Water vapor Uptake Across the Cocoon Wall of the introduced Pine Sawfly Diprion similis (Hartig) (Hymenoptera: Diprionidae)

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Water Vapor Uptake Across the Cocoon Wall of the Introduced Pine Sawfly Diprion similis (Hartig) (Hymenoptera: Diprionidae).

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BACKGROUND

- For many Holartic insect species, overwintering in a tightly spun cocoon provides protection against predators and pathogens, physical damage, and extreme fluctuations in environmental conditions.
- Research with insect cocoons has shown that the physical properties of the cocoon wall play an important role in regulating the diffusion of water vapor and respiratory gases.
- In an attempt to better understand properties of water vapor diffusion across the cocoon wall, we measured relative amounts of water uptake or loss in overwintering cocoons of the introduced Pine Sawfly Diprion similis (Hartig) in Maine. We also characterized the relative density of silk fibers arranged along the interior and exterior cocoon wall.

METHODS

**Directionality of Water Vapor Diffusion**
- Diprion similis cocoons were collected on and around Eastern White Pine (Pinus strobus L) saplings in Windham, Maine in late November of 2014 through February of 2015.
- Larvae were extracted through a small hole (approx. 3 mm dia) cut into one end of each cocoon.
- A tube (3 mm dia) was inserted into the extraction hole and sealed around the insertion site with melted paraffin wax. The open end of the tube was then inserted through the rubber septum in the cap of a 1.5 mL target vial containing either desiccant (0.7 g, 10-20 mesh, Drierite) or deionized water (DI) (0.5 mL). Vials and single cocoons were placed inside a 50 mL plastic vial containing either water (5mL) (N=10), providing a saturated environment, or desiccant (4g) (N=10), providing a dry environment (<15% relative humidity). Sealed target vials with either desiccant (N=5) or water served as controls (N=5) (Figure 1). The experiment was run for 65 hrs.
- Water uptake or loss was measured gravimetrically using a semi-microbalance. Mass data were square-root transformed and analyzed via ANOVA and Tukey HSD post hoc analysis using Statistica (10) (StatSoft, Tulsa, OK).

**Contact Angle**
- Cocoons were cut length-wise and secured on an adjustable platform with the external or internal surface facing up in a convex configuration.
- The inner surface was manually inserted after submerging the cocoon in water until it was pliable. It was then allowed to dry fully before analysis.
- A 50 µL droplet of DI water was placed on the inner or outer surfaces of individual cocoons (N=10) and evaporation was monitored through a USL Digital endoscope camera (2 mpx) connected to a PC. Contact angles were analyzed for digital images at 1 minute intervals using ImageJ (1.48I) (NIH, MD) and the DropSnake plugin.

**Silk Fiber Density**
- Micrographs at multiple focal points were obtained for inner and outer cocoon surfaces using a compound microscope (100X magnification). Circular oblique illumination of the cocoon surface was achieved using a novel microscope illuminator referred to as the Gorham Lamp and developed by our lab USM (Patent Pending).
- Multiple images were merged into one clear image using a stacking software.
- Density of silk fibers was determined visually by counting fibers along a 0.5 mm transect. Fiber density was assessed statistically via a two sample t-test.
- Scanning Electron Microscope images were captured at multiple magnifications using a Hitachi TM-1000 SEM (Clarksburg, MD).

**RESULTS**

**Directionality of Water Vapor Diffusion**
- The relative rate of water uptake was greater for cocoons exposed to higher external humidity relative to internal humidity (i.e. higher flow across the cocoon wall from outside to inside) compared to cocoons with higher internal humidity (i.e. lower flow across the cocoon wall from inside to outside) (Figure 2). Both treatments showed significantly higher mass changes compared to analogous controls (ANOVA; F(3,26)=423.18, p < 0.001; Tukey HSD (unequal N), df=26, p < 0.001).
- Contact Angle
  - The wicking and evaporation rate of a 50 µm droplet as determined by contact angle was higher on the outer surface of the cocoon compared to the inner surface (Figure 3).

**Silk Fiber Density**
- Observations of the cocoon surface from light microscope (100x magnification) and SEM images show clear differences between the outer and inner surfaces of the cocoons, with the outer cocoon surface appearing more porous and composed of fewer rough, narrow, round silk fibers compared to the higher density, smoother and wider flattened fibers of the inner surface. (t(66) = 9.36, p < 0.001) (Figure 4 and Figure 5).

**DISCUSSION**

**Directionality of Water Vapor Diffusion**
- We found that there is a significantly higher rate of water uptake relative to water loss in overwintering D. similis cocoons. These results suggest that under humid conditions, as may occur in the forest floor or within the forest canopy where D. similis cocoons are commonly found, there would be a net uptake in water from vapor into the cocoon.
- Contact Angle
  - Comparisons of contact angles for droplets deposited on the inner and outer surface of cocoons showed distinct hydrophobic and hydrophilic properties, respectively. These results also correlate with the directional diffusion data observed in this study.

**Silk Fiber Density**
- Although the exact chemical and physical properties of the cocoon structure remain to be determined, the greater silk fiber density shown for the inner surface compared to the outer surface suggests that the physical features of the cocoon likely play a fundamental role in regulating water flux and vapor uptake and diffusion.
- SEM and light micrographs of the outer and inner surface of the cocoon further show that the outer surface of the cocoon is more porous with a lower fiber density compared to the inner surface. The analysis of micrographs images suggest that the structure of the cocoon plays an important role in water balance and gas exchange between the external and internal environment.

**Conclusions and Future Work**
- In the variable climate conditions characteristic of the northeastern U.S., D. similis pupae will likely experience environmental conditions ranging from extreme cold and extreme dry and hot, to saturated conditions. Here we have a first look at the role that D. similis cocoons play in regulating balance. Future research planned for the summer 2015 and beyond will examine the chemical and physical properties of insect cocoons based on age and specific environmental conditions.

**ACKNOWLEDGEMENTS**

S. Monroe-Dudzin, PhD. and Kerri McNeil, M.S. for SAM Image!-University of Southern Maine, Applied Medical Sciences.
Karen Woods, Student researcher in the Environmental Entomology Laboratory, University of Southern Maine
Matthew Jones and Victoria Hill-Department of Environmental Science and Policy, University of Southern Maine

**REFERENCES**


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