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Rising air humidity during spring does not trigger leaf-out in temperate woody plants

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Letters

Rising air humidity during spring does not trigger leaf-out in temperate woody plants

Introduction

The timing of spring leaf emergence in temperate regions directly influences global biogeochemical cycles and species interactions (Richardson *et al.*, 2013). Understanding the environmental drivers of leaf-out is thus essential to forecasting ecosystem responses to global climate change. These drivers have long been thought to be species-specific combinations of spring temperature, winter chilling, and increasing day length during spring (Heide, 1993a,b; Laube *et al.*, 2014a; Polgar *et al.*, 2014; Zohner & Renner, 2015; Zohner *et al.*, 2016, 2017). Temperature-related increases in air humidity were suggested as an alternative trigger of the spring bud break recently, based on experimental results from nine Eurasian and American tree species, including two needle-leaved trees (Laube *et al.*, 2014b). The new hypothesis was cast into doubt, however, by experiments on 15 further species, again from both Eurasia and America, with one, *Cornus mas*, being used in both studies (Zipf & Primack, 2017). These contradictory conclusions raise basic questions about (1) the type of experiments in use for studying environmental leaf-out triggers, and (2) the extent of species differences in leaf-out triggers and hence the influence of the identity of species studied on the results.

In their initial experiment, Laube *et al.* (2014b) found that high humidity (90% relative air humidity compared to 40% control) advances leaf-out by, on average, 7 d. The experimental set-up relied on twig cuttings (*c.* 25 cm length) obtained on 3 March 2012 from a forest near Freising (a city near Munich, Germany), that were cleaned and placed in glass bottles filled with tap water. For each species, 20 twigs (from three individuals) were kept in climate chambers for 30 d, half in each humidity treatment. Temperatures were gradually increased, but day-length was kept constant at 16 h. As a physiological explanation for their results, the authors proposed that plant tissue dehydrates during the chilling period in winter and rehydrates in spring by absorbing water directly through the buds. They concluded that absolute air humidity, not temperature, triggers leaf unfolding in spring. In a follow-up study, Zipf & Primack (2017) again used twig cuttings in glass vials placed in climate chambers, but a gradient of humidity (20–94% relative air humidity) and constant temperature (23°C) and light conditions (12 h d⁻¹). Leaf-out in three of the 15 species studied was significantly affected by higher humidity: *Carya tomentosa* leafed out significantly earlier, while *Berberis thunbergii* and *Rhamnus frangula* leafed out significantly later. The remaining 12 species, including *Cornus mas* in which Laube *et al.* (2014a,b) had found a

significant effect of higher humidity, were unaffected. Because both studies relied on the twig-cutting experimental set-up in which the integrity of the hormone and water transport is compromised, Zipf & Primack (2017, p. 2216) concluded that ‘more work must be done on this topic before researchers can properly interpret the results of Laube *et al.* (2014b) and determine the effect of humidity on the leafing out process for woody species.’

Outdoor experiments in which twig cuttings were placed in water-filled vials next to the trees from which they had been cut have shown that leaf-out times between donor trees and cut twigs do not differ (Vitasse & Basler, 2014), supporting the utility of the twig-cutting experimental approach for studying temperature and day-length effects on leaf unfolding. For addressing potential effects of soil and air moisture on plant phenology, however, twig cuttings may be problematic because the water supply to buds differs from that experienced by plants rooted in soil. Due to more negative water potential in the xylem, twig cuttings exhibit reduced water uptake (Ikeda & Suzuki, 1986). Only once roots are developed, does the water potential of dormant cuttings increase (Puri & Thompson, 2003). Water uptake in rootless cuttings further depends on the water’s gas and nutrient composition (Erstad & Gislørød, 1994), while intact roots allow plants to adjust their water uptake depending on the shoot’s demands. Whether twig cuttings receive sufficient water might thus depend on their ability to take up water from the surrounding air. As far as we are aware, only two studies have used rooted saplings (of *Picea glauca*) to study how air humidity affects leaf-out, and they obtained opposite results: Marsden *et al.* (1996) found delayed bud flush under high air humidity, whereas Roberts & Zwiazek (2001) found advanced bud flush. In a twig-cutting experiment, air humidity had no effect on leaf-out in *Picea abies* (Laube *et al.*, 2014b). Further research is therefore needed to study the role of air humidity *per se* as a regulator of spring leaf-out in temperate trees.

We here report on experiments addressing the effect of air humidity on leaf-out in potted saplings of broad-leaved species, including one also studied by Laube *et al.* (2014b). The first experiment compared leaf-out timing between a control and a high-humidity treatment under natural spring light and temperature fluctuations. The second experiment compared saplings kept under fully-controlled environmental conditions under 20%, 50%, and 80% air humidity. These experiments allow us to detect effects of air humidity on leaf-out timing without possible artefacts introduced by the modified water uptake in twig cuttings.

Materials and Methods

Experiment 1

Our first experiment tested the effect of elevated air humidity under natural spring light and temperature fluctuations in glasshouse

chambers. It was conducted in the Munich Botanical Garden (48°09' N, 11°30' E; 501 m above sea level (asl)) between 1 March and May 2018 on 2-yr old saplings of 10 temperate tree species (Table 1). From autumn until the start of the experiment, plants were kept outdoors under natural conditions. Between 12 and 28 individuals per species were exposed to two treatments in climate-controlled glasshouse chambers with an openable top, and the effects on phenology were then followed until the end of May. In the humid chamber, relative air humidity was elevated to $96.0 \pm 0.6\%$ (mean \pm SE) using a humidifier placed at plant height (Luftbefeuchter CEZIO, C. Unger, Scheidt, Germany), resulting in an average absolute air humidity of $11.5 \pm 0.5 \text{ g m}^{-3}$ (Fig. 1a). Absolute air humidity was calculated as a function of temperature and relative air humidity, using the Clausius–Clapeyron equation implemented in the humidity R-package. In the control chamber, plants were watered if needed, but air humidity was otherwise not modified, resulting in an average relative air humidity of $70.0 \pm 1.4\%$ and an average absolute air humidity of $8.3 \pm 0.3 \text{ g m}^{-3}$. All other factors (temperature, light intensity, day-length) were identical between chambers, exposing plants to natural fluctuations in temperature and sunlight. Temperatures were *c.* 3°C warmer relative to outdoor temperatures and followed the natural outdoor fluctuations (mean temperature humid chamber = $13.4 \pm 0.6^\circ\text{C}$; mean temperature control chamber = $13.5 \pm 0.8^\circ\text{C}$). We observed bud phenology twice a week from 15 March until 22 May 2018. Individual leaf-out was defined as the date when the first leaf had fully unfolded. We used climate loggers (HOBO PRO v.2, Onset Computer Corporation, Bourne, MA, USA) to monitor temperature and humidity in both chambers.

Experiment 2

Our second experiment tested the effects of air humidity on spring leaf-out under fully-controlled environmental conditions in climate chambers. This experiment was conducted in Zurich (47°37' N, 8°55' E; 452 m asl) between 22 March and May 2019 on 2-yr old saplings of *Carpinus betulus*, *Fagus sylvatica*, and *Quercus robur*. From December 2018 until the start of the

Table 1 Species list and sample sizes (number of individuals).

Species	Expt 1			Expt 2			
	Total	Control	Humid	Total	20%	50%	80%
<i>Acer campestre</i>	25	12	13	—	—	—	—
<i>Acer palmatum</i>	21	10	11	—	—	—	—
<i>Aesculus hippocastanum</i>	18	9	9	—	—	—	—
<i>Carpinus betulus</i>	21	10	11	32	11	10	11
<i>Corylus avellana</i>	22	12	10	—	—	—	—
<i>Euonymus europaeus</i>	12	6	6	—	—	—	—
<i>Fagus sylvatica</i>	24	12	12	19	7	6	6
<i>Quercus robur</i>	25	12	13	18	6	6	6
<i>Sorbus torminalis</i>	23	10	13	—	—	—	—
<i>Viburnum opulus</i>	22	11	11	—	—	—	—
All	213	104	109	69	24	22	22

experiment, plants were constantly kept at 4°C, 50% relative air humidity, and 8-h days. Between 18 and 32 individuals per species were exposed to three treatments. Relative air humidity in the chambers was set to 20%, 50%, and 80% (1). Actual air humidities were within $\pm 10\%$ of the prescribed values (see Fig. 1b). All plants were regularly watered to ensure that soil moisture did not affect leaf-out timing. In all chambers, temperature was set to 20°C during the day and 8°C during the night. Actual temperatures were within $\pm 0.8^\circ\text{C}$ of the prescribed values. Daily mean temperatures in the 20%, 50%, and 80% chambers were 14.4 ± 0.9 , 14.3 ± 0.9 , and $14.4 \pm 0.9^\circ\text{C}$, respectively (see Fig. 1b). Daily mean absolute air humidities were 2.6 ± 0.2 , 6.4 ± 0.3 , and $10.4 \pm 0.5 \text{ g m}^{-3}$, respectively. Day-length was set to 12 h at the start of the experiment and extended in weekly intervals following the natural day-length progression. We observed bud phenology every 3 d. Individual leaf-out was defined as the date when at least three unfolded leaves, pushed out all the way to the petiole, were visible on the respective individual.

Statistical analysis

For both experiments, linear mixed effects models including species as random effect using the NLME R-package were performed to test for differences in leaf-out dates between the treatments across all species. Additionally, we performed multivariate analysis of variance (ANOVA) including an interaction term between species and treatment to test whether the effect of air humidity on leaf-out differs among species. To test for significant differences in leaf-out dates between the two treatments within each species in Expt 1, a Kruskal–Wallis *H* test was performed. To test for differences in leaf-out dates between the three treatments in Expt 2, the Kruskal–Wallis *post hoc* test (multiple comparison after Kruskal–Wallis) was used (Siegel & Castellan, 1988). All statistical analyses were performed with the R software (R 3.4.1, R Core Team 2019).

Results and Discussion

Our results are not in agreement with the study of Laube *et al.* (2014b) who inferred that, instead of temperature fluctuations, seasonal fluctuations in absolute air humidity are the main regulator of spring phenology in temperate woody plants. In our first experiment, increased air humidity led to slightly (by 1.5 d) delayed leaf-out when including all 10 species in a linear mixed effects model ($P = 0.02$, $n = 213$). There was a significant interaction between treatment effect and species (analysis of covariance (ANCOVA): $P < 0.01$), that is, the effect of air humidity on leaf-out dates differed across species. Separate analysis for each species revealed significantly earlier leaf-out under elevated air humidity in one of the 10 study species: *Aesculus hippocastanum* advanced leaf unfolding in the humid chamber by, on average, 3 d (Table 1; Fig. 2). High air humidity significantly delayed leaf unfolding in *Acer campestre*, *Sorbus torminalis*, and *Carpinus betulus* by, on average, 6, 5, and 3 d, respectively. In the remaining six species, no significant differences were detected (Fig. 2; Table 1). In our second experiment, no significant difference in leaf-out dates across treatments could be detected across the three species used in the

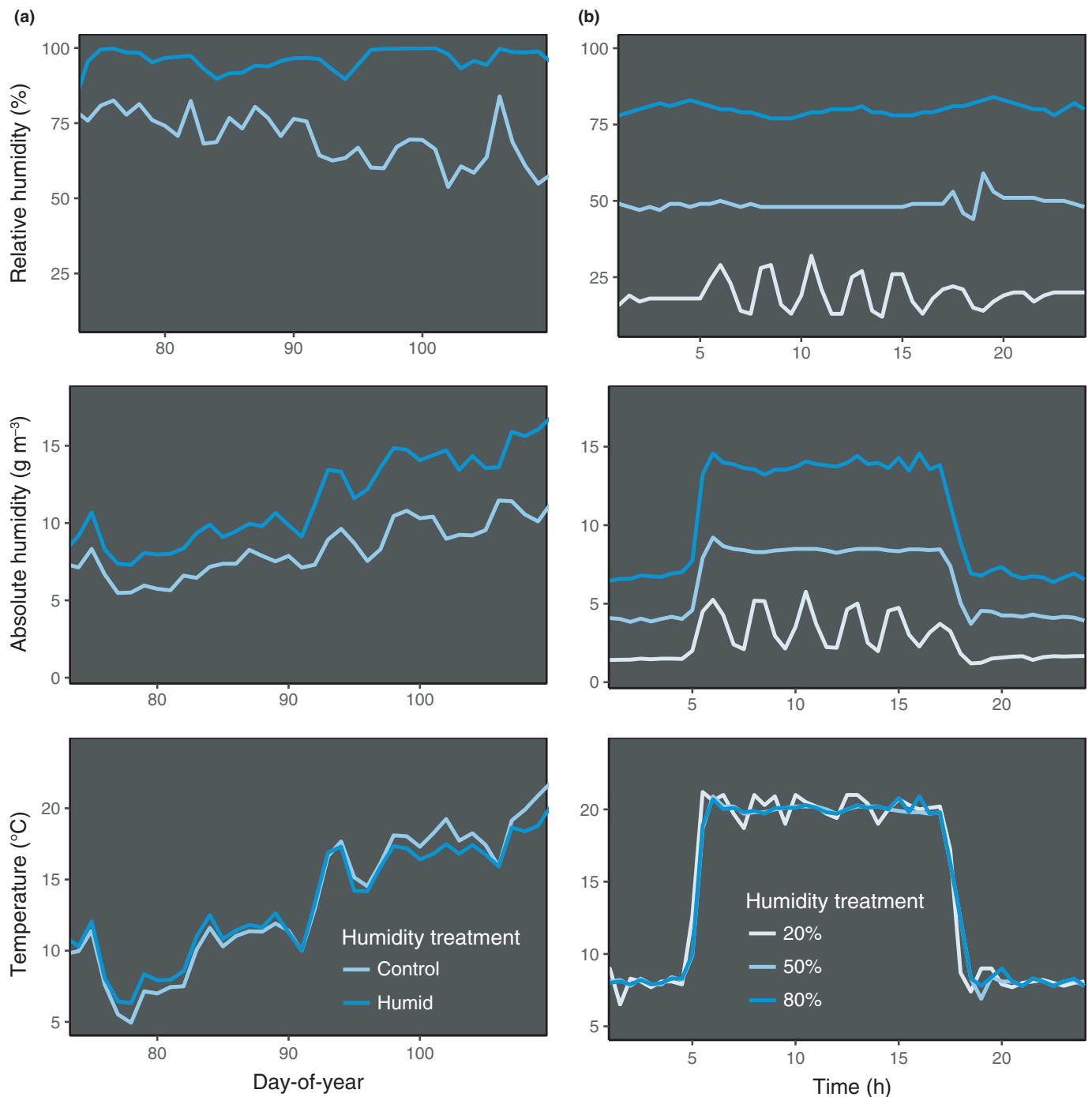


Fig. 1 Temperature and humidity profiles of the treatments applied in Expt 1 (a) and Expt 2 (b). (a) Mean daily relative humidity (top panel), absolute humidity (middle panel), and temperature (bottom panel) from 14 March until 1 May for the control and elevated humidity treatment in Expt 1. (b) Daily temperature and humidity profiles for the 20%, 50%, and 80% humidity treatments of Expt 2.

experiment (linear mixed effects model: $P = 0.62$, $n = 69$). Separate analysis for each species also showed no significant differences among treatments (Fig. 3).

The effects of air humidity detected for *Acer campestre*, *Aesculus hippocastanum*, *Carpinus betulus*, and *Sorbus torminalis* in Expt 1 show that air humidity can affect leaf-out to some degree, but the effect is of inconsistent direction (Fig. 2). The delays in leaf-out under high humidity found in two species by Zipf & Primack (2017 using cut twigs) and in three species in our study (using

rooted saplings) may be the result of a stress reaction. Although physiological responses to elevated air humidity before leaf unfolding have never been tested, there is evidence that high humidity affects plant behaviour once the leaves are out. For instance, thermoregulation in plants exposed to high air humidity is impaired because of lower transpiration rates (van de Sanden & Veen, 1992; Fordham *et al.*, 2001), leading to reduced photosynthesis (Mortensen, 1986; Rezaei Nejad & van Meeteren, 2008). Water-stress hormone concentrations, such as abscisic acid 8'-

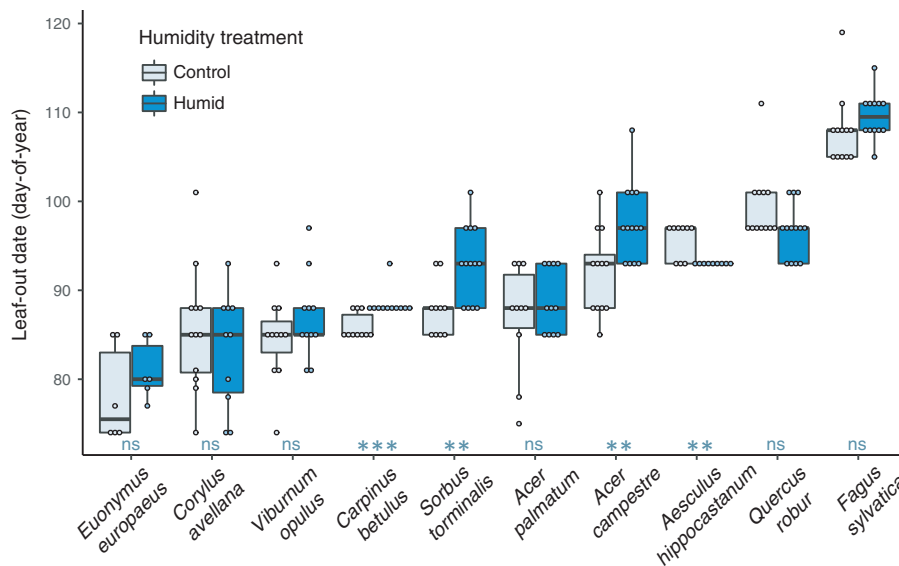


Fig. 2 Results of Expt 1 in which saplings of 10 woody species were exposed to ambient and elevated air humidity. Boxplots show the quartiles and medians of the days to leaf-out (from 1 January) for each species and treatment. Points show individual leaf-out dates. Significance levels from Kruskal–Wallis H test: ns, $P \geq 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

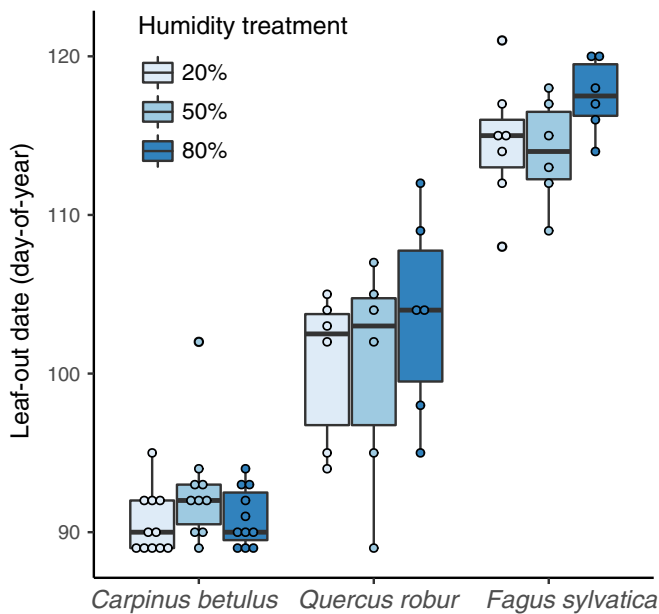


Fig. 3 Results of Expt 2 in which saplings of three woody species were exposed to 20%, 50%, and 80% air humidity. Boxplots show the quartiles and medians of the days to leaf-out (from 1 January) for each species and treatment. Points show individual leaf-out dates. No significant differences were found across treatments (Kruskal–Wallis H test: $P \geq 0.05$).

hydroxylase (ABA), are lower when exposed to high humidity, leading to changes in stomatal behaviour (Rezaei Nejad & van Meeteren, 2008; Okamoto *et al.*, 2009). As a result, leaves kept under high humidity fail to close their stomates when needed (Fordham *et al.*, 2001), resulting in shoot and leaf elongation (Mortensen, 1986; Roberts & Zwiazek, 2001) and possibly a longer time to full leaf unfolding of the newly developed shoots. Slightly delayed leaf-out under elevated air humidity might thus not result from physiologically delayed dormancy release, but

instead be due to a slowed leaf development during the initial stages of bud opening.

Conclusion

Here, we tested the effects of air humidity on leaf-out in potted saplings, not twig cuttings, to keep the integrity of the hydraulic and hormonal systems. We exposed saplings of 10 woody species to elevated or ambient air humidity in glasshouse chambers under natural light, and exposed three of the species to 20%, 50%, and 80% air humidity in light- (and temperature) controlled chambers. Our results, in agreement with those of Zipf & Primack (2017), using a different experimental set-up, refute the hypothesis that air humidity is a main trigger of spring leaf unfolding in temperate woody plants. By using rooted plants instead of cuttings to study the effects of air humidity on leaf-out phenology, we rule out artefacts associated with water supply and hormonal responses in plants disconnected from their root system. As such, our results uphold the overriding role of temperature (chilling and forcing) and day-length in triggering spring leaf-out, increasing our confidence in projections of future ecosystem responses based on the existing phenological models.

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Author contributions


The study was conceived and developed by CMZ. The experiments were performed by CMZ, AFTS and FB. Analyses were performed by CMZ and AFTS. The manuscript was written by CMZ with




assistance from SSR. YV and FB reviewed and provided input on the manuscript.

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