seed dispersal for any plant species. We hypothesized that dispersal with mucilage should be promoted under wet conditions. Specifically, we predicted that more seeds of *P. asiatica* would attach to shoes in wet conditions, due to mucilage formation, than in dry conditions.

(**a**) (**b**) (**c**)

Figure 1. Photographs of seeds of Asian plantain (*Plantago asiatica* L.) before and after hydration. (**a**) Pyxises (fruits); seeds can be seen inside the bell-shaped husks. (**b**) A seed before hydration. The length of each seed was usually 1–2 mm. (**c**) A seed after hydration. Mucilage can be seen, having formed around the surface of the seed. Photographs were taken in 2021 by Nanako Abe. The original high-resolution images are available as Supplementary Materials.

2. Materials and Methods

2.1. Study Site

We conducted this study at Tamokuteki Hiroba in Midorigaoka Park (45°55′ N 143°11′ E, altitude: 49 m a.s.l.) in Obihiro City, Hokkaido, Japan, where *P. asiatica* is a common weed. The mean annual temperature and precipitation were 7.4 °C and 922 mm, respectively (2002–2021), according to the Obihiro Weather Station, which is located 2.6 km from the study site [89].

2.2. Walking Experiment

We performed walking experiments for 6 days between August and October 2021, when seeds were matured, following a modified protocol from a combination of previous studies [29,33,34,37]. The exact dates of the experiments are shown in the dataset provided in the Supplementary Materials. The selected walking lanes were located inside a flat and sandy sports ground, where most of the surface was bare. Some sparse, small-stature weeds (e.g., *Polygonum aviculare* L. subsp. *aviculare*) were present, but were avoided when selecting the walking lanes.

Each of the eight authors repeatedly performed the same experimental trials, each of which involved the following steps (Figure 2): (1) One small part of the *Plantago* stand was selected. (2) The author put on a pair of commercially available work shoes (DCM-Kakkusu shoes, sole length: 24.5 cm) with synthetic rubber soles. (3) The selected part of the stand was trampled by stepping on it 20 times. The trampled parts were labeled with colored tape so as not to be used again. (4) The shoes were carefully removed while ensuring that the seeds were not dropped. The author then carried the shoes to the starting point (hereafter called the "0 m point") of one of the walking lanes. (5) The number of seeds attached to the shoes (including the soles and all the other part of the shoes) was counted; if none were found, the trial was stopped with the initial seed number recorded as zero. (6) The author put on the shoes again, started walking at a constant, normal speed, and stopped walking at each observational point (1, 2, 5, 10, 20, 50, 100, 200, 500, and 1000 m from the starting point). The shoes were carefully removed and all the seeds attached to the shoes were counted. The location of each seed on each shoe, including the sole and the other part of the outer surface, was recorded using the map to avoid double-counting or miscounting. The walking lanes were straight and 50 m long. Observational points that were over 50 m from the start were achieved by making round trips (i.e., the 100 m point was reached by turning at the end of the 50 m walking lane and returning to the beginning). The trial ended when 1000 m was reached, even if seeds remained on the shoes.

(**a**) (**b**) (**c**)

Figure 2. (**a**) The walking experiment of seed dispersal performed in 2021. (**b**) The work shoe used in the experiment. (**c**) Seeds of Asian plantain (*Plantago asiatica* L.) attached to the sole of the shoe. A red, bell-shaped husk was also attached, adjacent to the seed on the right. The photographs were taken in 2021 by Kohei Koyama.

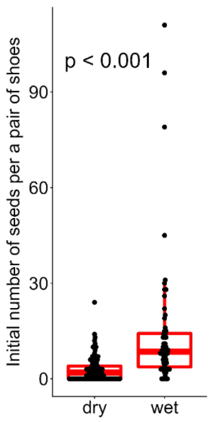
Field conditions during each experimental trial were classified as wet or dry. "Wet" conditions were defined as rain having occurred on the previous day or early in the morning of the day of the experiment; otherwise, the condition was classified as "dry". We could not rule out the possibility that some seeds may have become wet due to morning dew even on dry days. A total of 1171 seeds were examined during 162 trials in the experiment over the course of two dry and four wet days.

2.3. Data Analysis

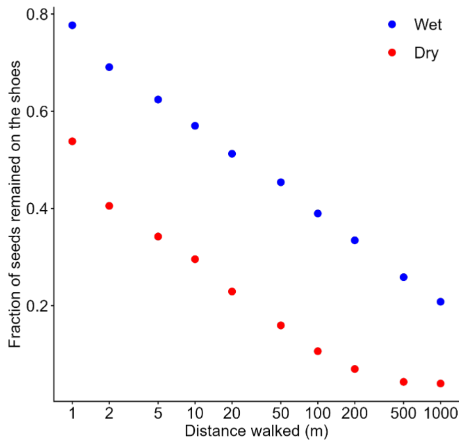
Statistical analyses were performed using R ver. 4.12 [90] with the packages *cowplot* [91], *ggbeeswarm* [92], and *ggplot2* [93]. To test the hypothesis that more seeds attach to shoes in wet conditions due to mucilage formation, we compared the mean number of seeds attached to shoes after trampling the stands (i.e., before walking) in the two conditions (wet and dry), using a generalized linear mixed model (GLMM). This analysis was performed using the R function *glmer* [94], setting the field condition (either dry or wet) as the fixed effect. We used a Poisson distribution (family = poisson (link = "log")) to predict positive and discrete dependent variables [95,96]. To test the second hypothesis, that seeds are more likely to remain attached for longer distances in wet conditions, we compared the fraction of seeds remaining after walking 1000 m using the logistic regression model of GLMM (family = binomial (link = "logit")) [47]. Following the arguments by Barr et al. [97], all factors (person and date) were included as random slopes and random intercepts, whenever justified by the design. Applying only random intercepts (no random slopes) also provided highly significant results ($p < 0.001$). The dataset is available in the Supplementary Materials.

3. Results

Significantly more seeds attached to shoes after trampling *P. asiatica* stands under wet conditions, compared with those observed under dry conditions ($p < 0.001$). The median numbers of attached seeds after trampling but before walking were 8.5 and 2.0 in wet and dry conditions, respectively (Figure 3a). In dry conditions, we often found no seeds attached to the shoes during trials (recorded as zero in Figure 3a). Seeds were significantly more likely to remain on shoes after 1000 m of walking in wet conditions, compared with those observed in dry conditions ($p < 0.001$). The mean fractions of seeds remaining at the 1000 m observation point were 20.8% and 3.99% in wet and dry conditions, respectively (Figure 3b). These seeds remained intact (i.e., not crushed) on shoes after walking.



(**a**)



(**b**)

Figure 3. Adhesion of the seeds of Asian plantain (*Plantago asiatica* L.) to shoes in different field conditions (wet vs. dry). (**a**) Initial number of seeds attached to a pair of shoes after trampling *P. asiatica* stands for 20 steps, just prior to each of the walking trials. The closed black circles indicate the results of one trial. This analysis includes cases where no seed attachment to shoes after trampling was observed, which was recorded as zero. Significantly more seeds attached under wet conditions (after rainfall) than dry conditions (no rainfall) (Poisson GLMM: $p < 0.001$). (**b**) Fraction of seeds (i.e., the total number of seeds at each distance divided by the total number of seeds at the 0 m point) remaining after walking for a given distance. Data from all trials were pooled separately for each condition (wet or dry), and trials where no seeds attached to the shoes after trampling were excluded. The seeds were significantly more likely to remain on shoes after the 1000 m walking experiment under wet conditions than under dry conditions (logistic GLMM: $p < 0.001$). The dataset is available in the Supplementary Materials.

4. Discussion

4.1. Role of Diaspore Mucilage in Human-Mediated Dispersal

Human-mediated dispersal plays an important role in the dispersal of many plant species [4,8,9,11,13,15,30,32,35–37,41,45]. It is thought that seed mucilage facilitates seed dispersal [33,43,45,46,54,56,59,63,65–71], including human-mediated dispersal. Kreitschitz et al. [54,56,71] found that the seed polysaccharide mucilage of two congeneric species, *P. ovata* and *P. lanceolata*, acts as a glue, causing seeds to become sticky when wet and to remain strongly adhered to a glass surface even after drying out. Therefore, seed mucilage can be considered analogous to the polysaccharide glue used for postal stamps [81], which also becomes sticky upon hydration and subsequent drying. Schaber et al. [98] found a similar property for the seeds of other species. However, neither of these authors investigated how the mucilage actually facilitates seed dispersal, as their work was laboratory based. The results of the present study provide the first field-based quantitative evidence supporting the laboratory findings of Kreitschitz et al. [54,56,71] and Schaber et al. [98], showing that seed mucilage facilitates seed dispersal by acting as a glue that contributes to the adhesion of seeds to shoes.

The weed *P. asiatica* is common on and around footpaths in the Midorigaoka park. Our study demonstrated that *P. asiatica* seeds can be dispersed more than 1000 m by walkers. In the park, there are bus stops and parking lots within 1000 m of identified *P. asiatica* stands. It is thus possible that seeds are dispersed to greater distances by remaining attached to the shoes of visitors who use buses or other vehicles, as has been suggested for *Brassica* spp. [29]. Therefore, our findings highlight a possible route for the inflow and outflow of seeds into and out of the park.

4.2. Limitations of the Present Study

The present study has several limitations. First, seed mucilage has been suggested to play versatile roles beyond seed dispersal [58,68,78,99], which were not investigated in our experiment. Seed mucilage anchors seeds in the soil, allowing them to remain in a favorable location (a phenomenon called antitelochory) [56,62,65,68,69,71,83,100,101]. Seed mucilage also facilitates water absorption and retention to promote germination (especially in arid environments) [56,61,68–70,73,74,78,81,83,102,103] and to facilitate repairing of damaged DNA [102]. It also prevents or reduces herbivory by protecting seeds [60,68,78,83,104,105], provides lubrication inside the digestive tracts of animals, and protects seeds during endozoochory [61,68,78,83,106,107]. Furthermore, it promotes seed floating on water during hydrochory [65,68], facilitates seed development inside fruits [68], provides carbohydrate resources to soil microbes [58,108], and acts as a reward for dispersal agents [48]. Although these additional roles of mucilage are not mutually exclusive to our hypothesis that mucilage facilitates epizoochory, further studies are needed to evaluate the relative significance of the role of mucilage in seed dispersal in comparison to its other roles.

Second, our finding that *P. asiatica* seeds are dispersed by attachment to shoes does not preclude the existence of alternative dispersal mechanisms for this species. Several species from the genus *Plantago* are known to attach to a variety of vectors. For example, the seeds of *P. major* and *P. lanceolata* are dispersed by attaching to both clothing and vehicles [12,41,88]. The seeds of *P. lanceolata* are dispersed by attaching to animals and being contained in dung [88]. The seeds of *P. lagopus* are dispersed by attaching to sheep fleece [42], and the seeds of *P. depressa* attach to vehicles [15]. Hence, it is likely that the seeds of *P. asiatica* also attach to non-human vectors. Therefore, future studies should evaluate the relative contribution of other non-human vectors.

Third, the present results are based on limited experimental conditions; the adherence property of mucilage depends on the materials of the surface of clothing [30,31,34] and, probably, on soil properties. Furthermore, we investigated only one species; patterns of seed retention on clothing also depend on seed traits, such as the type of adhesive structure of each species, which can include mucilages, sticky spines, and hairs [30,37]. Therefore, further studies are warranted to explore the potential dispersal of additional species with different seed traits under various field conditions, in order to make broader inferences.

5. Conclusions

Plantago asiatica seeds produce mucilage when they absorb water. Seeds are more likely to attach to the shoes of walkers who step on *P. asiatica* stands in wet conditions (after rainfall) than in dry conditions. The seeds are also more likely to remain on shoes when they attach during wet conditions, as compared to dry conditions. Our study provides the first empirical evidence that seed mucilage facilitates epizoochory and human-mediated dispersal. However, because the present dataset is limited to one species in one particular field, further studies that cover a wide range of species and field conditions are needed in order to draw generalizations from our results.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su14116909/s1.

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