

## Behavioral analysis of the interaction between the spitting spider *Scytodes globula* (Araneae: Scytodidae) and the harvestman *Discocyrtus invalidus* (Opiliones: Gonyleptidae)

Luanda Abrão Carvalho, Elene da Silva Souza, and Rodrigo H. Willemart<sup>1</sup>: LESCA (Laboratório de Ecologia Sensorial e Comportamento de Artrópodes), Escola de Artes Ciências e Humanidades, Universidade de São Paulo, Rua Arlindo Bétio, 1000 – Ermelino Matarazzo – 03828-000, São Paulo, SP, Brazil

**Abstract.** Spitting spiders (Scytodidae) have a distinct predatory strategy in which they eject a sticky secretion from their cheliceral fangs to immobilize prey. This behavior could potentially allow the spider not only to avoid defensive secretions but also to bite specific vulnerable spots of a potential prey such as a harvestman. We used an ethogram, a fluxogram and an experiment to analyze the interaction between the harvestman *Discocyrtus invalidus* Piza 1938 (Arachnida: Opiliones) and the syntopic spider *Scytodes globula* (Nicolet 1849) (Arachnida: Araneae). These spiders, while readily taking crickets as prey, seldom spat at and never bit the harvestmen, which apparently did not exude repugnatorial secretions. We therefore tested, by clogging the glands and using appropriate controls, whether non-visible amounts of secretions could cause the rejection, but the harvestmen were still refused. This is the first detailed and quantified description of an interaction between a spitting spider and a harvestman. The general conclusions are that *S. globula* avoids preying on *D. invalidus*, *S. globula* behaves differently when attacking harvestmen and crickets and the scent gland secretions of *D. invalidus* do not play a direct role in this predator-prey interaction.

**Keywords:** Chemical defense, foraging, Laniatores, prey capture, scent gland

Spitting spiders (Arachnida: Araneae) are unique among the more than 40,000 species of spiders in the way they capture prey. They spit a sticky secretion produced by cheliceral glands and extruded by their cheliceral fangs. This secretion is a mixture of glue, venom and silk (Suter & Stratton 2009; but see Clements & Li 2005) and is used to immobilize a wide variety of arthropods used as prey (Nentwig 1985; Li et al. 1999).

Potential prey items for spitting spiders are harvestmen (Arachnida: Opiliones) in the suborder Laniatores. Harvestmen are usually nocturnal and have poor eyesight (Willemart et al. 2009) and thus do not detect predators such as spiders visually. They have a combination of defenses that includes the use of chemicals, specifically an exudation of droplets of repugnatorial secretions from the scent glands located dorso-laterally on the prosoma; a heavy armature covering most of the body; and physical retaliation such as pinching with pedipalps, chelicerae, and spined legs (Gnaspini & Hara 2007; Pomini et al. 2010; Souza & Willemart 2011). Some harvestman species are preyed upon by some spiders, but others are rejected (Gnaspini & Hara 2007).

We hypothesized that spitting spiders could overcome the defenses of such harvestmen (1) by spitting from a distance instead of biting so that they would not come into contact with the harvestmen's repugnatorial secretions and (2) by spitting their viscous secretion, the spiders would limit the harvestmen's ability to move, allowing the spiders to bite specific vulnerable areas (i.e., where not protected by the heavy armature). Though spitting spiders have a delicate body and weak chelicerae, some laniatorid harvestmen like *Discocyrtus invalidus* Piza 1938 (Laniatores: Gonyleptidae) are often eaten by the syntopic recluse spider *Loxosceles* sp. (Fischer et al. 2006; Willemart & Souza pers. observ.), a spider of small body size and weak chelicerae similar to *Scytodes*. Moreover, in the

field, spitting spiders have been observed eating the laniatorid harvestman *Mischonyx cuspidatus* (Roewer 1913), which is very similar to *D. invalidus* in size (Mestre & Pinto-da-Rocha 2004).

Therefore, we studied the interaction between the spitting spider *Scytodes globula* (Nicolet 1849) (Scytodidae) and the syntopic harvestman *D. invalidus*. Both occur in the same microhabitat, using dead palm fronds on soil and fallen trunks as shelter during the day and their surroundings at night as foraging areas. No previous studies have been conducted on the interaction between these two species, so it was unknown whether or not the spider preyed upon or rejected this harvestman species. Based on our hypotheses, we first predicted a similar mortality rate of harvestmen and crickets when paired with the spiders. However, our first experiment revealed that the spiders attacked crickets significantly more readily, so we conducted an investigation as to why the spiders rejected these harvestmen. By performing a careful descriptive analysis, we noticed that the spiders behaved differently when interacting with harvestmen than with crickets. We did not detect the release of defensive secretions from the scent glands of the prey. Consequently, we experimentally tested the hypothesis that harvestmen were protected by the release of small amounts of secretions not visible to the human eye (see Machado et al. 2005 for further discussion), but found no evidence of such.

### METHODS

**Species studied.**—The harvestman *Discocyrtus invalidus* is found in tropical rainforests in southeastern Brazil, State of São Paulo, where it hides under logs and dead palm fronds during the day and wanders on tree trunks, on the ground or on bushes at night. When we insistently disturbed individuals of this species, they released conspicuous droplets of secretion from the scent glands, the openings of which can be seen with the naked eye. The defense secretion of this species is

<sup>1</sup> E-mail: willemart@usp.br

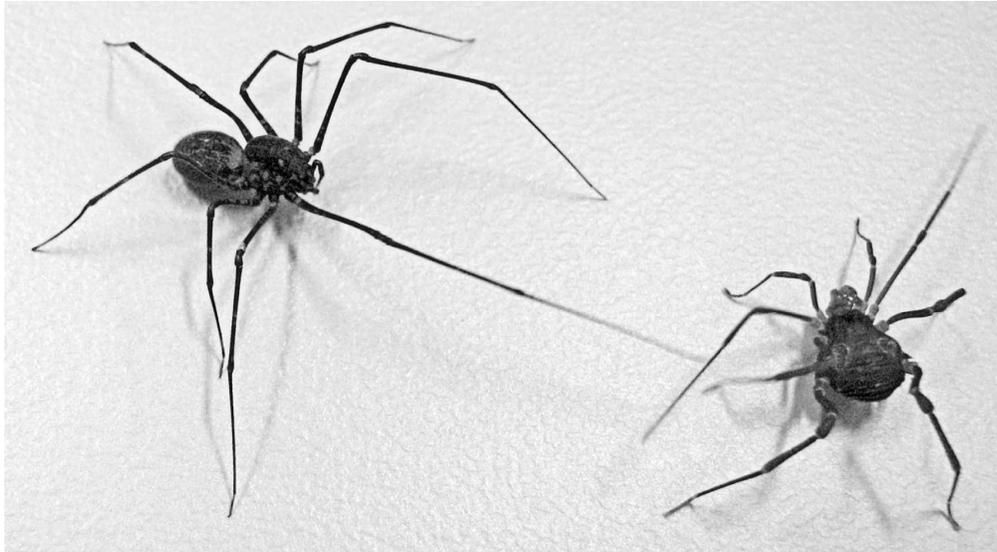


Figure 1.—*Scytodes globula* displaying “approach” and “contact leg” (see Table 1) with the harvestman *Discocyrtus invalidus* (~0.5cm length), in a staged set up for photography that also provides an idea of their comparative sizes.

composed mainly by 2,3-dimethyl-1,4-benzoquinone (Hara et al. 2005).

The spider *Scytodes globula* is a nocturnal, sit-and-wait predator retreating to the underside of palm fronds inside rotting logs during the day. These spiders feed on a variety of arthropods, including other spiders and insects (Nentwig 1985; Li et al. 1999). *Scytodes globula* is slightly larger than the harvestman (Fig. 1).

**Collection and maintenance in the laboratory.**—We manually collected 106 adult male and female spiders and 114 harvestmen at the Parque Esporte Para Todos in the University of São Paulo, São Paulo City, State of São Paulo, southeastern Brazil (23°32′51″ S, 46°38′10″ W), from February to April (end of rainy season) 2009. We numbered the animals, maintained them in individual plastic boxes (12 cm × 8 cm × 4 cm height) and fed them on moistened dog food (for harvestmen) and crickets (for spiders) once a week. We used crickets both as a food source for the spiders and as a control during the experiments. Because the spiders were collected as adults and therefore have probably eaten a wide variety of prey throughout their lives, we do not believe that the few crickets they have eaten in our laboratory influenced their behavior in the experiment. We provided water in a cotton ball for both species and maintained both an ambient temperature (25–30°C) and a natural light cycle (approximately 12:12 light: dark cycle). After the study, we fixed some animals in 70% ethanol and deposited them in the Museum of Zoology of the University of São Paulo, and released others at the same site where we had collected them.

**Experiments.**—We starved the spiders for 25–30 days before the trials to maximize their motivation to attack the prey (protocol previously tested in Souza & Willemart 2011). We used each animal only once. We used Sony Handycam DCR-TRV361 and DCR-HC65 NTSC, both with ‘nightshot’ (dim light), with no tripod to pick better angles for the movies. The arenas used in all experiments were 12 × 8 × 4 cm in height, with moist soil on the bottom.

*Experiment 1, survival rate:* To test if *S. globula* preys on *D. invalidus*, we randomly assigned 32 spiders to either harvestmen (8 males and 8 females) or 16 crickets used as a control (approximate body length equal to that of harvestmen – see Fig. 1). We left each pair for 5 d in the test arena and monitored the animals daily for prey capture between 12:00 and 13:00. We recorded the number of prey still alive on the fifth day, comparing the cricket and the harvestman groups. Because our interest was in testing the efficiency of spitting, we used a cotton ball to clean the arenas every day at the time we checked them for predation, specifically to remove the silk that some spiders had left.

*Experiment 2, details of the interactions:* Here we were interested in describing details of the behavior of *S. globula* ( $n = 20$ ) against either *D. invalidus* ( $n = 7$ ) or *Gryllus* sp. ( $n = 13$ ). We randomly assigned spiders to one of the treatments. To reduce stress, we introduced the spider into the test arena 8 h before the trial. We ran the trials between 18:00–23:00 (nocturnal period). We introduced the harvestman in a vial as far as possible from the spider, allowed it to acclimate for 2 min, and then released it. We waited 10 min before declaring a trial ended with no interactions. We monitored and digitally recorded behaviors related to the approach between the two animals, and any physical interactions occurring for 10 s after all encounters, a period sufficient to detect whether the spider would start eating the prey after biting it. After the trial ended, spiders were returned to where they were being maintained, and uneaten prey were discarded. From the resulting videos, we created behavioral categories, quantifying and comparing their occurrences between treatments.

*Experiment 3, testing the possible repellent effect of invisible secretions:* The prior experiment and observations suggested that although we could not see anything, this species could be producing some kind of defensive barrier. We therefore tested the hypothesis that harvestmen regularly secrete small amounts of defensive secretions from the scent glands, invisible to the human eye. This would explain why *S. globula*

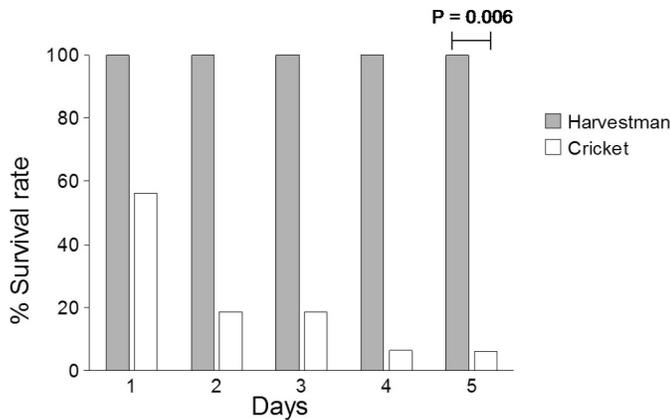


Figure 2.—Results of Experiment 1: survival rate of crickets (*Gryllus* sp.) and harvestmen *Discocyrtus invalidus* when paired with *Scytodes globula* for five days. The *P* value contrasts the number of prey that were still alive in the fifth day in the two groups.

would reject *D. invalidus*. We used four different treatments: harvestmen with glands experimentally obstructed with glue (*n* = 13), harvestmen with glue on the dorsum 3 mm from the gland (*n* = 13), crickets with glue on the dorsum (*n* = 14) and crickets with no glue (*n* = 14). We applied the glue 15 d before the trial to minimize the influence of residual odors. Because harvestmen secretions are extremely volatile (Gnaspini & Hara 2007), it is highly improbable that residual effluents were still on their body when we ran the trials. These were run between 18:00–23:00 (nocturnal period). All remaining procedures were the same as described for Experiment 2. From the videos, we quantified behavioral categories and compared their occurrences across treatments.

RESULTS

**Experiment 1.**—By the end of the fifth day, even after the spiders were severely starved and subsequently offered no other choice but harvestmen as a food choice, all the harvestmen were alive and more than 90% of the crickets were dead with their body contents emptied ( $X^2_1 = 7.69, P = 0.006$ ) (Fig. 2). The spiders preyed upon 81% of the crickets during the first day of the experiment. We did not detect injuries among harvestmen, and all spiders were alive after the experiment.

**Experiment 2.**—Because experiments 2 and 3 involved recordings of interactions among spiders, harvestmen and crickets, and because the glue in experiment 3 did not affect the behavior of either prey or predator, we pooled observations of experiments 2 and 3 in this specific analysis to better describe such interactions. Whenever possible, we employed behavioral categories previously used in the literature, such as those of Gilbert & Rayor (1985), Li et al (1999) and Souza & Willemart (2011). When spiders interacted with crickets (*n* = 41), the usual predatory sequence involved contact, spitting, and biting (Table 1, Fig. 3A). Biting was followed by “shake” in 40% of the observations, and all the trials ended with spiders eating the crickets. In contrast, spiders interacting with harvestmen (*n* = 33) seldom spat (*n* = 3) and never bit, generally touching the harvestmen and remaining still (Table 1, Fig. 3A). We did not observe emission of defensive secretions from the scent glands by the harvestmen or mechanical defenses such as pinching with chelicerae, pedipalps, or with the spines of legs IV (Fig. 3B).

**Experiment 3.**—As in experiments 1 and 2, data from Experiment 3 revealed a significant difference in the survival rate between harvestmen and crickets ( $X^2_1 = 21.03; P < 0.001$ ) (Fig. 4). Obstructing the glands did not interfere with the survival rate of the harvestmen (Fisher exact test, *P* = 1). The glue per se had no influence on the results (crickets with glue vs. crickets without glue: Fisher exact test, *P* = 1).

DISCUSSION

Contrary to our expectation, *Scytodes globula* did not take advantage of its specialized spitting mechanism to prey upon the harvestman *D. invalidus*, even in starvation and with both sharing a small arena for five days. To understand why the spider rejected the prey, we conducted further detailed observations and experiments. Secondary defenses (defensive mechanisms used only after the predator is detected – Edmunds 1974) did not play a role. We observed no mechanical defense in Experiment 2 and, according to Experiment 3, rejection was not mediated by chemicals from the scent glands.

Chemicals from the scent glands have been extensively studied and identified as responsible for some predators rejecting harvestmen (Gnaspini & Hara 2007). *Discocyrtus invalidus* is known to release mainly 2,3-dimethyl-1,4-benzoquinone (Hara

Table 1.—Behavioral repertoire of the spider *Scytodes globula* when interacting with potential prey.

Category	Definition
Approach	To move towards prey
Bite	To lean forward and pinch prey with the chelicerae
Contact leg	Active or passive contact between a spider leg and prey
Dorso-ventral step	With the body off the substrate, to rapidly move the femur or tibia of legs II, III and IV up and down, as if it was walking but without displacement
Motionless	Not moving the body or the appendages
Retreat	To walk away from the prey after touching it
Pull	After extending legs I and placing tarsi I on the prey, to draw the prey in by flexing legs I
Shake	To move legs II, III and IV back and forth with short quick movements so that the body trembles, while touching the prey with legs I
Spit	To eject a sticky secretion from its chelicerae
Orient to prey	To rotate the body without displacement, ending with the anterior portion of the body facing the prey
Wave	To move legs I dorso-ventrally, slower than “Dorso-ventral step” and without contact with the substrate
Wrap	With alternated movements of legs IV, to take silk from the spinnerets and wrap the prey in silk

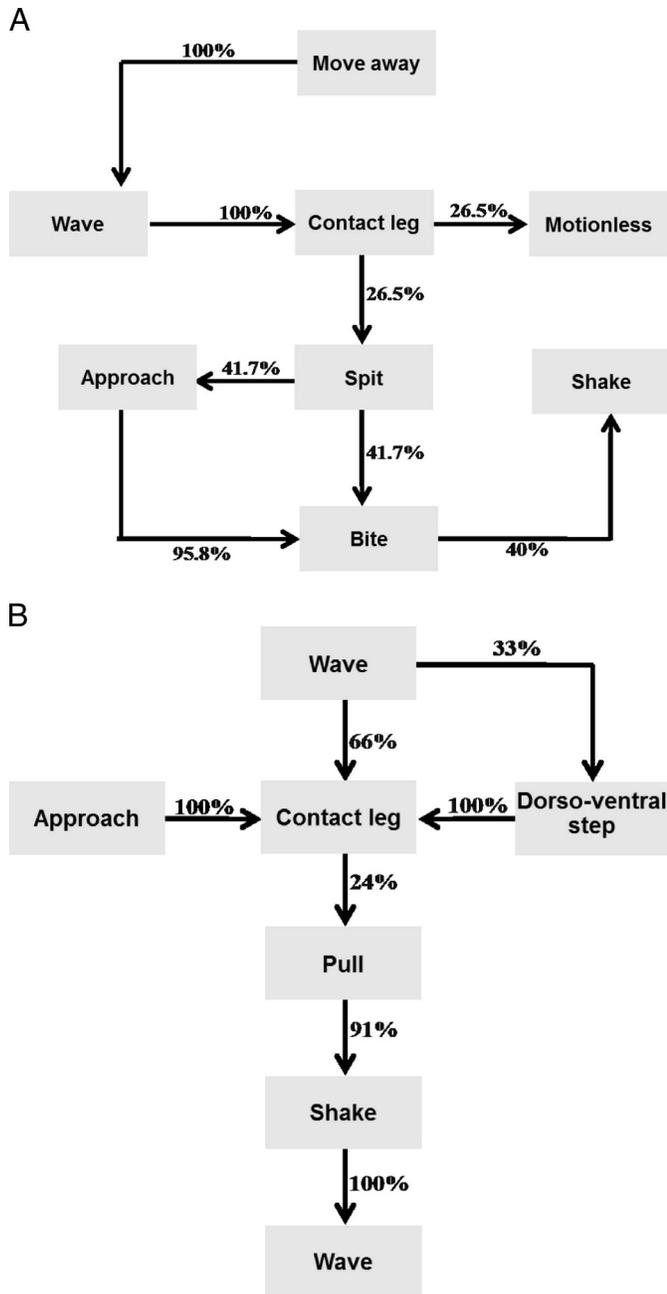


Figure 3.—Fluxograms of the predatory interaction between (A) the spider *Scytodes globula* and the cricket *Gryllus* sp. and (B) the spider and the harvestman *Discocyrtus invalidus* (see behavioral categories in Table 1). For the sake of clarity, only behavioral sequences with frequencies higher than 10% are included.

et al. 2005) by emitting a droplet that may run backwards in the grooves of the dorsal scutum (Hara & Gnaspini 2003), but this was never observed in our trials. Sabino & Gnaspini (1999), Eisner et al. (2004) and Willemart & Pellegatti-Franco (2006) also describe laniatorid harvestmen known to exude secretions when handled that failed to do so when attacked by spiders. Hara & Gnaspini (2003) triggered the exudation of secretions by holding *D. invalidus* with forceps, which is probably interpreted

as more threatening than contact with the legs or even the glue spat by *Scytodes globula*.

Spiders have been reported to change their predatory strategies according to the type of prey (reviewed in Clements & Li 2005; Pekár & Lubin 2009). The same behavioral categories were used by *S. globula* with harvestmen and crickets, but with different frequencies. Though it does not appear in the fluxogram because they were very rare behaviors, “dorso-ventral step” and “pull” were observed twice each (distinct spiders and distinct harvestmen) against crickets. Similarly to Li et al. (1999) and Ades & Ramires (2002), crickets and harvestmen were often attacked or rejected after contact, so that behavioral categories like “contact leg,” “pull,” “wave,” and “shake” probably inform the spider on the size/profitability/danger offered by the prey. Since our data suggest that chemicals from the scent gland secretions do not play a role in the harvestmen’s response, spiders may be responding to the hard integument or, alternatively, repellent chemicals embedded in the cuticle. Whatever the explanation, a primary defense (defensive mechanisms present even in the absence of predators – cf. Edmunds 1974) could be playing a role, rather than the well-known chemicals from the scent glands.

Spiders spat on harvestmen in only three cases, and in those cases the harvestmen seemed unaffected except that they had to flee, dragging soil and pieces of dry leaves glued to their legs. Since capturing prey is costly, and spiders control the amount of venom they use according to the prey (Wigger et al. 2002; Wullschlegel & Nentwig 2002; Nelson & Jackson 2011), an alternative hypothesis to explain the rejection is that the benefits provided by capturing a harvestman may not outweigh the cost of producing the amount of glue/venom necessary to subdue the harvestman. Moreover, a harvestman dragging leaves produces substrate-borne vibrations and air displacement, and the odor of the glue/venom may even act as a kairomone. These mechanical and chemical cues could potentially attract spider predators.

We have studied the predatory interactions between two harvestmen and spider species in our laboratory so far. The harvestmen *D. invalidus* and *Mischonyx cuspidatus* are avoided by the large ctenid *Enoploctenus cyclothorax* (Bertkau 1880) (Willemart & Pellegatti-Franco 2006; Souza & Willemart 2011) and the spitting spider *S. globula* (this study), both under starvation conditions. Factors such as being larger (*E. cyclothorax*) and having the ability to capture prey from a distance by spitting (which we had hypothesized would allow *S. globula* to avoid secretions and pick exact spots to bite) are therefore not indications that these spiders will feed on such harvestmen. If overcoming the thick cuticle were the issue, we would expect that even larger spiders, such as *Ctenus ornatus* (Keyserling 1877), would eat the harvestmen if they are able to pierce the prey’s integument. We would also expect that spiders with weak chelicerae that build sheet webs and actually prey upon these harvestmen (such as the recluse spider *Loxosceles* sp.), would take advantage of an immobilized prey to pick specific vulnerable spots (such as articulations) to bite the prey. We are currently studying these spiders in our laboratory. Such studies, in addition to this and previous papers, may add to the understanding of proximate causation of prey acceptance and rejection in spiders.

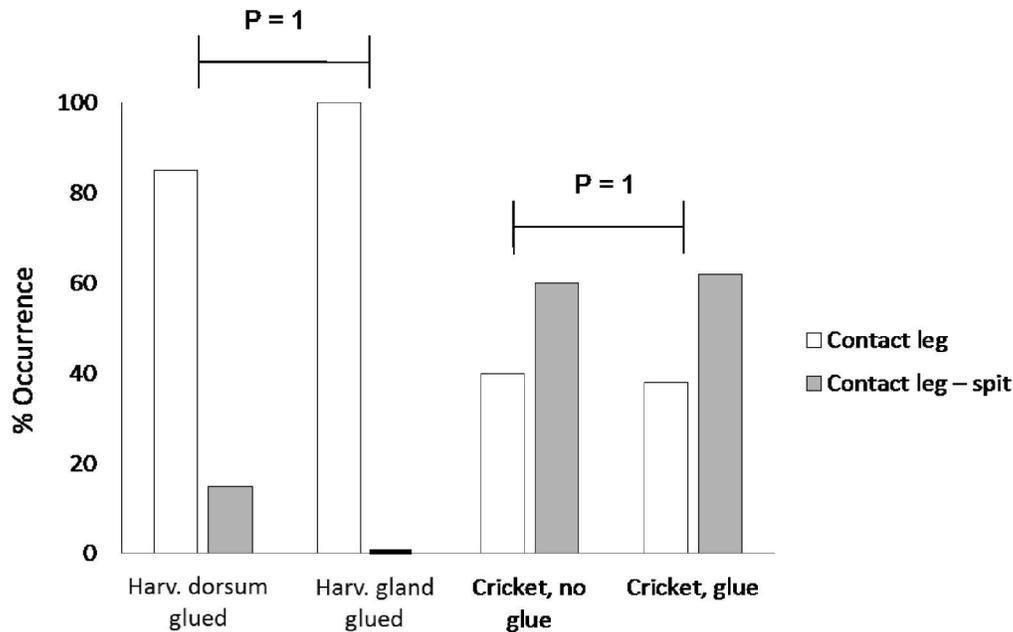


Figure 4.—Behavior of *Scytodes globula* when interacting with crickets with or without glue on the dorsum (control groups) and harvestman *Discocyrtus invalidus* with glue on the dorsum (control group) and glue clogging the scent gland opening (treatment group). Harv. = harvestman.

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