How-To-Do-It

What's So Bad About Being Wet All Over

Investigating Leaf Surface Wetness

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Background

A stroll through a mountain meadow, or even across your lawn, on a crisp summer morning provides moist evidence that many terrestrial plants start the morning covered with water. In fact, accumulation of water on leaf surfaces is a common phenomenon for plants in a wide variety of habitats because most regions of the world receive some form of predictable precipitation on an annual basis. Precipitation in the form of rain, ground fog, cloud mist, or dewfall results in the accumulation of moisture on photosynthetic leaf surfaces. Surprisingly enough, both upper and lower leaf surfaces are often wet after a night of heavy dewfall or rain!

What is so bad about waking up with wet leaves? The physiological significance of leaf wetness stems from the fact that CO_2 diffuses about 10,000 times more slowly through a film of water than through air (Weast 1979). Because of the slow transport of CO_2 through a film of water, CO_2 uptake should be slowed dramatically when a large portion of the leaf surface is covered by a film of water. In fact, evidence suggests that photosynthesis in many species may be much reduced because of wet leaf surfaces (Benzing & Renfrow 1971; Brewer & Smith 1994, 1995).

The frequent occurrence of wet leaf surfaces has been the subject of considerable agricultural research. Infection of leaves by pathogens (Reynolds et al. 1989), especially fungus, depends on periods of leaf surface wetness. Moreover, studies have also addressed concerns for pollutant deposition on wet leaves, especially from acidic precipitation (Adams & Hutchinson 1987; Pearcy & Baker 1991). Thus, considerable effort has been made to predict daily and seasonal patterns of leaf wetness, especially for important crop species (Barr & Gillespie 1987).

Leaves of plants show a broad range of interactions with liquid water. Very wettable leaves (e.g. poinsetta) may be covered by a film of water or many large, flattened droplets. Nonwettable leaves repel water (e.g. strawberry plants) by forming round droplets that roll off the leaf surface in a gentle breeze or when leaves are oriented at steep angles. In general, when leaves form very spherical droplets, they are considered nonwettable (Brewer et al. 1991). When moisture spreads out into films, or large, flattened drops, leaves are considered wettable. Trichomes (leaf "hairs") have a very important influence on leaf wettability. Very hairy leaves tend to repel moisture from the leaf epidermis; thus, interference with stomatal pores is reduced (Brewer et al. 1991). In addition, recent studies have shown that the leaf surface with the greatest concentration of stomata typically has the lowest leaf wettability (Smith & McClean 1989; Brewer et al. 1991; Brewer & Smith 1994, 1995). Furthermore, Ishibashi and Terashima (1995) have shown that stomata of some species close in as few as two minutes when exposed to simulated rainfall. Consequently, interference by water with CO_2 uptake for photosynthesis is significant.

In natural habitats, there are clear differences in leaf wettability and droplet retention (the tendency to retain water on leaf surfaces) that correspond to natural patterns of moisture deposition (Brewer & Smith, unpublished data). For example, leaves in open meadows typically are hairy, and have low wettability and low droplet retention. These characteristics are clearly adaptive because plants in open meadows are especially susceptible to dewfall and rainfall during the summer growing season. In contrast, plants in the understory (i.e. under a canopy of trees) invest little energy in repelling moisture, possibly because they are less likely to be affected by dewfall or summer rain showers.

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Experimental Procedures

Investigations of leaf surface wetness provide ideal opportunities for students to explore the relationships between leaf form and function, to study surface conditions of leaves and plant physiology, and to make predictions about plant adaptation in different environments. Three simple procedures for exploring questions related to the ecological significance of leaf wettability are described below. Suggestions are made for using these procedures in both classroom and outdoor settings, and as guided or open inquiries. Ideally, a variety of leaf types or species can be presented to students to highlight differences between wettable and nonwettable leaves (e.g. Table 1). Examples of plants with nonwettable leaves are strawberry (*Fragaria virginiana*) and columbine (*Aquilegia* sp.). Wettable species include the common geranium (*Pelargonium* sp.) and honeysuckle (*Lonicera* sp.). Leaves of many common grasses fall somewhere in-between.

Leaf Wettability

A simple apparatus for measuring leaf wettability, an angular value, can be constructed using the head of a dissecting microscope or simple inspection microscope, a lab jack, pipet and protractor (Figure 1A). The scope is attached to a clamp so that it is oriented horizontally facing the lab jack. Leaves are attached to the lab jack by taping each end of the leaf, thus insuring that they remain positioned horizontally. To use the protractor for measuring leaf wettability, the bottom of the protractor is pierced at the "zero point." A piece of thread is attached that can move freely from 0°–180°.

Table 1. Mean values for contact angles (degrees), droplet retention (degrees), and stomatal density (#/mm²) for wettable and nonwettable species of montane wildflowers (Brewer, unpublished data). Values for each leaf surface are reported separately (T = top surface; B = bottom surface).

	Contact Angle		Droplet Retention		Stomatal Density	
Species Name	*T*	*B*	*T*	*B*	*T*	*B*
Wettable						
Fragaria virginiana (strawberry)	149	147	42	34	0	258
Lupinus argenteus (wild lupine)	150	158	26	23	71	121
Nonwettable						
Geranium richardsonii (sticky geranium)	81	82	90	90	53	267
Dodecatheon pulchellum (shooting star)	66	65	45	76	18	89

Droplets of a standard size (5 μl recommended volume) are placed on the leaf surface with a calibrated pipet or dropper. Then, leaf wettability is determined by measuring the contact angle (θ) of a droplet with the leaf surface (Adam 1963). The angle theta, θ, of a line tangent to the droplet through the point of contact between the droplet and the leaf surface is measured (Figure 1B). Theta is measured relative to the epidermis for horizontally positioned leaves (even when droplets rest above the epidermis on trichomes). The modified protractor is held behind the droplet near the leaf surface (Figure 1A). By viewing the droplet and protractor through the microscope, it is possible to measure the contact angle by simply changing the angle of the string until it matches the angle at which the droplet contacts the leaf surface. At least five replicates per leaf surface and five leaves per species should be measured. Students should avoid making measurements on parts of the leaf that were handled and possibly damaged. Leaves with θ < 110° are considered wettable; those with θ > 130° are nonwettable (Crisp 1963). Given a droplet of a certain volume, θ also provides an index of the amount of the droplet that is in contact with the leaf surface, and the area of leaf surface covered (see Brewer et al. 1991 for an empirical description of these relationships). This procedure can be repeated with droplets of different sizes (e.g. 50 μl, 100 μl, etc.) to simulate drops resulting from rain, mist, dew, or a garden sprinkler.

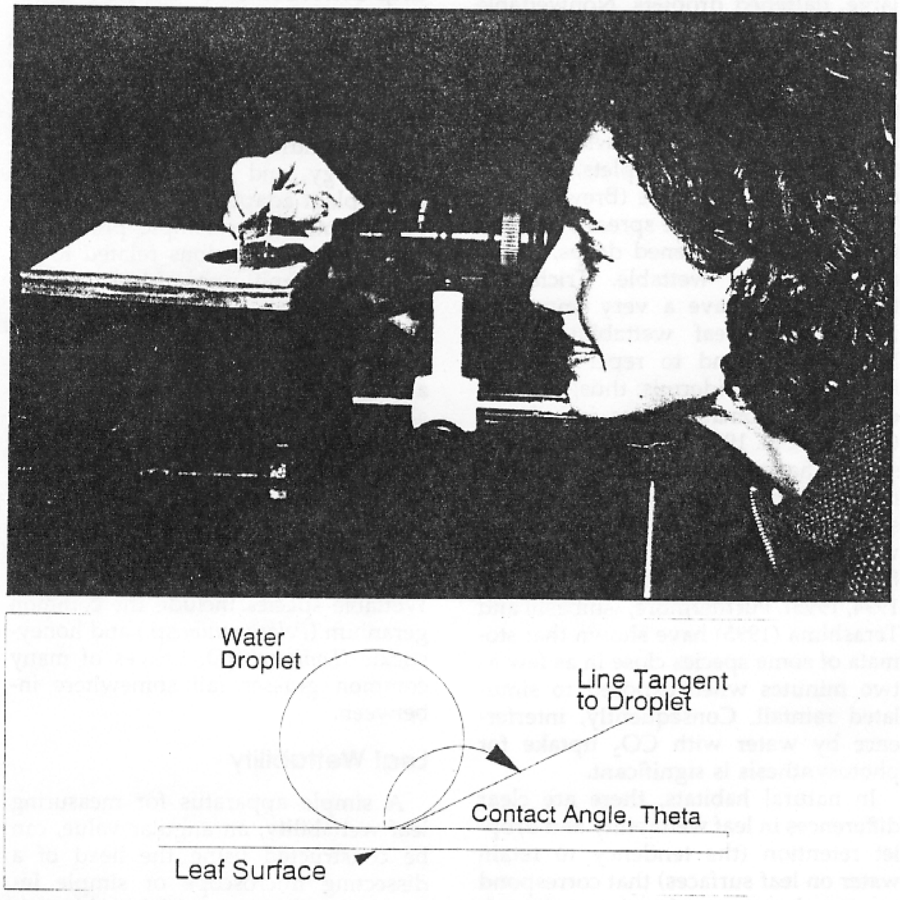

Figure 1. Setup for measuring wettability (contact angle, θ) on leaf surfaces. A. Experimental materials, including modified protractor, inspection scope and mounts. Leaf is taped to the platform for viewing droplet contact angle. B. Leaf wettability is angle, θ, of a line tangent to the droplet through the point of contact between the droplet and the leaf surface.

Droplet Retention

Droplet retention, an angular value, is an index of the "stickiness" of a leaf surface for water (Brewer et al. 1991). A simple "droplet retention meter" (Figure 2A) is constructed using an angle level (e.g. multi-function level from a local hardware store) and a metal plate (e.g. 4 × 4-inch piece of sheet metal). On one side, the metal plate is taped to a table surface, and then the leaf is taped (ends only) to the plate. Droplet retention is measured by placing a 50 μl droplet (recommended standard volume from a calibrated pipet or dropper) on the horizontal leaf surface and measuring the angle of inclination at which the droplet first begins to roll down the leaf surface (Figure 2B). At least five replicates per leaf surface and five leaves per species should be measured. As with measurements for leaf wettability, droplets of different sizes also can be compared.

High angular values (>60°) indicate a greater tendency to retain droplets while low values (<20°) indicate leaf surfaces that readily shed droplets.

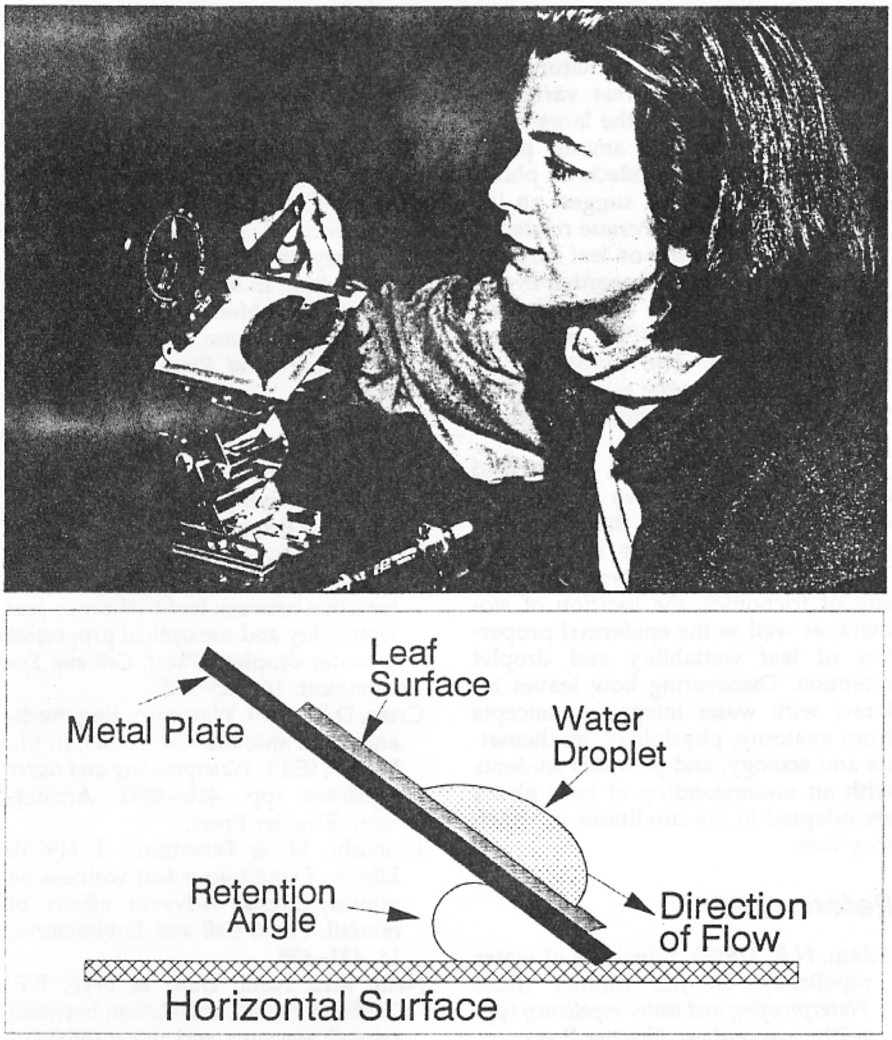

Figure 2. Setup for measuring droplet retention on leaf surfaces. A. Experimental materials, including angle level, moveable metal plate, pipet, and lab jack. Leaf is taped to the moveable plate for measuring retention angle. B. Droplet retention is the angle of inclination at which a droplet first begins to roll down the leaf surface.

Note that a high value of θ (i.e., non-wettable leaf) does not always correspond to low droplet retention (Brewer et al. 1991).

Stomatal Density

Stomatal density is estimated using surface impressions made from both top and bottom leaf surfaces (modified from Neill et al. 1990; Brewer 1992). Surface impressions are prepared by painting the leaf surface with clear fingernail polish. It is important that nail polish only be applied to leaves with no visible surface moisture. Leaves can be dried by gently blotting moisture with absorbent tissue paper. Otherwise, surface replicas will be cloudy and may not dry properly. When leaves are extremely hairy, they may have to be shaved prior to making surface impressions. A simple, plastic, single blade razor is sufficient for most hairy leaf surfaces. Care should be taken to avoid damaging the leaf epidermis when shaving trichomes. Allow the impressions to dry for 5–10 minutes (after 15 minutes, surface impressions will be very difficult to remove). To remove the surface impression, a piece of scotch tape, approximately 1.0-cm long, is cut. The tape is folded over on itself leaving about 0.5 cm of the sticky surface exposed. The sticky end of the tape is placed on the leaf so that it sticks to the nail polish impression. Using the remaining tape as a handle, the impression is carefully peeled from the surface of the leaf. Finally, the impression is taped to a microscope slide. Nail polish impressions from both leaf sides can be placed side by side on a microscope slide for easy comparisons. It is important that students clearly label all microscope slides to indicate "leaf top" and "leaf bottom". No cover slips are needed and stomata are viewed on the part of the surface impression not covered by tape. Stomatal density is calculated by counting the number of stomata within a calibrated micrometer grid (e.g. 2.5 × 2.5 mm), or within a field of view if ocular grids are not available. Typically, counting is facilitated by using a 40× objective. At least five replicates per leaf surface and five leaves per species should be measured.

Summarizing & Interpreting Data

Differences in leaf wettability, droplet retention, and stomatal density between leaf sides can be visually represented in a number of ways. Bar graphs conveniently illustrate differences in means for wettability, droplet retention and stomatal density among species, or between leaf sides. Students may also plot stomatal density as a function of wettability, or droplet retention. Interesting patterns also result when wettability is plotted as a function of droplet retention. For more advanced students, simple statistical procedures (e.g. correlation analysis, analysis of variance, student's T-test) can be used to explore relationships in more detail, as well as model variance within and among species.

Extending Beyond the Classroom—Microhabitat Patterns of Leaf Wetness

The procedures described above can be used in both laboratory and field applications. Techniques can be perfected in the laboratory in guided investigations where the learning objectives may be related to understanding leaf surface morphology, or stomatal anatomy and distribution. After students are provided with background information on the ecological and physiological implications of leaf surface wetness, they can formulate and test hypotheses about the kinds of species that might be wettable and nonwettable. Furthermore, they can predict which leaf surface should be the least wettable. In a field setting, students can look for correspondence between susceptibility to leaf wetting events and adaptations to avoid being wet all over.

For example, interesting hypotheses related to susceptibility to leaf wetting and adaptations for avoiding leaf soaking can be tested easily on the school grounds or in natural habitats. A hypothesis about avoiding leaf wetness for plants in habitats with frequent leaf wetting events might involve collection of the following data: moisture accumulated on leaves of plants in different habitats; leaf wettability; droplet retention; stomatal density; and a simple index of leaf hairiness.

The amount of moisture accumulated on leaves after heavy dewfall (early in the morning), rainfall, or experimental misting to simulate either of these weather phenomena can easily be determined in the following way. To prepare for field work, students number a suitable quantity of Ziploc® baggies (prepare for five replicate samples from the top surface and bottom surface of each leaf type in each habitat of interest). A piece of tissue paper (i.e. chem wipe, or other non-oily absorbent tissue) is placed in each bag, and the bags are carefully sealed after excess air is squeezed out. Then, the bags plus tissue paper are weighed.

Leaf surface moisture is collected separately from top and bottom leaf surfaces by blotting moisture from each surface with the pre-weighed tissue paper, and then returning the paper to the appropriate bags, which are sealed carefully to avoid evaporation. Samples are returned to the laboratory where they are reweighed. The amount of water on each leaf surface is determined by calculating the difference in wet and dry weights. If students are interested, data on leaf orientation and height above the ground can be recorded. Finally, sampled leaves are collected for determination of surface area. With data for leaf area, values can be standardized so that data on leaf surface moisture accumulation are expressed on a "per unit leaf area" basis.

Students also may wish to explore relationships between fungal infection of leaves, frequency of leaf wetting events, and surface characteristics of leaves. In many schoolyards, sprinkler systems provide a predictable source of moisture which accumulates on leaf surfaces. The occurrence of infected leaves may be explored as a function of leaf wetness duration, distance from sprinklers, or even height above the ground.

Conclusions

The high frequency of natural leaf wetting events in a great variety of habitats, coupled with the large range in surface wettability among plants and potentially strong effects on photosynthesis and growth, suggest an important evolutionary avenue related to the dynamics of water on leaf surfaces. Dewfall, rain and other weather events leading to leaf surface wetness are extremely common during a typical growing season. The probability of wetting events may be especially high for plants in open meadows or agricultural field habitats.

Many common native, exotic and agricultural species have leaf surface features that minimize the amount of surface area in contact with water. These features include type and density of trichomes, the location of stomata, as well as the epidermal properties of leaf wettability and droplet retention. Discovering how leaves interact with water integrates concepts from anatomy, physiology, mathematics and ecology, and provides students with an understanding of how plants are adapted to the conditions in which they live.

References

Adam, N.K. (1963). Principles of water repellency. In J.L. Molliet (Ed.), *Waterproofing and water repellency* (pp. 1–23). Amsterdam: Elsevier Press.

Adams, C.M. & Hutchinson, T.C. (1987). Comparative abilities of leaf surfaces to neutralize acidic raindrops II. The influence of leaf wettability, leaf age and rain duration on changes in droplet pH and chemistry on leaf surfaces. *New Phytologist, 106,* 437–456.

Barr, A. & Gillespie, T.J. (1987). Maximum wetness duration for water drops on leaves in the field. *Agricultural and Forest Meteorology, 41,* 267–274.

Benzing, D.H. & Renfrow, A. (1971). Significance of patterns of CO_2 exchange to the ecology and phylogeny of the Tillandsioideae (Bromeliaceae). *Bulletin of the Torrey Botanical Club, 98,* 322–327.

Brewer, C.A. (1992). Evaluation of patchy stomatal response to leaf microenvironmental conditions. In C.A. Goldman (Ed.), *Tested studies for laboratory teaching: Proceedings of the 13th Workshop/Conference of the Association for Biology Laboratory Education Manual* (pp. 67–75). Canton, NY: Association for Biology Laboratory Education (ABLE).

Brewer, C.A. & Smith, W.K. (1994). Influence of simulated dewfall on photosynthesis and above ground production in soybean isolines (*Glycine max* [L.] Merr. cv Williams) with different trichome densities. *International Journal of Plant Sciences, 155,* 460–466.

Brewer, C.A. & Smith, W.K. (1995). Leaf surface wetness and gas exchange in the pond lily *Nuphar polysepalum* (Nymphaeaceae). *American Journal of Botany, 82,* 271–1277.

Brewer, C.A., Smith, W.K. & Vogelmann, T.C. (1991). Functional interaction between leaf trichomes, leaf wettability and the optical properties of water droplets. *Plant, Cell and Environment, 14,* 955–962.

Crisp, D.J. (1963). Waterproofing mechanisms in animals and plants. In J.L. Molliet (Ed.), *Waterproofing and water repellency* (pp. 416–481). Amsterdam: Elsevier Press.

Ishibashi, M. & Terashima, I. (1995). Effects of continuous leaf wetness on photosynthesis: Adverse effects of rainfall. *Plant, Cell and Environment, 18,* 431–438.

Neill, R.L., Neill, D.M. & Frye, B.F. (1990). Is there a correlation between rainfall amounts and the number of stomata in cottonwood leaves? *The American Biology Teacher, 52,* 48–49.

Pearcy, K.E. & Baker, E.A. (1991). Effects of simulated acid rain on needle wettability and rain retention by two Sitka spruce (*Picea sitchensis*) clones. *Canadian Journal of Forest Research, 21,* 694–697.

Reynolds, K.M., Madden, L.V., Richard, D.L. & Ellis, M.A. (1989). Splash dispersal of *Phytophthora cactorum* from infected strawberry fruit by simulated canopy drip. *Phytopathology, 79,* 425–432.

Smith, W.K. & McClean, T.M. (1989). Adaptive relationship between leaf water repellency, stomatal distribution, and gas exchange. *American Journal of Botany, 76,* 465–469.

Weast, R.C. (1979). *Handbook of chemistry and physics.* Boca Raton, FL: CRC Press.