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Consumption of fresh and buried seed by ground beetles (Coleoptera: Carabidae)

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Abstract. Ground beetles (Carabidae: Coleoptera) are predators of the seed of herbaceous plants scattered on the ground, but prefer that of certain species. Foraging beetles encounter both freshly dispersed and seed exhumed from the soil bank. The predation on seed from the soil bank has never been studied and the effect of burial on seed acceptability is unknown. The preferences of two generalist granivorous carabids, *Harpalus affinis* and *Pseudoophonus rufipes*, were investigated by offering them fresh (stored frozen after dispersal) and buried (for 6 months in the soil under field conditions) seed of six common weed species. Significantly more of the buried seed of *Tripleurospermum inodorum* and significantly less of that of *Taraxacum officinale* was eaten than fresh seed. For four other weed species the consumption of both kinds of seed did not differ. The preferences were similar in both species of carabid. The change in preference probably occurred because the seed of *T. officinale* was partially decayed and the repellent surface of *T. inodorum* seed abraded. Provided the seed in the soil bank does not decay it may have a similar or better food value for carabids than fresh seed.

INTRODUCTION

In Central and Western Europe, ground beetles (Carabidae) are the most important invertebrate consumers of the seed of herbaceous plant scattered on the ground (Honěk et al., 2003; Westerman et al., 2003). The seed eating (granivorous) species typical of arable land belong mainly to the tribes Harpalini (represented by several genera, e.g. *Anisodactylus*, *Harpalus*, *Ophonus*, *Pseudoophonus*, *Stenolophus*) and Zabrini (*Amara*, *Zabrus*) (Lindroth, 1949; Thiele, 1977; Hůrka, 1996). Granivory, typical of adults (Goldschmidt & Toft, 1997; Jorgensen & Toft, 1997a; Hůrka & Jarošík, 2003), is also essential for successful larval development in some species (Jorgensen & Toft, 1997b; Saska & Jarošík, 2001). Seed predators aggregate in weedy agricultural fields (Kokta, 1988). Their role in decreasing the seed output of weeds on farmland is generally recognized (Luff, 1987; Lövei & Sunderland, 1996; Kromp, 1999; Tooley & Brust, 2002; Honěk et al., 2005). The adults (Tooley et al., 1999; Honěk et al., 2003) and larvae (P. Saska, unpubl.) eat particular seed species selectively. The choice differs between carabid tribes and at the species level is determined by the relative size of the seed to that of the carabid and other still incompletely understood qualities of the seed (Honěk et al., in prep).

In nature, ground beetles may encounter not only "fresh" recently shed seed, but also seed that entered the soil and was successfully brought to the surface by cultivation, water or wind erosion, soil movements caused by freezing, etc. This seed is referred to here as "buried". The beetles might also find buried seed when entering soil cracks in search of shelter. Burial is likely to change the qualities of seed, particularly that of its surface. The relative attractiveness of buried seed for ground beetles has not yet been investigated.

In this paper the differences in carabid preference for "fresh" and "buried" seed are investigated. The hypothesis that burial changes the acceptability of the seed was tested. The predators used were two generalist species of the tribe Harpalini, which differ in body size, *Harpalus affinis* (Schrank) (dry body mass 13.4 mg) and *Pseudoophonus rufipes* (DeGeer) (29.6 mg). Both species are abundant seed predators in central and western Europe (Honěk & Jarošík, 2000; Westerman et al., 2003) and seeds make up a considerable part of their diet under natural conditions (e.g. Sunderland et al., 1995). For preference experiments we selected the seed of six common species of weed known to differ in their attractiveness for the two carabid species (Honěk et al., 2003). The preferences of adults of both carabids for fresh and buried seed was studied using a cafeteria experiment.

MATERIAL AND METHODS

Seed

The seed of six species of weed, *Capsella bursa-pastoris* (L.) Medik, (seed mass 0.23 mg), *Cirsium arvense* (L.) Scop. (0.79 mg), *Melandrium album* (Mill.) Garcke (0.79 mg), *Taraxacum officinale* Weber ex Wiggers (0.48 mg), *Thlaspi arvense* L. (0.97 mg) and *Tripleurospermum inodorum* (L.) Schultz-Bip. (0.32 mg) was used in the experiments (seed mass: Honěk et al., 2003). All these weeds are common in fields, field margins, roadsides and anthropogeneous habitats (Dostál, 1989). The seed is produced in July–October (*C. arvense, M. album, T. inodorum*) or throughout the whole vegetative season, starting from April (other species) (Fisjunov, 1984). All species have long-term soil seed banks except *T. officinale*, which has a transient seed bank (Grime et al., 1990). The species of weeds were selected because in an earlier experiment (Honěk et al., 2003) their seed was less or more preferred by both species of carabid. The expectation was that the seed may reveal positive effects (i.e. increasing consumption of the less preferred seed) or negative effects (i.e. decreasing consumption of the more preferred seed) of burial. The seed was collected in August–October, 2002 at Praha Ruzyně (50°06´N, 14°06´E), dried and stored in a room at 25°C and 40% relative humidity. On November 11, one half of the seed of each species was frozen at –20°C, and the other half buried in a field. The buried seed was mixed with sieved soil (mesh diameter 1 mm) and wrapped in pieces of nylon fabric. The fabric (0.24 mm mesh size, 56% open area) allowed water to enter from the surrounding soil. The packets of seed were buried at a depth of 20 cm under grass sward. The positions of samples were marked by string that connected the packets of seed to wooden labels at the soil surface. The samples were exhumed on May 30, 2003, dried under room conditions for one week, then kept at –20°C until used in the consumption experiment. The two batches of seed for each species (seed "kinds") are called "fresh" and "buried" seed, respectively. Using frozen seed as "fresh" seed excluded the variation in quality that would arise if "fresh" seed is collected just before the start of a preference experiment, i.e. one year later than "buried" seed. Freezing was preferred although it might slightly change seed quality. Freezing is the recommended method for storing "orthodox" seeds that tolerate dessiccation and are stored dry (Anonymous, 1994). Because of absence of water in seed tissues freezing does not affect their morphology, ultrastructure or chemical composition and, therefore, may not change the acceptability of seeds to carabids.

Beetles

The seed consumption of two generalist granivorous species of the tribe Harpalini, *H. affinis* and *P. rufipes*, was determined. The species were selected because they are locally abundant polyphagous granivores. Adults of both species were collected using pitfall traps placed in an abandoned field 3–5 days before the start of the experiment. The traps were plastic cups 7.5 cm in diameter (orifice 44.2 cm^2) and 12 cm deep, embeded in the soil with the rim at the soil surface, screened from rain and direct sunshine by a dish wrapped in aluminium foil. No bait was used. A few lumps of soil at the bottom of the cups provided shelter for trapped arthropods. The traps were emptied at 2 or 3 day intervals. The beetles used in the experiment were immediately brought to the laboratory, placed in 0.5 l plastic bottles filled with folded moist filter paper and stored in the dark at temperatures of 5–7°C. This cold storage prevented cannibalism and standardised the level of hunger.

Consumption

The experiments were done in Petri dishes (250 mm in diameter, 50 mm height), each containing a 2 cm layer of sieved soil (mesh diameter 4 mm) dug from a depth of > 0.5 m in order to avoid contamination with natural seed. Each Petri dish contained twelve trays, 28 mm in diameter (area 6.2 cm^2) and 6 mm deep, in which particular species and kinds of seed were offered (Honěk et al., 2003). The trays were filled with white plasticine (JOVI®, Barcelona) and the seeds pressed into the plasticine to a depth of half their transverse diameter. Although this plasticine is eaten by different animals including slugs or mice, it is not attractive to carabid beetles, which never aggregated around the trays or ate plasticine. Thirty fresh or buried seeds of one species were placed in each tray. The trays of seed were inserted into the soil in the Petri dishes so that the plasticine surface was level with that of the soil. The seeds were thus accessible to beetles walking accross the surface. In each Petri dish the trays were arranged in a ring of regularly (c. 4 cm) spaced trays containing seed in a standard order around the edge of a Petri dish: (1) *T. inodorum* – fresh, (2) *T. officinale* – buried, (3) *T. arvense* – fresh, (4) *M. album* – buried, (5) *C. arvense* – fresh, (6) *T. inodorum* – buried, (7) *C. bursa-pastoris* – fresh, (8) *T. arvense* – buried, (9) *T. officinale* – fresh, (10) *C. arvense* – buried, (11) *M. album* – fresh, (12) *C. bursa-pastoris* – buried.

The consumption of seed by *H. affinis* was determined on July 6–11, and that of *P. rufipes* on August 25–30, 2003. Groups of 10 beetles of a species were selected from the stock collected from the field. They were not sexed because the objective was to measure the consumption of natural populations, including both sexes. Although consumption may differ between sexes average consumption was not influenced because the beetles were selected randomly. Each group of 10 beetles were placed in a Petri dish where it was presented with a set of 12 trays containing particular species and kinds of seed of weeds (see above). For each species of carabid the experiment was replicated in 12 Petri dishes. After introducing the beetles the soil in each Petri dish was moistened with 50 ml tap water and a piece of moist cotton wool provided for the beetles. The experiments were continued for five days, at 25–27°C, a relative humidity of 100% and natural photoperiod. Every day the numbers of intact seeds in each tray were counted, missing seeds were considered eaten. The trays were replaced if > 50% of the seeds were eaten. The seed of each weed species was thus provided in excess because the seeds in none of the trays were completely eaten. None of the beetles died during the experiment.

Data processing

The numbers of each kind ("fresh" vs. "buried") of seed of each species consumed were summed over the five-day period of the experiment. The differences between carabid species were tested first using a two-way ANOVA with consumption of seed species in particular replicates as a response variable and carabid and seed species as factors. The analyses were made separately for "fresh" and "buried" seed. As in both analyses there were significant interactions between carabid and seed species, the differences between "fresh" and "buried" seed were tested separately for each carabid species. The differences were tested using a two-way ANOVA with consumption of seed species in particular replicates as a response variable and seed kind ("fresh" vs. "buried") nested within seed species, as factors. The differences between seed kinds within each species were tested by a post-hoc LSD test. Average consumption of each kind of each species of seed (as shown Fig. 1) was calculated as the arithmetic mean (± SE) of its consumption in particular Petri dishes (replicates). The calculations were made using Statistica for Windows (StatSoft, 1994).

RESULTS

The consumption (Fig. 1) significantly varied with seed species (fresh seed: $F_{5,132} = 58.5$, $p < 1.00\ 10^{-9}$; buried seed: $F_{5,132} = 117.1$, $p < 1.00\ 10^{-9}$). The consumption was not significantly affected by carabid species (fresh: $F_{1,132} = 0.235$, $p = 0.63$; buried: $F_{1,132} = 0.541$, $p = 0.46$) but the carabid × seed species interaction was significant (fresh: $F_{5,132} = 3.32$, $p = 7.40\ 10^{-3}$; buried: $F_{5,132} = 5.67$, $p = 9.06\ 10^{-5}$). Both species had similar preferences for particular seed species (Fig. 1). The differences in consumption of "fresh" and "buried" seed was significant in both *H. affinis* ($F_{6,132} = 9.80$, $p = 6.53\ 10^{-9}$) and *P. pubescens* ($F_{6,132} = 7.74$, $p = 3.83\ 10^{-7}$). The differences were insignificant ($p = 0.11$–0.99) for the less preferred seed of *C. bursa-pastoris*, *M. album* and *T. arvense* and the most preferred seed of *C. arvense* (Fig. 1). In contrast, the differences were significant for *T. officinale* and *T. inodorum*. The "fresh" seed of *T. officinale* was more readily eaten than the "buried" seed and the reverse was the case

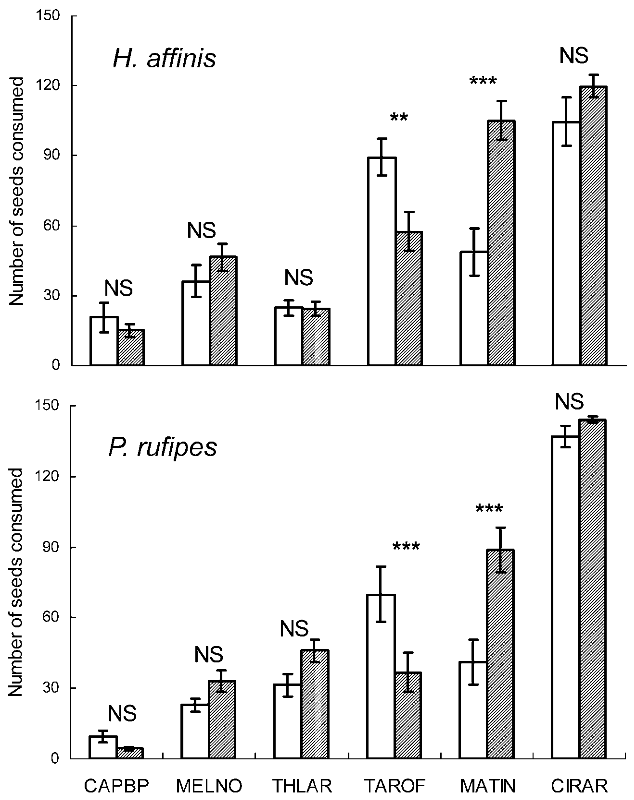

Fig. 1. Average numbers (± SE) of "fresh" (open bars) and "buried" (hatched bars) seeds of particular weed species consumed per replicate over a five day experimental period. The species of seed are ranked according to increasing carabid consumption and indicated by acronyms (European Weed Research Society codes): CAPBP – *C. bursa-pastoris*, MELNO – *M. album*, THLAR – *T. arvense*, TAROF – *T. officinale*, MATIN – *T. inodorum*, CIRAR – *C. arvense*. The differences in consumption of "fresh" and "buried" seed of weed species is indicated *** (significant at $p < 0.001$), ** (significant at $p < 0.005$) and NS (not significant).

for the seed of *T. inodorum* (Fig. 1). The differences were highly significant in both *H. affinis* (*T. officinale*: $p = 1.51\ 10^{-3}$; *T. inodorum*: $p = 7.53\ 10^{-8}$) and *P. rufipes* (*T. officinale*: $p = 3.31\ 10^{-4}$; *T. inodorum*: $p = 4.85\ 10^{-7}$). The appearance of "fresh" and "buried" seed differed little. While the "buried" seeds of *T. officinale* were somewhat eroded, the fresh and buried seeds of *T. inodorum* were similar.

DISCUSSION

The differences in consumption of particular species of seed were essentially similar to that reported in an earlier paper (Honěk et al., 2003). There were large differences in consumption of particular species of seed but little difference between the two species of carabid. The preferences are generally determined by the relative size of the carabid and the seed (Honěk et al., 2003). The smaller *H. affinis* accepted small seed of species like *C. bursa-pastoris* and *M. album*, which were rejected by the larger *P. pubescens*. In the ANOVA this difference was manifested as a significant carabid × seed species interaction.

In contrast to preferences for particular species of seed the preferences for freshly dispersed vs. soil bank stored seed were never considered nor experimentally investigated. There was no difference in the consumption of "fresh" and "buried" seed of *C. bursa-pastoris*, *M. album*, *T. arvense* and *C. arvense*. That is, burial for half a year did not change the quality of this seed for the two carabids. There was a significant difference in the consumption of fresh and buried seed of the two species of the family Asteraceae, *T. officinale* (0.48 mg) and *T. inodorum* (0.32 mg). This difference may result from a change in seed quality. The consumption of *T. officinale* seed decreased because it deteriorated during burial when some of the seeds germinated or rotted. Although only perfect seeds were selected for preference experiments burial affected their characteristics. This was expected because *T. officinale* has a transient seed bank, which does not persist in the soil for more than a year (Grime et al., 1990). In contrast, the consumption of *T. inodorum* seed increased following burial. The cause of this change is not obvious because the seed appeared unchanged. The change was possibly caused by the removal of a repellent chemical from the surface of the testa, which may protect the freshly dispersed seed from predation by carabid beetles. The chemical protection of seed against predation by carabids would be advantageous as the peak in *T. inodorum* seed dispersal occurs at the same time as the maximum activity of *P. rufipes* (mid July – mid September), the most efficient seed predator at this time (Honěk & Martinková, 1991). Erosion of the testa would also make it easier for the beetles to access the edible contents of the seed.

In the majority of species tested (4 out of 6) burial for half a year did not change the qualities of the seed important in determining carabid preference. However, it is premature to conclude that burial has a small effect on the acceptability of seed. The number of seed species tested is too small for estimating the proportion of species of seed affected by burial in the soil. Moreover, seed may persist buried in the soil for several years and such prolonged burial may affect seed quality more than burial for just half a year. Consumption of non-persistent species will be affected negatively because of the increased proportion of eroded seed. In contrast, the consumption of persistent seeds may be less affected or may even increase. Increased consumption will occur of seeds whose surfaces loose the repellent or antifeedant substance in the testa.

The results confirm the importance of carabid predation as a major mortality factor of weed seeds. Ground beetles consume large numbers of freshly dispersed seed, up to 2000 seeds m^{-2} day^{-1} (Honěk et al., 2003). This is particularly important in reducing the numbers of seed of species that do not persist in the soil bank (Honěk et al., 2005). Here it is demonstrated that seeds entering the soil bank do not escape carabid predation. After re-exposure they may be eaten even more readily than previously. This is especially important because rodents, another important predator of seed, cannot smell seed buried in the soil (Van der Wall, 1998). Carabid eating of buried seed may be an important cause of seed mortality when recently dispersed seed is scarce. This might be important in spring when few plants produce seed.

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