A high-throughput approach for quantifying turgor loss point in wine grapes

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Abstract

Quantifying drought tolerance in crops is critical for agricultural management under environmental change, and drought response traits in wine grapes have long been the focus of viticultural research. Turgor loss point (π_{tlp}) is gaining attention as an indicator of drought tolerance in plants, though estimating π_{tlp} often requires the construction and analysis of pressure-volume (P-V) curves which is time consuming. While P-V curves remain a valuable tool for assessing π_{tlp} and related traits, there is considerable interest in developing high-throughput methods for rapidly estimating π_{tlp}, especially in the context of crop screening. We tested the ability of a dewpoint hygrometer to quantify variation in π_{tlp} across and within 12 varieties of wine grapes (*Vitis vinifera*) and one wild relative (*Vitis riparia*) and compared these results to those derived from P-V curves. At the leaf-level, methodology explained only 4–5% of the variation in π_{tlp} while variety/species identity accounted for 39% of the variation, indicating that both methods are sensitive to detecting intraspecific π_{tlp} variation in wine grapes. Also at the leaf level, π_{tlp} measured using a dewpoint hygrometer significantly approximated π_{tlp} values (r^2 = 0.254) and conserved π_{tlp} rankings from P-V curves (Spearman's ρ = 0.459). While the leaf-level datasets differed statistically from one another (paired *t*-test p = 0.01), average difference in π_{tlp} for a given pair of leaves was small (0.1 ± 0.2 MPa (s.d.)). At the species/variety level, estimates of π_{tlp} measured by the two methods were also statistically correlated (r^2 = 0.304), did not deviate statistically from a 1:1 relationship, and conserved π_{tlp} rankings across varieties (Spearman's ρ = 0.692). The dewpoint hygrometer (taking ~ 10–15 minutes on average per measurement) captures fine-scale intraspecific variation in π_{tlp}, with results that approximate those from P-V curves (taking 2–3 hours on average per measurement). The dewpoint hygrometer represents a viable method for rapidly estimating intraspecific variation in π_{tlp}, and potentially greatly increasing replication when estimating this drought tolerance trait in wine grapes and other crops.

Background

Increases in the prevalence and duration of drought events due to climate change are a major concern agricultural production faces, with potentially destabilizing consequences for food security and sustainability at local through to global scales [1–3]. As a result, managing and predicting crop yield under drought is among the most pressing goals for agricultural management under environmental change, and has been for decades [4–9]. In aiming to understand the mechanistic basis of yield change under drought, one primary theme in crop science has been to better understand how plants cope with periods of water stress [10, 11].

Several reviews exist that summarize the myriad of short- and long-term drought tolerance mechanisms and strategies in plants globally, which spans from short-term physiological responses such as biochemical signaling that triggers stomatal closure, through to longer-term responses including changes in leaf-, root-, or whole-plant traits [2, 3, 12–15]. Indeed, this deep literature demonstrates how

and why drought tolerance represents among the most complex and multifaceted plant characteristics, and is dictated by the cumulative expression of multiplebiochemical, anatomical, architectural, and morphological traits [13]. Owing to its complex nature, studies often use different approaches or metrics to quantify drought tolerance. These metrics utilize various functional traits including instantaneous water-use efficiency (measured as a ratio of photosynthetic carbon gain [*A*] to water loss through transpiration [*E*] [e.g., 16]), stomatal traits including conductance (g_s) and sensitivity [e.g., 17, 18], traits associated with root phenotypes [e.g., 19], among others [e.g., 20].

One trait employed to characterize drought tolerance in plants is the turgor loss point (π_{tlp}), which represents the leaf water potential (Ψ_{leaf}) at which wilting occurs [21–23]. This trait is widely considered an indicator of drought tolerance, as plants expressing a more negative π_{tlp} are able to maintain leaf turgor and physiological functioning including sustained rates of g_s, *A*, and *E*, across a wider range of moisture availability [23]. In the field of comparative plant and functional trait ecology, turgor loss point is considered a “higher-level” drought tolerance trait that can be used to infer leaf- and plant-level environmental tolerances [22]. As such, π_{tlp} and its plasticity have been employed to characterize drought tolerance across large groups of species [21, 22, 24, 25]. In turn, in studies on unmanaged ecosystems, inter- and intraspecific variation in π_{tlp} is used to support hypotheses surrounding the environmental determinants of plant species distributions [26, 27] and species and community-level responses to changing water availability [e.g., 18, 24, 28].

More recently, and largely as an extension of the literature on “wild” plants, π_{tlp} has received attention as a hypothesized index of drought tolerance in crops. For example, in detecting variation in π_{tlp} within and among eight cultivars of spring wheat (*Triticum aestivum* L.), Mart et al. [29] argued that π_{tlp} represents a trait that could be used to rapidly screen crop drought tolerance. Employing π_{tlp} as a crop drought tolerance trait is relatively new, since prior to this time, there was a widespread assumption that crop physiological functioning ceased below a certain soil-based permanent wilting point [as cited by 29, 30]. Furthermore, researchers have noted that relationships between π_{tlp} and crop growth and yield remain an unresolved area of research, leading to uncertainties as to whether or not this trait represents a viable screening tool in breeding programs that are relevant for agricultural management [31]. Nonetheless, because π_{tlp} is a metric that integrates multiple aspects of osmotic adjustment in plants, quantifying π_{tlp} within and among crop species and varieties appears an increasingly important component of the wider literature on quantifying crop drought tolerance.

To date pressure-volume (P-V) curves have been the classical method for estimating π_{tlp} in plants, species, or genotypes [reviewed by 32], with P-V curve-derived data then supporting literature focused on plant drought tolerance and its ecological implications [33]. A P-V curve is constructed by progressively drying a fully rehydrated leaf at set pressure or drying intervals, and then assessing the statistical relationship between Ψ_{leaf} and relative water deficit (RWD) [summarized in Fig. 1 of 22]. Data necessary for P-V curve construction and associated π_{tlp} estimation are generated with the use of Scholander-type pressure chambers or “pressure bombs” [34], generally following either a bench drying method where

leaves are air dried on a bench top and both Ψ_{leaf} and RWD are measured at set time intervals, or a "squeeze method" where the mass of expressed sap is weighed at set pressure intervals.

Despite being widely applied in both basic and applied plant sciences for decades [34, 35], P-V curve generation is associated with major time constraints [33], often taking hours to complete for a single leaf, and certain assumptions embedded within P-V curve-based methods have motivated critical reviews on their accuracy across studies [32, 33, 36]. For example, Rodriguez-Dominguez et al. (2022) elucidated how key assumptions related to sample preparation, storage, and pressurization techniques (among other factors) may lead to variability in Ψ_{leaf} measurements, and ultimately P-V curve construction from pressure chambers. At the same time, estimating π_{tlp} for a single leaf through a P-V curve can take hours, even when using the relatively fast squeeze method [36]. So, while P-V curves remain a critical aspect of plant and crop science, there are major limitations associated with this technique for either 1) screening drought tolerance across multiple crops and varieties, or 2) for assessing variation in drought tolerance traits at the individual plant level. The latter is especially important in the fields of crop science and agroecology, where intraspecific trait variation—i.e., variation existing below the species level—is likely a key determinant of agroecosystem processes [37].

In response to these limitations, researchers have begun developing methods for high-throughput estimation of Ψ_{leaf} and π_{tlp}, which have gained considerable interest in studies of woody and herbaceous species in unmanaged ecosystems [33, 36, 38–40], and more recently of crops [29]. Notably, studies have employed both vapour pressure osmometers [33, 38, 39] and—the focus of our study here—dewpoint hygrometers [36, 40] to estimate π_{tlp} in plants by estimating this trait directly as a function of Ψ_s at full turgor. Specifically, a dewpoint hygrometer measures the sum of matric potential (Ψ_m) and osmotic potential (Ψ_s) using the chilled-mirror dewpoint technique, while the vapour-pressure osmometer measures osmolality which is then converted into Ψ_s following the Van't Hoff equation [33, 36]. In both these methods gravitational potential (Ψ_g), matric potential (Ψ_m), and Ψ_p are considered negligible when leaves are fully hydrated, and as a result Ψ_s in fully hydrated leaf samples can then be correlated to π_{tlp} [33].

The use of dewpoint hygrometers for measuring leaf bulk water relations dates back decades [41, 42] and recent studies using these techniques in high-throughput assessments of plant ecophysiology are promising. Specifically, Petruzellis et al. [40] found this method could capture interspecific variation in π_{tlp} across 27 Mediterranean woody species (which ranged from ~-4.5 to -0.5 MPa), with dewpoint hygrometer-based measurements of Ψ_s at full turgor linearly predicting (adjusted $r^2 = 0.46$) π_{tlp} values derived from P-V curves. Within species, Banks and Hirons [36] found this technique was able to quantify fine-scale differences in π_{tlp} that exists among five *Acer* genotypes, with mean π_{tlp} ranging between roughly − 1.5 to -2.0 MPa: differences that were masked when π_{tlp} was measured using P-V curves alone. While these studies and foundational theory [41, 42] point to the dewpoint hygrometer as a viable

technique for high-throughput π_{tlp} estimation, no studies have yet tested if this technique is able to quantify variation in π_{tlp} across crop varieties.

Wine grapes (*Vitis vinifera* subsp. *vinifera*) represent among the world's most economically important crops, with the environmental conditions necessary for wine grape production varying widely across the world's over 6,000 varieties [43–46]. Many varieties require a narrow range of climatic conditions for optimum plant performance, physiological functioning, fruit quality, and yield [45, 46]. Indeed, authors have argued that wine grapes are among the most sensitive to climatic shifts with projections indicating that due to alterations in water availability and temperature regimes, wine production will likely shift considerably in the future [47, 48]. Wine grape drought tolerance is an integrated characteristic comprised of multiple genetic, morphological, physiological, and phenological traits [49]. Additionally, the viability of wine grape production under a shifting climate is mediated not just by drought tolerarnce, but also by other agronomic considerations such as berry size, yield, and flavor profiles. No less, high-throughput measurements of π_{tlp} would be valuable towards holistic prediction and modelling of climatic suitability of varieties into the future [47, 50].

In this study, we assess whether or not a high-throughput technique based on the use of a dewpoint hygrometer, is able to quantify variation in π_{tlp} both within and among wine grape varieties. To address this, we specifically compare differences in π_{tlp} generated through P-V curves and a dewpoint hygrometer, at both the individual leaf- and variety-scale. While the primary focus of our study is on π_{tlp} variation within and among 12 widely cultivated varieties of wine grapes, our study also includes measurements on a wild relative of wine grapes, namely *Vitis riparia*, in order to assess the wider application of this technique towards quantifying π_{tlp} in wild crop relatives [51]. Our study was designed to address the following research questions: 1) do varieties of wine grapes vary significantly in their π_{tlp}, and if so, can these differences be quantified using a high-throughput technique? Then, we asked 2) which varieties are most drought-tolerant as per π_{tlp} values, and if inferences regarding varietal drought tolerance rankings change depending on π_{tlp} methods? Finally, we ask 3) do cultivated wine grapes differ in their π_{tlp} vs. wild relatives?

Methods

Field site and sample collection

Wine grape plant materials for this study were collected at the Niagara College Teaching Vineyard, located in Niagara-on-the-Lake, Ontario, Canada (43.1522° N, 79.1652° W). This 16.2 ha operational vineyard established in 1996 is situated within the Niagara Peninsula Appellation in Southern Ontario, Canada. At the vineyard, branches of 12 cultivated *Vitis vinifera* L. varieties were sampled, including riesling (varieties 23 and 171), pinot noir (varieties 89 and 828), merlot (varieties 384 and 181), cabernet sauvignon (varieties 29 and varieties 412), cabernet franc (varieties 327 and 314), viognier (variety 642), and sauvignon blanc (variety 906). Our study also sampled a wild relative of cultivated wine grapes *V.*

riparia growing in a forest edge ecosystem situated immediately adjacent to the vineyard (i.e., within ~ 10 m south of the vine rows at the south end of the vineyard).

From each variety and wild relative species, one shoot was sampled from three different individual vines. Each shoot was ~ 30–50 cm in length and included a minimum of three pairs of oppositely arranged leaves that were fully expanded, visually healthy, and of similar size and vigor. Once collected, all shoots were immediately recut underwater to avoid desiccation before being transported to the lab at the University of Toronto Scarborough, which occurred within 2 hours of field sample collection. In the lab, shoots were again recut under water at least 1 cm from the base and stored in the dark for 12 hours to fully rehydrate. Following this step, two adjacent leaves in the same growth conditions from each branch were then used for estimating π_{tlp} following two different methods: 1) P-V curves executed using a SAPS II portable plant water status console (Soilmoisture Equipment Corp., CA, USA), and 2) direct measurements of Ψ_s at full turgor and π_{tlp} using a WP4C dewpoint hygrometer (METER Environmental, Washington, USA).

Estimating π_{tlp} using pressure-volume curves

All P-V curves were generated via the pressure chamber method following protocols described by Sack et al. (2022). First, we removed one leaf from each rehydrated branch and recorded leaf area using an LI-3600C leaf area meter (LICOR Bioscience, Lincoln, Nebraska, USA). Then a cut was made at the base of the petiole using a razor blade, and the leaf was immediately weighed and sealed in the pressure chamber. The chamber was then pressurized until an initial balance pressure was reached (≥ 0.2 MPa) which was determined by the first appearance of sap expressed from the cut surface viewed under a digital microscope affixed to the SAP II console. The expressed sap was then collected using pre-weighed low-lint absorbent tissue paper inside a 1.5 ml Eppendorf tube. To prevent evaporation the opening time of the tube was minimized during sap collection. The tube and tissue paper were re-weighed after sap collection to determine the weight of water exuded. The pressure then was increased in 0.2 MPa intervals, and the sap collection procedure was repeated at least 10 times to obtain enough data points to construct a full P-V curve. In sum, this procedure took approximately 2–3 hours for each individual leaf.

Based on this data, we used a series of functions in the ‘pvcurveanalysis’ R package [52] to estimate π_{tlp} for each leaf. First, we used the 'FMSaturated’ function to estimate saturated fresh mass (FM) for each leaf, which is calculated as an extrapolation of a linear regression model fit between leaf mass and Ψ_{leaf} values above the estimated π_{tlp}. Based on this FM estimate, we used the ‘RelativeWaterDeficit” function to calculate the RWD at each pressure interval as:

RWD = 100–100 * ((FM – DM) (FMs-DM)-1) (Eq. 1)

where DM is dry mass measured at the end of each P-V curve by drying each leaf at 65°C to constant mass, and FMs is FM at water saturation. Then, π_{tlp} was estimated for each leaf using the ‘OsmoticPot’

function in the ‘pvcurveanalysis’ R package [52].

Estimating π_{tlp} using a dewpoint hygrometer

For high throughput estimates of π_{tlp} we used the WP4C dewpoint hygrometer to measure Ψ_s at full leaf turgor (with plants being rehydrated as described above), and subsequently convert this value to π_{tlp} estimates. In these analyses, leaves immediately adjacent to those used in P-V curve analyses were selected, and we collected three leaf discs per leaf (or pseudo-replicates) that were 35 mm in diameter from the base of each lobe using a circle cutting blade. Leaf discs were immediately wrapped in tinfoil to avoid water loss, flash frozen in liquid N_2 for five minutes, and then gently abraded using 600 grit sandpaper to remove the cuticle before being placed in the dewpoint hygrometer chamber. Measurements of Ψ_s were collected using the WP4C continuous reading mode and recorded after 10–15 minutes when the measuring chamber reached vapor equilibrium. The dewpoint hygrometer was calibrated prior to every 10 measurements using 0.5 mol KCl solution. Based on values of Ψ_s at full turgor, we then estimated π_{tlp} following the model described by Bartlett et al. [33] as:

$\pi_{tlp} = (\Psi_s * 0.832) - 0.63$ (Eq. 2)

Statistical analysis

Statistical analysis and data visualization were performed using R v. 4.2.2 statistical software (R Foundation for Statistical Computing, Vienna, Austria). Our first analysis made use of our dataset that included a total of $n = 39$ measurements of π_{tlp} estimated using both P-V curves and the dewpoint hygrometer, such that all leaf-level dewpoint hygrometer π_{tlp} were calculated as the mean of three π_{tlp} pseudo-replicates per leaf. Using this data, we first performed a *t*-test to evaluate differences in leaf-level π_{tlp} across the two datasets, and followed with a linear regression model (where $n = 39$ leaves) to test whether π_{tlp} derived from the high-throughput method predicts π_{tlp} derived from P-V curves. Additionally, we also used a linear hypothesis test implemented in the ‘car’ R package [53] to compare this linear regression model with a 1:1 relationship where π_{tlp} from the high-throughput method perfectly corresponds to π_{tlp} from P-V curves. This linear regression and hypothesis test analysis was augmented by a Spearman rank correlation test, to evaluate the degree to which rankings of π_{tlp} change as a function of methodology.

Using this same leaf level dataset, we fitted effects mixed models coupled with variance partitioning techniques to quantify the proportion of variation in π_{tlp} values that were attributable to variety identify (e.g., Riesling vs. Cabernet franc), clone identity (e.g., Riesling 23 vs. 171), and methodology (i.e., dewpoint hygrometer vs. P-V curves). Due to sample size limitations, we performed this analysis by fitting a series of mixed models versus using a single model. Specifically, we used the ‘lme’ function in the ‘nlme’ R package [54] to fit mixed models that predicted variation in π_{tlp} as a function of: 1) clone identity as a fixed effect and methodology as a random effect; 2) methodology as a fixed effect and clone identity as a random effect; and 3) variety identity as a fixed effect and methodology as a random

effect. For each of these models we applied the 'r.squaredGLMM' function in the 'MuMIn' R package [55] to estimate the proportion of variation in π_{tlp} associated with both fixed effects (or the marginal r^2 values) and fixed and random effects combined (or the conditional r^2 values) [56].

For additional analysis, we replicated our leaf-level analysis at the variety/species level. To do so, we calculated mean π_{tlp} values for each variety/species-by-method combination thereby generating a variety/species-level dataset based on $n = 3$ observations of π_{tlp} in total for each method (except in the case of cab. franc 314, cab. sauv. 412, and riesling 23 where one P-V curve failed). Then, the same *t*-test, linear regression, linear hypothesis test, and Spearman rank correlation procedures described above were performed on this species/variety-level dataset (where $n = 13$ for each test).

Results

Across the leaf-level dataset mean π_{tlp} values were − 1.7 ± 0.3 (s.d.) MPa (interquartile range = 0.3 MPa) when estimated using the P-V curve method, compared to a mean π_{tlp} of -1.8 ± 0.2 MPa (interquartile range = 0.3 MPa) using the high throughput method (Fig. 1). Observed π_{tlp} values derived from P-V curves ranged from − 2.4 to -1.2 MPa, and from − 2.2 to -1.2 MPa using the dewpoint hygrometer (Fig. 1B). Differences in π_{tlp} for individual vines calculated as P-V curve π_{tlp} minus dewpoint hygrometer π_{tlp} values, ranged from − 0.5 MPa to 0.8 MPa and averaged 0.1 ± 0.2 (s.d.) MPa (Fig. 1). A paired *t*-test indicated that these two datasets differed statistically from one another ($t = 2.96$, d.f.=35, $p = 0.006$), though these differences owe largely to a π_{tlp} value from P-V curves from one *V. riparia* leaf that was the most negative π_{tlp} value in our dataset (Fig. 1A).

Spearman rank correlation analysis indicated that π_{tlp} rankings among individual vines was significantly correlated across methodologies (Spearman's $\rho = 0.5$, $p = 0.002$). Similarly, a linear regression analysis found that π_{tlp} estimates generated by the dewpoint hygrometer explained 25.4% of the variation in π_{tlp} generated by P-V curves (regression model $p = 0.002$, intercept=-0.59 ± 0.32 (s.e.), slope = 0.61 ± 0.18; Fig. 1A). However, the relationship between paired π_{tlp} measurements did diverge significantly from a 1:1 relationship (linear hypothesis test $F = 7.3$, $p = 0.002$; Fig. 1).

Our mixed effects model analyses found that across the leaf-level dataset, variety/species identity accounted for the largest proportion of variation in π_{tlp} observations. Specifically, a mixed effects model that included variety/species identity as a fixed effect and method as a random effect detected statistically significant differences in π_{tlp} across varieties/species ($F_{12, 61}=4.32$, $p < 0.001$; Fig. 2). In this model, variety/species identity explained 38.6% of the variation in π_{tlp} across all observations (i.e., based on the marginal r^2 value), while the method (incorporated as a random effect) explained only an additional 6.0% of the variation in π_{tlp} (i.e., based on the conditional r^2 value). These results were largely robust when leaf-level data was analyzed with method as a fixed effect (marginal $r^2 = 0.043$) and variety

as a random effect (conditional r^2 = 0.394), though here method was a statistically significant predictor of π_{tlp} values ($F_{1, 61}$=5.25, p = 0.025).

When data was analyzed at the species/variety level (n = 13) we did not detect significant differences in mean π_{tlp} across methods (t = 1.74, d.f.=13, p = 0.107; Fig. 3A). Specifically, across species/varieties mean π_{tlp} values measured using P-V curves were − 1.7 ± 0.2 (s.d.) MPa and ranged from − 2.2 ± 0.3 (s.e.) in cabernet sauvignon 412 to -1.2 ± 0.1 (s.e.) MPa in riesling 23 (Fig. 3A). When π_{tlp} was based on dewpoint hygrometer measurements, mean π_{tlp} across all varieties averaged − 1.8 ± 0.2 (s.d.) MPa, ranging from − 2.1 ± 0.1 (s.e.) in *V. riparia* to -1.6 ± 0.1 (s.e.) MPa in viognier 642 (Fig. 3).

The ranking of species/varieties based on their mean π_{tlp} values did not differ statistically based on methodology (Spearman's ρ = 0.69, p = 0.011), with the rank of a number of varieties at both the most (e.g., riesling 171) and least negative π_{tlp} values (i.e., pinot noir clone 89) being robust towards methodology (Fig. 3B). And while the ranking of certain varieties did shift across methods (e.g. Cabernet franc 372), most rank changes were relatively limited to two to three positions on a π_{tlp} ranking scheme (Fig. 3B). Finally, a linear regression model found that variety-level mean π_{tlp} values based on dewpoint hygrometer measurements explained 30.4% of the variation in π_{tlp} values from P-V curves (regression model p = 0.05, intercept=-0.18 ± 0.7 (s.e.), slope = 0.84 ± 0.39), and at the species/variety level this relationship did not differ statistically from a 1:1 relationship (linear hypothesis test F = 1.49, p = 0.267; Fig. 3A).

Discussion

Estimating π_{tlp} using a dewpoint hygrometer presents a promising avenue for high-throughput assessments of wine grape drought tolerance. This technique closely approximates values of the same trait derived from traditional P-V curve techniques (Figs. 1–3), being able to quantify relatively fine-scale variation that exists among closely related varieties and species. Our results contribute to the literature on high-throughput π_{tlp} estimation, which gained considerable popularity with the development of osmometry-based methods [25, 33], extending to recent analyses employing dewpoint hygrometers [36, 40, 57]. Our results align with this previous work indicating that a dewpoint hygrometer, alongside established models correlating π_{tlp} and Ψ_s at full turgor [33], support the generation and analysis of an important drought tolerance trait in plants. However, our work extends this literature to suggest high-throughput methods are equipped to capture the fine-scale variation in π_{tlp} that exists among individual plants or varieties of the same species: a key consideration for studies on crop functional trait variation [37].

One consistent finding in our results is that at either the leaf- or variety/ species level, π_{tlp} values derived from the dewpoint hygrometer were on average 0.11 or 0.1 MPa lower (more negative) than paired observations from a P-V curve: values corresponding to average declines of π_{tlp} by 5–6% as one moves from traditional to high-throughput methods (Figs. 1, 3). This trend is similar to results obtained by Banks

and Hirons [36] in their efforts to quantify intraspecific variation in π_{tlp} across five maple (*Acer*) genotypes, where the same hygrometer model generated more negative π_{tlp} values in comparison with those derived from P-V curves. Similarly, one study on a single wine grape variety also found π_{tlp} from a dewpoint hygrometer strongly correlated with values from P-V curves, with more negative values derived from the high-throughput technique [57].

A proposed explanation for this trend is related to methods associated with P-V curve generation. Specifically, potential water loss during petiole excision, solute accumulation in undamaged tissue, and the possibility that Ψ_{leaf} is lower than water potential in the air (i.e., conditions of high ambient humidity), could all lead to higher π_{tlp} estimates derived from P-V vs. dewpoint hygrometer measurements [36, 57, 58]. In our study though, absolute and relative differences in π_{tlp} across methods were smaller (i.e., 5–6%) compared to observations in other studies (e.g.,, ~ 26.5% on average [36, 57]. No less the growing literature on high-throughput approaches to π_{tlp} estimation, including our own results, indicate these methods provide a viable alternative to P-V curves especially in situations where large sample sizes are an important consideration.

In relation to issues of sample size, at the leaf-level our high-throughput approach resulted in a slightly narrower range of π_{tlp} values (-1.2 to -2.18 MPa) compared to those generated by P-V curves (-1.2 to -2.41 MPa). This trend then scaled-up to support similar trends at the species/ variety-scale, where the dewpoint hygrometer results supported a more restricted range of mean π_{tlp} from values (-1.56 to -2.1 MPa) vs. P-V curve-based data (-1.28 to -1.16 MPa) (Fig. 3). Here, the (pseudo-)replication afforded by the high-throughput method may provide more robust π_{tlp} for individual varieties.

Related, the most notable is the difference in time required to generate data using these different methods. Each of the 39 P-V curves executed in our study required 2–3 hours of processing time, with three of them failing; analytical steps including visual inspection of all P-V curves prior to final curve fitting further added to this time requirement. Comparatively, each of the dewpoint hygrometer values took ~ 12–15 minutes to generate a single π_{tlp} data point. This time consideration is clearly of interest for plant and crop scientists, and indeed is the foundation of multiple high-throughput approaches to screening crop ecophysiological responses to environmental conditions [e.g., 59]. But despite the inherit value of high-throughput screening in terms of time and potential for greater (pseudo-)replication, there remain limitations. Since the WP4C model requires a leaf disc to be 35 mm, plants and crops with leaves smaller than 35 mm diameter cannot be analyzed by this instrument, and species with thicker cuticles or succulents are likely to require specialized preparation methods before measurement. Lastly, P-V curves also return highly informative metrics associated with leaf water relations, including for example estimates of the bulk elastic modulus or the apoplastic fraction: key traits contributing to plant and crop drought tolerarnce and stress responses [21, 60, 61].

Conclusions

Crop responses to drought conditions have long been the focus of applied agricultural research, with crops showing complex responses to reduced water availability [2, 3]. These responses span above and belowground biophysical processes, and synthetically incorporate phenology, multiple functional traits, biochemical signaling pathways, and genes across leaves, roots, stems, and reproductive structures [49, 62]. While drought represents only one part of wider discussions surrounding threats to food security [63], identifying drought tolerant crops and genotypes is clearly of importance for maintaining yields under a shifting climate [64]. High-throughput estimation of functional traits associated with drought tolerarnce, both within and among crop species, varieties, and genotypes, services this goal in part. Traditional techniques associated with quantifying plant-water relations remain invaluable in estimating certain traits. Though high-throughput techniques such as those evaluated here, especially when coupled with other techniques, appear well suited and able to rapidly quantify components of drought responses in crops, at data acquisition rates that match the urgency of global change science.

Declarations

Availability of Data and Materials

Upon publication, the dataset supporting the conclusions of this article will be made available upon request to the corresponding author, and in the TRY Functional Trait Database.

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Competing Interests

The authors declare they have no competing interests.

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Author contributions

A.R.M., G.L., and B.C. planned and designed the research; A.R.M., G.L., and B.C. performed field and lab analyses; A.R.M., R.O.M., and K.V. performed statistical analyses; A.R.M. and G.L. wrote the draft manuscript; B.C., R.O.M., K.V., A.F., G.R., and K.C. edited the manuscript; K.C, G.R., and A.R.M. secured funding related to this research.

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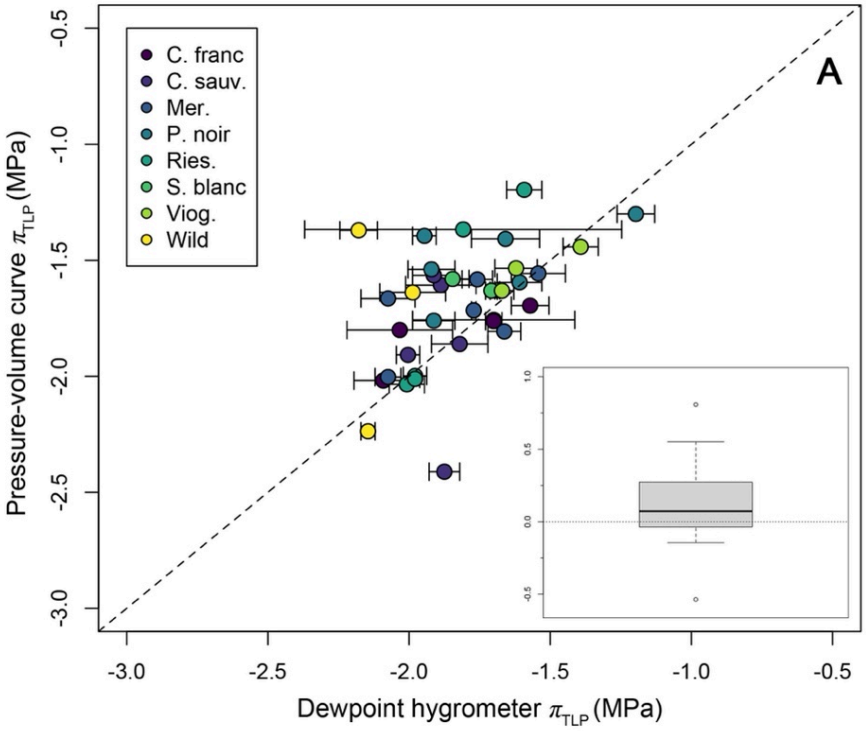
Figures



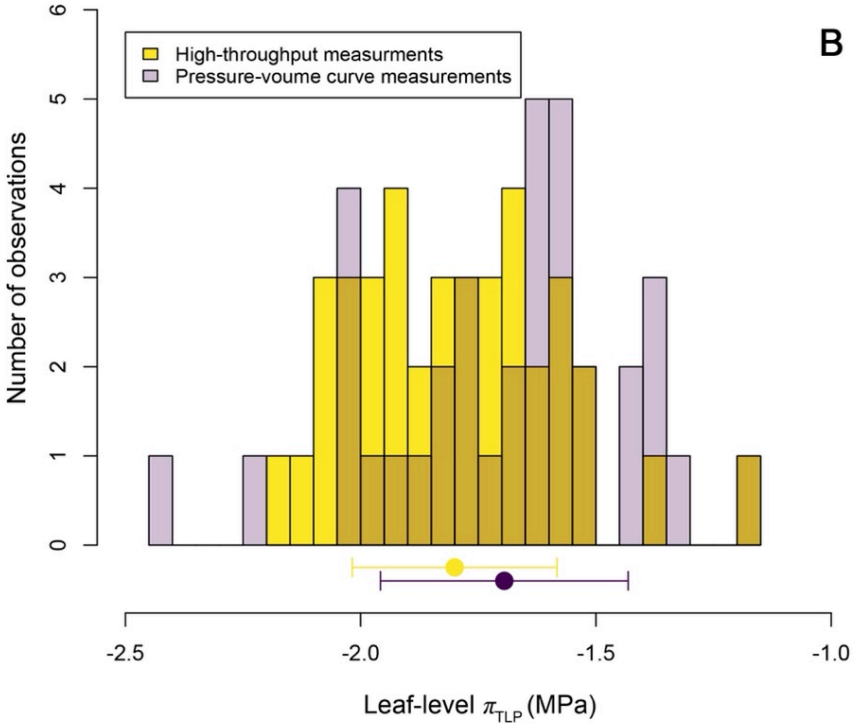


Figure 1

Turgor loss point (π_{tlp}) of leaves from 12 wine grape varieties and one wild relative (*Vitis riparia*) estimated through high throughput (dewpoint hygrometer) and traditional (pressure-volume curve) techniques. Panel A displays estimates of π_{tlp} derived through both methods measured on paired leaves from the same branch of individual vines. Dewpoint hygrometer-based π_{tlp} estimates for each individual leaf are derived as the mean of three π_{tlp} measurements per leaf (with error bars representing ± 1 s.e.). For clarity, points are colored according to their primary cultivar (or species in the case of the wild relative *V. riparia*), and the solid dashed line represents a 1:1 relationship. Inset box plot in Panel A presents the distribution of pairwise differences in π_{tlp} for all leaves in the dataset (where the average differences across n=36 leaves is 0.1 MPa), such that values above 0 represent leaves where high-throughput π_{tlp} values are more negative than paired measurements derived through P-V curves. Panel B displays histograms of π_{tlp} values estimated with high-throughput (dewpoint hygrometer) and traditional (P-V curve) techniques. Points below the histograms correspond to the overall mean π_{tlp} values (± 1 s.e.) across the two methods.

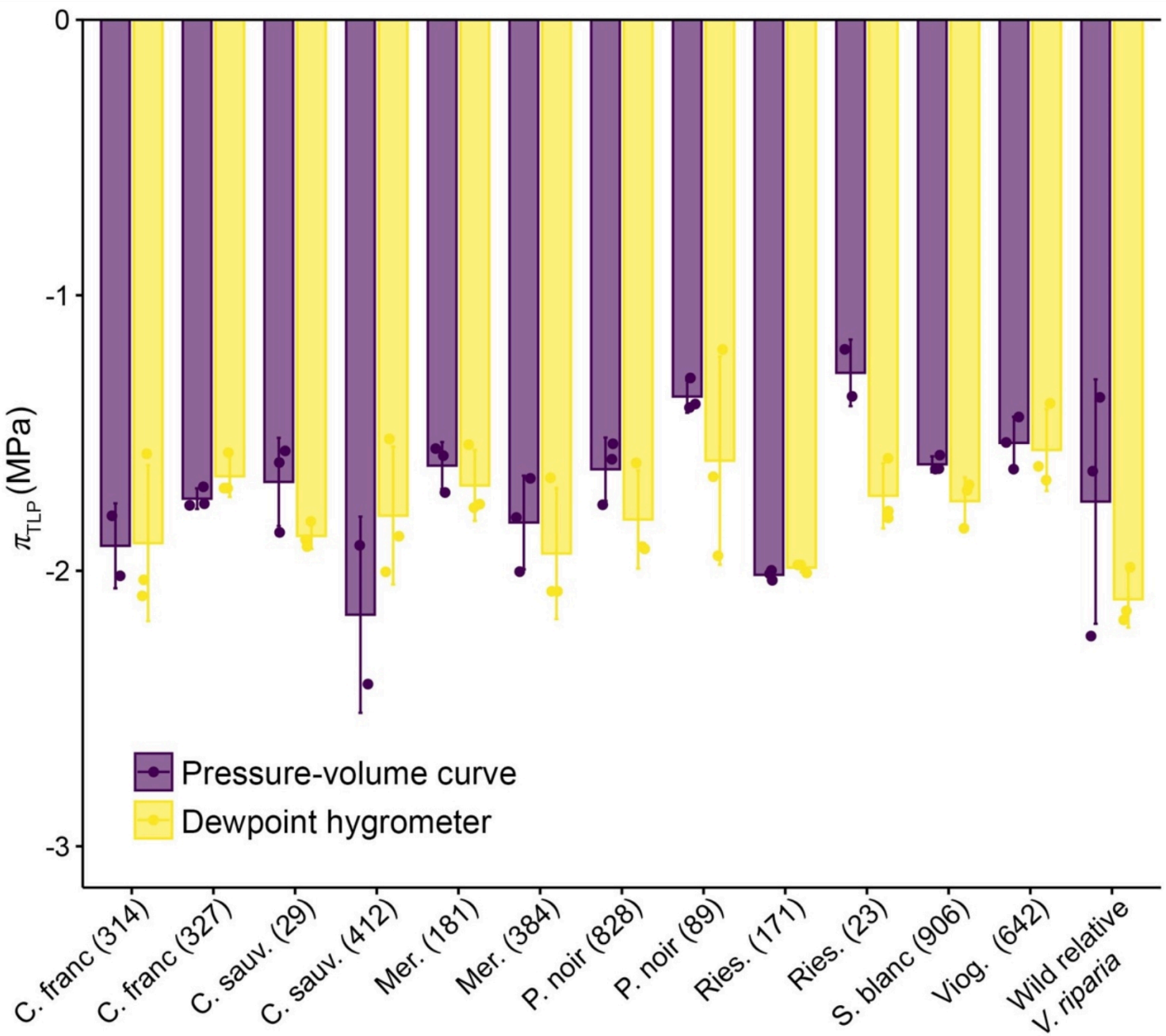


Figure 2

Leaf- and variety-level turgor loss point (π_{tlp}) estimates for 12 wine grape varieties and one wild relative derived through high throughput and pressure volume curves methodology. Individual points correspond to individual leaf-level observations of π_{tlp}. Leaf-level π_{tlp} values associated with the dewpoint hygrometer method correspond to the mean of n=3 pseudo-replicates per individual leaf, while leaf-level π_{tlp} values associated with pressure-volume curves are derived from one curve per leaf. Bars correspond to variety-level average π_{tlp} values (± 1 s.e.) associated with n=3 measurements for each variety-by-method combination.

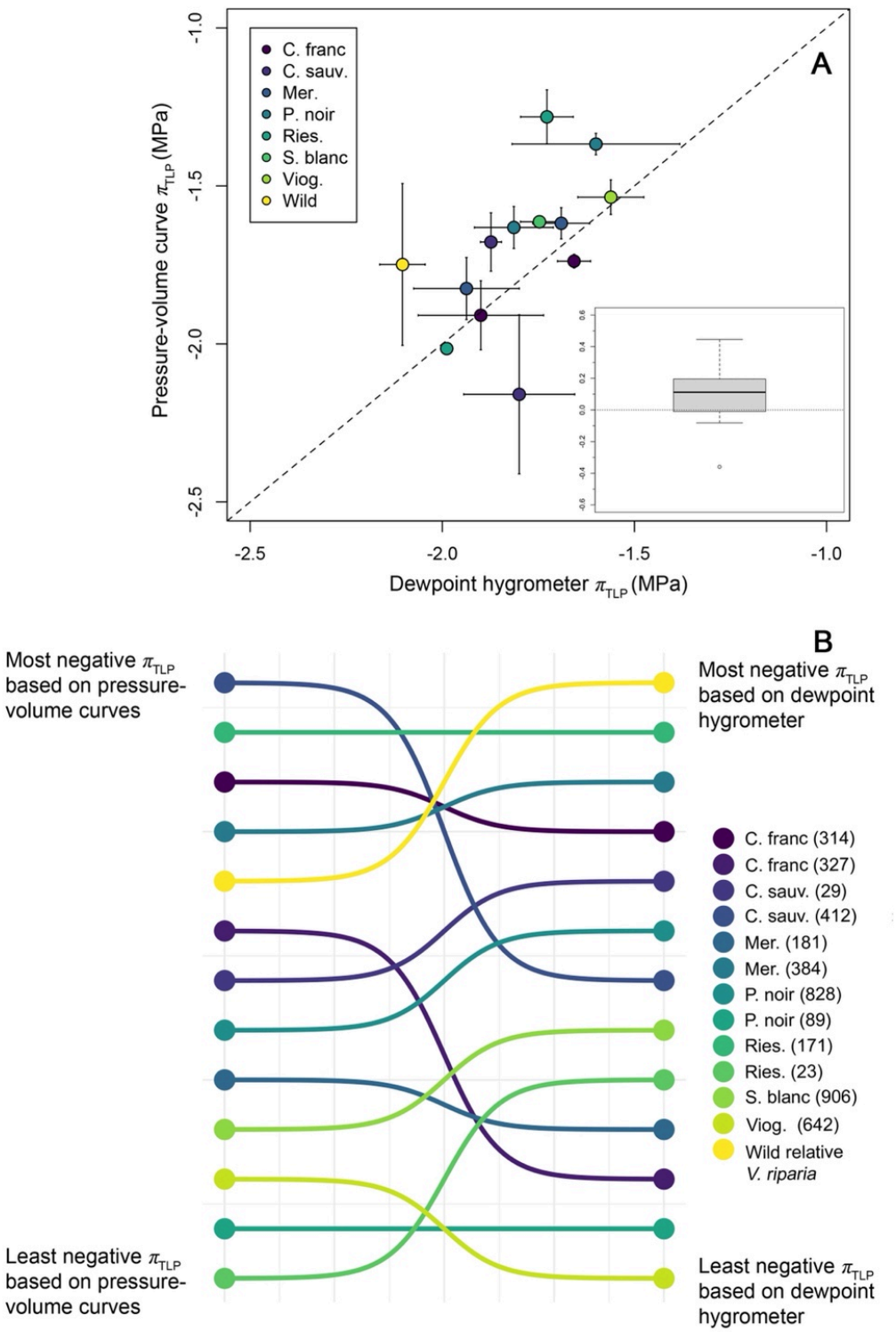


Figure 3

Average turgor loss point (π_{tlp}) of 12 wine grape varieties and one wild relative (*Vitis riparia*) estimated through high throughput (dewpoint hygrometer) and traditional (pressure-volume curve) techniques. Panel A displays estimates of variety-level mean π_{tlp} derived through both methods (with error bars representing ± 1 s.e.). The solid dashed line represents a 1:1 relationship. The inset box plot presents the distribution of pairwise differences in π_{tlp} for all varieties in the dataset, such that values above 0

represent leaves where high throughput π_{tlp} values are more negative than paired measurements derived through pressure-volume curves. Panel B represents rank shifts in estimated π_{tlp} for varieties when estimated using different techniques. Ordering of the left-hand side points denotes π_{tlp} rankings based on pressure-volume curves, while ordering of the right-hand side points denotes π_{tlp} rankings based on high throughput methods.