Effects of Water Vapour Pressure Deficit (VPD) on Plant Transpiration

– a review of experiments under controlled environments –

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I ABSTRACT

Drought is a major limiting factor in crop production and expected to become even more important due to climate change. Feeding the world's growing population necessitates breeding germplasm adapted to tolerate drought conditions. One trait suggested to increase drought tolerance is the restriction of transpiration (E) in response to a high water vapour pressure deficit (VPD) and the existence of this trait has been reported in several species. Textbook knowledge suggests that C4-plants might be more likely to restrict E in response to increasing VPD than C3-plants, but reports were inconclusive. The environmental conditions, gas exchange equipment and analysis used in the literature varied considerably, and information regarding them was patchy. There was a large intra- and interspecific variation in the response of E to VPD, both in E rate and restriction of E under increasing VPD. No significant relationship between photosynthesis pathway and rate of E, restriction of E, or the VPD at which a restriction occurs was identified in this analysis. Variation in the VPD-value at which E is restricted seem to be caused by genotypical variations and genotypes restricting E in response to increasing VPD were reported in all species. Expression of this trait seems to be independent of PS pathway, but dicotyledons were less likely to express it compared to monocotyledons.

II LIST OF ABBREVIATIONS

Abscisic acid (ABA)	3
Actual vapour pressure (e_a)	1
Breakpoint (BP)	6
Carbon dioxide (CO_2)	3
Infrared gas analyser (IRGA)	9
Oxygen (O_2)	4
Phosphoenolpyruvate carboxylase (PEP carboxylase)	4
Photosynthesis (PS)	3
Potassium ions (K^+)	3
Relative humidity (RH)	4
Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO)	4
Saturation vapour pressure (e_s)	1
Stomatal conductance (g_s)	3
Temperature (T)	1
Transpiration (E)	1
Water vapour pressure deficit (VPD)	1

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1 INTRODUCTION

Almost one third of global arable land is affected by water scarcity, a figure expected to double by 2050 (Vicente-Serrano et al. 2012). Drought is already a major limiting factor for many important crop species (Parida et al. 2007; Choudhary et al. 2013). Agriculture is especially vulnerable to the effects of climate change (Allen et al. 2010), for example, it is suspected that *Triticum spp.* and *Zea mays* production has already been negatively impacted by it between 1980 and 2008 (Lobell et al. 2011). Globally, temperature (T) is expected to rise and precipitation is likely to become more infrequent, with lengthened intervals between rainfall events and lower rainfall in the cropping season (Allen et al. 2010). Increasing food production, to feed the world's growing population, under these circumstances necessitates breeding adapted germplasm for drought tolerance.

One promising option for rain-fed cropping systems in environments with cyclic drought periods has been the identification of traits conserving water until the next rain episode. It has been suggested that limiting water use early in the season may increase water availability during grain filling (Zaman-Allah et al. 2011). Transpiration (E) constitutes the majority of evapotranspiration (Wei et al. 2017). Using their stomata to control E, plants can influence the rate of soil water depletion (Damour et al. 2010). It has been hypothesised that early season water conservation could be achieved by plants limiting E under high levels of air water vapour pressure deficit (VPD);(Sinclair et al. 2005).

At a given T, VPD describes the difference between the saturation vapour pressure (e_s) of air and its actual vapour pressure (e_a) (Fig. 1);(Anderson 1936). e_s is a curvilinear function of air T (Lawrence 2005). Mean air T has been continually rising since 1850 (Fig. 2);(IPCC 2019), this has led to an increased water vapour pressure deficit, because e_a has not increased at the same pace as e_s (Hatfield and Prueger 2015).

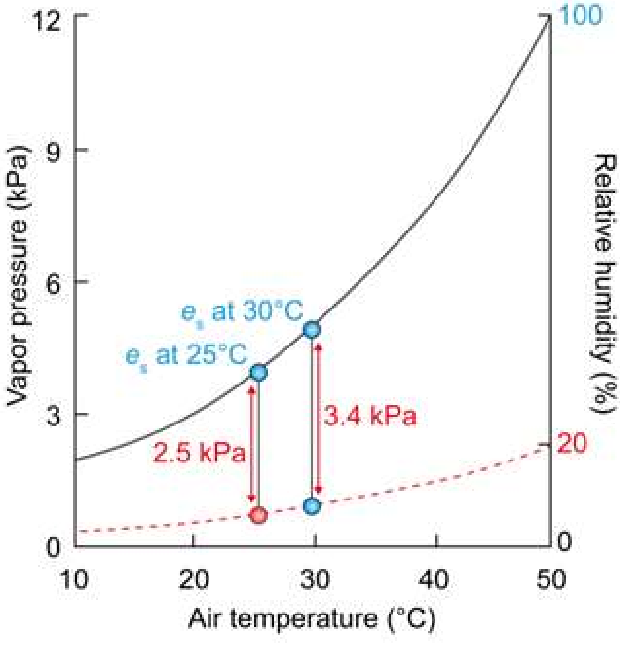


Figure 1: Vapour pressure deficit (VPD, kPa) is the difference between saturation vapour pressure (e_s, bold line) and actual vapour pressure (e_a, dotted line). A rise in temperature increases VPD if relative humidity is stable. Adapted from Grossiord et al. 2020.

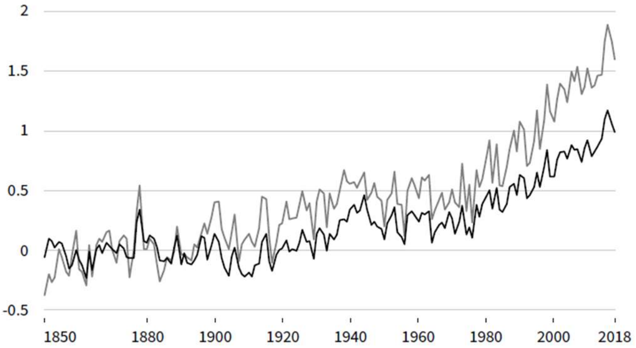


Figure 2: Change in surface air temperature (T,°C) over land (gray line) and global (land-ocean) mean surface T (black line);(IPCC 2019).

Water evaporating from a plant's leaves creates a tension in the xylem, by which the column of water in the xylem is drawn upward toward regions of lower hydrostatic pressure (Nobel 2020). Along the way of this soil-plant-atmosphere continuum the water faces several resistances. For liquid water, soil resistance is usually the largest resistance. This is followed by the resistance of the root epidermis, cortex, and endodermis, as water enters and moves through the root, while xylem resistance, on the way to the leaves, is proportional to its length (Nobel 2020). After liquid water diffuses into the mesophyll, as water vapour, it encounters mesophyll resistance, and then cuticular or stomatal resistance. In the proximity of the stomata there is a build-up of moisture in the air, the boundary layer, which represents another resistance for water to overcome (Nobel 2020).

An increased leaf-to-air VPD increases E (Pantin and Blatt 2018). When water loss from E exceeds water supply from the roots, the plant loses turgor, causing the stomata to close (Urry et al. 2019). Stomata are small pores on surfaces of leaves and stems. They are flanked by guard cells, which control the gas exchange between the plant and the atmosphere. Gas exchange is regulated by controlling the aperture of the stomatal pore and the number of stomata on the epidermis. Stomata are a major contributor to the plant's ability to balance its water loss with carbon gains (Hetherington and Woodward 2003). Even though stomata only make up one to two percent of leaf area, they account for about 95 percent of E (Urry et al. 2019).

When the guard cells take in water from surrounding cells via osmosis, they become more turgid. In most angiosperms, guard cells have a thickened cell wall at the stomatal pore, which is further reinforced by microfibrils (Fig. 3a). When the guard cells become turgid, the part of the cell wall bordering the neighbouring cells expands further than the reinforced cell wall bordering the stomatal pore, causing the guard cell to bend and the stomatal pore to open (Urry et al. 2019).

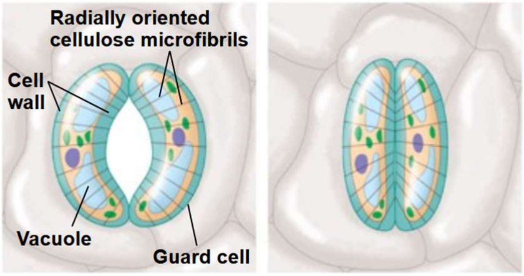


Figure 3a: Stoma in turgid (left) and flaccid (right) state. The reinforced cell wall at the stomatal pore and the radially oriented microfibrils cause the stoma to open when turgid (Urry et al. 2019).

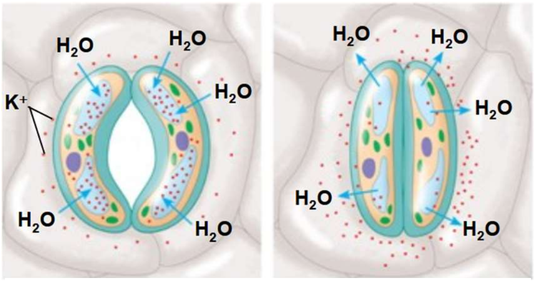


Figure 3b: Stoma in turgid (left) and flaccid (right) state. Absorbing or discharging potassium ions (red dots) influences turgor and opens or closes the stoma (Urry et al. 2019).

It is important to understand how stomata work because they play an important role in regulating E. Stomatal regulation happens in two ways. First, a passive process, in which stomatal conductance (g_s) is controlled by changing guard cell turgor, due to evaporative demand (Merilo et al. 2018). Secondly, by an active process where plants can change guard cell turgor independently from whole plant turgor, mainly by absorbing or discharging potassium ions (K^+) from/to neighbouring cells. The K^+ movement follows a membrane potential actively built up by a proton pump. Taking in K^+ lowers water potential in the guard cells and causes water to move into the guard cells from the neighbouring cells, by osmosis (Fig. 3b). Active stomatal closure is also influenced by root water potential. Low root water potential induces abscisic acid (ABA) synthetisation. ABA acts as a stress hormone, which is transported from the root to the leaf through the xylem and influences the proton pumps of guard cell membranes (Urry et al. 2019).

Aquaporins also play a role in controlling hydraulic conductance generally and g_s specifically. They are water channel proteins and substantially increase membrane water permeability (Medina et al. 2018; Verkman 2013). Aquaporins, and with them whole plant E, may be influenced by various environmental factors. It has been shown in several species e.g. *Juglans spp.* (Cochard et al. 2007), *Z. mays* (Ionenko et al. 2010), and *Oryza sativa* (Murai-Hatano et al. 2008) that their phosphorylation and gene expression are influenced by T.

While lowering g_s prevents excess water loss, it also leads to decreasing leaf carbon dioxide (CO_2) concentrations (Urry et al. 2019). This decreases the rate of photosynthesis (PS), because PS utilises light, water and CO_2 to synthesise carbohydrates (Jauregui et al. 2018). The loss of evaporative cooling exacerbates this effect, as high T denatures enzymes necessary for PS. Since cell enlargement depends on water, plant growth is also reduced (Urry et al. 2019).

If water conserved early in the cropping season was available at critical developmental phases, like grain filling, limiting E once VPD reaches a certain level may benefit crop yield. The exhibition of this trait has been simulated to increase yield for Australian grown *Sorghum spp.* and US grown *Glycine max* in 75% and 70-80%, respectively, of growing seasons (Sinclair et al. 2005; Sinclair et al. 2010). Genotypes exhibiting this trait, as well as intra-specific variation, have been identified in various crop species and it has been suggested that there is more than one genetic source for this trait (Choudhary et al. 2013; Sadok and Sinclair 2009b).

VPD effects on plant responses are difficult to isolate from other climate drivers on plant function like T and radiation. These usually occur alongside high VPD in the form of heatwaves and drought and have been thought to be responsible for the main effect on plant physiology (Grossiord et al. 2020). This may explain why understanding the independent physiological effect of VPD on vegetation dynamics has not received as much attention as plant responses to high T, reduced precipitation and rising atmospheric CO_2 concentrations.

The relative role of VPD as a stressor responsible for limiting crop yield may be much higher than previously thought (Grossiord et al. 2020). Fluctuations in environmental factors are difficult to eliminate in the field, but it is possible to control them in whole plant gas exchange systems (Jauregui et al. 2018). These closed environmental chambers are used to create a stable environment, in which individual factors, like relative humidity (RH), T or irradiance can be controlled and thus their effects isolated (Jauregui et al. 2018).

Most plants fix carbon by affixing CO_2 to ribulose 1,5-bisphosphate, using ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) as a catalyst in the calvin-benson-cycle (Urry et al. 2019). The first stable product of this reaction are two three-carbon 3-phosphoglycerate molecules, leading to the name C3. When plants close their stomata, the decreasing CO_2 concentrations lead to increasing photorespiration, which means that RuBisCO binds oxygen (O_2) instead of CO_2. One of the resulting products, 2-phosphoglycolate, is toxic and needs to be metabolised, leading to a net loss in energy (Urry et al. 2019).

Some plants have adapted to use phosphoenolpyruvate carboxylase (PEP carboxylase) to pre-fix CO_2 before it is metabolised by RuBisCO in the bundle-sheath cells. It binds CO_2 with phosphoenolpyruvate to create the four-carbon oxaloacetate, hence the name C4 (Urry et al. 2019). PEP carboxylase has a higher binding affinity to CO_2 than RuBisCO and does not bind O_2. This allows C4-plants to fix carbon more efficiently than C3-plants under low CO_2 and high O_2 concentrations, which usually occur when stomata are closed (Urry et al. 2019).

Stomata regulate their conductance to match CO_2 entry with the photosynthetic capability of the mesophyll (Nobel 2020). C4-plants generally have lower mesophyll CO_2 concentrations than C3-plants because CO_2 saturation regarding PS happens at a lower concentration. Keeping g_s higher than necessary to achieve the CO_2 saturation point would not benefit C4-plants with a higher rate of PS, but lead to higher E. Therefore, C4-plants are able to keep their optimal rate of PS at lower g_s, compared to C3-plants, and are likely to do so at a lower rate of E (Nobel 2020).

Oren et al. 1999 define stomatal sensitivity as the absolute reduction in g_s with increasing VPD. The absolute change in g_s is greater in plants with a high g_s at a VPD of 1.0 kPa (Ocheltree et al. 2014). There have been contrary reports on whether C4- and C3-plants differ in their stomatal sensitivity. Some report a lower sensitivity for C4-plants (Maherali et al. 2003), while others suggest similar behaviour (Oren et al. 1999; Ocheltree et al. 2014).

The aim of this review is to provide an overview of studies on E response to VP in controlled environments, of species' E response to VPD in a controlled environment and to examine whether C3- and C4-plants differ in their E response to VPD.

It is hypothesised that C4-plants are more likely to restrict E in response to increasing VPD and have a lower stomatal sensitivity, compared to C3-plants.

2 MATERIAL AND METHODS

2.1 LITERATURE REVIEW

The initial search for relevant literature was conducted using the Scopus (Elsevier, The Netherlands) database and Google Scholar (Google LLC, USA), searching for the tags “Chamber”, “Controlled”, “Transpiration”, “VPD” and “Vapour pressure deficit”. Literature fitting the criteria for this thesis (Controlled environment, E response to VPD) were checked for relevant citations both in text and bibliography to find more studies.

2.2 STATISTICAL ANALYSIS

18 of 29 relevant studies found reported their results as a linear or bilinear regression slope, with a breakpoint (BP) used to indicate a restriction in E. These studies were chosen for comparison, to provide as comprehensive an overview as possible. If necessary, data was converted to the unit most used (mg H_2O m^{-2} s^{-1}) using the conversion factors in table one.

Table 1: Conversion factors used in this study. Adapted from Nobel 2020.

Unit	mg H_2O m^{-2} s^{-1}
g H_2O cm^{-2} h^{-1}	2780
kg H_2O m^{-2} day^{-1}	$23\,{}^{1}/_{6}$

The statistical analysis was conducted using the software SAS 9.4 (SAS Institute Inc., USA). Testing the factors was done in a network meta-analysis while the likelihood of E restriction was analysed by a chi-squared test. Network meta-analysis allows the testing of whether a factor has a significant effect in a model and can be suitable for comparing trials with different designs (Piepho 2014).

The model for the network meta-analysis was:

$$y_{ijkt} = \mu_t + \alpha_{it} + \beta_{ijt} + \gamma_{ijkt} + e_{ijkt}$$

With:

y_{ijkt} = The value of the t-th trait (linear slope, bilinear slope below and above breakpoint, breakpoint value) for i-th PS pathway with the j-th species and the k-th genotype.

μ_t = The mean for the t-th trait.

α_{it} = The effect of the i-th PS pathway for the t-th trait.

β_{ijt} = The effect of the j-th species with the i-th PS pathway for the t-th trait.

γ_{ijkt} = The effect of the k-th genotype with the j-th species and i-th PS pathway for the t-th trait.

e_{ijkt} = The error of the t-th trait for i-th PS pathway with the j-th species and the k-th genotype.

As genotypes are limited to a species, which themselves are limited to one PS pathway, the effects are “nestled” into each other.

The analysis of the traits was weighted by their inverse variance to take into account the precision of the effect estimates of the studies (Marín-Martínez and Sánchez-Meca 2010). Where no variance data was provided, the data was assigned a calculated species variance.

Due to the nature of the network meta-analysis, e.g. uneven replication and different standard error of means, there are different standard errors of difference for pairwise comparisons. This may lead to one difference being significant while another, larger, difference is not.

The chi-squared analysis was conducted using C3/C4 and BP/No BP as qualitative characteristics using the studies which had tested BP for significance. After accounting for studies not testing significance, the number of observations was still >200 allowing the use of the chi-squared, rather than Fisher’s exact test.

For both tests the α-error was set at 5%, therefore a p-value < 0.05 is deemed significant.

3 RESULTS

3.1 OVERVIEW

Studies on E response to VPD under controlled environments have been done as early as 1973 (Tab. 2). However, most of the studies found were conducted after Sinclair et al. 2005 simulated an increased crop yield for *Sorghum spp.* in Australia for 75% of cropping seasons for genotypes expressing E limitation in response to high VPD and many have been performed by his working group.

Table 2: An overview of studies examining transpiration response to water vapour pressure deficit under controlled environments.

Lead author	Species
Beardsell et al. 1973	*Glycine max*
Kawamitsu et al. 1993	*Oryza sativa, Panicum maximum*
Thomas et al. 1998	*Musa sp.*
Clifton-Brown and Jones 1999	*Miscanthus giganteus*
Leuschner 2002	*Brachypodium sylvaticum, Campanula trachelium, Digitalis purpurea, Geum urbanum, Hieracium sylvaticum, Hordelymus europaeus, Rumex sanguineus and Scrophularia nodosa*
Fletcher et al. 2007	*Glycine max*
Sinclair et al. 2008	*Glycine max*
Shibuya et al. 2009	*Cucumis sativus*
Sadok and Sinclair 2009a	*Glycine max*
Sadok and Sinclair 2009b	*Glycine max*
Kholova et al. 2010	*Pennietum glaucum*
Gholipoor et al. 2010	*Sorghum bicolor*
Devi and Sinclair 2011	*Arachis hypogaea*
Zaman-Allah et al. 2011	*Cicer arietinum*
Yang et al. 2011	*Zea mays*
Schoppach and Sadok 2012	*Triticum aestivum*
Will et al. 2013	*Celtis occidentalis, Cercis canadensis, Juniperus virginiana, Morus rurbra, Platanus occidentalis, Prunus americana, Quercua macrocarpa, Quercus stellata, Quercus shumardii*
Choudhary et al. 2013	*Sorghum bicolor*
Ben-Asher et al. 2013	*Zea mays*
Gholipoor et al. 2013	*Zea mays*
Ocheltree et al. 2014	*Agrostis hyemalis, Andropogon gerardii, Bouteloua dactyloides, Bromus inermis, Chloris verticillata, Dactylis glomerata, Dichanthelium oligosanthes, Digitaria californica, Festuca sucverticillata, Hordeum pusillum, Koeleria macrantha, Panicum virgatum, Pascopyrum smithii, Poa pratensis, Setaria pumila, Spartina pectinata, Sporobolus cryptandrus, Sporobolus heteroplepis and Tripsacum dactyloides*
Shekoofa et al. 2014	*Glycine max*
Rashed 2016	*Panicum maximum*
Ryan et al. 2016	*Zea mays*
Devi and Reddy 2018	*Gossypium spp.*
Jauregui et al. 2018	*Hordeum vulgare, Triticum aestivum*
Karthika et al. 2019	*Sorghum bicolor*
Medina et al. 2019	*Triticum turgidum*
Choudhary et al. 2020	*Pennietum glaucum, Sorghum bicolor, Zea mays*

3.1.1 Gas exchange systems

The gas exchange systems used in the literature examined in this review can be grouped by their way of measuring E. Of the 29 studies reviewed, 22 used weight loss to determine E and seven an infrared gas analyser (IRGA). Only two studies conducted their measurements on single leaves, the remaining 27 used whole plant gas exchange systems.

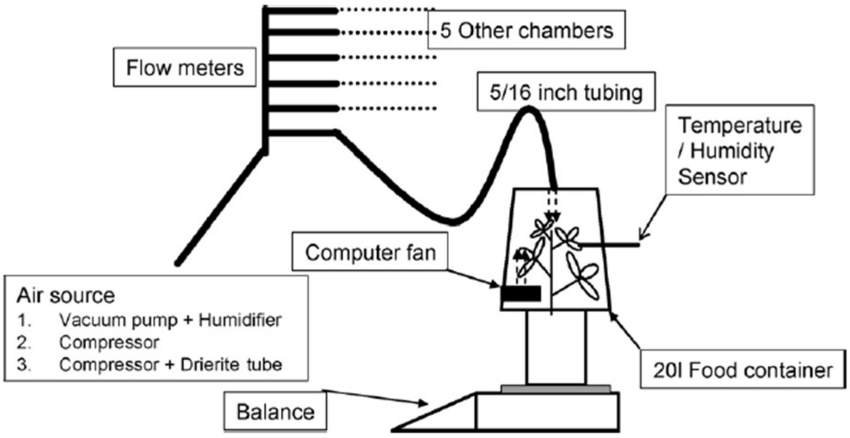


Figure 4: A diagram illustrating a gravimetric whole plant gas exchange system. Weight is registered at set intervals and the difference attributed to transpiration (Fletcher et al. 2007).

In the weight loss system, a balance is used to determine weight at set intervals and the difference attributed to E. This system can be a single plant chamber, as in the example by Fletcher et al. 2007 (Fig. 4), where the whole chamber is located on the balance or a multi-plant chamber with the balances inside. RH is usually controlled by changing the composition of incoming humidified or dehumidified air streams. Inside the chamber a fan mixes the air to disturb the boundary layer and sensors for T and humidity, which are used to calculate VPD.

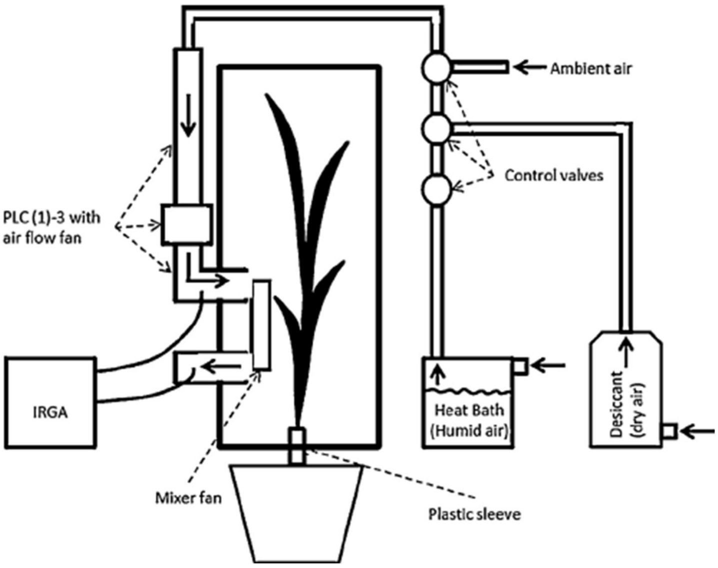


Figure 5: A diagram illustrating a whole plant gas exchange chamber using an infrared gas analyser (IRGA) to measure the concentration difference between ingoing and outgoing air (Ryan et al. 2016).

Gas exchange systems using an IRGA to determine E function very similar to weight loss systems, but instead measure gas concentrations in the incoming and outgoing air (Fig. 5). This has the advantage of being able to measure other factors as well, like net assimilation by the

CO_2 difference. Not using a balance also eliminates false readings due to plant movement from the fan. This chamber type is limited to one observation per chamber i.e. to obtain individual data for each plant, one chamber per plant is needed.

3.1.2 Data models

The form of the data in which the results were reported varied. Beardsell et al. 1973, Leuschner 2002, Ben-Asher et al. 2013 and Will et al. 2013 reported it as the value of E for a specific VPD-value. Clifton-Brown and Jones 1999 on the other hand provided the difference of E between two values for VPD. The remaining studies used a regression to demonstrate E response to VPD. While Kawamitsu et al. 1993, Thomas et al. 1998 and Ocheltree et al. 2014 used a quadratic model, Rashed 2016 used both linear and quadratic models and the other authors only linear models.

To represent whether a genotype reacted to a changing VPD, 19 of the 20 authors using a linear model grouped genotypes in those represented by a single-linear model and a bilinear model. Ryan et al. 2016 used the following method to decide which model fitted a genotype. A single-linear regression was fitted through the replicate data of E versus VPD and residual calculated. Then the VPD-value at which the maximum residual E occurred was calculated by plotting a third order polynomial through the residuals and finding the maximum of the function. Ryan et al. 2016 calls the maximum the change point, whereas most of the other authors (e.g. Fletcher et al. 2007, Sadok and Sinclair 2009a, Gholipoor et al. 2010) call it a BP. Data below and above the BP each has a single-linear regression fitted through it and if these are found to be significantly different, a bilinear model is used with slopes below and above the BP, otherwise a single-linear model is used.

3.1.3 Measurement temperature

Of the 29 studies examined in this paper, 17 kept T at a fixed level, six had a variable T, one had experiments with both and the remaining five did not provide information regarding measurement T. Most studies kept their measurement T around the 30 °C level (Fig. 6). The notable exception, with four experiments at or below 20 °C is Clifton-Brown and Jones 1999. An interesting result reported by Devi and Sinclair 2011, for two experiments examining *Arachis hypogaea* genotypes with a higher (28-36 °C) and lower (22-28 °C) T range, is that measurement T seems to impact the likelihood of a genotype expressing a breakpoint. While eight of nine genotypes expressed a BP in their high T experiment, none did so in their low T experiment. Yang et al. 2012 also reported an effect of measurement T on E response to VPD. In their experiment E response to VPD in *Z. mays* genotypes was measured at a stable 30 °C and 25 °C over a range of VPD. Measurement T only had a small effect on the slope of the response, but the plants measured at 30 °C expressed their BP at a significantly higher VPD.

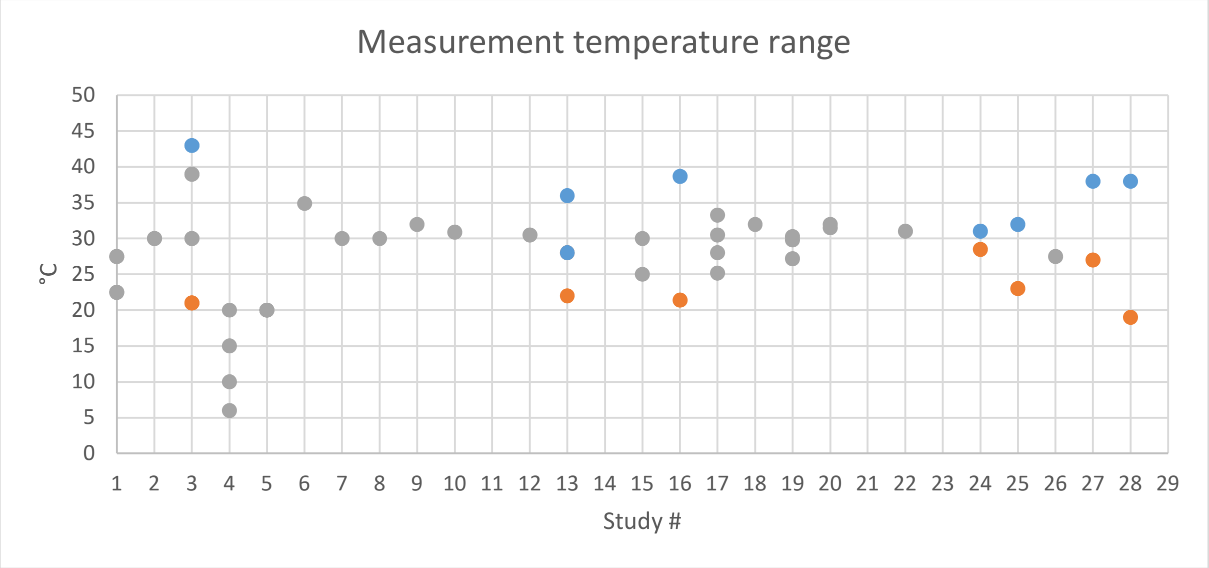


Figure 6: The range of measurement temperature (T) in °C. Gray dots indicate a stable T, while blue and orange dots indicate the maximum and minimum, respectively, of a range. Studies with missing data for these values were included to illustrate this problem in comparing different studies.

3.1.4 Measurement VPD

Only five studies measured E response to VPD at fixed VPD, 20 measured over a range of VPD, one did both and three did not report the VPD of their measurements. Most studies conducted their measurements in a range of about 0.7 kPa and 3.8 kPa (Fig. 7).

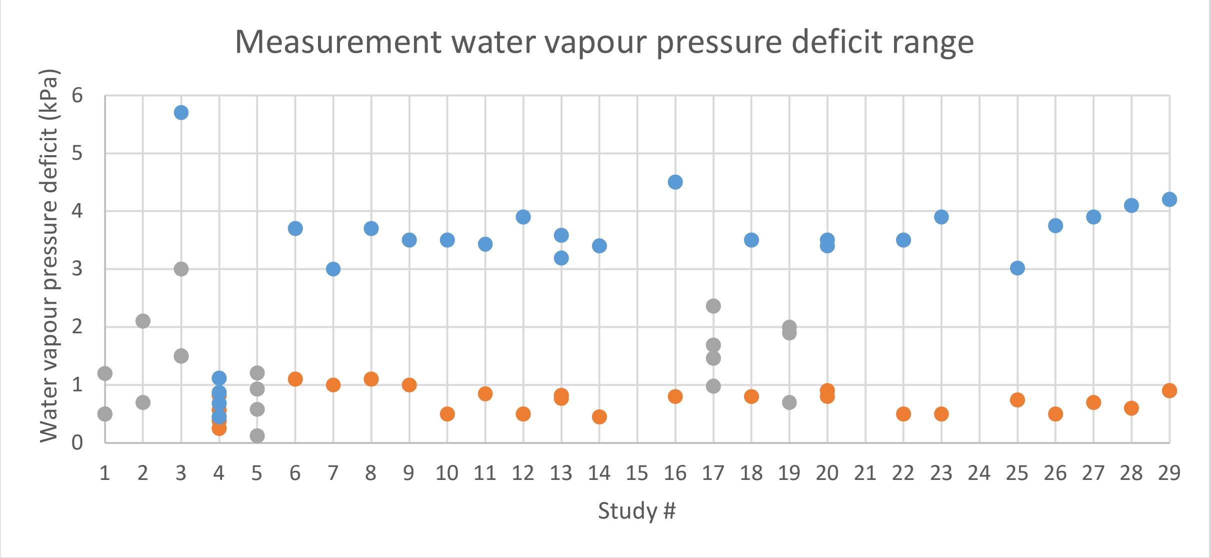


Figure 7: The range of measurement water vapour pressure deficit (VPD). Gray dots indicate a stable VPD, while blue and orange dots indicate the maximum and minimum, respectively, of a range. Studies with missing data for these values were included to illustrate this problem in comparing different studies.

3.1.5 Measurement relative humidity

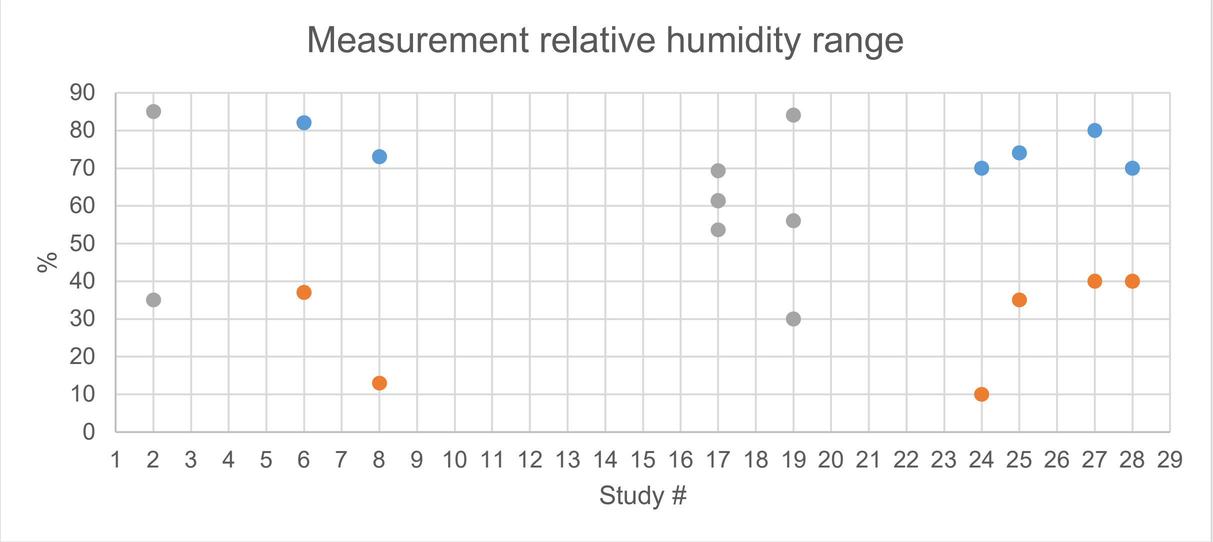


Figure 8: The range of measurement relative humidity (RH). Gray dots indicate a stable RH, while blue and orange dots indicate the maximum and minimum, respectively, of a range. Studies with missing data for these values were included to illustrate this problem in comparing different studies.

Information on measurement RH is sparse with 20 studies failing to provide any data. Of the nine authors providing data, three kept it stable, while six adjusted it. The reported data ranges from about 40% to 75%, with some as low as 10% (Fig. 8). Presumably, most of the authors not providing data adjusted it to achieve a VPD range, as many reported a fixed T. It is not clear why so many studies fail to provide data for this crucial parameter, as it needs to be recorded to calculate VPD. The data from Ben-Asher et al. 2013 suggests that RH might influence E independently from VPD. In their comparison of E response to VPD in one *Z. mays* genotype at different RH-levels they reported a difference of 11.58 mg H_2O m^{-2} s^{-1} in E between their 84% and 56% RH levels, with 30.3 °C and 29.8 °C and a VPD of 0.7 kPa and 1.9 kPa respectively. In their 30% RH treatment, air T was only 27.7 °C, with a VPD of 2 kPa, yet the difference of E between the 30% and the 56% treatments was 57.92 mg H_2O m^{-2} s^{-1}.

3.2 SPECIES

3.2.1 Slopes of E response to VPD

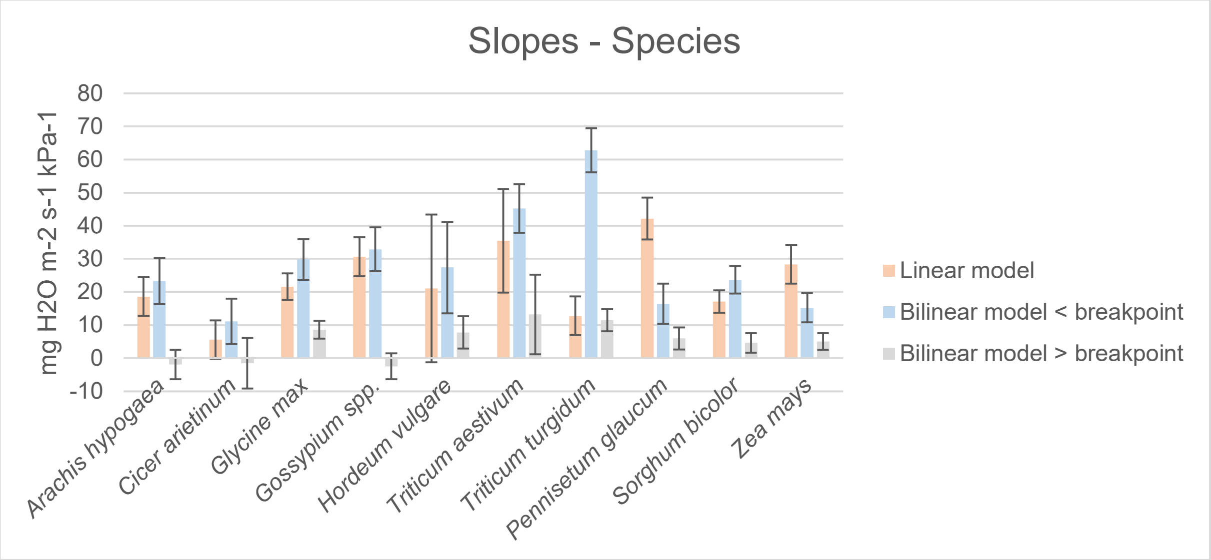


Figure 9: Estimated slopes for transpiration response to water vapour pressure deficit for different species in linear and bilinear models.

There is a large variation in species' E response to VPD (Fig. 9). Slopes for the linear model range from 5.62 ± 5.75 mg H_2O m^{-2} s^{-1} kPa^{-1} in *Cicer arietinum* to 42.16 ± 6.31 mg H_2O m^{-2} s^{-1} kPa^{-1} in *Pennisetum glaucum*. In the bilinear model the smallest slope below the BP was 11.32 ± 6.88 mg H_2O m^{-2} s^{-1} kPa^{-1} in *C. arietinum* and the biggest 62.78 ± 6.65 mg H_2O m^{-2} s^{-1} kPa^{-1} in *Triticum turgidum*, and the slope above the BP ranged from -2.45 ± 3.90 mg H_2O m^{-2} s^{-1} kPa^{-1} for *Gossypium spp.* up to 13.18 ± 12.01 mg H_2O m^{-2} s^{-1} kPa^{-1} in *Triticum aestivum*. Only three paired tests found significantly different slopes and only for the slope below the BP in the bilinear model (Tab. 3). The variation within species, i.e. between genotypes, was also very large, as illustrated by the error bars in figure nine, however neither species nor genotype was deemed a significant factor by the analysis.

Table 3: Species pairs with a significant difference for the slope of transpiration response to water vapour pressure deficit below the breakpoint in a bilinear model.

Significant difference pairs - slope < breakpoint		p-Value
Hordeum vulgare	*Triticum turgidum*	0.0465
Triticum turgidum	*Pennisetum glaucum*	0.0310
Triticum aestivum	*Pennisetum glaucum*	0.0460

Most species have a slightly lower slope in the linear model than the slope below the BP in the bilinear model with the mean for this difference being -5.92 mg H_2O m^{-2} s^{-1} kPa^{-1}. There are three species with a different behaviour. For *P. glaucum* and *Z. mays* the slope for the linear model is steeper than the slope below the BP in the bilinear model. While the difference between genotypes expressing a BP and not expressing a BP could lead to this result, it is likely to be an inaccuracy caused by the different methods used in the studies examined and is discussed in chapter 4. In *T. turgidum* the linear slope is 50 mg H_2O m^{-2} s^{-1} kPa^{-1} smaller than the first slope in the bilinear model. While the difference in the closely related *T. aestivum* is also higher than the median, with 9.73 mg H_2O m^{-2} s^{-1} kPa^{-1} it is far smaller. Further research is needed to understand the physiological reasons for this differing behaviour.

The significant differences in the slope below the BP between *P. glaucum* and *T. aestivum* ($p = 0.0460$) and *T. turgidum* ($p = 0.0310$) respectively could be explained by their different PS pathways. While not significantly different from either *T. aestivum* or *T. turgidum*, the other C4-species, *Sorghum bicolor* and *Z. mays*, have similar values for this slope as *P. glaucum*. However, this factor was not found to be significant by the analysis ($p = 0.0912$).

3.2.2 BP of E response to VPD

The median frequency of BP expression was 49.47%, but there was large interspecific variation (Fig. 10). Only 25% of *C. arietinum* genotypes were found to have a BP while in *P. glaucum* 75% of genotypes had a BP. Other species with a large difference from the median include *G. max* with 34.29% and *T. turgidum* with 70%. Interestingly, only monocotyledons were above the median and the dicotyledons were all below the median. *S. bicolor*, as the only monocotyledon below the median was only 0.53% below it, while the dicotyledons were 2.41%-24.47% below the median. This could be due to their differing stomatal anatomy and would be an interesting area for further research.

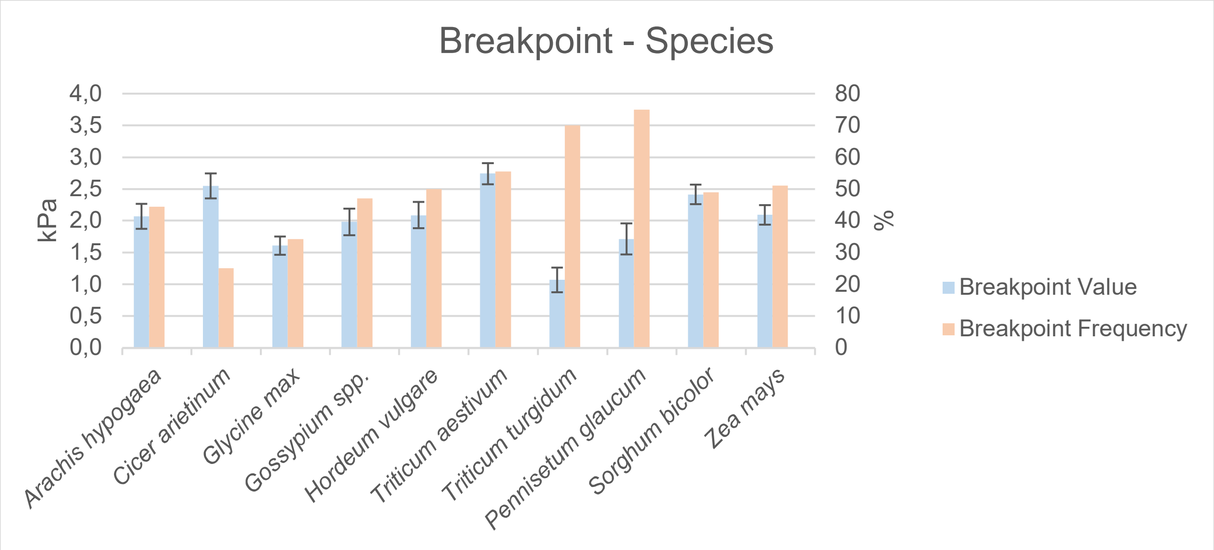


Figure 10: Estimated breakpoint (BP) and BP frequency in the transpiration response to water vapour pressure deficit for different species in bilinear models.

The lowest BP value is with 1.07 ± 0.19 kPa, in *T. turgidum* almost half of the median value of 2.08 kPa, while *T. aestivum* has the highest with 2.74 ± 0.17 kPa. That the two species most closely related to each other have the largest difference ($p = 0.0128$) suggests that BP value is less affected by species, but rather by genotype. The results of the statistical analysis indicate the same. Several species were found to have significant differences in their BP value (Tab. 4) in a pairwise comparison, yet the factor species was not deemed significant ($p = 0.5600$) while the factor genotype was ($p = 0.0233$).

Table 4: Species pairs with a significant difference for breakpoint value for the transpiration response to water vapour pressure deficit in a bilinear model.

Significant difference pairs - breakpoint value		p-Value
Triticum turgidum	*Zea mays*	0.0397
Triticum turgidum	*Sorghum bicolor*	0.0257
Triticum aestivum	*Pennisetum glaucum*	0.0210
Triticum aestivum	*Triticum turgidum*	0.0128
Hordeum vulgare	*Triticum turgidum*	0.0258
Hordeum vulgare	*Triticum aestivum*	0.0050
Glycine max	*Sorghum bicolor*	0.0296
Glycine max	*Triticum aestivum*	0.0084
Cicer arietinum	*Glycine max*	0.0402

3.3 PHOTOSYNTHESIS PATHWAY

3.3.1 Slopes of E response to VPD

The slope for the linear model was almost 50% steeper in C4-plants with 20.82 ± 3.08 mg H_2O m^{-2} s^{-1} kPa^{-1} than in C3-plants with 29.21 ± 3.06 mg H_2O m^{-2} s^{-1} kPa^{-1} (Fig. 11), but this difference was not statistically significant ($p = 0.3038$). For C4-plants this slope is 7.88 mg H_2O m^{-2} s^{-1} kPa^{-1} above the median value and for C3-plants it is 0.51 mg H_2O m^{-2} s^{-1} kPa^{-1} below. While it is possible that C4-plants express more extreme differences in their E response to VPD, it is likely that the slope for the C4 linear model contained the same inaccuracy as the linear model slopes for *P. glaucum* and *Z. mays*, which was mentioned in chapter 3.2.1 and discussed in chapter 4.

For the slope below the BP in the bilinear model, C3-plants transpired 33.21 ± 3.06 mg H_2O m^{-2} s^{-1} kPa^{-1} and C4-plants 18.43 ± 4.02 mg H_2O m^{-2} s^{-1} kPa^{-1}. This difference of 14.78 mg H_2O m^{-2} s^{-1} kPa^{-1} was evenly distributed around the median value of 25.54 mg H_2O m^{-2} s^{-1} kPa^{-1}. While not statistically significant ($p = 0.0936$), this difference could be explained by the physiological differences in CO_2 saturation due to which C4-plants can maintain their optimal rate of PS at a lower g_s than C3-plants (Nobel 2020), concurs with reported lower stomatal sensitivity for C4-plants (Maherali et al. 2003) and would be in line with the hypothesis that C4-plants have lower stomatal sensitivity compared to C3-plants.

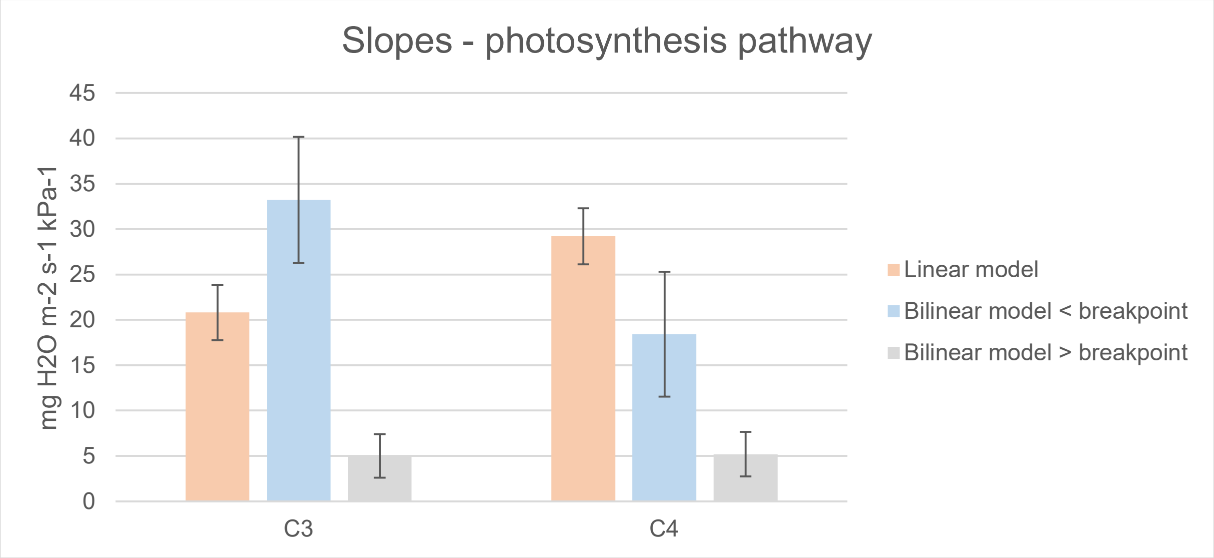
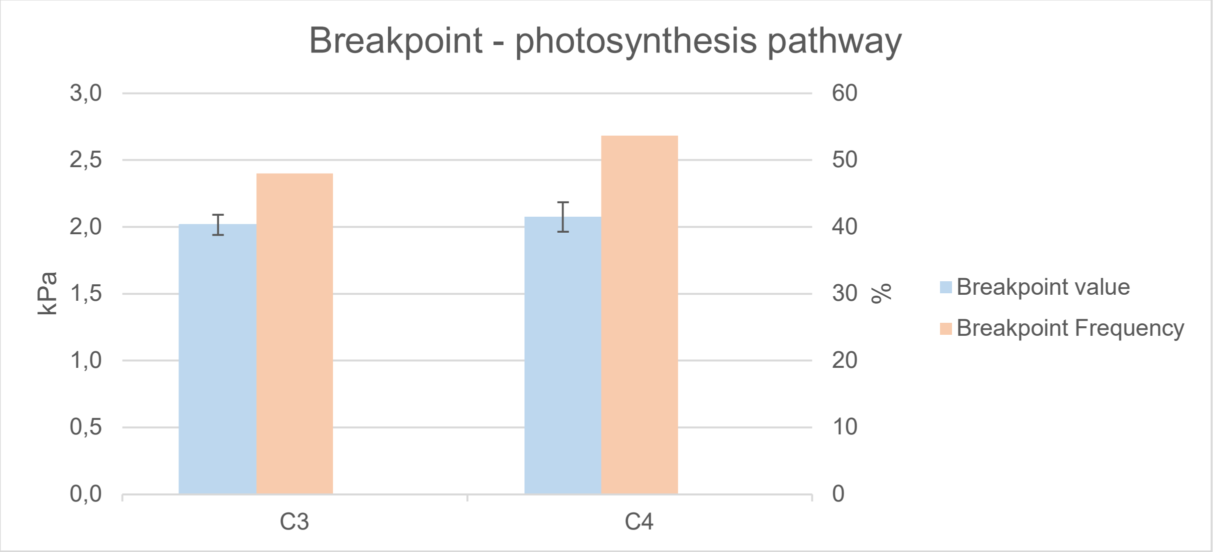


Figure 11: Estimated slopes for transpiration response to water vapour pressure deficit for different photosynthesis pathways in linear and bilinear models.

Above the BP, the slopes were very similar with 5.03 ± 2.40 mg H_2O m^{-2} s^{-1} kPa^{-1} for C3-plants and 5.20 ± 2.46 mg H_2O m^{-2} s^{-1} kPa^{-1} for C4-plants (p = 0.9620), which could be explained by plants regulating g_s for E above the BP, rather than for CO_2 concentration. It has been reported that C4-species' advantage in PS diminishes under drought due to converging g_s (Taylor et al. 2011), lending some support to this explanation.

3.3.2 BP of E response to VPD

With 53.66% the frequency of BP expression in C4-plants was higher than the 49.47% median and C3-plants with 48% (Fig. 12). This finding would support the hypothesis that C4-plants are



for different photosynthesis (PS) pathways in bilinear models.

more likely to restrict E in response to VPD than C3-plant and could be explained by C4-plants' physiology, which allows them to have a higher rate of PS under low CO_2 concentrations (Nobel 2020). However, no significant relationship for BP frequency and PS pathway was found by a chi-squared test (p = 0.4038). A previous report comparing *S. bicolor*, *P. glaucum* and *Z. mays* supports this independence by suggesting that drought tolerance strategy within these C4-species differs (Choudhary et al. 2020).

For the BP value, there is only a 0.05 kPa difference with C4-plants restricting E at 2.07 ± 0.11 kPa and C3-plants at 2.02 ± 0.07 kPa. This difference (p = 0.6823) and the factor PS pathway (p = 0.3852) were not found to be significant, supporting the indication in chapter 3.2.2 that of the factors examined in this thesis, genotype seems to determine BP-value.

4 DISCUSSION

4.1 METHODS

Conducting a meta-analysis and using data from different studies, with different hypothesises, replications, number of observations, and environments inevitably leads to inaccuracy. This is represented in this thesis by large standard errors of mean and high p-values. There is also a large variation in reported environmental data or accuracy. Only few studies reported their plants' developmental stage or date after sowing, some did not even indicate their data's accuracy by reporting their standard error of mean. While this makes it difficult to draw conclusions from the data reviewed in this thesis, it is possible to identify some possible connections.

Of the 18 studies analysed statistically, nine include T. R. Sinclair as an author. While this provides a certain comparability, due to a similar set-up and environment, it could also lead to a certain bias, where the same methodical errors are repeated and overrepresented. It could be interesting to do a similar analysis if/after more working groups have published data on this topic.

Most chambers are relatively small and constrained to measuring plants at early developmental stages. It would be interesting to see if/how plants' E response to VPD changes with different developmental stages and if these results match those found in the field. Another interesting question is whether the response of single plants is comparable to those of a plant community, with a canopy layer. It has been reported that in *Cucumis sativus* seedling communities, the negative effect of high VPD on PS and g_s was mitigated by a higher planting density, because of the development of a thicker canopy layer (Shibuya et al. 2009). It would not be surprising if this effect were replicated in other species and lead to a different E response to VPD in the field compared to a single plant gas exchange chamber.

Several of the studies reviewed pre-screened their genotypes for BP expression, e.g. by selecting for high canopy T, hypothesised to result from lower E (Sadok and Sinclair 2009a), by choosing genotypes staying green for longer in drought conditions (Choudhary et al. 2013) or by measuring E in field conditions (Shekoofa et al. 2014). Therefore, the frequencies of BP expression shown in this thesis are unlikely to have represented species' true expression. The possibility of using canopy T as an indicator for E restriction is intriguing. While the reported success of using canopy T for pre-screening is inconclusive and it has been suggested that there is at least one other factor responsible for higher canopy T (Sadok and Sinclair 2009b; Zaman-Allah et al. 2011), examining a possible correlation between canopy T and restricting E in response to VPD may be interesting.

4.2 SPECIES

P. glaucum and *Z. mays* have a linear slope which is steeper than the slope below the BP for the bilinear model. This is likely to be an error due to comparing different studies with different methods. Due to physiological restrictions E will not become infinitely greater and is better described by a quadratic function, i.e. an open bottomed parable, than a linear one. In the literature examined this was simplified by fitting either a linear or a bilinear model over E. Doing this for a single parable, a single-linear model will always be less steep than the first curve of a bilinear model and steeper than the second curve of the bilinear model. The regression curves were reported for different genotypes, so it is theoretically possible, that all genotypes that do not express a BP have a much higher rate of E than those who do. It is, however, very unlikely. While the standard error of mean is large for most species, it is especially large for *Hordeum vulgare* as only two genotypes were examined, one with a linear and one with a bilinear model. While the data used is a mean consisting of several observations, it is very difficult to draw any conclusions from this data. It is unlikely to significantly have influenced the result of the PS pathway comparison, because of the weighting and was included for a general indication how *H. vulgare* E might respond to VPD.

4.3 PHOTOSYNTHESIS PATHWAY

Not all studies using a bilinear model fitted an individual BP, in two studies (Choudhary et al. 2020; Sinclair et al. 2008) a bilinear model was used and regression slopes fitted below and above a set VPD for all genotypes. As these were not tested for significance in the difference between the two slopes, the data from these studies was not used for the chi-squared test for frequency of BP expression. Using this data to compare slopes may have influenced the comparison, as described in chapter 4.2.

The treatments applied in the studies may also have affected the data for the comparisons. For most studies, genotype was the only treatment used, but seven studies applied additional ones. Treatments included different measurement dates (Gholipoor et al. 2013; Karthika et al. 2019; Kholová et al. 2010), growth T (Devi and Sinclair 2011; Yang et al. 2012), measurement T (Yang et al. 2012), pressurised rhizosphere (Sinclair et al. 2008) and growth VPD (Choudhary et al. 2020). Some of these treatments led to significant differences in BP expression and regression slope (Choudhary et al. 2020; Devi and Sinclair 2011; Sinclair et al. 2008; Yang et al. 2012) and may have influenced the results of this thesis. The treatments of growth T and VPD seem to have led to plant acclimatisation and would be an interesting area for further research.

In the data used in this comparison, only three C4-species were included, but seven C3-species, which may have led to a certain inaccuracy. This might have led to the factor PS pathway not being found to be significant in any of the traits. However, the fact that practically all studies reviewed in this thesis found inter- and/or intraspecific variability, suggests that PS pathway is not a relevant factor for these traits.

5 CONCLUSION

Studies on E response to VPD are conducted in different set-ups, including different environmental conditions, gas exchange equipment and analysis methods, and therefore not all experiments are comparable. Authors publishing literature on E response to VPD could help comparability by reporting their environmental conditions and data accuracy in more detail. There was a large variation in intra- and interspecific response, though this thesis found few significant differences. The behaviour of species generally followed the same pattern, with the regression slope for the linear model found in between the two slopes in the bilinear model. The exceptions to this were *P. glaucum* and *Z. mays*, where the slope in the linear model was the steepest slope. The biggest difference between the linear and bilinear model was found in *T. turgidum*, which also restricted E at the lowest VPD and was most likely to express this restriction. Genotypes restricting E in response to increasing VPD were reported in all species and seems to be independent of PS pathway, but dicotyledons were less likely to express this trait. The VPD at which a BP occurs seems to be determined by genotype, rather than species and is practically the same for C3- and C4-species. To facilitate the use of this trait in drought tolerance breeding more research regarding the economic benefits and in situ-response, as well as the development or identification of phenotypic markers is necessary.

6 PUBLICATION BIBLIOGRAPHY

Allen, C. D.; Macalady, A. K.; Chenchouni, H.; Bachelet, D.; McDowell, N.; Vennetier, M. et al. (2010): A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. In *Forest Ecology and Management* 259 (4), pp. 660–684. DOI: 10.1016/j.foreco.2009.09.001.

Anderson, D. B. (1936): Relative Humidity or Vapor Pressure Deficit. In *Ecology* 17 (2), pp. 277–282. DOI: 10.2307/1931468.

Beardsell, M. F.; Mitchell, K. J.; Thomas, R. G. (1973): Transpiration and Photosynthesis in Soybean: EFFECTS OF TEMPERATURE AND VAPOUR PRESSURE DEFICIT. In *Journal of Experimental Botany* 24 (80), pp. 587–595. Available online at www.jstor.org/stable/23687398.

Ben-Asher, J.; Garcia y Garcia, A.; Flitcroft, I.; Hoogenboom G. (2013): Effect of atmospheric water vapor on photosynthesis, transpiration and canopy conductance: A case study in corn. In *Plant Soil Environ.* Vol. 59 (No. 12), pp. 549–555.

Choudhary, S.; Guha, A.; Kholova, J.; Pandravada, A.; Messina, C. D.; Cooper, M.; Vadez, V. (2020): Maize, sorghum, and pearl millet have highly contrasting species strategies to adapt to water stress and climate change-like conditions. In *Plant science : an international journal of experimental plant biology* 295, p. 110297. DOI: 10.1016/j.plantsci.2019.110297.

Choudhary, S.; Mutava, R. N.; Shekoofa, A.; Sinclair, T. R.; Prasad, P. V. V. (2013): Is the Stay-Green Trait in Sorghum a Result of Transpiration Sensitivity to Either Soil Drying or Vapor Pressure Deficit? In *Crop Science* 53 (5), pp. 2129–2134. DOI: 10.2135/cropsci2013.01.0043.

Clifton-Brown, J. C.; Jones, M. B. (1999): Alteration of transpiration rate, by changing air vapour pressure deficit, influences leaf extension rate transiently in Miscanthus. In *Journal of Experimental Botany* 50 (337), pp. 1393–1401. DOI: 10.1093/jxb/50.337.1393.

Cochard, H.; Venisse, J.-S.; Barigah, T. S.; Brunel, N.; Herbette, S.; Guilliot, A. et al. (2007): Putative role of aquaporins in variable hydraulic conductance of leaves in response to light. In *Plant Physiology* 143 (1), pp. 122–133. DOI: 10.1104/pp.106.090092.

Damour, G.; Simonneau, T.; Cochard, H.; Urban, L. (2010): An overview of models of stomatal conductance at the leaf level. In *Plant, Cell and Environment* 33 (9), pp. 1419–1438. DOI: 10.1111/j.1365-3040.2010.02181.x.

Devi, M. J.; Sinclair, T. R. (2011): Diversity in Drought Traits among Commercial Southeastern US Peanut Cultivars. In *International Journal of Agronomy* 2011, pp. 1–7. DOI: 10.1155/2011/754658.

Fletcher, A. L.; Sinclair, T. R.; Allen, L. H. (2007): Transpiration responses to vapor pressure deficit in well watered ‘slow-wilting’ and commercial soybean. In *Environmental and Experimental Botany* 61 (2), pp. 145–151. DOI: 10.1016/j.envexpbot.2007.05.004.

Gholipoor, M.; Choudhary, S.; Sinclair, T. R.; Messina, C. D.; Cooper, M. (2013): Transpiration Response of Maize Hybrids to Atmospheric Vapour Pressure Deficit. In *Journal of Agronomy and Crop Science* 199 (3), pp. 155–160. DOI: 10.1111/jac.12010.

Gholipoor, M.; Prasad, P. V. V.; Mutava, R. N.; Sinclair, T. R. (2010): Genetic variability of transpiration response to vapor pressure deficit among sorghum genotypes. In *Field Crops Research* 119 (1), pp. 85–90. DOI: 10.1016/j.fcr.2010.06.018.

Grossiord, C.; Buckley, T. N.; Cernusak, L. A.; Novick, K. A.; Poulter, B.; Siegwolf, R. T. W. et al. (2020): Plant responses to rising vapor pressure deficit. In *New Phytologist*. DOI: 10.1111/nph.16485.

Hatfield, J. L.; Prueger, J. H. (2015): Temperature extremes: Effect on plant growth and development. In *Weather and Climate Extremes* 10, pp. 4–10. DOI: 10.1016/j.wace.2015.08.001.

Hetherington, A. M.; Woodward, F. I. (2003): The role of stomata in sensing and driving environmental change. In *Nature* 424 (6951), pp. 901–908. DOI: 10.1038/nature01843.

Ionenko, I. F.; Anisimov, A. V.; Dautova, N. R. (2010): Effect of temperature on water transport through aquaporins. In *Biol Plant* 54 (3), pp. 488–494. DOI: 10.1007/s10535-010-0086-z.

IPCC (2019): Summary for Policymakers. In: Climate Change and Land: an IPCC special report on climate change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems [P.R. Shukla, J. Skea, E. Calvo Buendia, V. Masson-Delmotte, H.- O. Pörtner, D. C. Roberts, P. Zhai, R. Slade, S. Connors, R. van Diemen, M. Ferrat, E. Haughey, S. Luz, S. Neogi, M. Pathak, J. Petzold, J. Portugal Pereira, P. Vyas, E. Huntley, K. Kissick, M. Belkacemi, J. Malley, (eds.)]. Available online at https://www.ipcc.ch/site/assets/uploads/sites/4/2020/02/SPM_Updated-Jan20.pdf, checked on 11/8/2020.

Jauregui, I.; Rothwell, S. A.; Taylor, S. H.; Parry, M. A. J.; Carmo-Silva, E.; Dodd, I. C. (2018): Whole plant chamber to examine sensitivity of cereal gas exchange to changes in evaporative demand. In *Plant Methods* 14 (1), p. 97. DOI: 10.1186/s13007-018-0357-9.

Karthika, G.; Kholova, J.; Alimagham, S.; Ganesan, M.; Chadalavada, K.; Kumari, R.; Vadez, V. (2019): Measurement of transpiration restriction under high vapor pressure deficit for sorghum mapping population parents. In *Indian Journal of Plant Physiology*.

Kawamitsu, Y.; Yoda, S.; Agata, W. (1993): Humidity Pretreatment Affects the Responses of Stomata and CO_2 Assimilation to Vapor Pressure Difference in C_3 and C_4 Plants. In *Plant Cell Physiol* 34 (1), pp. 113–119. DOI: 10.1093/oxfordjournals.pcp.a078384.

Kholová, J.; Hash, C. T.; Kumar, P. L.; Yadav, R. S.; Kocová, M.; Vadez, V. (2010): Terminal drought-tolerant pearl millet Pennisetum glaucum (L.) R. Br. have high leaf ABA and limit transpiration at high vapour pressure deficit. In *Journal of Experimental Botany* 61 (5), pp. 1431–1440. DOI: 10.1093/jxb/erq013.

Lawrence, M. G. (2005): The Relationship between Relative Humidity and the Dewpoint Temperature in Moist Air: A Simple Conversion and Applications. In *Bull. Amer. Meteor. Soc.* 86 (2), pp. 225–234. DOI: 10.1175/BAMS-86-2-225.

Leuschner, C. (2002): Air humidity as an ecological factor for woodland herbs: leaf water status, nutrient uptake, leaf anatomy, and productivity of eight species grown at low or high vpd levels. In *Flora - Morphology, Distribution, Functional Ecology of Plants* 197 (4), pp. 262–274. DOI: 10.1078/0367-2530-00040.

Lobell, D. B.; Schlenker, W.; Costa-Roberts, J. (2011): Climate trends and global crop production since 1980. In *Science* 333 (6042), pp. 616–620. DOI: 10.1126/science.1204531.

Maherali, H.; Johnson, H. B.; Jackson, R. B. (2003): Stomatal sensitivity to vapour pressure difference over a subambient to elevated CO2 gradient in a C3/C4 grassland. In *Plant, Cell and Environment* 26 (8), pp. 1297–1306. DOI: 10.1046/j.1365-3040.2003.01054.x.

Marín-Martínez, F.; Sánchez-Meca, J. (2010): Weighting by Inverse Variance or by Sample Size in Random-Effects Meta-Analysis. In *Educational and Psychological Measurement* 70 (1), pp. 56–73. DOI: 10.1177/0013164409344534.

Medina, S.; Vicente, R.; Nieto-Taladriz, M. T.; Aparicio, N.; Chairi, F.; Vergara-Diaz, O.; Araus, J. L. (2018): The Plant-Transpiration Response to Vapor Pressure Deficit (VPD) in Durum Wheat Is Associated With Differential Yield Performance and Specific Expression of Genes Involved in Primary Metabolism and Water Transport. In *Frontiers in Plant Science* 9, p. 1994. DOI: 10.3389/fpls.2018.01994.

Merilo, E.; Yarmolinsky, D.; Jalakas, P.; Parik, H.; Tulva, I.; Rasulov, B. et al. (2018): Stomatal VPD Response: There Is More to the Story Than ABA. In *Plant Physiology* 176 (1), pp. 851–864. DOI: 10.1104/pp.17.00912.

Murai-Hatano, M.; Kuwagata, T.; Sakurai, J.; Nonami, H.; Ahamed, A.; Nagasuga, K. et al. (2008): Effect of low root temperature on hydraulic conductivity of rice plants and the possible role of aquaporins. In *Plant Cell Physiol* 49 (9), pp. 1294–1305. DOI: 10.1093/pcp/pcn104.

Nobel, P. S. (2020): Physicochemical and environmental plant physiology. Fifth edition. London, United Kingdom: Academic Press.

Ocheltree, T. W.; Nippert, J. B.; Prasad, P. V. V. (2014): Stomatal responses to changes in vapor pressure deficit reflect tissue-specific differences in hydraulic conductance. In *Plant, Cell and Environment* 37 (1), pp. 132–139. DOI: 10.1111/pce.12137.

Oren, R.; Sperry, J. S.; Katul, G. G.; Pataki, D. E.; Ewers, B. E.; Phillips, N.; Schäfer, K. V. R. (1999): Survey and synthesis of intra- and interspecific variation in stomatal sensitivity to vapour pressure deficit. In *Plant Cell Environ* 22 (12), pp. 1515–1526. DOI: 10.1046/j.1365-3040.1999.00513.x.

Pantin, F.; Blatt, M. R. (2018): Stomatal Response to Humidity: Blurring the Boundary between Active and Passive Movement. In *Plant Physiology* 176 (1), pp. 485–488. DOI: 10.1104/pp.17.01699.

Parida, A. K.; Dagaonkar, V. S.; Phalak, M. S.; Umalkar, G. V.; Aurangabadkar, L. P. (2007): Alterations in photosynthetic pigments, protein and osmotic components in cotton genotypes subjected to short-term drought stress followed by recovery. In *Plant Biotechnol Rep* 1 (1), pp. 37–48. DOI: 10.1007/s11816-006-0004-1.

Piepho, H.-P. (2014): Network-meta analysis made easy: detection of inconsistency using factorial analysis-of-variance models. In *BMC Med Res Methodol* 14 (1), pp. 1–9. DOI: 10.1186/1471-2288-14-61.

Rashed, M. R. U. (2016): Substrate Effects on Plant Transpiration Rate under Several Vapour Pressure Deficit (VPD) Levels. In *J Plant Pathol Microbiol* 7 (8). DOI: 10.4172/2157-7471.1000369.

Ryan, A. C.; Dodd, I. C.; Rothwell, S. A.; Jones, R.; Tardieu, F.; Draye, X.; Davies, W. J. (2016): Gravimetric phenotyping of whole plant transpiration responses to atmospheric vapour pressure deficit identifies genotypic variation in water use efficiency. In *Plant science : an international journal of experimental plant biology* 251, pp. 101–109. DOI: 10.1016/j.plantsci.2016.05.018.

Sadok, W.; Sinclair, T. R. (2009a): Genetic variability of transpiration response to vapor pressure deficit among soybean (Glycine max [L.] Merr.) genotypes selected from a recombinant inbred line population. In *Field Crops Research* 113 (2), pp. 156–160. DOI: 10.1016/j.fcr.2009.05.002.

Sadok, Walid; Sinclair, T. R. (2009b): Genetic Variability of Transpiration Response to Vapor Pressure Deficit among Soybean Cultivars. In *Crop Sci.* 49 (3), pp. 955–960. DOI: 10.2135/cropsci2008.09.0560.

Shekoofa, A.; Balota, M.; Sinclair, T. R. (2014): Limited-transpiration trait evaluated in growth chamber and field for sorghum genotypes. In *Environmental and Experimental Botany* 99, pp. 175–179. DOI: 10.1016/j.envexpbot.2013.11.018.

Shibuya, T.; Sugimoto, A.; Kitaya, Y.; Kiyota, M. (2009): High Plant Density of Cucumber (Cucumis sativus L.) Seedlings Mitigates Inhibition of Photosynthesis Resulting from High Vapor-pressure Deficit. In *HortScience* 44 (6), pp. 1796–1799. DOI: 10.21273/HORTSCI.44.6.1796.

Sinclair, T. R.; Hammer, G. L.; van Oosterom, E. J. (2005): Potential yield and water-use efficiency benefits in sorghum from limited maximum transpiration rate. In *Functional Plant Biol.* 32 (10), p. 945. DOI: 10.1071/FP05047.

Sinclair, T. R.; Messina, C. D.; Beatty, A.; Samples, M. (2010): Assessment across the United States of the Benefits of Altered Soybean Drought Traits. In *Agron. J.* 102 (2), pp. 475–482. DOI: 10.2134/agronj2009.0195.

Sinclair, T. R.; Zwieniecki, M. A.; Holbrook, N. M. (2008): Low leaf hydraulic conductance associated with drought tolerance in soybean. In *Physiologia Plantarum* 132 (4), pp. 446–451. DOI: 10.1111/j.1399-3054.2007.01028.x.

Taylor, S. H.; Ripley, B. S.; Woodward, F. I.; Osborne, C. P. (2011): Drought limitation of photosynthesis differs between $C_3$and C_4grass species in a comparative experiment. In *Plant, Cell and Environment* 34 (1), pp. 65–75. DOI: 10.1111/j.1365-3040.2010.02226.x.

Thomas, D. S.; Turner, D. W.; Eamus, D. (1998): Independent effects of the environment on the leaf gas exchange of three banana (Musa sp.) cultivars of different genomic constitution. Scientia Horticulturae, 75(1-2), 41-57. In *Scientia Horticulturae* 75 (1), pp. 41–57. DOI: 10.1016/S0304-4238(98)00114-9.

Urry, L. A.; Cain, M. L.; Wasserman, S. A.; Minorsky, P. V.; Reece, J. B. (2019): Campbell Biologie. 11., aktualisierte Auflage. Edited by Achim Paululat, Jürgen J. Heinisch. Hallbergmoos: Pearson.

Verkman, A. S. (2013): Aquaporins. In *Current biology : CB* 23 (2), R52-5. DOI: 10.1016/j.cub.2012.11.025.

Vicente-Serrano, S. M.; Beguería, S.; Lorenzo-Lacruz, J.; Camarero, J. J.; López-Moreno, J. I.; Azorin-Molina, C. et al. (2012): Performance of Drought Indices for Ecological, Agricultural, and Hydrological Applications. In *Earth Interactions* 16 (10), pp. 1–27. DOI: 10.1175/2012EI000434.1.

Wei, Z.; Yoshimura, K.; Wang, L.; Miralles, D. G.; Jasechko, S.; Lee, X. (2017): Revisiting the contribution of transpiration to global terrestrial evapotranspiration. In *Geophys. Res. Lett.* 44 (6), pp. 2792–2801. DOI: 10.1002/2016GL072235.

Will, R. E.; Wilson, S. M.; Zou, C. B.; Hennessey, T. C. (2013): Increased vapor pressure deficit due to higher temperature leads to greater transpiration and faster mortality during drought for tree seedlings common to the forest-grassland ecotone. In *New Phytologist* 200 (2), pp. 366–374. DOI: 10.1111/nph.12321.

Yang, Z.; Sinclair, T. R.; Zhu, M.; Messina, C. D.; Cooper, M.; Hammer, G. L. (2012): Temperature effect on transpiration response of maize plants to vapour pressure deficit. In *Environmental and Experimental Botany* 78, pp. 157–162. DOI: 10.1016/j.envexpbot.2011.12.034.

Zaman-Allah, M.; Jenkinson, D. M.; Vadez, V. (2011): Chickpea genotypes contrasting for seed yield under terminal drought stress in the field differ for traits related to the control of water use. In *Functional plant biology : FPB* 38 (4), pp. 270–281. DOI: 10.1071/FP10244.