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Nanostructural and mechanical property changes to spider silk as a consequence of insecticide exposure

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HIGHLIGHTS

- Spiders are beneficial organisms on arable lands that insecticides affect adversely.
- We performed mechanical and nanostructural analyses on exposed spider silks.
- The insecticides affected spider silk mechanics, nanostructures and composition.
- The effects on silk and webs render the insecticides detrimental to spiders.

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ABSTRACT

Neonicotinoids are one of the world's most extensively used insecticides, but their sub-lethal influences on non-target and beneficial organisms are not well known. Here we exposed the orb web spider *Parawixia audax*, which is found on arable lands in Uruguay, to a sub-lethal concentration of the broad spectrum insecticide Geonex (thiamethoxam + lambda-cyhalothrin) and monitored their web building. We collected their major ampullate silk and subjected it to tensile tests, wide-angle X-ray diffraction (WAXS) analysis, and amino acid composition analysis. Around half of the exposed spiders failed to build webs. Those that built webs produced irregular webs lacking spiral threads. The mechanical properties, nanostructures, and amino acid compositions of the silk were all significantly affected when the spiders were exposed to insecticides. We found that silk proline, glutamine, alanine and glycine compositions differed between treatments, indicating that insecticide exposure induced downregulation of the silk protein MaSp2. The spiders in the control group had stronger, tougher and more extensible silks than those in the insecticide exposed group. Our WAXS analyses showed the amorphous region nanostructures became misaligned in insecticide exposed silks, explaining their greater stiffness. While the insecticide dose we subjected *P. audax* to was evidently sub-lethal, the changes in silk physicochemical properties and the impairment to web building will indelibly affect their ability to catch prey.

1. Introduction

Neonicotinoids and pyrethroids are broad spectrum, biodegradable, neurotoxic insecticides that are effective at eliminating insect pests such as aphids, whiteflies, plant-hoppers and thrips from arable lands (Asquith and Hull, 1973; Honda et al., 2006; Ishaaya et al., 2007; Elbert et al., 2008). Compared to many other insecticides these are less toxic to birds and mammals than insects (Tomizawa and Casida, 2005). Compounds such as imidacloprid, acetamiprid and thiamethoxam act by disrupting insect nicotinic acetylcholine receptor synaptic transmission within invertebrate central nervous systems (Tomizawa et al., 1995; Jones and Sattelle, 2010). Accordingly they adversely affect insect cognition, learning,

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orientation, decision making and feeding (Tomizawa et al., 1995). Due to their broad spectrum of efficacy and distinct mode of action, neonicotinoid and pyrethroid use by agriculturalists is expanding worldwide (Honda et al., 2006; Dai et al., 2010). In Uruguay use of these insecticides has increased exponentially of late due to the recent arrival of new crops, such as soybean (Ministry of Agriculture and Fisheries of Uruguay, 2013; Benamú et al., 2013; Lacava, 2014).

While neonicotinoids, pyrethroids and other insecticides effectively decrease pest populations in the short term, their continuous use may induce secondary environmental damage, loss of biodiversity, and interrupt ecological processes. Furthermore, they can negatively affect non-target invertebrates, including pollinators and the natural enemies of crop pests (Pisa et al., 2015; Michalko and Kosulic, 2016). Spiders, for instance, can be negatively affected by insecticide applications (Benamú, 1999; Sunderland, 1999; Landis et al., 2000; Symondson et al., 2002; Hoefler et al., 2006; Öberg et al., 2007). In addition to direct lethal effects (Pekar, 2013; Michalko and Kosulic, 2016), insecticides have sublethal effects on spiders, including various developmental, biochemical, physiological, and behavioural impairments (Landis et al., 2000; Symondson et al., 2002; Desneux et al., 2007; Benamú et al., 2007, 2013; Benamú, 2010; Pekar, 2013; Royaute et al., 2015).

All spiders secrete silk (Breslauer and Kaplan, 2012). Orb web spiders (Orbiculariae) have the most impressive silk toolkits, secreting up to seven types of silk (major and minor ampullate, tubuliform, aciniform, pyriform, aggregate and flagelliform silks) from specialized glands (Blackledge and Hayashi, 2006; Heim et al., 2009; Blamires et al., 2017). These silks may combine to perform specific functions as a component of the prey-catching web or as components of eggsac cocoons (Blamires et al., 2017). Of these silks, major ampullate silk (MAS), the silk comprising the supporting frame and radial treads of orb webs, has the most impressive properties, with a strength and toughness exceeding most high performing synthetic materials, even Kevlar® (Vollrath et al., 2013; Blamires et al., 2017).

MAS is hierarchically organized with a lipid and glycoprotein-rich skin layer covering a fibrous outer- and inner-core (Papadopoulos et al., 2009; Heim et al., 2010; Blamires et al., 2017). The core is composed of two types of proteins (conventionally called spidroins); MaSp1 (derived from Major ampullate Spidroin 1) and MaSp2 (Major ampullate Spidroin 2). These proteins arrange as ordered crystalline regions dispersed among disordered semi-crystalline and amorphous regions. The crystalline regions contain stacked pleated β–sheet nanostructures while the semi-crystalline and amorphous regions arrange as matrices of 3_{10}-helices, β-turns or β –spirals nanostructures depending on the amino acid composition of the silk (Jelinski, 1998; Sponner et al., 2007; Jenkins et al., 2013; Blamires et al., 2016).

MAS is secreted from the major ampullate gland, which consists of a tail, sac and duct region (Andersson et al., 2013; Rising and Johansson, 2015; Blamires et al., 2017). The spidroins are secreted into the tail of the major ampullate gland and stored in the sac as a solution called dope (Heim et al., 2009; Vollrath et al., 2013). The dope flows into the duct where biophysical actions induce the silk proteins to form the different nanostructures (Hagn et al., 2011; Schwarze et al., 2013). Unfortunately the energetic, enzymatic or other biochemical processes facilitating protein nanostructural formation are not well known. However, we know that the nanostructures and the subsequent mechanical properties of MAS are sensitive to variations in temperature and the spider's diet (Craig et al., 2000; Tso et al., 2005; Blamires et al., 2015), thus suggesting nanostructure formation is a metabolically costly process.

Here we performed an experiment exposing the South American orb web spider *Parawixia audax* (Araneae, Araneidae) to a sub-lethal concentration of a broad spectrum commercially available insecticide. We then performed chemical and physical measurements on their silks to test whether their mechanical properties, nanostructures and/or amino acid compositions changed as a consequence of exposure to the insecticides. We predicted that the biochemical and neurophysiological stresses induced by insecticide exposure will affect spinning processes and, as a consequence, induce variability in the mechanical properties, nanostructures and amino acid composition of the silk.

2. Material and methods

2.1. Spider collection and pre-treatment

We collected 60 adult female *P. audax* (body mass ≈ 0.1–0.2 g) from the Rivera region, Uruguay. We collected these spiders from elevated forests outside of arable land so they were free from any prior insecticide or pesticide exposure.

To ensure that all spiders used were of approximately equal size we measured each spider's body length to ±0.1 mm, using digital Vernier calipers (Caliper Technologies Corp., Mountain View, CA, USA), and mass to ±0.001 g, using an electronic balance (Ohaus Corp., Pine Brook, NY, USA) upon collection before placing them in 115 mm (wide) x 45 mm (high) plastic circular containers and returning them to the Centro Universitario de Rivera, in Rivera, Uruguay. Here they were individually placed in 60 mm (wide) x 15 mm (high) Petri® dishes and maintained at 25 ± 5° C, 75± 5% relative humidity, and a 12:12 h (L: D) photoperiod for 5 days. During this pre-experimentation phase the spiders were fed one laboratory reared *Tenebrio molitor* (Coleoptera, Tenebrionidae) daily.

2.2. Experiment

Immediately following the pre-experimentation phase all spiders were reweighed to ensure they were of approximately similar mass to that ascertained upon capture. They were then placed in wooden frames (25 cm × 20 cm x 5 cm) with front and back glass barriers, through which we could view the spiders, and randomly allocated into one of two treatment groups: (1) insecticide exposed, or (2) control. The frame dimensions approximated the maximum dimensions of *P. audax* webs in the field (ML, LFG, SJB pers. obs.) so did not inhibit web building. We considered the day that the spiders were moved into the frames as day one of experimentation. The experiment was terminated after day 15 of experimentation, since this is approximately the length of time it takes for nutrients fed to spiders to effectively influence spider silk properties (Townley et al., 2006; Blamires et al., 2014, 2015).

For our experiment we used the commercially available insecticide Geonex (Geonex Commercial Insecticides, Tafirel SA, Uruguay), a broad spectrum insecticide composed of two complementary active ingredients (the neonicotinoid thiamethoxan and the pyrethroid lambda-cyhalothrin). Thiamethoxam is a synthetic organic insecticide that is effective at controlling most sucking and chewing insect pests. It is delivered to a plant by foliar application and ingested by sucking/chewing insects while feeding. Lambda-Cyhalothrin acts in a similar way as thiamethoxam except it targets a broader array of insects. Spiders are likely to imbibe thiamethoxam and lambda-cyhalothrin in nature by consuming affected insects or exposure to aerosols.

We prepared a 1.41 mg l^{-1} (i.e. 5% of the maximum field registered nominal concentration) solution of the insecticide by dilution in analytical grade acetone, as is standard for insecticide exposure experiments. The exposed spiders received 1.0 μl of the solution