

Contributions to integrated management of synanthropic spiders

by

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Abstract

Arachnophobia, an irrational fear of spiders, is a prevalent anxiety disorder causing harmless synanthropic spiders to be viewed as pests that must be controlled. The objective of my thesis was to explore tactics other than pesticide applications for managing synanthropic spiders. Studying cues that affect settling decisions by cob-web spiders, I found that web architecture, rather than spider silk or silk-borne chemicals, affects settling decisions by females of the false black widow spider, *Steatoda grossa*. Investigating potential natural repellents for spiders in a multi-trophic context, I found that herbivore-induced plant volatiles are deterrent to *S. grossa*, but not to other spiders. As ants prey on spiders, I also explored the effects of ant chemical cues on avoidance responses of spiders. I found that chemical deposits of European fire ants, *Myrmica rubra*, deterred *S. grossa*, Western black widows, *Latrodectus hesperus*, hobo spiders, *Eratigena agrestis*, and – to some extent – cross spiders, *Araneus diadematus*.

Keywords: Integrated Pest Management, Arachnophobia, Arrestant, Repellent

Dedication

I dedicate this Master of Pest Management thesis to my wife Alexandra Fischer. You have not only been the balance to my work, the love of my life, and the mother of my children, but also the reason I finish this program. You encourage me to reach beyond the horizon of my imagination, and cheer me on during the journeys we embark on. Your love and caring support enabled me to conduct my research, study classes, and write papers that led to this thesis. But much more important than any academic achievement, I am grateful to co-parent our children with you. I would also like to thank my son Raphael and my daughter Lily for their joy and love of life giving happiness to the world around them.

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β -farnesene, (*E/Z*)-nerolidol, (\pm)-jasmonic acid, methyl salicylate at equal proportions]. Control stimuli consisted of the corresponding volume and type of solvent(s) (see Table 3.1 for details). Number in bars indicate the number of spiders responding to treatment or control stimuli, whereas numbers in square inserts denote the number of non-responding spiders. There was no statistically significant deterrent effect caused by any test stimulus.

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List of Acronyms

IPM: Integrated Pest Management

HIPV: Herbivore-induced plant volatiles

Glossary

Synanthropic organism: An organism that lives in proximity with people and benefits from them.

Arachnophobia: The irrational fear of spiders.

Repellent: Chemical that causes avoidance behavior.

Semiochemical: Message-bearing chemical.

Pheromone: Intra-specific chemical that benefits the emitter and receiver.

Chemical cues: passive, biological and environmental stimuli that inherently provide the recipient with information.

Kairomone: Interspecific chemical that benefits the receiver (at the cost of emitter).

Allomone: Interspecific chemical that benefits the emitter (at the cost of the receiver).

Chapter 1. Introduction

1.1. Motivation for spider control: Arachnophobia

The irrational fear of spiders (arachnophobia) is one of the most frequent clinical phobias (Marks 1987). In the UK, 55 % of women and 19 % of men are arachnophobic (Davey, 1991). Fear of serious injury or even death resulting from conceived spider attacks is a common explanation for arachnophobia (Vetter and Isbister, 2008). Historically, spiders have been falsely accused of causing diseases such as the Black Death (Davey 1994) and mass hysterical anxiety such as “tarantism” (Gloyne 1950). Arachnophobia is prevalent across cultures; however, its cause remains poorly understood (Mammola et al. 2022). The ‘Preparedness Theory’ predicts that an inherent avoidance response evolves into a serious threat resulting in likely death (Seligman 1971). However, no spider poses such a prevalent threat (Vetter and Isbister 2008; Hauke and Herzig 2017). Of the 50,000 spider species known to date, only 0.5 % (n = 246) are considered ‘medically relevant’ (Hauke and Herzig 2017). Media coverage of spiders fuels widespread arachnophobia with alarming misinformation about the risks (product of ‘hazard’ and ‘exposure’) that spiders pose to humans (Mammola et al. 2020, 2022). The hazard of spiders is limited, and the exposure or likelihood of being bitten is even more limited. Most spiders are reluctant to bite, attempt to flee, and usually inject venom only when provoked or squeezed (Vetter and Isbister 2008; Hauke and Herzig 2017). Spider bites in humans may cause medical symptoms such as muscle spasms (e.g., *Latrodectus*) and localized tissue necrosis (e.g., *Loxosceles*) (Vetter and Isbister 2008; Hauke and Herzig 2017), but rarely cause mortality. In contrast, snake bites were significantly more lethal, accounting for >63,000 deaths globally in 2019 (Roberts et al. 2022). Even ‘dangerous’ spiders are rarely lethal, but their bites may cause death in small children (Lüddecke et al. 2022). Black widows, *Latrodectus* spp. (Theridiidae) are among the best known spiders. Globally, these synanthropic spiders comprise 31 species, of which several occur in North America, such as the endemic western black widow, *L. hesperus*, and the invasive brown widow, *L. geometricus*. Of 23,000 *Latrodectus* bites recorded over a period of eight years, 50% of bites were asymptomatic, only 1.4% were deemed severe (Monte et al. 2011), and none was lethal. Bites of the ‘violin’ or Brown Recluse spider, *Loxosceles reclusa*, cause local tissue necrosis, and while they are painful, they are not lethal (Hauke and Herzig 2017).

Misidentification of arachnids is common. For example, the harmless false widow spiders, *Steatoda* spp., are commonly misidentified as true widow spiders, and the harmless yellow sac spider, *Cheiracantium inclusum*, is often mistaken for the Brown Recluse spider.

Spiders play crucial roles in global ecosystems (Foelix 2015). Spiders are estimated to kill 400-800 million tons (fresh weight) of arthropod prey per year, with insects and collembolans composing >90% of the captured prey (Nyffeler and Birkhofer 2017). In agriculture, spiders serve as natural biocontrol agents and contribute to the reduction of pest insect populations (Nyffeler and Birkhofer 2017). Likewise, synanthropic spiders reduce many urban pest populations (Mallis 2011; Vetter 2011), and have even been suggested for use as biocontrol agents (Mallis 2011; Schal 2011).

In summary, spiders are beneficial, and harmless with very few exceptions. Nevertheless, spiders are considered hazardous and obnoxious in cities. Many homeowners are concerned about spiders, and they call pest management professionals to eliminate spiders with broad-spectrum pesticides in and around their homes (Ramires et al. 2011).

1.2. Overview of study spiders

Spiders occur in many ecosystems, and some species inhabit urban (synanthropic) settings. Spiders are generally classified as web building or cursorial. Web-building spiders build either two-dimensional (2D) orb webs or three-dimensional (3D) funnel, sheet, or cobwebs. Orb weavers recycle their webs by ingesting and re-building them at other locations, whereas 3D web builders are almost exclusively sessile and do not recycle their silk (Janetos 1982; Opell 1998). Cursorial spiders such as grass spiders (Agalenidae) actively forage for prey. Thus, cursorial spiders are more likely to enter buildings than web-building spiders; however, they are sessile web-building spiders that remain indoors longer. Furthermore, their silken webs are perceived as nuisance in buildings (Vetter, 2011).

Urban spiders are diverse and have distinct community compositions in specific geographic locations. While widow spiders, *Latrodectus* spp. (Theridiidae), are globally distributed, each species is present only within a certain geographic range (see Figure 1 in Vetter and Isbister 2008). In British Columbia, *L. hesperus* is endemic, whereas the harmless look-a-like

false black widow, *Steatoda grossa* (Theridiidae), is globally invasive and commonly found in buildings (Bradley 2012). Both are cob-web spiders that build 3D webs on which they remain sessile for most of their lives.

The common house spider, *Parasteatoda tepidarium* (Theridiidae), can be encountered indoors around the globe (Bradley 2012). Like other theridiids, it is sessile on its large cobweb throughout its life as an adult (Bellmann 2010).

The large, globally invasive cross spider, *Araneus diadematus* (Araneidae), builds 2D orb webs in gardens and rarely indoors (Bradley 2012). Females sit on the hub of their web during the day.

The cellar spider, *Pholcus phalangioides* (Pholcidae), is another globally distributed spider. It is one of the most frequently encountered spiders indoors (Bradley, 2012). Although the spider is harmless, its large size is scary, and its web can be a nuisance. The cellar spider is often named ‘daddy-long-legs’ causing it to be confused with harvestmen or even crane flies.

Cursorial spiders are frequently found outdoors. Some are building-invasive during the mating season in fall. The hobo spider, *Eratigena agrestis* (Agelinidae), is harmless but is perceived as dangerous (Vetter and Isbister 2008; Hauke and Herzig 2017). *Eratigena agrestis* holds the record as the fastest-running spider (Mammola et al. 2017).

The selection of study spiders was guided by their body size and presence in British Columbia. All study species are large, with body sizes (without legs) ranging from 1 cm (*S. grossa*) to 1.5 cm (*E. agrestis*). Smaller species were not considered here, because they draw less attention and cause fewer arachnophobic responses.

1.3. Integrated Pest Management

Pests are broadly defined as living organisms having a feature that is a nuisance to people (Flint and van den Bosch 1981). These features range from severe ecological or economic impacts on agriculture (Eigenbrode et al. 2018) to being disliked for no apparent reason, as in the case of spiders (Vetter 2013). Exclusively pesticide-based pest control has severe ecological and public health impact. Integrated Pest Management (IPM) is based on multiple tactics to efficiently

control pests, concurrently limiting or excluding the use of broad-spectrum pesticides (Flint and van den Bosch 1981). Common tactics for managing urban arthropods include physical space modifications, the release of biological control agents, such as natural enemies, and the application of select chemicals. Chemicals may be earth-friendly semiochemicals that induce certain behaviors, such as dispersal, or broad-spectrum pesticides as a last resort (Mallis 2011).

Integrated management tactics for spiders are currently limited in number and scope (Vetter 2011). Physical modifications of urban structures, such as plugging holes in external walls, would help reduce, but not exclude, invasions of common arthropods, such as spiders, that can ‘squeeze’ through tiny openings (Vetter 2011). Mechanical elimination of spiders from homes by vacuuming is possible, but cumbersome and requires the removal of egg sacs to prevent population establishment and buildup.

The biological control of spiders in urban settings is not feasible. The natural enemies of spiders include birds, certain wasps, and other spiders (Foelix 2015). Birds cannot prey on spiders once they have entered urban structures. Mud Dauber wasps are specialized parasitoids of spiders, but spiders do not avoid these wasps or their nesting sites (Obin 1982). Moreover, the low humidity of urban epicenters is not suitable for these wasps (Shafer, 1949). Finally, the introduction of large arthropods to potentially control other arthropods would not relieve those who have zero tolerance for critters in their homes (Schal 2011).

The chemical control of spiders is presently based on the application of broad-spectrum pesticides, with different pesticides registered in various countries (Ramires et al. 2011). Alternative tactics for spider management are currently unavailable and are the focus of my thesis. Various non-pesticidal control tactics have been proposed and will be discussed in segments below. Spiders rely on chemicals for communication, prey discrimination and predator avoidance. Intra-specific chemical communication signals (pheromones) play a role in the reproductive biology of spiders, but spiders also sense interspecific chemicals, such as allomones and kairomones, or certain environmental cues (Fischer 2019). Allomones benefit the emitter at the cost of a receiver. For example, defensive secretions from prospective prey can deter spiders. Kairomones benefit the receiver at the expense of the emitter. Spiders benefit from avoiding natural predators based on their scent. Chemical environmental cues have no signal function, but provide recipients with information such as the availability of essential resources, including food,

as well as potential mate-encounter and nesting sites. Various chemical signals and cues can be used to attract spiders to traps or to expel them from human dwellings, as is commonly practiced for arthropod pests (Mallis, 2011).

1.4. Arrestants for cob-web spiders

Habitat selection is particularly costly for web-building spiders because their settling behavior requires significant nutrient and time investments. Both refugia size (Vetter and Rust 2008) and silk presence (Vetter and Rust 2010) are significant determinants for habitat selection (arrestment) decisions of Brown Recluse spiders. During the day, they stay in refugia, such as narrow cracks, which then become lined with the spider's silk. At night, Brown Recluse spiders are cursorial. The integrated management of spiders could be improved by exploiting certain aspects of spider biology for spider control. For example, targeting artificial refugia of Brown Recluse spiders for pesticide applications would reduce adverse pesticide effects on non-targets (Ramires et al. 2011). The biology of Widow spiders differs from that of Brown Recluse spiders in that they build webs for days to weeks and remain on these webs. Whether silk presence affects settling decisions by Widow spiders, as it does for Brown Recluse spiders (Vetter and Rust 2010), is not yet known. As the web-building biology of 'medically relevant' *Latrodectus* spp. and harmless *Steatoda* spp. is comparable (Benjamin and Zschokke 2003), I will use a representative species of the genus *Steatoda* – *S. grossa* – as a model organism to investigate settling decisions and the importance of silk presence in these decisions.

1.5. Ecologically-motivated search for spider repellents

The search for naturally occurring spider repellents drew on folklore, anecdotal accounts, and reports in scientific literature (Fischer 2019). According to a Google search, chestnuts, lemon oil, and mint oil are the top three commonly suggested natural repellences against urban spiders, but all failed to consistently deter all my study spiders across three genera (*L. geometricus*, *S. grossa*, *A. diadematus*) (Fischer et al. 2018).

Herbivore-induced plant volatiles (HIPV) reportedly affect hunting-site choices by the nursery web spider *Pisaura mirabilis* (Junker et al. 2011). Plants that are fed upon by herbivores

emit HIPVs that attract herbivore predators in this tri-trophic interaction (McCormick et al. 2012). Third-trophic-level predators are often specialists in second-trophic-level herbivores, but third-level generalist predators might pose a threat to spiders that also hunt on plants. Plants would benefit from HIPVs deterrent to spiders because many spiders commonly prey on pollinators, which, in turn, reduces the reproductive fitness of plants (Brechtbühl et al. 2010).

1.6. Search for spider repellents from natural enemies

Natural selection favors animals that can avoid natural predators (Davies et al. 2012). The primary predators of many spiders are other spiders (Foelix 2015). In predator-prey systems, the ability of prey spiders to sense the chemical cues of predatory spiders has evolved, enabling predator avoidance behavior. For example, in predator-prey systems of two wolf spiders, *Pardosa milvina* is preyed upon by *Hogna helluo* (reviewed in Fischer 2019) and responds to airborne and substrate-borne chemicals from *H. helluo*, recognizing the age of *H. helluo* chemical deposits, and the hunger state and relative size of *H. helluo* (Persons and Rypstra 2001; Barnes et al. 2002; Schonewolf et al. 2006).

The search for sources of natural chemicals that are repellent to urban spiders should include ants as generalist predators. At the population level, spider occurrence is negatively correlated with the presence of ants (Halaj et al. 1997; Sanders and Platner 2007). As many ants are urban pests, niche overlap and coevolution with urban spiders is likely (Mallis 2011). Urban pest ants include (i) carpenter ants, *Camponotus* spp., which can cause structural damage to homes; (ii) invasive European red fire ants, *Myrmica rubra*, which nest in high density and aggressively outcompete native ants (Naumann and Higgins 2015); and (iii) black garden ants, *Lasius niger*, which are often perceived as nuisance.

1.7. Overview of research chapters

In Chapter 2 (Research Chapter 1) of my thesis, I tested the hypothesis that *S. grossa* females select sites for their webs based, in part, on the presence of conspecific or heterospecific webs, sensing both physical and chemical web cues. In bioassays, we offered female *S. grossa* a choice between an empty control frame and a frame bearing the web of a conspecific female or

that of a female common house spider, *P. tepidarium*. We also tested the effects of (1) silk micro- and macrostructure, (2) plastic webs, and (3) silk semiochemical extract on the responses of *S. grossa* females. Females settled on both conspecific and heterospecific webs and chose test stimuli based on their chemical and physical characteristics. Even plastic webs in cobweb-like arrangements readily prompted settling behavior in females. Based on these data, I conclude that web architecture, rather than web silk, mediates settling responses by female *S. grossa* on pre-existing webs, which may provide structural support for a new web and indicate habitat suitability.

In Chapter 3, I explored whether the underlying assumption of optimal foraging models, in which animals are behaviorally, morphologically, and physiologically adapted to maximize their net energy intake, applies to web-building spiders in a multi-trophic context. If a spider were to build her web next to herbivore-fed-on plants that signal the herbivores' enemies for help by emitting herbivore-induced plant volatiles (HIPVs), that spider may maximize web captures in the short term. However, she would also risk predation by *generalist* predators that "listen" to signaling plants to find both herbivore and spider prey, likely resulting in lower overall reproductive fitness for the spider. I tested the hypothesis that HIPVs trigger avoidance responses by web-building spiders. We selected seven common HIPVs and one HIPV elicitor, and in two-choice olfactometers bioassayed their effect on four synanthropic spider species: *S. grossa*, *P. phalangioides*, *E. agrestis*, and *L. hesperus*. The 8-component HIPV/HIPV elicitor blend had a weak deterrent effect on *S. grossa* but the effect did not extend to *P. phalangioides*, *E. agrestis*, and *L. hesperus*. Our findings imply that there was insufficient selection pressure for these spiders to recognize HIPVs in a multi-trophic context, where spiders themselves could become prey if generalist predators or spider-hunting parasitoid wasps were to respond to signaling plants.

In chapter 4, I tested the hypothesis that ant-derived semiochemicals deter synanthropic spiders. To generate stimuli, we exposed filter paper for 12 h to workers of *M. rubra*, *L. niger*, and *C. modoc*, and then offered select urban spiders in 3-chamber olfactometer bioassays a choice between ant-exposed filter paper and unexposed control filter paper. Semiochemical deposits of *M. rubra*, but not of *L. niger* or *C. modoc*, had a significant deterrent effect on sub-adults of *S. grossa*, *L. hesperus*, and *E. agrestis*, as well as a moderate (but statistically not significant) deterrent effect on *A. diadematus*. The deterrent effect caused by semiochemical deposits of *M. rubra* may be attributable to the aggressive nature and efficient foraging of *M. rubra* in its

invaded North American range, exerting selection pressure on community members to recognize *M. rubra* semiochemicals and avoid micro-locations occupied by *M. rubra*.

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Chapter 2: Female false black widow spiders, *Steatoda grossa*, recognize webs based on physical and chemical cues¹

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2.1 Abstract

Females of the false black widow, *Steatoda grossa* CL Koch (*Araneae: Theridiidae*), invest significant energy and time weaving cobwebs. We tested the hypotheses that *S. grossa* females select sites for their webs based, in part, on the presence of con- or heterospecific webs, sensing both physical and chemical web cues. In bioassays, we offered female *S. grossa* a choice between an empty control frame and a frame bearing the web of a conspecific female or that of a female common house spider, *Parasteatoda tepidarium* CL Koch (*Araneae: Theridiidae*), recording (1) the time she spent, and (2) the time she spent inactive (a proxy for settling behaviour) on each frame. We also tested the effect of (1) silk micro- and macrostructure (wrapped-up silk or intact web, each semiochemical-deprived), (2) plastic webs, and (3) silk semiochemical extract on the responses of *S. grossa* females. Females settled on both conspecific and heterospecific webs and chose test stimuli based on their chemical and physical characteristics. Even plastic webs in cobweb-like arrangement readily prompted settling behaviour by females. Our results suggest that web architecture, rather than web silk, mediates settling responses by female *S. grossa* on pre-existing webs which may provide structural support for a new web and indicate habitat suitability.

Keywords: habitat choice, web recognition, structural support hypothesis, *Araneae, Theridiidae*, semiochemicals, habitat suitability

2.2 Introduction

Habitat choice by animals is affected by both habitat quality and conspecific presence. There is consensus among ecologists that animals should settle in habitats of high ‘intrinsic quality’ that offer ample resources, facilitate survival, and allow reproduction (Griffen and Drake 2008; Davies et al. 2012). Conversely, the effects of conspecifics on habitat selection are equivocal (Stamps 1991; Sanza et al. 2012). As conspecifics are resource competitors, the fitness of individuals may decline as a function of conspecific density (Arcese and Smith 1988; Sams et al. 1996). Consequently, the presence of many conspecifics in a habitat should discourage new arrivals from selecting that habitat. On the other hand, newcomers may prefer to settle in a habitat already occupied by conspecifics, thus saving time and energy associated with inspecting a habitat or settling in an inferior habitat (Smith and Peacock 1990). In this case, conspecifics serve as indicators of habitat quality and newcomers may benefit from joining them because animals living in a group can be more effective in defending resources, protecting against predators (Davies et al. 2012), ‘diluting’ the risk of predator or parasitoid attacks, or attracting prospective mates (Brown and Farabaugh 1991). Also, instant habitat information gathered by an individual animal may not sufficiently or accurately reflect habitat quality given the temporal variation in resource availability and other stochastic variables (e.g., weather conditions) (Dall et al. 2005).

The presence of conspecifics may convey the suitability of a (micro)habitat, resulting in a clumped distribution pattern, with some patches occupied by many individuals and other patches remaining unoccupied (Stamps 1988, 1991, 1992). There is a growing body of literature demonstrating the effect of conspecifics as cues for habitat quality. For example, territorial grasshoppers orient more strongly towards bushes occupied by conspecifics or associated with play-back calls of conspecifics than to unoccupied bushes or bushes associated with play-back calls of heterospecific grasshoppers (Muller 1998). Similarly, females of the Western black widow spider, *Latrodectus hesperus* Chamberlin & Ivie, select and move between potential websites (web-building locations) based on conspecific cues, being less likely to relocate when they are in close proximity to conspecifics (Salomon 2009).

For web-building spiders seeking new habitats, the presence of webs may serve as an indicator of conspecific presence, informing them about the suitability of a website (Hoffmaster 1986). Spider webs function as territory, hunting, mating, and nesting sites (Foelix 2015), and are costly investments of time and energy (4.5 cal mg⁻¹ of silk) (Janetos, 1982; Tanaka, 1989). Orb

weavers may complete their two-dimensional webs in less than a day (Townley and Tillinghast 2013), ingesting old silk, recycling 90 % of the amino acids, and thus mitigating web-building costs (Peakall 1971; Opell 1998). Unlike orb-weavers, cobweb weavers build their three-dimensional webs over the course of several days, not recycling any silk (Knoflach and Pfaller 2004) and thus facing higher web-building costs. When threatened by a predator, cobweb weavers typically drop out of their webs (Uma and Weiss 2012). If their webs are damaged or destroyed by a predator, cobweb weavers may rebuilt their old webs, or build new webs at a new site after assessing its quality (Janetos 1982; Salomon 2009). The preference of cobweb weavers for websites with vacant webs over those without them can be explained by the structural-support hypothesis (Hodge and Storfer-Isser 1997) which postulates that pre-existing webs function as a structural basis for new webs. As most spider taxa have poor vision and rely primarily on chemical and vibratory cues and signals (Uhl and Elias 2011), it follows that cobweb weavers likely sense the presence of webs based on silk-borne semiochemicals (message-bearing chemicals).

The model species of our study are the false black widow spider, *Steatoda grossa* CL Koch, and the common house spider, *Parasteatoda tepidariorum* CL Koch (both Araneae: Theridiidae). Often co-inhabiting the same habitat (A Fischer, pers. obs.), these spiders build their cobwebs in or near human dwellings where the likelihood of disruption and destruction of their webs is amplified by human activity. *Steatoda grossa* belongs to the Latrodectinae subfamily, whereas *P. tepidariorum* is a member of the phylogenetically more distant Theridiinae (Liu et al. 2016). Females of *S. grossa* build webs with peripheral retreats, whereas female *P. tepidariorum* situate their retreats in the web centre (Benjamin and Zschokke 2003). When webs are destroyed, females are potentially faced with seeking a new website and re-building their webs. We tested the hypotheses that mated *S. grossa* females (1) prefer websites with con- or heterospecific webs over potential websites without webs, and (2) recognize webs based on the micro- and macrostructure of silk or silk-like material (a) and web-borne semiochemicals (b).

2.3 Material and methods

2.3.1 Experimental spiders

Adult females of *S. grossa* and *P. tepidariorum* were collected from hallways and near

buildings of Simon Fraser University (49°16'36.35"N, 122°55'4.65"W). All specimens originated from locations most often occupied by a single spider at the time of collection. Females that had laid egg sacs with spiderlings hatching were considered mated. Each spider was kept in a separate transparent 300-ml plastic cup (Western Family, Canada) which was maintained at 22 ± 2 °C under a reversed light cycle (L12:D12 h) and a relative humidity of 41 %. Spiders were fed weekly with adult black blow flies, *Phormia regina* Meigen (Diptera: Calliphoridae), and larvae of the mealworm beetle, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae). All spiders had access to water in cotton wicks which were secured to the top of the cup and re-moistened once a week. Spiders remained in the insectary for 6 months prior to experiments which were run during the dark phase under red light. All bioassay spiders were randomly selected from the laboratory colony and tested only once in each experiment.

2.3.2 Hypothesis 1: Females prefer websites with con- or heterospecific webs over potential websites without webs (experiments 1-3)

Each web stimulus was prepared by allowing a mated female *S. grossa* or *P. tepidariorum* 2 days to construct a web on a frame (30 × 25 × 22 cm) of bamboo skewers (GoodCook, Rancho Cucamonga, CA, USA) (Scott et al., 2018). After removing the female, the web-bearing frame and an empty control frame were placed without delay at opposite ends of a large T-rod (Figure 2.1A), consisting of a horizontal beam (72 × 0.44 cm) and a vertical beam (19 × 0.44 cm) interconnected with a piece of laboratory tape (3 × 1.9 cm) (Fisher Scientific, Ottawa, ON, Canada) (Fischer et al. 2018b; Scott et al. 2018). We deployed a longer horizontal beam than previously described (Scott et al. 2018) to accommodate the two rather large frames of bamboo skewers. The bottom of each frame was placed on top of a Petri dish in a water-filled tray to prevent the bioassay female *S. grossa* (different from web-producing females) from escaping the experimental area.

To test the response of *S. grossa* females to web-bearing or empty control frames, each female was introduced on a stick (15 × 0.4 cm) to the vertical beam of the T-rod, and the following parameters were recorded: (1) first choice of a frame, (2) time spent on each frame, and (3) time spent inactive on each frame. Females on empty frames, but not on web-bearing frames, readily engaged in web-building activity as evidenced by their locomotion and silk deposition. As silk deposition on well-established webs is difficult to quantify (Salomon 2009), but is always

coupled with locomotion, we associated locomotion with web-building activity and motionlessness (inactivity) with absence of web-building activity. We considered inactivity as a proxy for settling (web-accepting) behaviour. For each replicate, a new T-rod was deployed, the position of the treatment and the control frame was reversed, and a web previously touched by a bioassay spider was replaced with a new one. Each bioassay was run for 15 min but was terminated sooner if the bioassay female fell into the water and did not return to the T-rod. Using the above design, we tested the responses of female *S. grossa* to sets of two frames each bearing (1) a web of *S. grossa* or no web (exp. 1; n = 60), (2) a web of *P. tepidariorum* or no web (exp. 2; n = 40), and (3) a web of *S. grossa* or a web of *P. tepidariorum* (exp. 3; n = 40) (Table 2.1).

2.3.3 Hypothesis 2a: Females recognize webs based on the micro- and macrostructure of silk or silk-like material (experiments 4-6)

To test the effect of silk microstructure, in the absence of silk semiochemicals, on responses of spiders, each web of a female *S. grossa* (see above) was wrapped around a glass rod (20×0.6 cm) and transferred to a 2-ml vial. Each web was extracted separately in sequence, for 24 h each, in methanol (50 μ l) and hexane (100 μ l) (solvents: 99.9 % HPLC grade; Fisher Chemical, Ottawa, ON, Canada), air-drying the silk between extractions. Sequential web extractions with a polar solvent (methanol) and a nonpolar solvent (hexane) ensured that both polar and nonpolar web semiochemicals were extracted. Dried silk was placed on one of two pieces of filter paper (2 cm² each) affixed to either end of the horizontal beam (25×0.4 cm) of a small (bamboo skewer) T-rod (Figure 2.1B). The control filter paper received no silk. The vertical beam (30×0.4 cm) of this T-rod was inserted into a ball (33.5 cm³) of plasticine inside a tray of water to prevent the bioassay spider from escaping. Using the above design, we tested the responses of female *S. grossa* to filter paper which did, or did not, carry the solvent-extracted silk (exp. 4; n = 60) (Table 2.1), recording the same parameters as in experiments 1-3.

To test the effect of three-dimensional web macrostructure, in the absence of silk semiochemicals, on responses of spiders, we allowed mated female *S. grossa* 7 days to build their web in an inverted Ziploc cup (473 ml; Twist'N Loc small, Ziploc, USA). We used mated instead of virgin females to minimize the presence of silk-borne pheromone. After dislodging each female from her web by opening the cup's lid and gently knocking on the cup, the web was submerged in sequence, for 24 h each, in methanol (500 ml) and hexane (500 ml), air-drying the

web between extractions. The solvents were syringed into, and drained from, the cup through a hole near the top. For each bioassay replicate, we placed each of the two cups on a burette stand such that the cup opening faced the end section of a horizontal T-rod beam (Figure 2.1C). Using small T-rods (see above) for bioassays and recording the same parameters as in preceding experiments, we tested the responses of female *S. grossa* to cups that contained (1) a solvent-extracted *S. grossa* web or no web (exp. 5; n = 18), and (2) a web of *S. grossa* which had, or had not, been solvent-extracted (exp. 6; n = 18) (Table 2.1).

To address the possibility that residual web-semiochemicals may have remained on solvent-extracted webs and thus affected the spiders' responses, we further tested the effect of web macrostructure deploying material completely void of *S. grossa* web-semiochemicals. Using large T-rods (see above) for bioassays and recording the same parameters as in preceding experiments, we offered *S. grossa* females a choice between a frame carrying polyester Halloween spider web-decoration silk (0.5 g; generic brand) arranged in three-dimensional web macrostructure and a control frame with the web-decoration silk (0.5 g) tightly wrapped around the frame (exp. 7; n = 30) (Table 2.1).

2.3.4 Hypothesis 2b: Females recognize webs based on web-borne semiochemicals (exp. 8)

To isolate the effect of silk semiochemicals from physical silk properties on the responses of spiders, we tested methanol extracts of webs (see exp. 4) in small T-rod bioassays, with a piece of filter paper affixed to either end of the horizontal beam (Figure 2.1B). In each replicate of experiment 8, we offered the *S. grossa* female a choice between filter paper treated with either web methanol extract (1 web equivalent) or a methanol control (Table 2.1) and recorded the same response parameters as in preceding experiments.

2.3.5 Statistical analysis

Data were statistically analysed with IBM SPSS v.23 (Unicom Systems, Mission Hills, CA, USA). In all (web) experiments, first-choice responses to test stimuli were analysed with a χ^2 test. Both the proportion of total time spiders spent on test stimuli and the proportion of time they spent inactive on test stimuli were analysed with a Mann–Whitney U test.

2.4 Results

2.4.1 Hypothesis 1: Females prefer websites with con- or heterospecific webs over potential websites without webs (experiments 1-3)

Spiders chose first equally often the frame bearing a female *S. grossa* web (n = 30) and the empty control frame (n = 30). Fifteen of the 30 females that first chose the control frame reversed their decision and subsequently went to the web-bearing frame, whereas only a single female that first chose the web-bearing frame subsequently went to the control frame. Spiders spent more time, and more time being inactive, on the web-bearing frame than on the empty control frame (proportion time spent: $U = 2\,461$, $N_1 = N_2 = 60$, $p < 0.0001$; proportion time spent inactive: $U = 2\,809.5$, $N_1 = N_2 = 60$, $p < 0.0001$; Figure 2.2, exp. 1).

Female *S. grossa* also chose first similarly often the frame bearing a female *Parasteatoda* web (n = 18) and the empty control frame (n = 22). Eight of the 22 spiders that chose first the control frame subsequently chose the web-bearing frame, whereas only three of the 18 spiders that first chose the web-bearing frame subsequently chose the empty frame. Spiders spent similar proportions of time on the web-bearing frame and the empty frame ($U = 911.5$, $N_1 = N_2 = 40$, $p = 0.27$), but they spent more time being inactive on the web-bearing frame ($U = 1\,099$, $N_1 = N_2 = 40$, $p = 0.002$) (Figure 2.2, exp. 2).

Female *S. grossa* chose first equally often the frame bearing the *S. grossa* web (n = 20) and the frame bearing the *Parasteatoda* web (n = 20). Five of the 20 spiders that chose first the *Parasteatoda* web subsequently reversed their decision. All 20 spiders that chose first the *S. grossa*-bearing frame remained there. Females spent more time on the conspecific web than on the heterospecific web but spent similar proportions of time being inactive on either type of web (proportion time spent: $U = 1\,002$, $N_1 = N_2 = 40$, $p = 0.012$; proportion time spent inactive: $U = 908$, $N_1 = N_2 = 40$, $p = 0.12$; Figure 2.2, exp. 3).

Throughout experiments 1-3 (and 5-7, see below), we noticed that females at the beginning of a bioassay typically investigated the entire macrostructure of one, or both, of the two bamboo frames, depositing silk lines in the process. When females encountered a web on a frame, they often became inactive (settled) for the remainder of the bioassay. On empty frames, in contrast, females usually engaged in intermittent web-building activity for the entire bioassay period, sharply contrasting the sustained inactivity on web-bearing frames.

2.4.2 Hypothesis 2a: Females recognize webs based on the micro- and macrostructure of silk or silk-like material (experiments 4-6)

Spiders chose first similarly often the frame bearing filter paper with double-extracted *S. grossa* silk (n = 33) and the frame bearing only filter paper (n = 27). All spiders reversed their decision several times without eventually settling on one stimulus. Spiders spent more time, but not more time being inactive, on the filter paper with silk than on the control filter paper without silk (proportion time spent: $U = 2\ 214.5$, $N_1 = N_2 = 60$, $p = 0.030$; proportion time spent inactive: $U = 1\ 941$, $N_1 = N_2 = 60$, $p = 0.45$; Figure 2.2, exp. 4).

Spiders chose first equally often the cup containing an intact double-extracted *S. grossa* web (n = 9) and the empty control cup (n = 9). Four of the nine females that first chose the control cup reversed their decision, whereas only one of the nine females that first chose the double-extracted intact web reversed her decision. Females spent more time, and more time being inactive, in the cup with a web than in the empty control cup (proportion time spent: $U = 227$, $N_1 = N_2 = 18$, $p = 0.040$; proportion time spent inactive: $U = 226$, $N_1 = N_2 = 18$, $p = 0.044$; Figure 2.2, exp. 5).

There was no significant difference in the number of females that first chose the cup housing an intact untreated web (n = 12) and the cup housing an intact but double-extracted web (n = 6). No female reversed her decision. Females spent similar periods of time, and of time being inactive, in the cup housing the treated or the untreated web (proportion time spent: $U = 124.5$, $N_1 = N_2 = 18$, $p = 0.24$; proportion time spent inactive: $U = 107.5$, $N_1 = N_2 = 18$, $p = 0.085$; Figure 2.2, exp. 6).

There was no significant difference in the number of spiders that first chose the frame with the polyester web (n = 20) and the empty frame (n = 10). Three of the 10 females that chose first the control frame reversed their decisions, whereas only one of the 20 spiders that chose first the frame with the polyester web reversed her decisions. Females spent more time, and more time being inactive, on the frame with the polyester web than on the empty frame (proportion time spent: $U = 666.5$, $N_1 = N_2 = 30$, $p = 0.001$; proportion time spent inactive: $U = 717$, $N_1 = N_2 = 30$, $p < 0.0001$; Figure 2.2, exp. 7).

2.4.3 Hypothesis 2b: Females recognize webs based on web-borne semiochemicals (exp. 8)

In (small) T-rod bioassays without frames, more spiders chose first the filter paper treated with web extract (n = 38) than the control filter paper (n = 22) ($\chi^2 = 4.23$, d.f. = 1, p = 0.039). All spiders repeatedly reversed their decisions. Females spent more time, and more time being inactive, on the treatment than on the control filter paper (proportion time spent: U = 2 475.5, $N_1 = N_2 = 60$, p < 0.0001; proportion time spent inactive: U = 2 194, $N_1 = N_2 = 60$, p = 0.036; Figure 2.2, exp. 8).

2.5 Discussion

Our data support the conclusion that female *S. grossa* (1) recognize the presence of webs based on both physical and chemical web cues, and (2) discriminate between con- and heterospecific webs.

The three-dimensional web macrostructure plays a key role in web recognition. Females spent more time on frames bearing conspecific webs than on empty control frames and settled on both con- and heterospecific webs (exps. 1, 2). Females also spent more time, and settled more often, in cups enclosing solvent-extracted (but otherwise intact) webs than in empty cups (exp. 5). Amazingly, they also spent more time inactive and more time overall on frames with polyester webs than on empty control frames (exp. 7). Apparently, the three-dimensional web macrostructure has a stronger effect on the females' acceptance and settling responses than the silk microstructure because even the three-dimensional polyester webs, but not the bundled-up and solvent-extracted *S. grossa* silk (exp. 4). Our observations that females became inactive (settled) only after they had investigated a potential website, and only after they had encountered a web or web-like structure, support the interpretation that settling behaviour is a good indicator of website acceptance.

One might argue that methanol as a polar solvent may have denatured the silk proteins and thus altered the silk microstructure. However, methanol apparently affects spider silk less than water which is more polar (Shao and Vollrath 1999) and to which webs are frequently exposed during rainfalls and in form of dew. It follows that methanol treatment of silk, although extracting the sex pheromone (Scott et al. 2018), is not likely to significantly alter the

microstructure of silk.

Chemical cues associated with webs contribute to web recognition by *S. grossa* females. Females preferred conspecific webs to heterospecific (*P. tepidariorum*) webs (exp. 3), and methanol extract of *S. grossa* webs to methanol controls (exp. 8). Preferential selection of conspecific webs, however, could also have been modulated by structural differences between con- and heterospecific webs. Female *P. tepidariorum* and *S. grossa* build their retreats in the web center and web perimeter, respectively (Benjamin and Zschokke 2003).

Our observations that female *S. grossa* preferred frames with webs over those without (exp. 1) support the concept that they seek a pre-existing three-dimensional web, or web-like configuration (here, polyester webs), as a structural foundation upon which to build their own webs (Hodge and Storfer-Isser 1997; Pruitt et al. 2009). By selecting a pre-existing foundation for web construction, *S. grossa* females may accrue energy (protein) and time-saving benefits and gain protection from predators during web construction. The inference of energy and time savings accrued by settling on pre-existing webs is supported by our data showing that females engaged in web-building activity more on web-bearing frames than on empty frames, as indicated by the relative time they spent being inactive or being in locomotory and silk-depositing modes on these respective frames. All spiders used in this study to prepare a web stimulus for bioassays readily constructed a web on empty frames, implying that bioassay spiders indeed selected web-bearing frames rather than aversed empty frames.

Selecting and settling on a frame with a pre-existing web, *S. grossa* females choose a website previously found suitable by conspecifics, supporting the concept of website choice being based, in part, on the presence of cues from conspecifics (Stamps 1992). It appears, however, that female *S. grossa* select websites based on the presence of either con- or heterospecific webs (exps. 1, 2), even though they prefer conspecific webs (exp. 3). Exploiting the presence of spider webs, rather than the presence of conspecific webs, as a website suitability cue would enable *S. grossa* females to choose from a broader range of potential websites, provided that the heterospecific spiders have overlapping prey spectra or criteria for habitat selection.

Physical (three-dimensional structure) and chemical web cues elicited settling behaviour in bioassayed *S. grossa* females, but these cues generally did not induce attraction of females (see results of exp. 8 for an exception). Although it is possible that females sense the presence of con- or heterospecific webs primarily upon physical contact, it seems plausible that they can sense the presence of pheromone-emitting webs over some distance and then orient towards them. This

inference is supported by findings that male *S. grossa* were attracted to pheromone-containing extracts of virgin female *S. grossa* webs but not to solvent controls (Scott et al. 2018). It is conceivable that the sex attractant pheromone of virgin females, that signals to males the presence of a prospective mate, reveals to females the presence of another female, and thus habitat suitability. That females exhibited settling but not attraction behaviour in this study could be attributed to several parameters, including greater spacing between test stimuli (exps. 1-3), silk experimentally stripped of its semiochemicals (exps. 4, 5), restricted emanation of semiochemicals from cup-enclosed webs (exp. 6), and polyester webs lacking any semiochemicals (exp. 7).

In conclusion, we demonstrate that *S. grossa* females sense webs based on physical and chemical web cues. As both natural webs and fake polyester webs with cobweb-like macrostructure readily prompted settling responses by females, it seems that web architecture, rather than silk microstructure, is the main cue for web recognition by *S. grossa* females. Settling by female *S. grossa* on both con- and heterospecific webs supports the concept that pre-existing webs may provide a structural foundation for new webs (Hodge and Storfer-Isser 1997). Furthermore, this behaviour suggests that the presence of both con- and heterospecific webs informs website selection by female *S. grossa*, expanding a previous concept that the presence of conspecifics, or their webs, signals microhabitat suitability (Stamps 1992). Deployment of (closable) traps fitted with fake polyester webs may encourage settlement decisions by habitat-seeking *S. grossa* females and facilitate their capture and safe removal from homes.

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Table 2.2 Details of the bioassay design – with large or small T-rods (see Material and methods for details), and female *Steatoda grossa* (*S.g.*) spiders –, stimuli presented, and number of replicates (N) run in experiments 1-8, testing three hypotheses: (1) females prefer websites with con- or heterospecific webs over micro-habitats without webs, (2a) females recognize webs based on the micro- and macrostructure of silk or silk-like material, and (2b) females recognize webs based on web-borne semiochemicals.

Hypothesis	Exp.	Bioassay design	Test stimuli		N
			Stimulus 1	Stimulus 2	
1	1	Large T-rod + female	Frame (empty)	Frame + <i>S.g.</i> web	60
	2	Large T-rod + female	Frame (empty)	Frame + <i>P.t.</i> web ^a	40
	3	Large T-rod + female	Frame + <i>P.t.</i> web ^a	Frame + <i>S.g.</i> web	40
2a	4	Small T-rod + female	Filter paper (2 cm ²)	Filter paper + <i>S.g.</i> silk ^g (2× extracted)	60
	5	Small T-rod + female	Cup (empty)	Cup + <i>S.g.</i> web (2× extracted)	18
	6	Small T-rod + female	Cup + <i>S.g.</i> web (2× extracted)	Cup + <i>S.g.</i> web	18
	7	Small T-rod + female	Frame + 1-D polyester ‘web’	Frame + 3-D polyester ‘web’	30
2b	8	Small T-rod + female	Filter paper + MeOH ^b	Filter paper + <i>S.g.</i> web MeOH ^b extract	60

^a*P.t.*, *Parasteatoda tepidarium*; ^bMeOH, methanol.

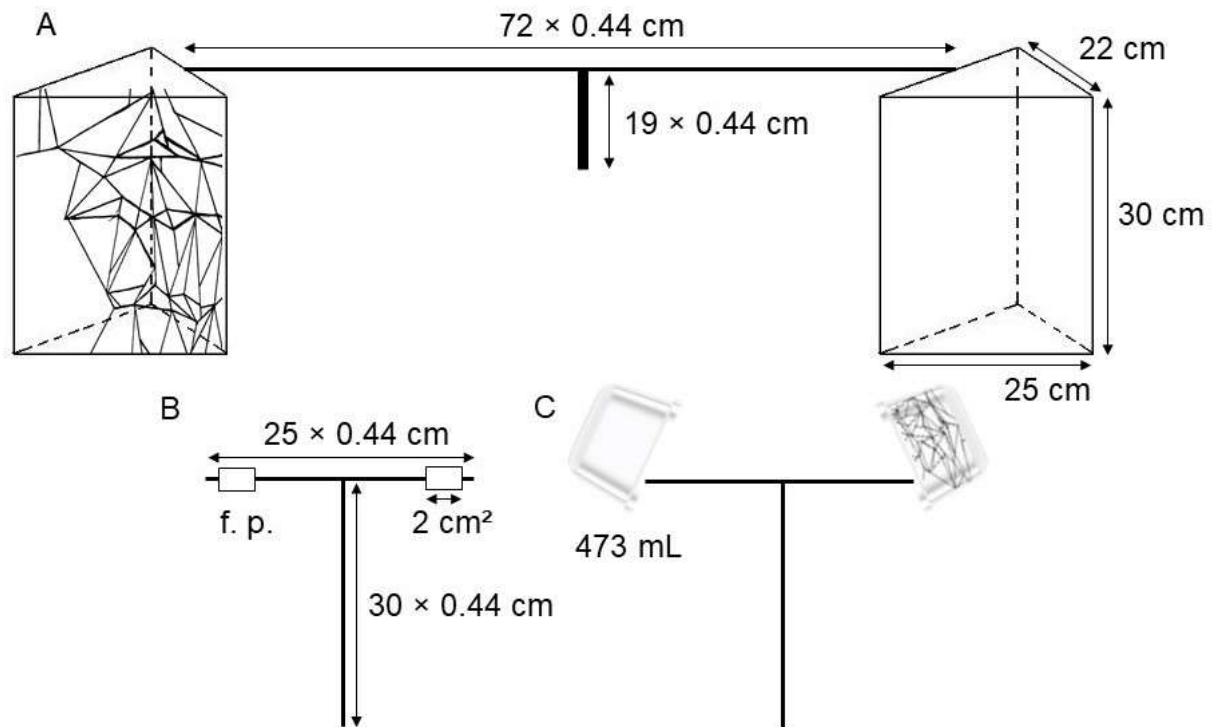


Figure 2.1 Experimental design for experiments 1-8, that tested website choices by *Steatoda grossa* females, displaying (A) a large T-rod interconnected with a web-bearing or empty control bamboo frame, (B) a small T-rod with two pieces of filter paper (f. p.), and (C) a small T-rod with two 473-ml Ziploc cups housing a web, or not (control).

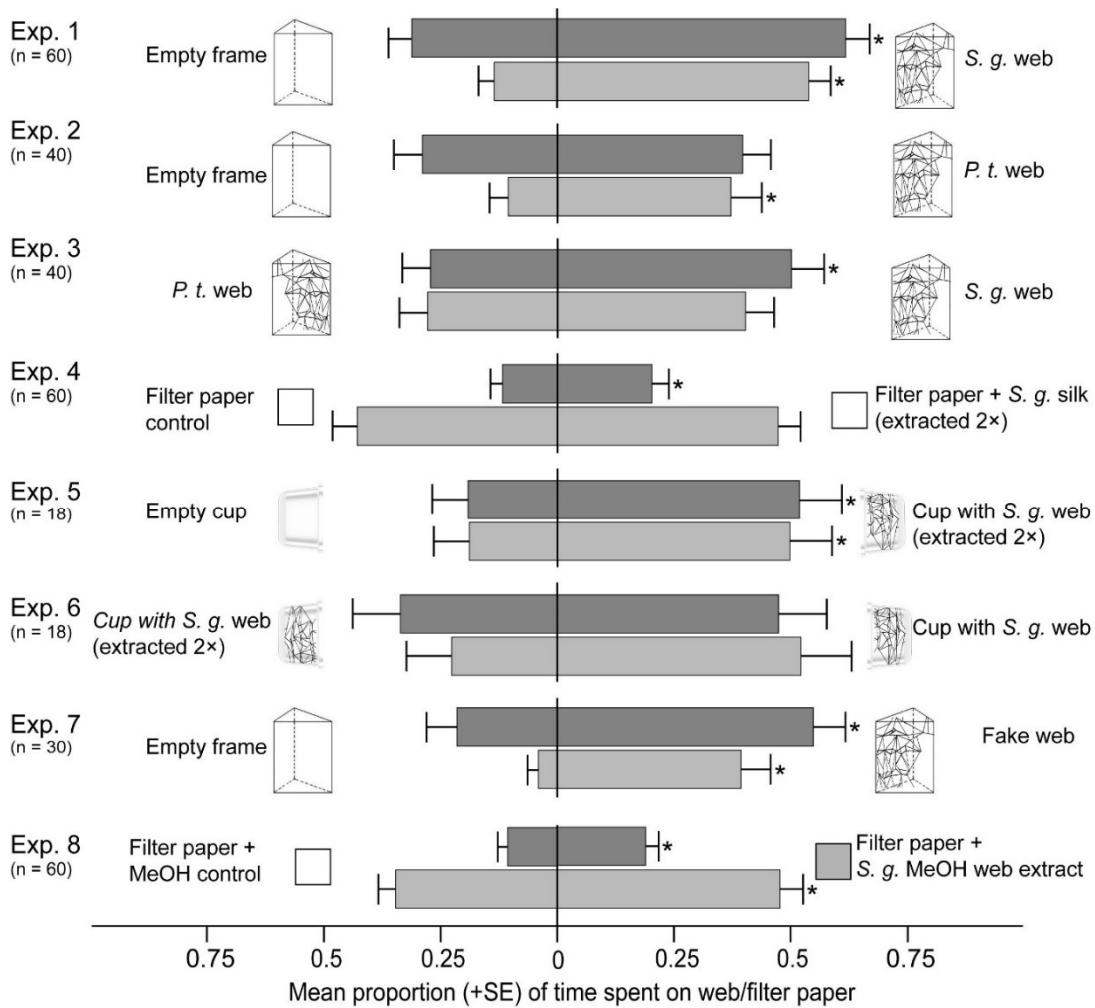


Figure 2.2 Mean (+ SE) proportion of time (upper bars), and of time being inactive (as a proxy for settling behaviour; lower bars), female *Steatoda grossa* (*S. g.*) spent in response to a choice of test stimuli (see Table 2.1 for details) presented in each of experiments 1–8. In each experiment, an asterisk indicates the test stimulus on which spiders spent a significantly greater proportion of time or of time being inactive (Mann-Whitney U test: $p < 0.05$); *P. t.*, *Parasteatoda tepidarium*; MeOH, methanol.

Chapter 3 Herbivore-induced plant volatiles do not affect settling decisions by synanthropic spiders¹.

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3.1 Abstract

An underlying assumption of optimal foraging models is that animals are behaviorally, morphologically, and physiologically adapted to maximize their net energy intake. Here we explored whether this concept applies to web-building spiders in a multi-trophic context. If a spider were to build her web next to herbivore-fed-on plants that signal the herbivores' enemies for help by emitting herbivore-induced plant volatiles (HIPVs), that spider may maximize web captures in the short term. However, she would also risk predation by *generalist* predators that "listen" to signaling plants to find both herbivore and spider prey, likely resulting in lower overall reproductive fitness for the spider. We tested the hypothesis that HIPVs trigger avoidance responses by web-building spiders. We selected seven common HIPVs and one HIPV elicitor, and in two-choice olfactometer bioassays tested their effect on four synanthropic spider species (false black widow, *Steatoda grossa*; common cellar spider, *Pholcus phalangioides*; hobo spider, *Eratigena agrestis*; western black widow, *Latrodectus hesperus*). The 8-component HIPV/ HIPV elicitor blend had a weak deterrent effect on *S. grossa*, but the effect did not extend to *P. phalangioides*, *E. agrestis*, and *L. hesperus*. Our findings imply that there was insufficient selection pressure for these spiders to recognize HIPVs in a multi-trophic context, where spiders themselves could become prey if generalist predators or spider-hunting parasitoid wasps were to respond to signaling plants.

Keywords Synanthropic spiders · Herbivore-induced plant volatiles · Multi-trophic interaction · HIPV-based spider deterrents

3.2 Introduction

An underlying assumption of optimal foraging models is that animals are behaviorally, morphologically, and physiologically adapted to maximize their net energy intake (Schoener 1971; Cowie 1977). A behavioral adaptation of spiders would be to select foraging patches such as plants that are frequently visited by insect prey (Krebs et al. 1974; Shafir and Roughgarden 1998). Foraging-site choices by the nursery web spider, *Pisaura mirabilis*, and the crab spider, *Thomisus spectabilis*, are indeed guided, at least in part, by phytochemicals (Heiling et al. 2004; Junker et al. 2011). Whether the same concept applies to web-building spiders which are important members of terrestrial food webs (Polis and Hurd 1995) is largely unexplored. If a spider were to build her web next to herbivore-fed-on plants that signal the herbivores' enemies for help by emitting herbivore-induced plant volatiles (HIPVs) (McCormick et al. 2012), that spider may trade-off a greater likelihood of prey captures against increased apparency to generalist predators that "listen" to signaling plants to find (spider) prey. In this type of trade-off, spiders that avoid becoming prey themselves should have greater overall reproductive fitness than spiders that maximize their own predation success in the short term.

The underlying assumptions in this multi-trophic signaling system are (1) that web-building spiders can intercept parasitoid wasps that respond to signaling plants in search for herbivore hosts, and (2) that spiders themselves can become prey or host to generalist predators or parasitoids. Both assumptions are supported by literature, at least in part. For example, parasitoid wasps, which respond to signaling plants under herbivore attack (Braasch et al. 2012) and parasitize many herbivores (Gols et al. 2012; Wist et al. 2015), could indeed be readily intercepted by web-building spiders (Bellmann 2010; Foelix 2015; Nyffeler and Birkhofer 2017). Spiders, in turn, are preyed upon not only by other spiders, lizards and frogs (Foelix 2015), they are also pursued by many insect natural enemies including ants (Gillespie and Reimer 1993), ichneumonid and pompilid wasps as well as sarcophagid and phorid flies (Foelix 2015; Gillung and Borkent 2017; Nyffeler and Birkhofer 2017). While some species of these wasp and fly taxa are known to respond to HIPVs (Braasch et al. 2012), it is not yet known whether responses to HIPVs afford additional opportunities for capturing spider prey.

There are some reports in the peer-reviewed literature and in anecdotal accounts about natural chemicals that repulse spiders (Pekár 2012; Fischer 2019) or trigger their dispersal. Some

of the hunting spiders disperse in response to chemical cues from *Lasius* spp. or *Formica* spp. ants (Mestre et al. 2014), and the garden spider, *Araneus diadematus*, as well as the brown widow, *Latrodectus geometricus*, avoid both chestnut fruit, *Castanea sativa*, and mint oil (Fischer et al. 2018a). However, the semiochemicals mediating the avoidance responses are not known.

Working with four web-building synanthropic spider species (false black widow, *Steatoda grossa*; common cellar spider, *Pholcus phalangioides*; hobo spider, *Eratigena agrestis*; western black widow, *Latrodectus hesperus*), we tested the hypothesis that HIPVs and an HIPV elicitor trigger avoidance responses by these spiders.

3.3 Materials and Methods

3.3.1 Spiders

Adults of *S. grossa* and subadults of *P. phalangioides* were collected in buildings on the Burnaby campus of Simon Fraser University [Burnaby, British Columbia (BC), Canada; 49°16'40.6"N 122°54'57.8"W]. Field-collected specimens were directly tested in bioassays (*P. phalangioides*) or were used to rear F1 and F2 generation offspring (*S. grossa*) for bioassays.

Subadults of *E. agrestis* and *L. hesperus* were collected in Centennial Beach Boundary Bay Regional Park (Delta, BC; 49°01'10.9"N 123°02'32.1"W) and in Island View Beach Regional Park (Saanichton, BC; 48°34'29.712"N 123°22'4.8468"W). Field-collected specimens were tested in bioassays.

Single specimens of the spider species were housed in separate labelled petri dishes (100 × 20 mm) containing a moist piece of cotton (1 cm³). Weekly, all spiders were provisioned with food. *Pholcus phalangioides* and *E. agrestis* received *Drosophila* vinegar flies, whereas *S. grossa* and *L. hesperus* – body size-dependent – received *Drosophila* vinegar flies or *Phormia regina* blow flies.

3.3.2 General experimental design

The effects of HIPVs [ocimene, (–)-linalool, (*E*)- β -caryophyllene, (*E*)-4,8-dimethyl-1,3,7-nonatriene, (*E*)- β -farnesene, (*E/Z*)-nerolidol, methyl salicylate] and one HIPV elicitor [(\pm)-jasmonic acid] (Dudareva et al. 2004; McCormick et al. 2012) on responses of spiders were tested in still-air, dual-choice olfactometers (Takács and Gries 2001) kept at room temperature and a 12 L:12 D photoperiod. Olfactometers ($n = 20$; Figure 3.1) consisted of three Pyrex glass chambers (each 3.5×10 cm ID) with removable glass lids linearly interconnected by glass tubes (each 2.5×1 cm ID). Treatment and control stimuli were assigned to lateral chambers such that the treatment stimulus was equally often presented in the left and right lateral chamber of an olfactometer to minimize any effect of side bias.

Experimental replicates were initiated 2 h into the photophase. To this end, the lateral chambers of olfactometers received a piece of filter paper (Whatman, Maidstone, UK) cut to size (2.0 cm^2) and labelled treatment or control. Then, 10- μL aliquots of either the treatment stimulus (an HIPV dissolved in solvent), or the solvent control stimulus (Table 3.1) were pipetted on the filter paper, the solvent was allowed 3 min to evaporate, and the chambers were closed with a lid. Finally, a single spider was introduced into the central chamber, which was then also closed with a lid. Eighteen to 21 h later when spiders had clearly settled for a web-building site (as indicated by deposition of silk which is energetically costly to produce and reflects the spider's decision to stay in that site (Janetos 1982; Fischer et al. 2019)), their position in the treatment chamber, control chamber, or central chamber (Figure 3.1) was recorded, with spiders in control and treatment chambers revealing a stimulus avoidance or tolerance response, respectively. Spiders in the central chamber were considered non-responders and were excluded from statistical analyses. Following the completion of each bioassay, olfactometers were washed with soap and water and air-dried. Each spider was tested only once.

HIPVs and the HIPV elicitor (\pm)-jasmonic acid differed in polarity and accordingly were dissolved in different types of solvent. Ocimene (>90 %, Sigma-Aldrich, Oakville, ON, Canada), (–)-linalool (97 %, Sigma-Aldrich), (*E*)- β -caryophyllene (>80 %, Sigma-Aldrich), (*E*)-4,8-dimethyl-1,3,7-nonatriene (“DMNT”; synthesized in Gries-lab adapting the protocol of (Maurer et al. 1986)), (*E*)- β -farnesene (>95 %, Bedoukian Research Inc., Canbury, CT, USA), and (*E/Z*)-nerolidol (98 %, Sigma-Aldrich) were all dissolved in pentane (99.9 % HPLC grade, Fisher

Chemical, ON, Canada), whereas (\pm)-jasmonic acid (synthesized in Gries-lab from methyl jasmonate (Sigma-Aldrich)) and methyl salicylate (98 %, Sigma-Aldrich) were dissolved in ethyl ether (99.9 %, anhydrous, Fisher Chemical, ON, Canada) (see Table 3.1 for details).

3.3.3 Specific experiments

Experiments 1-11: Testing for repellent effects of HIPVs/HIPV elicitor on *S. grossa*

Experiments 1-11 (n = 30 each) were designed to determine the HIPV(s)/HIPV elicitor deterrent to *S. grossa* and worthy of further testing with other spiders (*P. phalangioides*, *E. agrestis*, *L. hesperus*). In experiments 1-8, each of eight compounds [ocimene, (–)-linalool, (*E*)- β -caryophyllene, DMNT, (*E*)- β -farnesene, (*E/Z*)-nerolidol, (\pm)-jasmonic acid, methyl salicylate] was tested singly at 10 μ g for the responses of subadult *S. grossa*. Follow-up experiments 9-10 then tested the effect of these eight compounds when presented at equal proportions in a blend at a total amount of 10 μ g (Exp. 9) or 100 μ g (Exp. 10). To ascertain that the solvents (pentane and ethyl ether) used to dissolve the test compounds had no effect on their own (negative control), experiment 11 tested the effect of filter paper with or without solvent application on responses of *S. grossa*.

Experiments 12-19: Testing for repellent effects of HIPV/HIPV elicitor blends on *S. grossa*, *P. phalangioides*, *E. agrestis*, and *L. hesperus*

Experiments 12-19 (n = 28 each) aimed to determine whether the three compounds [(–)-linalool, (*E/Z*)-nerolidol, (\pm)-jasmonic acid] which singly were somewhat (but not significantly) deterrent to *S. grossa* were more deterrent to *S. grossa*, *P. phalangioides*, *E. agrestis*, and *L. hesperus* when presented in a ternary blend. Accordingly, a 10- μ g blend with equal proportions of (–)-linalool, (*E/Z*)-nerolidol, and (\pm)-jasmonic acid was tested for its effects on behavioral responses of *S. grossa* (Exp. 12), *P. phalangioides* (Exp. 13), *E. agrestis* (Exp. 14), and *L. hesperus* (Exp. 15). Moreover, to determine whether compounds other than (–)-linalool, (*E/Z*)-nerolidol, and (\pm)-jasmonic acid are needed to induce a deterrent effect, an 8-component blend (see above) at 10 μ g was tested for its effect on *S. grossa* (Exp. 16), *P. phalangioides* (Exp. 17), *E. agrestis* (Exp. 18) and *L. hesperus* (Exp. 19) (Table 3.2).

3.3.4 Statistical analyses

Using R (R Core Team 2020), a one-sided binominal test was used to analyze data for the hypothesized repellent effect of test compounds on spiders in each of two-choice experiments 1-19 (Ruxton and Neuhäuser 2010). According to research objectives (see above), experiments were then assigned to five groups [Group (G) 1: Exps. 1-8; G2: Exps. 9-10; G3: Exp. 11; G4: Exps. 12-15; G5: Exps. 16-18], and the p-values of experiments within each group were adjusted using the Benjamini-Hochberg method to account for multiple comparisons (Chen et al. 2017).

3.4 Results

Experiments 1-11: Testing for repellent effects of HIPVs/HIPV elicitor on *S. grossa*. Of eight compounds tested singly for behavioral effects on *S. grossa*, none caused significant deterrence: ocimene (Exp. 1: spiders in treatment chamber (6) vs spiders in control chamber (8); $p = 0.527$); (-)-linalool (Exp. 2: 4 vs 12; $p = 0.102$); (*E*)- β -caryophyllene (Exp. 3: 8 vs 8; $p = 0.684$); DMNT (Exp. 4: 5 vs 8, $p = 0.465$); (*E*)- β -farnesene (Exp. 5: 10 vs 5; $p = 0.940$); (*E/Z*)-nerolidol (Exp. 6: 3 vs 11; $p = 0.102$); (\pm)-jasmonic acid (Exp. 7: 4 vs 13; $p = 0.102$); and methyl salicylate (Exp. 8: 5 vs 8; $p = 0.465$) (Figure 3.2).

The 8-component HIPV/HIPV elicitor blend both at the 10- μ g dose (Exp. 9) and the 100- μ g dose (Exp. 10) expressed deterrence to *S. grossa* (Exp. 9: 3 vs 11; $p = 0.029$; Exp. 10: 0 vs 10; $p = 0.002$) (Figure 3.2).

Pentane and ethyl ether as a test stimulus had no effect on the response of *S. grossa* (Exp. 11: 12 vs 9; $p = 0.808$) (Figure 3.2).

A large number of spiders in various experiments remained in the central chamber of the olfactometer and did not move the farthest possible away from the HIPV or HIPV elicitor stimulus in the treatment chamber, providing further evidence that these compounds had little (if any) effect on settling decisions by bioassay spiders.

Experiments 12-19: Testing for repellent effects of HIPV/HIPV elicitor blends on *S. grossa*, *P. phalangioides*, *E. agrestis*, and *L. hesperus*.

The 3-component HIPV/HIPV elicitor blend (10 µg total) of (-)-linalool, (*E/Z*)-nerolidol and (±)-jasmonic acid had no deterrent effect on *S. grossa* (Exp. 12: 9 vs 9; $p = 0.790$), *P. phalangioides* (Exp. 13: 9 vs 11; $p = 0.780$), *E. agrestis* (Exp. 14: 1 vs 3; $p = 0.790$), and *L. hesperus* (Exp. 15: 15 vs 10; $p = 0.885$) (Figure 3.3).

The 8-component HIPV/HIPV elicitor blend (10 µg total) had a moderate (but statistically not significant) deterrent effect on *S. grossa* (Exp. 16: 5 vs 15; $p = 0.083$), and no deterrent effect on *P. phalangioides* (Exp. 17: 5 vs 10; $p = 0.302$), *E. agrestis* (Exp. 18: 3 vs 2; $p = 0.876$), and *L. hesperus* (Exp. 19: 16 vs 11; $p = 0.876$) (Figure 3.3).

Like in experiments 1-11, many spiders remained in the central chamber of the olfactometer and were obviously not deterred by the nearby HIPV/HIPV elicitor blend in the treatment chamber.

3.5 Discussion

An 8-component HIPV/HIPV elicitor blend deterred *S. grossa* (Figure 3.2), but the deterrent effect did not extend to any of the other three spider species tested. These data do not support our hypothesis that HIPVs trigger generic avoidance responses by web-building spiders. Nonetheless, testing this hypothesis was well justified.

Spiders have co-evolved with other members of their community (Uhl and Elias 2011) and thus appertain to a communication or eavesdroppers' network, with member interactions mediated, in part, by semiochemicals (message-bearing chemicals). Plants as autotrophic community members emit semiochemicals that, e.g., attract pollinators (Valenta et al. 2017), inform other plants of incipient attack (Witzany 2006; Babikova et al. 2013), and alleviate the impact of herbivores (Unsicker et al. 2009). Under herbivore attack, many plants emit HIPVs that recruit herbivore predators and parasitoids (War et al. 2011; Braasch et al. 2012; McCormick et al. 2012). The plants' signaling for help to eliminate the herbivores attacking them (McCormick et al. 2012) tend to recruit specific natural enemies of these herbivores but generalist predators may also respond (Braasch et al. 2012; Naranjo-Guevara et al. 2017; Turlings and Erb 2018). These predators may then potentially prey on both the plant herbivores and the spiders that have built their webs near the signaling plant, possibly to increase web prey captures. As spiders that

avoid becoming prey themselves have greater overall reproductive fitness than spiders that maximize their immediate predation success, it was reasonable to predict that spiders avoid plants releasing HIPVs and to test HIPVs as spider deterrents. However, our findings that HIPVs had only a modest deterrent effect on *S. grossa*, and did not deter *P. phalangioides*, *E. agrestis*, and *L. hesperus*, imply that there was insufficient selection pressure for these spiders to recognize HIPVs in a multi-trophic context, where spiders themselves could become prey when generalist predators respond to signaling plants. However, more studies on semiochemical signaling in a multi-trophic context with spiders are needed to arrive at a definitive conclusion whether and to what extent (web-building) spiders respond to HIPVs of signaling plants.

(*E*)- β -Caryophyllene and (*E/Z*)-nerolidol which we tested in our study as HIPVs serve additional roles as floral odorants. For example, they elicit avoidance behavior by the nursery web spider *Pisaura mirabilis* (Junker et al. 2011) in the context of resource partitioning (rather than multi-trophic signaling). *Pisaura mirabilis* and flower (crab) spiders are sit-and-wait spider predators that occupy dissimilar foraging sites, with *P. mirabilis* hunting predominantly in leafy vegetation and flower spiders ambushing prey in inflorescences (Bellmann 2010). Preference of *P. mirabilis* for leaves and leaf extracts over flowers and floral extracts, and avoidance of substrates treated with the floral scent components (*E*)- β -caryophyllene and nerolidol (Junker et al. 2011), suggest that (*E*)- β -caryophyllene and nerolidol help partition foraging sites between different taxa of sit-and-wait ambush spiders.

Floral semiochemicals often serve the dual functions of attracting pollinators while deterring general arthropod predators (Pichersky and Gershenzon 2002; Gershenzon and Dudareva 2007; Unsicker et al. 2009) that prey on pollinators and thus lower the plants' reproductive fitness (Dukas and Morse 2003; Heiling and Herberstein 2004; Brechbühl et al. 2010). As spider webs that are near inflorescences also intercept pollinators, it was conceivable that floral semiochemicals such as (*E*)- β -caryophyllene and nerolidol deter web building spiders. However, our findings that three of four study species (*P. phalangioides*, *E. agrestis*, *L. hesperus*) were not deterred by HIPV blends containing (*E*)- β -caryophyllene and nerolidol suggest that these spiders have not co-evolved with plants and their pollinators or that they have learned to tolerate the predator-deterrent scent of inflorescences. Alternatively, not all spiders may have yet evolved the ability to recognize plant odorants that are harmful or beneficial to their survival (Calbiague et al. 2017).

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Table 3.1 List of seven herbivore-induced plant volatiles (HIPVs) and an HIPV elicitor [(±)-jasmonic acid] tested singly or in an 8-component blend in 3-chamber olfactometers (Figure 1) for the responses of *Steatoda grossa* in experiments 1-11 (n = 30 each).

Exp. #	Stimuli tested		Amount (µg) tested in 10 µL	Number of spiders responding
	Treatment ¹	Control ¹		
1	ocimene	pentane	10	14
2	(-)-linalool	pentane	10	16
3	(E)-β-caryophyllene	pentane	10	16
4	DMNT ²	pentane	10	13
5	(E)-β-farnesene	pentane	10	15
6	(E/Z)-nerolidol	pentane	10	14
7	(±)-jasmonic acid	ethyl ether	10	17
8	methyl salicylate	ethyl ether	10	13
9	8-component blend ² (low dose)	pentane	10	14
10	8-component blend ² (high dose)	pentane	100	10
11	pentane; ethyl ether	no solvent	none	21

¹Treatment and control stimuli received the same type and volume of solvents.

²DMNT = (E)-4,8-dimethyl-1,3,7-nonatriene

³The 8-component blend of ocimene, (-)-linalool, (E)-β-caryophyllene, DMNT, (E)-β-farnesene, (E/Z)-nerolidol, (±)-jasmonic acid and methyl salicylate contained all components at equal proportion.

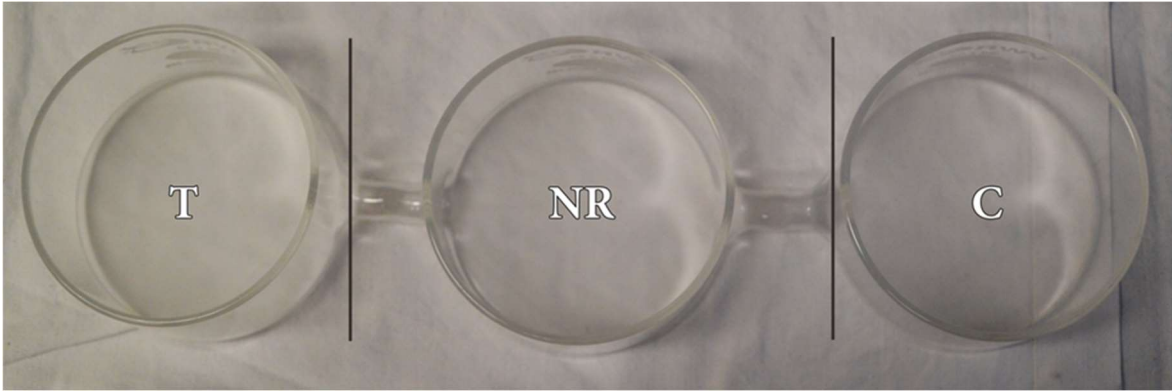


Figure 3.1 Photograph of a 3-chamber Pyrex glass olfactometer (with lids removed from each chamber) depicting the central chamber (3.5×10 cm ID) interconnected by glass tubes (each 2.5×1 cm ID) to two lateral chambers. For each replicate, a single spider was introduced into the central chamber, and 18 - 21 h later its position in the Treatment (T) or the Control (C) chamber was recorded. A spider found in the central chamber or the interconnecting glass tubes (an area delineated by the two black lines) was deemed a 'non-responder' (NR) and was excluded from statistical analyses.

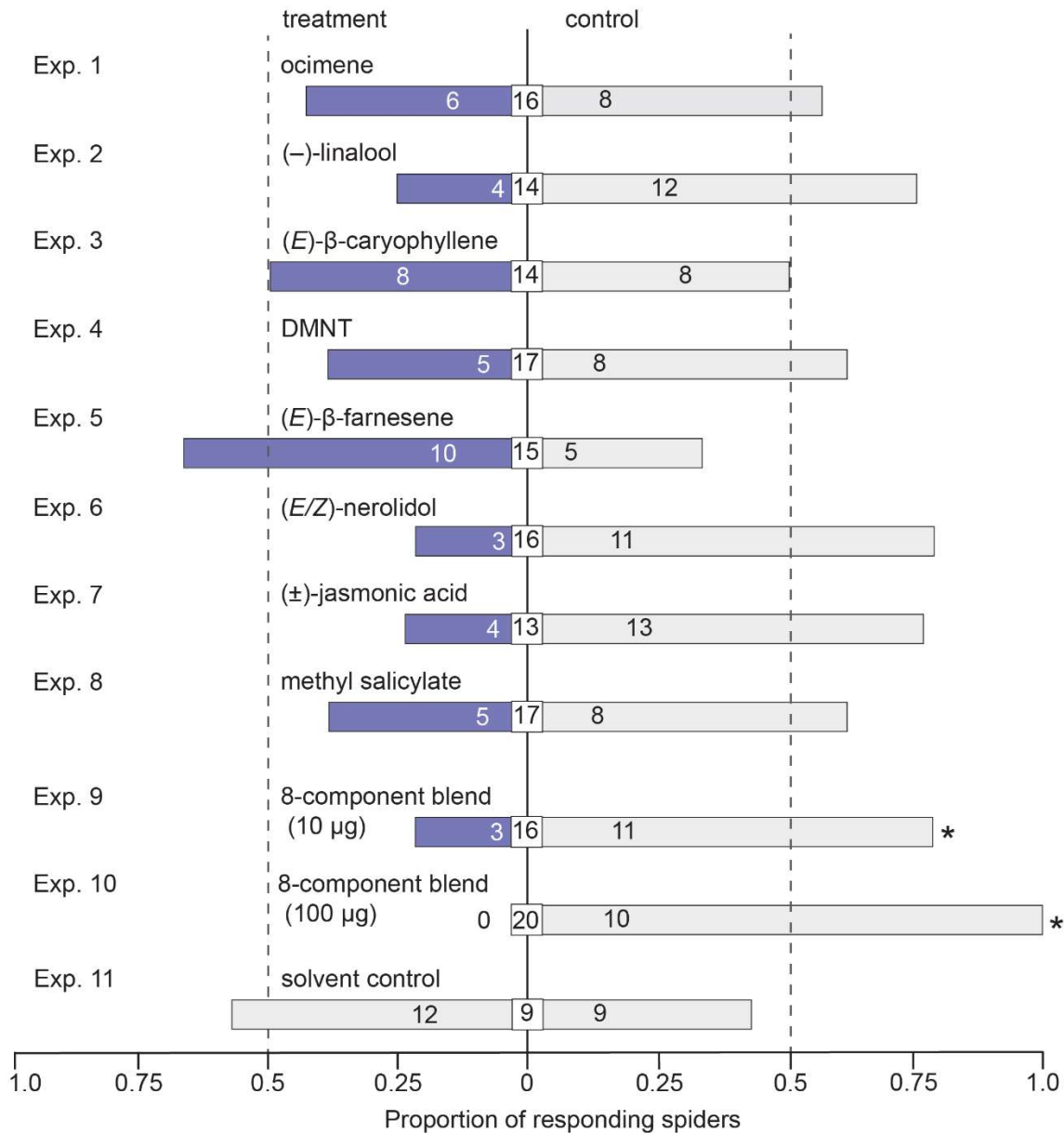


Figure 3.2 Responses of *Steatoda grossa* in experiments 1-11 (n = 30 each) in 3-chamber olfactometers (Figure 3.1) to treatment stimuli consisting of seven herbivore-induced plant volatiles (HIPVs) and an HIPV-elicitor [(\pm)-jasmonic acid] tested singly (10 μ g/10 μ L) or as an ‘8-component blend’ [ocimene, (-)-linalool, (E)- β -caryophyllene, (E)-4,8-dimethyl-1,3,7-nonatriene (= DMNT), (E)- β -farnesene, (E/Z)-nerolidol, (\pm)-jasmonic acid, methyl salicylate] (10 μ g/10 μ L), with all components at equal proportion. Control stimuli consisted of the corresponding volume and type of solvent, or no solvent (Exp. 11) (see Table 3.1 for details). Number in bars indicate the number of spiders responding to treatment or control stimuli, whereas numbers in square inserts denote the number of non-responding

spiders. For experiment, an asterisk (*) denotes a statistically significant treatment effect ($p < 0.05$; one-tailed binominal tests with Benjamini-Hochberg adjustment for each test group (Exps. 1-8, 9-10, and 11, respectively)).

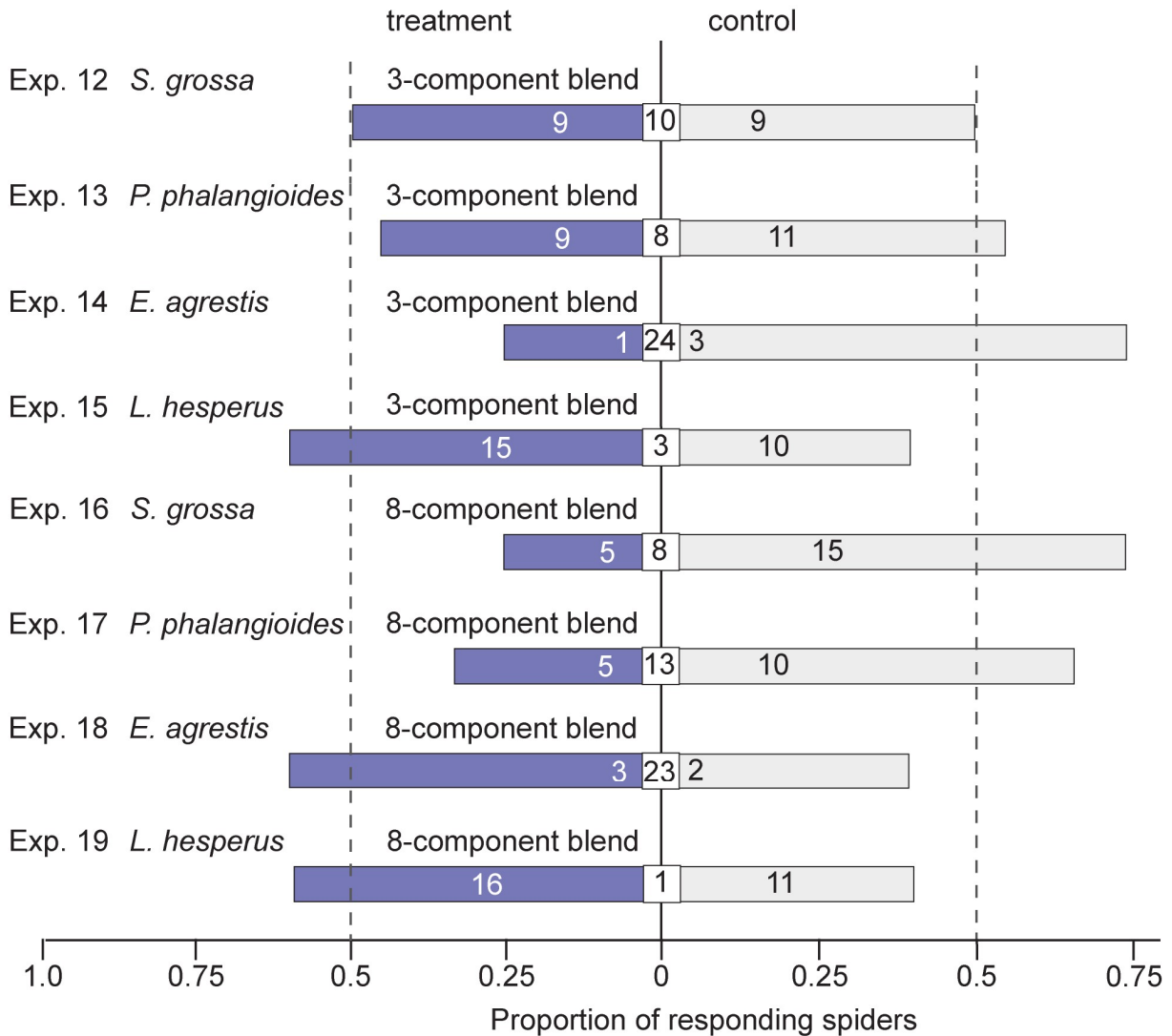


Figure 3.3 Responses of *Steatoda grossa*, *Pholcus phalangioides*, *Eratigena agrestis*, and *Latrodectus hesperus* in experiments 12-19 (n = 28 each) in 3-chamber olfactometers (Figure 3.1) to treatment stimuli consisting of herbivore-induced plant volatiles (HIPVs) and an HIPV-elicitor [(±)-jasmonic acid], tested as a ‘3-component blend’ (10 µg/10 µL) [(–)-linalool, (E/Z)-nerolidol, (±)-jasmonic acid at equal proportions], or as an ‘8-component blend’ (10 µg/10 µL) [Ocimene, (–)-linalool, (E)-β-caryophyllene, (E)-4,8-dimethyl-1,3,7-nonatriene (= DMNT), (E)-β-farnesene, (E/Z)-nerolidol, (±)-jasmonic acid, methyl salicylate at equal proportions]. Control stimuli consisted of the corresponding volume and type of solvent(s) (see Table 3.1 for details). Number in bars indicate the number of spiders responding to treatment or control stimuli, whereas numbers in square inserts denote the number of non-responding spiders. There was no statistically significant deterrent effect caused by any test stimulus.

Chapter 4. Know your foe – Synanthropic spiders are deterred by semiochemicals of European fire ants¹

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4.1 Abstract

Many ants prey on spiders, suggesting that web-building spiders may avoid micro-locations near ant colonies or frequented by foraging ants. Here we tested the hypothesis that ant-derived semiochemicals deter synanthropic spiders. To generate stimuli, we exposed filter paper for 12 h to workers of European fire ants, *Myrmica rubra*, black garden ants, *Lasius niger*, or western carpenter ants, *Camponotus modoc*, and then offered select urban spiders in 3-chamber olfactometer bioassays a choice between ant-exposed filter paper and unexposed control filter paper. Semiochemical deposits of *M. rubra*, but not of *L. niger* or *C. modoc*, had a significant deterrent effect on sub-adults of the false black widow, *Steatoda grossa*, the black widow, *Latrodectus hesperus*, and the hobo spider, *Eratigena agrestis*, as well as a moderate (but statistically not significant) deterrent effect on the cross spider, *Araneus diadematus*. The deterrent effect caused by semiochemical deposits of *M. rubra* may be attributable to the aggressive nature and efficient foraging of *M. rubra* in its invaded North American range, exerting selection pressure on community members to recognize *M. rubra* semiochemicals and avoid micro-locations occupied by *M. rubra*.

Keywords: ant cue, spider deterrent, integrated pest management, *Myrmica rubra*

4.2 Introduction

Widespread arachnophobia (Marks 1987; Davey 2008) is fuelled, in part, by fear of the few neurotoxin-producing spiders (Vetter 2011; Hauke and Herzig 2017). This fear has inspired the development of tactics to physically and chemically discourage synanthropic spiders from settling in and around human dwellings (Ramires et al. 2011). Proposed physical tactics include sealing holes and cracks in building walls, removing webs, reducing moisture, and changing exterior lighting that attracts insect prey for spiders (Vetter 2011). Chemical tactics such as insecticide applications (Ramires et al. 2011) are largely ineffective because spiders can avoid insecticides by abandoning their web and re-building one elsewhere (Vetter 2011; Pekár 2012). Natural repellents of spiders, such as chestnuts and lemon oil, are widely advertised in anecdotal accounts but only a few have been experimentally tested (Fischer et al. 2018a, 2021), and none effectively repelled all species of spiders tested (Fischer 2019). Moreover, there is no immediate ecological reason why these materials are repellent to spiders. In contrast, there is every reason for spiders to avoid natural predators such as ants that prey on both web-building or cursorial spiders (Gillespie and Reimer 1993; Henschel 1998; Jackson et al. 2004). At the population level, there is a negative correlation between the density of ant populations and the total biomass of spiders (Halaj et al. 1997; Sanders and Platner 2007). Cobweb spiders, *Phylloneta impressa*, tend to disperse in response to chemical cues derived from black garden ants, *Lasius niger*, and the formicine ant *Formica clara* (Mestre et al. 2014). Sensing chemical cues of potentially predatory ants is particularly adaptive for sub-adult web-building spiders which seek suitable micro-locations for settling and building their webs (Foelix 2015). As web building is a significant time and energy investment (Janetos 1982; Tanaka 1989), sub-adult spiders are thought to explore, and ultimately select, primarily those microhabitats that have no or few threats for survival, such as the presence of predatory ants. Flat rock spiders, *Morebilus plagusius*, e.g., avoid ant-scented rocks when selecting retreat sites (Penfold et al. 2016).

Here we tested the hypothesis that ant-derived semiochemicals deter spiders. As model organisms for our study, we selected three synanthropic ant species [European fire ants, *Myrmica rubra*; black garden ants, *Lasius niger*; western carpenter ants, *Camponotus modoc* (all Formicidae)] and four synanthropic web-building spider species [false black widow, *Steatoda grossa*; western black widow, *Latrodectus hesperus* (both Theridiidae); cross spider, *Araneus*

diadematus (Araneidae); hobo spider, *Eratigena agrestis* (Agelenidae)], all of which are commonly found in and around human dwellings in North America (Bradley 2012; Chalissery et al. 2019).

4.3 Materials and methods

4.3.1 Ants

Myrmica rubra workers (Figure 4.1) were collected from nests at Inter River Park (49°19'10.9"N 123°01'43.7"W) in North Vancouver, British Columbia (BC), Canada, whereas workers of *L. niger* and *C. modoc* (Figure 4.1) were collected from nests located on the Burnaby campus of Simon Fraser University (SFU, 49°16'33"N 122°54'55"W), BC. All ants were kept in jars (1 - 4 L) filled with soil from collection sites and were provisioned with tubes of sugar water retained with a cotton ball. To standardize the presentation of test stimuli according to weight equivalent of ants, 75 workers of each species were weighed in groups of five using a microbalance (TR-204, Denver Instrument Comp., Arvada, CO 80004, USA). Body weights (mean \pm SE) of individual workers of *M. rubra*, *L. niger* and *C. modoc* amounted to 3.51 ± 5.56 mg, 3.02 ± 4.44 mg, and 43.7 ± 52.7 mg, respectively.

4.3.2 Spiders tested

All specimens of *S. grossa* (Figure 4.1) were F1 sub-adult offspring of mated females captured on SFU's Burnaby campus (Fischer et al. 2018b), whereas specimens of *L. hesperus* and *E. agrestis* (Figure 4.1) were F1 subadult offspring of mated females collected on Centennial Beach Boundary Bay Regional Park, Delta, British Columbia (49°01'10.9"N 123°02'32.1"W). Spiderlings were housed singly in a petri dish (100 \times 20 mm) containing a moist cotton wick and – based on body size – were provisioned with *Drosophila* vinegar flies or *Phormia regina* blow flies once a week.

All *A. diadematus* were sub-adults, collected on the day of bioassays on SFU's Burnaby campus. Following bioassays, they were released into a designated non-collection zone on campus.

4.3.3 General experiments design

The effects of ant-derived deposits on aversion responses by spiders were tested in still-air, dual-choice olfactometers (Takács and Gries 2001; Fischer et al. 2021) kept at room temperature and a 12 L:12 D photoperiod. Olfactometers (see Figure 4.1 in [8] for a photographic illustration) consisted of three circular Pyrex glass chambers (3.5×10 cm inner diameter) with removable glass lids linearly interconnected by glass tubes (each 2.5×1 cm ID). The bottoms of lateral chambers were lined with circular filter paper (Whatman, Maidstone, England). Treatment and control stimuli were assigned to lateral chambers such that the treatment stimulus was equally often presented in the left and right lateral chamber of an olfactometer to minimize any potential effect of side bias. To prepare a treatment stimulus, ants were placed in one lateral chamber and prevented from leaving by a wet cotton ball inserted in the glass tube interconnecting the lateral and central chamber. The wet cotton ball not only blocked the chamber exit, but also provided a source of moisture for the ants. To ensure symmetry of the experimental design, a wet cotton ball was also inserted in the glass tube interconnecting the central chamber and the second lateral chamber. As the quantity of semiochemicals deposited by ants was likely correlated with their body size or weight, equal weight equivalents of ants were used to standardize the preparation of treatment stimuli; hence, 37 *M. rubra*, 43 *L. niger* and 3 *C. modoc* were confined in the treatment chamber. After 12 h of (overnight) confinement, the ants and the cotton balls removed. Then, a bioassay spider was introduced into the central chamber and kept in darkness for 24 h, following which its final position was scored under red light. Spiders positioned in lateral chambers were classed as responders to treatment or control stimuli, whereas those in the central chamber were recorded as non-responders. Spiders located in an interconnecting glass tube were scored as non-responders if they were closer to the central chamber than to the respective lateral chamber. All spiders were tested only once, and olfactometers were washed in detergent water (Sparkleen, Fischerbrand, Toronto, Canada) and oven-dried between replicates.

4.3.4 Specific experiments

Experiment 1 (Table 4.1) was designed to reveal potential side bias associated with olfactometers. It tested the response of *S. grossa* to two control stimuli (untreated filter paper) which were presented in the lateral chambers of the olfactometer.

As there was no side bias in experiment 1 (see Results), experiments 2-4 (Table 4.1) then tested whether semiochemicals deposited by *M. rubra* (Exp. 2), *L. niger* (Exp. 3), or *C. modoc* (Exp. 4), have a deterrent effect on *S. grossa*.

As only semiochemical deposits of *M. rubra*, but not of *L. niger* or *C. modoc*, deterred *S. grossa* (see Results), follow-up experiments 5-8 (Table 4.1) focused on *M. rubra* semiochemicals, and tested whether they deter only *S. grossa* (Exp. 5), or also deter *L. hesperus* (Exp. 6), *E. agrestis* (Exp. 7), and *A. diadematus* (Exp. 8).

With evidence that *M. rubra* semiochemicals deter at least three spider heterogeners (see Results), experiments 9 and 10 (Table 4.1) then tested dose-dependent effects of deterrent semiochemicals by offering *S. grossa* a choice between filter paper left untreated (control) or soiled with semiochemicals from either 37 *M. rubra* workers (Exp. 9; same dose as in Exps. 3, 5-8) or 111 *M. rubra* workers (Exp. 10; 3-fold higher dose).

4.3.5 Statistical analysis

R (R Core Team 2020) was used to perform one-sided binominal tests to analyse data for the hypothesized repellent effect of ants on spiders in two choice experiments 1-10 (Ruxton and Neuhäuser 2010). Subsequently, the p-values of experiments were adjusted using the Benjamini-Hochberg method to account for multiple comparisons (Chen et al. 2017).

4.4 Results

When subadult *S. grossa* were offered a choice between two lateral olfactometer chambers, each containing a control stimulus (untreated filter paper), they chose the right and left chamber 11 and 10 times, respectively, revealing no evidence for a side bias ($p = 0.50$; Exp. 1, Figure 4.2). Semiochemicals deposited by *M. rubra* had a significant deterrent effect on *S. grossa* (Exp. 2: spiders in treatment chamber (5) vs. spiders in control chamber (16), $p = 0.004$; Figure 4.2). In contrast, semiochemicals deposited by *L. niger* (Exp. 3) or *C. modoc* (Exp. 4) failed to deter *S. grossa* (Exp. 3: 11 vs. 12; $p = 0.50$; Exp. 4: 9 vs. 13, $p = 0.393$; Figure 4.2). In parallel experiments 5–8, semiochemicals deposited by *M. rubra* had a significant deterrent effect on *S. grossa* (Exp. 5: 4 vs. 17; $p = 0.007$, Figure 4.3), *L. hesperus* (Exp. 6: 5 vs. 14; $p =$

0.042, Figure 4.3) and *E. agrestis* (Exp. 7: 4 vs. 18; $p = 0.007$, Figure 4.3), but not on *A. diadematus* (Exp. 8: 6 vs. 13; $p = 0.084$, Figure 4.3). There was a dose-dependent effect of the amount of semiochemicals deposited by *M. rubra* on behavioural responses of *S. grossa*. The amount of semiochemicals deposited by 111 *M. rubra* had a deterrent effect on *S. grossa* (Exp. 10: 5 vs. 15, $p = 0.041$, Figure 4.4) but the aversion effect caused by deposits of only 37 *M. rubra* was not statistically significant in this experiment (Exp. 9: 8 vs. 15; $p = 0.105$, Figure 4.4).

4.5 Discussion

Our data support the conclusion that semiochemical deposits of *M. rubra* worker ants have a significant deterrent effect on three spider species (*S. grossa*, *L. hesperus*, *E. agrestis*) and that they express a moderate deterrent effect on a fourth spider species tested in our study, the cross spider *A. diadematus*. Conversely, semiochemical deposits of *L. niger* and *C. modoc* worker ants failed to induce a discernible behaviour-modifying effect on the spiders tested.

Our findings that semiochemical deposits of *M. rubra* worker ants, but not of *L. niger* or *C. modoc* worker ants, prompted aversion responses by *S. grossa* have multiple potential explanations, such as contrasting life history traits of ants, niche overlap, or not, between ants and spiders, and the specifics of the experimental design.

As part of the experimental design to prepare ant semiochemical deposits as test stimuli for spiders, we selected diverse taxonomic species of ants that greatly varied in body size and weight. Assuming that larger ants deposit greater amounts of semiochemicals, we standardized the amount of deposits between experiments by testing equal weight equivalents of ants, using 37, 43 and 3 worker ants of *M. rubra*, *L. niger* and *C. modoc*, respectively, to generate a test stimulus. However, contrary to our assumption, the body weight of ants and the amount of semiochemical deposits may not be positively correlated, and equal numbers, rather than equal weights, of *M. rubra*, *L. niger* and *C. modoc* worker ants may have been required to generate standardized test stimuli. Alternatively, the semiochemicals deposited by *M. rubra* may have significantly greater potency as spider deterrents than those of *L. niger* and *C. modoc*. Worker ants of *M. rubra* are omnivorous scavengers and prey on many invertebrates (Reznikova and Panteleeva 2001). In their invaded North American range, populations of *M. rubra* occur in extremely high densities and appear more aggressive than their counterparts in Europe. These characteristics, coupled with

efficient foraging and aggressive nest defence, have enabled *M. rubra* to outcompete native ants and lower the arthropod biodiversity in invaded communities (Naumann and Higgins 2015). It is conceivable then, that over evolutionary time arthropod community members, including spiders which may fall prey to *M. rubra*, have learned to respond to semiochemical cues of *M. rubra* and to settle in (micro) habitats void of *M. rubra*. If so, this would provide ecological rationale for our data showing that semiochemical deposits of *M. rubra* have deterrent effects on *S. grossa*, *L. hesperus* and *E. agrestis*.

Insufficient niche overlap between *M. rubra* and *A. diadematus*, and thus a lack of opportunity to learn each other's semiochemical signals or cues, may explain why semiochemical deposits of *M. rubra* had only a weak (and statistically not significant) deterrent effect on *A. diadematus*. As orb-weavers, *A. diadematus* females build their webs above ground (Bellmann 2010), physically well separated from the subterranean colonies of *M. rubra*. Females of *S. grossa*, *L. hesperus* and *E. agrestis*, in contrast, build their 3-dimensional cobwebs near ground level (Bellmann 2010) with greater likelihood of frequent encounters with foraging *M. rubra* workers.

The identity of the deterrent semiochemical(s) deposited by *M. rubra* workers remains unknown. Communication signals such as trail or alarm pheromones (Morgan and Wadhams 1972; Cammaerts-Tricot 1973; Cammaerts et al. 1978, 1981; Evershed et al. 1982) are least likely to be the deterrent(s) because the sets of 37 *M. rubra* workers used to generate test stimuli in the confines of olfactometers had no immediately obvious incentive to release pheromone and coordinate activities. Yet, signaling in ants is complex and we are just beginning to grasp that complexity. While the functional role of most exocrine glands in *M. rubra* (Morgan 2008) is still unknown, any gland may have released the semiochemical(s) that prompted the deterrent effect on spiders. Alternatively, the semiochemicals are not released from glands but originate from the ants' body surface.

Irrespective, the rather remarkable deterrence of *M. rubra* semiochemical deposits against *S. grossa*, *L. hesperus* and *E. agrestis* warrant the identification of these deterrents through proven-effective techniques in arthropod chemical ecology (Millar and Haynes 1998). Once identified, the origin of these deterrents could be traced to a specific exocrine gland and/or the body surface of ants. Moreover, synthetic replica of these deterrents could be developed, together

with concurrently known spider deterrents (Fischer 2019), for earth-friendly manipulation of synanthropic spiders.

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Table 4.1 List of test stimuli consisting of filter paper with chemical deposits from the ants *Myrmica rubra*, *Lasius niger*, or *Camponotus modoc*, of control stimuli invariably consisting of filter paper without chemical deposits by any ants, and of the synanthropic spiders *Steatoda grossa*, *Latrodectus hesperus*, *Eratigena agrestis* and *Araneus diadematus* in binary choice olfactometer experiments.

Exp. no	Test stimulus ¹	Control stimulus	Spider species bioassayed	n ²
<i>Bioassays with S. grossa to test for potential side bias of olfactometers</i>				
1	No deposits	No deposits	<i>S. grossa</i>	24 (3)
<i>Effect of ant species-specific chemical deposits on havioural responses of S. grossa</i>				
2	37 <i>M. rubra</i>	No deposits	<i>S. grossa</i>	24 (3)
3	43 <i>L. niger</i>	No deposits	<i>S. grossa</i>	24 (1)
4	3 <i>C. modoc</i>	No deposits	<i>S. grossa</i>	24 (2)
<i>Effect of M. rubra chemical deposits on behavioural responses of 4 synanthropic spiders</i>				
5	37 <i>M. rubra</i>	No deposits	<i>S. grossa</i>	30 (9)
6	37 <i>M. rubra</i>	No deposits	<i>L. hesperus</i>	30 (11)
7	37 <i>M. rubra</i>	No deposits	<i>E. agrestis</i>	30 (8)
8	37 <i>M. rubra</i>	No deposits	<i>A. diadematus</i>	30 (11)
<i>Effect of M. rubra chemical deposit amonts on havioural responses of S. grossa</i>				
9	37 <i>M. rubra</i>	No deposits	<i>S. grossa</i>	30 (7)
10	111 <i>M. rubra</i>	No deposits	<i>S. grossa</i>	30 (10)

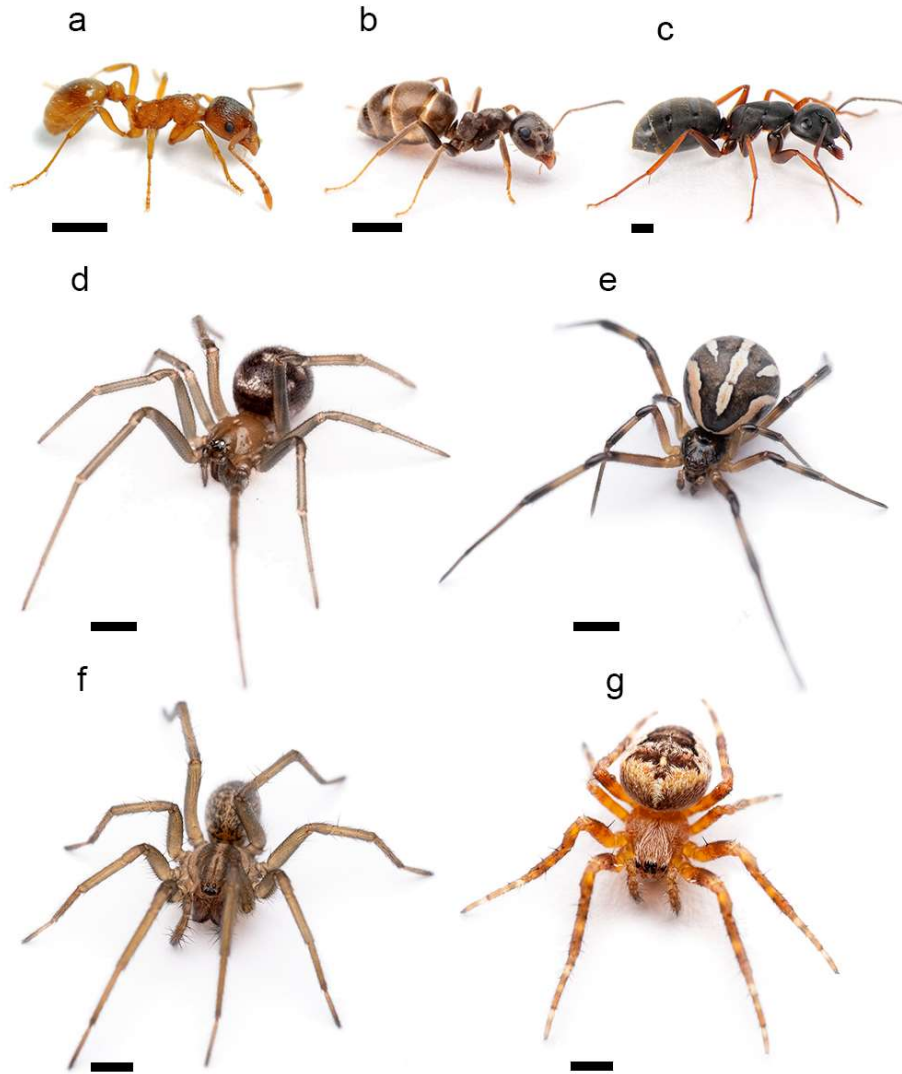


Figure 4.1 Photographs of worker ants of *Myrmica rubra* (a), *Lasius niger* (b), and *Camponotus modoc* (c) that were used to prepare test stimuli, and of subadult female spiders of *Steatoda grossa* (d), *Latrodectus hesperus* (e), *Eratigena agrestis* (f), and *Araneus diadematus* (g) that were tested in laboratory experiments. Bar length: 1 mm.

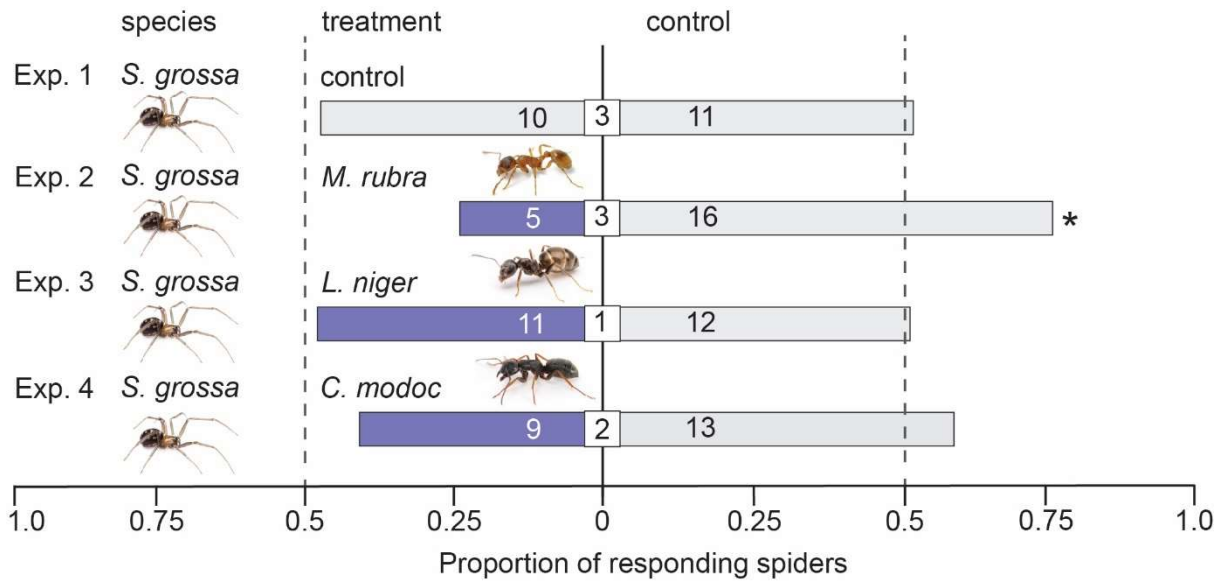


Figure 4.2 Responses of subadult *Steatoda grossa* that were given a choice in 3-chamber olfactometers (Takács and Gries 2001) between two test stimuli, both being untreated filter paper (Exp. 1), or one, being untreated filter paper, and the other being filter paper previously exposed to worker ants of *Myrmica rubra* (37; Exp. 2), *Lasius niger* (43; Exp. 3), or *Camponotus modoc* (3; Exp. 4). Shown within bars and square inserts are the number of spiders responding to treatment or control stimuli, and not responding to stimuli, respectively. For each experiment, an asterisk (*) denotes a statistically significant treatment effect (one sided binomial tests; $p < 0.05$).

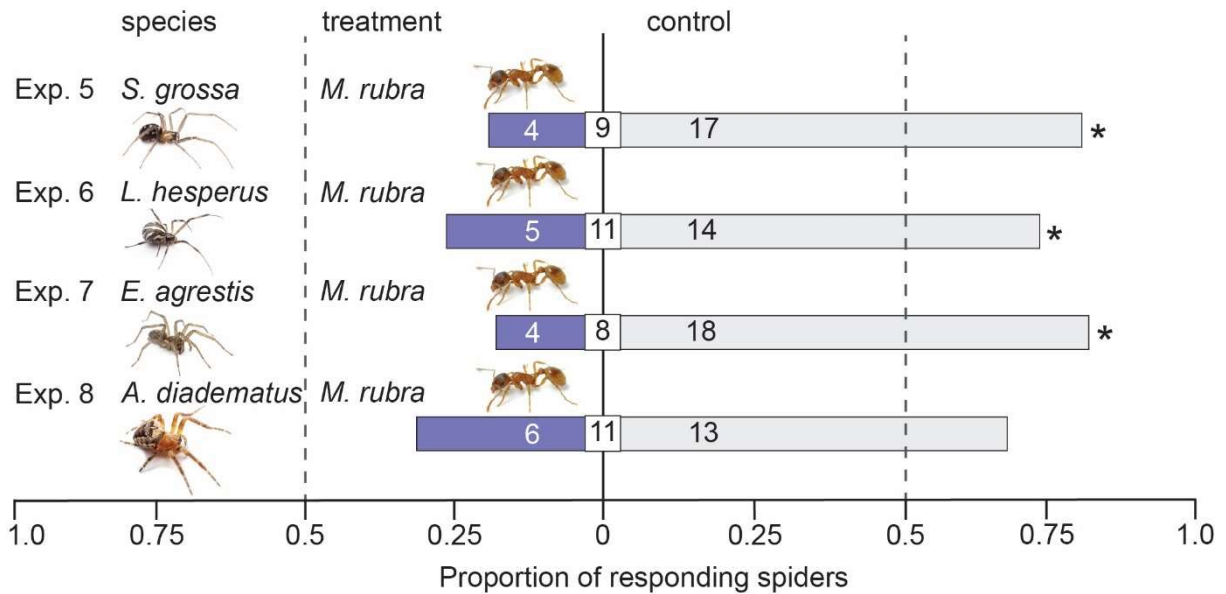


Figure 4.3 Responses of subadult *Steatoda grossa* (Exp. 5), subadult *Latrodectus hesperus* (Exp. 6), subadult *Eratigena agrestis* (Exp. 7), and subadult *Araneus diadematus* (Exp. 8) that were given a choice in 3-chamber olfactometers (Takács and Gries 2001) between two test stimuli, one being untreated filter paper and the other being filter paper previously exposed to 37 worker ants of *Myrmica rubra*. Shown within bars and square inserts are the number of spiders responding to treatment or control stimuli, and not responding to stimuli, respectively. For each experiment, an asterisk (*) denotes a statistically significant treatment effect (one sided binomial tests; $p < 0.05$).

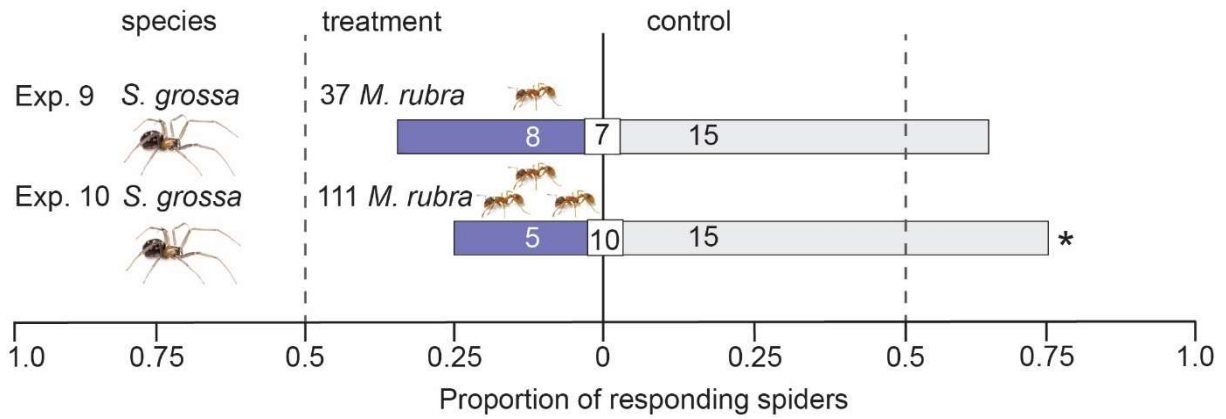


Figure 4.4 Responses of subadult *Steatoda grossa* that were given a choice in 3-chamber olfactometers (Takács and Gries 2001) between two test stimuli, one being untreated filter paper or and the other being filter paper previously exposed to 37 or 111 worker ants of *Myrmica rubra* (Exp. 9 and 10, respectively). Shown within bars and square inserts are the number of spiders responding to treatment or control stimuli, and not responding to stimuli, respectively. For each experiment, an asterisk (*) denotes a statistically significant treatment effect (one sided binomial tests; $p < 0.05$).