# Water Transport in Azaleas: Who Will Be Left High and Dry?

*By Sharon Danielson—Kirtland, Ohio*

As any gardener will tell you, providing the right amount of water for your plants is pivotal. Avid gardeners meticulously measure rainfall and soil moisture. Most folks know the crucial role that the root system plays in accessing water from the soil for the plant to use. But have you ever wondered about what happens to the water after it enters the roots? If you are like the researchers at the Holden Arboretum, you may be very curious about how water flows through the plant, and how different species use water. We are particularly interested in the role that leaves play in utilizing and moving water through the plant. Working in the laboratory of Dr. Juliana Medeiros at the Holden Arboretum, I have been studying how azaleas from various climates differ in the way they use water. The following is an account of the preliminary results for three azaleas I studied.

## Leaf Hydraulics Background

To inspect the way water is used by each species, I used a measurement called leaf hydraulic conductance. Leaf hydraulic conductance quantifies how easily water flows through the leaf. During photosynthesis, small pores on the leaf called stomata open to allow $CO_2$ to enter. At the same time, water evaporates from the leaf surface in the form of water vapor, because the air is dryer outside of the leaf than inside it.

This places the plant in a precarious balancing act between maintaining open stomata to photosynthesize and potential desiccation, or closing the stomata to reduce water loss but risking starvation. If the plant maintains open stomata to keep exchanging gas for photosynthesis, it risks losing too much water and drying out, unless it is able to continuously replenish water to the leaf. Simultaneously, this process of water evaporating from the leaf also pulls the water from the soil up through the plant and into the leaves. This makes leaf hydraulic conductance such an important physiological trait because it determines the ability of the plant to replenish the water lost during photosynthesis.

## Measuring Leaf Hydraulic Conductance in the Lab

To measure leaf hydraulic conductance in the laboratory, we determine how quickly water evaporates from a leaf. In fact, measuring evaporation rate is quite difficult, because there is always water in the air, so determining how much came specifically from the leaf is problematic. So, we use a clever technique: we determine how much water flows into an evaporating leaf, which will be equal to the amount of water lost to evaporation. The evening before measurements are taken, a branch is cut from the plant and then it is allowed to hydrate in a vase of water overnight. The next day, leaves are cut off the branch and measured one at a time on a system specifically designed to measure the rate of water flow into the leaf. The system consists of tubing leading from the leaf to a cylinder filled with water. The cylinder sits atop a balance, which weighs the water. Next, the leaf is placed under a grow light, causing stomata to open and water to evaporate from the leaf surface. The balance records the amount of water that is lost every 30 seconds until a steady flow rate is reached. A fan keeps the leaf from overheating and both leaf and air temperature are continuously monitored. Trials last approximately 30 minutes to an hour. After the leaf is taken off the system, its water status is measured and recorded. The leaf size is also measured and used in the calculation of leaf hydraulic conductance. In our study, we measured three plants per species, and at least five leaves from each plant. Leaf hydraulic conductance is calculated as the flow rate (at steady state) divided by the water status, and normalized by leaf size.

## Leaf Hydraulic Conductance is Related to Environment and Leaf Morphology

Hydraulic conductance is not a static trait. It is also impacted by environmental factors including the evaporative demand (i.e. how warm and dry the air is), light level, concentration of $CO_2$ in the air, and the water status of the leaf (Table 1). Environmental factors like increased light and elevated $CO_2$ levels will increase the photosynthetic rate, and water supply will need to ramp up in order to accommodate the drying leaves. Hotter, dryer air should also be associated with high water flow because the water is quickly evaporating from the leaves during photosynthesis.

Leaf features also play a key role in how the leaf interacts with the environment. Since water transport in plants is driven by the difference in humidity inside the leaf and outside the leaf, even small differences in leaf traits can shield or expose the stomata to the outside air and impact water supply. It is perhaps easiest to think of the leaf the way we think of topography. The environmental conditions that we experience differ if we are in a field, in a valley, or in a forest. For example, leaf hairs can be thought of as creating a tiny forest around the stomata. Much like a dense forest of trees would hold humidity and shield the wind, these fine hairs create a layer of humid air around the stomata, reducing the rate of water loss from the leaves. On the flip side, if we were to stand in an open field we could immediately sense any changes in temperature, humidity, and wind. On a smooth leaf, the stomata are essentially sitting in a field, easily exposed to differences in water vapor or temperature. Therefore, smooth leaves should have higher hydraulic conductance.

| Table 1: Summary of Factors that Impact Leaf Hydraulic Conductance | |
|---|---|
| **High Conductance** | **Low Conductance** |
| Warm Climates | Cool Climate |
| Low Humidity | High Humidity |
| High Light | Low Light |
| Low $CO_2$ | High $CO_2$ |
| Leaf Fully Hydrated | Leaf Dehydrated |
| Smooth Leaf | Hairy or Rough Leaf |

I have determined a hydraulic profile for three species of azalea—*Rhododendron occidentale*, *R. austrinum*, and *R. yedoense*, each originating from very different native climates.

## Section *Pentanthera:* Deciduous Azaleas

*Rhododendron occidentale*, a deciduous shrub from Section *Pentanthera*, is a coastal species native to the Pacific Northwestern United States. This species had the highest maximum leaf hydraulic conductance of any of the azaleas that were measured. This is in sync with the leaf traits that we observed in this species. It has smooth leaves void of hairs or rough texture (see Fig. 1). Its stomata are more exposed to differences in humidity and temperature, so when I applied light and reduced humidity, plants of this species exhibited higher leaf hydraulic conductance. While *R. occidentale* has a relatively high hydraulic conductance, a closely related species, *R. austrinum* has a different strategy.

*Rhododendron austrinum*, another deciduous azalea, hails from the Southeastern United States. We had hypothesized that species originating from warmer climates would, on average, have higher maximum leaf hydraulic conductance. What I found is that *R. austrinum* had a much lower hydraulic conductance. This is a rather curious state for a shrub from a warm climate.

Why might a plant coming from a warm location not have as high of a leaf hydraulic conductance as one from a colder location? Surely, the leaves from a hot climate are experiencing a higher evaporative demand and would therefore need to increase water flow to the leaf. There may be a clue as to why I found this result, based on the native climate for these two species. Some new research has shown that leaf hydraulic conductance is related to the elevation of the plant. *Rhododendron* growing at higher elevations have been shown to have higher maximum leaf hydraulic conductance, compared to those from lower elevations.[1] While Taneda et al. found this trend in evergreen species of *Rhododendron*, it is worth noting that *R. austrinum* is found only up to elevations of 330 ft. while its sister, *R. occidentale*, is found in elevations up to 9,000.[2] At higher elevations plants typically experience lower atmospheric $CO_2$ and lower humidity outside the leaf, compared to lower elevations, so high elevation plants are predicted to have higher rates of evaporation during photosynthesis. Since water exits the leaf at a higher rate, the water needs to be replaced. This could explain why a plant found in much higher elevations would have a higher maximum leaf hydraulic conductance than its relative from lower elevations.

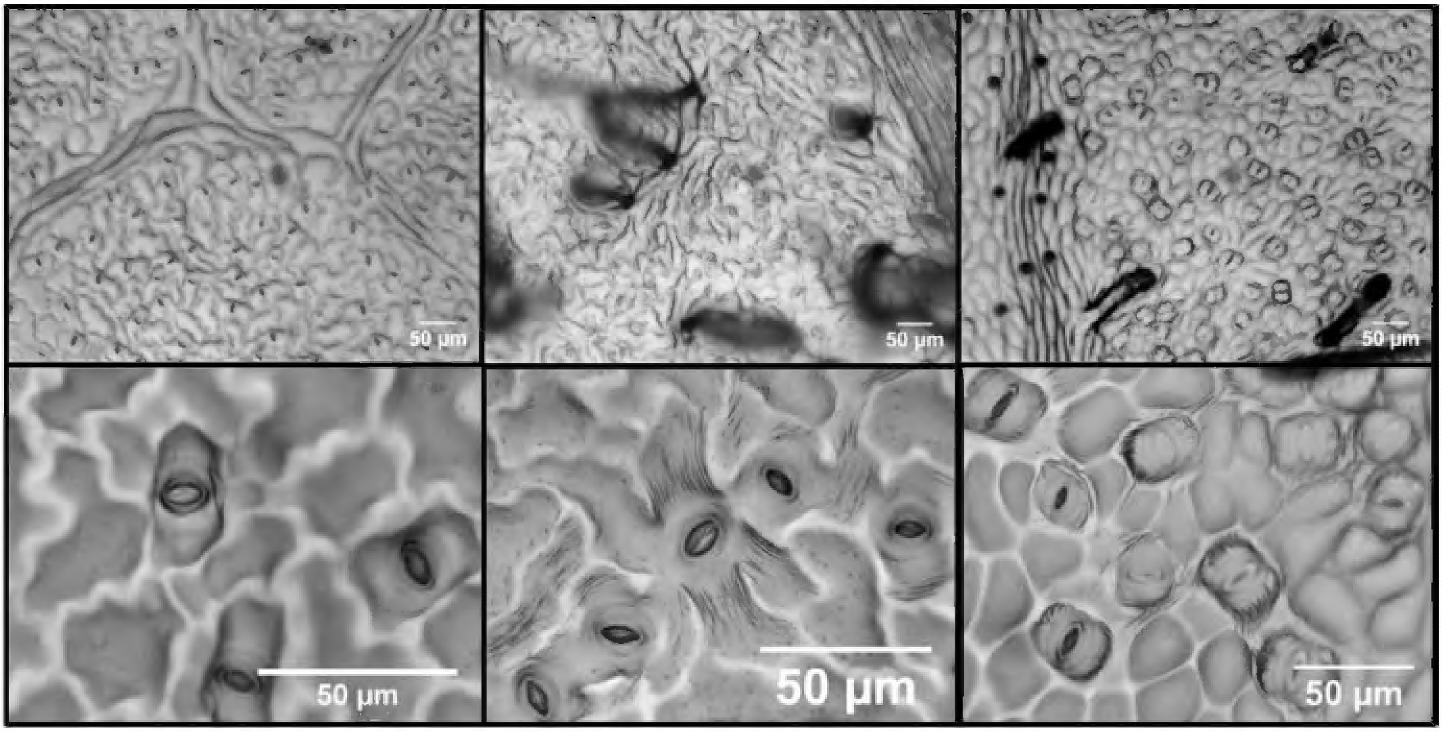
The leaf morphology of *R. austrinum* may also provide some explanation for our water transport results. *Rhododendron austrinum* has leaf hairs and a rough leaf surface (see Fig. 1). The hairs have created a thin shield around the stomata, slightly increasing the humidity directly outside of the leaf. Additionally, some of the stomata are in tiny valleys because of the rough texture. All of these traits can work together to decrease the leaf hydraulic conductance.

## Section *Tsutsusi:* Semi-Evergreen Azaleas

*Rhododendron yedoense* is native to Korea and Japan where the minimum temperatures dip to approximately -26°C. A particularly durable azalea, it is often hybridized with other species in order to increase hardiness.[2] Our research showed that it was moderate in maximum leaf hydraulic conductance compared to the other two azaleas I measured.

Recall that hydraulic conductance is a dynamic trait that changes with the water status of the leaf. As a leaf dries out, small air bubbles begin to form in the plant veins, or xylem, which are the cells that transport water to the leaves. These bubbles, called embolisms, block the movement of water within the veins. Just like an embolism in your veins can block the flow of blood, embolisms in the plant reduce or eliminate the leaf water supply. Some species are very susceptible to embolisms as the leaf dehydrates, but some species are resistant to embolisms, such that the leaves can still transport water, even when they are very dehydrated. The way in which leaf hydraulic conductance declines as the leaves dehydrate is called "drought vulnerability," and much attention has been paid to how it differs among species to understand their relative susceptibility to drought.

Interestingly enough, while the maximum hydraulic conductance in *R. yedoense* is not particularly high, as the leaf dehydrates the leaves of this plant maintain higher hydraulic function than *R. occidentale* or *R. austrinum*. This means that these leaves are relatively less vulnerable to drought embolism than the other azaleas measured (although it should be noted that, compared to arid-land plants, no azaleas are considered drought tolerant). As seen in Fig. 1, the leaf surface of *R. yedoense* is somewhat rough, but the roughness is imparted by subsidiary cells. These cells are often found on plants from dry locations, and it has been hypothesized that they could play a part in water conservation. So, once again, our work points to leaf surface features in *R. yedoense* as a key component that could make it a more stress-tolerant species. For a hardy plant that still manages to succeed in cold




▲ Figure 1. Leaf surfaces of azaleas showing features that could influence leaf hydraulic conductance. The upper row shows the leaf landscape, and the bottom row shows a close-up of stomata. *R. occidentale* (top left) has a fairly smooth leaf landscape, with a small leaf vein running through the landscape like a river. *R. austrinum* (top center), has a rough landscape, with large hairs that can be seen as black filaments rising up from the leaf. *R. yedoense* (top right) also has hairs, but they are small and stiff, often located on top of the veins, which are the long cells along the left-hand side of the image. Zooming in, note the stomata (bottom row), which look like small, dark beans. The stomata of *R. occidentale* (bottom left) have a raised border surrounding them, but the leaf surface appears very smooth compared to *R. austrinum* (bottom center) and *R. yedoense* (bottom right). In *R. austrinum* the stomata are found on mountains and in valleys, and the surfaces of the epidermal cells are covered in small raised dots, imparting a rough feel to the leaves. *R. yedoense* also has a rough leaf landscape, but the roughness is imparted by raised subsidiary cells, which are the small rectangular cells surrounding the bean-shaped stomata. Rougher leaves should have lower leaf hydraulic conductance, because greater roughness increases the humidity at the leaf surface.

conditions, adding an extra benefit of drought tolerance is certainly a bonus.

## Conclusions and Implications

Leaf hydraulic conductance helps us to understand how a plant functions under specific environmental conditions. Ultimately, that information can be extrapolated to predict which species will do best in horticultural practice and in a changing climate. When choosing plants to use in gardens, ideally, we want a plant that has it all—beauty, cold hardiness or heat tolerance (depending on where you live), longevity, and drought resistance. Each of these things has its importance, and the importance varies with location.

Plants that have some mechanism to provide them with drought tolerance will make for a flexible watering schedule. *R. yedoense* is less vulnerable to changes in water status, so the impact of mild drought will be less intense on this species compared to the deciduous azaleas measured in this study. So, as a plant that is already known to be cold hardy and is less vulnerable to changing water status, it is likely to provide a gardener with a little peace of mind. The deciduous azaleas *R. occidentale* and *R. austrinum* are more vulnerable to changes in water status making them potentially more fussy plants to have in the garden.

Perhaps of bigger concern, however, is that as the climate warms overall, weather events are predicted to become more extreme. Cold and warm temperature shifts alike are expected to become more dramatic. Further, droughts are predicted to become more frequent and last longer. Our data thus far suggests that the deciduous azaleas will be will be more affected by these extreme shifts than *R. yedoense*. Even though *R. occidentale* has a high maximum hydraulic conductance to support high rates of photosynthesis, it has little protection against drought. Its leaves are at risk of desiccation. With a relatively low maximum hydraulic conductance, *R. austrinum* may have difficulty supplying its leaves with water under hot or dry conditions, and it is more sensitive to changes in water status, leaving it in a precarious state for a warming climate. Again, *R. yedoense* is the apparent winner of the three species to manage the stress of a warmer climate because of its ability to continue to replenish water to its leaves, thereby maintaining photosynthesis, even as the leaves become dehydrated.

## Future Work

There is still a lot we do not know about leaf hydraulic conductance in azaleas, and it turns out this trait is even more complex and interesting than we first expected. The main finding of this work is that many leaf traits interact with the environment, to cause unexpected patterns of hydraulic conductance in azaleas. This initial work has shown us that leaf surface characteristics certainly play a role in the water flow pathway. Rough leaf texture and the thickness of the indumentum can potentially increase the fine layer of humidity surrounding the leaf, leading to lower hydraulic conductance even when climate tolerance is similar. Azaleas can vary widely in their leaf surface characteristics—for example, *R. canescens* is pubescent, while *R. periclymenoides* is predominantly glabrous. It will take further study to fully understand how these features affect water transport. Currently, I am quantifying the arrangement and size of stomata on the leaf surface, which will provide insight into the rate of evaporation that would be possible while controlling for other surface traits like indumentum.

In addition to examining leaf surface features, I also plan to assess the leaf anatomy to see if internal leaf traits may account for the difference in leaf hydraulic conductance I observed. Perhaps the most important puzzle piece in understanding differences in hydraulic conductance, is the venation network. Since the veins provide the pathway of water across the leaf, they play a key role in determining the conductance of water. I will be quantifying different traits of the leaf veins. I have also been measuring leaf hydraulic conductance from many species of *Rhododendron* in the garden over different seasons to track how this trait changes with differences in precipitation, light, and temperature. All of these factors together, will help us to better understand this dynamic trait and how plants have such differences in their water use so that, ultimately, we can make informed planting decisions.

## Literature Cited


1 Taneda, H., Kandel, D. R., Ishida, A., & Ikeda, H. 2016. "Altitudinal Changes in Leaf Hydraulic Conductance across Five *Rhododendron* Species in Eastern Nepal. *Tree Physiology*, tpw058. http://doi.org/10.1093/treephys/tpw058

2 Cox, Peter A. & Cox, Kenneth N.E. 1997. *The Encyclopedia of Rhododendron Species*. Glencarse, Perth, Scotland: Glendoick Publishing, Glendoick Gardens Ltd. p. 212, 384.



Lead researcher: Juliana Medeiros is a researcher at the Holden Arboretum. Juliana received her PhD in Biology from the University of New Mexico, and conducted postdoctoral research at the University of Kansas.

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# ASA Seed Exchange

*By Lindy Johnson*

## Contributing Seed

Seed contributions will be accepted from ASA members and other sources throughout the year until December 31.

The seed from each plant should be described by the:

- contributor's name
- seed parent name
- pollen parent name
- plant type (evergreen, deciduous, azaleodendron)
- pollination type (open pollinated, hand pollinated or wild cutting)
- where collected (geographic feature or town)
- notes

This information can be written on seed envelopes, or we have a seed data form to describe the seed, as a short (4KB) file to down load and print.

If you have digital pictures of the parents, please e-mail them to Dave Banks, with the name, date and location taken, for posting on the web linked to your seed. dfbanks@earthlink.net.

Seed should be current year production and can be cleaned or not. Put the seed from one plant into one paper envelope with one completed form (or write the information on the envelope), and mail to: Lindy Johnson, 843 Wallace Rd, Trade, TN 37691.

When we receive the seed, it is cleaned and distributed into #1 coin envelopes, and each lot of envelopes is assigned a number and stored until it is ordered.

## Ordering Seed

The seed list will be posted online on or about January 1st. The notice also gives the address to request a hard copy list of seed available.

Seed is shown on the web on a seed list page, where it is listed alphabetically by seed parent name with the information provided by the seed contributor, including links to any pictures of the parent plants.

After January 1st seed is distributed to contributors and ASA members on a first come, first served basis. After April 1st seed is distributed to anyone on a first come, first served basis.

All seed is packaged in #1 coin envelopes, and costs $2.00 for approximately 50 seeds. Shipping and handling is an additional $3.00 for all the envelopes in one order. Orders can be placed by e-mail to appalnativeplants@gmail.com or by a letter addressed to the Lindy's address provided above.

All seed not distributed before the annual convention will be offered for sale there.

Seed orders can be paid for with a check made out to "ASA" with "seed exchange" on the memo line, or by a credit card payment through PayPal using the form on the Seed Exchange 2016 page.