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The effect of leaf morphological traits and acidic wet deposition on hydrophobicity

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Cover Page Footnote

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Introduction

Leaf environment interactions are important for plant growth and survival. Leaves have a variety of important functions including photosynthesis as well as gas exchange. In addition to hosting important functions, leaves can also absorb water through the stomata and epidermis (Limm *et al*, 2009). The absorption of aqueous solutions can have positive or negative effects on leaf function (Holder, 2007). Some plants such as, redwood trees in the western United States use fog and foliar water uptake to supplement rainfall (Limm *et al*, 2009). However, for most plant species wet deposition can inhibit leaf functions, as well as encourage the growth of fungal and microbial pathogens (Taylor, 2011, Dawson and Goldsmith, 2018). Damage to the plant could also come from droplets contaminated with acidic compounds (Singh *et al*, 2008).

Leaf morphological traits are known to affect how a droplet interacts with the leaf. Leaf characteristics such as stomatal density, cuticle thickness and trichome density (Brewer *& Smith*, 1976; Smith & McClean, 1989) all affect the hydrophobicity of a leaf or how well a leaf can repel a droplet (Wagner *et al*, 2003). Understanding the relationship between leaf morphology and hydrophobicity across species can inform us about phylogenetic relationships, ecophysiological interactions, and future climate interactions.

In this study we examined how leaf morphological traits affect hydrophobicity as well as how an acidic solution differs from pure water. We hypothesized that as the density of trichomes and stomata increases, the hydrophobicity of the leaf would decrease due to trichomes and stomata disrupting the surface tension and water cohesion of the droplet (Smith & McClean, 1989; Taylor, 2011). Additionally, as cuticle thickness increased the hydrophobicity of the leaf would also increase (Neinhuis & Barthlott, 1997). We further predicted that a pure water solution would be more hydrophobic than an acidic solution because an acidic solution would have dissolved ions that could affect the water cohesion of the droplet (Rosado *et al*, 2013).

Methods

Five study species were sampled from the Michigan State University Learning and Conservation greenhouses where we were able to sample plant species from two different climates. The species were chosen to have variation in the density of trichomes, cuticular thickness as well as general growth and form. The species measured were *Elaeagnus umbellata*, *Malus sp.*, *Ilex sp.*, and two different *Manilkara sp*. From each species we sampled 30 leaves to measure traits and test hydrophobicity for an acidic solution and pure water (Holder 2013).

We measured cuticle thickness, trichome density and stomatal density as our leaf traits. To measure stomatal density, we applied nail polish on a section of the leaf and allowed it to dry, once dry we covered the nail polish patch with a piece of clear tape. We then removed the clear tape and counted the number of guard cells imprinted into the nail polish. We only found stomata on the abaxial side of the leaf samples. No stomata were observed on the adaxial side of the leaf samples. For trichome density we manually counted the trichomes on both adaxial and abaxial sides of the leaf with a dissecting scope with a viewing area of $15mm^2$. (Wagner *et al*, 2004).

Cuticular thickness was measured by cutting a small section of the leaf, less than 0.2mm, with a microtome. Each leaf section was stained for one hour with Sudan IV to stain the cuticular wax orange (DeLucia *et al*, 1984). After staining, each leaf section was viewed under a microscope and a photograph was taken using spot software (version 3.1, Diagnostic instruments, 2001). We then used a scale for the microscope to measure the size of the cuticle in the picture.

Each sample was a flat surface cut from an individual leaf and hydrophobicity was measured on the abaxial and adaxial side of the leaf (Holder, 2013). A 10 µl samples of pure water at 7.1 pH and H_2SO_4 at 3.4 pH were pipetted separately onto each sample. We used sulfuric acid due to sulfur's common occurrence in acid rain and historical pH data for acid rain pH (Menz & Seip 2004). A photograph was taken of each water droplet, with the camera (Nikon D40) at the same level as the water droplet. Each photograph was analyzed using ImageJ (version 1.51k Schneider *et al*, 2012), using the LB_ADSA drop analysis software (version 1.45, Stalder *et al*, 2010) to measure the contact angle of the droplet. Contact angle has an inverse relationship to hydrophobicity.

Data was analyzed by using R, (Version 3.4.2, R studio team 2015.) Linear regression models were used to model trichome density, stomatal density, and cuticle thickness individually against hydrophobicity. An ANOVA was performed to compare the contact angles of acidic and pure water samples on the adaxial and adaxial side as well as to compare the contact angle of species with trichomes to species without trichomes.

Results

From our results there was no significant relationship between hydrophobicity and stomatal density (**Figure 1**, $p = 0.306$, $r^2 = 0.001$, N=150). The density of stomata for the species observed does not appear to affect the contact angle of water droplets on the abaxial of leaves.

We also found no significant relationship between cuticle thickness and hydrophobicity (**Figure 2**, $p = 0.529$ $r^2=0.032$, N=30). The thickness of the cuticle layer does not appear to affect the contact angle of leaves on the abaxial or adaxial side of leaves.

We found that as trichome density increased the contact angle increased (**Figure 3**, Linear regression, $p<0.001$ $r^2= 0.057$, N=150, t value=3.345). Trichomes were found on both sides of the leaves. We found that across all species there was a significant difference in contact angle between leaves with trichomes and leaves without trichomes (Student's *t*-test, $p<0.001$). Trichomes appear to affect contact angle.

We found that the sulfur solution had a lower contact angle or was more hydrophobic than pure water (p<.001). The was no significant difference in contact angle for the abaxial and adaxial sides for either pure water or sulfuric acid solutions solutions(p=0.391) (**Figure 4**).

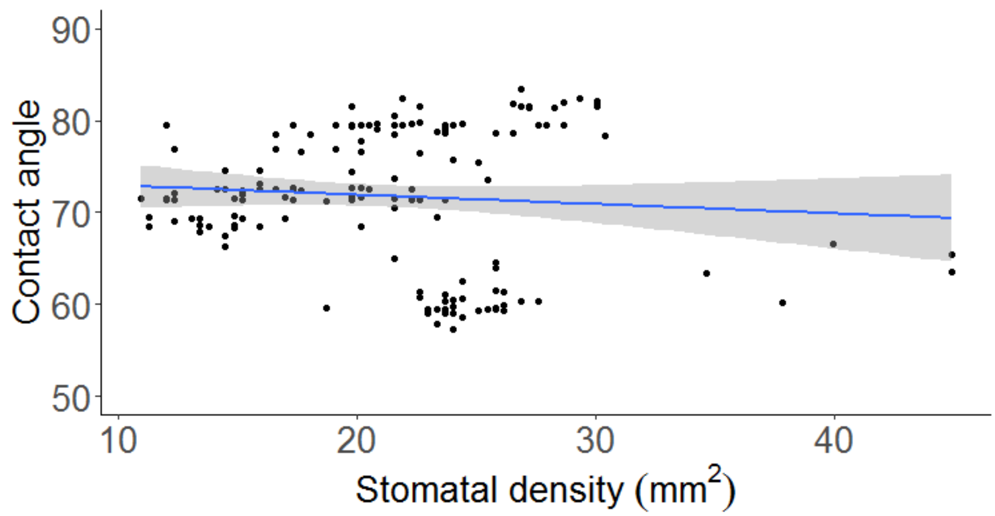

Figure 1. Linear regression between stomatal density of the leaves and contact angle between the water droplet and the leaf. For the chosen plant species, no stomata were found on the abaxial side of leaf surfaces. As a result, all data is of adaxial stomata. ($p = 0.306$, $r^2 = 0.001$, $N=150$)

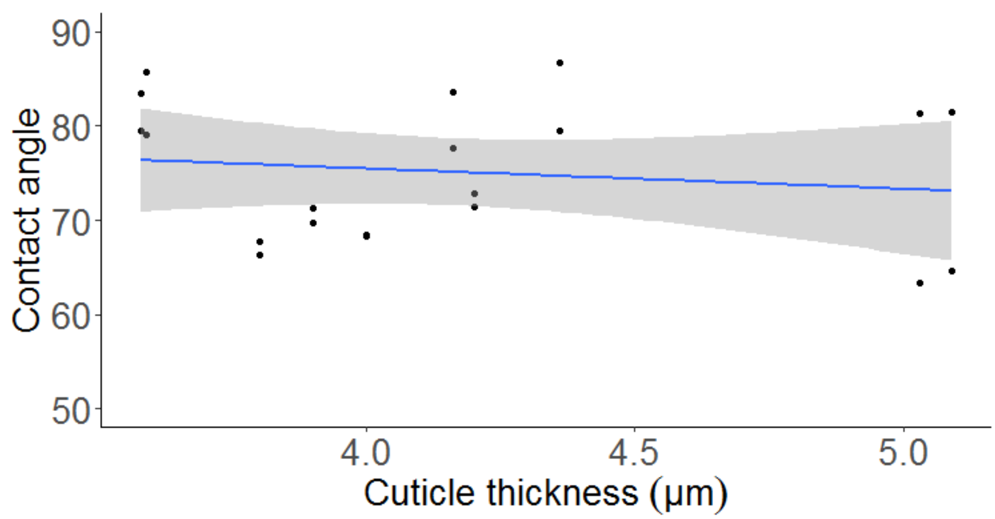

Figure 2. Linear regression between cuticle thickness of the leaves and contact angle between the leaf and the water droplet. The average between the adaxial and abaxial sides was used for each data point. ($p= 0.529$ $r^2=0.032$, $N=30$)

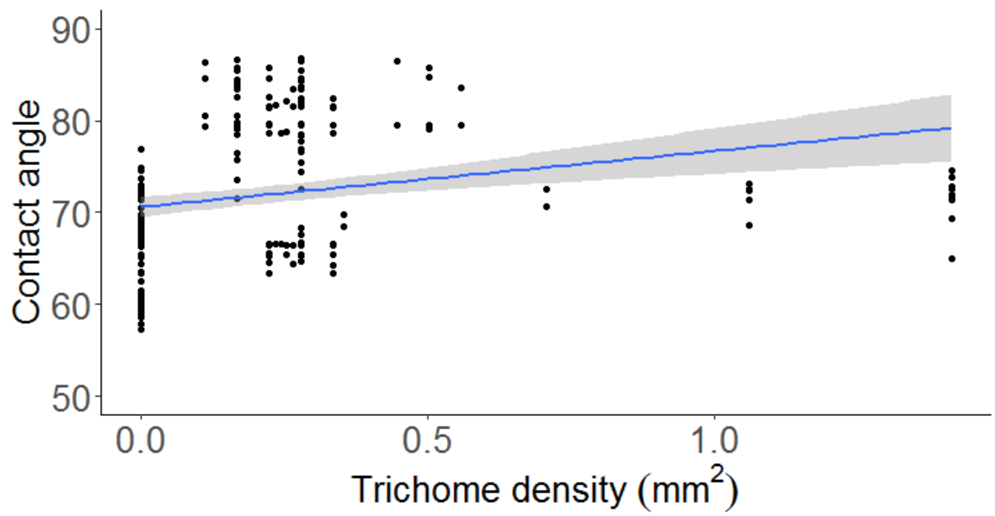

Figure 3. Linear regression between trichome density of the leaves and contact angle between the leaf and the water droplet. (p=>.5) on either side. (p=<0.001 r^2= 0.057, N=150)

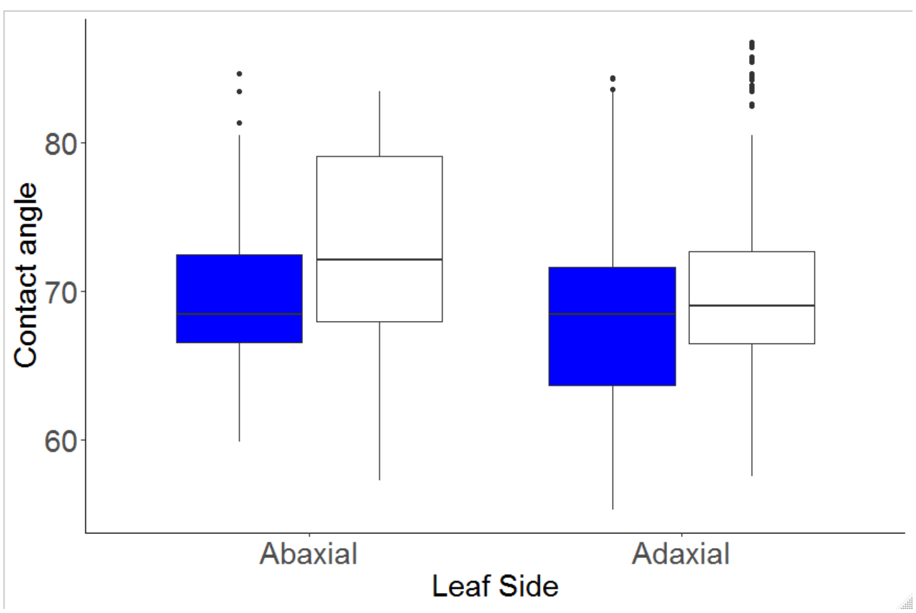

Figure 4. Box and whisker plot comparing leaf side with contact angle, blue boxes represent the Pure water treatment of and white boxes represent the acid treatment. Treatment (P-Value:= <0.001 Leaf side P-Value: 0.401)

We found that the species chosen had variation in the number of stomata, trichomes and cuticle thickness (**Table 1**). We also found that there was variation in the hydrophobicity for both the test and control treatments for the adaxial and abaxial sides of the leaves across all the species.

Table 1. Mean and standard error for cuticle thickness (CT), stomatal density (SD) and trichome density (TD) for each of the five species.

Species	**SD mm^2**	**TD mm^2**	**CT µm**
Malus sp.	17.41 ± 0.99	1.18 ± 0.01	1.75 ± 0.09
Ilex sp.	25.32 ± 0.68	0.31 ± 0.03	3.50 ± 0.12
Manilkara chicle	24.63 ± 0.25	0	5.01 ± 0.1
Elaeagnus umbellata	21.27 ± 0.40	0.25 ±0.02	3.83 ± 0.11
Manilkara sp.	13.87 ± 0.32	0	4.51 ± 0.12

Table 2. Mean and standard error for hydrophobicity for each side and experiment treatment for all five species. PW and A correspond to pure water and acidic treatments respectively.

Species	**PW (adaxial) Degrees**	**A (adaxial) Degrees**	**PW (abaxial) Degrees**	**A (abaxial) Degrees**
Malus sp.	70.76 ± 0.14	68.76 ± 0.19	72.01 ± 0.31	67.79 ± 0.36
Ilex sp.	65.89 ± 0.10	62.69 ± 0.28	80.12 ± 0.34	80.04 ± 0.42
Manilkara chicle	64.18 ± 0.69	63.58 ± 0.45	59.00 ± 0.22	58.26 ± 0.38
Elaeagnus umbellata	79.70 ± 0.65	81.40 ± 0.25	78.21 ± 0.44	74.60 ± 0.66
Manilkara sp.	73.10 ± 0.37	69.90 ± 0.47	70.01 ± 0.77	69.04 ± 0.55

Discussion

The relationship between trichome density and contact angle was congruent with our hypothesis (Brewer *et al* 1991, Johnson 1975, Holder 2013). While we used five species with a wide range of morphological values, it is possible we did not capture the full range of trait variation. Table 1 indicates that cuticle thickness and trichome density had a range of averages. Due to the limited availability of plants available for testing the most ideal range of trait variation could be achieved. The variation in our chosen morphological traits allowed for investigation of possible patterns. Brewer *et al* (1991) examined species with a higher trichome density. By having a larger number of species with and without trichomes we could explore the possible effect of contact angle on trichomes. The presence of trichomes appears to disrupt the surface area of the droplet and prevent the leaf's surface from being water repellant.

Our cuticular thickness results were non-significant. Due to our limited selection of plant species the variation in plant cuticle thickness was not captured and may have affected our results. Other research in this field found that a large density of wax crystals on the leaf will increase the water repellency of the leaf suggesting that a thick cuticular layer could also allow for a leaf to be self-cleaning (Neinhuis *&* Barthlott, 1997).

Stomatal density results were non-significant and did not follow previous work. Other research found that an increased stomatal density resulted in a surface that prevented the formation of water droplets (Smith *et al*, 1989). All species used for sampling in our study were trees and only had stomata on the abaxial side of the leaf. Past research used herbs and shrubs as well as trees and these differences in leaf structure could have affected stomatal density.

The results from the hydrophobicity trials contradicted our predictions. Past research has shown that surface tension allows for a droplet to retain its shape and dissolved compounds within a droplet reduces surface tension (Burkhardt *et al*, 2012). Our results suggest something contradictory in that a dissolved solid doesn't seem to negatively affect the ability of the water droplet to retain its shape. This could be due to the sulfuric acid used in our study. Other research focused on salts instead of acids in solution with water. Past research also suggests that the abaxial side of the leaf would be more hydrophobic than the adaxial side (Smith *et al*, 1989, Brewer *et al*, 1991, Taylor, 2011) Although, from our results we saw no significant difference.

Trichome density had a significant positive trend with hydrophobicity and there was a significant difference between an acidic solution and a pure water solution's hydrophobicity across a variety of leaves. The results from our study contribute to the body of literature examining the interactions between leaf morphology and water in the natural environment. This information can be valuable to the agriculture and the field of plant ecology.

Understanding the effects of different leaf characteristics on hydrophobicity can be useful for fertilizer and pesticide application in agriculture as the duration of foliar contact with aqueous solutions is important, too long and the leaf surface could burn, too short and the desired result may not be achieved (Bryla *et al.* 2015). When irrigating crops, it would also be useful to know leaf hydrophobicity because water droplets on a leaf can disrupt photosynthesis (Smith *et al*, 1989).

The results from our study contribute to the body of literature examining the interactions between leaf morphology and water in the natural environment. This information can be valuable to both agriculture and plant ecology disciplines. Our research improves our understanding of how multiple leaf traits can affect hydrophobicity. However due to our small sample size it may have been difficult to observe patterns and relationships between traits and hydrophobicity. But, by examining a variety of species, we may uncover how plants interact with water on the surface of leaves.

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