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

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BACKGROUND

- For many Holarctic 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 cocoon 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 Statisitca (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 inverted 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=3) and evaporation was monitored through a USB Digital endoscope camera (2 mp) connected to a PC. Contact angles were analyzed for digital images at 1 minute intervals using ImageJ (1.48t) (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 at 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).

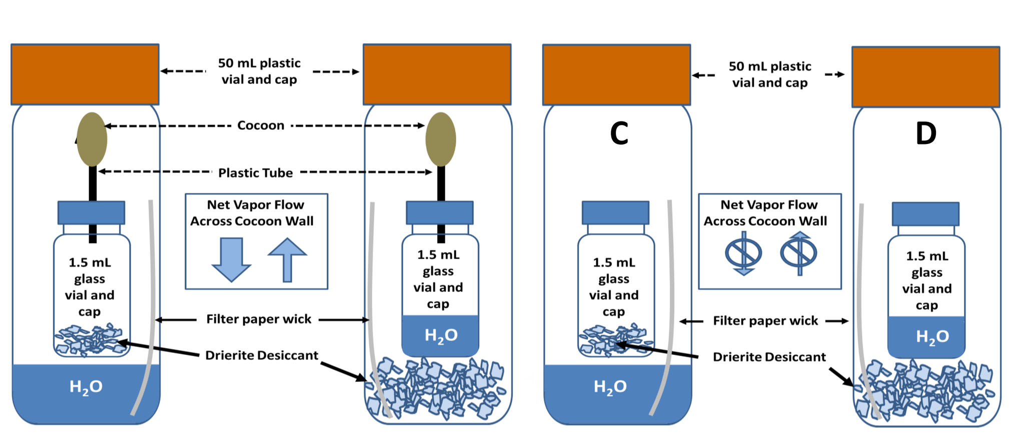

Figure 1: Experimental setup for treatments measuring the directionality of water vapor diffusion. A illustrates experimental setup with water vapor that is higher on outer surface relative to the inner surface, B illustrates experimental setup with water vapor that is lower on outer surface relative to the inner surface, and C and D control treatments consisting of sealed vials setup as described above.

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 (100 x magnification) and SEM images show clear differences between the outer and inner surfaces of the cocoon, 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. ($t(86) = 5.36$, $p < 0.001$) (Figure 4 and Figure 5).

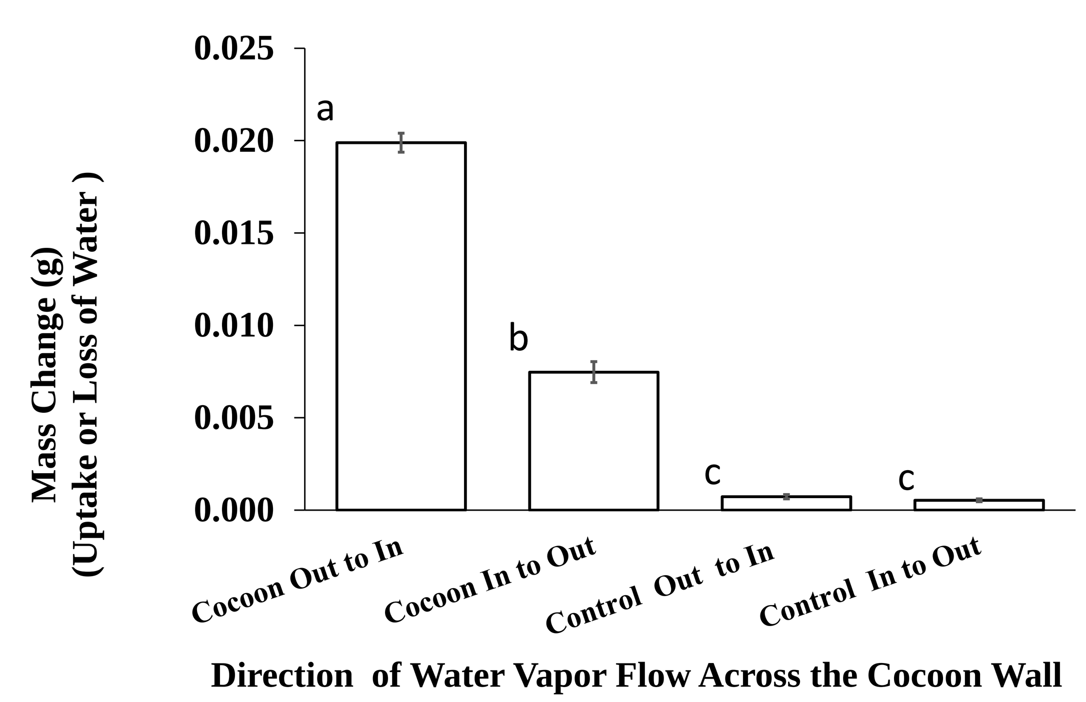

Figure 2. Average mass change (g) in vials resulting from water vapor diffusion across the cocoon wall. Treatments consist of individual treatments and controls. Error bars refer to standard error. **Cocoon Out to In =** treatments with higher humidity on the outside of the cocoon. **Cocoon In to Out =** treatments with higher humidity on the inside of the cocoon. **Control Out to In =** higher humidity on the outside of a closed vial. **Control In to Out =** vials with higher humidity on the inside of closed vial. Letters indicate statistically distinct groups as determined by *post hoc* analysis of square-root transformed data (Tukey HSD (unequal N), $df= 26$, $p < 0.001$).

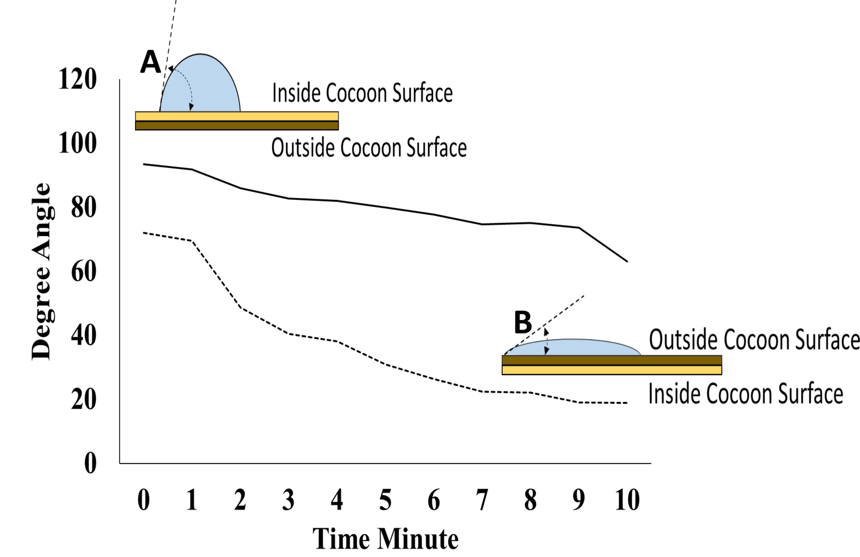

Figure 3. Contact angles for the internal surface and the external surface of the cocoon over a ten minute period of time. Insert A and B are idealized images of the experimental design for contact analysis.

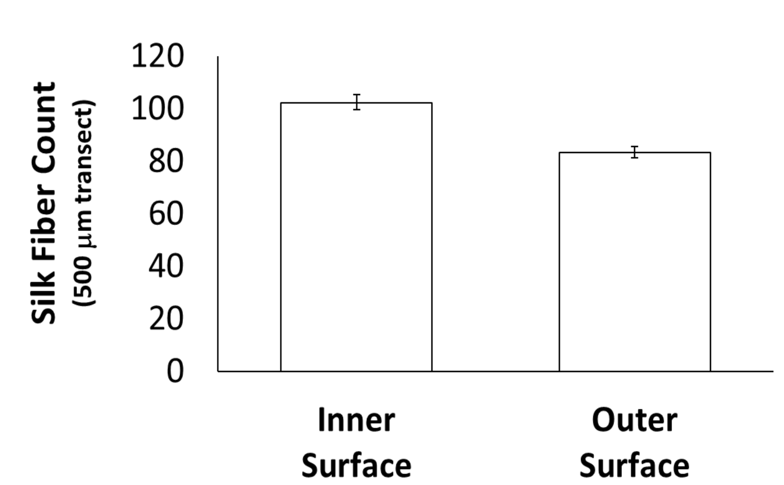

Figure 4. Silk fiber density for digital images of the inner surface and outer surface of the cocoon. Error bars refer to standard error.

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 liquid water and water 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 a 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 northeast 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 water 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.

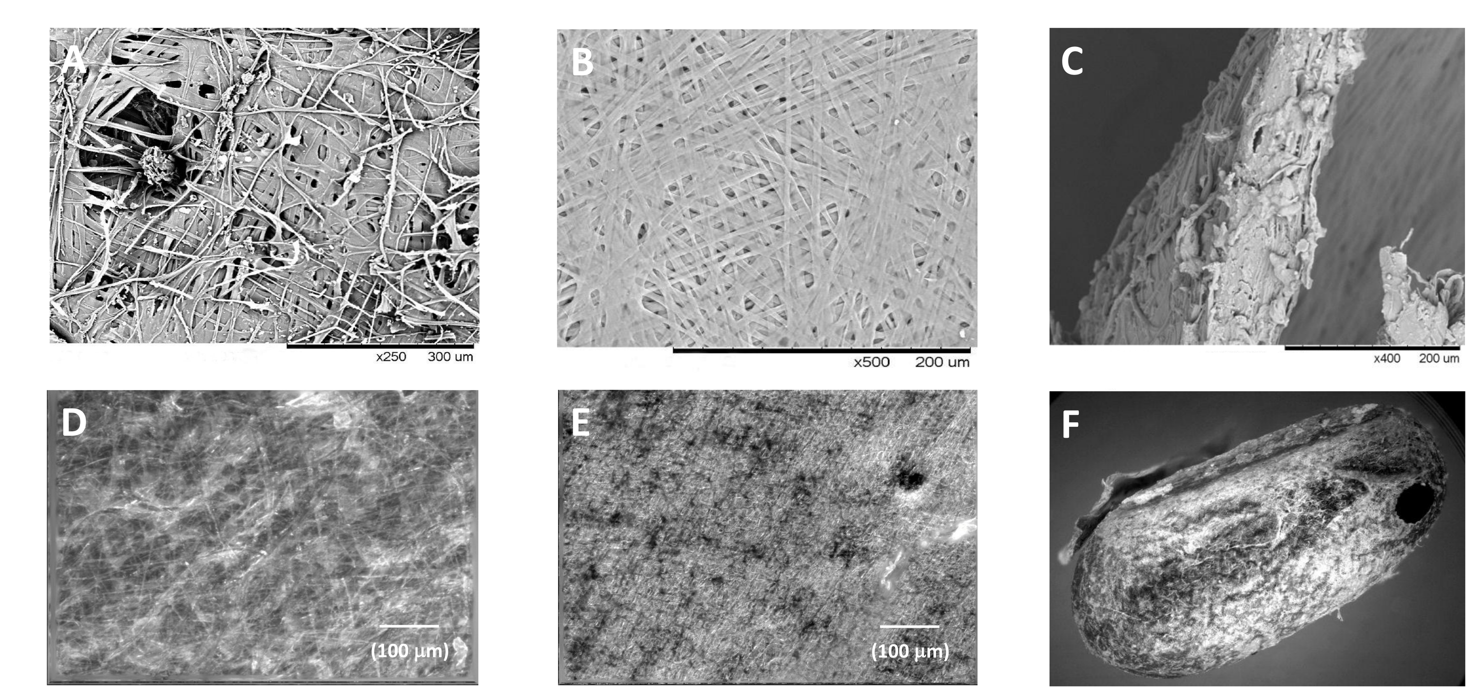

Figure 5. Series of SEM and light micrographs of *D. similis* cocoons. SEM micrographs of outside (**A**) and the inside surfaces a cocoon (**B**) showing the distinct differences in fiber patterns. An SEM micrograph of a cross section of the cocoon (**C**) wall suggests greater porosity near the outer wall relative to the inner wall. Examples of stacked light microscope images (100X magnification) of the outer cocoon (**D**) and the inner cocoon wall (**E**) used to determine silk fiber counts. A whole, parasitized cocoon (**F**).

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