SHORT COMMUNICATION

The impact of UVA on the glycoprotein glue of orb-weaving spider capture thread from a diurnal and a nocturnal species (Araneae: Araneidae)

Sarah D. Stellwagen, Brent D. Opell and Mary E. Clouse: Department of Biological Sciences, Virginia Tech, Blacksburg, Virginia 24061, USA; E-mail: stellw@vt.edu

Abstract. We compared the effect of Ultraviolet A radiation on the adhesive droplets of the diurnal orb-web weaver Argiope trifasciata Forskål, 1775 and the nocturnal orb-web weaver Neoscona crucifera (Lucas, 1838). We hypothesized that glycoprotein glue within A. trifasciata droplets will either be unaffected or will benefit from UVA exposure, whereas the glycoprotein of N. crucifera will be degraded by UVA. In both species, the volume of fresh droplets did not differ from that of droplets that were exposed to UVA for four hours, or from the volume of droplets kept in the dark for four hours. This documented that UVA did not affect compounds that confer droplet hygroscopicity. Both dark and UVA treatments reduced the relative toughness of droplet glycoprotein, though the reductions were not statistically significant, with the dark treatment exhibiting a greater decrease in relative toughness. This study suggests that ecologically relevant levels of UVA exposure do not affect the glycoprotein glue of orb-weaver capture silk.

Keywords: Adhesion, biomaterials, toughness, silk, ultraviolet

Ultraviolet radiation (UVR) comes in several forms including UVA and UVB, the latter of which is more damaging, although more UVA enters the atmosphere than the other forms of UVR (Rizzo et al. 2011). Ultraviolet radiation induces cross-linking in proteins (Bhat & Karim 2009; Hu et al. 2013), and low doses may enhance spider silk performance by further aligning proteins, similar to the “improve-ment phase”, during which molecule alignment is hypothesized to continue after a silk strand is extruded (Aagnarsson et al. 2008). Non-adhesive major ampullate spider silk continues to improve mechanically after several hours of natural UVA exposure, hypothesized to be the result of free radical generation that induces cross-linking, however longer exposures do eventually result in degradation (Osaki 2004; Osaki & Osaki 2011; Perea et al. 2015). UVA at ~700 W/m² reduces the molecular weight of spider dragline silk from Nephila clavipes Linnaeus, 1767 more than 30% during the first hour, and more than 80% after 5 hours (Matsuhira et al. 2013). However the radiation intensity used was more than 20x the natural incident UVA striking spider webs in the summer. Osaki & Osaki (2011) demonstrated that ecologically relevant doses of UVA actually serve to mechanically strengthen dragline silk of Argiope bruennichi (Scopoli, 1772) while weakening that of Neoscona nautica (L. Koch, 1875).

The capture spiral threads of araneoid orb weaver webs differ from the dragline threads that form their radial and frame threads. These adhesive threads are composed of a pair of supporting axial flagelliform fibers that are covered by aqueous aggregate gland material. Originally deposited as a cylinder of dope, this material coalesces into regularly spaced droplets, each containing a glycoprotein glue core (Sahni et al. 2010, 2014). Inorganic salts and low molecular mass organic (LMMC) compounds within the aqueous layer that surrounds this glycoprotein core attract atmospheric moisture, causing droplet volume to fluctuate with environmental humidity (Edmonds & Vollrath 1992; Opell et al. 2013; Townley & Tillinghast 2013). We have shown that diurnal species preferring habitats that expose their webs to full or partial sun produce viscous threads with droplets that are not only resistant to degradation, but also contain glycoprotein that exhibits an increase in relative toughness when exposed to UVB (Stellwagen et al. 2015). This results in more work being required to extend these UVB exposed droplets. Species of the genus Argiope Audouin, 1826 (Orbiculariae), including A. trifasciata Forskål, 1775, are found in habitats where their webs are exposed to full sun. Others, such as Neoscona crucifera (Lucas, 1838), are nocturnal and forage from the center of their webs at night and monitor their webs from an adjacent cryptic retreat during the day. The web’s radial threads extend from the center like the spokes of a wheel and are largely responsible for absorbing the force of prey impact (Sensenig et al. 2012), whereas the prey capture spiral retains flying insects (Apstein 1889; Sekiguchi 1952; Sahni et al. 2014). The measure of a capture thread’s ability to overcome the efforts of a prey struggling to escape is the droplet’s toughness, the work required to extend a droplet (Sensenig et al. 2012). This is determined by the extent of droplet stretching (the droplet’s extensibility) and the force required to extend the droplet until it pulls from a surface, that is, to overcome the droplet’s adhesion.

To complement our previous UVB study (Stellwagen et al. 2015), we investigated the effects of UVA radiation on the performance of viscous glue droplets from A. trifasciata and N. crucifera (family Araneidae). We tested the hypothesis that the performance of droplets from the webs of the full-sun diurnal species, A. trifasciata, is enhanced by UVA exposure; whereas that of droplets from webs of the primarily nocturnal N. crucifera, is either not affected or is degraded by UVA. We did this by comparing several performance metrics. First, the duration of droplet extension before droplet pull-off, a measure of the time over which the work of extension occurs, and second, the angle of axial line deflection, a measure of the force on an extending droplet. Together, these metrics can be used to compute relative toughness, a measure of the energy required to extend a droplet to pull-off.

Fresh threads collected in the early morning (A. trifasciata) or late evening (N. crucifera), soon after they were spun, were compared with those that were aged in the dark for 4 hours and droplets that were exposed to 4 hours of UVA. We photographed each thread droplet prior to extension, permitting us to compare the effect of aging and UVA on droplet volume. This allowed us to test a conditional hypothesis that differences observed in droplet performance can be explained by the impact of UVA on droplet hygroscopicity through its effect on LMMC and salts in a droplet’s aqueous layer, a conclusion that would make it more difficult to ascribe UVA action to its effect on the droplet’s glycoprotein core.
Table 1.—Droplet length (l m), width (m3), extension phase times (seconds), relative toughness (N/m3), and log relative toughness for both species and treatments (¼ matched pair, WP = Total Loaded time). Droplet volume was computed as described in Liao et al. (2015).

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>(Log RT)</th>
<th>Droplet Extension (DE)</th>
<th>Loaded Time</th>
<th>Volume</th>
<th>MP</th>
<th>DE</th>
<th>Log RT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>A. trifasciata</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh</td>
<td>62.0</td>
<td>9.7</td>
<td>31.5 ± 6.2</td>
<td>24.1 ± 7.5</td>
<td>3.9 ± 2.5</td>
<td>4.8 ± 2.5</td>
<td>3.9 ± 2.5</td>
<td></td>
</tr>
<tr>
<td>UVA</td>
<td>58.1</td>
<td>7.5</td>
<td>31.3 ± 6.1</td>
<td>24.5 ± 7.1</td>
<td>3.5 ± 2.5</td>
<td>3.6 ± 2.5</td>
<td>3.5 ± 2.5</td>
<td></td>
</tr>
<tr>
<td><strong>N. crucifera</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh</td>
<td>32.0</td>
<td>10.8</td>
<td>23.4 ± 7.8</td>
<td>10.2 ± 11.4</td>
<td>9.237 ± 9.834</td>
<td>4.8 ± 2.5</td>
<td>3.9 ± 2.5</td>
<td></td>
</tr>
<tr>
<td>UVA</td>
<td>31.4</td>
<td>9.5</td>
<td>23.1 ± 8.8</td>
<td>10.3 ± 11.4</td>
<td>9.241 ± 9.830</td>
<td>4.8 ± 2.5</td>
<td>3.9 ± 2.5</td>
<td></td>
</tr>
</tbody>
</table>

Thread samples from webs constructed by 13 adult female A. trifasciata and 11 adult female N. crucifera were collected on and near the Virginia Tech campus in Blacksburg, Montgomery County, Virginia, USA, from 15 August to 25 September 2014. Each A. trifasciata web sample was collected between 05:30h and 08:30h and all images and videos captured by 16:00h the same day. Threads of N. crucifera webs were collected between 21:30h and 23:00h and their study was completed by 16:00h on the following day. Except for differences in irradiance described below, all methods and analyses are those described by Stellwagen et al. (2015).

Two 15.0 watt, 352 nm spectral peak UVA fluorescent tubes (F15T8BL; 440.4 mm length, 25.4 mm diameter; USHIO Inc., Cypress, CA, USA) were used to irradiate samples. Irradiance was measured using a photometer radiometer (Solar Light Co., Inc. PMA2200) equipped with a UVA detector (PMA2110, Glenside, Pennsylvania, USA) with a spectral sensitivity from 320-400 nm, calibrated traceable to the National Institute of Standards and Technology (NIST) on 18 August 2014. Threads were irradiated for 4 hours at ~13 W/m² (the maximum level produced by the UVA lamps), which is two-thirds the maximum level of full sunlight received in Blacksburg in late summer. Conditions in the dark treatment cylinder and the UV cabinet were recorded every 30 seconds for two hours by temperature/relative humidity data loggers (Hobo® model U23-002, Onset Computer Corp., Bourne, Massachusetts, USA). The dark treatment cylinder maintained ambient temperature at 24°C ± 0.13°C (mean ± SD) and relative humidity at 55% ± 1.3%; for the UV cabinet, the corresponding values were 24°C ± 0.08°C and 55% ± 1.7%.

We used JMP (SAS Institute, Cary, North Carolina) to analyze data and considered comparisons with ¼ ≤ 0.05 as significant. Shapiro-Wilk W tests were used to determine normality of the data (¼ ≥ 0.05). Normally distributed values were compared with matched pair t-tests. Non-normal values were log-transformed, and again tested for normality. Remaining non-normal values were compared using Wilcoxon pair tests. Each treatment value was compared to the fresh thread value, which served as the control. Our best gauge of similarity of control and treatment droplets was droplet length and width, which, within each species, were quite similar (Table 1).

Neither the dark nor the UVA exposure treatments had an effect on the droplet volumes of either species (Table 1). This failed to support the contending hypothesis that salts and LMMC in a droplet’s outer aqueous layer are affected by UVA exposure and indicate that any observed effect of aging or UVA exposure on droplet performance must be attributed to the effects of these treatments on the glycoprotein core within each droplet.

Total loaded time began when an axial line was at 180° and experienced no pull from a droplet, and ended when the droplet had extended and had either released from the contacting probe or had become so thin that it no longer exerted a measurable force on the axial line, which had returned to a 180° configuration. This time was divided into the pre-extension phase, before force on the droplet was sufficient to extend the droplet’s glycoprotein core, and the extension phase, during which the glycoprotein elongated (fig. 2 in Stellwagen et al. 2015). Extension times provided an index of the extensibility of the glycoprotein within droplets (Table 1). For the full sun species, A. trifasciata, droplet extension time was reduced by 29% after UVA exposure. For N. crucifera, extension time was unaffected by UVA exposure.

Plotting the force on an extending droplet against extension time depicts the performance of an extended droplet. The area under this curve represents relative toughness and is an index of the energy required to extend a droplet (Table 1, Fig. 1). Compared with fresh threads, the energy absorbed by threads of A. trifasciata and N. crucifera decreased 55% and 43%, respectively, after dark treatments and 45% and 31%, respectively, after UVA exposure. Neither treatment difference was significant and, although both treatment...
values were less than the control, there was no evidence to suggest that that UVA exposure degraded droplet performance beyond the aging that occurred during this time.

Retention time is important for prey capture success, and a reduction of only a few seconds can mean a lost feeding opportunity for an orb-weaving spider (Blackledge & Zevenbergen 2006). This study hypothesized that droplets from webs of the diurnal, full sun species *Argiope trifasciata* would be more likely to be positively affected by UVA than those from the nocturnal species *Neoscona crucifera*, or at least would be more resistant to UVA degradation. However, although droplets from *A. trifasciata* webs showed a reduction in droplet extension time after exposure to UVA, this did not affect the overall energy absorption of the droplet during its extension.

Interestingly, the relative toughness of both species’ droplets decreased more after dark exposure than after UVA exposure, although these differences were not statistically significant. Threads of both species experienced a 4-hour, dark aging treatment, however, for *A. trifasciata* this occurred approximately 3 hours after a web was constructed, but for *N. crucifera* it occurred approximately 11 hours after a web was constructed. This difference may explain the smaller decrease in energy absorption by *N. crucifera* droplets. This decrease in toughness with aging may indicate that the chemical cross-linking mechanism hypothesized to strengthen dragline silk (Osaki 2004) either does not affect the glycoprotein, or that higher doses of UVA not typically experienced by spider threads are needed to see this effect and sufficiently counteract the effect of aging. Similar to UVB, UVA does not appear to impact the LMMC in droplets, which supports the observation that these compounds resist degradation (Opell et al. 2015). Thus, our study suggests that ecologically relevant doses of UVA have little impact on spider capture spiral thread function.

**ACKNOWLEDGEMENTS**

Carlyle C. Brewster assisted with statistical analyses. Author contributions: S.D.S. collected and prepared thread samples, performed droplet extensions and image measurements, analyzed data, and prepared the manuscript and figures. B.D.O. designed and constructed the instrumentation used in this study, collected and helped prepare thread samples, and contributed to data analysis, and manuscript and figure preparation. M.E.C. assisted with droplet extension, image measurements, and data entry. Funding: Funds from the State Council for Higher Education for Virginia provided...
the digital camera used in this study. This study was supported by National Science Foundation grant IOS-1257719.

LITERATURE CITED


Manuscript received 13 November 2015, revised 25 April 2016.