Universidade Federal de Uberlândia
Instituto de Biologia

INTERAÇÃO PREDADOR-PRESA ENTRE A ARANHA *Loxosceles gaucho* (SICARIIDAE) E O OPILIÃO *Mischonyx cuspidatus* (GONYLEPTIDAE)

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Dissertação apresentada à Universidade Federal de Uberlândia, como parte das exigências para obtenção do título de Mestre em "Ecologia e Conservação de Recursos Naturais".

Orientador
Prof. Dr. Kleber del Claro
Co-orientador
Prof. Dr. Rodrigo Hirata Willemart

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APROVADO em 28 de Fevereiro de 2014

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Fevereiro - 2014

DEDICATÓRIA

Dedico esta dissertação ao meu Pai, que me contava entusiasmado sobre cada novo bicho que avistava. Em nossa última conversa, me perguntou sobre este projeto, os objetivos do trabalho e fez comentários bastantes motivantes, que para mim serviram como reconhecimento e me deram força para seguir trabalhando após o seu falecimento. Com muito carinho e enorme saudade.

Em memória.

AGRADECIMENTOS

- Agradeço à minha Mãe, por toda ajuda, apoio, exemplos e ensinamentos que me deu ao longo da vida e durante o período de Mestrado. Uma pessoa que possui um valor imensurável para mim e certamente foi fundamental para minha formação, em todos os sentidos.
- Agradeço à Andressa, por todo seu o apoio durante o mestrado, pelas muitas ajudas e por sua presença, durante parte do período experimental, que foi decisiva para que eu conseguisse conduzir os experimentos em determinados momentos. Bem como à sua família pelo apoio, confiança e pelo trato sempre gentil em relação a mim.
- Agradeço aos meus irmãos Caio e Vitor pela amizade, apoio e pelos momentos agradáveis da vida que compartilhamos.
- Agradeço ao meu professor Lito, por tudo que com ele aprendi, que certamente influenciou de forma positiva minha personalidade.
- Agradeço a todo pessoal do LESCA: Nathy, Thay e prof. Rodrigo, por ajudarem nas coletas iniciais da fase de experimentos pilotos, a todos os membros do laboratório pelas sugestões e críticas, sempre com muito boa vontade. Ao Gabriel por me ajudar na filmagem dos pilotos. Ao Nortão pela ajuda nas coletas de opiliões, sempre com muito boa vontade. À Elene e ao Gui por terem sido muito legais quando entrei no laboratório.
- Agradeço ao pessoal do LECI pelas sugestões e críticas e pela gentileza durante as reuniões, em especial a Alexandra, Estevão, Gudryan e Vanessa.
- Ao prof. Kleber Del Claro por ter aceito me orientar, pela forma como me recebeu e incentivou no momento em que o procurei, por ter acreditado em mim e pela orientação.
- Agradeço o prof. Rodrigo Willemart, pela co-orientação. Por toda a ajuda no desenvolvimento do projeto, nos experimentos, pelas dicas, correções gerais e de inglês. Por permitir que eu desenvolvesse o trabalho em seu laboratório e até mesmo pelos Papers que me mandava às pencas aos 44 do segundo tempo. Todo este suporte foi fundamental para o desenvolvimento deste trabalho.
- Agradeço o professor Heraldo Vasconcelos pelos ensinamentos durante a disciplina de estatística, que foi uma das disciplinas que mais contribuíram para minha formação.
- Agradeço o Professor Luiz Andrioli pelo empréstimo do equipamento de CO_2.
- Agradeço o professor Marcelo Gonzaga pelo auxílio na identificação da espécie de *Loxosceles* e também por ter aceito fazer parte da Banca Examinadora.

- Agradeço o professor Michael Hrncir por ter aceito fazer parte desta banca.

- Agradeço a técnica de laboratório Ana Machado pelo auxílio com os cuidados dos opiliões e ser muito gentil em todas as ajudas adicionais.

- Agradeço a Vanessa Penna do instituto Butantan pelas valiosas dicas de cuidado com as *Loxosceles*.

- Agradeço ao Elmo e Giancarlo pela convivência pacífica e agradável, pelas discussões e trocas de experiências durante o Mestrado. Ao Rafael pelas discussões de artigos e Ciência, algumas vezes calorosas, mas certamente produtivas. A estes três também agradeço pelas brincadeiras e risadas, que ajudaram a deixar este período mais leve.

- Agradeço a toda minha turma de Mestrado pelos momentos que compartilhamos e pelas contribuições de cada um em minha formação, mesmo que em alguns casos de maneira inconsciente.

- Agradeço à secretária da pós graduação, Maria Angélica, pelas muitas ajudas ao longo do Mestrado de forma sempre muito eficiente e educada.

- Agradeço ao Instituto de Biologia da UFU e ao Programa de Pós Graduação em Ecologia e Conservação de Recursos Naturais por terem me proporcionado este período de aprendizado.

- Agradeço pelo apoio FAPESP 2010/00915-0, ao laboratório (LESCA) coordenado pelo professor Rodrigo H. Willemart.

- Agradeço à FAPEMIG pela bolsa concedida durante o período de Mestrado.

ÍNDICE

RESUMO GERAL

A interação entre presas e predadores é uma relação ecológica assimétrica e antagônica visto que, para predadores, capturar presas é vantajoso devido ao ganho de energia e, para presas, ser capturado, frequentemente significa morrer. A interação predador-presa é tida como uma pressão evolutiva que tende a aperfeiçoar tanto os mecanismos de caça de predadores como as defesas das presas. Para esta dissertação, utilizamos como modelo de predador a aranha *Loxosceles gaucho* e como presa o opilião *Mischonyx cupidatus*, ambos noturnos e sintópicos. No primeiro capítulo investigamos o uso de modalidades sensoriais por *L.gaucho* na busca por um sítio adequado de forrageio e na detecção de presas, bem como qual comportamento possibilita que estas aranhas consumam opiliões, presas com uma cutícula altamente esclerotizada. Foram testadas as seguintes hipóteses: (1) *L. gaucho* passa mais tempo em áreas com químicos de suas presas; (2) a vibração é uma informação essencial no processo de predação por *L. gaucho*; (3) o lençol de teia permite que *L. gaucho* prede opiliões. Realizamos também uma análise comportamental em que foi descrito um etograma e um fluxograma. Todas as hipóteses foram rejeitadas. O que aparentemente possibilita que *L. gaucho* capture esta presa é sua habilidade de encontrar e morder os pontos fracos do corpo do opilião, como juntas e partes distais das pernas. Contrariando vários estudos que demonstram que aranhas rejeitam opiliões Laniatores, este foi o primeiro estudo que demonstrou uma alta taxa de sucesso de predação de aranhas sobre opiliões e, além disso, demonstrou evidências comportamentais das estratégias que permitem a predação. No segundo capítulo, investigamos os comportamentos defensivos de *M. cuspidatus* em uma situação de risco intermediário (frente aos químicos dos predadores) e alto risco (frente aos predadores), visto que a teoria conhecida como "Threat sensitive hypothesis" postula que para as presas é vantajoso modular comportamento defensivo de acordo com o nível de ameaça. Adicionalmente, testamos se lesões no abdômen de *L. gaucho*, provocadas por apófises do fêmur da perna IV de um opilião, podem influenciar na sobrevivência da aranha. Encontramos comportamentos defensivos nos opiliões apenas na situação de alto risco. A sobrevivência das aranhas não foi alterada após serem lesionadas pelas apófises dos opiliões. Surpreendentemente, não observamos comportamentos de defesa muito conhecidos entre opiliões (ex. defesa química, retaliação com quelícera e pedipalpos) em uma situação de alto risco. Por fim, destacamos a importância de abordagens comportamentais descritivas de comportamento para compreender especificidades das interações entre predadores e presas.

Palavras-chave: Comportamento predatório, Comportamento defensivo, Ecologia sensorial, Opiliones, Araneae

INTRODUÇÃO GERAL

A interação entre presas e predadores é um tema que desperta grande interesse, provavelmente por estar diretamente relacionado à fronteira entre continuar vivo ou morrer, seja por meio da manutenção da vida (por meio da ingestão de presas) ou de evitar ser morto por predadores. Dentro das ciências biológicas, a interação predador-presa é estudada em diversos campos, tais como: ecologia de populações, ecologia de comunidades, ecologia comportamental e evolução. Devido a abrangência deste tipo de interação, livros-texto básicos de várias áreas discutem o tema (por exemplo Begon et al., 2007; Ricklefs, 2003; Davies & Krebs, 2012; Ridley, 2004; e outros), além de existirem títulos dedicados especialmente aos mecanismos de defesa contra predadores (Edmunds, 1974) ou à ecologia deste tipo de interação (Barbosa & Castellanos, 2013).

No que diz respeito à ecologia de populações, trabalhos clássicos demonstram oscilações sincronizadas nos ciclo populacionais de presas e predadores (Elton, 1924). Entretanto, o impacto da predação em uma população de presas pode variar dependendo do tipo de predador, uma vez que diferentes estratégias de captura podem resultar em uma taxa de sucesso de captura distinta (veja Cresswell & Quinn, 2010). Outro ponto importante é que os predadores podem exibir preferências em sua dieta. A preferência por presas pode se basear em fatores como: o seu valor energético, a proporção peso do predador em relação ao tamanho da presa e em experiências anteriores com as presas (Spitz et al., 2010, McQuaid, 1994, Pearson & Rypstra, 2000; Punzo, 2002). Além destes fatores, podem existir mudanças ontogenéticas no tipo de dieta (Cardona et al., 2010).

Os predadores possuem um importante papel na estruturação de comunidades, pois podem possibilitar que espécies competidoras coexistam prevenindo que determinadas espécies superiores na hierarquia competitiva monopolizem os recursos. Este fenômeno é conhecido como coexistência mediada pelo predador (Paine, 1966). Uma meta-análise realizada por Gurevich et al., (2000), que comparava o efeito da competição na presença e ausência de predadores, demonstrou que de fato os competidores tiveram uma maior influência em parâmetros como o crescimento, sobrevivência e massa, quando não havia predadores do que quando os predadores estavam presentes. No entanto, determinar o efeito da predação em uma comunidade natural pode ter complicadores, como os efeitos emergentes, um fenômeno em que o efeito de predadores múltiplos pode ser diferente da soma do efeito destes predadores separadamente (Sih et al., 1998).

A interação entre presas e predadores ao longo do tempo dá origem a uma relação coevolutiva conhecida como “corrida armamentista” (e.g. Thompson, 2013e refêrencias neste). Esta relação possui um caráter assimétrico e antagônico, pois para os predadores, capturar presas é vantajoso devido ao ganho de energia. Para as presas ser capturado significa morrer (Dawkins & Krebs, 1979). Assim, a pressão da predação seleciona presas com melhores mecanismos defensivos, e predadores com melhores estratégias de caça (Davies & Krebs, 2012).

Os predadores apresentam várias adaptações para capturar presas, como estruturas sensoriais bem desenvolvidas, adaptações para escolha do sítio de forageio, mecanismos de aproximação, habilidades motoras, estratégias e estruturas ofensivas (Davies & Krebs, 2012). As presas, por sua vez, possuem adaptações morfológicas como um tegumento espesso e espinhos, além de mecanismos comportamentais como aposematismo, anacorese, tanatose, camuflagem e defesas químicas (Edmunds, 1974; Eisner, 2005; Davies & Krebs, 2012). Outra estratégia de alto valor adaptativo conhecida entre as presas é a capacidade de modular os comportamentos defensivos de acordo com o nível de ameaça imposto pelo predador (Helfman, 1989).

Esta breve introdução objetivou tratar sucintamente alguns dos domínios nos quais a interação presa-predador está direta ou indiretamente ligada. A presente dissertação aborda este tipo de interação sob uma perspectiva ecológico-evolutiva, buscando compreender quais as estratégias que possibilitam que a aranha *Loxosceles gaucho* consuma opiliões, que são conhecidos por sua secreção defensiva e por possuírem um tegumento espesso bastante esclerotizado (Primeiro capítulo, formatado de acordo com as normas da revista Animal behaviour). O Segundo capítulo (que segue as normas da revista Behaviour) investiga como o opilião *Mischonyx cuspidatus* reage a este predador em uma situação de risco moderado (frente aos químicos do predador) e em uma situação de alto risco (frente à aranha *L.gaucho*). Adicionalmente, este estudo contribuirá para o conhecimento das aranhas *Loxosceles*, um gênero de importância médica (Veter, 2008), uma vez que, até onde temos conhecimento, este será o primeiro estudo a descrever o comportamento de captura de presas em uma das espécies do gênero.

REFERÊNCIAS

Barbosa, P. & Castellanos, I. (eds) (2013). *Ecology of predator–prey interactions*. Oxford, UK: Oxford University Press.

Begon, M., Townsend, C. R., & Harper, J. L. (2007). *Ecologia: de indivíduos a ecossistemas*. (4th ed.) Porto Alegre, Brazil, Artmed.

Cardona L., Campos P., Levy Y., Demetropoulos A. & Margaritoulis D. (*2010).* Asynchrony between dietary and nutritional shifts during the ontogeny of green turtles (*Chelonia mydas*) in the Mediterranean. *Journal of Experimental Marine Biology and Ecology*, 393, 83–89.

Cresswell W & Quinn J.L. (2010). Attack frequency, attack success and choice of prey group size for two predators with contrasting hunting strategies. *Animal Behaviour*, 80, 643-648.

Davies, N. B., Krebs, J. R., & West, S. A. (2012). *An introduction to behavioural ecology*. (4th ed.) Chichester, UK: Wiley-Blackwell.

Dawkins, R., & Krebs, J. R. (1979). Arms races between and within species. *Proceedings of the Royal Society of London*. Series B. Biological Sciences, 205, 489-511.

Edmunds, M. (1974). *Defence in animals: a survey of anti-predator defences*. London, UK: Longman.

Eisner, T., Eisner, M., & Siegler, M. V. (2005). *Secrets Weapons: Defenses of Insects, Spiders, Scorpions, and Others Many-legged Creatures*. Cambridge, USA: Harvard University Press.

Elton, C. S. (1924). Periodic fluctuations in the numbers of animals: their causes and effects. *Journal of Experimental Biology*, 2, 119-163.

Fenk, L. M., Hoinkes, T., & Schmid, A. (2010). Vision as a third sensory modality to elicit attack behavior in a nocturnal spider. *Journal of Comparative Physiology A*, 196, 957-961.

Gurevitch, J., Morrison J. A., & Hedges L. V. (2000). The Interaction between Competition and Predation: A Meta analysis of Field Experiments. *The American Naturalist*, 155, 435-45.

Helfman, G. S. (1989). Threat-sensitive predator avoidance in damselfish-trumpetfish interactions. *Behavioral Ecology and Sociobiology*, 24, 47-58.

McQuaid, C.D. (1994). Feeding behaviour and selections of bivalve prey by *Octopus vulgaris* Cuvier. *Journal of experimental marine biology and ecology*, 177: 187-202.

Paine, R.T. (1966). Food Web Complexity and Species Diversity. *The American Naturalist*, 100: 65-75.

Persons, M. H. & Rypstra A. L. (2000). Preference for chemical cues associated with recent prey in the wolf spider *Hogna helluo*. (Araneae: Lycosidae) *Ethology*, 106 27-35.

Punzo, F. (2002). Early Experience and Prey Preference in the Lynx Spider, *Oxyopes salticus* Hentz (Araneae:Oxyopidae). *Journal of the New York Entomological Society*. 110, 255-259.

Ridley, M. *Evolution*. (2004). (2nd ed.), USA, Oxford University Press.

Ricklefs, R. E. (2003). *A economia da natureza*. Rio de Janeiro, Brazil, Guanabara-Koogan.

Sih, A., Englund, G., & Wooster, D. (1998). Emergent impacts of multiple predators on prey. *Trends in Ecology & Evolution*, 13, 350-355.

Spitz J., Mourocq E., Leauté J.P., Quéro J.C. & Ridoux. (2010). Prey selection by the common dolphin: fulfilling high energy requirements with high quality food. *Journal of Experimental Marine Biology and Ecology*, 390, 73–77.

Thompson, John N. (2013). *Relentless evolution*. Chicago, USA: University of Chicago Press.

Vetter, R. S. (2008). Spiders of the genus Loxosceles (Araneae, Sicariidae): a review of biological, medical and psychological aspects regarding envenomations. *Journal of Arachnology*, 36, 150-163.

FIRST CHAPTER

Delicate fangs, smart killing: the predation strategy of the brown recluse spider overcomes heavily sclerotized prey

ABSTRACT

Consuming prey involves finding an adequate site to forage, detecting and manipulating it. We investigated these three steps in interactions between the delicate recluse spider *Loxosceles gaucho* (Araneae) and a heavy bodied and armored harvestman, here represented by the harvestman *Mischonyx cuspidatus* (Opiliones). The hard integument of such harvestmen had been previously reported to protect them from spiders larger than *Loxosceles*. We tested the following hypotheses: (1) spiders prefer areas with cues from their prey, (2) vibratory cues are essential information in the predatory process and (3) the web sheet allows handling prey adequately so that the vulnerable spots of the prey can be bitten. To understand exactly how a delicate predator can overcome the defenses of a heavy bodied and well-defended prey, we also quantitatively described the spider´s behavior. To test hypothesis (1), we compared the time spent in areas with harvestmen, crickets and no cues. For hypothesis (2), we compared latency to bite and number of bites in the presence or absence of vibratory cues, and for hypothesis (3) we compared latency to detect, to capture and predation success. All hypotheses were rejected. *Loxosceles gaucho* seems to be exceptional among spiders by not needing its web, indirect prey chemical cues, or prey´s substrate borne vibrations to hunt it. What seems to enable *Loxosceles* to prey upon *M. cuspidatus* is its totally different hunting strategy, compared to previously studied spiders attempting to eat these armored harvestmen. The spider quickly touches the prey with its tarsi, finding and biting several times the weak parts of the prey body, such as joints and distal parts of the legs. With several previous papers reporting spiders rejecting these harvestmen, this is the first that describes how a spider overcomes the defenses of an armored harvestman.

Keywords: Foraging, Prey handling, Sensory ecology, Arachnida, *Loxosceles*, Opiliones

INTRODUCTION

Prey-predator interactions shape each other so that both prey defences and predatory strategies improve in evolutionary time in a process known as arms races (Dawkins & Krebs 1979). The predatory process encompasses the phases of search, capture and handling prey (Davies et al 2012). The search phase begins with the choice of a foraging site. Distinct pieces of information are used to assess the quality of a spot, such as the presence of conspecifics (review in Danchin et al 2004; Valone 2007), environmental characteristics that potentially attract prey, the presence of prey, prey cues or physical characteristics that facilitates prey capture (Chien & Morse 1998; Clark et al 2000; Hopcraft et al 2005; Johnson 2011; Hanna & Eason 2013).

After picking an adequate foraging site, predators need to detect prey using distinct sensory modalities. These include vision in frogs (González-Bernal et al 2011); the perception of substrate-borne vibration in scorpions (Mineo & Del Claro 2006); echolocation in bats (Schnitzler & Kalko 2001); tactile sense in star-nosed moles and shrews (Catania & Remple 2005; Anjum et al 2006); thermal sensitivity in snakes (Buning 1983) or chemoreception in salamanders (Placyk & Graves 2002), for example. Very often, multiple sensory modalities are important for prey detection (Piep et al 2008).

After detecting prey, predators must capture and handle them, which involve distinct structures and behaviors. To give a few examples, snakes use venom or constriction (Greene 1978; Kardong 1986), chameleons rapidly extend their sticky tongues (Herrel et al 2000; de Groot & Leween 2004), insects may move over the prey and directly bite quickly (De la Mora et al 2008), or use raptorial legs (Correte 1990; Betz & Mumm 2001). Among arachnids, the structures used to capture and handle prey may be chelicerae, pedipalps (Casper 1985; de Andrade & Gnaspini 2002; Ladle & Velandes 2003; Beccaloni 2009; Del Claro & Tizo-Pedroso 2009; Santer & Hebets 2009; Willemart et al 2011), and adhesive setae on the legs (Rovner 1980), sometimes with the help of venom in scorpions, pseudoscorpions and spiders (Weygoldt 1969; Bub & Bowerman 1979; Foelix 2011).

Several but not all spiders are generalists (Foelix 2011; Nelson & Jackson 2011) and their diet often includes arthropods in general. In the phase of choice of a suitable spot to forage, spiders use multiple cues to locate prey depending on the species: one that has been shown to be of particular importance is the use of chemicals (Persons & Uetz 1996; Persons

& Rypstra 2000; Jackson et al 2002). In the following stage, when the prey is close and specifically in the phase of capture, substrate-borne vibrations have a fundamental role in web spiders, wandering spiders and even in spiders that capture prey on aquatic environment (Hergenröder & Barth 1983; Bleckmann & Barth 1984; Masters 1984; Barth 2002).

Among the several species eaten by spiders, harvestmen are certainly an interesting prey from a behavioral point of view. Harvestmen exhibit many kinds of defences such as cripsis, thanatosis, anachoresis, aposematism, mimicry, deimatic behavior and fleeing (see Gnaspini & Hara 2007). Chemical defence has been the most studied, with harvestmen releasing defensive secretions through glandular openings positioned latero-dorsally on the body (Eisner et al 2004; Hara & Gnaspini 2003; Hara et al 2005; Machado & Pomini 2008; Pomini et al 2010). Such chemicals have been shown to be efficient against spiders (eg. Machado et al 2005), but they do not seem to be always used. Instead, a thick cuticle of adults seems to be protective against predatory some spiders (Souza & Willemart 2011; Dias & Willemart 2013). Unless they are strong enough to pierce the thick cuticle, spiders are left only a few vulnerable spots to bite, such as the distal parts of legs, mouth and articulations (Souza & Willemart 2011). Wandering spiders previously tested with heavy bodied laniatorid harvestmen as prey have a low success rate (Eisner et al. 2004; Souza & Willemart 2011; Carvalho et al 2012; Dias & Willemart 2013) despite their large size or ability to spit venom. The large *Enoploctenus cyclothorax,* for example, did not feed on the harvestman *Mischonyx cuspidatus* even after sharing a small terrarium with it for about 70 days and with no alternative food source (Willemart & Pellegatti-Franco 2006). In contrast, we often find dead harvestmen on the horizontal sheets of web of recluse spiders (*Loxosceles*) (Fischer et al 2006; Fig. 1). Despite being world famous for causing severe skin wounds and being of medical importance (Cardoso et al 2009), there are no detailed studies on how recluse spiders hunt their prey. Because these spiders have delicate body and chelicerae, Carvalho et al (2012) hypothesized that they could prey upon harvestmen because the sheet of web would help immobilizing the prey, allowing the spiders to bite the vulnerable parts of the harvestman body.

We were interested in looking at the distinct phases of prey capture in interactions between a recluse spider and a heavy bodied harvestman. Due to the selective advantages of adequate site selection, we first hypothesized that, given the choice, they would prefer areas with chemical cues from their prey. After picking a good foraging site, predators need to detect their prey: we hypothesized that vibratory cues would be essential information in the predatory process. We also tested the hypothesis of Carvalho et al (2012), that the web sheet would allow to handle prey adequately so that the vulnerable spots would be bitten. In order to understand exactly how a delicate predator can overcome the defences of a heavy bodied and well-defended prey that is rejected by much larger predators (Souza & Willemart 2011; Dias & Willemart 2013), we also quantitatively described the spider´s behavior.

METHODS

Species studied

The harvestmen *Mischonyx cuspidatus* (Roewer 1913) and spiders of the genus *Loxosceles* are active mainly at night (Pereira et al 2004; Fischer et al 2006) and can be found under tree trunks, dead palm fronds and anthropized spots, often under bricks (Mestre & Pinto-da-Rocha 2004; Fischer & Vasconcelos-Neto 2005). *Loxosceles* spiders are polyphagous, feeding on a wide variety of arthropods (Fischer et al 2006).

In our study, the spiders *Loxosceles gaucho* Gertsch 1967 were collected in Mairiporã city, São Paulo State Brazil (23◦19 S, 46◦35 W), within building material, between November 2012 and February 2013. We found individuals of *M. cuspidatus* on the exact same bricks where the spiders were collected, but not in enough quantity for the experiments. The harvestmen *M. cuspidatus* used in the experiments were therefore collected at the "Parque Ecológico do Tietê - São Paulo city, São Paulo State, Brazil (23◦25 S, 46◦28 W) between December, 2012 and May 2013, under tree trunks.

Laboratorial conditions

We maintained the animals in a room under ambient temperature and following the natural light-dark cycle but with a weak red light (15 watts) alight 24 hours per day. That allowed us to work at night without disturbing the animals. We maintained spiders and harvestmen individually in plastic recipients (12x8x4 cm height) with soil on the bottom. Water was provided with a wet cotton ball. We fed the spiders according to the experiments, as described below, and harvestmen were given moistened dog food once a week.

Figure 1. The recluse spider *Loxosceles* sp. in the field, in a dead palm frond, with three harvestmen carcasses (white setae). In this case the web sheet where the spider and prey carcasses are on is very thin and can hardly be seen.

Experiment 1: chemicals of prey as information for the predator

This experiment was conducted to test whether *L. gaucho* uses chemicals left on substrate by its prey when foraging. We predicted that the spiders would spend more time in places with chemicals of harvestmen (*M. cuspidatus*) and crickets (*Gryllus* sp.) than on white controls.

The spiders used were previously fed on crickets and harvestmen to minimize biases, because the previous feeding experience can influence future decisions about what to eat (Persons & Rypstra 2000). The food deprivation period before the trials was between 37-38 days.

The testing arenas (18 cm diameter x 6 cm height) had two pieces of filter paper and a neutral central area (5.5 cm diameter) on the bottom. We tested 3 groups in this experiment, each with two treatments: harvestmen chemicals/ blank filter paper (*N* = 17, 14 females and 3 males); cricket chemicals/ blank filter paper (*N* = 17, 14 females and 3 males); harvestmen chemicals/ cricket chemicals (*N* = 19, 14 females and 5 males).

To impregnate the filter paper with chemicals of prey (harvestmen or crickets), 15 individuals were left for 24 hours in a recipient similar to the arenas, with two pieces of filter paper on the substrate. The filter papers were removed immediately before the tests.

The spider was introduced in the central neutral place in a vial (5cm diameter x 6 cm height) and acclimated for 3 minutes. After releasing the spider we covered the arena with a sheet of glass. We recorded the animals for 30 minutes in each test. After each trial, we horizontally turned the arena 45° clockwise to minimize biases related to the position of the arena in relation to the laboratory. We used a Sony Handycam HDXR550V in nightshot mode to register the time spent in each side of the arena. The arena was cleaned with 70% ethanol between trials and each piece of filter paper was only used once. The data were collected in May 2013, between 8 pm and 5 am. We used paired *t*-tests to analyze the data, and therefore measures of central tendency presented here are for the differences between the time spent in each side of the arena.

Experiment 2: substrate vibration as information for predators and predatory behavior with distinct prey

In this experiment we tested whether substrate-borne vibrations are important in the predatory process for *L. gaucho* with harvestmen and crickets, tested separately. We predicted that predatory success would be higher and latency to attack would be lower in spiders tested in a substrate that transmits vibration (filter paper) than in spiders tested in granite, a substrate that greatly reduces the transmission of substrate-borne vibrations (Elias et al 2004; Hebets 2004). Because harvestmen have fewer vulnerable spots to bite than crickets (Souza & Willemart 2011) so that spiders would not be able to pick body regions more sensitive to venom, we also predicted that handling time would be larger and more bites would be used against harvestmen.

Prior to the experiments, we fed the spiders with a beetle larvae (*Zophobas* sp.) in the same terrarium where that the spiders were maintained (12 x 8 x 4 cm). Prey uneaten after 48h were removed. Only spiders that ate the larvae were used. The food deprivation period before the trials was between 25-31 days. The granite substrate was a piece of light granite (40 x 40cm width x 2cm thickness). The filter paper substrate was a squared piece of paper filter (21 x 21 cm). The arena was cleaned with 70% alcohol after each trial.

We tested the spiders offering prey in four different situations, all of them in a circular arena (18 cm diameter x 6 cm height) made of plastic walls, open on the bottom, placed either on the filter paper or on the granite block: (i) harvestman on filter paper substrate (*N* = 14, all females); (ii) harvestman on granite substrate (*N* = 14, all females); (iii) cricket on filter paper substrate (*N* = 14, all females); and (iv) cricket on granite substrate (*N* = 14, 13 females and 1 male);. Each spider was tested only once. Both spider and prey were introduced in the arena and acclimated for 3 minutes in separate recipients (5 cm diameter x 6 cm height) and in opposite sides of the arena. The animals were released together and the arena was covered with a sheet of glass.

The experiments were recorded with a camera Sony Handycam HDXR550V, in nightshot mode. The time of recording was standardized in 40 minutes, starting when contact was established. If prey and predator did not touch each other after 40 min, we considered there was no predation. The trials were conducted in April 2013, from 8PM to 5AM. We compared predation success between substrates with a Chi-Square, latencies to attack with a Mann-Whitney, time between first bite and long bite and number of bites with a *t*-test

Experiment 3: the role of silk in predation

This experiment was conducted to test the hypothesis that the sheet of silk of *L. gaucho* is necessary to capture the harvestmen. We predicted that the spiders tested with the sheet of silk (control group) would have a greater success in capturing and that the latency to detect and capture prey would be shorter in the absence of silk (treatment group). Spiders in both the no silk group (nsg) (*N* = 19, 17 females and 2 males) and the silk group (sg) (*N* = 19, 13 females and 6 males) were offered 10 females and 9 male harvestmen, totalizing 38 harvestmen offered as food. Twenty five to 29 days before the trials, we offered the spiders one cricket nymph (*Gryllus* sp.) (≅1.5 cm) and one beetle larva (*Zophobas* sp.), in order to standardize hunger. Only spiders that ate at least one of the prey were tested. Prey uneaten after 48h were removed. The terraria of the experiments (18 cm diameter x 6 cm height) were the same used to maintain the spiders. During the tests we covered the terrarium with a sheet of glass to prevent spiders from fleeing. Spiders in the control group were simply removed and put back in their terrarium. For spiders in the treatment group, we took them out of the terrarium, we removed all the soil, cleaned the whole terrarium with a dry paper towel to remove remnants of silk and then put new soil in it. Changing the soil was necessary to completely remove the silk of the terrarium. We then reintroduced the spider. In both groups, we allowed the spider to walk freely in the terrarium for 15 minutes before we introduced a harvestman as far as possible from the spider.

We registered the trials using a Sony Handycam HDXR550V in the nightshot mode. We started recording after introducing the harvestman and recorded for 40 minutes after the predatory process started (defined as first bite). Each spider was tested only once. We collected the data between February and March 2013 at night time (Between 9 PM and 5 AM). We compared capture success with a Chi-Square, latencies between behavioral categories with a Mann-Whitney and number of bites with a *t*-test.

RESULTS

Experiment 1: chemicals of prey as information for the predator

The time (in seconds) spent in each side of the arena with chemicals of harvestmen/crickets did not differ (median of the difference harvestmen-cricket: -127; min: -1800, max: 1219; $t = 0.190$; $df = 18$; $P = 0.852$). For the combination harvestmen/blank there was also no difference (median of the difference harvestmen-blank: 255; min: -1071, max: 1518; $t = 0.977$; $df = 16$; $P = 0.343$). But spiders spent slightly more time in blank filter paper than on cricket cues (median of the difference cricket-blank: -217, min: -897, max: 1122; $t = 2.387$; $df = 16$; $P = 0.03$).

Experiment 2: substrate vibration as information for predators

Ten out of 14 harvestmen were preyed upon in each of the substrates ($\chi^2 = 0$; $df = 1$; $P = 1$). Among crickets, nine were preyed upon in filter paper (fp) and seven on granite (g) ($\chi^2 = 0.583$; $df = 1$; $P = 0.699$) ($N = 14$ for each treatment). The latency to bite compared between the substrates of filter paper (median = 268.5; min: 111, max 745, $N = 10$) and granite (median = 156; min: 21, max 643, $N = 11$) was not different within the harvestmen (U = 45; $N_{fP} =$ 10; $N_g = 11$; $P = 0.481$). Latency to bite crickets also did not differ between filter paper (median = 157; min: 52, max 1358, $N = 9$) and granite (median = 411; min: 48, max 950, $N = 9$) (U = 43; $N1_{fP} = 9$; $N2_g = 9$; $P = 0.825$).

Because substrate was unimportant, we pooled the data from granite and filter paper to compare the predation rate between crickets and harvestmen and found no differences ($\chi^2 = 1.244$; $df = 1$; $P = 0.265$). However, the time between the first bite and the first long bite was longer for harvestmen ($t = 2.116$ $df = 28$ $P = 0.043$) (Figure 2), and the number of bites on harvestmen was higher (U = 27.500 $N_{cricket} = 18$ $N_{harvestman} = 21$; $p < 0.001$) (Figure 3).

Because there were no differences in the predation rate of harvestmen among treatments, we used the videos of both substrate groups to analyse where the spiders bit the harvestmen's body (Figure 4).

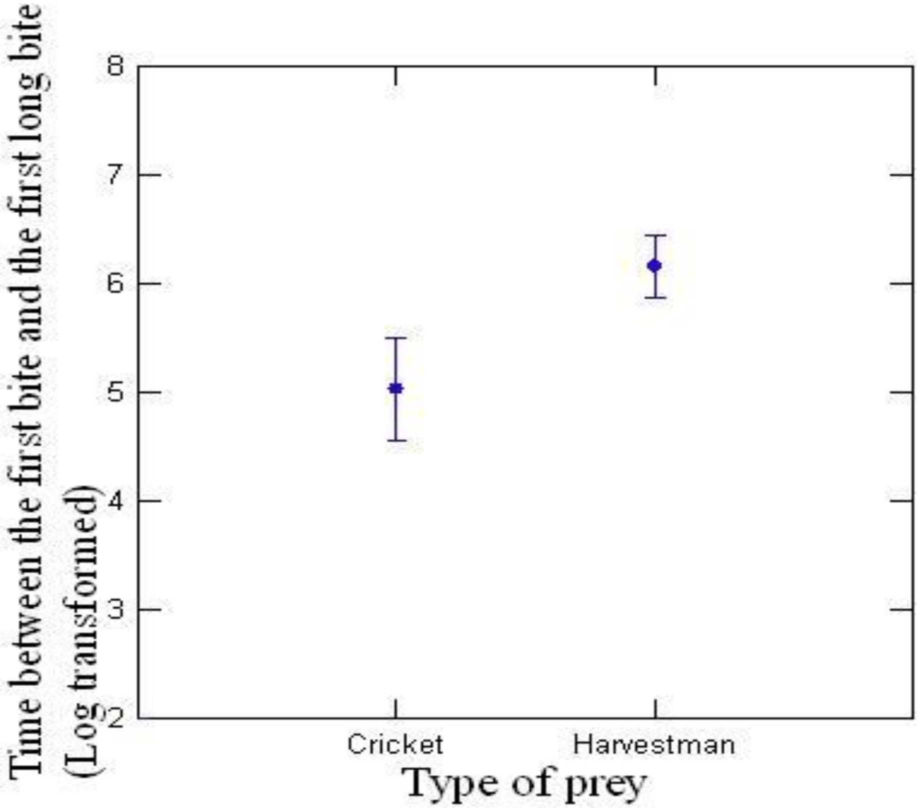

Figure 2. Time between the first bit and the first long bit in harvestmen ($N = 15$) and crickets ($N = 15$). The data was transformed in logarythm to suit the premisses of parametrical analyses.

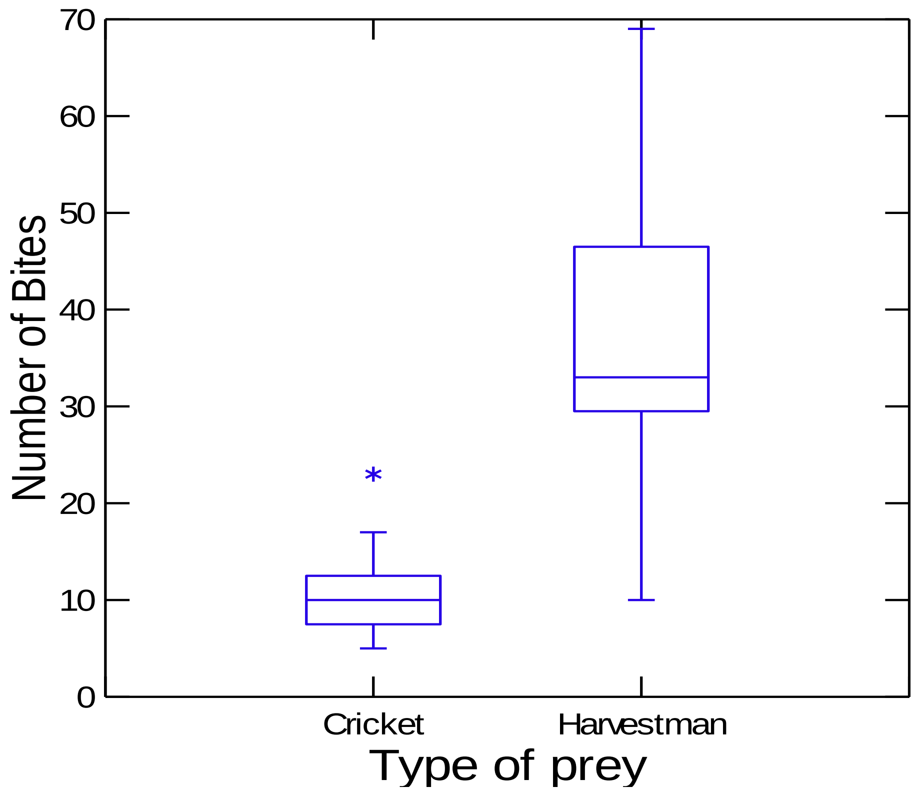

Figure 3. Number of bites performed by *Loxosceles gaucho* when preying upon crickets (*N* = 16; max = 23; min = 1) and harvestmen (*N* = 20; max = 69; min = 1).

Experiment 3: the role of silk on predation

Contrary to our expectations, the presence of silk did not influence the capture success of harvestmen by the spider *L. gaucho*. We described 17 behavioral categories of the spider when interacting with the harvestman (Table 1). A typical capture sequence (Figure 6) starts with prey detection either at a distance or after “Passive contact”, with either the prey or both walking. The spider would then “Approach”) and/or “Touch prey". After biting the prey several times (Table 1) until the harvestman would stop attempting to flee, the spider would start to "Weave silk threads". Then the spider display "Short bites" and a "Long bite" that can last several minutes. Not shown in the fluxogram for the sake of clarity. "Wa-zari" was preceded by "Short bite" and Touch with legs. "Motionless" was preceded by "Weaves silk", "Approach", "Detection at a distance", "Handling silk" and "Drag". "Leg threading" was preceded by "Active contact". Spiders that did not prey upon the harvestmen would usually flee after touching the harvestmen.

Seventeen spiders preyed upon the harvestmen in the silk group (*N* = 19) and 14 in the no silk group (*N* = 19) (χ^2 = 1.576; *df* = 1; *P* = 0.209). The number of bites performed by the spiders ($\bar{X}_{sg}$ = 24.8 ± 11.9; min = 9; max = 52; $\bar{X}_{nsg}$ = 20.5 ± 9.1; min = 1; max = 34; *t* = 1.136; *df* = 30; *P* = 0.265) did not differ between the groups. Latency to bite, the time between the first touch and the first long bite, and the time between the first bite and the first long bite also did not differ between the two groups (Table 2).

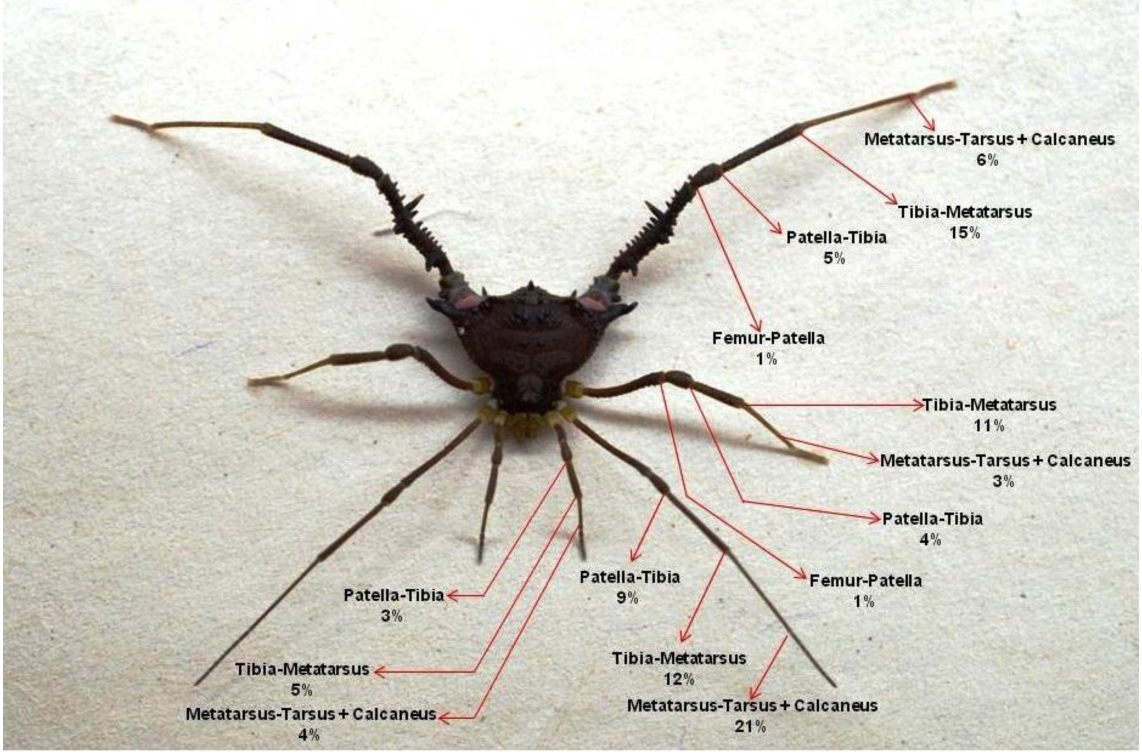

Figure 4. Spots where the spiders bit the harvestmen's body (sum of data from both sides of the body). Percentuals based on 176 bites in 20 events of predation of harvestmen and spiders tested only once.

Table 1

Behavioral repertory of the spider *Loxosceles gaucho* when interacting with the harvestman *Mischonyx cuspidatus.*

Behavioral category	Definition
Active contact	Spider moves mainly its legs I and II and touches the prey with the distal portion
Approach	Spider moves towards the prey, either following a straight way or in a zigzag manner
Detection at a distance	The spider raises any of its legs in a slow dorso-ventral movement, often of small amplitude and without touching the substrate. A variation of this category is to quickly move the legs
Drag	The spider drags the harvestman with their first pair of legs or the chelicerae, holding the prey by its legs
Handling silk	Spider performs repeated movements with its first, second or third pair of legs, extending and flexing them sideways, in a movement with short amplitude. Some variations of this category include rubbing legs I, II e III between them in dorso-ventral movements and quick antero-posterior movements with pedipalps
Leg-threading	Spider passes the leg of the second pair between its chelicerae
Long bite	Spider pinches the prey with the fangs of chelicerae for ten or more seconds (the spider can hold the prey for minutes) Sometimes the spider weaves silk threads and/or apparently handle threads of silks often with its third pair of legs (Figure 5)
Move away	Spider walks away from the prey.

Move pedipalps quickly	Fast dorso-ventral movements of pedipalps
Motionless	Not moving the body or the locomotory appendages
Orient to prey	To rotate the body without displacement, ending with the anterior portion of the body facing the prey
Passive contact	While walking a prey touches a spider
Short bite	Spider pinches the prey with the fangs of the chelicerae for less than 10 seconds. This behavior can happen while the spider is touching the prey (mainly with its legs) and/or while the spider taps the harvestmen and/or weaving threads of silk
Touch with legs	Spider maintains the legs on the body of the harvestman
	Sometimes the spider taps the prey with its legs and/or pedipalps
Tremble	Spider performs movements of small amplitude and flexion of legs without withdrawing the legs of the ground
Wa-zari	Spider pulls the harvestman mainly with its first pair of legs grabbing it by the fourth pair of legs close to the patella or to the femur-trochanter joint and slowly throws the harvestman with the dorsum on the floor
Weave silk	Spider weaves silk threads, sometimes while maintaining the appendages (mainly the legs) on the harvestmen body touching it and/or handling threads of silk, often with the third pair of legs. In a variation of this category the spider moves away from the harvestman and weaves silk threads around the body of the harvestman

Table 2

Time (in seconds) spent to some acts of predation by *Loxosceles gaucho* when preying upon *Myschonix cuspidatus*

	With silk				Without silk				P	U
	Med	Min	max	N	Med	Min	Max	N		
Latency to bite	205	5	2252	17	581.5	1	2173	16	0.428	158
First touch-first long bite	132.5	3	1435	16	279	58	1960	13	0.236	131
First bite-first long bite	84	0	1378	16	125	48	1866	13	0.219	132

Figure 5. Spider exhibiting “Long bite” on the tibia-patella joint of the third right leg of the harvestman.

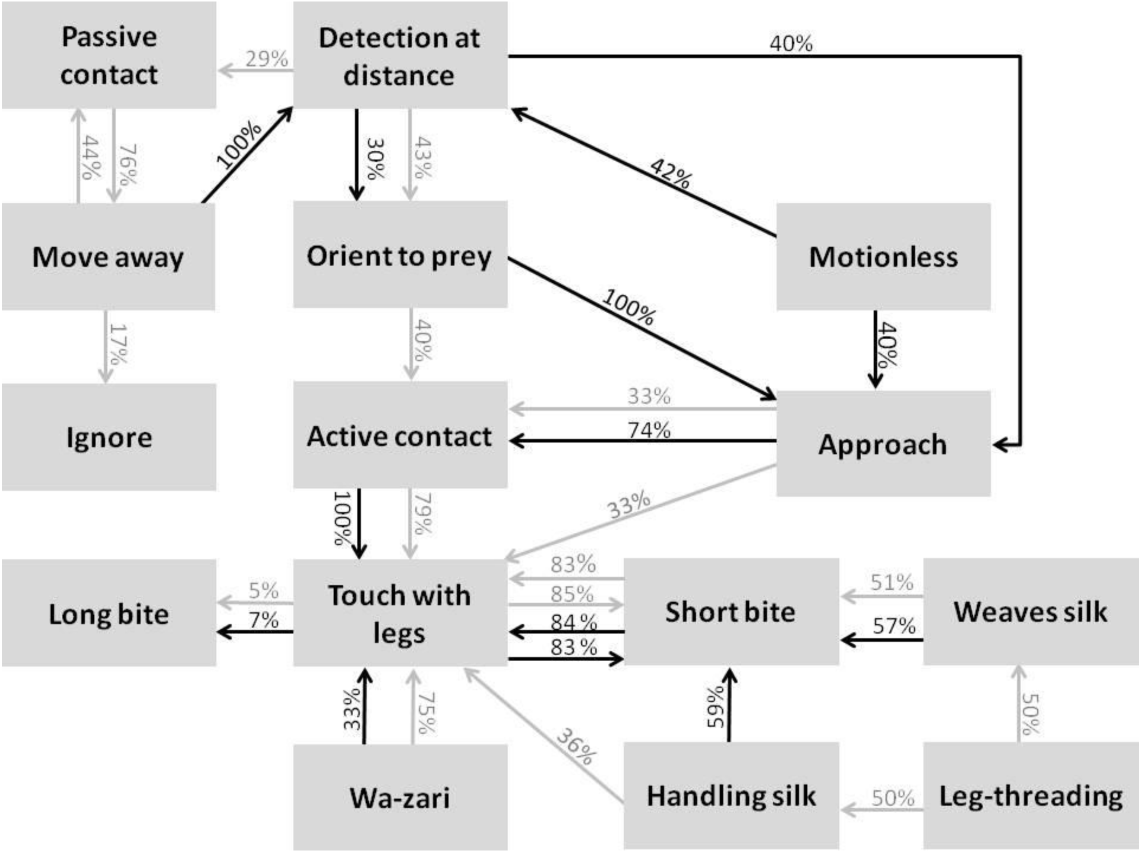

Figure 6. Fluxogram showing behavioral sequence of spiders that preyed upon harvestmen in the silk group (arrows and percentage in black – *N* = 17) and in the no silk group (arrows and percentage in grey – *N* = 14). Arrows represent the percentage of times behaviour A was followed by behaviour B. For the sake of clarity, only behavioral sequences with frequencies higher than 25% are included (except the final categories "Long bite" and "Ignore"). The behaviors "Move pedipalps quickly", "Drag " and "Tremble" were omitted because they occurred just in one group or in both groups.

DISCUSSION

Loxosceles gaucho do not use harvestmen´s or cricket's chemical cues to choose where to forage, do not rely on vibratory cues to detect the tested prey and exhibits a high predation rate of heavy bodied laniatorid harvestmen. These spiders are not dependent on the sheet of silk to capture harvestmen and behave very similarly in the presence or absence of silk, at least against this specific prey.

Our results showed that the rate of predation and the time of handling before subduing the prey were not different comparing the spiders of silk group to no silk group. These results clearly show that the presence of the sheet of silk is not decisive. Moreover, we found a high predation rate, showing that the spider *L. gaucho* do prey upon harvestmen, contrasting with much more robust spiders such as *Enoploctenus cyclothorax* (Bertkau 1880) and *Ctenus ornatus* (Keyserling, 1877) (Willemart & Pellegatti-Franco 2006; Souza & Willemart 2011; Dias & Willemart 2013) or the spitting-spider *Scytodes globula* Nicolet 1849 that cannot prey on the harvestman (Carvalho et al 2012).

In the fluxogram, a strong association between the categories "Touch with legs" and "short bite" was found in the silk and no silk groups. This behavior probably allows the spider to find the weak spots of the harvestman´s body, such as joints and the distal portions of the tarsi (Souza & Willemart 2011). Corroborating the suggested correlation between these two behavioral categories, *C. ornatus* and *E. cyclothorax* do not choose where to bite and do not display “Touch with legs” (Souza & Willemart 2011; Dias & Willemart 2013). *Loxosceles gaucho* seem therefore to have a behavioral strategy that allows it to prey upon well armored prey, differently from some spiders of the genus *Dysdera* (Dysderidae) which, in addition to distinct behavior, also have modified chelicerae that allow them to prey upon armored woodlouse (Isopoda: Oniscoidea) (Řezáč et al 2008). The fluxogram also show that similar behavioral sequences are efficient to subdue the harvestmen in the presence or absence of silk. We suggest this is due to the fact that these harvestmen are not fast runners or jumpers, so that silk is not necessary for the venomous bites to take place at the exact spots.

Because we did not find differences in the latency to attack in a substrate that transmits vibration compared to one that does not, we rejected our hypothesis that substrate-borne vibrations are fundamental information in the capture process of *L. gaucho*. This was quite interesting not only because this is a main sensory modality among spiders (Hergenröder & Barth 1983; Barth 2002; Foelix 2011) but also because recluse spiders are known to use vibrations on the web sheet for intra-specific communication (Rinaldi & Stropa 1998; Fischer et al 2009). It is known that at least in the spider *Cupiennius salei* (Keyserling 1877), a single sensory modality may release capturing alone, air displacement and vision being examples (Fenk et al 2010; Klopsch et al 2013). Since *Loxosceles intermedia* Mello-Leitão 1934 (Fischer et al 2009) and *L. gaucho* (Rinaldi & Stropa 1998) are each found on several kinds of substrates that probably have a different potential of vibration transmission, not relying only on this substrate-borne vibration may be advantageous. So it is possible that more than one sensorial modality could release the predatory behavior in this species.

Our results show that the time between the first bite and first long bite and the number of bites performed by the spiders was greater in harvestmen than in crickets. These results are probably related to the barrier imposed by the thick cuticle of the harvestmen (Souza & Willemart 2011; Dias & Willemart 2013), which can prevent the spiders to access more vulnerable areas such as the main areas of the nervous system. The spider would compensate that by biting more, maximizing the chances of immobilizing the prey.

Due to the greater time of handling and greater number of bites it is probable that *L.gaucho* spends more energy attacking harvestmen than crickets, potentially leading to prey preference. But our experimental set up was testing prey separately and in this case both prey were equally eaten. The same set up with a less intense starvation period could also increase preference for crickets. That could be related to the low proportion of harvestmen found on the webs of *L.intermedia,* in spite of the great number of harvestmen using the same microhabitat of the spider (Fischer et al 2006).

No preference for substrates with prey chemicals were detected in our study. This was similar to results found for salticid spiders (Hoefler et al 2002) but contrasts with the results obtained for lycosid and oxyopid spiders (Persons & Uetz 1996; Punzo & Kukoyi 1997; Persons & Rypstra 2000). It is possible that the use of chemicals as a source of information to find prey is restricted to some spider species. On the other hand, our results showed that the time spent in the area with no chemicals was greater that the time spent in the area with cricket chemicals. In this way, apparently these spiders can detect chemicals on the substrate, but for some reason do not use this information to pick a foraging spot.

Our data have shown a predator that do not use indirect prey cues to choose a foraging site, that do not rely on substrate-borne vibrations to find prey, that builds a so called “trap” (web) that is useful for intra-specific communication but not for capturing every kind of prey, and how a delicate predator can subdue a well-defended and heavy bodied prey by handling it carefully, finding the weak spots and biting lots of times. Our data also clearly suggests the importance of tactile cues to find such weak spots. Taken together, our study shows the importance of avoiding generalizations when studying prey-predator interactions, since several of our results contrasted to what would be expected based on previous studies of spider behaviour.

REFERENCES

Anjum, F., Turni, H., Mulder, P. G., van der Burg, J., & Brecht, M. (2006). Tactile guidance of prey capture in *Etruscan shrews*. *Proceedings of the National Academy of Sciences*, 103, 16544-16549.

Barth, F. G. (2002). *A spider's world: senses and behavior*. Springer.

Betz, O., & Mumm, R. (2001). The predatory legs of *Philonthus marginatus* (Coleoptera, Staphylinidae): functional morphology and tarsal ultrastructure. *Arthropod Structure & Development*, 30, 77-97.

Bleckmann, H., & Barth, F. G. (1984). Sensory ecology of a semi-aquatic spider (*Dolomedes triton*). *Behavioral Ecology and Sociobiology*, 14, 303-312.

Bub, K., & Bowerman, R. F. (1979). Prey capture by the scorpion *Hadrurus arizonensis* Ewing (Scorpiones: Vaejovidae). *Journal of Arachnology*, 7, 243-253.

Buning, T. D. C. (1983). Thermal sensitivity as a specialization for prey capture and feeding in snakes. *American Zoologist*, 23, 363-375.

Cardoso, J. L. C., França, F. D. S., Wen, F. H., Malaque, C. M. S., & Haddad Jr, V. (2003). *Animais peçonhentos no Brasil: biologia, clínica e terapêutica dos acidentes*. Sarvier (Almed).

Carvalho, L.A., Souza, E.S. & Willemart R.H. (2012). Behavioral analysis of the interaction between the spitting spider *Scytodes globula* (Araneae: Scytodidae) and the harvestman *Discocyrtus invalidus* (Opiliones: Gonyleptidae). *Journal of Arachnology*, 40, 332-337.

Casper, G. S. (1985). Prey capture and stinging behavior in the emperor scorpion, *Pandinus imperator* (Koch) (Scorpiones, scorpionidae). *Journal of Arachnology*, 13, 277-283.

Catania, K. C., & Remple, F. E. (2005). Asymptotic prey profitability drives star-nosed moles to the foraging speed limit. *Nature*, 433, 519-522.

Chien, S. A. & Morse, D. H. (1998). The roles of prey quality in the choice of hunting sites by adult male crab spiders *Minusena vatia* (Araneae, Thomisidae). *The Journal of Arachnology*, 26, 238-23.

Corrette, B. J. (1990). Prey capture in the praying mantis Tenodera aridifolia sinensis: coordination of the capture sequence and strike movements. *Journal of Experimental Biology*, 148, 147-180.

Clark, R. J., Jackson, R. R., & Cutler, B. (2000). Chemical cues from ants influence predatory behavior in *Habrocestum pulex*, an ant-eating jumping spider (Araneae, Salticidae). *Journal of Arachnology*, 28, 309-318.

Danchin, E., Giraldeau, L. A., Valone, T.J. & Wagner, R.H. (2004). Public information: from nosy neighbors to Cultural evolution. *Science*, 305, 487-491.

Davies, N. B., Krebs, J. R., & West, S. A. (2012). *An introduction to behavioural ecology.* (4th ed.) Chichester, UK: Wiley-Blackwell.

Dawkins, R., & Krebs, J. R. (1979). Arms races between and within species. *Proceedings of the Royal Society of London Series B. Biological Sciences*, 205, 489-511.

de Andrade, R., & Gnaspini, P. (2002). Feeding in *Maxchernes iporangae* (Pseudoscorpiones, Chernetidae) in captivity. *Journal of Arachnology, 30*, 613-617.

De la Mora, A., Pérez-Lachaud, G., & Lachaud, J. P. (2008). Mandible strike: The lethal weapon of *Odontomachus opaciventris* against small prey. *Behavioural processes*, 78, 64-75

Del-Claro, K., & Tizo-Pedroso, E. (2009). Ecological and evolutionary pathways of social behavior in Pseudoscorpions (Arachnida: Pseudoscorpiones). *Acta Ethologica*, 12, 13-22. de Groot, J. H., & van Leeuwen, J. L. (2004). Evidence for an elastic projection mechanism in the chameleon tongue. *Proceedings of the Royal Society of London-B*, 271, 761.

Dias, B.C. Willemart, R.H. (2013). The effectiveness of post-contact defenses in a prey with no pre-contact detection. *Zoology*, 116, 168-174.

Eisner, T., Rossini, C., González, A., & Eisner, M. (2004). Chemical defense of an opilionid (*Acanthopachylus aculeatus*). *Journal of Experimental Biology*, 207, 1313-1321.

Fenk, L. M., Hoinkes, T., & Schmid, A. (2010). Vision as a third sensory modality to elicit attack behavior in a nocturnal spider. *Journal of Comparative Physiology*, 196, 957-961.

Fischer, M. L., & Vasconcellos-Neto, J. (2005). Microhabitats Occupied by *Loxosceles intermedia* and *Loxosceles laeta* (Araneae: Sicariidae) in Curitiba, Paraná, Brazil. *Journal of Medical Entomology*, 42, 756-765.

Fischer, M. L., Vasconcellos-Neto, J., & dos Santos Neto, L. G. (2006). The prey and predators of *Loxosceles intermedia* Mello-Leitao 1934 (Araneae, Sicariidae). *Journal of Arachnology,* 34, 485-488.

Fischer, M. L., Čokl, A., Ramires, E. N., Marques-da-Silva, E., Delay, C., Fontana, J. D., Donatti, L., Schneider, V.F. & Marques, F. D. A. (2009). Sound is involved in multimodal communication of *Loxosceles intermedia* Mello-Leitão, 1934 (Araneae; Sicariidae). *Behavioural Processes*, 82, 236-243.

Foelix, R. (2011). *Biology of spiders*. Oxford University Press.

Gnaspini, P. & Hara, M. R. 2007. Defense mechanisms. In: *Harvestmen: the Biology of Opiliones* (Ed. by R. Pinto-da-Rocha, G. Machado & G. Giribet), pp. 374e399. Cambridge, Massachusetts: Harvard University Press.

González-Bernal, E., Brown, G. P., Cabrera-Guzmán, E., & Shine, R. (2011). Foraging tactics of an ambush predator: the effects of substrate attributes on prey availability and predator feeding success. *Behavioral Ecology and Sociobiology*, 65, 1367-1375.

Greene, H. W., & Burghardt, G. M. (1978). Behavior and phylogeny: constriction in ancient and modern snakes. *Science*, 200, 74-77.

Hanna, C. J., & Eason, P. K. (2013). Juvenile crab spiders (*Mecaphesa asperata*) use indirect cues to choose foraging sites. *Ethology Ecology & Evolution*, 25, 161-173.

Hara, M. R., & Gnaspini, P. (2003). Comparative study of the defensive behavior and morphology of the gland opening area among harvestmen (Arachnida, Opiliones, Gonyleptidae) under a phylogenetic perspective. *Arthropod Structure & Development*, 32, 257-275.

Hara, M. R., Cavalheiro, A. J., Gnaspini, P., & Santos, D. Y. (2005). A comparative analysis of the chemical nature of defensive secretions of Gonyleptidae (Arachnida: Opiliones: Laniatores). *Biochemical Systematics and Ecology*, 33, 1210-1225.

Herberstein, M. E. (Ed.). (2011): *Spider behaviour: flexibility and versatility.* Cambridge University Press.

Hergenröder, R., & Barth, F. G. (1983). Vibratory signals and spider behavior: How do the sensory inputs from the eight legs interact in orientation?. *Journal of comparative physiology*, 152, 361-371.

Herrel, A., Meyers, J. J., Aerts, P., & Nishikawa, K. C. (2000). The mechanics of prey prehension in chameleons. *Journal of Experimental Biology*, 203, 3255-3263.

Hopcraft, J. G. C., Sinclair, A. R. E., & Packer, C. (2005). Planning for success: Serengeti lions seek prey accessibility rather than abundance. *Journal of Animal Ecology*, 74, 559-566.

Hoefler, C. D., Taylor, M., & Jakob, E. M. (2002). Chemosensory response to prey in *Phidippus audax* (Araneae, Salticidae) and *Pardosa milvina* (Araneae, Lycosidae). *Journal of Arachnology*, 30, 155-158.

Jackson, R. R., Clark, R. J., & Harland, D. P. (2002). Behavioural and cognitive influences of kairomones on an araneophagic jumping spider. *Behaviour*, 139, 749-776.

Johnson, A., Revis, O., & Johnson, J. C. (2011). Chemical prey cues influence the urban microhabitat preferences of Western black widow spiders, *Latrodectus hesperus*. *Journal of Arachnology*, 39, 449-453.

Kardong, K. V. (1986). Predatory strike behavior of the rattlesnake, *Crotalus viridis oreganus*. *Journal of Comparative Psychology*, 100, 304.

Klopsch, C., Kuhlmann, H. C., & Barth, F. G. (2013). Airflow elicits a spider's jump towards airborne prey. II. Flow characteristics guiding behaviour. *Journal of The Royal Society Interface*, 10.

Ladle, R. J., & Velander, K. (2003). Fishing behavior in a giant whip spider. *Journal of Arachnology*, 31, 154-156.

Machado, G., Carrera, P. C., Pomini, A. M., & Marsaioli, A. J. (2005). Chemical defense in harvestmen (Arachnida, Opiliones): Do benzoquinone secretions deter invertebrate and vertebrate predators? *Journal of Chemical Ecology*, 31, 2519-2539.

Machado, G., & Pomini, A. M. (2008). Chemical and behavioral defenses of the neotropical harvestman *Camarana flavipalpi* (Arachnida: Opiliones). *Biochemical Systematics and Ecology*, 36, 369-376.

Masters, W. M. (1984). Vibrations in the orbwebs of *Nuctenea sclopetaria* (Araneidae). *Behavioral Ecology and Sociobiology*, 15, 207-215.

Mestre, L. A. M., & Pinto-da-Rocha, R. (2004). Population dynamics of an isolated population of the harvestman *Ilhaia cuspidata* (Opiliones, Gonyleptidae), in Araucaria Forest (Curitiba, Paraná, Brazil). *Journal of Arachnology*, 32, 208-220.

Mineo, M. F., & Del Claro, K. (2006). Mechanoreceptive function of pectines in the Brazilian yellow scorpion *Tityus serrulatus*: perception of substrate-borne vibrations and prey detection. *Acta Ethologica*, 9, 79-85.

Pereira, W., Elpino-Campos, A., Del-Claro, K., & Machado, G. (2004). Behavioral repertory of the neotropical harvestman *Ilhaia cuspidata* (Opiliones, Gonyleptidae). *Journal of Arachnology*, 32, 22-30.

Persons, M. H., & Uetz, G. W. (1996). The influence of sensory information on patch residence time in wolf spiders (Araneae: Lycosidae). *Animal Behaviour*, 51, 1285-1293.

Persons, M. H., & Rypstra, A. L. (2000). Preference for chemical cues associated with recent prey in the wolf spider *Hogna helluo* (Araneae: Lycosidae). *Ethology*, 106, 27-35.

Piep, M., Radespiel, U., Zimmermann, E., Schmidt, S., & Siemers, B. M. (2008). The sensory basis of prey detection in captive-born grey mouse lemurs, *Microcebus murinus*. *Animal Behaviour*, 75, 871-878.

Placyk Jr, J. S., & Graves, B. M. (2002). Prey detection by vomeronasal chemoreception in a plethodontid salamander. *Journal of Chemical Ecology*, 28, 1017-1036.

Pomini, A. M., Machado, G., Pinto-da-Rocha, R., Macías-Ordóñez, R., & Marsaioli, A. J. (2010). Lines of defense in the harvestman *Hoplobunus mexicanus* (Arachnida: Opiliones): Aposematism, stridulation, thanatosis, and irritant chemicals. *Biochemical Systematics and Ecology*, 38, 300-308.

Punzo, F., & Kukoyi, O. (1997). The effects of prey chemical cues on patch residence time in the wolf spider *Trochosa parthenus* (Chamberlin) (Lycosidae) and the lynx spider *Oxyopes salticus* Hentz (Oxyopidae). *Bulletin of the British Arachnological*, Society, 10, 323-326.

Řezáč, M., Pekár, S., & Lubin, Y. (2008). How oniscophagous spiders overcome woodlouse armour. *Journal of Zoology*, 275, 64-71.

Rinaldi, I. M. P., & Stropa, A. A. (1998). Sexual behaviour in *Loxosceles gaucho* Gertsch (Araneae, Sicariidae). *Bulletin of the British Arachnological Society*, 11, 57-61

Rovner, J. S. (1980). Morphological and ethological adaptations for prey capture in wolf spiders (Araneae, Lycosidae). *Journal of Arachnology*, 8, 201-215.

Santer, R. D., & Hebets, E. A. (2009). Prey capture by the whip spider *Phrynus marginemaculatus* CL Koch. *Journal of Arachnology*, 37, 109-112.

Schnitzler, H. U., & Kalko, E. K. (2001). Echolocation by Insect-Eating Bats. *Bioscience*, 51, 557-569.

Souza, E. D. S., & Willemart, R. H. (2011). Harvest-Ironman: heavy armature, and not its defensive secretions, protects a harvestman against a spider. *Animal Behaviour*, 81, 127-133.

Valone, T. J. (2007). From eavesdropping on performance to copying the behavior of others: a review of public information use. *Behavioral Ecology and Sociobiology*, 62, 1-14.

Willemart, R. H., & Pellegatti-Franco, F. (2006). The spider *Enoploctenus cyclothorax* (Araneae, Ctenidae) avoids preying on the harvestman *Mischonyx cuspidatus* (Opiliones, Gonyleptidae). *Journal of Arachnology*, 34, 649-652.

Willemart, R.H. Santer, R.D., Spence, A.J & Hebets, E.A. (2011). A sticky situation: solifugids (Arachnida, Solifugae) use adhesive organs on their pedipalps for prey capture. *Journal of Ethology*, 29, 177-180.

SECOND CHAPTER

Defences of a Neotropical harvestman against different levels of threat by the recluse spider

ABSTRACT

The threat sensitive hypothesis predicts that animals modulate the defensive behaviour with the level of threat. Therefore, responses to predator cues may differ from responses to the actual predator in close range. Also, in high threat situations, prey would be expected to use their most dangerous defences. The recluse spider *Loxosceles gaucho* (Araneae, Sicariidae) is known to prey upon well defended harvestmen such as the laniatorid *Mischonyx cuspidatus* (Opiliones, Gonyleptidae), which has been reported to use tanathosis, chemical defences, pinching with sharp apophyses on legs, chelicerae and pedipalps. Because of harvestmen´s dependence on chemical stimuli, we tested if *M. cuspidatus* would change its locomotory behaviour in the presence of chemicals of the recluse spider (medium threat situation; spider vs blank vs chemical control; one at a time). Subsequently, we tested harvestmen behaviour in the presence of the spider in close range, a high-threat situation. Finally, we looked at the survival rate of spiders after being pierced by sharp apophyses *M. cuspidatus* that have on the legs. The harvestmen only showed defensive behaviours in the high threat situation. Surprisingly, their mostly known defensive behaviours (chemical defence, tanathosis, pinching with chelicerae and pedipalps) were not seen even in the high threat situation. This is the first evidence that these behaviours are not used against a natural predator that has an almost 80% predation success when attacking harvestmen. Pinching with the sharp legs IV apophyses may perforate but do not kill the spiders. We highlight the importance of the traditional descriptive approach with natural predators to understand the specificities of defensive behaviours against different types of predator.

Keywords: Arachnida, Laniatores, Defensive behaviour, deimatic, chemoreception

INTRODUCTION

According to the threat sensitive hypothesis, modulating the defensive behaviour with the level of threat is evolutionarily advantageous (Helfman, 1989), and previous studies have indeed shown that many prey react differently depending on the threat imposed: these reactions can be physiological responses of stress (Monclús et al., 2009), increased vigilance time (Mathoth et al., 2009), thanatosis (Gyssels & Stocks, 2005) and distinct behaviours according to the distance of the predator (Kleindorfer et al., 2005). Prey can exhibit a threat sensitive behaviour related to the size of the predator, for example by avoiding only larger individuals (Chivers et al., 2001). In mites, latency to lay the first egg and the total number of eggs laid can also be affected by the level of threat by the presence of predator cues as well as to the predator species (Ferrari & Schausberger, 2013). Finally, some monkeys can modulate their behaviour according to the threat imposed by human presence (Papworth et al., 2013).

When animals face a moderate threat like chemical cues of predators, they can avoid the site, increase locomotion, decrease movements and flee (Kats & Dill, 1998 for a review). After the predator gets closer or grasp the prey, defensive behaviours such as retaliation come into play (Edmunds, 1974). Among the weapons used to retaliate predators, some seem to have evolved from mechanisms of prey capture, others from functions related to intra-specific interactions and others are supposed to have evolved only for defensive reasons (Edmunds, 1974).

Harvestmen, arachnids belonging to the order Opiliones, have several anti-predator behaviours. When facing an intermediate threat situation such as the presence of predator chemical cues, *Eumesossoma roweri* (Eupnoi: Sclerosomatidae) alters its locomotory pattern (Chelini et al., 2009). A presumably high threat situation such as a human attempt to hold it causes harvestmen to release defensive secretions produced by glands that open dorso-ventrally, to autotomise locomotory appendages, to shake the body, to flee, and to pinch the potential predator with apophyses on the legs, the so called 'nipping behaviour' (the harvestman flexes legs IV so that the apophyses of the femur of the fourth pair of legs pinch against the body of the attacker) (Gnaspini & Hara, 2007).

Despite the great variety of defences that harvestmen possess, many animals prey upon individuals of this group. The list includes birds, frogs, opossums, hemipterans and spiders (Gnaspini & Hara, 2007). Among neotropical spiders, *Ctenus fasciatus* (Araneae, Ctenidae) feeds on harvestmen of the subfamily Goniosomatinae (Opiliones, Gonyleptidae) (Gnaspini, 1996; Machado et al., 2000) but there are no detailed studies on this prey-predator system. The only pairs of spiders-harvestmen that have been studied in details were spiders that do not usually eat harvestmen. These were carried out exactly with the purpose of understanding the proximal causes of rejection (Eisner et al., 2004; Souza & Willemart, 2011; Carvalho et al., 2012; Dias & Willemart, 2013; see also Machado et al., 2005). It has been shown that the hard integument is a highly important defence in these harvestmen belonging to the suborder Laniatores, and overcoming or avoiding it somehow is a prerequisite to feed on these animals. We have shown that the delicate recluse spider *Loxosceles gaucho* (Gertsch, 1967) (Sicariidae) bites such prey at the few vulnerable spots of the integument and is a very efficient predator of such harvestmen (JMGS, KDC and RHW, unpublished data). It seemed therefore to be an excellent system to understand how prey react to different levels of threat by predators. In addition, we expected that most known defensive behaviours in laniatorid harvestmen would be revealed in interactions with such efficient predators. We therefore conducted a detailed behavioural analysis of the interaction between the recluse spider and the syntopic harvestman *Mischonyx cuspidatus* (Roewer, 1913) (Gonyleptidae). We first tested whether these harvestmen changed their locomotory behavioural pattern when exposed to chemical cues of dangerous predators (a situation of intermediate threat). Then we looked at the defensive behaviours of the harvestmen when the spider is approaching, after spider's touches and after spider's bites (all situations considered to be of high threat based on unpublished data by JMGS, KDC and RHW (unpublished data). Since preliminary data showed that the sharp spines of legs IV in *M. cuspidatus* may perforate the abdomen of a spider and because prey are expected to use their most dangerous weapons in situations of high threat, we also tested the hypothesis that such sharp spines are lethal when the abdomen of the spider is perforated.

MATERIAL AND METHODS

Species studied

Loxosceles gaucho is a predator that includes harvestmen in its diet because it is able to find and bite the weak points of the thick integument of laniatorid harvestmen (JMGS, KDC and RHW, unpublished data). It typically builds a web sheet in which it is usually found, but it can also leave the web and capture prey out of it (Fischer et al., 2006). In laboratory experiments of predation under different substrate conditions and in the presence or absence of their web sheets, the success rate of prey capture was 77.3% with pooled data, combining substrates and presence/absence of web (N = 66) (JMGS, KDC and RHW, unpublished data).

Mischonyx cuspidatus has defensive behaviours that include the release of defensive secretions by the ozopores (Hara et al., 2005), pinching with sharp apophyses on legs IV (Figure 1), thanatosis by flexing the legs and keeping them close to the body (Pereira et al., 2004) and vibrating legs II, a putative defensive behaviour recently described (ILT or intense leg tapping, see; Dias et al., 2014). In the field, dead carcasses have been found on the webs of *Loxosceles gaucho* and theridiid spiders (Mestre & Pinto da Rocha 2004).

Collection and maintenance

Loxosceles gaucho was collected in a terrain located in the city of Mairiporã-SP, within a pile of bricks (23◦19 S, 46◦35 W). They were collected between 24 November of 2012 and 26 February of 2013. *Mischonyx cuspidatus* was also found in that same area, but for this study they were collected at Parque Ecológico do Tietê- São Paulo (23◦25 S, 46◦28 W) between 20 December of 2012 and 8 May of 2013. Both species co-occur in several locations (e.g. Dias and Willemart, 2013).

In the laboratory, they were both individually maintained in plastic recipients (12x8x4 cm height) with soil on the bottom. Water was provided with a wet cotton ball. We fed the spiders with larvae of the tenebrionid beetle *Zophobas* sp. and nymphs of crickets *Gryllus* sp. according to the schedule of the experiments, and harvestmen once a week with moistened dog food.

During the experiments, the animals were maintained in a room with a weak red light (15 watts) permanently on 24 hours per day. The natural daylight cycle was maintained and temperature was ambient. All experiments were carried out from 8:30 pm to 5 am.

Figure 1. A harvestman *Mischonyx cuspidatus* (Arachnida, Opiliones) male, dorsal view. Black arrows show the sharp apophyses of the femur of the fourth pair of the legs.

Behaviour of the harvestman in an intermediate threat situation

This experiment was conducted to test the hypothesis that harvestmen react to chemicals left on the substrate by dangerous predators. We predicted that the harvestmen would change their locomotory behaviour in the presence of the spider chemicals (see Chelini et al., 2009). To test it, the harvestmen (N = 20) were exposed to filter paper in the following conditions, with one single stimulus per trial: (1) chemicals of spider; (2) a chemical control (mate tea) and (3) a blank control. Each harvestman was tested three times in a design of repeated measures with interval of 24 hours between the tests for each individual. Individuals in all treatments were tested in distinct hours of the night.

To impregnate the filter paper with the spider cues we maintained one adult female of *L. gaucho* (5-10 days after it received its last meal) for 24 hours in a recipient (8 cm diameter x 10 cm height) with filter paper on the bottom. We removed the spider immediately before the test. We then removed silk deposited on the bottom with forceps, to prevent that harvestmen would get stuck within it. In the chemical control group we covered the filter paper with a thin layer of mate tea and maintained it there for 24h, removing it immediately before the trials. In the blank control, the filter paper was not impregnated. In each group the recipient was closed while impregnating (the 24h before the test).

Tests were run in the same recipient where we impregnated the filter paper with one of the chemicals (or control with no cues). We placed the filter paper on the bottom and a neutral central disc of filter paper (4 cm diameter) in the center. We first acclimated the harvestman in a vial (4 cm diameter x 7.5 cm height) placed in the center of arena (in the neutral disk). After a 2 min, the harvestman was released and we recorded it for 20 minutes with a Sony Handycam HDXR550V in nightshot mode. The harvestmen were submitted to each of the three treatments in a systematic alternated order.

We compared three behavioural categories between the treatments: the time harvestmen spent: 1- stationary (Motionless), 2- stand waving (stationary but leg waving, and 3- walking (displacing around the arena) (cf Chelini et al., 2009). We used the Friedman test (because the data did not follow a normal distribution), followed by post hoc test Student Neuman Keuls.

Behaviour of the harvestman in a high threat situation

To describe the behaviour of the harvestmen in a high threat situation, we reanalyzed videos used in an experiment for another project, which were conducted to test, from the spider point of view, the role of silk on predation (JMGS, KDC and RHW, unpublished data). Spiders in both the "no silk" group (N = 19, 17 females and 2 males) and the "silk group" (N = 19, 13 females and 6 males) were offered 10 females and 9 male harvestmen (total = 28 harvestmen used). Twenty five to 29 days before the trials (following Souza & Willemart, 2011), we offered one cricket nymph (*Gryllus* sp.) (≅ 1.5 cm) and one beetle larvae (*Zophobas* sp.) simultaneously to the spiders, to standardize hunger. Only spiders that ate at least one prey were tested. Prey uneaten after 48h were removed. The terraria of the experiments (18 cm diameter x 6 cm height) were the same used to maintain the spiders. During the tests we covered the terraria with a sheet of glass to prevent that spiders would flee. Spiders in the treatment group were simply removed and put back in their terrarium. For spiders in the control group, we took them out of the terrarium, we removed all the soil, cleaned the whole terrarium with a dry paper towel to remove remnants of silk and then put new soil in it. This was done because removing silk was not possible without removing the soil. We then reintroduced the spider. In both groups, we allowed the spider to walk freely in the terrarium for 15 minutes before we introduced a harvestman as far as possible of the spider.

We registered the experiments using a Sony Handycam HDXR550V in the nightshot mode. We recorded for 40 minutes after the predatory process (defined as first bite) and the recording time was usually one hour. Each spider was tested only once. We collected the data between 21 February and 07 March of 2013, between 9 pm and 5 am. We used both the videos with the presence and absence of silk because the predation success between the two treatments was the same (JMGS, KDC and RHW, unpublished data). We only analyzed videos in which the spider bit the harvestman. The behavioural analysis started about 5 seconds before the spider touched the harvestman and finished 2 minutes after the first bite by the spider. We looked at the behavioural categories displayed by the harvestmen. Preliminary analyses of ten videos (5 silk group and 5 no-silk group) showed that increasing this time did not result in more behavioural categories observed.

Potential lethality of a harvestman defence under high threat

Nipping behaviour has been observed in interactions between *M. cuspidatus* and the spiders *Ctenus ornatus* (Keyserling, 1891) (Ctenidae) and *L. gaucho* (Dias & Willemart, 2013; this paper). This behaviour is potentially dangerous for a predator since *M. cuspidatus* has a sharp apophysis on the retrolateral portion of the femur of both legs IV. When they rapidly flex their legs at the coxa-trochanter articulation (= 'nipping'), the two apophyses pinch what is in between the legs. By experimentally holding the spider by the prosoma and allowing a male *M. cuspidatus* to naturally pinch the abdomen of the spider with these apophyses, we found that the harvestman is capable of perforating the abdomen of *L. gaucho,* which loses hemolymph (Figure 2). Because losing hemolymph seem to potentially cause death in arachnids (e.g. Willemart, 2002), we tested the hypothesis that a perforation caused by these apophyses negatively affects the survivorship of the spiders *L. gaucho*. We predicted that perforated spiders would have a lower survival rate than the spiders that were not perforated.

We used two groups in this experiment. In the treatment group (N = 10) we anesthetize the spiders with CO_2 by having them in a closed vial with a small aperture on the top in which the gas was injected for 1 minute and a half. We then held the spider within the femora of a male adult harvestman so that we could manually flex its legs, pinch and perforate the spider´s abdomen. We used a different harvestman for each spider and the same person held the harvestmen in every test. In the control group (N = 10), we used the same procedures, but we pinched the abdomen with a cushioned forceps, again always by the same person. The experiment was carried out in May 2013. The harvestmen were then maintained in plastic recipients (12 x 8 x 4 cm height) with soil on the bottom. Water was provided with a wet cotton ball. In the 5 subsequent days we verified the survivorship of the spiders between 10 and 11 pm and after 40 days we monitored again. We compared the survival rate at the end of the experiment using a X^2.

RESULTS

Behaviour of the harvestman in an intermediate threat situation

There were no differences when comparing the controls (blank and mate tea) and the treatment group (spider chemicals) in the time that the harvestman spent: stationary ($p = 0.071$), stand waving ($p = 0.170$) or walking ($p = 0.744$). The animals spent more time stand waving in all groups ($p < 0.05$): mate tea, blank and spider chemicals (Table 1). No defensive behaviour was observed.

Behaviour of the harvestman in a high threat situation

We found behaviours that can be considered as potentially defensive in *M. cuspidatus* in different phases, as detailed below (see Table 2 - for descriptions). The results are divided in 'no-silk group' (N = 15) and 'silk group' (N = 17).

Phase 1- pre-contact: In the no-silk group we did not find behaviours considered as defensive. In the silk group we have observed the behaviour 'Pull the leg' (N = 6).

Phase 2- post contact, before biting: In the no-silk group, we have observed 'Move away from the spider' (N = 3); 'Motionless' (N = 2); 'ILT' (N = 1) and 'Move toward the spider' (N = 1). In the silk group we have observed 'Motionless' (N = 4); 'ILT' (N = 1); 'Move away from the spider' (N = 1) and 'Move pedipalps' (N = 1).

Phase 3- post contact after biting: In the no-silk group we have observed 'Move toward the spider' (N = 13); 'Move away from the spider' (N = 8); ' Motionless' (N = 2); 'Move pedipalps' (N = 1); 'ILT' (N = 1) and ‘Nipping' (N = 1). In the silk group we have observed 'Pull the leg' (N = 9); 'Motionless' (N = 2); 'Move away from the spider' (N = 2) and 'Nipping' (N = 1).We did not observe the release of the defensive secretions, thanatosis, and pinching with pedipalps and chelicerae in any phase analyzed.

Table 1. Time spent by the harvestman *Mischonyx cuspidatus* on three behavioral categories when on filter paper without stimuli; with mate tea (chemical control) or with predator chemicals. The harvestmen were recorded for 1200 seconds. Each treatment was tested separately. MIN = minimum; MAX = maximum.

	time spent: median; MIN and MAX (seconds)		
Behavioral category/treatment	Blank	Chemical control	Spider chemicals
Stationary	49; 0; 1087	0; 0; 424	1; 0; 1200
Stand waving	903; 0; 1200	1018; 361; 1200	1024; 0; 1200
Walking	23; 0; 452	78; 0; 714	51; 0; 399

Table 2. Behavioural repertory of the harvestman *Mischonyx cuspidatus* when interacting with the recluse spider *Loxosceles gaucho*, based on 32 predatory events.

Behavioural Category	Description
Intense leg tapping (ILT)	Intense dorso-ventral movements of legs II
Move away from the spider	The harvestman walks or try to walk away from the spider
Move toward the spider	The harvestman walks or try to walk toward the spider, often when being held by the spider
Move pedipalps	Harvestman displays dorso-ventral movements with the pedipalps
Nipping	Pinching with legs IV that bear sharp apophyses
Motionless	Not moving the body or the locomotory appendages
Pull the leg	Harvestman quickly moves its legs dorso-ventrally, probably trying to get rid of silk threads
Self grooming	Harvestman passes legs and or pedipalps between the chelicerae

Potential lethality of a harvestman defence under high threat

One individual died in the control group in the first day and one died in the treatment group in the second day. All the others stayed alive until the end of the 5 days in both treatments. There were no differences in the survival rate after 40 days ($\chi^2 = 0.00$ df = 1 p = 1.00).

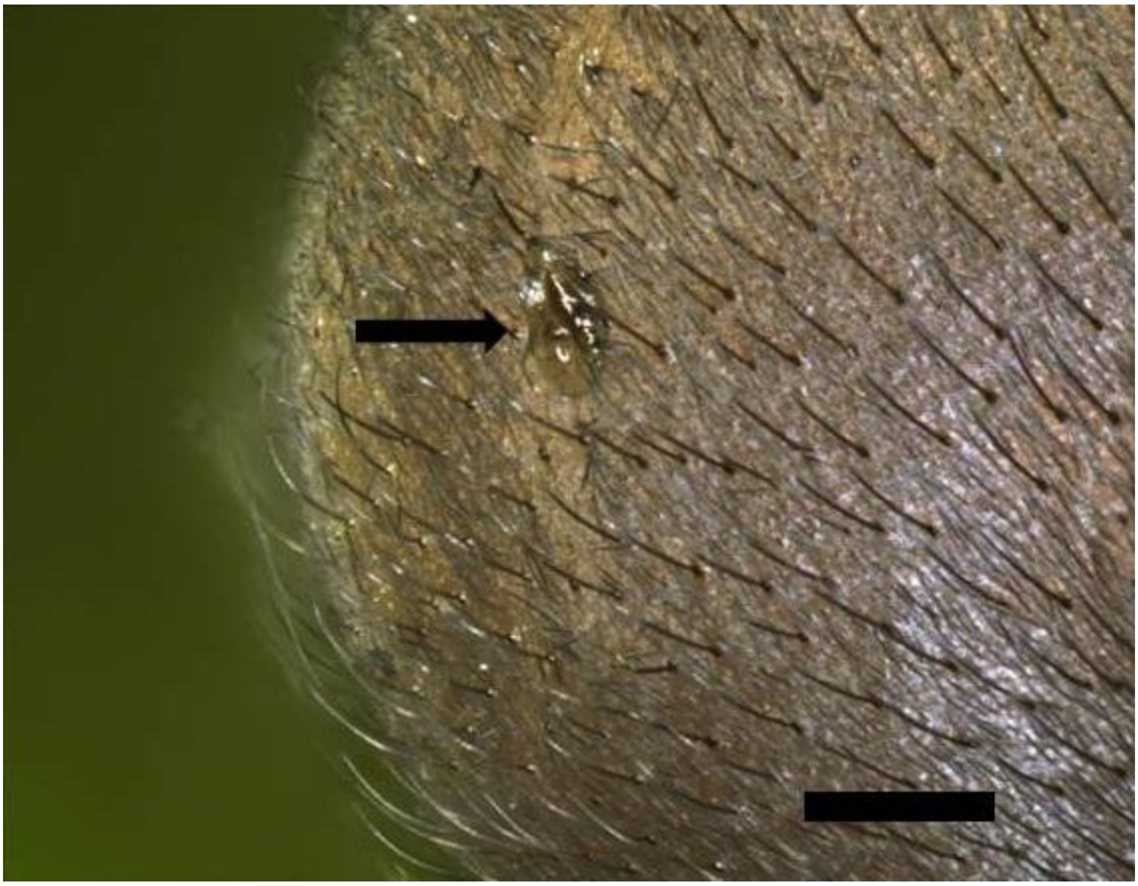

Figure 2. Opisthosoma of the recluse spider *Loxosceles gaucho* female injured by the retrolateral apophysis of the femur of the harvestman *Mischonyx cuspidatus* male after a nipping. A droplet of hemolymph is shown by the arrow. Scale bar = 500µm.

DISCUSSION

We observed defensive behaviours in *M. cuspidatus* in the high threat situation but not in an intermediate threat situation, in which we also did not detect differences in the time the harvestmen spent stand waving, walking or stationary. Perforation of the spider´s body by the harvestman did not affect its survival.

Because no defensive behaviour at all could be noticed in the presence of predator chemicals, this is partially in accordance with previous studies that show prey changing its behaviour according to the level of threat (Sih et al 1986; Bothan et al 2008; Walzer & Schausberger 2011). An example in arachnids is the spider *Latrodectus hesperus* (Theridiidae) that can modulate it's defensive bites, doing it more frequently in a high threat situation than in medium and low threat (Nelsen et al., 2014). But the lack of reaction of *M.*

cuspidatus to chemical cues of *L. gaucho* contrasts with the results obtained for spiders (e.g. Persons et al., 2001, 2002; Wilder and Rypstra, 2004) and for the harvestman *Eumesosoma roeweri (Chelini et al., 2009)* that exhibited defensive behaviours in the presence of chemicals of the spider *Schizocosa ocreata (Lycosidae) (Chelini et al., 2009).* A possible explanation for this lack of defensive behaviour in an intermediate threat situation is that the harvestmen did not detect the spider chemicals, but that seems unlikely since laniatorids have chemoreceptors in the first and second pair of legs and are capable of detecting chemicals at close range or contact (Willemart & Chelini, 2007; Willemart et al., 2009; Costa & Willemart, 2013). An alternative hypothesis is that harvestmen do not avoid the chemicals because they cannot determine the spider species only by their chemicals. Indeed, most syntopic spiders are not efficient predators of *M. cuspidatus*, and unnecessary predator avoidance may result in less weight gain (Chelini et al., 2009). Moreover, the hard integument plays a great role in defence in species of the suborder Laniatores (Souza & Willemart, 2011; Carvalho et al., 2012; Dias & Willemart, 2014; JMGS, KDC and RHW, unpublished data*).* The soft integument in Eupnoi could partially explain why *E. roeweri* show defensive behaviours in the presence of predator chemicals only *(*Chelini et al., 2009) but *M. cuspidatus* (with a hard protective integument) do not. In any case, it should be considered that *p*rey perform defensive behaviours more often towards specialists than towards generalist predators (Crawford et al., 2012), such as *Loxosceles* spiders (Fischer et al., 2006).

In the presence of the spider, in the pre contact phase, we did not detect any defensive behaviour in the 'no-silk group'. Only in the silk group we observed the behaviour 'Pull the leg', suggesting it is related to attempts of getting rid of the silk. Moving away from the spider is probably an attempt to escape, one of the most common defensive behaviours among animals (Edmunds, 1974). We also observed ILT (intense leg tapping), a behaviour first described in male-male fights (Willemart et al., 2009) and then in a defensive context (Dias et al., 2014). In our study, in one case *L. gaucho* stopped the attack temporarily after this behaviour and in the other case the spider was touching the leg of *M. cuspidatus* and did not change its behaviour after ILT.

In the phase 3 (post contact after biting), the most common behaviours we have observed were 'Move toward the spider' and 'Move away from the spider'. We have also observed 'Motionless', which is expected to be important against spiders since these typically rely also on vibratory cues to detect prey (Barth, 2002). But the recluse spiders attacked in all cases after the harvestmen displayed this behaviour, which is in accordance with our data that vibratory cues may not be necessary for *L. gaucho* at least for capturing harvestmen without silk (JMGS, KDC and RHW, unpublished data). Further studies should be conducted to test which sensory modality is mainly used by these spiders to detect their prey. Visual stimuli are known to be used for prey capture by spiders not known for their visual acuity such as ctenids (Pirhofer et al., 2007; Fenk et al., 2010). Air displacements detected by trichobothria are another possibility (Barth, 2002).

Nipping behaviour has long being cited as an anti-predator behaviour (see references in Gnaspini & Hara, 2007) but only recently has it been observed against a natural predator (Dias & Willemart, 2013). Now we have shown that it has the potential to pierce the body of a predator so it may not only be deimatic, since losing hemolymph can be costly. At least in males of some crickets, the loss of hemolymph can decrease reproductive success (Sakaluk et al., 2004). Nonetheless we do not know if the amount of hemolymph lost by the spiders in our study is enough to affect reproduction. Although our results provided no evidence of survival costs, in nature such injuries could affect their behaviour or reduce the spider's survivorship since animals are more exposed to pathogens, and therefore open wounds can act as entry for bacteria (Loof et al., 2010).

Some of the mostly known defences of laniatorids are the release of repugnatory chemicals and mechanical retaliation such as pinching with chelicerae and pedipalps (Gnaspini & Hara, 2007). *Mischonyx cuspidatus* in particular is also known for displaying thanatosis for several minutes, bringing the legs close to the body. This is the first evidence that none of these behaviours are observed against such a successful predator (almost 4 in each 5 attacking *L. gaucho* successfully feed on *M. cuspidatus*), and typically not against other spiders too (Souza & Willemart, 2011; Carvalho et al., 2012; Dias & Willemart, 2013). Since most descriptions of chemical defences in harvestmen were made based on human manipulation (see references in Gnaspini & Hara, 2007), chemicals may be mainly used against vertebrate predators at least in some species that are more reluctant to release secretions, such as *M. cuspidatus*. Some anurans with toxic glands flex their legs when being attacked and swallowed by predators, which often regurgitate them. Flexing the legs may be a way to minimize the chances of injuries to the legs (see Toledo et al., 2010). The same could be happening in *M. cuspidatus*, but it has never been tested against any natural vertebrate predator. At least the repugnatory chemicals of one laniatorid have been tested and proved to be effective against toads, but not opossums (Machado et al., 2005). A domestic dog has also been observed to be deterred by the secretions of *Acanthogonyleptes fulvigranulatus* (Gonyleptidae) (GF Pagoti, pers comm). After the dog sniffed on the harvestman, it released secretions on the dog´s nose. The dog subsequently rubbed its nose against the substrate and did not chase the harvestman. Since the dog only sniffed and did not bite, the possibility that CO2 from the predator influences the release of defensive secretions remains to be tested.

We have shown that some of the most known defences of laniatorid harvestmen such as releasing repugnatorial chemicals, pinching with chelicerae and pedipalps and thanatosis are surprisingly not used against a very dangerous spider. Studies with arthropod defence and specifically chemical defences follow a tradition of manipulating with tweezers or by hand (e.g. Eisner et al., 2005). Great advances were made, but several defensive behaviours seen against tweezers or human hands are not used against some natural predators. They are probably specific to some predators. A clear methodological message of this and previous studies (Souza & Willemart, 2011; Carvalho et al., 2012; Dias & Willemart, 2013; Cook et al., 2013) is that ethologists have to be careful when designing experiments of prey-predator interactions. For example, knowing that a species releases defensive secretions does not means it will release against any predator. Testing the effect of secretions on predators against which secretions are not used is unnatural, and this also applies to the huge number of arthropods and other animals that use chemical defences. The same apply to other defences. Finally, the traditional descriptive approach is crucial to unravel such specificities. As noted by Tinbergen (1963), “We must hope that the descriptive phase is not going to come to a premature ending”.

REFERENCES

Barth, F. G. (2002). A spider's world: senses and behavior. Springer.

Botham, M. S., Hayward, R. K., Morrell, L. J., Croft, D. P., Ward, J. R., Ramnarine, I. & Krause, J. (2008). Risk-sensitive antipredator behavior in the Trinidadian guppy, *Poecilia reticulata*. – Ecology. 89: 3174-3185.

Carvalho, L.A., Souza, E.S. & Willemart R.H. (2012). Analysis of the interaction between the spitting spider *Scytodes globula* (Araneae: Scytodidae) and the harvestman *Discocyrtus invalidus* (Opiliones: Gonyleptidae). – J. Arachnol. 40: 332-337.

Chelini, M. C., Willemart, R. H. & Hebets, E. A. (2009). Costs and benefits of freezing behaviour in the harvestman *Eumesosoma roeweri* (Arachnida, Opiliones). – Behav. Proc. 82: 153-159.

Chivers, D. P., Mirza, R. S., Bryer, P. J. & Kiesecker, J. M. (2001). Threat-sensitive predator avoidance by slimy sculpins: understanding the importance of visual versus chemical information. – Can. J. Zool. 79: 867-873.

Cook, D.R., Smith AT, Proud D.N., Víquez, C. &Townsend, V.R. Jr (2013). Defensive responses of Neotropical harvestmen (Arachnida, Opiliones) to generalist invertebrate predators. – Caribb. J. Sciences 47: 334-343.

Costa, T.M. & Willemart, R.H. (2013). First experimental evidence that a harvestman (Arachnida, Opiliones) detects odors of non-rotten dead prey by olfaction. – Zoologia 30: 359-361.

Crawford, B. A., Hickman, C. R. & Luhring, T. M. (2012). Testing the Threat Sensitive Hypothesis with Predator Familiarity and Dietary Specificity. – Ethology 118: 41-48.

Dias, B.C. & Willemart, R.H. (2013). The effectiveness of post-contact defenses in a prey with no pre-contact detection. – Zoology 116: 168-174.

Dias, B.C., Souza, E.S., Hara M.R. & Willemart, R.H. (2014). Intense leg tapping by the harvestman *Mischonyx cuspidatus* (Gonyleptidae): An undescribed defensive behavior in Opiliones? – J. Arachnol. 42:123–125.

Edmunds, M. (1974). Defence in animals: a survey of anti-predator defences. Harlow: Longman.Eisner, T., M. Eisner & M. Siegler. 2005. Secret Weapons: Defenses ofInsects, Spiders, Scorpions, and Other Many-legged Creatures. Harvard University Press, Cambridge, Massachusetts.

Eisner, T., Rossini, C., González, A. & Eisner, M. (2004). Chemical defense of an opilionid (*Acanthopachylus aculeatus*). – J. Exp. Biol. 207: 1313-1321.

Fenk, L. M., Hoinkes, T. & Schmid, A. (2010). Vision as a third sensory modality to elicit attack in a nocturnal spider. – J. Comp. Physiol. 196: 957-961.

Ferrari, M. C. F. & Schausberger, P. (2013). From repulsion to attraction: species-and spatial context-dependent threat sensitive response of the spider mite *Tetranychus urticae* to predatory mite cues. – Naturwiss. 100: 541–549.

Fischer, M. L., Vasconcellos-Neto, J. & dos Santos Neto, L. G. (2006). The prey and predators of *Loxosceles intermedia* Mello-Leitao 1934 (Araneae, Sicariidae). – J. Arachnol. 34: 485-488.

Gnaspini, P. (1996). Population ecology of *Goniosoma spelaeum* a cavernicolous harvestman from south-eastern Brazil (Arachnida: Opiliones: Gonyleptidae). – J. Zool. 239: 417-435.

Gnaspini, P. & Hara, M.R. (2007). Defense mechanisms. In: Pinto-da-Rocha, R., Machado G., Giribet, G. (Eds), Harvestmen: the Biology of Opiliones. Harvard University Press, Cambridge, MA, pp. 374-399.

Gyssels, F. G. & Stoks, R. (2005). Threat-sensitive responses to predator attacks in a damselfly. – Ethology 111: 411-423.

Hara, M. R., Cavalheiro, A. J., Gnaspini, P. & Santos, D. Y. (2005). A comparative analysis of the chemical nature of defensive secretions of Gonyleptidae (Arachnida: Opiliones: Laniatores). – Bio. Syst. Ecol. 33: 1210-1225.

Helfman, G. S. (1989). Threat-sensitive predator avoidance in damselfish-trumpetfish interactions. – Behav. Ecol. Sociobiol. 24: 47-58.

Kats, L. B. & Dill, L. M. (1998). The scent of death: chemosensory assessment of predation risk by prey animals. – Ecoscience 5: 361-394.

Kleindorfer, S., Fessl, B. & Hoi, H. (2005). Avian nest defence behaviour: assessment in relation to predator distance and type, and nest height. – Anim. Behav. 69: 307-313.

Loof, T. G., Schmidt, O., Herwald, H. & Theopold, U. (2010). Coagulation systems of invertebrates and vertebrates and their roles in innate immunity: the same side of two coins? – J. Innat. Imm. 3: 34-40.

Machado, G., Raimundo R.L. G. & Oliveira P.S. (2000). Daily activity schedule, gregariousness, and defensive behaviour in the Neotropical harvestman *Goniosoma longipes* (Arachnida: Opiliones: Gonyleptidae). – J. Nat. Hist. 34: 587-596.

Machado, G., Carrera, P. C., Pomini, A. M. & Marsaioli, A. J. (2005). Chemical defense in harvestmen (Arachnida, Opiliones): Do benzoquinone secretions deter invertebrate and vertebrate predators? – J. Chem. Ecol. 31: 2519-2539.

Mathot, K. J., van den Hout, P. J. & Piersma, T. (2009). Differential responses of red knots *Calidris canutus* to perching and flying sparrowhawk *Accipiter nisus* models. – Anim. Behav. 77: 1179-1185.

Mestre, L. A. M. & Pinto-da-Rocha, R. (2004). Population dynamics of an isolated population of the harvestman *Ilhaia cuspidata* (Opiliones, Gonyleptidae), in Araucaria Forest (Curitiba, Paraná, Brazil). – J. Arachnol. 32: 208-220.

Monclús, R., Palomares, F., Tablado, Z., Martínez-Fontúrbel, A. & Palme, R. (2009). Testing the threat-sensitive predator avoidance hypothesis: physiological responses and predator pressure in wild rabbits. – Oecologia158: 615-623.

Nelsen, D. R., Kelln, W. & Hayes, W. K. (2014). Poke but don't pinch: risk assessment and venom metering in the western black widow spider, *Latrodectus hesperus*. – Anim. Behav. 89: 107-114.

Papworth, S., Milner-Gulland, E. J. & Slocombe, K. (2013). Hunted woolly monkeys (*Lagothrix poeppigii*) show threat-sensitive responses to human presence. – PloS One 8: e62000.

Pereira, W., Elpino-Campos, A., Del-Claro, K. & Machado, G. (2004). Behavioral repertory of the neotropical harvestman *Ilhaia cuspidata* (Opiliones, Gonyleptidae). – J. Arachnol. 32: 22-30.

Persons, M.H., Walker, S.E., Rypstra, A.L.& Marshall, S.D. (2001). Wolf spider predator avoidance tactics and survival in the presence of diet-associated predator cues (Araneae, Lycosidae). – Anim. Behav. 61: 43–51.

Persons, M.,Walker, S.E. & Rypstra, A.L. (2002). Fitness costs and benefits of antipredator behavior mediated by chemotactile cues in the wolf spider *Pardosa milvina* (Araneae: Lycosidae). – Behav. Ecol. 13: 386–392.

Pirhofer-Walzl, K., Warrant, E. & Barth, F. G. (2007). Adaptations for vision in dim light: impulse responses and bumps in nocturnal spider photoreceptor cells (*Cupiennius salei* Keys). – J. Comp. Physiol. 193: 1081-1087.

Sakaluk, S. K., Campbell, M. T., Clark, A. P., Johnson, J. C. & Keorpes, P. A. (2004). Hemolymph loss during nuptial feeding constrains male mating success in sagebrush crickets. – Behav. Ecol. 15: 845-849.

Sih, A. (1986). Antipredator responses and the perception of danger by mosquito larvae. – Ecology 67: 434-441

Souza, E. D. S. & Willemart, R. H. (2011). Harvest-Ironman: heavy armature, and not its defensive secretions, protects a harvestman against a spider. – Anim. Behav. 81: 127-133.

Toledo, L.F., Sazima, I. & Haddad, C.F.B. (2010). Is it all death feigning? Case in anurans. – J. Nat. Hist. 44: 1979-1988.

Walzer, A. & Schausberger, P. (2011). Threat-sensitive anti-intraguild predation behaviour: maternal strategies to reduce offspring predation risk in mites. – Anim. Behav. 81: 177-184.

Wilder, S.M. & Rypstra, A.L. (2004). Chemical cues from an introduced predator (Mantodea, Mantidae) reduce the movement and foraging of a native wolf spider (Araneae, Lycosidae) in the laboratory. – Environ. Entomol. 33: 1032–1036.

Willemart, R. H. (2002). Cases of intra- and inter-specific food competition among Brazilian harvestmen, in captivity (Opiliones, Laniatores, Gonyleptidae). – Rev. Arachnol. 14: 49–58.

Willemart, R.H. & Pellegatti-Franco F. (2006). The spider *Enoploctenus cyclothorax* (Araneae: Ctenidae) avoids preying on the harvestman *Mischonyx cuspidatus* (Opiliones: Gonyleptidae). – J. Arachnol. 32: 649-652.

Willemart, R. H. & Chelini, M. C. (2007). Experimental demonstration of close-range olfaction and contact chemoreception in the Brazilian harvestman, *Iporangaia pustulosa*. – Ent. Exp. App. 123: 73-79.

Willemart, R. H., Farine, J. P. & Gnaspini, P. (2009). Sensory biology of Phalangida harvestmen (Arachnida, Opiliones): a review, with new morphological data on 18 species. – Acta Zool. 90: 209-227.

CONCLUSÃO GERAL

No primeiro capítulo, em que o foco foi direcionado para as estratégias comportamentais e modalidades sensoriais de *Loxosceles gaucho* para capturar opiliões, encontrou-se que esta aranha é capaz de predá-los por sua habilidade de morder pontos moles em seu corpo, tais como juntas e extremidades distais dos apêndices locomotores. Por outro lado, ao contrário do que foi hipotetizado na literatura, o lençol de seda construído por essa aranha não é essencial neste processo. Os experimentos que foram realizados para testar a função das pistas químicas na escolha do sítio de forrageio e da vibração de substrato na captura por *L. gaucho* não evidenciaram o papel destas modalidades sensoriais. Estes resultados opõem-se a estudos com outras espécies de aranhas em que estas são informações importantes na captura de presas.

O segundo capítulo foi conduzido para investigar de que modo as defesas do opilião *Mischonyx cuspidatus* atuam em uma situação de nível intermediário de ameaça (frente aos químicos da aranha *L. gaucho*) e a um nível alto de ameaça (quando interagindo diretamente com a aranha predadora). Não foram percebidas mudanças no comportamento do opilião quando em contato com os químicos das aranhas. Por outro lado, quando interagindo com as aranhas, alguns comportamentos defensivos foram observados, entre eles "Nipping" "Intense leg tapping" e "Erratic movements". Adicionalmente demonstrou-se pela primeira vez que o "Nipping" realizado por um opilião, representado por *M.cuspidatus* é capaz de perfurar o tegumento de um predador natural e causar perda de hemolinfa. Contudo, o furo causado pelas apófises do fêmur do quarto par de pernas dos opiliões não teve influencia sobre a sobrevivência das aranhas. Alguns dos comportamentos defensivos de opiliões mais característicos não foram observados, como a emissão de secreção defensiva e retaliação com pedipalpo e quelíceras.

Estes resultados evidenciam a importância de estudos comportamentais em interação entre predadores e presas, visto que somente assim pode se conhecer quais as estratégias utilizadas por um predador para capturar suas presas e as defesas das presas contra um predador específico. A forma como esta interação ocorre, influenciará o sucesso da taxa de captura das espécies, que terá implicações sobre as espécies de presas e a disponibilidade de alimento, influenciando as populações de ambas. É importante também mencionar que algumas extrapolações acerca de modalidades sensoriais utilizadas em um táxon baseiam-se em dados de poucas espécies, sendo assim, devem ser vistas com cautela, já que particularidades da autoecologia das espécies podem ter moldado diferentemente a forma em que modalidades atuam, visto que passam por diferentes desafios, a exemplo do que nossos resultados sugerem para as aranhas.