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Effects of Human Handling of Seeds on Seed Removal by Rodents

Abstract.—Many studies have examined seed removal by rodents without regard to possible biases from inadvertent olfactory cues left by the researcher. I compared removal rates of seeds that had direct contact with human skin (scent treatment) with those that had no direct contact (no-scent treatment). Seeds of two species were alternated at 5 m intervals along a straight transect in a native grassland in northwestern Illinois. Each treatment had 100 seeds, equally divided among two woodland species that are regularly dispersed by birds into grasslands. Seeds were monitored for removal for 30 d. The rate of seed removal was significantly faster for the scent than no-scent treatment. At the end of the study 88% of scented seeds had been removed compared with 55% of the no-scent seeds. Researchers should minimize unintentional olfactory cues during seed removal studies in order to get more accurate estimates of the impact of seed removers.

Introduction

Experiments with seeds are often used to evaluate the relationship between seed foragers and plant community dynamics. Such studies include those that compare seed removal rates or total removal among plant species, locations, or seasons (reviewed by Price and Jenkins, 1986; Crawley, 1992; Hulme, 1993). Seeds are usually handled by researchers during preparation of experiments, but few studies have even mentioned the possible effects of unintentional olfactory cues (*e.g.*, Vander Wall, 1998). Rodents are the most abundant consumers of large seeds (>2 mm diam) in many terrestrial ecosystems and use olfactory cues to find food (Price and Jenkins, 1986; Hulme, 1993; Vander Wall, 1998). Thus, unintentional olfactory cues left by researchers may influence rates of seed removal by rodents. Olfactory cues can influence bird nest predation (Whelan *et al.*, 1994; Rangen *et al.*, 2000; but *see* Skagen *et al.*, 1999) and researcher visitation can influence herbivory (Cahill *et al.*, 2001), but biases introduced by researchers have not been examined in relation to seed removal.

I tested the effect of human scent on seed removal by comparing removal rates and the total amount of removal between seeds that had been handled with bare hands (hereafter scented seeds) and seeds that had not been handled directly (hereafter no-scent seeds). This comparison was intended to imitate a typical seed removal study in which the researcher takes few precautions in leaving olfactory cues.

Study Area

The study was conducted at the Lost Mound Unit of the Upper Mississippi River National Fish and Wildlife Refuge in Jo Daviess Co., Illinois (42°12′ N, 90°20′ W). The site is a former military installation with restricted access so human activity during this study was virtually nonexistent. The major habitat is dry-mesic sand prairie including over 150 plant species (Robertson *et al.*, 1997). Fire suppression and cattle grazing during the past 50 y have contributed to considerable invasion by trees and shrubs including the two focal species. Scattered individuals of *Prunus serotina* and isolated clumps of *Cornus drummondii* occur throughout the site. Both species produce fleshy fruits typically dispersed by birds (Willson, 1986). Small rodents, as opposed to ants or birds, are the primary post-dispersal seed consumers (Whelan *et al.*, 1991). Mean seed size (n = 10 for each species) are as follows: *Prunus*, length of longest axis 6.6 mm, mass 0.1 g; *Cornus*, length 3.9 mm, mass 0.02 g.

Methods

Seed preparation.—Ripe fruits were collected from trees and seeds separated from pulp by smashing fruits in a colander under running water. During seed preparation I wore latex gloves that had been rinsed in water. Any seeds with signs of insect attack or appearing discolored, cracked, misshapen or unusually small were discarded. Seeds were air-dried and then refrigerated in plastic bags until the experiment. I divided the seeds of each species into two bags, one for each treatment, and ran my bare hands through the seeds in the scent treatments for several minutes each day for a week before setting them out in the field.

Experimental design.—Seed stations were established 10 d before setting out seeds. Stations were located at 5-m intervals along a 995-m transect 10 m from, and parallel with, a seldom used gravel road. During transect establishment, seed placement, and all seed monitoring I wore tall rubber boots and sprayed my boots and clothes with a human scent neutralizer (Hunter's Specialties Scent-A-Way Spray™, Cedar Rapids, Iowa). Each station consisted of a 10-cm diameter clearing and slight depression in the sandy soil made with a boot heel. Because visual cues may also affect seed removal studies (Vander Wall, 1994), I used a minimum of visual cues to identify seed locations. The closest vertical plant stem was bent over to mark each station. In addition, a 1-m-long boot-scrape was made in the road opposite each station to help find the stations. The transect was divided into four sections of 50 stations each. The first and third sections had the no-scent seeds and the second and fourth had the scented seeds. Within each section the two species were alternated. This transect arrangement was used instead of a randomized design in order to keep the scent and no-scent treatments as distinct as possible without incorporating confounding habitat effects. Scented seeds were placed at stations with bare hands whereas no-scent seeds were only handled with forceps that had been rinsed in water and sprayed with neutralizer.

One seed was set out at each station on 25 October 1999, and monitored for removal on days 1, 3, 8, 16 and 30. Typically, seeds were easily visible from a distance of about 1 m. Aside from a light rain on day 28, no precipitation occurred during the study. On the last day (24 November) all remaining seeds were collected and examined for signs of predation and viability. Of these remaining seeds, 10 of each species were cracked open to estimate viability. All *Prunus* appeared normal and all *Cornus* had at least one filled locule (the dispersal unit of *Cornus* is a two-seeded stone), so I assumed seed condition did not influence removal.

Statistical analyses.—Data were analyzed with Systat 8.0 (SPSS, 1998). Removal rates were compared between treatments and species with survival analysis. This procedure compares observed and expected "failure" (*i.e.*, seed removal) for each census interval and then computes an overall test statistic, the log-rank Tarone-Ware χ^2 (Pyke and Thompson, 1986). Because removal rates can differ even when total removal does not (*e.g.*, Wenny, 2000), I also compared the total amount of removal with Yates-corrected χ^2 test. To determine if removal was random or sequential along transects (*e.g.*, if rodents followed the transect because of some other unintentional cues I left) I used Wald-Wolfowitz runs tests on each 50-station section of transect separately (Zar, 1999). Doing the sections separately allowed a test of removal independent of the scent treatments. I did the runs tests on removal after days 1, 3 and 8. Most seed removal took place early in the experiment so nonrandom seed removal would be most apparent during this period.

RESULTS

Removal rate of scented seeds was significantly higher than for no-scent seeds (Tarone-Ware χ^2 = 32.9, df = 1, $P < 0.001$; Fig. 1A). Overall, 88% of scent seeds were removed compared with 55% of no-scent seeds. Most of this difference occurred in the first week; 74% of scented compared with 34% of no-scent seeds had been removed by day 8. Removal rates did not differ between the two species (Tarone-Ware χ^2 = 0.9, df = 1, P = 0.32; Fig. 1B).

The total number of seeds removed was significantly greater for scented than for no-scent seeds (Yates-corrected χ^2 = 25.13, df = 1, $P < 0.001$). More *Prunus* than *Cornus* seeds were removed, but the difference was not significant (Yates-corrected χ^2 = 2.45, df = 1, P = 0.12).

None of the runs tests were significant (all tests $\alpha > 0.05$), indicating that removal was random within each of the four 50-station sections of transect during any of the three time intervals examined (days 1, 3 and 8).

DISCUSSION

In this study seeds handled with bare hands had higher removal rates and higher total number of seeds removed than no-scent seeds. These differences were consistent between the two seed species tested. Rodents appeared not to sequentially remove seeds from the transect, thus the fate of a seed was independent of the fate of adjacent seeds. Therefore, differences in removal were attributable to presence or absence of human scent rather than from species or habitat effects. These results indicate that studies in which researchers take no precautions to minimize olfactory cues may overestimate seed removal. This bias would apply to estimates of seed predation as well as estimates of secondary dispersal (for example, by rodents that scatterhoard seeds). The effect of olfactory cues on removal rates may

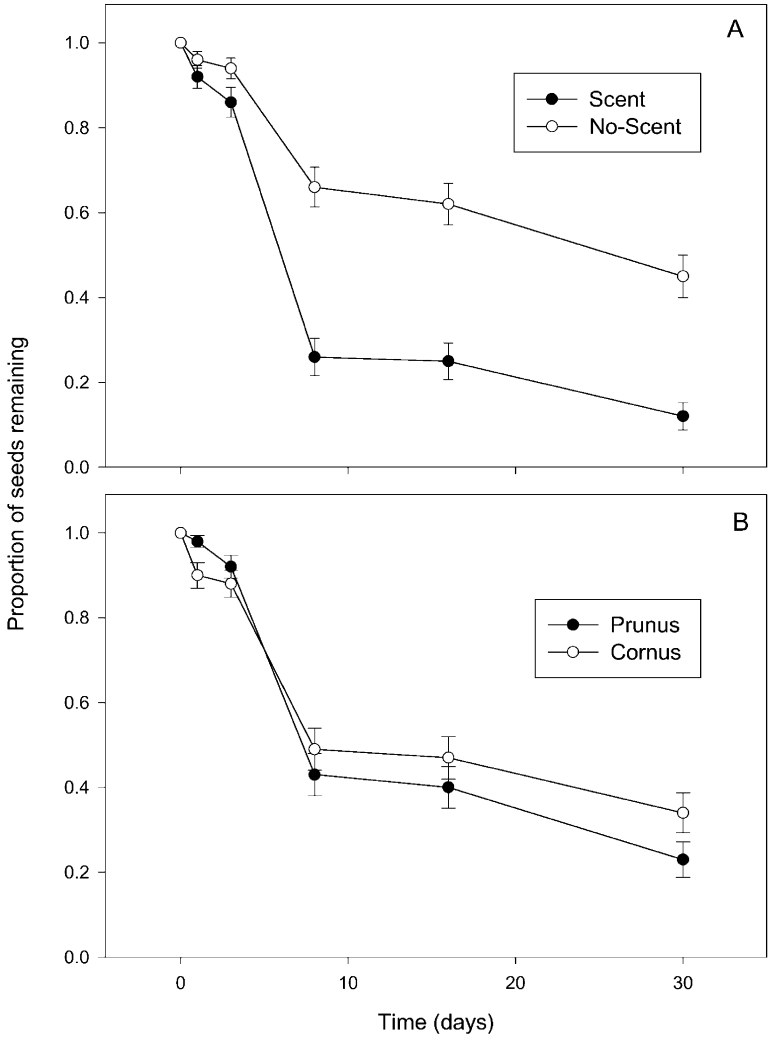

FIG. 1.—Proportion of seeds remaining (± SE) based on the Kaplan–Meier survival function compared between (A) scent treatments and (B) species. N = 100 seeds in each treatment divided equally among species

not apply to plant species for which ants, birds or fungal pathogens are the main seed removers or mortality agents. Although olfactory cues may bias estimates of removal, studies that compare relative removal among seed species, habitats or other treatments may still be valid if there are no interactions between olfactory cues and the treatments compared.

Overall, it is increasingly clear that more attention to experimental design is warranted for seed removal studies. Although the use of visual cues by foraging rodents has been noted in a few studies (Vander Wall, 1994; Blate *et al.*, 1998; Duncan and Jenkins, 1998; Vander Wall, 1998), the impact of olfactory cues remains poorly understood. Seeds emit volatile chemicals and rodents detect seeds using olfactory cues (Vander Wall, 1998; Jorgensen, 2001). The extent to which human scent interacts with or interferes with the natural odor from seeds deserves further study.

Based on this study of seed removal in a grassland area, unintended olfactory cues may increase seed removal by 60%. However, because the woodland and grassland habitats have different small mammal communities (Hofmann *et al.*, 2000), the effects of olfactory cues may vary among habitats. Areas with greater abundance or diversity of seed predators may be most susceptible to error from olfactory cues. Thus, it would be useful to compare the effects of olfactory cues on seed removal among habitats. It would also be useful to repeat this experiment in tropical habitats where seed removal is often very high (Crawley, 1992; Hulme, 1993; Wenny, 2000). Some other questions that could be explored in future studies include: To what extent does an overestimate of seed removal affect the current view of the role of seed removal and seed predation in community dynamics? Do unintended olfactory cues influence removal of seeds cached in the soil? Do artificial cues have more influence on seed predators than on secondary dispersers? Does the effect of these cues differ between frequency-dependent and density-dependent foragers? Answering these questions will help us understand if and how experimental procedures bias the results and how we can limit these biases in future studies.

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