Received: 14 February 2023 | Accepted: 15 August 2023

DOI: 10.1002/aps3.11565

Applications in Plant Sciences

PROTOCOL NOTE

Thallus hydrophobicity: A low-cost method for understanding lichen ecophysiological responses to environmental changes

Natália Mossmann Koch[1] | Raúl Díaz Dominguez[2] | Ana Fávaro[1] | Daniel Stanton[1]

[1]Department of Ecology, Evolution and Behavior, University of Minnesota, Saint Paul, Minnesota, USA

[2]Instituto de Investigaciones Biológicas y Tecnológicas, Centro de Ecología y Recursos Naturales Renovables, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Córdoba, Argentina

Correspondence

Natália Mossmann Koch, Department of Ecology, Evolution and Behavior, University of Minnesota, 140 Gortner Laboratory, 1479 Gortner Avenue, Saint Paul, Minnesota 55108, USA.
Email: nkoch@umn.edu

This article is part of the special issue "Resilient botany: Innovation in the face of limited mobility and resources."

Abstract

Premise: Methods to evaluate lichen thalli hydrophobicity have previously been described, but only recently has hydrophobicity been shown to be an important functional trait related to water regulation dynamics that could be used to predict future climate change effects. We describe a novel protocol to measure lichen thallus hydrophobicity that aims to be an easier and more affordable approach.

Methods and Results: Our protocol requires only a micropipette, distilled water, a tripod, and a smartphone or camera. Hydrophobicity is inferred from multiple metrics associated with the absorption times of standardized droplets (initial and total absorption time). We used a data set of 93 lichen taxa with different growth forms and from different biomes and demonstrated that this method is well suited for capturing different levels of hydrophobicity, including very hydrophilic species.

Conclusions: Our results show that this new protocol to measure lichen hydrophobicity is a rapid and low-cost method to assess an ecophysiologically based functional trait that can be used with almost no limitations, including in different climates, lichen species, and growth forms.

KEYWORDS

ecophysiology, functional traits, hydration, lichens, water absorption, wettability

Resumen

Premisa: En el pasado se han descrito métodos para evaluar la hidrofobicidad de los talos liquénicos, sin embargo, no fue hasta recientemente que se demostró la importancia de esta propiedad como rasgo funcional en relación a la dinámica de regulación hídrica de los talos, permitiendo utilizarla como herramienta para predecir futuros efectos del cambio climático. Describimos un nuevo protocolo para medir la hidrofobicidad de los talos liquénicos que pretende ser más fácil y asequible.

Métodos y Resultados: Nuestro protocolo requiere solamente de una micropipeta, agua destilada, un trípode y un teléfono o una cámara. La hidrofobicidad es inferida a partir de múltiples métricas asociadas a los tiempos de absorción de gotas de un volumen estandarizado (tiempo de absorción inicial y tiempo de absorción total). Utilizamos datos de 93 taxones de líquenes con diferentes formas de crecimiento y provenientes de distintos biomas, lo que permitió demostrar que este método es adecuado para capturar diferentes niveles de hidrofobicidad, incluyendo a especies altamente hidrofílicas.

Conclusiones: Nuestros resultados muestran que la medición de la hidrofobicidad de los talos es un método rápido y de bajo costo que permite evaluar un rasgo funcional basado en la ecofisiología de líquenes y que puede ser utilizado prácticamente sin limitaciones, incluyendo en diferentes climas, especies y formas de crecimiento.

Functional traits are measurable attributes that show the ecological responses of an organism's phenotype to biotic or abiotic, spatial, or temporal gradients (Ellis et al., 2021). Although there is a rapidly growing set of studies of lichen functional traits, mirroring advances in other taxa (Gauslaa, 2014; Matos et al., 2015; Ellis et al., 2021; Koch et al., 2022), quantitative functional traits that are more widely applicable (suitable for different thallus morphologies or growth forms) and accessible (that do not require expensive equipment to measure) are still lacking. While easily applied methods have been developed for water storage (water-holding capacity; Asplund and Wardle, 2017), low-cost approaches have not, until now, been proposed in detail for water uptake traits. Furthermore, water storage trait protocols have been focused on large, easily detached taxa, which introduces taxonomic and ecological biases in trait coverage.

Surface properties of lichens have been shown to be ecologically important and to vary greatly among species (Lakatos et al., 2006; Honegger, 2007). Although lichens have different growth forms, the upper surface is present in all lichen species (Büdel and Scheidegger, 2008). Because lichens are poikilohydric, their upper surface plays key roles in their water uptake, and thus thallus hydrophobicity (i.e., the ability to repel water) has an impact on water-holding capacity and desiccation tolerance (Lakatos et al., 2006; Esseen et al., 2017). However, no simple and inexpensive protocol exists to measure thallus hydrophobicity, which may account for the fact that only a few ecological studies have used thallus surface hydrophobicity as an important lichen physiological trait (Hauck et al., 2008; Colesie et al., 2017; Díaz Dominguez et al., 2022). Hauck et al. (2008) measured the contact angle as a predictor of hydrophobicity and found correlations with species tolerance to sulfur dioxide. Colesie et al. (2017) showed that the lichen *Psora decipiens* (Hedw.) Hoffm. from European soil crust communities had faster water uptake in drier environments. In a study by Díaz Dominguez et al. (2022), the authors introduced an earlier version of the method we present here and demonstrated that lichen surface hydrophobicity can vary within species according to microenvironment in mountain regions in Argentina.

Here, we describe in detail and further evaluate the method used by Díaz Dominguez et al. (2022) to measure lichen thallus hydrophobicity, which consists in timing the initial and complete absorption of water droplets. This method aims to be an easier and more affordable approach to assess lichen thallus hydrophobicity than measuring the contact angle of those droplets, which often requires specialized equipment (e.g., Contact Angle Measuring System; DataPhysics Instruments USA, Charlotte, North Carolina, USA), adding costs to the method. Furthermore, the method proposed here can be applicable to a full range of surface characteristics, as some lichens take up water too fast to even show a droplet shape without high-speed photography (less than 0.05 seconds). The fact that all lichens have an upper surface does not mean all species have a cortex, but the method we describe here can be used on lichens with or without a cortical upper surface. Therefore, we have adapted a low-cost technique for surface hydrophobicity in leaves (Matos and Rosado, 2016) and modified it to be suitable for lichens of a wide range of sizes, lineages, and ecologies, as well as to include a focus on absorption rates rather than contact angles.

METHODS AND RESULTS

The detailed method presented in this paper started as an adaptation from a protocol used to measure surface hydrophobicity in leaves described by Matos and Rosado (2016). The protocol has been modified to be applicable to the lichen surface, as lichens have a more complex surface (with different structures and planes) than leaves. In addition to being applicable to lichens, this new method provides an easy and accessible way to measure a quantitative functional trait of lichens that could help understand water relations in all types of lichen growth forms. Requiring only a camera (digital or smartphone) with a tripod, a micropipette, and distilled water, this new method for measuring lichen hydrophobicity has the potential to be widely implemented and an important contribution for understanding water relations in lichens.

To determine hydrophobicity, it is important that the specimen is dry and healthy (not showing areas of chlorosis covering more than around 30% of the thallus, or tissue death). Wet specimens and specimens with many dead spots might not reflect the actual hydrophobicity of the thalli. If the specimen was stored in a freezer before the procedure, it needs to sit overnight in dry conditions before starting the protocol (Appendix 1).

The specimen must then be placed on a flat surface, with a camera installed at the same height as the specimen to capture the entire lichen surface in a horizontal plane for analyzing multiple droplets in the same video. A black background and a lamp are also helpful to better visualize the droplets when analyzing the video, but they are not absolutely necessary. For filming, we recommend using a shutter speed of 24 frames per second (fps) or higher. The specimen needs to be in focus and well-lit.

Using a micropipette, place 10-μL droplets of distilled water on the surface of the specimen (Figure 1A, Appendix 1, Video 1). Damaged areas, soredia, apothecia, or any other type of reproductive structure should be avoided (if possible), as such structures can vary from the main vegetative thallus in surface chemistry (Hamlett et al., 2011). Also, droplets must not be placed onto previously wetted surfaces (where a previous droplet was absorbed or too close to other droplets) or on areas where the droplet cannot be clearly seen in the video. Considering that properties change dramatically on wet surfaces, droplets that were accidentally added to a wet spot or that merged with another wet spot must be excluded

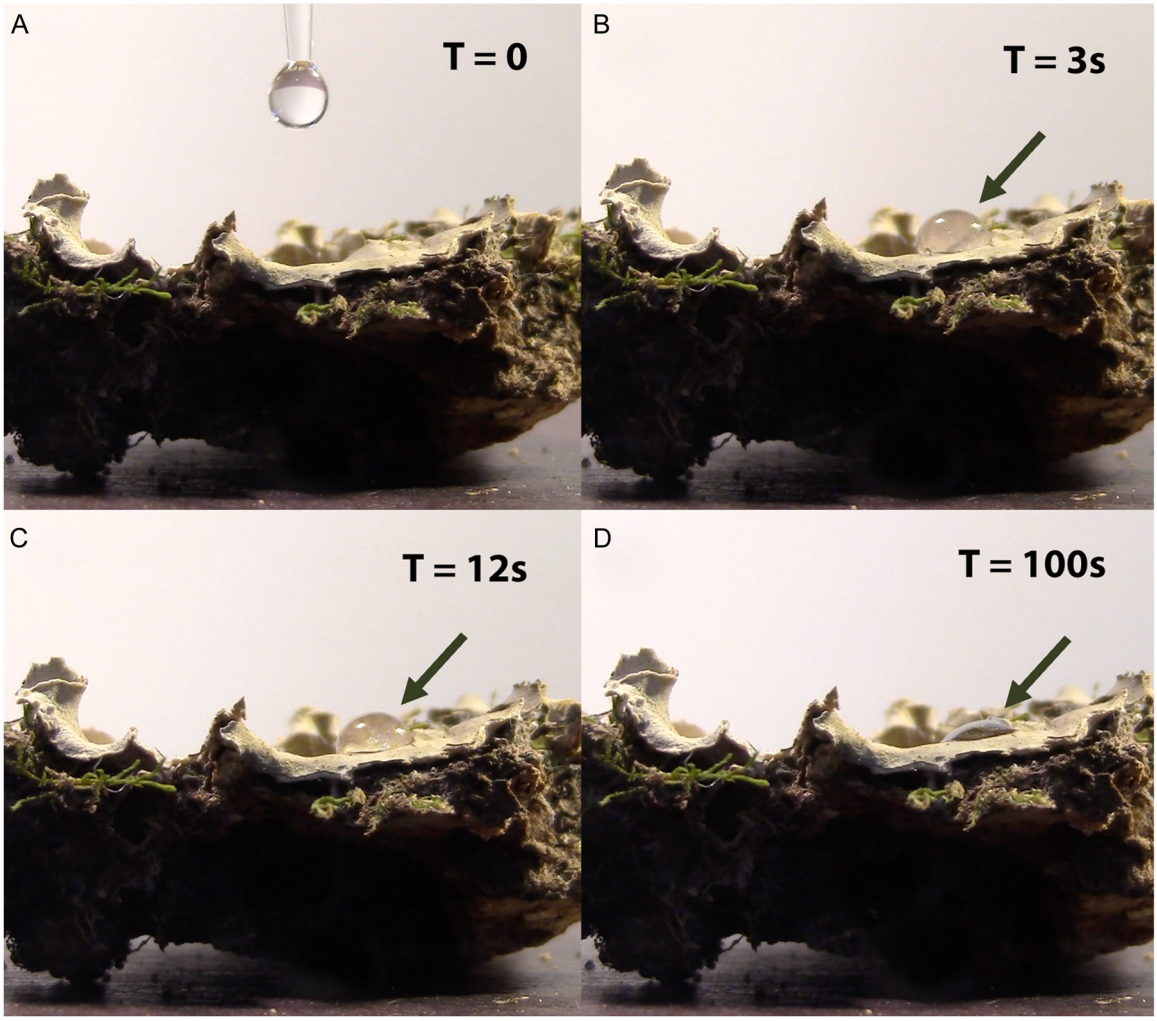
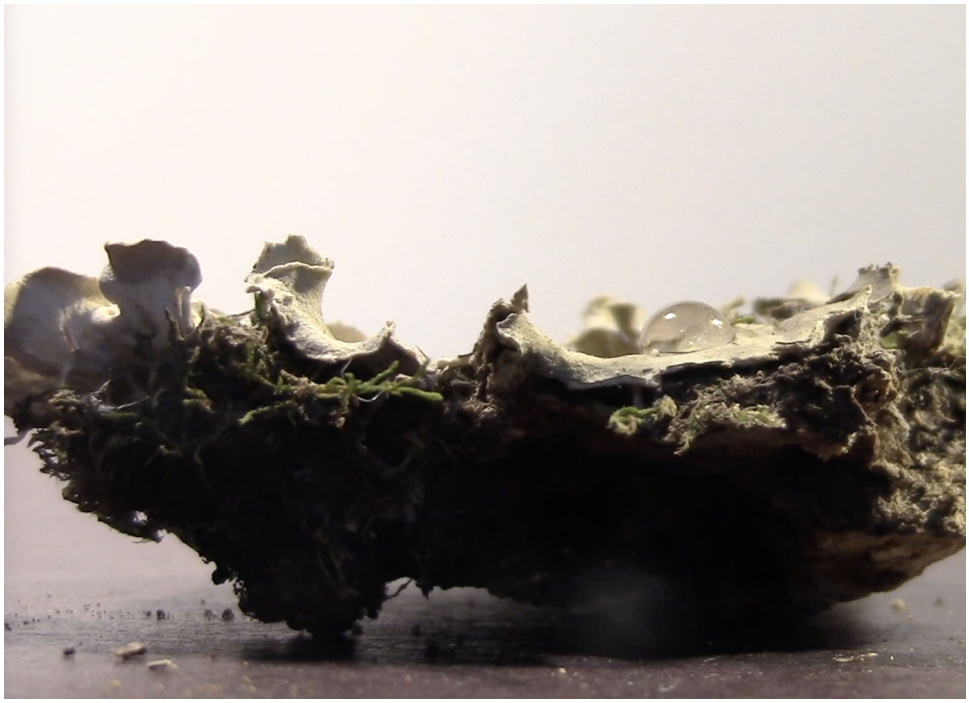


FIGURE 1 Sequence of 10-μL droplet absorption by the lichen species *Peltigera canina* (L.) Willd. Initial time (T = 0) represents when the droplet was placed on the thallus (A). Panel (B) shows the droplet before the start of absorption, and the different shape of the droplet in panel (C) indicates that absorption began at 12 seconds. Panel (D) shows the moment before droplet absorption was complete. The placement of the droplets is indicated by the arrows. The complete video can be found in Video 1.

from the analyses. Stop recording when all droplets are absorbed or 150 seconds after the last droplet was placed. Ideally, at least three droplets per specimen should be recorded; we recommend adding more (up to five) whenever possible, but it may not be feasible if the specimen is too small. Note that some species, such as members of the genera *Lepraria* and *Chrysothrix*, are highly hydrophobic and may not absorb droplets at all, so it is not productive to continue filming past 150 seconds. After completing the video, save the files using file names reflecting relevant specimen information. When processing the video, record for each droplet (a) the time (in seconds) between contact with the surface and the start of absorption (this can be 0 in very hydrophilic lichens), and (b) the time between contact with the surface and apparent complete absorption of the droplet (again, this can be 0 in very hydrophilic lichens). Also note whether or not the droplet was fully absorbed. The protocol is described in detail in Appendix 1.

Protocol feasibility

To demonstrate that measuring thallus hydrophobicity using the time of absorption is suited for capturing very hydrophilic species and to determine the optimal time for capturing the videos, we used a data set of 93 lichen taxa with different growth forms and from different biomes in the United States (boreal to subtropical) and a desert area in Chile (Alto Patache) (Appendix S1). For all specimens tested, hydrophobicity was measured within two years after sampling. Based on this data set, we built absorption curves (Figure 2) using the package 'survival' (Therneau and Grambsch, 2000; Therneau, 2023) in R (R Core Team, 2023), aiming to visualize the time of initial absorption of the water droplets and the time droplets were completely absorbed. Additionally, we ran analyses of correlation for each data set using the Pearson correlation coefficient to test whether the two metrics proposed (time of initial absorption and time of complete absorption) were correlated. A list of the



VIDEO 1 Video showing the absorption sequence of a 10-μL droplet on a sample of *Peltigera canina*.

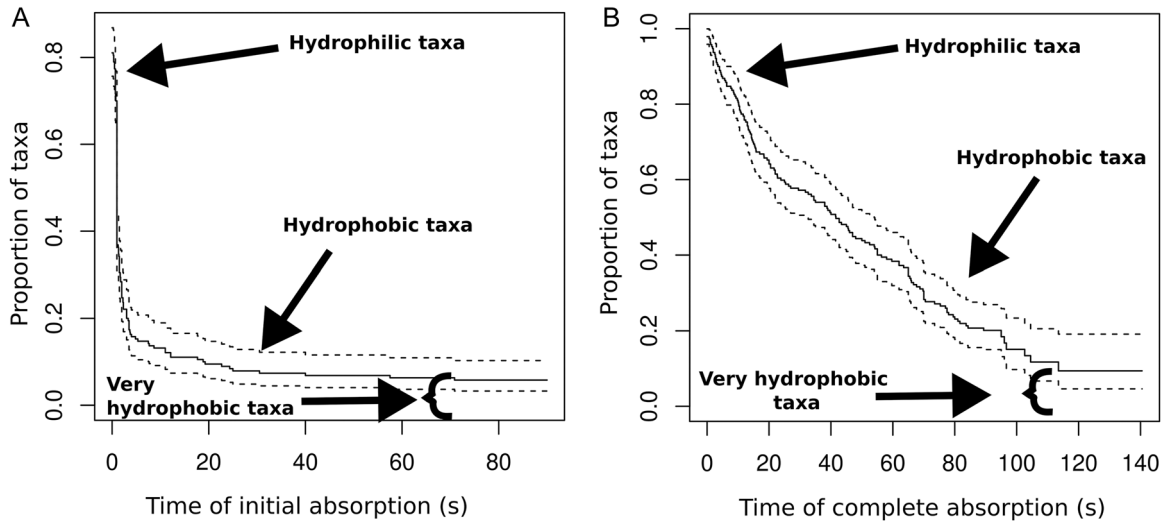


FIGURE 2 The proportion of taxa that have not begun (A) or completed (B) absorption over time, using a data set of 93 lichen species from varied biomes of the United States and Chile. While many taxa rapidly begin absorption (within the first minute), absorption can take up to 2 min depending on the taxon or never reach completion (very hydrophobic taxa). The dashed lines in the graphs represent the 95% confidence interval.

species used, the average time for complete droplet absorption, and the sites where they were collected can be found in Appendix S1. The complete data set is available in the Dryad Digital Repository (https://doi.org/10.5061/dryad.cc2fqz6bs; Koch et al., 2023).

It is notable that some species start absorbing the water droplets almost immediately (very hydrophilic species) (Figure 2A), while others never absorb the droplet (very hydrophobic species). Based on the wide variability in absorption times in our data set, we recommend 150 seconds (120 seconds + a 30-second buffer) as a sufficient length of time to record a video capturing most of the variability of absorption time of the water droplets (Figure 2B); however, it may be prudent to test typical absorption times before choosing a time limit for new sites or taxa. The results of the correlation analyses showed that

the time of initial droplet absorption and complete absorption was positively but not strongly correlated considering both data sets (United States: $r = 0.28$, $P = 0.006$; Chile: $r = 0.66$, $P < 0.001$).

CONCLUSIONS

The protocol described here to evaluate thallus hydrophobicity in lichens is a low-cost and efficient method for assessing lichen water relations. It is effective and applicable due to the fact that it can be used to evaluate nearly all members of any lichen community, including lichens from different biomes, lineages, growth forms, and surface characteristics. Because the surface properties of lichens do not change considerably with time, this method could potentially be applied in archival material. Furthermore, the protocol only strictly requires a camera (even a smartphone is viable, provided the droplets can be clearly seen), a tripod, a micropipette, and distilled water, and can be completed in just a few minutes, which contrasts with the currently existing methods that require expensive equipment and more time to be performed. We recommend that both the time of the beginning of droplet absorption and the time for complete absorption should be recorded, as the correlation of those two metrics is not very strong and may vary in different environments.

Applying this protocol to fine-branching lichens (e.g., some fruticose thalli) can be somewhat difficult, but it is possible. We also emphasize that the effect of incomplete drying on thallus hydrophobicity was not explored here, and this should be further tested. Furthermore, it is important to use caution in interpreting hydrophobicity measurements made on reproductive structures, as they may have very different surface properties than the thallus (e.g., apothecia). Because this method does not require expensive equipment and can be applied with almost no limitations, the use of this new protocol has the potential to drastically expand our understanding of water regulation dynamics and how lichens may be affected in climate change scenarios (Díaz Dominguez et al., 2022; Stanton et al., 2023), as well as applications to other taxa that absorb water through aerial surfaces, such as bryophytes, terrestrial biocrusts, and some vascular plants.

AUTHOR CONTRIBUTIONS

D.S. first designed the new protocol. N.M.K. led the writing of the manuscript with contributions from R.D.D., A.F., and D.S. D.S. and N.M.K. sampled lichen data, performed the statistical analyses, and prepared the figures and tables. All authors revised and approved the final version of the manuscript.

ACKNOWLEDGMENTS

The authors thank Dennis Schu (University of Minnesota) for helping to record some of the videos for the lichens of the boreal United States and Laima Liulevicius (University of Minnesota) for making Video 1. D.E.S. and N.M.K. were supported by start-up funding to D.E.S.

DATA AVAILABILITY STATEMENT

The complete data set is available in the Dryad Digital Repository (https://doi.org/10.5061/dryad.cc2fqz6bs; Koch et al., 2023).

ORCID

Natália Mossmann Koch http://orcid.org/0000-0001-6958-4495
Raúl Díaz Dominguez http://orcid.org/0000-0002-2674-1531
Ana Fávaro http://orcid.org/0000-0001-6430-9080
Daniel Stanton http://orcid.org/0000-0002-6713-9328

REFERENCES

Asplund, J., and D. A. Wardle. 2017. How lichens impact on terrestrial community and ecosystem properties. *Biological Reviews of the Cambridge Philosophical Society* 92: 1720–1738.

Büdel, B., and C. Scheidegger. 2008. Thallus morphology and anatomy. *In* T. H. Nash III [ed.], Lichen biology, 40–68. Cambridge University Press, Cambridge, United Kingdom.

Colesie, C., L. Williams, and B. Büdel. 2017. Water relations in the soil crust lichen *Psora decipiens* are optimized via anatomical variability. *The Lichenologist* 49: 483–492.

Díaz Dominguez, R., D. Stanton, M. Peralta, and J. M. Rodriguez. 2022. Water regulation dynamics of lichens as functional traits could predict future climate change scenarios in an elevational gradient from Central Argentina. *The Bryologist* 125: 466476.

Ellis, C. J., J. Asplund, R. Benesperi, C. Branquinho, L. Di Nuzzo, P. Hurtado, I. Martínez, et al. 2021. Functional traits in lichen ecology: A review of challenge and opportunity. *Microorganisms* 9: 766.

Esseen, P.-A., M. Rönnqvist, Y. Gauslaa, and D. S. Coxson. 2017. Externally held water—a key factor for hair lichens in boreal forest canopies. *Fungal Ecology* 30: 29–38.

Gauslaa, Y. 2014. Rain, dew, and humid air as drivers of morphology, function and spatial distribution in epiphytic lichens. *The Lichenologist* 46: 1–16.

Hamlett, C. A. E., N. J. Shirtcliffe, F. B. Pyatt, M. I. Newton, G. McHale, and K. Koch. 2011. Passive water control at the surface of a superhydrophobic lichen. *Planta* 234: 1267–1274.

Hauck, M., S.-R. Jürgens, M. Brinkmann, and S. Herminghaus. 2008. Surface hydrophobicity causes SO_2 tolerance in lichens. *Annals of Botany* 101: 531–539.

Honegger, R. 2007. Water relations in lichens. *In* G. Gadd, S. C. Watkinson, and P. S. Dyer [eds.], Fungi in the environment, 185–200. Cambridge University Press, Cambridge, United Kingdom.

Koch, N. M., D. Stanton, S. C. Müller, L. Duarte, A. A. Spielmann, and R. Lücking. 2022. Nuanced qualitative trait approaches reveal environmental filtering and phylogenetic constraints on lichen communities. *Ecosphere* 13: e4042.

Koch, N. M., R. Díaz Dominguez, A. Fávaro, and D. Stanton. 2023. Thallus hydrophobicity: A low-cost method for understanding lichen ecophysiological responses to environmental changes. Dryad dataset. https://doi.org/10.5061/dryad.cc2fqz6bs [accessed 14 December 2023].

Lakatos, M., U. Rascher, and B. Büdel. 2006. Functional characteristics of corticolous lichens in the understory of a tropical lowland rain forest. *New Phytologist* 172: 679–695.

Matos, I. S., and B. H. P. Rosado. 2016. Retain or repel? Droplet volume does matter when measuring leaf wetness traits. *Annals of Botany* 117: 1045–1052.

Matos, P., P. Pinho, G. Aragón, I. Martínez, A. Nunes, A. M. V. M. Soares, and C. Branquinho. 2015. Lichen traits responding to aridity. *Journal of Ecology* 103: 451–458.

R Core Team. 2023. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Website: http://www.R-project.org/ [accessed 14 December 2023].

Stanton, D. E., A. Ormond, N. M. Koch, and C. Colesie. 2023. Lichen ecophysiology in a changing climate. *American Journal of Botany* 110: e16131.

Therneau, T. 2023. A package for survival analysis in R. R package version 3.5-7. Website: https://CRAN.R-project.org/package=survival [accessed 14 December 2023].

Therneau, T., and P. M. Grambsch [eds.]. 2000. Modeling survival data: Extending the Cox model. Springer, New York, New York, USA.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1. List of taxa used in the analysis shown in Figure 2, mean time for complete droplet absorption (in seconds), and their sampling location.

How to cite this article: Koch, N. M., R. Díaz Dominguez, A. Fávaro, and D. Stanton. 2024. Thallus hydrophobicity: A low-cost method for understanding lichen ecophysiological responses to environmental changes. *Applications in Plant Sciences* 12(2): e11565. https://doi.org/10.1002/aps3.11565

Appendix 1. Detailed protocol to measure lichen hydrophobicity.

Materials

1. Digital camera or smartphone with video capacity
2. Micropipette (10 or 20 µL)
3. Micropipette tips (10 or 20 µL)
4. Distilled water
5. Stands/mounts for camera and specimen
6. A black background (optional)
7. Lights (optional)

Video recording

1. Before the analysis, make sure the specimens are completely dry. If the samples were in a freezer before the hydrophobicity analysis, take them out of the freezer and let them sit overnight in dry conditions.
2. Place the specimen on a flat surface and arrange the camera at the same height as the specimen. Place the specimen horizontally, in a way that maximizes the horizontal surface available for water droplets (e.g., by choosing the side with a bigger flat area). For smaller taxa, it can be possible to have multiple specimens in the same recording.
3. Make sure that the specimen is in focus and well-lit. A black background behind the specimen and diffuse photography lights can be used to improve visualization of the droplets when analyzing the video and to reduce shadows, but they are not strictly necessary.
4. Start recording. We recommend using a shutter speed of 24 frames per second (fps) or higher.
5. Gently place a 10-µL droplet of distilled water on the surface of the specimen, choosing an undamaged, dry, and (ideally) horizontal region. It is important that the specimen does not show areas of chlorosis covering more than 30% of the thallus and/or a similar amount of tissue death. Also, avoid placing droplets on apothecia as they are hydrophilic structures. The droplet needs to be formed before being placed on the lichen surface.
6. Add additional (ideally at least three) droplets to dry locations on the specimen, if possible. Properties change dramatically on wet surfaces; thus, if a droplet is accidentally added to one of these wet spots, take note of it and exclude it from the data analyses. If a droplet merges with another droplet, also exclude both droplets from the analyses.
7. Record the video until all droplets are absorbed or until 150 seconds after the last droplet is applied. Some highly hydrophobic species will take so long to absorb that it is unproductive to film past 150 seconds.
8. Save files with file names that reflect relevant specimen information.

Video processing

Video processing can be performed manually using any video playback software your operating system comes with. For each droplet, record (a) the time in seconds between contact with the surface and the start of absorption and (b) the time in seconds between contact with the surface and apparent complete absorption of the droplet. The beginning of the absorption is measured from when the shape of the droplet starts to change, which can be slow for some species or very fast for others. If the specimen is very hydrophilic, the time between the droplet touching the surface and the start of absorption (measurement a) can be 0 because the droplet is rapidly absorbed. If the specimen is very hydrophobic, the time between the droplet touching the surface and complete absorption (measurement b) can be 0 because the droplet is not absorbed in less than 150 seconds.