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THERMAL CHARACTERIZATION OF SOCIAL VESPID SILK

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Abstract

In this paper we try to establish a link between the microclimate in the wasp nest and the structure and thermal stability of vespid silk. We suggest that there are at least two types of water that is absorbed by the silk of Oriental hornets, namely, surface water and intrinsic structural water. The release of both types of water was found to be reversible. The enthalpy values of the endothermic peaks associated with the release of water from different silk samples do not differ substantially and are in the range of 106 to 130 J g^{-1} for the *Vespa orientalis* male larvae silk (sample #1), *Paravespula germanica* (yellowjacket) worker larvae silk (#3) and *Vespa orientalis* nest envelope(#4). For the *Vespa orientalis* worker larvae silk (sample #2), however, it is twice as large (228 J g^{-1}). This is in agreement with the increased total amount of absorbed water. The silk studied has a fibrilar structure with interconnecting surfaces overlying entire regions. It is assumed that the initial water loss stems from water evaporation from the coat of the fibers - a daily occurrence in the hornets' nest. Heating to above 70°C may result in structural changes in the silk core.

Keywords: thermal characterization, vespid silk

Introduction

The social wasps and hornets belong to the very large group of insects that undergo a complete metamorphosis; that is, insects that have larval and pupal stages between the egg and adult stages. The mature larva, prior to pupate, encases itself in a silk weave and inserts in its home cell [14]. The pupating larva first spins a silk cap, which, inter alia, seals the cell entrance and ensures the presence of the larva inside the cell. The walls of the cells have slits that enable light and possibly water to pass between the cells [23]. The present study assessed the response to the heating and cooling of the pupal silk caps of workers and males of the Oriental hornet *Vespa orientalis*, of workers of the German wasp *Paravespula germanica* and also of the

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nest envelopes of *V. orientalis.* The investigation was intended to elucidate some of the known facts regarding microclimate in the nest, such as self-thermoregulation and the contribution of the silk produced by larvae to this phenomenon. The above-mentioned types of silk are secreted both by the pupating larvae as well as by the worker hornets, the latter building envelopes to protect the nest. The materials involved, including the fibers that hold the protective envelopes together, are secreted by the hornets, both larvae and adults; these materials settle between the inner structure of the nest and its external surface and serve to protect against parasites and predators [1] and guard against any drastic, undesirable climatic disparity between the external milieu and the interior of the nest [2–6]. In the case of the larvae, the silk provides a microclimate to enable the development of the adult. One of the important factors in the maintenance of a hornet or wasp society is the relative humidity. This high relative humidity (RH) is maintained always, day and night. Ordinarily, hornets or wasps obtain needed water from their food, inasmuch as they consume insects or pieces of mammalian flesh, but for purposes of construction, including that of envelopes, they collect water from sources near the nest. The pupating larvae, which coat themselves with silk, secrete not only the silk but also a certain amount of water. The role of this water, the manner of its evaporation and the evaporation forces require investigation. Differential scanning calorimetry (DSC), thermogravimetric analysis, scanning electron microscopy (SEM), transmission electron microscopy (TEM) and X-ray ddiffraction (XRD) are widely used to analyze such biological systems [7–10].

Experimental

Three types of vespid silk were studied, namely: #1, that of *V. orientalis* pupating male larvae; #2, that of *V. orientalis* pupating worker larvae; #3, that of *P. germanica* pupating worker larvae and #4 – nest envelopes material produced by *V. orientalis* adult workers [2]. The materials were collected from the natural habitats both in the Tel-Aviv metropolitan area as well as in the Ein-Gedi region as described previously [11]. The study entailed TG and DTA, DSC, SEM and XRD. Processing of the samples for characterization was undertaken on strips of silk caps removed from puparia in the natural nest combs. Simultaneous TG and DTA tests were carried out with a TA Instruments (USA) module SDT 2960. TG/DTA runs were recorded at a scan rate of 0.5°C min^{-1} up to 70 and 150°C. The sample compartment was flushed at all times with dried, ultra-high-purity (UHP) argon. DSC tests were carried out with TA Instruments module 2920, and runs were recorded at a scan rate of 1.0°C min^{-1} up to 150°C. A liquid-nitrogen cooling accessory (LNCA) was used for sub-ambient temperature measurements. Three samples of each material were analyzed and good reproducibility of the curves was observed. XRD data were obtained by the use of a Θ–Θ Scintag powder CuK_α radiation diffractometer equipped with a liquid nitrogen germanium solid-state detector. Films were mounted on background-free single crystal quartz slide and analyzed over a 2Θ range of 5 to 70°. A JSM-6300 scanning microscope (Jeol Co.) equipped with a Link elemental analyzer and a silicon detector was used for studying surface topology. Samples were vacuum-plated by 60 nm gold to avoid charging. Philips transmission electron microscope, type CM 100, operated at 60 kV was used to analyze these biological systems. The experimental details are described by Jongebloed *et al.* [12] and Rosenzweig [13].

Results and discussion

Figure 1 depicts the mass loss (*WL*-curve 1), DTA (curve 2) and the derivative of the mass loss (d*W*/d*t* – curve 3) TG, DTA and DTG curves for the sample #1. On being heated, the sample immediately starts to lose mass. The first sharp 1% *WL* is observed at 29°C, which coincidentally is the optimal nest temperature for social wasps [3]. The slope of this step is 0.67% $°C^{-1}$ and is accompanied by a sharp endothermic peak. The second *WL* proceeds at two disparate rates of 0.37 and 0.29% $°C^{-1}$ and this up to 35 and 46°C, respectively. A broad endotherm with t_{max} of 34.7°C characterizes this process. Further slow mass loss of sample #1 (0.1% $°C^{-1}$) continues up to 70°C without visible phase transitions. Total mass loss of the sample is 7.3%.

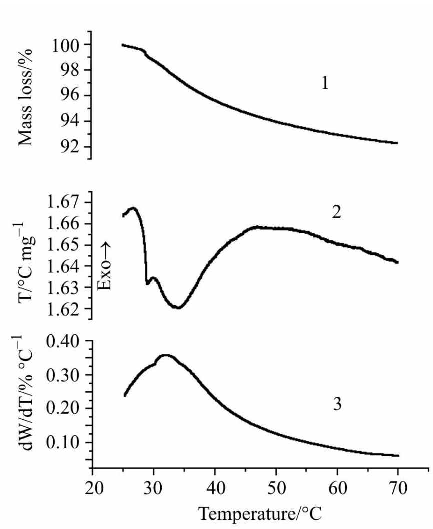

Fig. 1 TG, DTA and DTG curves of the silk of Oriental hornet male larvae #1; 1 – TG; 2 – DTA; 3 – DTG

Figure 2 shows comparative *WL* TG curves of the three silk types and the envelope. The TG and also the DTA curves for samples #1, 2 and 4 are similar and the total mass loss is 7.3–8.3%. The stabilization of the mass loss process of the envelopes, however, commenced at lower temperatures than those recorded for the male larvae of *V. orientalis* and *P. germanica*, while the rate of the second *WL* of envelopes is about two times faster. Silk of the *V. orientalis* worker larvae was found to change its mass more readily, the total *WL* approaching 11.1%. Upon heating up to 150°C this sample loses about 14.3% of its initial mass, but above 70°C the rate of the process is very slow. It should be mentioned that the initial white color of the silk samples does not change, whether on storage at room temperature (RT), or on being heated up to 70°C, nor on subsequent storage of the heated sample. The color of the silk becomes light brown after heating up to 150°C, presumably because of the partial decomposition. A primary and prominent feature of all the samples under investigation was the appearance of the first sharp *WL* (1 to 2.5%) at near-ambient temperature. Interestingly, the inflection point of this transition and the subsequent *WL* rate seem to depend on the temperature at the onset of heating. For instance, the detected change of mass in the silk of *P. germanica* proceeded more slowly (0.18% $°C^{-1}$) over the temperature range of 30 to 50°C when the thermal run commenced from 17°C then when it commenced from 24°C (0.37% $°C^{-1}$) (Fig. 3). The total *WL* of the silk, however, was almost unaffected by the starting temperature of heating.

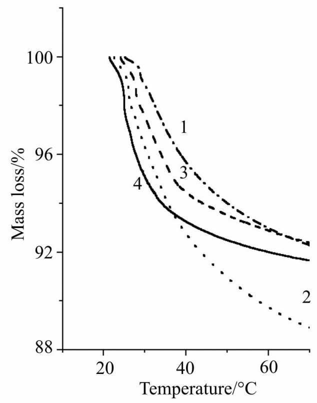

Fig. 2 TG curves of silk samples; 1 – silk of *V. orientalis* male larvae; 2 – silk of *V. orientalis* worker larvae; 3 – silk of *P. germanica* worker larvae; 4 – nest envelope of *V. orientalis*

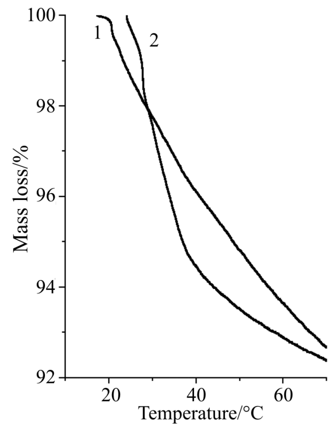

Fig. 3 Effect of the initial temperature on the rate of decomposition of the silk (#3) (silk of *P. germanica* worker larvae): 1 – run started at 17°C; 2 – run started at 24°C

An isothermal run on the silk of the *Oriental hornet* worker larvae performed at 30.7°C is shown in Fig. 4. It is clear that the primary *WL* occurs at the beginning of the heating. During the first 14 min the sample underwent about half the total mass loss recorded over the five hours of heating, and after 1 h of isothermal heating almost complete stabilization of the sample mass was observed.

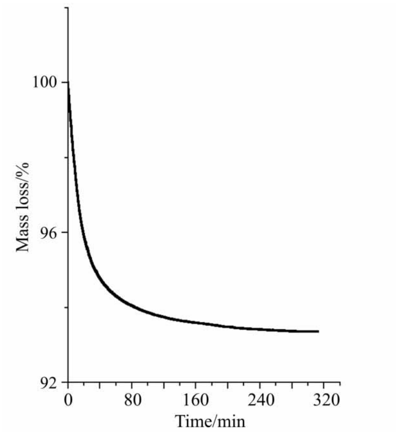

Fig. 4 Isothermal run on the silk of Oriental hornet worker larvae performed at 30.7°C

Ishay and Kirshboim [14] suggested that the observed mass loss may be associated with the desorption and evaporation of water. In order to examine this possibility four consecutive thermal runs with the intervening cooling to room temperature were conducted on the same sample. The *WL* of the first isothermal run at 29°C for 60 min was 5.5%. During the second isothermal run only 0.5% mass change was detected. Taking into account that the tests were performed in a flow of dry argon, the observed mass loss may indeed be attributed to the desorption of water. In subsequent runs up to 70 and up to 150°C, 2.4 and 8.4% *WL* was measured respectively. We believe that such *WL* may be related to both desorption of intrinsic structural water and partial decomposition of the silk.

It was of particular interest to elucidate whether the water loss is reversible or not. We have found that when stored at room atmosphere, the silk of the Oriental hornet worker larvae easily restores the amount of native water lost both under isothermal heating at 29 and at 70°C. This indicates high thermal stability of the silk secreted by worker hornets.

In order to study the hydrophilic properties of the silk, both pristine and preheated (to 70°C) silk and nest envelope samples were placed for prolonged period in a desiccator together with a beaker containing 50 mL of distilled water. The TG curves of the samples after 48 and 96 h of storage are shown in Fig. 5. From the *WL* of different samples (6.9 and 12.3%) it is clear that the amount of absorbed water is proportional to the time of storage under these conditions. Here, also, the most significant *WL* of samples stored in a humid atmosphere occurs at the onset of the heating run. The pre-dried sample absorbs

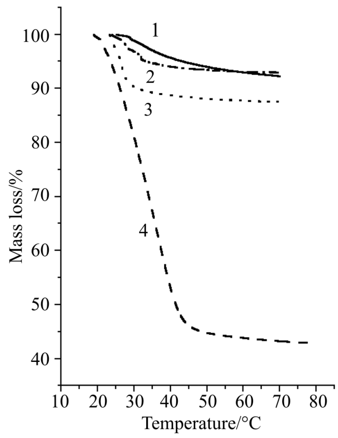

Fig. 5 Effect of storage in a humid atmosphere on the thermal behavior of silk #1 (*V. orientalis* male larvae); 1 – pristine sample; 2 – sample pre-dried at up to 70°C and then stored for 12 h in humid atmosphere; 3 – pristine sample stored for 48 h in humid atmosphere; 4 – pristine sample stored for 96 h in humid atmosphere

water much more strongly than does the pristine sample (the endothermic *WL* of the latter is 56% up to 60°C). Noteworthy is the appearance of a sharp first-step transition on the TG curves of all the samples. Another interesting observation is the lower hygroscopicity of the pristine silk of Oriental hornet worker larvae (#2) compared to that of the envelopes (#4). Thus when stored for more than one month in a humid atmosphere, the enve-

Table 1 Thermal stability of the silk samples

Sample	Start of heating T_0/°C	Inflection point of the 1st transit. T_1/°C	Total *WL*/%	Surface water/%	Structural water/%
Silk#1-pristine	25.1	28.9	7.3	15.3	84.7
Silk#2-pristine	22	26.1	11.1	20.5	79.5
Silk#3-pristine	24	27.8	7.6	17.0	83.0
Envelope#4-pristine	21.5	25.0	8.3	32.5	67.5
Silk#1-pristine+48 h in humid atmosph.	23.9	27.6	7.0	31.7	68.3
Silk#1-pristine+96 h in humid atmosph.	23.3	26.6	12.5	52.6	47.4
Silk#1-pre-dried+96 h in humid atmosph.	18.9	21.8	57.1	7.4	92.6
Silk#3-pre-dried+60 h in the room atmosph.	17.5	20.4	7.3	6.8	93.2
Silk#2-pre-dried+24 h in the room atmosph.			8.4	7.0	93

lopes absorbed about 14% of the water, which amounted to twice that absorbed by worker larvae silk. 50% of the absorbed water was released at about RT. The main *WL* data are presented in the Table 1.

In order to calculate the enthalpy (ΔH) of water release, DSC tests were conducted on the four samples, following pre-quenching to –30°C. Clear glass-transition points (T_g) were detected at –18.6, –14.1 and –0.3°C (see insert) for the three silk samples, respectively. The shift of T_g toward positive temperature as well as the narrow shape of the endothermic transition attests to the fact that the silk of *P. germanica* pupating worker larvae has a more ordered structure than does the silk produced by *V. orientalis* pupating male larvae and by the *V. orientalis* pupating worker larvae (Fig. 6). The enthalpy values of the endothermic peaks associated with the release of water do not differ substantially for the samples #1, 3 and 4, being within the range of 106–130 J g^{-1}, but for the sample #2, the enthalpy is twice as large (228 J g^{-1}). This is in agreement with the increased amount of water absorbed by worker larvae (Fig. 2 and Table 1).

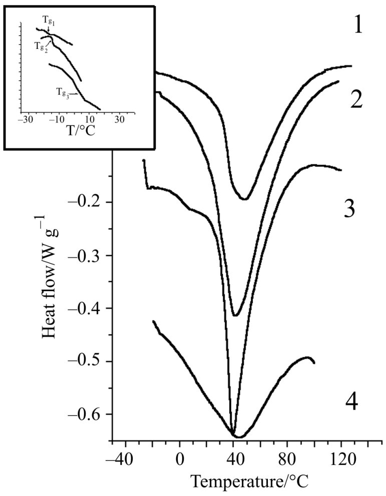

Fig. 6 DSC plots of silk samples; 1 – silk of *V. orientalis* male larvae; 2 – silk of *V. orientalis* worker larvae; 3 – silk of *P. germanica* worker larvae; 4 – nest envelope of *V. orientalis*

The broad maxima of the XRD patterns of the samples (#1 to #3) presented in Fig. 7 are typical of amorphous materials. The presence of sharp Bragg reflection peaks indicates that nest envelopes are composed of crystalline phases having long-range order, or, at least, contain crystalline inclusions.

The silk of *Hymenoptera larvae* is composed of fibers. Each fiber comprises a central fibril (core) and an outer envelope (coat) enwrapping it. The core is made up of fibroin proteins [15–21] whereas the coat is made up of sericin proteins [22]. While the fibroin fibrils extend longitudinally, the sericin coat extends transversely over the fibril, enveloping it [15]. In *V. orientalis larvae* silk fibers, we observed in-

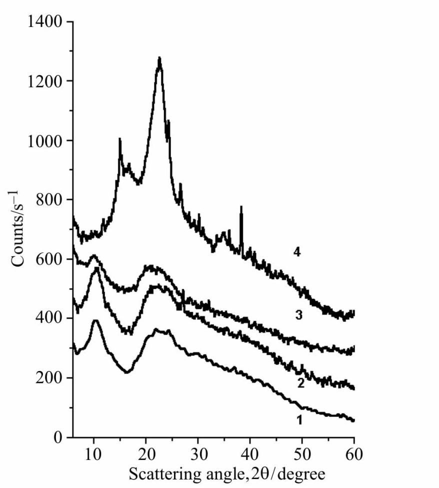

Fig. 7 XRD patterns of silk samples; 1 – silk of *V. orientalis* male larvae; 2 – silk of *V. orientalis* worker larvae; 3 – silk of *P. germanica* worker larvae; 4 – nest envelope of *V. orientalis*

terruptions in the coats, but such were not observed in *P. germanica*. SEM micrographs of the silk of *V. orientalis* and *P. germanica* are shown in Fig. 8. Apart from silk fibers and their criss-crossing, one notes also surfaces that interconnect and cover entire regions. In the silk of worker larvae the interconnecting plaque-like surfaces occupy about 40% of the total area. Even so, despite the interconnection of fibers in this part of the silk cap, there are still gaps, or 'windows' for gas exchange. As for the silk fibers, some of them are discrete and others are conjoined. The diameter of each fiber of *V. orientalis* is about 10 μm, whereas for *P. germanica* the diameter of the fiber does not exceed 5 μm. A thick cord is formed by the transverse conflation of several single fibers of 50–300 nm-thick (25000×), while a torn fiber, with the central core fibril denuded of its surrounding coat can also be seen.

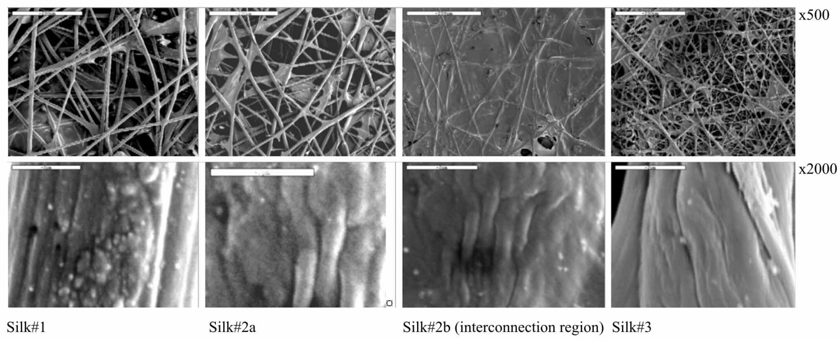

Fig. 8 SEM micrographs of the silk caps

From the SEM micrographs (Fig. 9) it is clear that isothermal treatment does not influence the structure of the silk of *V. orientalis* worker larvae. After heating up to 70°C only minor changes in the fibers can be observed, albeit heating up to 150°C results in a partial destruction of the fibers as well as of the interconnecting surfaces.

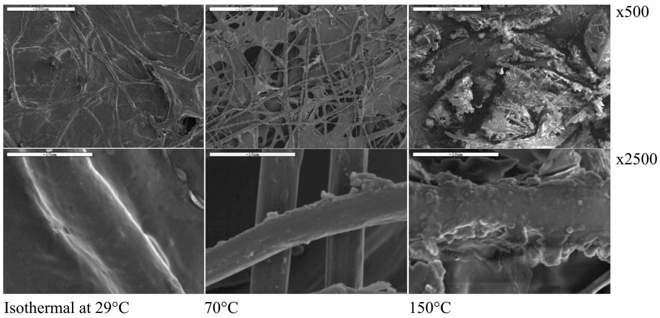

Fig. 9 SEM micrographs of the silk caps of *V. orientalis* worker larvae: Effect of temperature

A longitudinal section TEM view through a silk fiber of the Oriental hornet is shown in Fig. 10. A longitudinal duct, which is the canal at the center of the fiber, is seen in Fig. 10A. Around this canal there are longitudinally extending fibers composed of fibroin fibrils and outside these (on the left of the picture) – a sericin coat. At higher magnification (Fig. 10B), one notice the central canal inside the core fibril and around it – the fibrils extending longitudinally. The sericin coat, which appears to be made up of an amorphous material, can be seen on the right. Figure 11A presents a light photograph of a hornet comb with envelopes at the top, while Fig. 11B offers the SEM views of a nest envelope. In addition to carbon and oxygen the EDX analysis detected small concentrations of silicon, potassium and calcium. The envelope is relatively thin (not more than 100–200 μm thick) and seems likely to be composed of organic particles or minerals that have been fastened together by the saliva secreted by worker hornets (worker silk). As for the particles held together by the saliva, these are 100–200 μm in diameter. The saliva itself is a polymeric material that envelopes each particle, hardens rapidly and keeps the filling material in place. By analogy to the silk fiber, in the envelope there are the organic or mineral particles that make up the core, while the polymeric matter is the coat, but in this case the polymer interconnects and

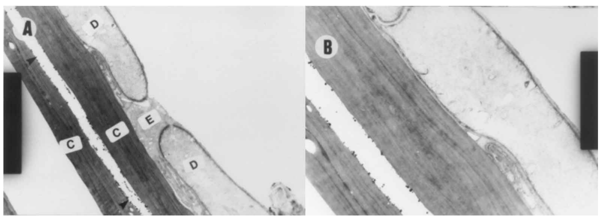

Fig. 10 A longitudinal section of a silk fiber of *V. orientalis* worker larvae; A – the canal in the center of the core fiber; C – core fiber; D – the sericin envelope; E – the gap in the envelope; A – bar=2 μm; B – bar=1 μm

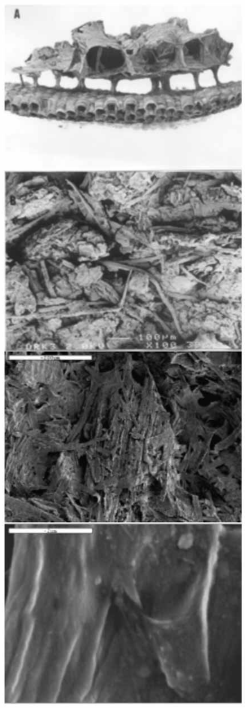

Fig. 11 SEM micrographs of the nest envelope of *V. orientalis*

binds all the envelope components, the envelope itself being very delicate and fragile. Based on the thermal analysis data and SEM micrographs we suggest that the mass loss following heating of the silk and nest envelope samples is associated with the loss of surface and structural water. As can be seen from Table 1 release of the surface water proceeds at near ambient temperature. The relative concentration of the surface water varies in the range of 15–20% in the pristine silk samples and ranges around 32% in the envelope. Heating up to 70°C is followed by almost total surface water loss and partial loss of structural water, the latter being restored in room atmosphere. Storage of the pre-heated samples in the humid atmosphere results in the enhanced adsorption of the surface water. The increased total water content in the silk of Oriental worker larvae is presumably associated with the relatively larger interconnection area that may absorb water.

We assume that the initial water loss stems from water evaporation from the coating of the fibers – a daily occurrence in the hornets' nest. Thus, when it is warmer (e.g. at noon), water evaporates from the silk and this results in cooling of the pupae within their silk cocoons. Such water evaporation during the warm hours of the day is also needed to boost the relative humidity as the temperature rises during the noon hours. In the afternoon or at night, when the temperature drops, there is reabsorption of water into the silk fiber coating, enabling restoration of the water in the coat and preparation of the coat for renewed evaporation the next morning. Additionally, it also prevents the accumulation of water droplets within the nest. We believe that the water from the deeper layers of the silk fiber (structural water) does not evaporate from the fiber core except under extreme conditions. The latter type of water loss may possibly be associated with structural changes in the silk. It seems likely that water is allowed to pass through the silk. This water may evaporate and thereby exert a cooling effect when needed. This water passage through the silk coat also regulates the relative humidity, which is critical both for the survival of the pupa as well as for the welfare of the entire nest. Our data are in a good agreement with [24], in which thermoelectric measurements were performed on strips of hornet silk of different humidity. Authors found that in the warm daytime hours, hornet silk can longitudinally transport water that serves to raise the relative humidity and to provide the vaporization needed for nest thermoregulation, whereas at night the very same silk provides an electric charge and longitudinally transfers current and heat. In addition, we assume that there is a thermal exchange between core of the silk fiber core and its coat. This assumption, however, requires verification by additional tests.

Conclusions

The present study attempted to clarify the relation between the microclimate within the hornets' nest and the moisture content of the vespid silk and envelopes. Thermal characterization showed that there are at least two types of water that is absorbed by the fibers and interconnecting surfaces in the silk of the Oriental hornet, namely, surface water and intrinsic structural water. The surface water evaporates at 25–35°C and the structural water starts to release at higher temperature. It seems likely that the release of water up to 70°C is reversible. Heating of the silk above 70°C may be followed by structural changes in the silk core. We believe, that in both hornet and wasp nest, release of the water vapors is crucial for maintaining the optimal temperature and relative humidity during the day.

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