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REVIEW

Review of the cuticular lipids of spiders (Araneae)

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Abstract. Cuticular lipids are essential for the functioning of all arthropods. They maintain water balance, are involved in intraspecific and interspecific chemical communication and prevent fungal and bacterial infections. Although, the insect's lipid layer is relatively well known, that of spiders is considerably less well known. Only a few species are studied in terms of the identification and composition of cuticular lipids. The results obtained so far are discussed in this article. Spiders are subjected to various forms of extraction and in the majority of cases the subsequent analysis using GC-MS identified hydrocarbons, carboxylic acids, esters, alcohols, aldehydes, ketones, methyl ethers and cholesterol. Among these lipids there are semiochemicals, mainly pheromones and kin recognition cues. Despite the few publications on spider cuticular lipids, this subject is significant in terms of searching for antimicrobial substances such as potential bio fungicides and medicines.

INTRODUCTION

Lipids are a large group of organic compounds characterized by high solubility in non-polar solvents and low solubility in water. They are essential for the functioning of all living organisms. Among their most important functions are energy storage in the case of triglycerides, their protective and hormonal role and also their structural function as cellular components (Gurr et al., 2016). However, this is far from the limits of the usefulness of lipids, especially in invertebrates. The surface of arthropods is covered by a thin layer of epicuticle, which is a complex mixture of lipids, which are nonpolar and polar compounds that can be extracted using organic solvents. It creates a barrier between the individual and its environment. A vital function of the outer lipid layer is protection against water loss and maintaining water balance (Cuber et al., 2016; Gołębiowski et al., 2014a; Chinta et al., 2010). Furthermore, cuticular lipids are significant in preventing microbial infections due to the fungicidal and bactericidal properties of the constituents of this layer (Gołębiowski et al., 2014b; González-Tokman et al., 2014; Paszkiewicz et al., 2016; Bojke et al., 2020), which can also have an important role in chemical communication. Some of the chemical compounds in the epicuticle can provide information on sex, location, physiological state and also facilitate mating, the ultimate purpose of procreation (Suter et al., 1987; Grinsted et al., 2011).

The cuticular lipids of insects are generally well investigated. There are numerous books and research on the constituents of the epicuticle and its various functions (Bagnères & Blomquist, 2010; Cerkowniak et al., 2013), which is not surprising because this topic is important from many points of view, ranging from fundamental insights into insect biology and ecology to practical applications in agriculture, pest control and environmental monitoring. The situation, however, is different when it comes to the research on spider's epicuticle. Only a few species of spiders have been thoroughly analysed in terms of the identification of their cuticular lipids: *Argiope bruennichi* (Gerbaulet et al., 2022), *Latrodectus geometricus* (Guimarães et al., 2018), *Argyrodes elevatus* (Chinta et al., 2016), *Brachypelma albopilosum* (Trabalon, 2011), *Anelosimus eximius* (Bagnères et al., 1997), *Tegenaria atrica* (Trabalon et al., 1996) and *Cosmophasis bitaeniata* (Allan et al., 2002). As there are more than 50 000 species of spiders in the world (World Spider Catalog, 2023), it is clear they are poorly investigated in this respect. There are reports of lipid semiochemicals in the cuticles of variety of species, such as, sex pheromones and recognition cues in *Allagelena difficilis* (Chen et al., 2018), *Araneus diadematus* (Fischer et al., 2021), *Steatoda grossa* (Fischer et al., 2022), *Latrodectus hasselti* (Jerhot et al., 2010), *Pholcus beijingensis* (Xiao et al., 2010), *Stegodyphus lineatus* (Grinsted et al.,

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2011), *Tegenaria domestica* (Trabalonet al., 1997), *Tegenaria pagana* (Trabalon et al., 1997), *Tetragnatha extensa* (Adams et al., 2021) and *Tetragnatha versicolor* (Adams et al., 2021). In addition to the lipids present in the cuticle and silk, spiders also produce volatile compounds, which are semiochemicals. The nature of these compounds in *Argiope bruennichi* (Chinta et al., 2010), *Steatoda grossa* (Fischer et al., 2022), *Araneus diadematus* (Fischer et al., 2021), *Loxosceles reclusa* (Foulks et al., 2021) and *Plectreurys tristis* is known (Foulks et al., 2021). To some extent, despite the relatively limited amount of research into spiders' lipids there are similarities in the composition and function of the outer lipid layer of insects and spiders, however, it seems that the of the lipids in spiders is more complex (Gerbaulet et al., 2022). There are excellent reviews on the cuticular lipids of spiders (Schulz, 1997; Trabalon, 2013), which focus mainly on chemical communication. This review does not replicate past achievements, but is a compilation of all of the information on the cuticular lipids of spiders along with the methods used to identify them and the results of the research into the pheromones and other semiochemicals. The object is to highlight what has been done and what needs to be done in the future in this important and underrated field.

LIPID SEMIOCHEMICALS

In spiders, the prevalent lipid group include straight-chain saturated hydrocarbons, methyl-branched hydrocarbons and esters (methyl esters, wax esters). Other components are carboxylic acids, methyl ethers, long-chain aliphatic aldehydes and alcohols, methoxyalkanes, glycerides and cholesterol (Trabalon, 2013; Gerbaulet et al., 2022). Some of these lipids play a crucial role in facilitating the chemical communication of a wide range of animals, ranging from small multicellular organisms to humans.

The substances used in communication are called semiochemicals, which are chemicals that induce a behavioural response in the receiver. In this case only the receiver has evolved a response to this cue (Fischer, 2019). For example, in *A. bruennichi* differences in long-chained wax esters enables individuals to distinguish between conspecific and heterospecific individuals (Weiss & Schneider, 2021). The signals are chemicals that have evolved in both receivers and emitters. They are divided into intraspecific pheromones and interspecific allelochemicals (Kost, 2008). Allelochemicals are distinguished based on their benefits. If there is an advantage for the emitter, as in the case when an individual uses a toxic compound to deter a predator, the semiochemical is called an allomone. The opposite situation, where only the receiver benefits, occurs in the case of different semiochemicals called kairomones. These compounds are perceived by a predator, which enables it to track the unaware emitter. The last type of allelochemical are synomones, which benefit both the producer and the recipient, but there are no reports of such chemical signals being produced by spiders (Fisher, 2019). Finally, there are pheromones, which are secreted by one individual and received by another of the same species. These two groups are often difficult to distinguish because a semiochemical can fulfil both functions. The primers induce long-term physiological changes in reproduction, development or recognition learning releasers immediately influence the receiver's behaviour. These signals can affect, for example, sexual attraction, aggression between individuals, or cannibalism (Kost, 2008; El-Shafie & Faleiro, 2017; Fischer, 2019).

Classification of semiochemicals depend on the distance at which they remain active and can be either volatile or contact substances. Spiders produce both types of semiochemicals, with contact semiochemicals mainly in the silk produced by spiders or deposited on a substrate, whereas volatile substances are present in the cuticle and silk of spiders (Trabalon, 2013). The transmission of contact (substrate-borne) semiochemicals requires physical contact. These compounds have a relatively high molecular weight and are on the surface of spider's silk, in the cuticle or on the substrate (Fischer, 2019). Spiders receive this chemical signal via chemoreceptors present on their legs and pedipalps (Tichy et al., 2001; Trabalon & Bagnères, 2010; Muller et al., 2020). The contact semiochemicals on the silk produced by spiders is transferred there by the nearby spinnerets during the weaving process (Gaskett, 2007). On the other hand, transmission of volatile (air-borne) semiochemicals do not require physical contact between the receiver and emitter. These compounds have a low molecular weight and use wind to transport them over long distances. This type of communication is often used during mating when males use them to find a mate or locate prey (Chinta et al., 2010; Fischer, 2019).

While our understanding of spider pheromones is limited, they are the most extensively researched category of semiochemicals in this group of arthropods. Of all the types of pheromones, the most studied are the contact sex pheromones. Although, they are present both in the cuticle and silk of spiders, it is the silk pheromones that are most studied (Schulz & Toft, 1993; Papke et al., 2000; Schulz, 2013). Using two-choice behavioural tests several dozen organic compounds involved in mating have been identified. In addition to improving the understanding of the communication system of arthropods, this information is very important in the case of species that have an adverse effect on humans or their environment (Bryan et al., 2019). The best example is the synanthropic venomous spiders of the genus *Latrodectus*, which are harmful to human health and can even be deadly for humans. Acylated serine derivatives such as *N*-3-methylbutyryl-*O*-(S)-2-methylbutyryl-L-serine, *N*-3-methylbutyryl-*O*-propionyl-L-serine-methyl ester and *N*-3-methyl-butynoyl-*O*-methylpropanoyl-L-serine-methyl ester are the contact sex pheromones of, respectively, *Latrodectus hasselti*, *Latrodectus geometricus* and *Latrodectus hesperus* (Jerhot et al., 2010; Scott et al., 2015; Baruffaldi, 2016). For these species pheromones can be used to control them by using species-specific pheromone traps (Bryan et al., 2019). Cuticular lipids identified as sex pheromones are listed in Table 1 and will be described below.

SILK LIPIDS

The chemistry of spider cuticular lipids and their roles in spider biology are presented in this review. Although silk lipids are well studied (Schulz, 1997, 2009, 2013; Trabalon & Bagnères, 2010), it is important to highlight their presence and close resemblance to cuticular lipids. Despite spider silk being primarily protein-based, lipids are important in preventing desiccation and degradation of the proteins (Schulz, 2001, 2013). However, their weak attachment to silk implies they have a minimal effect on its mechanical strength (Spooner et al., 2007; Wirth et al., 2019). Notably, silk lipids contribute to the transmission of semiochemicals, either originating from silk glands or the cuticle during web construction (Trabalon & Bagnères, 2010). Identification of these organic compounds involves techniques, such as, gas chromatography-mass spectrometry (GC-MS) and nuclear magnetic resonance (NMR) spectroscopy (Fisher, 2019). Sex pheromones, an important feature in silk semiochemical research, can be lipid or non-lipid compounds, with complex compositions that are species dependent (Trabalon & Bagnères, 2010; Jerhot et al., 2010; Fischer et al., 2022). Despite numerous studies on silk pheromones, ongoing research underscores the evolving nature of this field.

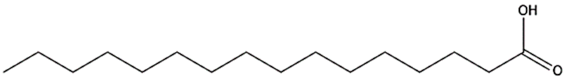
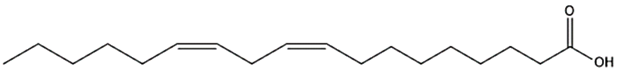
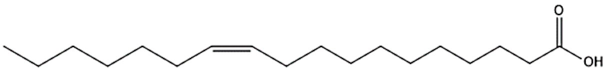
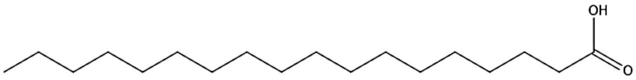
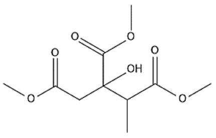
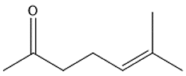
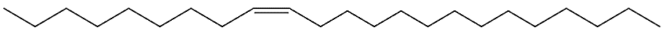
ANTIMICROBIAL PROPERTIES OF CUTICULAR LIPIDS

Arthropods, including spiders, have evolved a variety of cuticular defences against infections by potentially lethal pathogens, which are essential for the survival of arthropods, given that entomopathogenic fungi are a significant threat (Sharma et al., 2023). One key cuticular defence is simply to develop a structural barrier. The exoskeleton of many insects is made up of many layers, of which the epicuticle can be sclerotized and hard (Lockey, 1988). It is difficult for fungi to penetrate the lipid layer of this tough physical barrier. In addition this barrier prevents the entry of water and microorganisms, which require moisture for essential biochemical processes and reproduction, in order to become established and maintain colonies on the surface of arthropods (Oritz-Urquiza et al., 2013; Batalha et al., 2020). The chemical composition of the cuticle can also be protective, as although there is little information on the antimicrobial properties of the cuticle of spiders compared to that for insects the similarities in the properties of the cuticle of arthropods warrant serious consideration of cuticular lipids having a protective function. Arthropod cuticular lipids provide a diverse chemical arsenal, which along with the physical barriers, form a versatile defence system, including effective antimicrobial protection, which enables them to survive in various environments.

ANALYSIS OF CUTICULAR LIPIDS

There are three stages in the qualitative analysis of spiders' cuticular lipids: collection and handling biological material, extraction of cuticular lipids and identification of the chemical constituents in the lipid layer. Different methods are used at each of these stages. Nevertheless, they make it possible to identify the chemical composition of the lipid layer on the surface of spiders. Information on all the techniques used in this research is listed in Table 2.

Table 1. Pheromones present in the cuticle of four species of spider.

Species	Type	Name	Structure	References
Allagelena difficilis	Female contact sex pheromone	Palmitic acid		Chen et al., 2018
		Linoleic acid		
		Cis-vaccenic acid		
		Stearic acid		
Argiope bruennichi	Female volatile sex pheromone	Trimethyl methylcitrate		Chinta et al., 2010
Araneus diadematus	Female volatile sex pheromone	sulcatone		Fischer et al., 2021
Pholcus beijingensis	Male contact sex pheromone	(Z)-9-tricosane		Xiao et al., 2010

Handling biological material

Proper preparation of living biological material is essential before extraction. One commonly employed method is freeze killing. This involves subjecting the spider to low temperatures for a specific period of time. Temperatures used for freezing range from –18°C to –80°C, with typical temperatures being between –20 and –25°C (Grinsted et al., 2011; Fischer et al., 2021; Weiss & Schneider, 2021; Gerbaulet et al., 2022). One significant benefit of this method is the preservation of morphological features, as rapid freezing helps maintain the structural integrity of the specimen. In addition, freeze killing effectively halts enzymatic activities, minimizing the degradation of biological material and facilitating studies involving biochemical

Table 2. Techniques and methods used to extract cuticular lipids and identify their functions.

Spider taxa	Type of material	Purpose of study	Type of compounds	Killing method	Extraction method		Drying	Instrument used	Reference
					Solvent	Time			
Allagelena difficilis	female	Sex pheromones	Cuticular lipids	no data	DCM	2 h	stream of nitrogen	GC/MS	Chen et al., 2018
Anelosimus eximius	female	Identification	Cuticular lipids	no data	pentane	15 min	stream of nitrogen	GC/MS	Bagnères at al., 1997
Anelosimus eximius	female	Chemical differences in social spiders	Cuticula lipids	no data	pentane	15 min	stream of nitrogen	GC/MS	Pasquet et al., 1997
Araneus diadematus	females	Sex pheromones	Cuticular lipids	Freeze in –20°C	DCM	3 min (gently shaken)	stream of nitrogen	GC/MS	Fischeret al., 2021
Araneus diadematus	females	Sex pheromones	Volatile compounds	x	headspace technique		x	GC/MS	Fischeret al., 2021
Argiope bruennichi	female, male	Identification	Cuticular lipids	Freeze in –25°C	DCM	1 h	at room temperature	GC/MS	Gerbaulet et al., 2022
Argiope bruennichi	female, male	Family-specific chemical profiles	Cuticular lipids	Freeze in –80°C	DCM	2 h (gentle shaken)	at room temperature	GC/MS	Weiss & Schneider, 2021
Argiope bruennichi	female, male	Semiochemicals	Volatile compounds	x	headspace technique		x	GC/MS	Chinta et al., 2010
Argyrodes elevatus	female	Identification, sex recognition cues	Cuticular lipids	immobilized on ice	DCM	no data	no data	GC/MS	Chinta et al., 2016
Brachypelma albopilosa	female	Variations of cuticular lipids	Cuticular lipids	x	DCM-pentane (3:7)	5 min	stream of nitrogen	GC/MS	Trabalon, 2011
Cosmophasis bitaeniata	female, male	Mimicry cuticular lipids	Cuticular lipids	no data	hexane	10 min	stream of nitrogen	GC/MS	Allan et al., 2002
Latrodectus geometricus	female, juveniles	Identification	Cuticular lipids	no data	hexane	10 min (ultrasonic bath)	fume hood	GCxGC/MS	Guimaraes et al., 2018
Latrodectus geometricus	female, juveniles	Variations in cuticular lipids	Cuticular lipids	no data	hexane	30 min (ultrasonic bath)	fume hood	GC/MS	Guimaraes et al., 2016
Loxosceles reclusa	female, male, juveniles	Identification	Volatile compounds	x	SPME		x	GC/MS	Foulks et al., 2021
Pholcus beijingensis	female, male	Sex pheromones	Cuticular lipids	no data	DCM	24 h	no data	GC/MS	Xiao et al., 2010
Plectreurys tristis	female, male, juveniles	Identification	Volatile compounds	x	SPME		x	GC/MS	Foulks et al., 2021
Stegodyphus lineatus	female, male	Kin recognition cues	Cuticular lipids	Freeze in –18°C	pentane	5 min	fume hood	GC/MS	Grinsted et al., 2011
Tegenaria atrica	female	Effect of hormone on pheromones	Cuticular lipids	no data	pentane	15 min	stream of nitrogen	GC/MS	Trabalonet al., 2005
Tegenaria atrica	female	Variations of fatty acids	Cuticular lipids	no data	pentane	10 min	stream of nitrogen	GC/MS	Pourié et al., 2005
Tegenaria atrica	female	Variations of cuticular lipids	Cuticular lipids	no data	pentane	15 min	stream of nitrogen	GC/MS	Prouvost et al., 1999
Tegenaria atrica	female	Variations of cuticular lipids	Cuticular lipids	no data	pentane	15 min	stream of nitrogen	GC/MS	Trabalonet al., 1996
Tegenaria domestica	female	Sex recognition cues	Cuticular lipids	cold anesthetized 4°C 15 min	pentane	15 min	stream of nitrogen	GC/MS	Trabalonet al., 1997
Tegenaria pagana	female	Sex recognition cues	Cuticular lipids	cold anesthetized 4°C 15 min	pentane	15 min	stream of nitrogen	GC/MS	Trabalon et al., 1997
Tetragnatha extensa	female, male	Species recognition cues	Cuticular lipids	CO2	DCM	30 min	no data	GC/MS	Adams et al., 2021
Tetragnatha versicolor	female, male	Species recognition cues	Cuticular lipids	CO2	DCM	30 min	no data	GC/MS	Adams et al., 2021

components. Furthermore, unlike other methods that may induce stress in spiders, freeze killing is relatively quick. On the other hand, there are some concerns associated with the efficiency of lipid extraction. Specifically, after removing a dead spider from a freezer, water condenses on its body may affect lipid extraction using an organic solvent due to the formation of a barrier between the cuticle and the solvent.

An alternative method involves anesthetizing the spider, which can be achieved by either subjecting it to cold temperature (at 4°C) or CO_2 (Trabalon et al., 1997; Chinta et al., 2010; Adams et al., 2021). These methods are also rapid, safe and humane. In comparison to other methods, CO_2 anaesthesia can be advantageous in situations where water interfering with the extraction process needs to be minimized. However, the necessity for immediate extraction after death to prevent the degradation of biological tissues, may pose a logistical challenge.

Furthermore, it is worth considering alternative extraction methods, particularly in the case of endangered species where preserving the spider is essential. For instance, cuticular lipids can be extracted without causing harm by swabbing with sterile cotton previously moistened in an organic solvent. Following swabbing, the cotton is soaked in an organic solvent to extract the cuticular lipids (Trabalon, 2011). However, this method may result in uneven lipid extraction and hinder a precise quantitative analysis.

Extraction and analysis

Extraction facilitates the isolation of cuticular lipids and their subsequent identification. Spiders are typically subjected to an organic solvent or a mixture of organic solvents during the extraction process. Frequently utilized solvents include dichloromethane (Xiao et al., 2010; Chinta et al., 2016; Chen et al., 2018; Adams et al., 2021; Fischer et al., 2021; Weiss & Schneider, 2021; Gerbaulet et al., 2022), n-pentane (Trabalon et al., 1996, 1997, 2005; Bagnères et al., 1997; Pasquet et al., 1997; Grinsted et al., 2011; Pourié et al., 2005; Prouvost et al., 1999) and n-hexane (Guimarães et al., 2016, 2018; Allan et al., 2002), either individually or in combination, e.g. dichloromethan-pentane (3 : 7, v/v) (Trabalon, 2011). All solvents used for extraction have a low polarity, which makes them particularly suitable for isolating lipid components from the cuticle. However, it is essential to acknowledge that dichloromethane, despite falling within the category of low-polarity solvents, has a relatively high polarity when compared to n-alkane solvents. This distinction results in subtle variations in the outcome of the extraction. Dichloromethane has a greater affinity for lipids such as fatty acids and alcohols, while n-pentane and n-hexane have a preferential affinity for aliphatic hydrocarbons like alkanes (Rose et al., 2021; Simoes et al., 2022). Consequently, when selecting a solvent, taking into consideration toxicity and cost, researchers must also consider the specific type of lipid to be used.

The duration of extraction depends on the solvent and specific research objectives. For instance, for dichloromethane extraction it can range from 3 min with gentle shaking of the biological material (Fischer et al., 2021) up to 24 h (Xiao et al., 2010). Pentane extraction generally lasts 15 min (Trabalon et al., 1996, 1997, 2005; Bagnères et al., 1997; Pasquet et al., 1997; Prouvost et al., 1999), while hexane extraction takes 10 to 30 min with the assistance of an ultrasonic bath for enhancing the efficiency of the process (Guimarães et al., 2016, 2018; Allan et al., 2002). Determining the optimal extraction time is not a trivial problem. A too brief duration may result in an insufficient extraction of certain lipids, especially volatile hydrocarbons. Conversely, an extraction time exceeding one hour introduces ambiguity regarding whether the lipids originate exclusively from the cuticle or if some have been extracted from internal tissues and haemolymph. The attainment of equilibrium during extraction varies for different types of hydrocarbons and establishing a universal trend is challenging. Nevertheless, maintaining the extraction time below one hour is advantageous as it significantly mitigates the risk of internal lipid contamination (Belenioti et al., 2022).

Following lipid extraction, the solvent is evaporated, typically by drying samples in a stream of nitrogen (Bagnères et al., 1997; Trabalon, 2011). However, this method poses a risk of inadvertently losing volatile lipids due to the high gas exchange rate. Alternatively, researchers use evaporation within a fume hood (Grinsted et al., 2011; Guimarães et al., 2018) or at ambient room temperature (Weiss & Schneider, 2021; Gerbaulet et al., 2022).

Another approach to extracting external lipids, particularly volatile compounds, involves a headspace technique. Chinta et al. (2010) utilized this method for adsorbing volatiles from *A. bruennichi* using an activated charcoal filter. The volatiles adsorbed on the charcoal filter are then extracted with dichloromethane and analysed using GC-MS (Chinta et al., 2010). Another headspace technique for extracting volatile compounds is Solid Phase Microextraction (SPME), which is fast and solvent-free. Nowadays, SPME is the most frequently used method in research on headspace volatile compounds due to its simplicity, sensitivity and compatibility with the basic equipment used for chemical analyses like HPLC, LC-MS, GC and GC-MS (Cerkowniak et al., 2013; Ferreira-Caliman et al., 2014; Reitz et al., 2015).

Derivatization is frequently used in lipid analysis, which involves converting analytes into derivatives to enhance stability under chromatographic conditions. Silylation, employing *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA), is a common derivatization method. While derivatization has been used in arachnid research, particularly for silk lipid extracts (Schulz, 2001; Jerhot et al., 2010; Ruhland et al., 2017), not all studies on the lipids of spiders, including volatile cuticular lipids, have incorporated this step. The duration and temperature conditions for this process varies: 60 min at 60°C in studies by Ruhland et al. (2017) and Chinta et al. (2010), 60 min at 50°C in those of Jerhot et al. (2010) and 30 min at 50°C in the study of Schulz (2001). The time range between 30 and 60 min is consistently demonstrated to be adequate for achieving complete silylation of targeted lipids.

Gas chromatography combined with mass spectrometry (GC-MS) is the prevailing method for analysing lipid extracts in studies on arachnids, as it effectively separates complex mixtures and identifies individual compounds. Guimarães et al. (2018) pioneered the use of comprehensive two-dimensional gas chromatography (GCxGC-MS) to assess the cuticular chemical profile and demonstrated its effectiveness in identifying spider lipids.

COMPOSITION OF CUTICULAR LIPIDS

The first study on the cuticle lipid layer of spiders dates back to 1942, and was on the exocuticle and endocuticle of *T. atrica* spiders using a light microscope (Browning, 1942). Nearly four decades later, the cuticle of the spider *Latrodectus hesperus* was examined using scanning electron microscopy (SEM) (Hadley, 1981). However, this investigation focuses solely on elucidating the fine structure of the cuticle, not its lipid composition.

A breakthrough in the analysis of spider cuticular lipids occurred in 1996 when *T. atrica* spiders underwent the first-ever examination using gas chromatography-mass spectrometry (GC-MS). Subsequently, this research was extended to the cuticular lipid composition of nine spider families: Agelenidae, Araneidae, Eresidae, Heliophaninae, Pholcidae, Plectreuridae, Sicariidae, Tetragnathidae, Theraphosidae and Theridiidae. The lipids identified for each species of spider are listed in Table 3.

For the Agelenidae, there is information for three synanthropic species belonging to the genus *Tegenaria*: *T. atrica, T. domestica* and *T. pagana*. The initial study on cuticular lipids of *T. atrica* reports changes that occur during the gregarious period and post-dispersal of juveniles (Trabalon et al., 1996). The extract contains 75 compounds, predominantly hydrocarbons (51% n-alkanes) of from 17 to 39 carbon atoms, then 7 methyl esters and 4 carboxylic acids: palmitic acid, octadecenoic acid and two octadecenodioic acids, with notable differences between the different age groups. The principal constituents are n-alkanes ranging in carbon chain length from C25 to C29. Consistent lipid composition is reported in subsequent investigations on *T. atrica*, which report variations linked to sexual receptivity (Prouvost et al., 1999) and the effect of the ecdysteroid hormone 20-hydroxyecdysone (Trabalon et al., 2005).

T. domestica and *T. pagana* have similar lipid compositions to *T. atrica*, with the addition of cholesterol. However, there are differences in the quantities of methyl esters and dimethyloalkanes (Trabalon et al., 1997). *T. domestica* has a higher methyl ester content (2.4% vs. 0.4%) and lower dimethyloalkane content (9.1% vs. 18.5%) than *T. pagana*. Hydrocarbons, mainly n-alkanes, constitute the majority in both species.

In pursuit of the identity the sex pheromones of *Allagelena difficilis* (Agelenidae), a study by Chen et al. (2018) using GC-MS compares whole-body extracts from males, virgin females and mated females. Five carboxylic acids are unique to the female extract and among them, four compounds: palmitic acid, linoleic acid, cis-vaccenic acid and stearic acid, are identified as female sex pheromones (Table 1).

Currently, the cuticular lipids of two species of spiders belonging to the Araneidae have been studied. The study of Chinta et al. (2010) on *Argiope bruennichi,* using the headspace technique, reports volatile compounds. The significant sex-specific semiochemicals are hexacosane, heptacosane and nonacosane in females, and heptacosane and 2-methyloctacosane in males. In addition, this study reports the specific female sex pheromone (Table 1). The study of Weiss & Schneider (2021) on the cuticular lipids of *A. bru-*

Table 3. Group of lipids recorded in cicle of different species of spider.

Species	Lipid class										Most abundant group of lipids	References
	Hydrocarbons											
	n-alkanes	methyl alkanes	alkenes	carboxylic acids	esters	alcohols	aldehydes	ketones	methyl ethers	cholesterol		
Allagelena difficilis				+							carboxylic acids	Chen et al., 2018
Anelosimus eximius	+	+		+	+					+	propyl esters	Bagnères et al., 1997, Pasquet et al., 1997
Araneus diadematus	+	+			+	+					methyl alkanes	Fisher et al., 2021
Argiope bruennichi	+	+		+	+	+					wax esters	Gerbaulet 2022; Weiss & Schneider, 2021
Argyrodes elevatus		+			+	+				+	wax esters	Chinta et al., 2016
Brachypelma albopilosa	+			+	+						methyl esters	Trabalon, 2011
Cosmophasis bitaeniata	+	+									methyl alkanes	Allanet al., 2002
Latrodectus geometricus	+	+	+								methyl alkanes	Guimaraes et al., 2016, 2018
Pholcus beijingensis	+		+	+			+	+			aldehydes	Xiao et al., 2010
Stegodyphus lineatus	+	+									hydrocarbons	Grinsted et al., 2011
Tegenaria atrica	+	+		+	+						n-alkanes	Trabalon et al., 1996, 2005; Prouvost et al., 1999; Pourié et al., 2005
Tegenaria domestica	+	+		+						+	n-alkanes	Trabalon et al., 1997
Tegenaria pagana	+	+		+						+	n-alkanes	Trabalon et al., 1997
Tetragnatha extensa									+		methyl ethers	Adams et al., 2021
Tetragnatha versicolor									+		methyl ethers	Adams et al., 2021

ennichi reports 87 lipids in females and 37 in males. Major compounds include 4-methyltriacontane, 4-methyloctacosane and pentacosane. This study demonstrates that differences in chemical composition, especially in wax esters, influences individual recognition between sexes. Gerbaulet et al. (2022), on the other hand, reports the novel finding in arthropods of bishomomethyl-branched esters in *A. bruennichi*. Here, tridecyl 2,4-dimethylnonadecanoate is the primary component in both sexes.

Fischer et al. (2021) report sulcatone as a potential sex pheromone of female *A. diadematus* (Table 1). Composition of cuticular lipids varies with stage of moulting in terms of differences in long-chained methyl alkanes and n-alkanes. There is total of 55 compounds in the cuticular lipids of *A. diadematus*, which mainly consist of long-chained methyl alkanes, n-alkanes, alcohols and esters.

The cuticular lipids of the salticid spider *Cosmophasis bitaeniata* consist of 1 n-alkane (C27) and 13 methyl and dimethyl branched alkanes, which is similar to the chemical profile of *Oecophylla smaragdina*, an aggressive species of ant (Allan et al., 2002). This mimicry enables spiders to blend seamlessly with ants in their foraging columns and forage for food unhindered by the ants.

The cuticular lipids of the males and females of the pholcid spider, *Pholcus bijingensis,* which belongs to a widely distributed family, contain 20 compounds (Xiao et al., 2010). Major components include aldehydes (hexadecanal and tetradecanal) and a fatty acid (hexadecenoic acid). One unique compound, (Z)-9-tricosene, is present only in extracts of adult males. Electroantennography (EAG) and behavioural tests confirm it is the first male sex pheromone to be reported for spiders (Table 1).

There is a study on the intraspecific and interspecific chemical profiles of female and male *Loxosceles reclusa,* belonging to the Sicariidae and an interspecific comparison with *Plectreurys tristis* belonging to the Plectreuridae family (Foulks et al., 2021). A total of 17 chemicals are identified and quantified for *L. reclusa*, with 11 compounds successfully characterized. These compounds include carboxylic acids, aldehyde, terpenes and aromatic hydrocarbons, with 3-methylbutanoic acid, 2-methylbutanoic acid and butanoic acid major constituents.

In a study on *Tetragnatha extensa* and *T. versicolor,* belonging to the Tetragnathidae, using GC-MS, body lipids are species recognition cues and long-chain methyl ethers are species recognition cues in this genus (Adams et al., 2021). The 8,14,20-trimethylnonacosyl methyl ether is present in both species. In addition, in *T. extensa* there is 12,20-dimethylnonacosyl methyl ether and 6,14,20-trimethylnonacosyl methyl ether, and in *T. versicolor* only 14,20-dimethylnonacosyl methyl ether.

The composition of the cuticular lipids of *Brachypelma albopilosa*, a species belonging to Theraphosidae, is known (Trabalon, 2011). It consists of three lipid groups: 4 fatty acids, 4 methyl esters and 19 saturated hydrocarbons. Notably, the most abundant are methyl ester methyl palmitate, methyl stearate, methyl linoleate and methyl oleate, which make up 54% of the extract. The primary hydrocarbons are pentacosane, heptacosane and nonacosane, making up 26% and palmitic, stearic, linoleic and oleic acids collectively making up 20% of the extract.

A study of *Anelosimus eximius*, one of 15 species of social spiders, belonging to Theridiidae, reports differences in the chemical profiles of the colonies (Pasquet et al., 1997). A GC-MS analysis indicates the presence of 86 compounds, with 81 identified, including 31 esters, 47 saturated hydrocarbons, 2 carboxylic acids and cholesterol. Esters, predominantly propyl esters, make up 80% of the extract, while hydrocarbons account for 11%. Carboxylic acids and cholesterol make up 7% and 1.6%, respectively. Another study on *A. eximius*, specifically on identifying compounds, mainly propyl esters, which are notably abundant in the cuticles of spiders (Bagnères et al., 1997) reports 85 compounds, with the key components of the extract being propyl 4,20- and 4,30-dimethylhentriacontanoate (22.7%), propyl 6,20- and 6,30-dimethylhentriacontanoate (12.0%) and propyl 18- and 28-methylnonacosanoate (9.8%).

There are also sex-specific differences in the cuticular lipids of *Argyrodes elevatus*, a kleptoparasitic spider, belonging to the Theridiidae, (Chinta et al., 2016). The extract from females contain four major compounds, making up 77% of the extract: 2,8-dimethylundecyl 2,8-dimethylundecanoate (28.0%), heptadecyl 4-methylheptanoate (26.2%), 2-methylundecyl 2,8-dimethylundecanoate (11.3%) and 14-methylheptadecyl 4-methylheptanoate (10.3%), whereas in that from males the main compound, undecyl 2-methyltridecanoate, makes up 76%. Over 97% of the extracts consist of esters, along with hydrocarbons, alcohols, cholesterol and acetates.

There are changes in the composition of the cuticular lipids of *Latrodectus geometricus* (Theridiidae) associated with changes in their agonistic behaviour towards juveniles at different stages in their development (Guimarães et al., 2016). In the extract n-alkanes and methyl alkanes make up nearly 70%, with the n-alkanes C_{24}, C_{25}, C_{27} and C_{28} the most abundant. Results of subsequent studies, using GCxGC-MS, indicate that the composition of the cuticular lipids differs on different parts of the body of this spider (Guimarães et al., 2018). Of the 50 compounds identified, 41 are on the abdomen, 22 on the cephalothorax and 13 on the legs, with methyl-branched alkanes being the main constituents and the composition of the cuticular lipids on the abdomen the most diverse. That the highest number of compounds is associated with the abdomen may be attributed to the presence of ovaries and spinnerets, which produce chemical cues and sex pheromones. These findings indicate that the abdomen has an important role in both intra and interspecific chemical communication and understanding the behavioural interactions of spiders.

CONCLUSIONS

In summary, the results of studies on the cuticular lipids of spiders has revealed a diverse chemical landscape in the various spider families. Notable variations exist in the types and quantities of cuticular lipids between species and their compositions are intricate and species-specific. This diversity reflects the unique chemical strategies of spiders for communicating and their ecological interactions.

The extraction and analysis of cuticular lipids of spiders present a multifaceted challenge, with the different methods yielding different outcomes. Despite the different solvents and extraction durations employed, determining the most suitable method is challenging. The composition of cuticular lipids is consistently very variable with no discernible patterns. The ongoing exploration of techniques, including novel approaches like GCxGC-MS and SPME, however, are contributing to our understanding of the composition of the cuticular lipids of spiders and improving the methodology.

Despite the extensive research on insect cuticular chemistry, spider cuticles have received relatively little attention, but could reveal some interesting chemicals with various uses. Cuticular lipids, with antimicrobial properties, might be potential bio fungicides or pharmaceuticals. In addition, understanding the chemical communication system, including the intricate language of pheromones, could aid in devising traps for managing spiders and addressing potential threats to human health (Kačániová et al., 2022). Furthermore, a better understanding of spider behaviour, including communication based on cuticular lipids could be used to manage spider populations in natural environments. Given the predatory nature of spiders, which contributes to the suppression of insect populations and the maintenance of ecological balance, the regulation of insect numbers, particularly crop pests, could be facilitated. This ecological service, along with the potential use of lipids as bio fungicides may reduce the reliance on chemical pesticides and promote sustainable pest management practices. Thus, the study of the role cuticular lipids in the behaviour of spiders not only satisfies scientific curiosity but also has practical implications for conservation and pest control.

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REFERENCES

Adams S.A., Gerbaulet M., Schulz S., Gillespie R.G. & Uhl G. 2021: Chemical species recognition in a *Tetragnatha* spider (Araneae: Tetragnathidae). — *J. Chem. Ecol.* **47**: 63–72.

Allan R.A., Capon R.J., Brown V.W. & Elgar M.A. 2002: Mimicry of host cuticular hydrocarbons by salcid spider *Cosmophasis bitaeniata* that preys on larvae of tree ants *Oecophylla smaragdina*. — *J. Chem. Ecol.* **28**: 835–848.

Bagnères A.G. & Blomquist G.J. 2010: *Insect Hydrocarbons Biology, Biochemistry and Chemical Ecology*. Cambridge University Press, New York, 492 pp.

Bagnères A.G., Trabalon M., Blomquist G.J. & Schulz S. 1997: Waxes of the social spider *Anelosimus eximius* (Araneae, Theridiidae): abundance of novel n-propyl esters of long-chain methyl-branched fatty acids. — *Arch. Insect Biochem. Physiol.* **36**: 295–314.

Barabasz B. & Górz A. 1998: *Argiope bruennichi* (Scopoli, 1772) – rare and poorly studied spider species in Poland. — *Fragm. Faunist.* **41**: 255–267 [in Polish].

Barbero F. 2016: Cuticular lipids as a cross-talk among ants, plants and butterflies. — *Int. J. Mol. Sci.* **17**: 1966, 19 pp.

Baruffaldi L. 2016: *Function and Diversity of Sex Pheromones in Representative Species of the Black Widow Spiders (Genus:* Latrodectus*, Araneae: Theridiidae).* PhD Thesis, University of Toronto, 198 pp.

Batalha M.M.C., Goulart H.F., Santana A.E.G., Barbosa L.A.O., Nascimento T.G., da Silva M.K.H., Dornelas C.B. & Grillo L.A.M. 2020: Chemical composition and antimicrobial activity of cuticular and internal lipids of the insect *Rhynchophorus palmarum*. — *Arch. Insect Biochem. Physiol.* **105**: e21723, 15 pp.

Belenioti M., Roditakis E., Sofiadis M., Fouskaki M., Apostolaki M. & Chaniotakis N. 2022: Effect of solvent extraction time on the hydrocarbon profile of *Drosophila suzukii* (Diptera: Drosophilidae) and behavioural effects of 9-pentacosene and dodecane — *Eur. J. Entomol* **119**: 232–241.

Bojke A., Tkaczuk C., Bauer M., Kamysz W. & Gołębiowski M. 2020: Application of HS-SPME-GC-MS for the analysis of aldehydes produced by different insect species and their antifungal activity. — *J. Microbiol. Meth.* **169**: 105835, 7 pp.

Browning H.C. 1942: The integument and moult cycle of *Tegenaria atrica* (Araneae). — *Proc. R. Soc. (B)* **131**: 65–86.

Bryan S.A., Vink C.J., Barratt B.I.P., Seddon P.J. & van Heezik Y. 2019: Investigation of two new putative pheromone components of the invasive Australian redback spider, *Latrodectus hasseltii*, with potential applications for control. — *N. Z. J. Zool.* **46**: 189–200.

Cerkowniak M., Puckowski A., Stepnowski P. & Gołebiowski M. 2013: The use of chromatographic techniques for the separation and the identification of insect lipids. — *J. Chromatogr. (B)* **937**: 67–78.

Chen Z.W., Zhao Y.F., He S.C., Liu K.K., Liu J.H. & Xiao Y.H. 2018: Particular levels of odors released by virgin females attract conspecific males of the funnel-web spider *Allagelena difficilis*. — *J. Chem. Ecol.* **44**: 905–914.

Chinta S.P., Goller S., Lux J., Funke S., Uhl G. & Schulz S. 2010: The sex pheromone of the wasp spider *Argiope bruennichi*. — *Angew. Chem. (Int. Ed. Engl.)* **49**: 2033–2036.

Chinta S.P., Goller S., Uhl G. & Schulz S. 2016: Identification and synthesis of branched wax-type esters, novel surface lipids from the spider *Argyrodes elevatus* (Araneae: Theridiidae). — *Chem. Biodivers.* **13**: 1202–1220.

Cuber P., Urbanek A., Naczk A., Stepnowski P. & Gołębiowski M. 2016: Seasonal changes in the fatty acid profile of the tick *Ixodes ricinus* (Acari, Ixodidae). — *Exp. Appl. Acarol.* **69**: 155–165.

El-Shafie H.A.F. & Faleiro J.R. 2017: Semiochemicals and their potential use in pest management. In Shields V.D.C. (ed.) *Biological Control of Pest and Vector Insects*. InTech, London, pp. 3–22.

Ferreira-Caliman M.J., Andrade-Silva A.C.R., Guidetti-Campos M.C., Turatti I.C.C, Do Nascimento F.S. & Lopes N.P. 2014: A non-lethal SPME method for insect cuticular analysis by GC-MS. — *Anal. Methods* **6**: 8823–8828.

Fischer A. 2019: Chemical communication in spiders – a methodological review. — *J. Arachnol.* **47**: 1–27.

Fischer A., Schulz S., Ayasse M. & Uhl G. 2021: Pheromone communication among sexes of the garden cross spider *Araneus diadematus*. — *Sci. Nat.* **108**: 38, 11 pp.

Fischer A., Gries R., Alamsetti S.K., Hung E., Torres A.C.R., Fernando Y., Meraj S., Ren W., Britton R. & Gries G. 2022:

Origin, structure and functional transition of sex pheromone components in a false widow spider. — *Commun. Biol.* **5**: 1156, 12 pp.

Foulks Z., Parks J., Stoecker W., Kristensen C., Hebets E.A. & Shi H. 2021: Identification and quantification of 11 airborne biochemicals emitted by the brown recluse and another primitive hunting spider using headspace solid-phase microextraction-GC/MS. — *Anal. Bioanal. Chem.* **413**: 6605–6615.

Gaskett A. 2007: Spider sex pheromones: Emmision, reception, structures and function. — *Biol. Rev.* **82**: 27–48.

Gerbaulet M., Möllerke A., Weiss K., Chinta S., Schneider J.M. & Schulz S. 2022: Identification of cuticular and web lipids of the spider *Argiope bruennichi*. — *J. Chem. Ecol.* **48**: 244–262.

Gołębiowski M., Sosnowska A., Puzyn T., Boguś M., Wieloch W., Włóka E. & Stepnowski P. 2014a: Application of two-way hierarchical cluster analysis for the identification of similarities between the individual lipid fractions of *Lucilia sericata*. — *Chem. Biodivers.* **11**: 733–748.

Gołębiowski M., Cerkowniak M., Urbanek A., Dawgul M., Kamysz W., Boguś M.I., Sosnowska D. & Stepnowski P. 2014b: Antimicrobial activity of untypical lipid compounds in the cuticular and internal lipids of four fly species. — *J. Appl. Microbiol.* **116**: 269–287.

González-Tokman D., Ruch J., Pulpitel T. & Ponton F. 2014: Cuticular antifungals in spiders: density and condition dependence. — *PLoS ONE* **9**(3): e91785, 6 pp.

Grinsted L., Bilde T. & D'Ettorre P. 2011: Cuticular hydrocarbons as potential kin recognition cues in a subsocial spider. — *Behav. Ecol.* **22**: 1187–1194.

Guimarães I.C., Cardoso C.A.L., Lima S.M., da Cunha Andrade L.H. & Antonialli W.F. Jr. 2016: Chemical signals might mediate interactions between females and juveniles of *Latrodectus geometricus* (Araneae: Theridiidae). — *Behav. Processes* **126**: 27–35.

Guimarães I.C., Cardoso C.A.L., Caramão E.B., Schneider J.K. & Antonialli W.F. Jr. 2018: Analysis of cuticular chemical profiles of *Latrodectus geometricus* (Araneae: Theridiidae) females and juveniles using GC×GC/QMS. — *CeN* **40**: e1, 13 pp.

Gurr M., Harwood J., Frayn K., Murphy D. & Michell R. 2016: *Lipids: Biochemistry, Biotechnology and Health. 6th ed.* Wiley-Blackwell, Chichester, 448 pp.

Hadley N.F. 1981: Fine structure of the cuticle of the black widow spider with reference to surface lipids. — *Tissue Cell* **13**: 805–817.

Jerhot E., Stoltz J.A., Andrade M.C.B. & Schulz S. 2010: Acylated serine derivatives: a unique class of arthropod pheromones of the australian redback spider, *Latrodectus hasselti*. — *Angew. Chem. Int. Ed.* **49**: 2037–2040.

Kačániová M., Terentjeva M., Kowalczewski P.Ł., Babošová M., Porhajašová J.I., Hikal W.M. & Fedoriak M. 2022: Bacteriota and antibiotic resistance in spiders. — *Insects* **13**: 680, 17 pp.

Kost C. 2008: Chemical communication. In Jorgensen S.E. & Fath B.D. (eds): *Encyclopedia of Ecology*. Elsevier, Oxford, UK, pp. 557–575.

Kullmann E. 1972: Evolution of social behavior in spiders (Araneae: Eresidae and Theridiidae). — *Am. Zool.* **12**: 419–426.

Lockey L. 1988: Lipids of the insect cuticle: origin, composition and function — *Comp. Biochem. Physiol. (B)* **89**: 595–645.

Muller C.H.G., Ganske A.S. & Uhl G. 2020: Ultrastructure of chemosensory tarsal tip-pore sensilla of *Argiope* spp. Audouin, 1826 (Chelicerata: Araneae: Araneidae). — *J. Morphol.* **281**: 1634–1659.

Ortiz-Urquiza A. & Keyhani N. 2013: Action on the surface: Entomopathogenic fungi versus the insect cuticle. — *Insects* **4**: 357–374.

Papke M., Schulz S., Tichy H., Gingl E. & Ehn R. 2000: Identification of a new sex pheromone from the silk dragline of the tropical wandering spider *Cupiennius salei*. — *Angew. Chem. (Int. Ed.)* **39**: 4339–4341.

Pasquet A., Trabalon M., Bagnères A.G. & Leborgne R. 1997: Does group closure exist in the social spider *Anelosimus eximius*? Behavioural and chemical approach. — *Insectes Soc.* **44**: 159–169.

Paszkiewicz M., Gołębiowski M., Sychowska J., Boguś M.I., Włóka E. & Stepnowski P. 2016: The effect of the entomopathogenic fungus *Conidiobolus coronatus* on the composition of cuticular and internal lipids of *Blatta orientalis* females. — *Physiol. Entomol.* **41**: 111–120.

Pourié G., Ibarra F., Francke W. & Trabalon M. 2005: Fatty acids mediate aggressive behavior in the spider *Tegenaria atrica*. — *Chemoecology* **15**: 161–166.

Prouvost O., Trabalon M., Papke M. & Schulz S. 1999: Contact sex signals on web and cuticle of *Tegenaria atrica* (Araneae, Agelenidae). — *Arch. Insect Biochem. Physiol.* **40**: 194–202.

Reitz M., Gerhardt H., Schmitt C., Betz O., Albert K. & Lammerhofer M. 2015: Analysis of chemical profiles of insect adhesion secretions by gas chromatography-mass spectrometry. — *Anal. Chim. Acta* **854**: 47–60.

Rose A., Jaczynski J. & Matak K. 2021: Extraction of lipids from insect powders using a one-step organic solvent extraction process. — *Future Foods* **4**: 100073, 5 pp.

Ruhland F., Schulz S. & Trabalon M. 2017: Variations of cocoon external lipids during wolf spiderlings' development. — *J. Comp. Physiol. (A)* **203**: 819–829.

Schulz S. 1997: The chemistry od spiders toxins and spider silk. — *Angew. Chem. (Int. Ed.)* **36**: 314–326.

Schulz S. 2001: Composition of the silk lipids of the spider *Nephila clavipes*. — *Lipids* **36**: 637–647.

Schulz S. 2009: Semiochemistry of spiders. In Carde R.T. & Millar J.G. (eds): *Advances in Insect Chemical Ecology*. Cambridge University Press, New York, pp. 110–150.

Schulz S. 2013: Spider pheromones – a structural perspective. — *J. Chem. Ecol.* **39**: 1–14.

Schulz S. & Toft S. 1993: Identification of a sex pheromone from a spider. — *Science* **260**: 1635–1637.

Scott C., McCann S., Gries R., Khaskin G. & Gries G. 2015: N-3-methylbutanoyl-o-methylpropanoyl-L-serine methyl ester – pheromone component of western black widow females. — *J. Chem. Ecol.* **41**: 465–472.

Sharma A., Sharma S. & Yadav P.K. 2023: Entomopathogenic fungi and their relevance in sustainable agriculture: A review. — *Congent Food Agric.* **9**: 2180857, 21 pp.

Simoes R., Miranda I. & Pereira H. 2022: The influence of solvent and extraction time on yield and chemical selectivity of cuticular waxes from *Quercus suber* leaves. — *Processes* **10**: 2270, 12 pp.

Sponner A., Vater W., Monajembashi S., Unger E., Grosse F. & Weisshart K. 2007: Composition and hierarchical organization of a spider silk. — *PloS One* **2**(10): e998, 8 pp.

Suter R., Shane C.M. & Hirscheimer A.J. 1987: Communication by cuticular pheromones in a Linyphiid spider. — *J. Arachnol.* **15**: 157–162.

Tichy H., Gingl E., Ehn R., Papke M. & Schulz S. 2001: Female sex pheromone of a wandering spider (*Cupiennius salei*): Identification and sensory reception. — *J. Comp. Physiol. (A)* **187**: 75–78.

Trabalon M. 2011: Agonistic interactions, cuticular and hemolymphatic lipid variations during the foraging period in spider females *Brachypelma albopilosa* (Theraphosidae). — *J. Insect Physiol.* **57**: 735–743.

Trabalon M. 2013: Chemical communication and contact cuticular compounds in spiders. In Nentwig W. (ed.): *Spider Ecophysiology*. Springer, Berlin, Heidelberg, pp. 125–140.

Trabalon M. & Bagnères A.G. 2010: Contact recognition pheromones in spiders and scorpions. In Blomquist G.J. & Bagnères A.G. (eds): *Insect Hydrocarbons Biology, Biochemistry, and Chemical Ecology*. Cambridge University Press, New York, pp. 344–374.

Trabalon M., Bagnères A.G., Hartmann N. & Vallet A.M. 1996: Changes in cuticular compounds composition during the gregarious period and after dispersal of the young in *Tegenaria atrica* (Araneae, Agelenidae). — *Insect Biochem. Mol. Biol.* **26**: 77–84.

Trabalon M., Bagnères A.G. & Roland C. 1997: Contact sex signals in two sympatric spider species, *Tegenaria domestica* and *Tegenaria pagana*. — *J. Chem. Ecol.* **23**: 747–758.

Trabalon M., Niogret J. & Legrand-Frossi C. 2005: Effect of 20-hydroxyecdysone on cannibalism, sexual behavior, and contact sex pheromone in the solitary female spider, *Tegenaria atrica*. — *Gen. Comp. Endocrinol.* **144**: 60–66.

Weiss K. & Schneider J.M. 2021: Family-specific chemical profiles provide potential kin recognition cues in the sexually cannibalistic spider *Argiope bruennichi*. — *Biol. Lett.* **17**: 202102, 7 pp.

Wirth M., Wolff J.O., Appel E. & Gorb S.N. 2019: Ultrastructure of spider thread anchorages. — *J. Morphol.* **280**: 534–543.

World Spider Catalog 2023: *World Spider Catalog. Ver. 24.5.* Natural Museum Bern, URL: http://wsc.nmbe.ch (last accessed 25 Sep. 2023).

Xiao Y.H., Zhang J.X. & Li S.Q. 2010: Male-specific (Z)-9-tricosene stimulates female mating behaviour in the spider *Pholcus beijingensis*. — *Proc. R. Soc. (B)* **277**: 3009–3018.

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