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Reply on RC2

Ana M. Sabater et al.

Author comment on "Technical note: Validation of Aleppo pine transpiration rate measurements using the heat ratio method under laboratory conditions" by Ana M. Sabater et al., Hydrol. Earth Syst. Sci. Discuss., https://doi.org/10.5194/hess-2022-328-AC2, 2022

RC2: 'Comment on hess-2022-328', Anonymous Referee #2

The main objective of the study is to validate sap flow laboratory-made sensors based on the HRM method, a method that is recommended mainly for reverse and low-intermediate sapflow rates and, therefore, recommended for plant species growing under limiting environmental conditions like Aleppo pine trees. Measurements are taken in three young trees under controlled conditions for about 60 days, and then compared to the readings of precision load cells for subsequent validation. In addition, the work has two other complementary objectives: the first is to study the effect of the dynamic calibration of the base flow (in the event that zero flow is not achieved under environmental conditions when it was expected) as a consequence of probe misalignment; to study the responses of sap flow to the environmental REW and DPV variables. In my opinion, the topic is of interest for HESS readers because it presents a methodology that has been used for Aleppo pine, but has not been validated under laboratory or field conditions. However, I am afraid that this work contains significant weaknesses that make its publication unsuitable for a journal like HESS unless the authors can improve their study by increasing tree sample size and the range of environmental conditions to be clearly representative of natural conditions for Aleppo pine. Moreover, some other aspects are indicated below. First, the few individuals used in the experiment does not allow any adequate quantification of tree variability to, therefore, properly test the method. Second, the environmental conditions during the experiment do not seem representative of the ranges observed/expected under field conditions, which makes the study incomplete to test the validity of sapflow sensors for natural Aleppo pine conditions, which is, in turn, one the main purposes of this study. Third, the study has the complementary objective of testing dynamic calibration for probe misalignment (that is recommended for periods longer than 3 months). In the end however, the work applies a systematic approach based on the study that the authors follow and it, therefore, seems similar to the classic approaches in which correction does not vary with time since installation. Finally, I consider the objective of studying the relations between the transpiration and environmental variables for the conditions presented in the work (not representative of natural ranges, nor for young individuals) because they do not contribute to the topic, especially when other published works have already described them for mature Aleppo pine trees growing under natural conditions.

Reply: *Dear reviewer,*

Thank you very much. We appreciate your comments and suggestions, which have been taken into account to improve the new version of the article.

We agree with the referee that this is the first time that an HRM methodology previously published in HESS has been tested in the laboratory. For this reason, we aimed to focus our article on testing this type of probes and correcting them by misalignment. The "gold standard" in this study would be transpiration measured by head cells. We hope that the improvement in the manuscript helps to focus the topic of this article.

(1) We agree with the referee that employing only three juvenile trees was a low replication design; yet for structural/technical reasons (i.e greenhouse space and design, facility to control transpiration by load cells), it was not possible to implement higher replication. Furthermore, the good functional variability shown by trees made us think that the results were consistent and robust (see Sect. Appendix D on the individual analysis for tree variability, Table D2 and Figure D2). For this reason, we also extended the sampling period to, thus, avoid short-term responses. Therefore, we have renamed the study as a test on HRM transpiration estimations more than a validation due to a small replication number. Nevertheless, (i) the long experimental period selected (57 days), (ii) the resolution of the transpiration rates methodology on a half-hourly basis and (iii) the measurement precision for observations by load cells can be considered a series of strengths in the study (lines 369-391)

(2) We are sorry if the word "validation" is confusing, which we have changed to "test" (in the title, Introduction (lines 23-25, 27-29, and 116-121), Material and methods (lines 169-172 and 182-185), Discussion (lines 370-393), Conclusions (lines 404-408 and 415-419)), which is more accurate according to the information that we provide. We thought that the information obtained in the test could serve as a precedent for future validations with greater individual replication and/or that use adult trees.

(3) Regarding the environmental conditions, it is true that the extreme VPD values that can occur in the field have not been reached (due to limiting technical greenhouse factors). We are aware of these limitations and have accordingly modified our assertions in the manuscript. Yet despite the limitations in the VPD range, we think that the collected transpiration range throughout the series is valid and sufficient to generate information for this test. Moreover, the soil moisture range could be perfectly controlled by irrigation pulses and mimic soil water availability in our ecosystems (lines 155-160).

(4) We apologise for the presentation of the main purpose about the probes misalignment correction is confusing. We only wished to test the method for probes misalignment correction, which is based on Burgess et al., (2001), following the same assumptions and procedures in Larsen et al. (2020) when selecting zero flows and calculating X1 and X2 terms. We did not wish to test the whole method in Larsen et al. 2020, which included a dynamic correction. As the referee mentions, if we aimed to test dynamic correction, we would need time series as long as 1 year at least, but this was not the actual purpose of our study.

(5) We thought that the relation between transpiration and the environmental variables (i.e. VPD and REW) would help to understand the manuscript and how the data (i.e. tree transpiration) match the climate variables as in other articles. However, we agree with the referee that this information is not strictly necessary for our study objective. For this reason, we have decided to move this part to Sect. Appendix D.

Title: "Technical note: Testing transpiration rates of juvenile Aleppo pine trees using the heat ratio method under laboratory conditions"

Lines 23-25: "Hence the study objective was to test transpiration rates by both sap flow *probes (i.e. the heat ratio method, HRM) and load cells in juvenile Aleppo pine trees.."*

Lines 27-29: "Probe misalignment correction was applied to test if the results would gain accuracy. These measurements were recorded in a greenhouse under controlled conditions."

Lines 116-121: "Therefore, the main objective of this study was to test the accuracy of HRM in a Mediterranean pine characterised by isohydric water-saver functioning with low transpiration rates. This study aimed to: i) test the Aleppo juvenile pine transpiration rates measured by the HRM technique compared to load cells as a direct method taken as the "gold standard" to measure plant transpiration rates under a variety of laboratory environmental conditions;"

Lines 152-156: "The VPD range went from 0.27 to 2.82 kPa. A wider range was not possible due to technical limitations. Soil moisture was better controlled by an irrigation system within the wide 0.14 to 0.40 m^3 m^{-3} range. As pines could not be moved for head cell installation reasons, one big fan was installed to homogenise air conditions and to avoid VPD gradients. Solar radiation followed the daily dynamics for the time of year."

Lines 169-171: "Therefore, T_{OBS} was considered to offer true values and were used to test sap flow probes transpiration measurements. Thus sap flow and water losses were the two independent approaches followed to estimate the transpiration rate per tree (T, kg h^{-1})."

Lines 182-185: "Transpiration was also tested by applying probe misalignment correction, which followed the criteria for detecting the zero-flow and heat pulse velocities equations described in Larsen et al. (2020) ($T_{HRM\ MIS}$, kg h^{-1}). The temporal change of the misalignment probes was tested."

Lines 370-393: "On the technical issues of this study, plant material contained only three pines in the juvenile life stage, which can be considered a poor replication design. However, for structural/technical reasons (i.e., greenhouse space and design, facility to control transpiration by load cells), it was not possible to implement higher replication and older trees and, consequently, a bigger size. According to recent review works about calibration experiments, these limitations fall in line with other studies. Not only are the cost of plant material, time and equipment challenges, but so are transport logistics and performing calibration with increasing tree size (Dix and Aubrey, 2021). The use of big containers was possible, and no root limitations were found in containers due to the size-tree age combination. On the contrary, big trees could pose root limitations for absorbing water and, therefore, an anomalous transpiration response with different water availabilities that are unrelated to the sap flow method. In addition, previous sap flow calibration studies have been done in juvenile trees, and found no effects caused by the followed method (Sun et al., 2012). Although some studies have reported intraspecific variability with individuals' status (Aranda et al., 2012; Delzon and Loustau, 2004), the variability herein observed could not be a weak point, but a strong one for testing the method and misalignment correction under different conditions. From our point of view, the confidence in our results for its use as a test in the Aleppo pine transpiration estimations by the HRM method is reinforced by: (i) transpiration was measured by head cells ("gold standard") with accurate precision; (ii) the long experimental period (57 days); (iii) the resolution of the transpiration rates methodology on a half-hourly basis; (iv) estimating individual-specific functional traits; (v) the range of environmental conditions. In fact the range of environmental conditions (VPD and REW) used in this paper is consistent with the range observed in fieldwork studies."

Lines 383-389: "Although some studies have reported intraspecific variability with individuals' status (Aranda et al., 2012; Delzon and Loustau, 2004), the variability herein observed could not be a weak point, but a strong one for testing the method and *misalignment correction under different conditions. From our point of view, the confidence in our results for its use as a test in the Aleppo pine transpiration estimations by the HRM method is reinforced by: (i) transpiration was measured by head cells ("gold standard") with accurate precision;"*

Lines 404-408: "This paper provides not only a test of transpiration rate measurements following the theory and transformation described in Burgess et al. (2001), but is also the first test of transpiration rates measurements corrected by probe misalignment correction following the heat pulse velocities calculations and zero-flow criteria described in Larsen et al. (2020)."

Lines 415-419: "The information obtained in this test can serve as a precedent for future validations with greater individual replication and/or using adult trees. Our results can also act as a starting point from which future research that addresses improvements in estimations at high flows can be promoted by either improving the technique or applying correction factors for these values."

Other suggestions:

L40-41: Please indicate for which region/s the authors indicate these expected changes.

Reply: *This has been done. The sentence is now as follows: "Global change scenarios not only predict increasing temperature and changes in precipitation patterns, but also higher drought intensity and longer drought duration in the Mediterranean Basin (IPCC, 2021)."*

L42-43: Climate will affect water use, but also other important processes related to ecosystem functioning and survival. This sentence should be improved.

Reply: *It now states: "Climate alterations will affect ecosystem function and survival by promoting plant water use and carbon assimilation changes (Allen et al., 2015; Lindner et al., 2010; Morcillo et al., 2022)."*

L45: Please consider this change to be made: "and, therefore, for a better definition of the required adaptive forest management".

Reply: *This has been done. Thank you!*

L47-48: Please delete "water fluxes to the atmosphere".

Reply. *This has been done.*

L49: I suggest "on an annual basis".

Reply: *Thank you. This has been changed.*

L49-51: This sentence requires supporting literature: "Additionally, transpiration provides plant-level information and is important in water status, leaf cooling and nutrient transport"

Reply: *This reference is added to support the sentence: Peguero-Pina, J. J., Vilagrosa, A., Alonso-Forn, D., Ferrio, J. P., Sancho-Knapik, D., Gil-Pelegrín, E. Living in drylands: Functional adaptations of trees and shrubs to cope with high temperatures and water scarcity, Forests, 11(10), 1028.2020.*

L52: I suggest changing "inferred" by "measured".

Reply: *This has been done.*

L52-61: This sentence is too general and lacks information about the main differences among approaches, the implications of using one method instead of others, other methods, etc. I would also suggest improving the citations to indicate the most significant works for each category. In my opinion, the authors should better explain why the sap flow technique is the most widely used one because the current explanation is too simple (please also take into account the mention of "leaf measurements" because the authors do not prviosuly mention any method based on this type of measurements, such as chambers or porometers).

Reply: *The paragraph has been rewritten (lines 56-71).*

Lines 56-71: "Tree or plant transpiration can be inferred by a wide range of methods, such as lysimeters (Swanson and Whitfield, 1981; Rana and Katerii, 2000), isotopic approaches (Scheidegger et al. 2000), atmospheric techniques (e.g. eddy covariance; Williams et al. 2004), models (Collatz et al., 1991) leaf chambers (e.g. infrared gas analyser, Moncrieff et al. 1997) or sap flow measurements (Granier, et al. 1987; Smith and Allen, 1996). Sap flow methods have been used since the start of the 20th century to trace water movement through xylem tissues (Huber, 1928). The sap flow technique is often followed in woody species at the whole plant level because it presents several advantages: (i) contrary to discrete readings in isotopic approaches or leaf chambers, the sap flow technique provides continuous readings and is not limited to single or a few leaf measurements (Smith and Allen, 1996); (ii) conversely to atmospheric techniques, such as the eddy covariance tower, the sap flow technique is not conditioned by either orographic or atmospheric conditions (Forster, 2017). Of the several methods available to estimate sap flow (Dissipation family, Pulse family, Field family, Balance family, etc), dissipation methods can be more appropriate for assessing relative sap flow, while pulse methods can be more suitable for quantifying absolute flows (Flo et al., 2019)."

L61: I am unable to follow this sentence. Please review it: "…can be applied irrespectively of both orographic and atmospheric conditions".

Reply: *This has been reviewed and modified (lines 66-68).*

Lines 65-67: "(ii) conversely to atmospheric techniques, such as the eddy covariance tower, the sap flow technique is not conditioned by either orographic or atmospheric conditions (Forster, 2017)."

L62-63: Please add references to support the statements made.

Reply: *This has been done. We have rewritten the sentence and added the following reference: Flo et al., (2019) (lines 68-71).*

Lines 67-70: "Of the several methods available to estimate sap flow (Dissipation family, Pulse family, Field family, Balance family, etc), dissipation methods can be more appropriate for assessing relative sap flow, while pulse methods can be more suitable for quantifying absolute flows (Flo et al., 2019)."

L65: Please replace "works" with "articles"; please also indicate more articles. The sentence "is quite reliable for determining transpiration" should be better defined because as it is imprecise as it is now worded. L66-72: A better explanation of the HRM method is required, as is indicating the advantages of using it versus other approaches. I think this entire paragraph should be rewritten.

Reply: *This has been done. "Works" has been replaced with "articles". The paragraph has been rewritten (lines 56-71).*

Lines 55-70: "Tree or plant transpiration can be inferred by a wide range of methods, such as lysimeters (Swanson and Whitfield, 1981; Rana and Katerii, 2000), isotopic approaches (Scheidegger et al. 2000), atmospheric techniques (e.g. eddy covariance; Williams et al. 2004), models (Collatz et al., 1991) leaf chambers (e.g. infrared gas analyser, Moncrieff et al. 1997) or sap flow measurements (Granier, et al. 1987; Smith and Allen, 1996). Sap flow methods have been used since the start of the 20th century to trace water movement through xylem tissues (Huber, 1928). The sap flow technique is often followed in woody species at the whole plant level because it presents several advantages: (i) contrary to discrete readings in isotopic approaches or leaf chambers, the sap flow technique provides continuous readings and is not limited to single or a few leaf measurements (Smith and Allen, 1996); (ii) conversely to atmospheric techniques, such as the eddy covariance tower, the sap flow technique is not conditioned by either orographic or atmospheric conditions (Forster, 2017). Of the several methods available to estimate sap flow (Dissipation family, Pulse family, Field family, Balance family, etc), dissipation methods can be more appropriate for assessing relative sap flow, while pulse methods can be more suitable for quantifying absolute flows (Flo et al., 2019)."

L73: Please consider including this part in the previous section.

Reply: *This has been done. Thank you!*

L108-L110: I am unable to follow this statement: "This study attempts to respond to the need to perform calibration on sap flow probes in an isohydric water-saver species (i.e. low transpiration rates) by providing high-quality sap flow data (Dix and Aubrey, 2021)".

Reply: *The paragraph has been rewritten lines 84-93.*

Lines 84-93: "Indeed several works have described the importance of quantitative calibrations in sap flow methods to guarantee the highest measurement accuracy (Dix and

Aubrey, 2021). In some scientific disciplines (e.g., ecohydrology) that require many probes, the cost of commercial probes can be one of the main disadvantages of this technique (Wiedemann et al., 2016). In addition, lab-built probes can be adapted to specific plant characteristics (e.g. tree size, bark width, wood density, etc.). Previous studies have used HRM lab-built probes to measure the sap flow of Aleppo pines and other Mediterranean species (Del Campo et al., 2014, 2019; Molina et al. 2021; Vicente et al. 2022). Additionally, Davis et al. (2012) made a guide for lab-built probes and provided accurate qualitative HRM calibration results."

L114-116: Please consider removing this as an "auxiliary" objective andin all the related sections (Material and Methods, Results in 3.2). As with any experimental environmental studyconducted under laboratory conditions, the authors should ensure obtaining representative data for the ranges of the environmental conditions to describe the expected field variations. Inded the authors should clearly explain and describe the environmental conditions of the conducted experiment. In the Discussion, they mention that the low REW and high VPD conditions were not simultaneously achieved during the experiment. Theregfore, this common drought pattern for Aleppo pine was not properly covered by their sapflow experiment (also indicated in Figure 2). Please consider removing this as an objective and better explain the environmental conditions during the experiment (irrigation doses, soil water content dynamics, solar and VPD dynamics). In addition, the transpiration relations with REW and VPD are widely described in several works for Aleppo pine under field conditions. Thus reporting them under laboratory conditions does not contribute relevant information for the subject.

Reply: *We agree that the relation between transpiration and the environmental variables should be in the background. This was our intention when we called it an auxiliary objective, or even considering it to form part of the methodology. Thus in the new version of the manuscript, the auxiliary objective and all the related sections have been removed. Instead the environmental conditions are better described in Sect. 2.3 and also in Fig. C1 (lines 244-250). The results of the transpiration response to environmental conditions per each tree are now in Appendix D. These last results responded to Referee 1 to explain that although there was intraspecific variation in juvenile pines, the transpiration of all the individuals was controlled by the interaction between VPD and REW (Figure D2 and Table D2).*

However, we partly agree with the comments about the range of environmental variables. It is true that low REW and high VPD conditions, which are indeed a common drought pattern for Aleppo pine, were not simultaneously achieved during the experiment. Although it could be interesting to achieve a wide range of conditions that are more similar to field conditions, the stomatal conductance of Aleppo pine lowers, which results in a sharp drop in transpiration rates under these conditions. Low transpiration rates due to simultaneous low REW and high VPD would have not added much more information when correlating HRM and load-cells measurements, but would have been important for achieving more generalized dependence on these environmental variables. We have added some sentences in the manuscript to explicitly clarify this concept (lines 392-399).

Lines 244-250: "The half-hourly REWs ranged from 0.14 to 1 (dimensionless), while the hourly VPDs obtained minimum and maximum values of 0.27 kPa and 2.82 kPa, respectively (Table 2, Fig. C1)."

Table 2. Environmental condition during the experiment. Abbreviations: VPD: vapour pressure deficit, REW: relative extractable water.

	Minimum	Mean ± SD	Maximum
VPD (kPa)	0.27	0.78 ± 0.47	2.82
Global solar radiation (W m^{-2})	0.00	109.55 ± 188.02	1012.00
REW			
Pine 1	0.20	0.31 ± 0.20	1
Pine 2	0.22	0.32 ± 0.26	1
Pine 3	0.28	0.41 ± 0.25	1

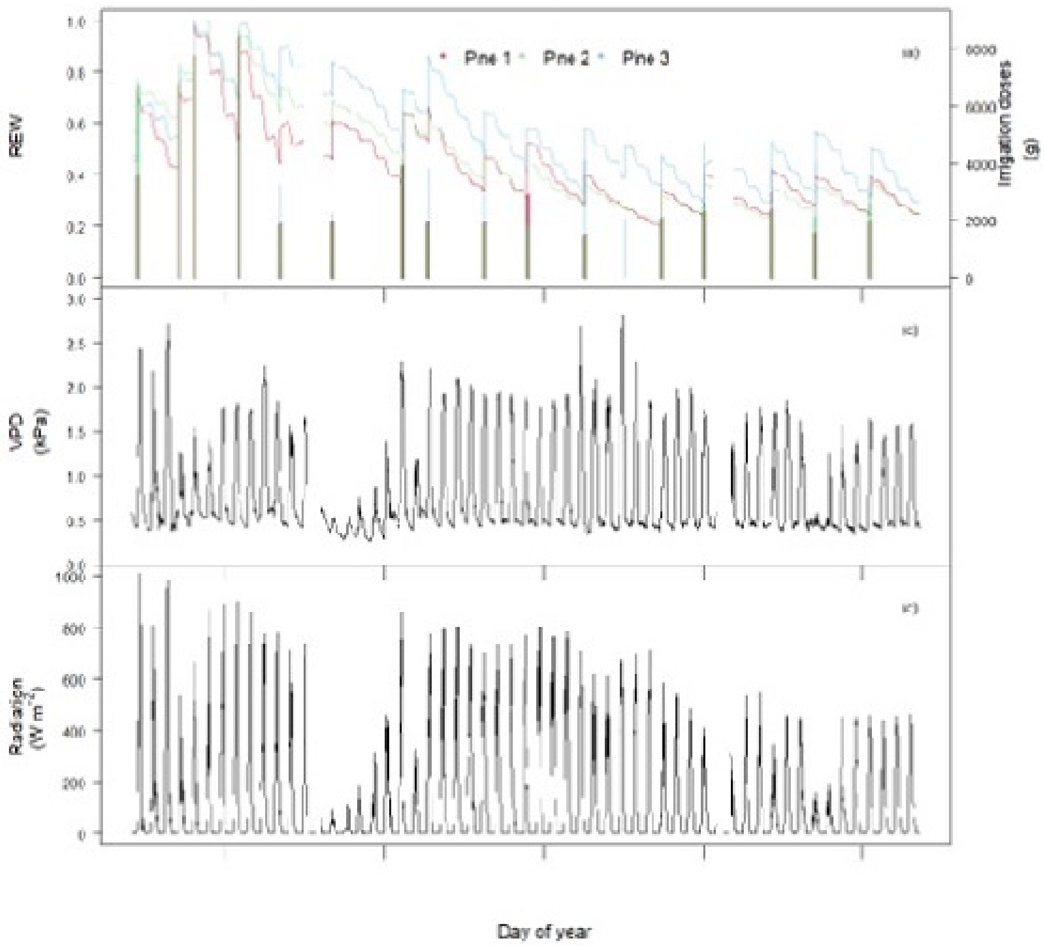

Figure C1. Representation of the transpiration and environmental conditions throughout the study period. Abbreviations: REW: relative extractable water; VPD: vapour pressure deficit.

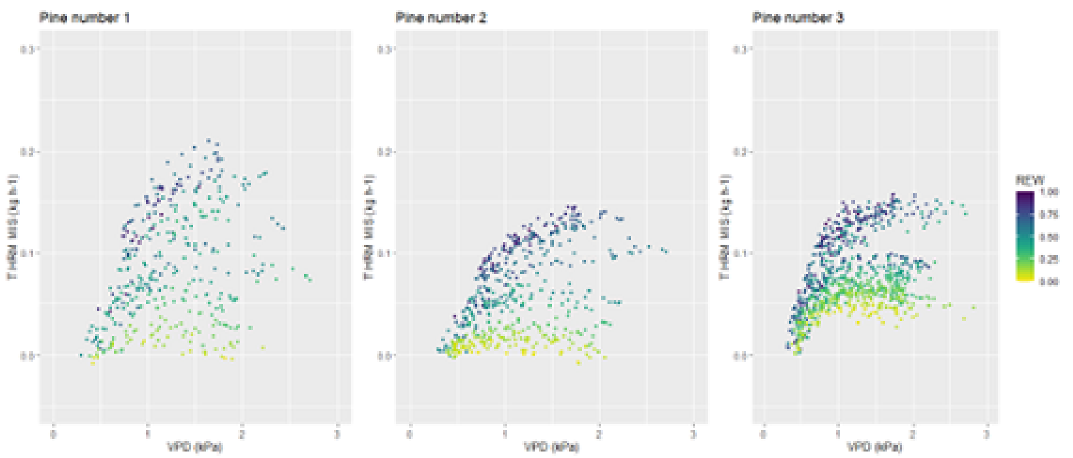

Figure D2. Representation of the response of the transpiration rate measured by the heat ratio method probes and corrected by Larsen's misalignment probe corrections (THRM

MIS) to the environmental variables interaction (VPDxREW, vapour pressure deficit x relative extractable water) along the three Aleppo pines. REW is represented as a gradient scale colour, where purple depicts wet conditions and yellow dry conditions. The figure is a graphical explanation of the models in Table D2.

Table D2. Summary statistics of the linear mixed-effects models of the transpiration measured by the heat ratio method probes and corrected by Larsen's misalignment probe corrections ($T_{HRM\ MIS}$) according to the environmental variables. Pine individuals are fitted as a random factor. Abbreviations: VPD: vapour pressure deficit, REW: relative extractable water. The cross indicates the VPDxREW interaction. Asterisk (*) depicts a p-value belown 0.05.

$T_{HRM\ MIS}$	Pine 1	Pine 2	Pine 3
Intercept	0.025 ± 0.012 *	0.016 ± 0.004 *	0.032 ± 0.004 *
VPD	-0.027 ± 0.009 *	-0.019 ± 0.003 *	0.001 ± 0.003
REW	0.001 ± 0.025	0.014 ± 0.007	0.008 ± 0.008
VPDxREW	0.165 ± 0.020 *	0.103 ± 0.006 *	0.075 ± 0.006 *
R^2	0.66	0.86	0.66

Lines 392-399: "In fact the range of environmental conditions (VPD and REW) used in this paper is consistent with the range observed in fieldwork studies. Although it is true that similar environmental conditions to those of a Mediterranean summer (high evaporative demand and scarce water availability) could not be simulated due to technical limitations, under the most extreme conditions (i.e. REW = ~ 0.1, VPD = ~ 4.5 kPa) Aleppo pine induces stomatal closure and transpiration rates drastically drop. This means that the study presented transpiration values that could correspond to Aleppo pine transpiration values under these summer conditions."

L130: I suggest including substrate in the title: "Plant and Substrate Materials". I also suggest moving all the basic parameters for the sap flow calculations to Section 2.3 to focus on the HRM method (wood density, sapwood area, etc.).

Reply: *This has been changed. Thank you for the appreciation.*

L132-133: Information about plant structure is required (height, diameter).

Reply: *We have rewritten Sect. 2.1. and included this type of information, which was missing in the previous version (lines 137-138).*

Lines 137-138: "Pines were 3 m high, with a basal diameter of 5 cm on average. Pines displayed the same juvenile maturation status and presented a few reproductive structures (cones)"

L134: Please replace "measured" with "estimated".

Reply: *This has been done and replaced.*

L150-152: I am unable to follow what you mean here; please remove the sentence if the authors indicate that they calibrate their load cells because this is obvious.

Reply: *We agree. This has been removed.*

L154-158: Please rewrite by removing any repeated information. I am also unsure about the way the authors explain their approach here. I understand that water loss measurements are the way to validate their sap flow sensor because they are the true values. I suggest modifying this part to highlight this fact and to not consider them at the same level as in the current form.

Reply: *Thank you for this appreciation. The paragraph has been rewritten (lines 164-171). As you mention, water loss measurements are an important aspect of this paper. We have considered adding your suggestion in lines 120-123 and 387-393.*

Lines 118-121: "i) test the Aleppo juvenile pine transpiration rates measured by the HRM *technique compared to load cells as a direct method taken as the "gold standard" to measure plant transpiration rates under a variety of laboratory environmental conditions;"*

Lines 164-171: "2.2. Transpiration estimations. Tree transpiration was measured by two simultaneous techniques: the HRM by sap flow probes (T_{HRM}) and weighing water losses by load cells (Lysimeter technique, $T_{OBS,}$ Swanson and Whitfield, 1981), which measured water loss by transpiration. The lysimeter technique by load cells allows high-resolution transpiration determinations (Table 1). Therefore, T_{OBS} was considered to offer true values and were used to test sap flow probes transpiration measurements. Thus sap flow and water losses were the two independent approaches followed to estimate the transpiration rate per tree (T, kg h^{-1})."

Lines 387-393: "From our point of view, the confidence in our results for its use as a test in the Aleppo pine transpiration estimations by the HRM method is reinforced by: (i) transpiration was measured by head cells ("gold standard") with accurate precision; (ii) the long experimental period (57 days); (iii) the resolution of the transpiration rates methodology on a half-hourly basis; (iv) estimating individual-specific functional traits; (v) the range of environmental conditions. In fact the range of environmental conditions (VPD and REW) used in this paper is consistent with the range observed in fieldwork studies."

L161: I am unsure about this explanation (the limitation of having only three individuals was overcome by including a long experimental period (57 days) using half-hourly measurements); are the authors sure that temporal variation is comparable to tree-to-tree variation?

Reply: *We are aware that technical limitations resulted in a small sample of tree-to-tree variation (N=3), and this topic has been addressed throughout the document. However, we wish to highlight that having long-term data during our experimental period with accurate half-hourly continuous recordings (Tobs) will provide good information and higher accuracy about the test between both methods.*

L164-168: The description of the sap flow sensor construction is further explained in Appendix A, but I think some significant information should also be indicated here (heat pulse energy, number and distance of radial measurements, aspects of where sensor is installed). Please consider adding heat pulse energy and distance of radial measurements to Appendix A.

Reply: *At each heat pulse, which typically lasted 3 seconds, the total energy density released at the central needle was approximately 30 Joules/cm. As we describe, there was one sap flow equipment per tree, made up of two parallel temperature probes, and lower and upper probes, that were inserted into a vertical trunk section and spaced every 0.6 cm from a central heating line. Probes were inserted at breast height, were north-facing and protected with insulation material attached to the trunk. We have added the following information for the distance of the radial measurements (lines 429-433): "The constantan (0.3 cm) and chromium wires (2.3 cm) were welded together and placed firstly inside a glass tube to a depth of ~1.15 cm (0.1 cm x 2.3 cm, MODEL), and were secondly placed inside a needle (0.13 cm x 2.3 cm, STERICAN Hypodermic Needles). This means that the distance of radial measurements fell within ~1.15 cm."*

L172-192: As Larsen et al. (2020) stated that their correction is suitable for being applied when measurements are taken for more than 3 months, I do not properly follow why the authors applied this approach here. I see they consider a fixed correction (L188-190) based on Larsen´s approach. Larsen’s approach is dynamic calibration based on changes in zero flow with time. I believe that the authors’ experimental design is, therefore, unsuitable to address this properly given the duration of the experiment and corresponding the fixed correction that they applied.

Reply: *It is true that it is suitable to follow Larsen et al. (2020) when measurements are taken for more than 3 months to provide dynamic calibration. In our study, we tested the temporal change of the misalignment probes and no significant temporal changes were observed (because the study period lasted less than 3 months). So as the referee mentions, we applied fixed correction for each found misalignment. Although we did not aim to look for dynamic correction, we achieved fixed values for misalignment following the same assumptions and procedures as in Larsen et al. (2020) when selecting zero flows and calculating terms X1 and X2 (lines 180-203). For these reasons, (i) we thought that Larsen’s work must be explicitly acknowledged in our study and (ii) our study could be useful as a quantitative test of the proposed methodology (lines 355-358 and Sect. Appendix B).*

Lines 180-203: "For a given tree, sap velocity (V_s, cm h^{-1}) was obtained following Eq. (B1), Eq. (B2) and Eq. (B3). Then the transpiration rate (T_{HRM}, kg h^{-1}) was upscaled by multiplying each sap wood area per tree (Table 1, Eq. (B4)). Transpiration was also tested by applying probe misalignment correction, which followed the criteria for detecting the zero-flow and heat pulse velocities equations described in Larsen et al. (2020) ($T_{HRM\ MIS}$, kg h^{-1}). The temporal change of the misalignment probes was tested. As expected, no significant changes were observed because the study period lasted less than 3 months. Therefore, the dynamics of Larsen’s research was not followed (Sect. Appendix B, Eq. (B5)). For these transpiration rate estimations, two different heat pulse velocities (V_{h1} and V_{h2})were obtained. For the V_{h1} calculations, it was assumedthat the distance between the heater and the downstream temperature probe (X_1) underwent misalignment (different to 0.6 cm), while the distance between the heater and the upstream probe (X_2) was set at -0.6 cm. The opposite was assumed to calculate the other sap velocity (V_{s2}). To evaluate X_1 and $X_{2,}$ it was necessary to achieve zero-flow conditions, which were identified as night episodes when null-weight variations were recorded by load cells per tree. Four events that met the requirements were clearly detected at night (23:00-02:30h) when the vapour pressure deficit came close to zero. An average of the half-hour temperature readings per event was performed and Eq. (B6) was applied. Finally, the averages of the resulting X_1 and X_2 values for each zero-flow event (Table D1) were used in Eq. (B5) to obtain V_{h1} and V_{h2}. As no temporal dependence of X_1 and X_2 was observed among the selected events, no temporal probe misalignment behaviour was considered. So V_{h1} and V_{h2} were averaged (. Then was applied to Eq. (B2) and (B3), and was upscaled to transpiration by multiplying per sap wood area (Table 1, Eq. (B4))."

Lines 355-358: "Another key step is probe alignment when once inserted into trunks. Larsen et al., (2020) introduced specific probe misalignment correction as a modification of that formerly proposed by Burgess et al. (2001). This correction resulted in higher accuracy compared to HRM without correction."

One important aspect that should be mentioned and clarified is that probe misalignment is not expected when sensor needles are properly installed. In line with this, the small differences shown in the Results section when comparing uncorrected and corrected lectures seem to be related to that fact and, therefore, the need to apply a correction factor is not justified. This would once again imply that the authors’ experimental design is not suitable for applying dynamic calibration.

Reply: *We disagree with this comment. Firstly because, once the probe is inserted into the trunk, we could not know if it was properly installed or not. According to the methodological corrections in Larsen et al. (2020), we could estimate how aligned or misaligned probes are, and then correct them. Secondly, as one of the main outcomes, we had already clarified and discussed that the larger the misalignment, the better the correction (lines 370-373). As a result, when we applied misalignment correction, values were corrected from negative (non-sense) values to positive ones. Therefore, these results would indicate that misalignments could be corrected and values would fall within what is considered normal. Thirdly, our study objective was not to test dynamic calibration. As mentioned in other comments, we tested transpiration rate estimations corrected by probe misalignment correction. We have clarified this information in lines 180-203 (mentioned in the comment above) and lines 354-368.*

Lines 355-369: "Another key step is probe alignment when once inserted into trunks. Larsen et al., (2020) introduced specific probe misalignment correction as a modification of that formerly proposed by Burgess et al. (2001). This correction resulted in higher accuracy compared to HRM without correction. In our study, probe misalignment correction not only provided better transpiration rate quantification accuracy (i.e. closer values to line 1:1), but also shifted negative transpiration rates to positive ones, while also correcting these deviations. When applying this correction, the underestimation at medium and high flows decreased, which conferred the transpiration rate estimations higher accuracy under wetter conditions. However, magnitude varied depending on the degree of probe misalignment. The error variability in the magnitude of the transpiration rate has been shown in previous reports (Sun et al., 2012; Rubilar et al., 2017). The greater probe misalignment is, the better the correction of the T_{HRM} *values; moreover, the smaller probe misalignment is, the poorer the correction of the* T_{HRM} *values. In line with this, the results in this paper support applying probe misalignment correction in all the sampled individuals to correct even minor misalignments, which produce undesirable errors."*

Another important point is that the authors do not mention anything about the wounding effect and the corrections proposed to address this matter. Did the authors apply the classic approach by Swanson and Whitfield (1981) or another one? Please, clarify this. If the authors do not correct their lectures, they also should justify why.

Reply: *We applied Equation 6 described in Burguess (2001), which is a modification of CHPM Swanson's (1983) solutions, where Vh is heat pulse velocity, Vc is the heat pulse velocity corrected by wounding effects and, a,b, and c are three coefficients for varying wound widths. As mentioned in other comments, we have included a new appendix that describes all the equations used in this study for transpiration estimations (Sect. Appendix B).*

L165: I am unsure about Williams et al. (2004) as the most appropriate reference here. I would indicate Marshal (1958) instead.

Reply: *This has been done. Thank you.*

Figure 1. What about the linear behaviour of residuals from 0.20 cm/h in subfigures c) and d), especially from pine 3? I think the authors should further discuss this aspect.

Reply: *We have discussed this now in lines 299-303.*

Lines 297-301: "The analysis of the residuals with T_{HRM} and $T_{HRM\ MIS}$ did not show any relevant changes (Fig. 1c and 1d). However, the residuals responses above the 0.20 kg h^{-1} T_{OBS} values were slightly turned aside from zero, which could provide information about the poor accuracy of the transpiration rates at high rates. The maximum underestimations (T_{OBS} - $T_{HRM\ MIS}$) per individual went from 0.08 to 0.15 kg h^{-1} (Fig. A3b, d, f)."

Please consider combining Tables 1, 2 and 3 to make comparisons easy.

Reply: *We agree. We have combined Tables 2 and 3 to better compare the results, but we consider that Table 1 could be left separate because it is related to technical and tree structural features, and is suitable for Sect. Material and methods.*

Table 3. Table 3. Summary statistics of the linear model of (a) the transpiration rate measured by the HRM probes ($T_{HRM,}$ kg h^{-1}) according to the transpiration rate measured by load cells ($T_{OBS,}$ kg h^{-1}); (b) the transpiration rates measured by the HRM probes corrected by the probe misalignment correction that Larsen et al. (2020) proposed ($T_{HRM\ MIS,}$ kg h^{-1}) according to T_{OBS}.

	Pine 1	Pine 2	Pine 3
(a) T_{HRM}			
Intercept	-0.030 ± 0.002	-0.026 ± 0.002	0.020 ± 0.002
T_{OBS}	0.853 ± 0.016	0.785 ± 0.014	0.475 ± 0.017
R^2	0.88	0.86	0.50
p-value	< 0.05	< 0.05	< 0.05
(b) $T_{HRM\ MIS}$			

Intercept	-0.002 ± 0.002	-0.012 ± 0.001	0.023 ± 0.002
T_{OBS}	0.871 ± 0.017	0.790 ± 0.014	0.476 ± 0.017
R^2	0.88	0.87	0.50
p-value	< 0.05	< 0.05	< 0.05

Please consider including "," after et al. for all the references.

Reply: *Thank you so much. We have included "," in the references in brackets, but not in the references which are embedded in the text, following the information described on the website: https://www.hydrology-and-earth-system-sciences.net/submission.html#references*

Although the text flows well, the English should be profoundly revised because there are several mistakes and sentences that should be rewritten.

Reply: *The manuscript has been revised by a professional native English editor. However, we have submitted this new version to another revision to improve its readability. A certificate of the new revision is included.*

Please also note the supplement to this comment:
https://hess.copernicus.org/preprints/hess-2022-328/hess-2022-328-AC2-supplement.pdf