Dual roles of terrestrial isopods in seed predation and seed dispersal in Phacellanthus tubiflorus (Orobancheaceae)

Suetsugu, Kenji

Nakanishi, Osamu

(Citation)
Entomological Science,27(4):e12586

(Issue Date)
2024-12

(Resource Type)
journal article

(Version)
Accepted Manuscript

Dual roles of terrestrial isopods in seed predation and seed dispersal in *Phacellanthus tubiflorus* (Orobancheaceae)

Kenji SUETSUGU[1,2,*], Osamu NAKANISHI[3]

[1]*Department of Biology, Graduate School of Science, Kobe University, 1-1 Rokkodai, Nada-ku, Kobe, Hyogo, 657-8501, Japan*

[2]*Institute for Advanced Research, Kobe University, 1-1 Rokkodai, Nada-ku, Kobe, Hyogo, 657-8501, Japan*

[3]*Kokubo, Akashi City, 673-0005, Japan*

Correspondence: Kenji Suetsugu, Department of Biology, Graduate School of Science, Kobe University, 1-1 Rokkodai, Nada-ku, Kobe, 657-8501, Japan.

Email: kenji.suetsugu@gmail.com

Running title: Isopod-mediated seed dispersal

Abstract

Terrestrial isopods are known to play significant roles in litter decomposition and seed predation. The present study examines the function of terrestrial isopods, particularly *Armadillidium vulgare* and *Armadillidium nasatum*, in the seed dispersal of a non-photosynthetic plant, *Phacellanthus tubiflorus*. These isopods were observed ingesting seeds in the natural habitat of *P. tubiflorus* in Hyogo Prefecture, Japan. Contrary to the prevailing belief that invertebrates do not serve as effective internal seed dispersers, our analysis demonstrated that some seeds ingested by *A. vulgare* and *A. nasatum* were excreted whole, with viability rates comparable to those of seeds directly sourced from fruits. This indicates that these isopods could act not only as seed predators but also as seed dispersers. The research highlights the importance of further exploration into the ecological contributions of isopods and other invertebrates as seed dispersers.

Key words: *Armadillidium*, isopods, seed dispersal, seed predation

Seed predation is a critical factor in plant reproductive cycles, significantly influencing seed mortality (Saska 2008; Honek *et al.* 2011). The guild of seed predators is diverse, encompassing both vertebrate and invertebrate groups, with their impact varying according to geographic location, season, and vegetation cover. In temperate climates, invertebrates such as ground beetles (Coleoptera: Carabidae), weevils (Coleoptera: Curculionidae), rove beetles (Coleoptera: Staphylinidae), isopods (Isopoda: Oniscidea), crickets (Orthoptera: Gryllidae), millipedes (Diplopoda: Julidae), slugs (Mollusca: Gastropoda), and ants (Hymenoptera: Formicidae) play significant roles in seed predation (Honek *et al.* 2003, 2009; Saska 2008; Koprdová *et al.* 2010; Lundgren *et al.* 2013). Notably, ground beetles and terrestrial isopods are prominent among these seed predators (Saska 2008; Honek *et al.* 2009).

During a field study of the seed dispersal system of the achlorophyllous plant *Phacellanthus tubiflorus* (Orobancheaceae) in Kobe City, Hyogo Prefecture, Japan, individuals of *Armadillidium vulgare* and *Armadillidium nasatum* (Isopoda: Armadillidiidae) were repeatedly observed feeding on its fruit in mid to late July 2013 (Fig. 1). Some of these terrestrial isopods seemed to be ingesting entire seeds, suggesting a potential role in seed dispersal. Typically, due to size-related biophysical constraints, invertebrates are not commonly regarded as endozoochorous (internal) seed dispersers (de Vega *et al.* 2011; Suetsugu 2018a,b). However, our observations imply that certain invertebrates may contribute not just to seed predation but also to seed dispersal, especially in plants with small seeds (Suetsugu 2020). For instance, Suetsugu (2018b) found that camel crickets were capable of dispersing intact seeds of all three investigated heterotrophic plants, including *P. tubiflorus*, while no intact seeds were found in the feces of ground beetles. This evidence challenges the preconceived notion that small

invertebrates consuming seeds are exclusively seed predators, highlighting their potential dual role in seed predation and dispersal.

Our research aimed to test the hypothesis that terrestrial isopods, specifically *A. vulgare* and *A. nasatum*, could function as seed dispersers for *Phacellanthus tubiflorus*, in addition to their role as seed predators. In our experiments, we captured *A. vulgare* and *A. nasatum* near *P. tubiflorus* fruit patches in Kobe City, Hyogo Prefecture, Japan in mid-July 2018. Although there might be intraspecific variations in seed dispersal effectiveness depending on developmental stage (King *et al.* 2011; Larsen & Burns 2012), here we focused only on adults with a standard body length (ca. 10 mm). The sex of the isopods was not identified in this study. The isopods were held for at least two days to clear any previously ingested seeds. To assess seed consumption, we extracted 50 seeds from field-collected fruits, mixing them with seedless fruit pulp. This mixture was placed in each Petri dish containing one individual of either *A. vulgare* or *A. nasatum* (each species: n = 12) on moist filter paper. After 24 hours, any remaining unconsumed seeds were counted and removed. Fecal pellets were collected every 12 hours for up to 48 hours after seed consumption, and the number of intact seeds in each pellet was determined using a dissection microscope. The viability of these intact seeds was tested using TTC viability staining. TTC is a white salt that turns from a colorless state to a pink/red formazan in living cells. Thus, as previously described (Thorogood *et al.* 2009; de Vega *et al.* 2011; Ren *et al.* 2011), orange- to red-colored seeds were counted as viable and uncolored seeds were considered to be non-viable. We then compared the viability of seeds defecated by isopods to those of non-defecated seeds using ANOVA, followed by a post hoc Tukey-Kramer test.

The results of our study indicated that while *A. vulgare* and *A. nasatum* primarily

act as seed predators of *P. tubiflorus*, a small percentage of the seeds they consume remain viable. Feeding experiments demonstrated that a minor fraction of consumed seeds (6.1 ± 5.3% for *A. vulgare* and 4.4 ± 3.4% for *A. nasatum*; mean ± SD%; n = 12 for each species) were excreted intact by the isopods, while three individuals of each species did not excrete any intact seeds (Table S1). There was no significant difference in the proportion of intact seeds between these two isopod species ($p = 0.39$). Although many broken seeds were observed in the feces under microscopic examination, when considering only intact seeds, there was no significant difference in viability between seeds defecated by isopods (52.0 ± 37.0% for *A. vulgare* and 53.7 ± 39.8% for *A. nasatum*; n = 9 for each) and those obtained directly from intact fruits (59.0 ± 9.6%; n = 12; $p > 0.8$ for both). This finding suggests a potential role for these isopods in seed dispersal, aligning with previous findings that some seeds of *Monotropastrum humile* consumed by the isopod *Porcellio scaber* remained viable (Suetsugu *et al.* 2024; Suetsugu & Yamasaki 2024).

Although seed dispersal is a crucial aspect of plant-animal mutualism (Wang & Smith 2002), it remains unknown whether *P. tubiflorus* benefits from interactions with the isopods *A. vulgare* and *A. nasatum*. While isopods do defecate viable seeds, their limited movement range and the relatively low proportion of intact seeds they excrete could potentially decrease the fitness of *P. tubiflorus*. This is especially true when compared to the more effective seed dispersal by camel crickets, which tend to excrete a higher proportion of intact seeds (ca. 80%) and distribute them over larger distances (Suetsugu 2018b). However, the low seed viability does not immediately exclude the possibility of mutualistic relationships. For instance, some fruits are commonly consumed by small mammals such as rabbits, which mostly destroy the seeds (germination rate less

than 5%) (Cosyns *et al.* 2005). Despite this, these mammals can still form mutualistic associations as seed dispersers (Janzen 1984; Cosyns *et al.* 2005; Suetsugu & Hashiwaki 2023). Thus, the interaction between the isopods and *Phacellanthus* probably ranges from mutualistic to commensal or antagonistic, depending on the presence and abundance of effective seed dispersers.

It is also important to note that *A. vulgare* and *A. nasatum* are exotic to Japan. Despite their non-native status, these widespread exotic species can significantly contribute to maintaining ecosystem functions. Some research indicated that exotic species often adapt effectively to human-altered environments and can provide ecosystem services that may be lost due to the decline of native species (Hobbs *et al.* 2006, 2009; Kiers *et al.* 2010; Pattemore & Wilcove 2012; Stavert *et al.* 2018). For example, Pattemore & Wilcove (2012) demonstrated that in New Zealand, invasive rats and recently colonized birds partially offset the loss of endemic pollinators. In the context of our study, the positive ecological roles of isopods, such as *A. vulgare* and *A. nasatum*, might become increasingly significant, especially in disturbed areas where native and more effective seed dispersers, like camel crickets, experience population declines. Actually, *A. vulgare* is known to occur with higher densities in artificial environments (Tanaka & Karasawa 2018; Karasawa 2022).

In conclusion, our study sheds light on the dual roles of *Armadillidium* species as both seed predators and potential dispersers in their interactions with *Phacellanthus*. Given the high densities of terrestrial isopods in some environments, which can reach hundreds or even thousands per square meter (Paoletti & Hassall 1999), their impact on seedling establishment and survival of *Phacellanthus* could be ecologically significant. Future research should aim to determine whether the mutualistic aspects of this

interaction outweigh the antagonistic ones.

ACKNOWLEDGMENTS

We thank Hidehito Okada and Kazuma Takizawa for their support in the laboratory work.

REFERENCES

Cosyns E, Delporte A, Lens L, Hoffmann M (2005) Germination success of temperate grassland species after passage through ungulate and rabbit guts. *Journal of Ecology* **93**, 353–361.

Hobbs RJ, Arico S, Aronson J *et al.* (2006) Novel ecosystems: theoretical and management aspects of the new ecological world order. *Global Ecology and Biogeography* **15**, 1–7.

Hobbs RJ, Higgs E, Harris JA (2009) Novel ecosystems: implications for conservation and restoration. *Trends in Ecology and Evolution* **24**, 599–605.

Honek A, Martinkova Z, Jarosik V (2003) Ground beetles (Carabidae) as seed predators. *European Journal of Entomology* **100**, 531–544.

Honek A, Martinkova Z, Saska P (2011) Effect of size, taxonomic affiliation and geographic origin of dandelion (*Taraxacum* agg.) seeds on predation by ground beetles (Carabidae, Coleoptera). *Basic and Applied Ecology* **12**, 89–96.

Honek A, Martinkova Z, Saska P, Koprdova S (2009) Role of post-dispersal seed and seedling predation in establishment of dandelion (*Taraxacum* agg.) plants. *Agriculture, Ecosystems & Environment* **134**, 126–135.

Janzen DH (1984) Dispersal of small seeds by big herbivores: foliage is the fruit. *The American Naturalist* **123**, 338–353.

Karasawa S (2022) Comparison of isopod assemblages (Crustacea: Isopoda: Oniscidea) among four different habitats—Evergreen forest, exotic bamboo plantation, grass and urban habitat. *Pedobiologia* **91–92**, 150805.

Kiers TE, Palmer TM, Ives AR, Bruno JF, Bronstein JL (2010) Mutualisms in a changing world: an evolutionary perspective. *Ecology Letters* **13**, 1459–1474.

King P, Milicich L, Burns KC (2011) Body size determines rates of seed dispersal by giant king crickets. *Population Ecology* **53**, 73–80.

Koprdová S, Saska P, Honěk A, Martinková Z (2010) Seed consumption by millipedes. *Pedobiologia* **54**, 31–36.

Larsen H, Burns KC (2012) Seed dispersal effectiveness increases with body size in New Zealand alpine scree weta (*Deinacrida connectens*). *Austral Ecology* **37**, 800–806.

Lundgren JG, Saska P, Honěk A (2013) Molecular approach to describing a seed-based food web: the post-dispersal granivore community of an invasive plant. *Ecology and Evolution* **3**, 1642–1652.

Paoletti MG, Hassall M (1999) Woodlice (Isopoda: Oniscidea): their potential for assessing sustainability and use as bioindicators. *Agriculture, Ecosystems & Environment* **74**, 157–165.

Pattemore DE, Wilcove DS (2012) Invasive rats and recent colonist birds partially compensate for the loss of endemic New Zealand pollinators. *Proceedings of the Royal Society B: Biological Sciences* **279**, 1597–1605.

Ren ZX, Li DZ, Bernhardt P, Wang H (2011) Flowers of *Cypripedium fargesii* (Orchidaceae) fool flat-footed flies (Platypezidae) by faking fungus-infected foliage. *Proceedings of the National Academy of Sciences of the United States of America* **108**, 7478–7480.

Saska P (2008) Granivory in terrestrial isopods. *Ecological Entomology* **33**, 742–747.

Stavert JR, Pattemore DE, Bartomeus I, Gaskett AC, Beggs JR (2018) Exotic flies maintain pollination services as native pollinators decline with agricultural expansion. *Journal of Applied Ecology* **55**, 1737–1746.

Suetsugu K (2018a) Seed dispersal in the mycoheterotrophic orchid *Yoania japonica*: Further evidence for endozoochory by camel crickets. *Plant Biology* **20**, 707–712.

Suetsugu K (2018b) Independent recruitment of a novel seed dispersal system by camel crickets in achlorophyllous plants. *New Phytologist* **217**, 828–835.

Suetsugu K (2020) A novel seed dispersal mode of *Apostasia nipponica* could provide some clues to the early evolution of the seed dispersal system in Orchidaceae. *Evolution Letters* **4**, 457–464.

Suetsugu K, Hashiwaki H (2023) A non-photosynthetic plant provides the endangered Amami rabbit with vegetative tissues as a reward for seed dispersal. *Ecology* **104**, e3972.

Suetsugu K, Kimura-Yokoyama O, Kitamura S (2024) Earwigs and woodlice as some of the world's smallest internal seed dispersal agents: insights from the ecology of *Monotropastrum humile* (Ericaceae). *Plants, People, Planet*, in press. https://doi.org/10.1002/ppp3.10519

Suetsugu K, Yamasaki M (2024) Influence of body size on the dual role of isopod *Porcellio scaber* in seed dispersal and predation of the mycoheterotrophic plant *Monotropastrum humile*. *Ecological Entomology*, in press.

Tanaka R, Karasawa S (2018) Life histories and soil water content preferences of sympatric exotic and native terrestrial isopods. *Entomological Science* **21**, 198–209.

Thorogood CJ, Rumsey FJ, Hiscock SJ (2009) Seed viability determination in parasitic broomrapes (*Orobanche* and *Phelipanche*) using fluorescein diacetate staining. *Weed Research* **49**, 461–468.

de Vega C, Arista M, Ortiz PL, Herrera CM, Talavera S (2011) Endozoochory by beetles: a novel seed dispersal mechanism. *Annals of Botany* **107**, 629–637.

Wang BC, Smith TB (2002) Closing the seed dispersal loop. *Trends in Ecology & Evolution* **17**, 379–386.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Table S1. The number of intact and viable seeds defecated by each individual of *Armadillidium vulgare* and *Armadillidium nasatum* or extracted from intact fruits.

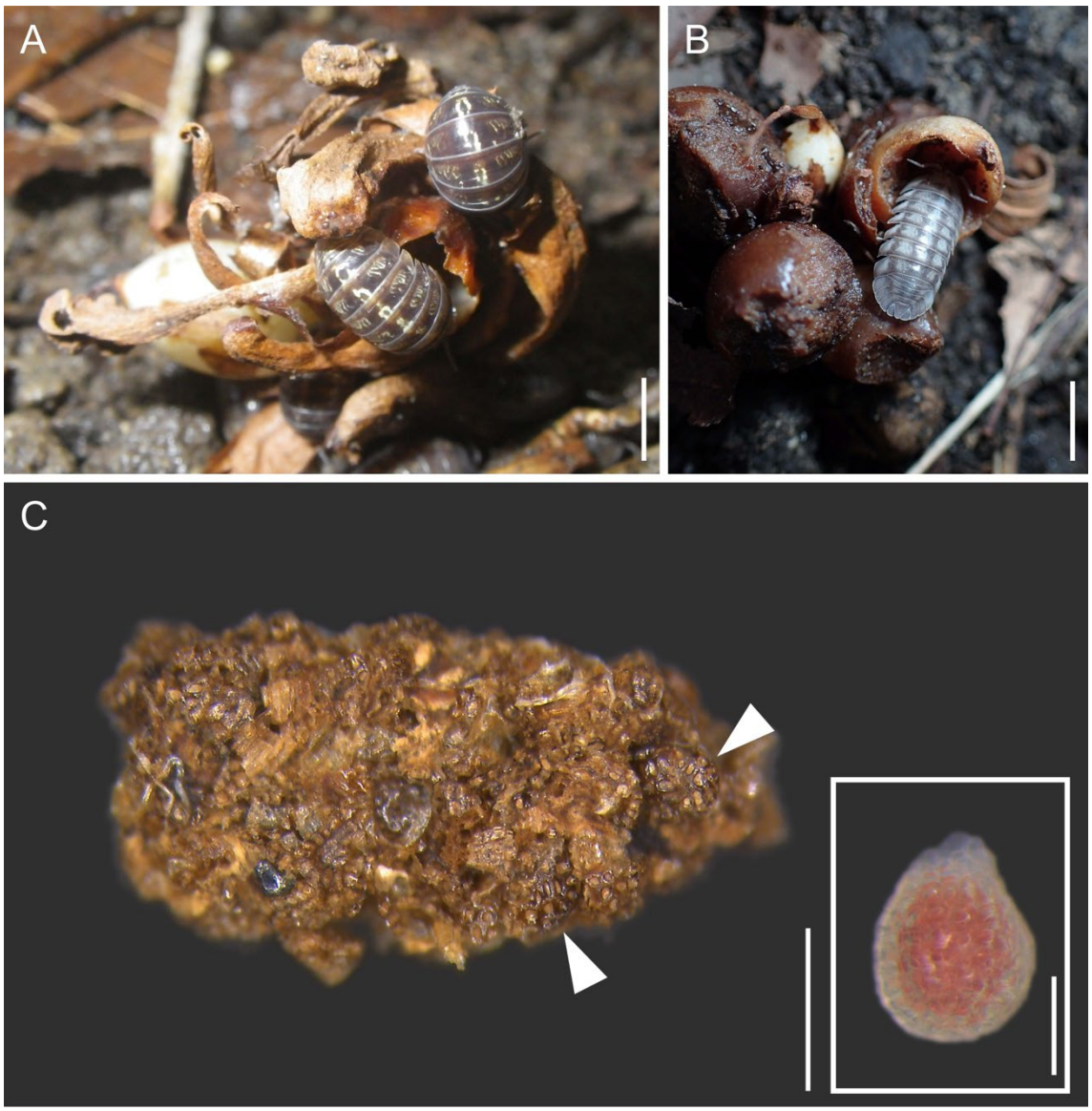

Figure 1 Interaction between *Monotropastrum humile* and its fruit-feeding isopods. (A) Some *Armadillidium vulgare* individuals consuming the fruit. (B) Some *A. nasatum* individuals consuming the fruit. (C) Feces defecated by an *A. vulgare* individual, with some seeds remaining intact (indicated by arrows). Inset: A viable seed isolated from the feces and stained with TTC. Scale bars: 5 mm (A–B), 500 μm (C), and 200 μm (inset).