Journal of Plant Hydraulics
INRA SCIENCE & IMPACT

Journal of Plant Hydraulics 6: e-002

XIM4 meeting report, Sept. 25-27 2019, Padua (Italy) The hydraulic community has taken a step forward towards non-invasive measurements

Hervé Cochard[1], Giai Petit[2], José M. Torres-Ruiz[1] and Sylvain Delzon[3]
[1]Université Clermont-Auvergne, INRA, PIAF; [2] Dept. TeSAF - University of Padua; [3]Université de Bordeaux, INRA, Biogeco.

Corresponding author: Hervé Cochard, herve.cochard@inra.fr

Abstract
The fourth edition of the international xylem meeting was held for the first time outside France. This represents an important step forward for the meeting and attests to the resolutely international dimension of this symposium. The conference was organized by the University of Padua in the green setting of the botanical garden. No less than 140 researchers from more than 21 countries attended these three intense days of seminars and discussions. For logistical reasons, the number of places had been limited by the organizers, which forced us to decline a number of registrations. This tends to prove, if there was any need, that the community of researchers in xylem physiology and plant hydraulic functioning is booming, and that these meetings are becoming an essential biennial event for our discipline.
For this edition, no less than a hundred communications were presented, including about fifty plenary talks. It is difficult to summarize all the richness of this work in a few lines, so we propose here to highlight some of the most salient points.

A scientific community in tune with its society
While we were discussing our scientific work in session, more than 20,000 peaceful demonstrators marched for climate in the streets of Padua. In a manifesto published on social networks and signed by all congresses, we wanted to remind that:
"We, as an international community of plant scientists, support climate change mitigation. Our investigations of trees, forests and climate dynamics clearly show that critical ecosystems are being threatened by increasingly frequent extreme climatic events. Large-scale tree mortality across the globe demonstrate the severity of the threat that we are facing and demands actions."
Our work on the hydraulic functioning of plants allows us to better understand the response of trees to future climate change, especially to drought events, and their ability to adapt or migrate. We must also take advantage of these scientific meetings to better communicate with the general public, the media and political decision-makers, and to explain what will be the consequences of the ongoing climate change based on scientific evidences. It is our responsibility to alert people of the serious risks that our current lifestyles pose to natural and anthropized ecosystems. We will try not to miss this opportunity during our next meeting.

The long history of plant hydraulics
The symposium began with a presentation by Mel Tyree on "A brief history of 'our' science". Mel Tyree, one of the founding fathers of the modern history of plant hydraulics, honored us with his presence this year. His presentation, punctuated by numerous anecdotes, made young researchers aware of the importance of placing our research in a more global historical context. From its origins in the 15th century until today, 'our' science has been marked by controversies about the mechanism of sap ascend in trees. Erroneous methods

or observations are most often at the root of these controversies, but when rigorous experiments have been conducted independently, Dixon's (1914) cohesion-tension theory has always been reinforced. We must therefore look at current controversies in the light of this historical dimension and be convinced that they are a sign of a very dynamic field of research.

“Nothing in life is certain except death, taxes, and the second law of thermodynamics” (Seth Lloyd)
The remarkable feature of sap ascent in plants is that it occurs under negative pressure in a conductive tissue formed by dead cells. This physically very unstable state is likely to evolve into a more stable gaseous state through a cavitation process when the sap tension overpasses a threshold value. Living cells are under positive pressure due to the presence of a plasma membrane that establishes an osmotic barrier. As can be seen from these selected examples, physics, and thermodynamics in particular, are at the heart of the water relations of plants. And you cannot escape the laws of thermodynamics! Mel Tyree's many interventions during this conference often alerted us to a lack of knowledge of the physics of the systems we are studying. It is therefore critically important to associate physicists to our projects, especially when we are to explore the controversial topics dealing with the ascent of sap and the process of xylem embolism and repair. The talk of Missy Holbrook on the collapse of minor leaf veins was another great illustration of the close link between physics (biomechanics) and hydraulics. It could be interesting to attract more physicists to our conference in future editions to benefit from their critical view of our work.

“If it matters, it produces controversy” (Jay Green)
As a matter of facts, if we can judge by the number of controversies that animate our discipline since its early hours, plant hydraulics matters! Three main controversies have driven our debates:

- The “Miraculous” refilling of xylem conduits

Science is progressing in small steps, but perhaps the most significant advance of this symposium is the consensus in our community that the "miraculous" ability to resorb embolism under negative pressure is certainly elusive. It is thanks to the development of new non-destructive observation methods (X-ray tomography, NMR, Optical technique) that we are now reaching this consensus. In other words, a conductive system that is highly vulnerable to embolism and that would lead to high exposure to the risk of cavitation is unlikely to have evolved. However, as Marciej Zwieniecky mentioned in his presentation, just because xylem refilling capacities are low does not mean that we should not focus more on the plants' ability to recover from stress. Plants are exposed to many drought events during their lifetime, so their ability to recover and how they prepare to recover more quickly and effectively should be carefully studied.

- Carbon starvation *vs* hydraulic failure

The mechanisms by which water stress leads to plant mortality have yet to be clarified. Nate McDowell in his presentation recalled several important points to consider in moving this issue forward. If hydraulic failure is detected in almost all drought-induced mortality events, correlation is not necessary causation. The two mechanisms are therefore not exclusive of each other and can interact, especially over long periods of time. However, the mechanical link between hydraulic failure and carbon starvation remains to be identified, as the "refilling cost" hypothesis now seems to be excluded. We must better take into account this carbon starvation hypothesis in our work, and in particular evaluate the carbon status of the key organs involved in plant survival (primary and secondary meristems). Jess Gersony showed in her presentation that Raman-spectroscopy technology could be used to measure several sugars at the cell level, which should lead to significant progress on this issue.

- News direct observational methods for less artefacts

In the history of our discipline, researchers have always been confronted with methodological biases that have led them to misinterpret their observations and therefore misunderstand the hydraulic functioning of plants. This is largely due to the very particular water status of the water present in the conductive tissue of plants (negative pressures) and therefore to the risk of strongly perturbing observations by too invasive methods. This explains why in many presentations, researchers have implemented less or non-invasive and more direct methods of observing embolism. Some techniques require very sophisticated and non-easily accessible equipment (NMR, micro-CT), others, such as the optical method, are much more accessible. The electrical resistivity tomography method presented by Stefan Mayr is also a promising and affordable non-invasive technique which has also the merit of being able to be used *in situ* and on large trees. We are only beginning to use these new techniques and we must still be cautious in our conclusions. Nevertheless, and at this stage of our knowledge, these non-invasive methods reveal a conductive tissue that is much more resistant to cavitation than can be suggested by invasive techniques (i.e. hydraulic methods). In particular, for all the species that have been studied with these new methods and presented at this symposium, cavitation events never occur under the normal operating range of water potential, but only under severe water stress. The hydraulic techniques (benchtop dehydration, air injection) no longer appear to be the gold standard methods to study species with long pipes and techniques allowing to work on intact plants must be preferred. Significantly, cavitation occurs after the closing point of the stomata, which also often corresponds to the point of loss of turgor of the leaves. Missy Holbrook and Scott McAdam showed in their presentation that even Bryophytes or earliest vascular plants like Lycophyte have conductive tissues that are remarkably resistant to cavitation. This very conservative nature of hydraulic functioning is therefore very old, and probably constituted an evolutionary lock to be lifted for the colonization of emerged lands.

This should lead us to be extremely critical when we encounter R-shaped curves in our work, as B. Schuldt enthusiastically demonstrated during his presentation on root hydraulic safety and efficiency. Without wishing to reject *a priori* all these curves, we must collectively require a minimum of experimental guarantees on the validity of such curves before publication. When these curves are produced by a new method (as with the Pneumatron currently), it should be cross-validated with a reference and non-invasive method. In all cases, and whatever the method used, it is essential to test the consistency of the curves obtained with the water relations of the plant. In particular, the cavitation pressure threshold (P_{12}) must be more negative than the sap pressure measured on a plant that is well supplied with water and transpiring heavily. Similarly, this P_{12} is always less than or equal to the sap pressure inducing stomatal closure (P_{gs90}) or the point of loss of foliar turgidity (P_{tlp}). If these conditions are not met, and therefore if a significant rate of embolism is predicted based on the vulnerability curve, then this rate should be validated by robust *in situ* embolism measurements. With these simple rules, we will produce more robust data and avoid repeating the mistakes we made in the past and stop blocking the progress our discipline because of these avoidable misconception.

Trees hiding the forest

Since the aim was to understand the rise of sap beyond the theoretical 10-metre barrier, the first work on plant hydraulics historically focused on trees. The development of our discipline at the end of the 20th century was also undertaken in institutes closely linked to trees, such as the Harvard-Forest department, the US-Forest Service and the INRA Forest department. The amount of information accumulated on tree hydraulics is now considerable, which has led to major cognitive advances in tree physiology, stress response and ecology. However, work on the hydraulics of non-woody plants is still very marginal, but some studies presented at this conference show that things are moving forward. We have seen work on the hydraulics of early vascular plants (Mosses, Ferns) and on wild or cultivated herbaceous plants (maize, wheat). We must intensify this work and encourage the crop and herb scientific communities to join our discipline. It is a mutually beneficial process. Tree hydraulics specialists will benefit from a better understanding of how their species work by placing their work in a broader phylogenetic context. Conversely, as pointed out by Sean Gleason in his presentation, considering hydraulic traits is a promising new way to improve the agronomic performance of crop species in a changing climate context.

A second important point that has been mentioned in several communications is the need to develop work on plants *in situ*, under natural growing conditions, and not only *ex situ* on potted plants placed under controlled but artificial conditions. The dehydration kinetics of potted plants are often not comparable to that of plants *in situ*, so the responses observed may not be representative of the natural functioning of these plants. If, in addition, the hydraulic features are more plastic than we currently think, as in the case of vines for example, pointed by Uri Hochberg and Laurent Lamarque, then we would underestimate in part the plants' ability to acclimatize to the constraints of the environment through these *ex situ* experiments. An important expectation of our work concerns our ability to understand and predict the risks of tree mortality in forests. Surprisingly enough, very little of the work of this symposium concerned the response to drought in mature trees under natural conditions. This type of study represents higher technical constraints and requires higher investments, but they remain unavoidable and must therefore be encouraged, especially to validate the predictions of some of our models.

On a completely different scale, there was remarkably little work presented on the molecular and genetic basis of the hydraulic functioning of plants (but see Grace John's presentation). There is a strong challenge in attracting researchers working on this scale to our discipline. Lukas Schreiber's presentation on cuticles is a good example of how work on well-targeted gene mutants could advance our understanding of structure/function relationships and, more importantly, establish causal and not simply correlative relationships. It is also surprising that researchers working on aquaporins do not participate more in our symposium and the scientists working on variable extra-xylary pathways did not incorporate more these water-channels in their study. We must ensure that we can better attract molecular biologists to our meetings in the future.

“All the models are wrong, but some are useful” (George Box)

Modelling has always been an integral part of the work on the hydraulic functioning of plants. It is often an essential tool for integrating processes in space or time or for understanding the interaction of complex processes. Surprisingly, modelling was not well represented in the work presented in this conference. For example, one would expect to see more attempts to integrate hydraulic traits into plant functioning models in order to better reconstruct or predict tree mortality *in situ*. Chris Blackman's presentation made it clear that the key functional traits of this mortality can now be identified and measured. Collaborations with modelers are now timely to predict mechanistically and globally the response of ecosystems to climate change. The people who walked this Friday in the streets of Padova are waiting for such work and we now have the tools to carry it out.

XIM4 was undoubtedly a new important step in the animation and cohesion of researchers around a common discipline. One of the challenges for the future of our discipline will be its ability to open up to other related field of research.

Acknowledgments

The meeting was supported, in part, by the University of Padua, Dept. TeSAF (University of Padua), Dept. DAFNAE (University of Padua), INRA-EFPA department, the Federation of European Societies of Plant Biology (FESPB), the Italian Society of Plant Biology (SIBV), the Italian Society of Silviculture and Forest Ecology (SISEF) and the New Phytologist Trust.