

Intraspecific Directed Deterrence by the Mustard Oil Bomb in a Desert Plant

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Summary

Plant secondary metabolites (SMs) acting as defensive chemicals in reproductive organs such as fruit tissues play roles in both mutualistic and antagonistic interactions between plants and seed dispersers/predators [1–5]. The directed-deterrence hypothesis states that SMs in ripe fruits deter seed predators but have little or no effect on seed dispersers [6]. Indeed, studies have demonstrated that birds are able to cope with fruit SMs whereas rodents are deterred by them [1, 7]. However, this mechanism was only demonstrated at the class level, i.e., between birds and mammals, based on differences in the vanilloid receptors [7]. Here we present experimental and behavioral data demonstrating the use of the broad-range, class-independent “mustard oil bomb” mechanism in *Ochradenus baccatus* fruits to force a behavioral change at an ecological timescale, converting rodents from seed predators to seed dispersers. This is achieved by a unique compartmentalization of the mustard oil bomb, causing activation of the system only upon seed and pulp coconsumption, encouraging seed dispersal via seed spitting by rodents. Our findings demonstrate the power of SMs to shift the animal-plant relationship from predation to mutualism and provide support for the directed-deterrence hypothesis at the intraspecific level, in addition to the interspecific level.

Results and Discussion

Glucosinolates (GLSs) are secondary metabolites (SMs) that are found in many plant species in the order Brassicales, including members of the Brassicaceae and Resedaceae families [8]. Generally, intact GLSs are harmless; however, when plant tissue is mechanically damaged, released myrosinases hydrolyze the GLSs, producing mainly thiocyanates, isothiocyanates, and/or nitriles [8, 9]. These compounds have been shown to induce toxicological and pharmacological effects on various organisms [9–12]. We examined ripe fruits from a wild population of *Ochradenus baccatus* from southern Israel for presence of GLSs.

Ochradenus baccatus (Resedaceae) is a Saharo-Sindian desert plant. Unlike most desert plants, it produces fleshy fruits with a high water and sugar content consumed by a wide variety of vertebrates [13, 14]. The fruit pulp is rich in carbohydrates (85.1% ± 0.4% dry mass; all values are means ± SE) but low in nitrogen (2.6% ± 0.09% dry mass), whereas the seeds are rich in protein (25% ± 0.09% dry mass). Each stem carries tens of fruits arranged in clusters (Figure 1C). The fruits are white berries, each ~4 mm in diameter (56.8 ± 3.9 mg fresh mass) and containing an average of 9.4 (± 0.5, n = 100 fruits) small black (viable) or white (inviable) seeds (average seed fresh mass 0.7 ± 0.03 mg, n = 40 fruits). *O. baccatus* organs (roots, leaves, stems, and fruit pulp) were found to be rich in GLSs. Moreover, in the fruits of *O. baccatus*, we found a compartmentalization between the GLSs, found only in the pulp (Figure 1A), and myrosinase enzyme, found only in the seeds (Figure 1B). The interaction of the myrosinase and GLSs during seed consumption hydrolyzes harmless GLSs in the pulp into toxic compounds, a mechanism known as the “mustard oil bomb” (Figure 1C). This activation of GLSs could deter seed predators but would not affect seed dispersers because they are not likely to damage the seeds and thus release the enzyme.

We tested the interaction between *O. baccatus* and *Acomys cahirinus* (Figure 2A), a nocturnal, predominantly seed-predating murid rodent [15], in situ. Using day/night motion-activated cameras, we documented multiple events of *A. cahirinus* climbing on *O. baccatus* bushes (n = 3 individuals; Movie S1A) and carrying an entire fruit cluster away from the parent plant (n = 3 individuals; Movie S1B). Moreover, we documented *A. cahirinus* consuming the fruits on the ground between the rocks (“rocky crevices”; 8 distinct individuals, 20 fruits per individual per session on average) and orally expelling *O. baccatus* seeds in the process (Movie S1C). When fruits were placed overnight in 90 mm Petri dishes in rocky crevices (n = 8 sites) or under *O. baccatus* bushes (n = 22 bushes), more seeds were left intact in the rocky crevices compared to under the bushes (25.7% ± 14.0% versus 6.2% ± 2.7%; Mann-Whitney U = 44.5, p < 0.05), further evidence that fruits are more likely to be moved away from the parent plant. These are conservatively low bounds of uneaten seeds, because we counted only seeds remaining inside the small dishes, and the video recordings showed that *A. cahirinus* may handle fruits away from the dish. Interestingly, rocky crevices may also provide favorable conditions for establishment of seedlings by blocking radiation and increasing humidity, factors which often limit plant growth in the desert ecosystem [14, 16]. Furthermore, compared to seeds dispersed by birds, seeds dispersed by *A. cahirinus* are more likely to remain in the wadi, which is a primary condition for *O. baccatus* germination success [14]. Seeds collected from the field that had been orally expelled by *A. cahirinus* germinated successfully in the laboratory (100% of all viable, black-colored seeds [n = 22] germinated). In laboratory experiments, 21 of 23 naive *A. cahirinus* individuals presented with whole fruits ate the pulp but left 73.8% ± 7.7% of the seeds intact, by either dropping the seeds or spitting them (Figure 2A; Movie S2). The germination rate of these seeds

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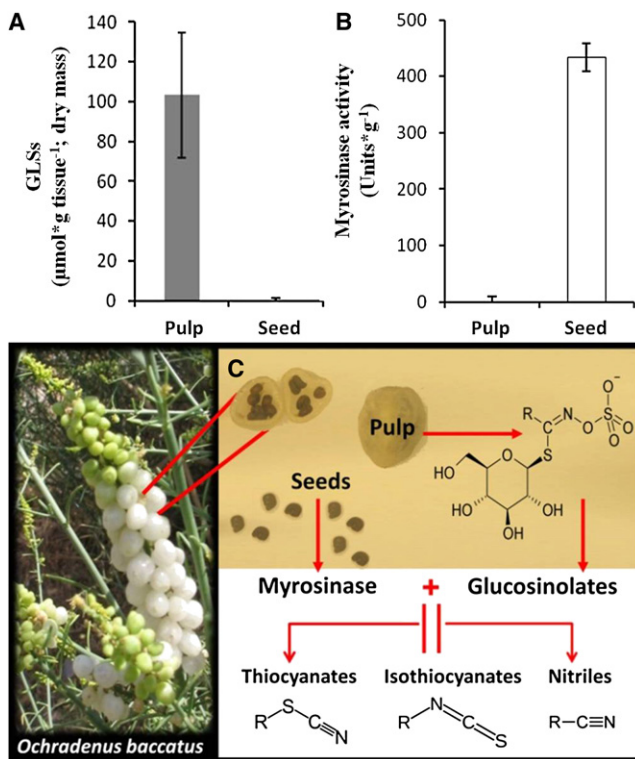


Figure 1. Compartmentalization of Glucosinolates and the Myrosinase Enzyme in Fruits of *Ochradenus baccatus*

(A) Glucosinolate (GLS) concentration in pulp (n = 8) and seeds (n = 8). Data in (A) and (B) are presented as means ± SE.

(B) Myrosinase activity in pulp (n = 8) and seeds (n = 8).

(C) Upon mechanical injury to seeds, GLSs are activated (hydrolyzed) by the enzyme myrosinase, producing toxic components [8, 9].

(83.1%, n = 65 seeds treated by n = 10 *A. cahirinus* individuals) did not differ from seeds that had been manually separated from the pulp by the experimenter (90.3%, n = 31 seeds; Z = -1.32, p = 0.18). The fruit-eating strategy of seed spitting improved germination by more than 2-fold compared to the reported germination rate of seeds within intact fruit [13, 14].

Other rodents, i.e., *Acomys russatus* (n = 4; **Figure 2B**; **Movie S3**) and *Sekeetamys calurus* (n = 1; **Figure 2C**), were also observed spitting the seeds while consuming *O. baccatus* fruits in the field, suggesting that this behavior is not limited to one species. Although seed dispersal via seed spitting has been shown in other mammalian taxa [17, 18], the present study is to our knowledge the first documentation in rodents, which are usually highly specialized granivores [19–21].

To examine the role of GLSs in modifying the behavior of *A. cahirinus*, we presented captive naive *A. cahirinus* (n = 21) with whole fruits containing seeds that had undergone treatment to deactivate myrosinase. In this experiment, less than 20% of the seeds were left intact by the rodents, compared to more than 73% for untreated seeds with natural myrosinase activity (Mann-Whitney U = 46.5, p < 0.001). Thus, when faced with a “disarmed” mustard oil bomb, *Acomys* behaved as a seed predator. To further examine the effects of consumption of different parts of the fruit, we performed feeding trials in which we monitored body mass of rodents fed for 4 days on one of five possible treatments (n = 8 individuals per treatment). Treatments 1 (pulp and seeds mashed together) and 2

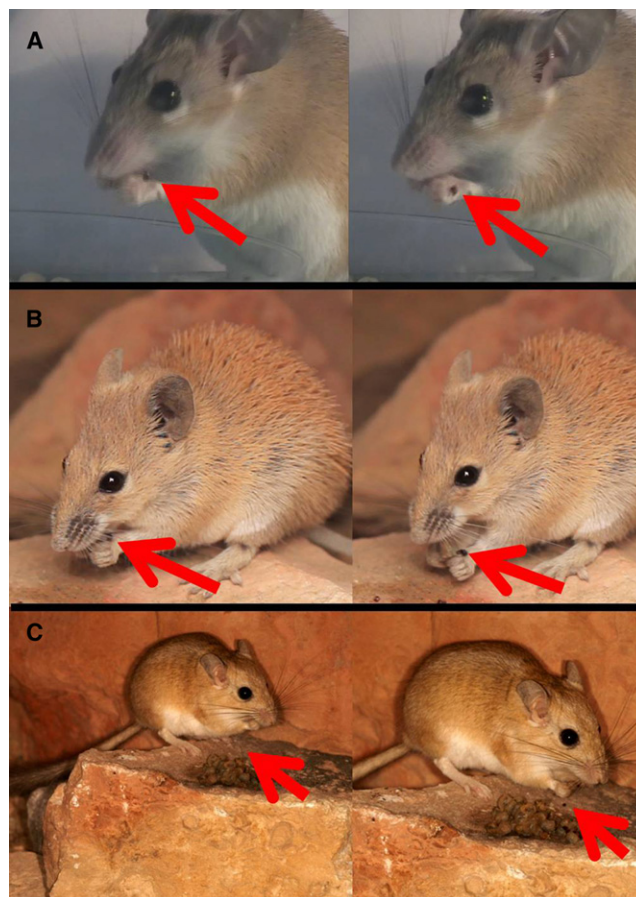


Figure 2. Three Rodent Species Spitting Intact Seeds of *Ochradenus baccatus* during Fruit Consumption

(A) Captive *Acomys cahirinus* (see **Movie S2**).

(B) Wild *Acomys russatus* (see **Movie S3**).

(C) Wild *Sekeetamys calurus*.

(purified GLSs mashed with seeds) contained all the necessary components to generate the mustard oil bomb, whereas the other treatments contained only some of the components. Only treatment 1 had a significant negative effect on the body mass of *A. cahirinus* (**Figure 3**). Treatment 2 also resulted in decreased body mass, although not significantly, relative to controls (treatments 3–5), probably because the pulp, which was missing in treatment 2, provides an optimal chemical environment for the myrosinase enzyme activity.

The mustard oil bomb is well established for its role in preventing herbivory [8, 12]. Generally, GLSs are found in all plant organs, and their concentration may vary between organs as well as between individuals of the same species ([22]; A.L. and I.L., unpublished data). The myrosinase enzyme is stored in cells separated from GLSs, presumably to avoid autotoxic effects [8, 22]. Some herbivores utilize this separation to avoid the mustard oil bomb. For example, green peach aphids, *Myzus persicae*, consume phloem containing GLSs while leaving the cells containing myrosinase that surround it intact [8, 23]. Here we demonstrate a new role for this mechanism in ripe fruits: a generalistic, species-independent, seed-dispersal-promoting mechanism resulting in an unusual relationship between a plant and a predominantly seed-predating rodent. We found that the granivorous rodent

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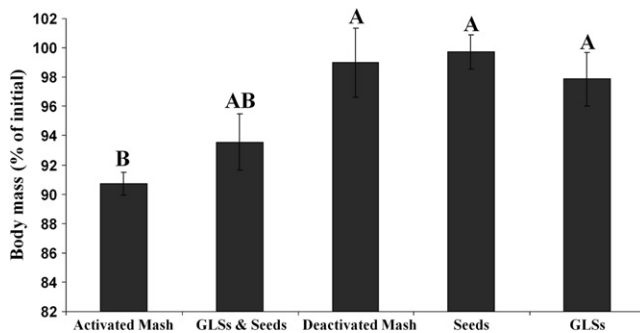


Figure 3. Feeding Trials

Change from initial body mass of *A. cahirinus* after 4 days of feeding on different *O. baccatus* diets. Only the activated mash diet had a significant negative effect on body mass. The effect of extracted GLSs combined with seeds did not differ significantly from that of the activated mash diet. Data are presented as means \pm SE ($n = 8$ animals per diet group). ANOVA showed a significant effect of the different *O. baccatus* diets ($F_{4,35} = 5.4$, $p < 0.005$); letters above columns indicate significant differences ($p < 0.05$, Bonferroni multiple comparison).

A. cahirinus circumvents the activation of GLSs found in *O. baccatus* fruits by spitting viable seeds, thus becoming a seed disperser. When the mustard oil bomb was disarmed by inactivation of the myrosinase enzyme, the rodent returned to its typical behavior as a seed predator. When offered diets containing a GLS-myrosinase combination, *A. cahirinus* were negatively impacted. No such effect was evident when feeding on GLSs, on seeds (containing myrosinase), or on pulp with seeds containing deactivated myrosinase. Given the importance of *O. baccatus* as a keystone species in the desert ecosystem [13], this mustard oil bomb mechanism and the ability of *A. cahirinus* to alter its behavior with respect to SMs in the fruits illustrates the flexibility of symbiotic relationships within an ecological timescale. Moreover, our findings suggest an intraspecific, in addition to interspecific, variation of the directed-deterrence hypothesis: SMs in ripe fruits deter individuals if they act as seed predators but have no such effect on individuals of the same species acting as seed dispersers. Our results suggest that SMs in ripe fruits play a deeper role in plant fitness by shaping plant-animal interactions much more than previously assumed.

Supplemental Information

Supplemental Information includes Supplemental Experimental Procedures and three movies can be found with this article online at doi:10.1016/j.cub.2012.04.051.

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