

## Experimental assessment of the ability of generalist predators to control *Opatrum sabulosum* (Coleoptera: Tenebrionidae)

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*Opatrum sabulosum* L. (Tenebrionidae: Coleoptera) is a widespread phytophagous insect in the steppe zone, which in years of mass reproduction can cause damage to numerous crops, but is most dangerous for vegetable seedlings, as adults readily feed on wilted plant organs. As insects are increasingly developing resistance to existing synthetic insecticides and the organic farming market is growing, it is becoming increasingly important to identify new, effective and environmentally friendly means of controlling pest populations, such as the use of entomophages. Unfortunately, there are no data on invertebrate predators that are capable of natural control populations of phytophages from the Tenebrionidae family and *O. sabulosum* in particular. We evaluated the ability of 17 species of predators from 7 families to prey on *O. sabulosum* adults in a laboratory experiment. The results show that generalist predators, especially ground beetles and representatives of some other families, are capable of predation against *O. sabulosum* adults. The highest predation rates were recorded for *Reduvius personatus*, *Rhynocoris iracundus*, *Staphylinus caesareus* (100% of attacks) and *Brosicus cephalotes* (73%). Lower predation rates were recorded for *Molops piceus* (40%), *Harpalus rufipes* (33%) and *Calathus ambiguus* (27%). A moderate percentage of attacks was recorded for *Hister quadrimaculatus* (20%) and *Harpalus affinis* (13%). Other entomophages did not prey on *O. sabulosum* during the experiments. Undoubtedly, the ground beetles *B. cephalotes*, *H. rufipes* and *C. ambiguus* have the potential to play a significant role in the natural control of the populations of the above-mentioned phytophagous. However, this topic undoubtedly requires further research, especially in the field.

Key words: pest, biological control, predation rate; predatory arthropods, biocontrol agents, generalist predators.

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### INTRODUCTION

*Opatrum sabulosum* L. is a small (7-10 mm) polyphytophage from the family

Tenebrionidae, widespread in the steppes of Eastern Europe (Brygadyrenko and Nazimov 2015; Carpaneto and Fattorini 2001; Iosob and Cristea 2022). The colour of

the beetle's covers is black, but due to the adhering soil crust, they are usually greyish. Being a clear xerophile, in Ukraine the species is most abundant in the southern steppe zone in Zaporizhzhia, Odesa, Kherson and Mykolaiv regions (Chernej, 2005). The beetles have an extremely wide range of feeding preferences and damage various crops, but are most dangerous for seedlings of row crops and vegetable seedlings in spring and early summer (Brygadyrenko & Nazimov 2014). In this period, from several dozen to hundreds of individuals can be concentrated on one square metre (Nazimov & Pahomov 2015). In years of massive reproduction, they cause relatively significant damage to sunflower, corn, tomato, cucumber, cabbage, onion, bean and soybean crops. It is known that *O. sabulosum* prefers to feed on wilted plant organs, so they are particularly dangerous for newly planted vegetable and tobacco seedlings (Nazimov & Brygadyrenko 2013). In cereals, they eat away parts of the leaf blade, and in sunflower, they damage the cotyledons. Damage to sprouted seeds of wheat, barley, oats and melons is also recorded (Chernej, 2005). The most destructive stage is the adult, while the larvae feed on rotting plant residues in the soil and hardly damage living plants (Brygadyrenko & Nazimov 2015).

They live for 1-2 years and overwinter among plant residues in fields and in the upper soil layers. In the steppe zone of Ukraine, they appear on the soil surface in late March or early April, depending on the degree of soil warming (Nazimov & Pahomov 2015). Mating usually takes place in late April or early May, depending on weather conditions. Females lay their eggs in the soil to a depth of 2-5 cm in clusters of several to a dozen. One female can lay up to 100 eggs per season. The adults of the new generation appear in July and continue to emerge from the soil throughout August (Fattorini, 2010). Larvae hatch from late

clutches pupate in late summer, and adults emerge in the following spring (Chernej, 2005).

Insecticides are still the most commonly used measures to control the number of phytophage populations in open field. However, their widespread use is both harmful to the environment and public health and can provoke the development of pest resistance. For example, there are already populations of *Tuta absoluta* (Van Lenteren et al. 2023), *Frankliniella occidentalis* (Rocha et al. 2015), *Trialeurodes vaporariorum* (Leman et al. 2020) and *Diabrotica virgifera* (Meinke et al. 2021) that are resistant to most insecticides, controlling their populations is a serious challenge. Today, biological pest control is an effective alternative to the use of chemicals and allows for the control of even pesticide-resistant populations (Kheirodin et al. 2020). Biological plant protection is based on the use of living organisms - natural enemies of harmful insects and entomopathogenic organisms such as nematodes, viruses, fungi, bacteria and their products. Generalist predators play an important role as agents of biological pest control (Snyder, 2019). Bioagents such as predatory beetle *Atheta coriaria* (Echegaray & Cloyd 2013) and coccinellids (Saleem et al. 2014; Tun et al. 2020; Gómez-Marco et al. 2022;), polyphagous bug *Macrolophus pygmaeus* (Zhang et al. 2019), mites *Stratiolaelaps scimitus* (Park et al. 2021) and *Amblyseius swirskii* (Dalir et al. 2021), lacewing *Chrysoperla carnea* (Boetzel et al. 2020; Fang et al. 2022) etc. are widely used in the plant protection market. When applied preventively, they do not allow pests to reach the level of economic damage (Bouvet et al. 2019). Despite the fact that the successful use of entomophages is mainly observed in protected ground conditions, generalist predators can help control phytophagous populations in open agricultural landscapes, as they do in wild

ecosystems (Martin-Chave et al. 2019; Perez-Alvarez et al. 2019).

Unfortunately, we are faced with a lack of information on predators that can reduce populations of *O. sabulosum* and phytophagous Tenebrionidae in general. However, numerous studies have shown the ability of generalist predators to reduce numerous pests in the open field. For example, positive data have been obtained on the ability of coccinellids, ground beetles, nabid bugs and wolf spiders to help control the raspberry beetle (*Oulema melanopus*) (Arus et al. 2012; Kheirodin et al. 2019). The ground beetles of the genera *Broscus*, *Pterostichus* and *Harpalus* (Nourmohammadpour-Amiri et al. 2022) are able to control slugs (El-Danasoury et al. 2017) and the larval stage of some dipteran pests. Numerous species of lacewings and ladybugs effectively control numerous pests, such as aphids, scale insects, psyllids, etc. (Gharbi, 2021; Francis et al. 2022). All of this suggests that generalist predators have great potential for controlling phytophages in the open field (Anjos et al. 2022).

The aims of our study were to (1) identify the range of the most abundant generalist predators living in ecosystems near *O. sabulosum* and (2) assess their potential to play the role of entomophages of darkling beetles of this species in the laboratory arena.

## MATERIAL AND METHODS

### Study area and collection of entomophages

Imagoes of *O. sabulosum* were collected in early June 2023 in 2 relatively undisturbed natural ecosystems (coordinates 48.4764, 34.8544 and 48.4665, 34.8520) and 2 agroecosystems (coordinates 48.3863, 35.0417 and 48.3489, 35.0283) on the outskirts of

Dnipro city. Predators were collected at the same time as darkling beetles in the same ecosystems, as well as in artificial forest plantations: a city park (coordinates 48.4320, 35.0389) and two shelterbelts (coordinates 48.4745, 34.8550 and 48.3865, 35.0409) within the city of Dnipro. We selected certain species of entomophages based primarily on their abundance in the ecosystems where the darkling beetles inhabited. Arthropods were collected using both soil traps and manual collection methods. The collected specimens were placed in plastic containers and transported to the laboratory for further research.

### Laboratory maintenance of insects

The imagoes of *O. sabulosum* were kept in 0.8-litre plastic containers and fed with fresh lettuce (*Lactuca sativa*) leaves of the Lollo Rosso and Lollo Blondo varieties. Every third day, the leaves were replaced to protect against the process of decay, which would have caused massive mortality of xerophilous beetles. The generalist predators were kept both together and in individual containers (0.4 litre containers), depending on their propensity for cannibalism (Rasekh & Osawa 2020). They were also fed with special food (earthworms and dipteran larvae), but stopped feeding the day before the experiment to ensure that they were motivated to hunt and that they were not too hungry to hunt any live prey. A range of biological and ecological characteristics, on the basis of which the ranking of the studied entomophages was conducted, such as the ecosystem affiliation of the species, prevalence in agroecosystems, and average body size indices, as well as the methods used for interpreting the obtained results, were taken from the monographs of experienced researchers (Zhukov 2009; Puchkov 2018; Sumarokov 2023).

### Predation trials

In the course of the study of predatory activity of *O. sabulosum* imagoes, 17 species of predators were tested. In each individual experiment, predatory arthropods were given the opportunity to hunt phytophages within a specific laboratory arena, which is a classic and widespread method for studying the feeding spectrum of entomophages (Brygadyrenko & Nazimov 2015; El-Danasoury & Iglesias-Piñeiro 2018; Schäfer et al. 2018; Pasquier et al. 2021). During the experiment, 1 entomophagous individual was offered 5 individuals of darkling beetles. Each of the series of experiments was conducted in 15 replicates (Nazimov & Brygadyrenko 2013). It was believed that predation occurred not only when

entomophages pierced the prey's coverings with their mouthparts, but also when they actively fed on prey (Jałoszyński & Olszanowski 2016). If no predation occurred within an hour, we left the subjects together for another 6 hours to make sure that the prey was ignored (Zhang et al. 2021). In cases where prey was not accepted, we fed the predators their usual food to make sure that the darkling beetles was not rejected for reasons other than hunger. The laboratory arena was a plastic container with dimensions of  $9 \times 15 \times 10$  cm. *O. sabulosum* individuals that were not eaten were used in subsequent experiments. A series of experiments was conducted at the same air humidity and temperature in the range of 24–26 °C.

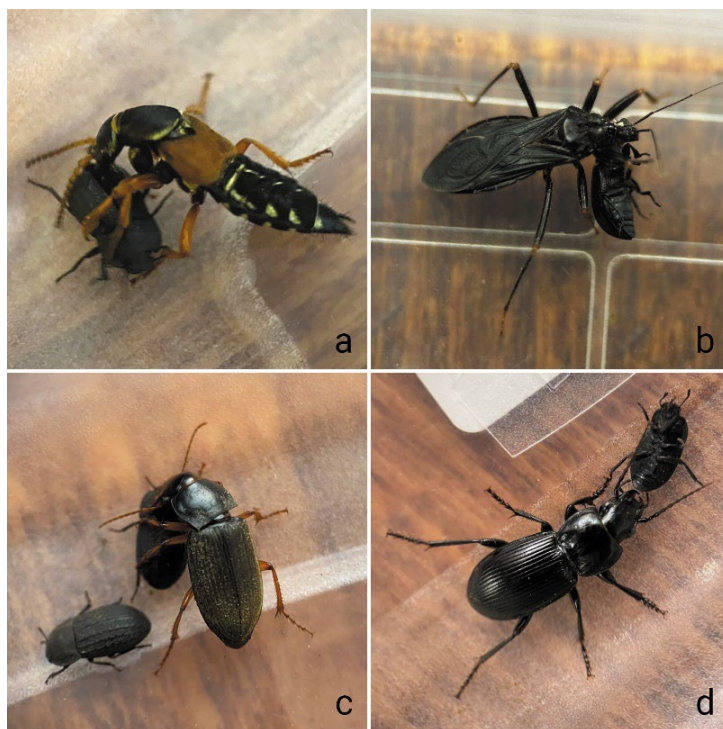


Fig 1. Attacks of generalist predators on *Opatrum sabulosum* imagoes in a laboratory experiment: a – *Staphylinus caesareus*; b – *Reduvius personatus*; c – *Harpalus rufipes*; d – *Molops piceus*.

## RESULTS

The results of a series of experiments on feeding generalist predators with adult *O. sabulosum* are presented in Table 1. Since the frequency of cases when a single predator captured more than one victim during the experiment was less than 5% of the total number of experiments, we do not present such data. The highest hunting rates were obtained in the experiments with *Brosicus cephalotes*, *Reduvius personatus*, *Rhynocoris iracundus* and *Staphylinus caesareus*, with the latter three having 100% attack and feeding success. Lower rates of eating of darkling beetles were recorded for the ground beetles *Molops piceus* (40%), *Harpalus rufipes* (33%) and *Calathus ambiguus* (27%). Also, a low percentage of attacks was observed in the experiments with *Hister quadrimaculatus* (20%) and *Harpalus affinis* (13%). Other experimental arthropods did not prey on *O. sabulosum* during the series of experiments.

From the taxonomic point of view, the highest rates of eating of black beetles were observed in the families Reduviidae, Histeridae and Staphylinidae (100%, 100% and 50% of species hunted *O. sabulosum* adults, respectively), but only a few representatives of these taxa participated in the experiments. They were closely followed by the ground beetles (Coleoptera: Carabidae), 44% of which fed on the darkling beetles. More than half of the predators participating in the experiments

belonged to the family Carabidae. Ground beetles that hunted *O. sabulosum* belonged to the tribes Broscini, Harpalini, Pterostichini and Sphodrini. Representatives of the Carabini and Platynini tribes did not feed on darkling beetles. They were also not preyed upon by tiger beetles (Cicindelidae), as well as wolf spiders of the family Lycosidae and spongy millipedes of the family Lithobiidae.

Based on the size characteristics, *O. sabulosum* was preyed upon by 50% of predators whose length exceeded 15 mm, 66% whose size was in the range of 10-15 mm, and only 33% whose size was less than 10 mm. According to the ecosystem affiliation of the predator species, imagoes of the darkling beetles were eaten by 75% of steppe species, 50% of meadow species, 40% of forest species and 100% of polytopic species. Meadow-forest and swamp-forest species did not feed on *O. sabulosum*.

If we consider the prevalence of a particular entomophage on arable land or in other types of agrocenoses (such as orchards and berry fields), 100% of species with massive and rare-common occurrence in agrocenoses were hunted by the phytophage under study. This value was 67% for the occasional-common species, 50% for the species not found on arable land, and 33% for the rare-occasional species. The common-massive species that participated in the study did not prey on *O. sabulosum* imagoes.

**Table 1.** Potential ability of generalist predators to feed on by *O. sabulosum* imagoes in a laboratory experiment.

| Predator species                    | Number of trials | Hunting efficiency, % | Average predator length (according to literature data), mm | Ecosystem affiliation of the species | Distribution in agrocenoses * |
|-------------------------------------|------------------|-----------------------|--|--------------------------------------|-------------------------------|
| Arachnida<br>( <b>Lycosidae</b> )   |                  |                       |  |                                      |                               |
| Lycosa singoriensis                 | 15               | 0                     | 25-30  | Steppe                               | OC                            |
| Myriapoda<br>( <b>Lithobiidae</b> ) |                  |                       |  |                                      |                               |
| Lithobius forficatus                | 15               | 0                     | 18-30  | Forest                               | NF                            |
| Insecta                             |                  |                       |  |                                      |                               |
| Hemiptera                           |                  |                       |  |                                      |                               |
| <b>Reduviidae</b>                   |                  |                       |  |                                      |                               |
| Reduvius personatus                 | 15               | 100                   | 17-22  | Steppe                               | NF                            |
| Rhynocoris iracundus                | 15               | 100                   | 15   | Steppe                               | RC                            |
| Coleoptera                          |                  |                       |  |                                      |                               |
| <b>Cicindelidae</b>                 |                  |                       |  |                                      |                               |
| Cylindera germanica                 | 15               | 0                     | 8-11   | Meadow                               | RO                            |
| <b>Carabidae</b>                    |                  |                       |  |                                      |                               |
| Carabini                            |                  |                       |  |                                      |                               |
| Carabus granulatus                  | 15               | 0                     | 16-23  | Forest                               | OC                            |
| Broscini                            |                  |                       |  |                                      |                               |
| Broscus cephalotes                  | 15               | 73.3                  | 15-25  | Meadow                               | RC                            |
| Harpalini                           |                  |                       |  |                                      |                               |
| Harpalus affinis                    | 15               | 13.3                  | 9-12   | Meadow                               | RC                            |
| Harpalus distinguendus              | 15               | 0                     | 9-11   | Meadow-forest                        | CM                            |
| Harpalus rufipes                    | 15               | 33.3                  | 11-16  | Polytopic                            | M                             |
| Platynini                           |                  |                       |  |                                      |                               |
| Agonum duftschmidi                  | 15               | 0                     | 8-9.5  | Swamp-forest                         | RO                            |
| Pterostichini                       |                  |                       |  |                                      |                               |
| Molops piceus                       | 15               | 40                    | 9-14   | Forest                               | RO                            |
| Sphodrini                           |                  |                       |  |                                      |                               |

|                                       |    |      |       |           |    |
|---------------------------------------|----|------|-------|-----------|----|
| Calathus<br>(Neocalathus)<br>ambiguus | 15 | 26.7 | 9-13  | Polytopic | M  |
| Dolichus<br>halensis                  | 15 | 0    | 15-19 | Meadow    | CM |
| <b>Histeridae</b>                     |    |      |       |           |    |
| Hister<br>quadrimaculatus             | 15 | 20   | 8-11  | Steppe    | OC |
| <b>Staphylinidae</b>                  |    |      |       |           |    |
| Philonthus<br>decorus                 | 15 | 0    | 14    | Forest    | NF |
| Staphylinus<br>caesareus              | 15 | 100  | 17-25 | Forest    | NF |

\*Explanation of the table on the distribution in agroecosystems: M - massive, CM - common-massive, OC - occasional-common, RC - rare-common, RO - rare-occasional, NF - not found.

## DISCUSSION

The issue of the feeding spectrum of invertebrate generalist predators and their ability to regulate pest populations and phytophages that could potentially be them is of considerable scientific and practical interest. There are many cases where phytophages that did not cause significant damage to crops have become dangerous quarantine pests under certain conditions (mass reproduction, introduction to new climatic conditions, etc.). Our study helped to identify for the first time entomophages from among generalist predators that can feed on imagoes of *O. sabulosum*, a moderately dangerous phytophagous pest that, in years of mass reproduction, can cause economically sensitive losses to agriculture in Eastern and some regions of Southern Europe (Chernej, 2005).

Theoretically, it was expected that the ground beetles would be the taxonomic group that would provide the largest number of species capable of preying on *O. sabulosum* imagoes. These expectations were partially met, as 44% of the Carabidae that participated in the surveys preyed on the darkling beetles to some extent. Other taxonomic groups (predatory bugs, clown

beetles and staphylinids) were represented by one or more species during the laboratory tests, and the results of their hunting cannot be relevant for their family as a whole. As expected, the highest predation results among the ground beetles were observed for species that are larger in size, inhabit steppe or meadow ecosystems, or are polytopic species and are common on arable land (Kamenova et al. 2015; Ballman et al. 2017). However, this statement is only true if we exclude beetles that did not feed on prey at all. Nevertheless, there are exceptions, such as in the case of the ground beetle *M. piceus*, which is a forest mesophile and is a rare-occasional species for agroecosystems. Apparently, in this case, the size of the predator played a key role in hunting. A similar positive correlation is observed, in general, for all species of entomophages whose size is more than 11-12 mm and which fed on *O. sabulosum* adults.

It should be noted that in 1 of the 15 experimental variants, *H. rufipes* failed to cope with the adult *O. sabulosum*: the predator held the victim for some time, trying to penetrate the hard cover of the darkling beetle, despite its active attempts to free itself, but after a while released it. It is unknown why the ground beetle failed to kill

the prey, or how much damage the predator caused to the darkling beetle's life. It is interesting to know what proportion of similar hunting outcomes occur in natural conditions, in larger samples, and whether phytophages survive such contacts.

The role of the ecosystem affiliation of the studied arthropods cannot be underestimated, because, as mentioned above, of all the taxonomic groups of generalist predators, most species belonged to steppe and meadow species (Nourmohammadpour-Amiri et al. 2022). Here, too, size was not a guaranteed advantage for the fact of hunting, as large forest predators such as *Philonthus decorus*, *Lithobius forficatus* and *Carabus granulatus* did not feed on darkling beetles at all. The same can be said for the relatively large (15-18 mm in length) and moisture-loving ground beetle *Dolichus halensis*. It is likely that *O. sabulosum* is not a common prey for inhabitants of wetter ecosystems, or this fact is explained by specialisation to feed on larger prey with less hard cover.

In the experimental variants with the clown beetle *H. quadrimaculatus*, a moderate percentage of attacks on the darkling beetle was recorded, although we believed that this species prefers less "armoured" prey with softer covers. It is also interesting that the forest species *S. caesareus* ate prey in all 15 experimental variants. This can be explained both by the fact that *O. sabulosum* adults are often found in windbreaks, the forest floor of which serves as a habitat for predators of this species, and by the wide range of staphylinid (Echegaray & Cloyd 2013). Thus, today, the entomophage from the Staphylinidae family *Dalotia coriaria* is widely used in biological plant protection against pests, which feeds on soil stages of thrips, larvae of the Ephydriidae and Sciaridae families, as well as many other insects from different taxonomic groups (Tourtois & Grieshop 2015; Herrick & Cloyd 2017). This suggests that *S. caesareus* may to some extent control

the darkling beetles species during its migration through forest belts.

We observed probably unusual attacks of bugs from the family Reduviidae on *O. sabulosum*. Numerous species of predatory bugs are currently used in organic crop production, primarily representatives of the families Miridae *Macrolophus pygmaeus* (De Backer et al. 2014) and *Nesidiocoris tenuis* (Hagler et al. 2004), and Anthocoridae *Anthocoris nemoralis* (Reeves et al. 2023; Horton, 2024), *Orius laevigatus* (Mendoza et al. 2022; Zuma et al. 2023) and *Orius majusculus* (Toft et al. 2020). These entomophages provide effective control of numerous pests, primarily whiteflies, thrips, spider mites, aphids and leafhoppers. Representatives of the Pentatomidae family, primarily *Perillus bioculatus* and species of the *Podisus* genus, are somewhat less popular (Plata-Rueda et al. 2022). They are larger predators capable of attacking large caterpillars and hard-covered beetles such as the Colorado potato beetle (*Leptinotarsa decemlineata*). Thus, the very fact that predatory bugs attack the darkling beetles is explained by the breadth of their feeding spectrum. On the other hand, Reduviidae bugs are predominantly chortobionts and hunt their prey in the upper levels of the grass cover, often waiting for prey on inflorescences (Franin et al. 2021), while *O. sabulosum* belongs to the herpetofauna (Chernej, 2005) and has little chance of encountering any predatory bugs outside the laboratory arena.

Spiders are an important group of generalist predators, whose impact on pests within agrocenoses has not been sufficiently studied (Sardar et al. 2020). There are data on the control of phytophagous populations by wolf spiders in agrocenoses in Latin America (García et al. 2021) and in Czech orchards (Michalko et al. 2022), but this topic still requires further factual material. Spiders *Lycosa singoriensis* in our series of



experiments did not prey on beetles, probably due to the small size of the prey, which made it uninteresting for the predator as a food object.

*B. cephalotes*, *H. rufipes* and *C. ambiguus*, which are massive on arable land, proved to be the most promising for controlling the imagoes of *O. sabulosum* by generalist predators. Probably, under natural conditions, the populations of these ground beetles limit the mass reproduction of this species of darkling beetles. Attracting and preserving populations of such entomophages is a priority to ensure crop protection from pests and reduce the cost of commercial synthetic plant protection products. Under natural conditions, the effectiveness of phytophagous population regulation is influenced not only by the selectivity of entomophage feeding on a particular prey species, but also by the total number of predators in the ecosystems where the prey lives (Cividanes, 2021). *H. affinis* and *H. quadrimaculatus* have a low percentage of attacks on *O. sabulosum*, but due to the high abundance of other natural enemies of the darkling beetles, they may increase pressure on its population density. Other studied predators probably do not affect the level of *O. sabulosum* populations in natural conditions, which is explained by the absence of their predation on the darkling beetles, due to their size characteristics and different habitat conditions.

In the future, it is important to compare the results with similar data from natural ecosystems. Further research should include molecular analysis of the gut contents of generalist predators that have been caught in the field hunting for darkling beetles in the laboratory. Another important issue that requires attention is the study of the predatory activity of entomophages in conditions of heavy metals and pesticides contamination, with an increase in the number of prey, in the presence of

alternative prey, and at different temperatures (Frank & Bramböck 2016).

This information will be key to the development of a comprehensive programme of biological control of phytophagous darkling beetles populations, which will take into account the ecosystem services provided by generalist predators, including the ground beetles.

## CONCLUSION

*B. cephalotes*, *H. rufipes*, *C. ambiguus* and some other ground beetles are able to actively prey on *O. sabulosum* imagoes, putting pressure on the populations of this phytophagous species. Other generalist predators such as *S. caesareus* and members of the Reduviidae family also actively prey on this species in the laboratory arena, but it is not known how often they occur in the wild. Based on the results, we can conclude that at least half of the studied species of predatory invertebrates show some degree of predatory activity against *O. sabulosum*. However, this topic requires further research, especially evidence of predation in natural conditions.

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