Investigating The Role Of Volatile Signalling In Plant Responses To Drought

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Contents

Abstract

Volatiles released by plants are becoming important to understand how plants may exchange information. With a wide chemical variety, plant derived volatiles have been shown to be used by plants for pollination, and defence against biotic stress. In a drought stress situation, where stomata play a central role in tolerance, plants have been observed to have their volatile emission decreased, associated with stomatal closure, as well as increased emission of other volatiles. However, the specific functions of the volatiles remain obscure.

In many studies on plant responses to drought and rehydration, but without volatile analysis, a particular phenomenon has been observed where the well-watered plants displayed a drought-like response similar to the water-stressed plants when co-located in the same environment. Indeed, while a reduction of stomatal conductance (g_s) of plants under water deficit is an expected response, it is not for plants with continuous adequate watering. Thus, the main hypothesis to be tested by this thesis is that volatiles are released by water-stressed plants that induce stomatal closure in nearby well-watered plants. Supposedly, the water-stressed plants would emit volatiles triggering a closure of stomata of the nearby plants in order to preserve water in the likely event of further reduced water availability.

To test the hypothesis, *Vitis vinifera* and *Arabidopsis thaliana* potted plants were examined in three configurations of drought/rehydration experiments: i) having well-watered (WW) and water-stressed (WS) treatments together, ii) separating the treatments with custom-made individual plastic chambers and, iii) having both treatments together and a separate growth cabinet for controls. For each configuration, volatiles were extracted with solid-phase micro-extraction (SPME) using DVB/CAR/PDMS coated fibres which were desorbed and analysed on a gas chromatogram combined with a mass spectrometer (GC-MS).

All results combined tend to support the hypothesis of the g_s of WW plants not being stable during the severe stress phase applied on the WS group, and supported by multilinear regression analysis showing a stronger effect of WS g_s on WW g_s than light or VPD. When WS grapevines were enclosed in chambers, this interaction was not evident. The volatile samples revealed a change in the emission profile during the drought stress phase and some volatiles showed strong significant correlations with WW and WS g_s. Especially, 1,2,3-trimethylbenzene was significantly negatively correlated with g_s for both WW and WS plants.

The last part of this study was to develop a method to test the effect of volatile(s) on single leaf stomatal regulations avoiding the use of leaf-attached chambers common for leaf gas exchange analysis that are restrictive with monitoring released or applied volatiles. By connecting the petiole of a detached leaf to a sensitive liquid flow meter, responses to volatiles could be determined while simultaneously monitoring some

alcohols in real-time with gas sensors. The placement of two sensors one close and one further from the leaf surface, allowed detection of changes in concentration of externally applied volatile alcohols as well as those released from the leaf. Results showed similar responses to normal conditions over time, light-to-dark transitions and revealed a strong effect of volatile methanol that induced a rapid closure of stomata. The measurements of flow (*Q*) into the leaf were also compared with transpiration (*E*) from the leaf using an attached infra-red gas-analyser (IRGA). This revealed a potential problem with measuring gas exchange in Arabidopsis due to restriction of the petiole xylem by the seals on the IRGA chamber that was not evident with measurements on *Vitis vinifera* leaves. For the latter the *E* and *Q* were not always well correlated also indicative of a capacitance in the water pathway to the stomata. Despite these interesting effects, the technique may be developed further to enable routine testing of potential volatile signalling molecules that impact stomatal regulation.

Résumé

L'étude des composés volatiles émis par les plantes est de plus en plus primordial pour comprendre comment elles s'échangent des informations. D'une grande variété, ces molécules sont connues pour être utilisées, par exemple, lors de la pollinisation et pour la défense contre les stress biotiques. En condition de stress hydrique, où les stomates jouent un rôle central dans la tolérance, on observe chez les plantes une diminution de l'émission de composés volatiles qui est associée à la fermeture des stomates, ainsi qu'une augmentation d'autres composés. Néanmoins, leurs fonctions spécifiques restent inconnues.

Dans beaucoup d'études sur les réponses des plantes au stress hydrique, sans analyse des composés volatiles, un phénomène particulier a été observé où les plantes suffisamment arrosées montrent une réponse similaire aux plantes stressées lorsqu'elles sont localisées dans le même environnement. Il est en effet attendu qu'une diminution de la conductance stomatique (g_s) soit observé chez des plantes sous stress hydrique, mais en théorie ce n'est pas le cas pour des plantes convenablement irriguées. Ainsi, la principale hypothèse testée dans cette thèse est que des composés volatiles sont relâchés par des plantes sous stress hydrique qui induisent la fermeture des stomates des plantes environnantes. Il est supposé que ces plantes stressées émettent dans l'air des composés déclenchant la fermeture des stomates pour se préserver dans le cas d'une réduction de la disponibilité en eau ultérieure.

Pour tester cette hypothèse, des plants en pots de *Vitis vinifera* et *Arabidopsis thaliana* ont été utilisés dans trois configurations : i) le traitement « bien-irrigué » (well-watered, WW) et le traitement « stress-hydrique » (water-stressed, WS) sont ensemble, ii) les traitements sont séparés par l'utilisation de chambres plastiques individuelles faites sur mesure et, iii) les deux traitements sont ensemble et une deuxième chambre de croissance est utilisée pour les contrôles. Pour chaque configuration, les composés volatiles ont été extraits par la méthode de micro-extraction sur phase solide (solid-phase micro-extraction, SPME) en utilisant des fibres enrobées de DVB/CAR/PDMS qui ont été désorbées et analysées par un chromatographe en phase gazeuse combiné à un spectromètre de masse (GC-MS).

Les résultats tendent à supporter l'hypothèse que la g_s des plantes WW n'est pas stable durant la phase de stress sévère appliqué au groupe WS, et est supporté par l'analyse de régression multilinéaire qui montre un effet de la g_s des WS sur la g_s des WW plus important que l'intensité lumineuse ou le déficit de pression de vapeur (VPD). Lorsque les plantes WS ont été placées dans les chambres plastiques, cette interaction n'était plus évidente. Les échantillons de composés volatiles ont révélé un changement de profil d'émission durant la phase de stress hydrique et certains composés ont montré de fortes corrélations significatives avec la g_s

de WW et WS. En particulier, le 1,2,3-trimethylbenzène est significativement et négativement corrélé avec les g_s des plantes WW et WS.

La dernière partie de cette étude a eu pour but de développer une méthode pour tester les effets de volatile(s) sur les régulations stomatiques de feuilles isolées, en évitant les appareils attachés aux feuilles communément utilisées dans les analyses d'échange gazeux qui restreignent le suivi de volatiles émis ou testés. En connectant le pétiole d'une feuille détachée à un débitmètre (liquid flow meter), les réponses induites par les volatiles ont pu être déterminées tout en monitorant simultanément certains alcools en temps réel par des capteurs. Le placement de deux capteurs, l'un proche de la feuille et l'autre plus éloigné, a permis la détection de variations de concentrations des alcools volatiles appliqués en externe ainsi que ceux émis directement par la feuille. Les résultats montrent des réponses similaires entre les feuilles dans le temps, ainsi que pendant des transitions d'intensité lumineuse (dark-to-light) et ont révélé que le méthanol induit une rapide fermeture des stomates. Les mesures de flux (*Q*) à l'intérieur de la feuille ont aussi été comparé avec la transpiration (*E*) de la feuille en utilisant un analyseur de gaz à infra-rouge (infra-red gas-analyseur, IRGA). Cela a révélé un problème pour les mesures d'échange gazeux chez Arabidopsis due à la compression des canaux de xylème par les joints d'étanchéité de la chambre de l'IRGA, ce qui n'était pas évident sur les mesures de feuilles de vigne. De plus, les mesures de *Q* et *E* n'étant pas toujours corrélées ont aussi révélé un effet de capacitance du passage de l'eau jusqu'aux stomates. Cette technique pourrait donc être utilisée pour tester d'autres molécules volatiles qui pourraient impacter les régulations stomatiques.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Signed.

Date. 11/08/2021

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1. Introduction and literature review

1.1. Introduction

Global warming linked to anthropogenic greenhouse emissions (IPCC, 2014) and evidenced by higher average temperatures year after year (Medhaug *et al.*, 2017) has resulted in increased abiotic stress for plants; i.e. high atmospheric carbon dioxide (CO_2), high temperatures (average, non-seasonal, and length and intensity of heatwaves), and secondary effects of warming of the earth system, e.g. drought, salinity, and nutrient imbalance. In addition, added pressures from higher UV radiation (Bais *et al.*, 2018; Bornman *et al.*, 2015; Williamson *et al.*, 2014), air pollution (Knippertz *et al.*, 2015; Pescott *et al.*, 2015; Vacek *et al.*, 2015), ozone (Fuhrer *et al.*, 2016) and pests and diseases (Burge *et al.*, 2014; Trebicki *et al.*, 2015) interact with and compound the impact of global warming.

Locally, the 2019-2020 summer in Australia was the hottest on record, based on the Australian Government Bureau of Meteorology data, alongside extreme and deadly bushfires that were predicted in 2008 (Garnaut, 2008). In 2017-2018, the Australian Bureau of Statistic (ABS) revealed that the agriculture sector increased its freshwater use to supply crops and pasture, due to 'changing water availability and poor forecasts'. With cotton being the most water avid and winegrape growing regions also greatly dependent on water availability for irrigation, water deficits will thus be a limiting element in wine production and quality (Webb *et al.*, 2007).

Plants respond to abiotic and biotic stress using complex intra- and intercellular signalling cascades (Peck & Mittler, 2020; Zandalinas *et al.*, 2020). Long distance communication of stress between organs (leaf, shoot, root) can occur through the vascular system with a variety of potential signals and combinations thereof (Heil & Ton, 2008; Thomas & Frank, 2019). These signalling pathways involve many kinds of signals which are chemical in nature (e.g. hormones) (Lacombe & Achard, 2016; Toyota *et al.*, 2018; Tripathi *et al.*, 2018), hydraulic (pressure) (Buckley, 2005), electrical (Huber & Bauerle, 2016), or possibly acoustical (Mishra *et al.*, 2016; Wu & Lin, 2002). It is possible for plants to bypass the vascular system for within plant signalling through volatile signals (Heil & Ton, 2008), which, for priming of resistance to herbivores can also be communicated to neighbouring plants (Baldwin & Schultz, 1983; Erb, 2018; Kessler *et al.*, 2006) (Figure 1). Even though, interplant signalling can occur via the roots and mycorrhiza (Babikova *et al.*, 2013; Song *et al.*, 2014), interplant volatile signalling that can communicate abiotic stress is less explored than that for biotic stress (Erb, 2018). Some examples exist for higher plants (Caparrotta *et al.*, 2018) and algae (Zuo *et al.*, 2012), and identified volatiles emitted under abiotic stress when applied to non-stressed plants can prime for stress resistance (Cofer *et al.*, 2018). Such volatile abiotic signalling could be important in adapting plant production

to climate change as suggested for biotic interactions (Brilli *et al.*, 2019; Peñuelas & Llusià, 2003; Pickett & Khan, 2016), in enclosed/protected horticultural production (Ingwell *et al.*, 2018; Tosh & Brogan, 2015), and design and interpretation of experiments in enclosed phenomics platforms.

A diverse terminology exists in the literature to refer to plant volatile compounds, e.g. airborne signal (Ton *et al.*, 2007), plant volatile (Pichersky *et al.*, 2006), volatile organic compound (VOC) (Possell & Loreto, 2013), biogenic volatile organic compound (BVOC) (Peñuelas & Staudt, 2010), herbivore-induced plant volatile (HIPV) (Yoneya & Takabayashi, 2014), microbe-induced plant volatile (MIPV) (Sharifi *et al.*, 2018), or airborne infochemicals (J. Keaton Wilson, Kessler, & Woods, 2015). Hence, as volatile compounds emitted by plants can be of a different nature (organic or inorganic), they will be referred to as volatiles in general.

Volatiles can be emitted by plant leaves and stems (Rissanen, Vanhatalo, Salmon, Bäck, & Hölttä, 2020) and involve many roles in diverse situations and ecological levels. At the Earth system level, plant emissions interact with chemical and physical properties of the atmosphere (Laothawornkitkul *et al.*, 2009; Lerdau, Guenther, & Monson, 1997), and some volatiles influence the process of cloud formation (Zhao *et al.*, 2017). At the ecosystem level, volatiles are involved in the interactions between plants and other organisms. During plant reproduction, some volatiles will attract pollinators (Pichersky & Gershenzon, 2002), or seed dispersers (Bolen & Green, 1997; Luft, Curio, & Tacud, 2003; Raguso, 2008). Plants also emit diverse volatiles in response to the inoculation with beneficial microbes such as rhizobia, mycorrhiza and plant growth promoting rhizobacteria (Schulz-Bohm *et al.*, 2018; Sharifi *et al.*, 2018). The most familiar volatiles are the most odorant ones that can be recognise by humans, for example the smell of pine, lemon or eucalyptus (e.g. pinene, limonene and 1,8-cineole respectively being the predominant volatiles present in these types of vegetation) (Šimpraga, Takabayashi, & Holopainen, 2016). Plants may also repel biotic threats such as bacteria (M. Huang *et al.*, 2012) or herbivorous insects (Pickett & Khan, 2016), or even by attracting natural predators of the herbivorous insect (Yoneya & Takabayashi, 2014). In this context, plants communicate responses to neighbouring plants, introducing this interaction as a plant-plant communication, also referred to as “plant vocabulary” (Trewavas, 2016), “language of plants” or “talking trees” (Baldwin, Halitschke, Paschold, von Dahl, & Preston, 2006; Šimpraga *et al.*, 2016). Not always beneficial, the plant-plant volatile exchange also plays a role in competition to detect neighbours or camouflage themselves (Effah, Holopainen, & McCormick, 2019). The first evidence of communication between plants was made by Baldwin and Schultz in 1983 (Baldwin & Schultz, 1983), showing the emission of ethylene by unharmed plants placed nearby mechanically damaged plants. Then, 10 years later, Sharkey and Loreto (Sharkey & Loreto, 1993) identified the emission of the most studied plant volatile, isoprene, and showed its high emission during heat stress in *Pueraria*

lobata. Since then, numerous studies have investigated the correlation between the emission of volatiles and environmental stresses. The majority of studies focused on biotic stress, some on the combination of biotic and abiotic stress (Catola *et al.*, 2018; Scott *et al.*, 2019) and on purely abiotic stress, but the trend might shift as climate change is suspected to increase the overall emission and changes in volatile profiles (Peñuelas & Staudt, 2010; Wilson *et al.*, 2018).

A form of chemical signalling that can communicate stress between plants (interplant signalling) that involves volatile compounds is relatively unexplored in abiotic stress signalling. Here, this literature review i) explores the components of plant water regulation, ii) describes plant volatile signalling for both biotic and abiotic conditions, by detailing the biosynthesis and storage, the membrane transport and emission, and the reception and stress-associated responses, iii) to contemplate the potential interplant volatile signalling of abiotic stress prompted by unpublished observations and those evident in the literature of leaf gas exchange responses of control plants to stressed plants contained in the same enclosed growth chamber or glasshouse.

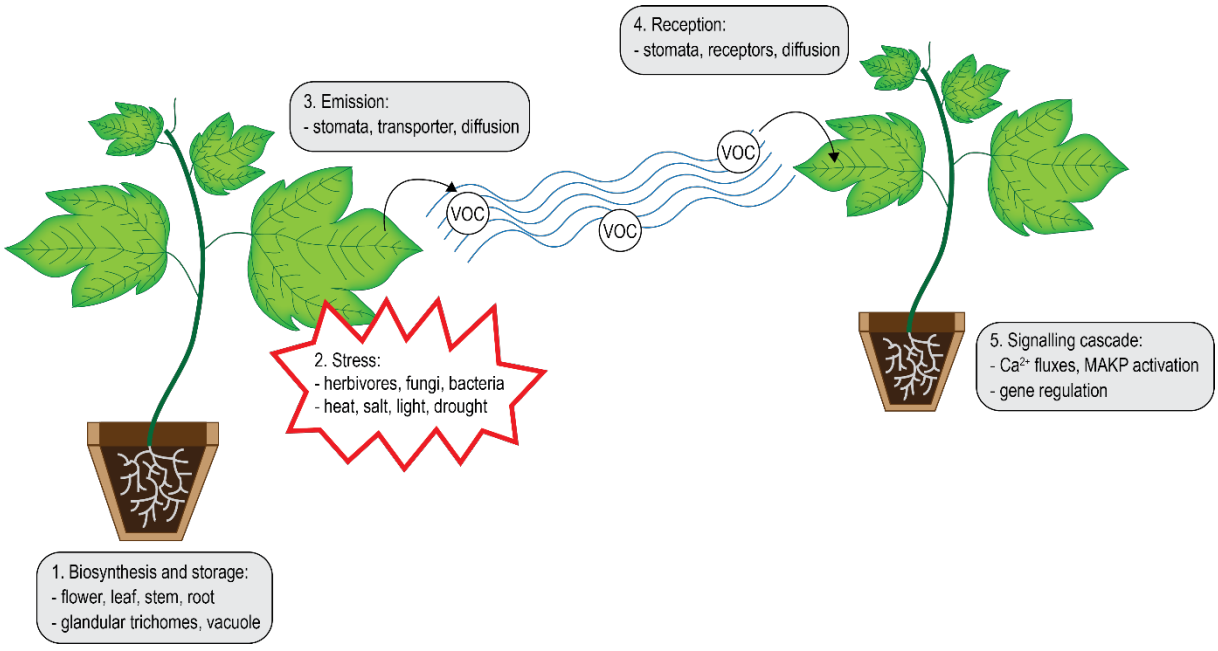


Figure 1. Plant volatile signalling cascade. Volatiles are biosynthesised and stored in the plant leaves and if a stress occurs, the plant will emit volatile organic compounds (VOCs) in the atmosphere escaping through the stomata, via specific membrane transporters or diffuse freely across membranes. These can be received by neighbouring plants inducing stress-specific responses, such as improving tolerance to abiotic stress or attract predators of herbivory insects.

1.2. Plant water relations

1.2.1. Components of water regulation

1.2.1.1. Plant vascular system

The plant vascular system serves as mechanical support and distribution of vital resources such as sugars, mineral nutrients and water to all organs. Moving from the roots to the leaves via the xylem vessels and tracheids, water then evaporates from cell wall surfaces into the intercellular air spaces of leaves and diffuses into the atmosphere through open stomata. According to the generally accepted cohesion-tension theory (Steudle, 2001), water is pulled under tension to the site of evaporation in the leaves by the capillary force established within the cell wall capillaries of the leaf at the top of the water column (Figure 2). These vascular conduits or xylem are derived from procambium, a primary meristematic tissue that develop from ground meristem cells and is a non-living cell structure when functional with lignified vertically oriented tracheary elements consisting of vessels and tracheids. These elements have pits that span the secondary wall to allow water to flow between tracheary elements and from tracheary elements to the leaf apoplast. In the leaf, the vascular system consists of a network of interconnecting veins with conducting tissues (xylem and phloem) and non-conducting supporting cells (parenchyma, sclerenchyma and fibres) (Lucas *et al.*, 2013).

The flow of water from a moist to a drier substrate is determined by the water potential (Ψ) gradient, which is the free energy of water per unit volume, and this drops from the rhizosphere to the leaves across hydraulic resistances in the pathway. During steady-state transpiration, Ψ at any given point in the plant depends on Ψ of the soil, the transpiration rate and the effective water transport resistance (Buckley, 2019). In most studies, the leaf water potential measured at predawn (Ψ_{pd}) constitutes a proxy for soil water potential (Ψ_s) since at night, stomata are closed and water equilibrates between the plant and the soil. The plant needs to maintain a leaf Ψ to a level that enough CO_2 is taken up for photosynthesis while the water flux from the soil to the leaves can be maintained (Buckley, 2019). This regulation is optimally based on a non-linear trade-off (Ratzmann *et al.*, 2019).

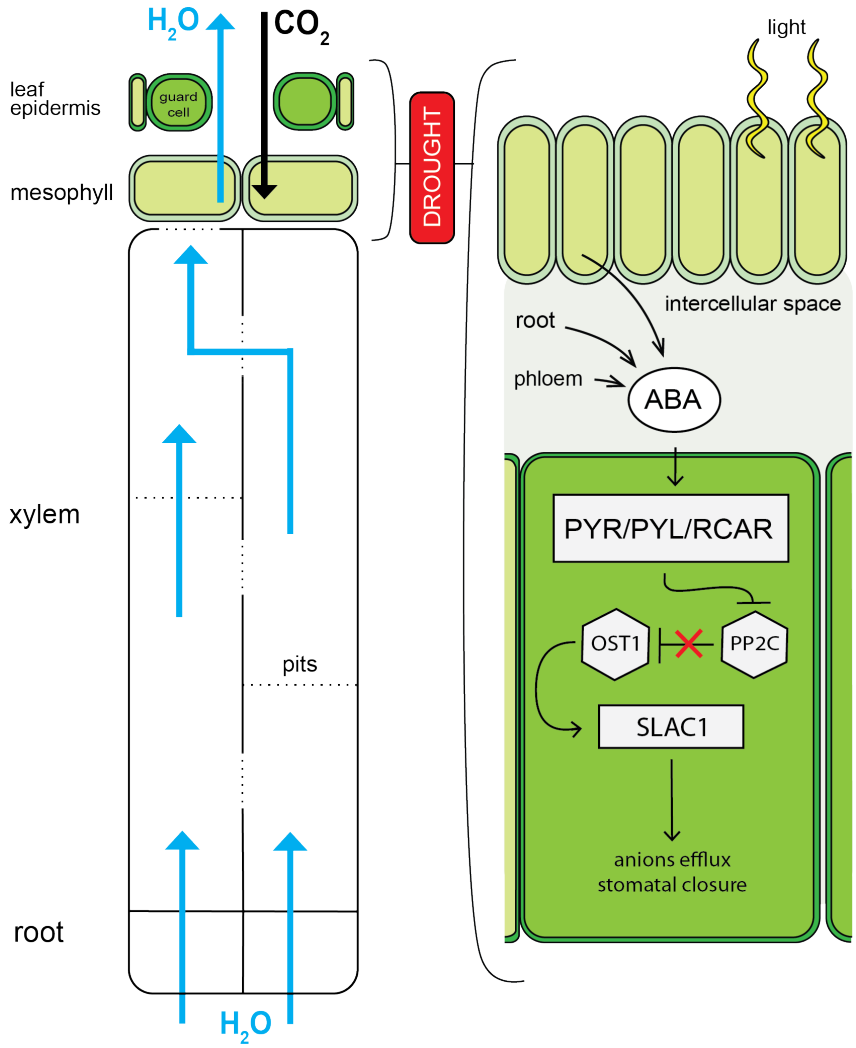


Figure 2. Transpiration is driven through a low-resistance network of dead xylem conduits by an energy gradient created by the tension at the surface of narrow pores in the cell walls. They are connected by pits allowing the long-distance bulk flow and protecting against air entry and cavitation. The guard cells actively regulate transpiration through opening and closing where the water-for-carbon exchange occurs. During drought, abscisic acid (ABA) may be synthesised at different sources along the root-shoot-leaf continuum and transported in the guard cells, and binds to the PYR/PYL/RCAR receptor, deactivating the PP2C phosphatase that inhibits the OST1 protein kinase. The SLAC1 channel is then phosphorylated leading to an efflux of anions. Then, membrane depolarisation leads to opening of potassium channels resulting in a net efflux of potassium chloride or malate, reduction of guard cell turgor and stomata closure.

1.2.1.2. Stomata

Stomata are small pores defined by two guard cells on the surface of leaves and stems. They are important in the context of volatile signalling since they may determine volatile release from an emitter plant and in receiver plants, and potentially also responsible for stomatal closure (Niinemets & Reichstein, 2003). Plants regulate water loss and CO_2 uptake by controlling the stomatal aperture and the number of stomata on the epidermis (Figure 2). There are two broad morphological types of stomata, the kidney-shaped for most species and the dumb-bell-shaped typical of grasses. They can range in size from about 10-80 µm in length, and have densities of between 5 and 1,000 per mm^2. They can be on one or both leaf surfaces (amphistomatous for both upper (adaxial) and lower (abaxial) sides, or hypostomatous) (Hetherington & Woodland, 2003). Highly sensitive to various environmental cues, stomata react to environmental signals such as humidity, soil moisture, light intensity, leaf internal CO_2 concentration, pollutants (e.g. ozone), hormones, and pathogens (Lawson & Matthews, 2020).

The two guard cells develop on mature leaves from protodermal cells, in a basipetal manner (from the leaf tip to the base) and following the one cell spacing rule to ensure that all stomata are separated by at least one pavement cell (Zoulias *et al.*, 2018). When guard cells are fully turgid, the pore stays open, and for the stomata to close, water has to exit the guard cells. This movement requires a highly precise signalling pathway involving hormones, protein receptors and signal cascades, ion fluxes through specific transport proteins, and has been described as a scale free network (Daszkowska-Golec & Szarejko, 2013; Hetherington & Woodland, 2003). This aperture is determined by the displacement of the guard cell walls or ventral walls, that is significantly counteracted by the volume changes in the adjacent epidermal cells (Buckley, 2019). New computational modelling and indentation techniques have explained the mechanics behind stomatal movements (Jezek *et al.*, 2019), enlightening the changes in the cell wall matrix of the guard cells that strain-stiffens during opening, especially at the poles (Woolfenden *et al.*, 2018).

Stomata movements are also ruled by hydromechanics. The stomatal aperture is related to the turgor pressure of the guard cells and the turgor pressure of the adjacent subsidiary epidermal cells. Those turgor pressures are related to water potentials and osmotic pressures of the cells. Furthermore, the osmotic pressure of the guard cells is characterised by the osmotic content, the volume of the cell and gas and temperature constants. Finally, the osmotic content of a cell can be easily modulated by electrogenic proton pumps, ion channels and intracellular synthesis of osmolytes (Buckley, 2005). Variations of the osmotic content of guard cells will affect the osmotic pressure and water potentials, causing water to move in or out of the guard cells, opening or closing the stomata respectively.

The stomata control over water status discriminates plants along a continuum. Plants that can maintain high water potentials under water stress by greater control of stomata are characterised as isohydric. In contrast, if plants are less conservative of their water use and develop lower leaf water potentials, they are anisohydric (Hochberg *et al.*, 2018; Tardieu & Simonneau, 1998). In *Vitis vinifera*, different cultivars can show these diverging characteristics, for instance, Grenache is considered more isohydric, and Shiraz (Syrah) more anisohydric (Schultz, 2003; Soar *et al.*, 2006). Thus, stomatal behaviour is pivotal for plants to regulate and respond to varying water availability and plays an important role in the emission of some volatiles depending on chemical properties (Niinemets *et al.*, 2004). This will be described in more detail below in section 1.3.1.3.

1.2.1.3. Growth regulators/hormones

An arsenal of growth regulators is available for plants to activate and regulate their responses to water stress, such as auxin, cytokinin, brassinosteroids, jasmonates, salicylic acid, ethylene, GABA, abscisic acid (ABA) and others (Acharya & Assmann, 2009; Palmer *et al.*, 2016). Abscisic acid ($C_{15}H_{20}O_4$) has extensively been studied as the main hormone that regulates the stomatal aperture (Munemasa *et al.*, 2015). It is synthesised via the methylerythritol phosphate (MEP) pathway from carotenoids in plastids, and the ABA2 (Abscisic acid deficient2) enzyme catalyses the conversion of xanthoxin to abscisic aldehyde and then ABA is released to the cytosol. As examples of genes involved in the synthesis, there is the 9-cis-epoxy carotenoid dioxygenase *NCED3*; and in catabolism, the cytochrome p450 monooxygenases *CYP707A3*. ABA has long been thought to be produced in the roots and then translocated to the shoot to induce the closure of stomata when under drought stress (Zhang *et al.*, 1987). However, evidence has accumulated that ABA is synthesised directly in the leaf (Manzi *et al.*, 2015; McAdam *et al.*, 2016), and that much of ABA present in roots may in fact originate in the leaves (Buckley, 2019). Another study has found that leaf-borne ABA was synthesised in guard cells and in phloem companion cells of the vasculature (Merilo *et al.*, 2018).

1.2.1.4. Aquaporins

To regulate water flow from cell to cell across a tissue, plants have membrane proteins that function as water channels, called aquaporins (AQPs). AQPs are divided into five subfamilies based on their sequences and generally named because of their membrane localisation, i.e. the plasma membrane intrinsic proteins (PIPs), the tonoplast intrinsic proteins (TIPs), the Nodulin26-like intrinsic proteins (NIPs), the small basic intrinsic proteins (SIPs), and the uncategorised intrinsic proteins (XIPs) (Chaumont & Tyerman, 2014; Kammerloher *et al.*, 1994). AQPs are localised in almost all cell membranes (plasma, tonoplast or chloroplast membranes) and are involved in the maintenance of cellular water homeostasis. They can also transport other compounds

such as neutral solutes (urea, silicic acid, boric acid, reactive oxygen species) or dissolved gas molecules like CO_2 or ammonia (NH_3) (Li *et al.*, 2014; Maurel *et al.*, 2015) and ions (Byrt *et al.*, 2017; Tyerman *et al.*, 2021).

For instance, AtTIP1;1 is a water-specific channel that facilitates dihydrogen monoxide (H_2O), hydrogen peroxide (H_2O_2) and urea transport (Maurel *et al.*, 2008) and AtPIP2:1 may function as a non-selective cation transporter of Na^+ in roots (Byrt *et al.*, 2017); it is also proposed to function as a H_2O_2 and CO_2 channel in stomata (Rodrigues *et al.*, 2017; Wang *et al.*, 2016). In addition, Arabidopsis and wheat TIP2 homologs appear to have significant permeability and contribute to the loading of NH_3 before being trapped as ammonium (NH_4^+) in vacuoles (Holm *et al.*, 2005; Loqué *et al.*, 2005). More than 30 aquaporin isoforms exist in different species and are involved in many functions such as osmoregulation, ROS detoxification, water transport, biotic interactions and stomatal regulation (Ding & Chaumont, 2020; Maurel *et al.*, 2015). Although aquaporins are primarily linked to water transport, there is increasing evidence for their role in gas transport. Adding to CO_2 and ammonia, it has been suggested that the bacterial aquaporin Z might transport ethanol (Soupene *et al.*, 2002) and the AQP AtPIP2:1 is indeed regulated by ethylene (Qing *et al.*, 2016). Thus, it places aquaporins as potential transport systems for small volatile molecules.

1.2.2. Drought stress

1.2.2.1. Effects of drought stress on plant physiology

When plants sense a reduction of soil water content or drought stress, commonly caused by low rainfall, salinity, high and low temperatures, dry wind or high intensity of light, they trigger multidimensional responses to reduce their water use and leading to changes in physiological, morphological, biochemical and molecular traits, affecting photosynthesis, growth and productivity (Salehi-Lisar & Bakhshayeshan-Agdam, 2016). One of the main pivots of water regulation is the stomata, which can close or open to control the transpiration rate but also the influx of CO_2 for photosynthesis. To achieve the closure of stomata, induced by water stress, plants respond through hydraulic and chemical signalling pathways (Comstock, 2002). At the leaf level, when a decrease in water supply occurs, stomata transiently 'pop open' before eventually closing. This biphasic response or "Wrong-Way Response", is followed by the "Right-Way Response" (RWR), observed in angiosperms (Buckley, 2019). To explain this phenomenon, the 'hydroactive local feedback' hypothesis invokes a metabolically mediated response of guard cells to local water status. This seems to be associated with the opposing effect of the adjacent epidermal cells also called 'mechanical advantage of the epidermis' and other signals such as strigolactones, which are predicted to be volatile (Buckley, 2019).

Drought, but also other stresses such as high temperatures, freezing and pathogen damages, can lead to cavitation or embolism. This phenomenon is induced by excessive tensions in the xylem inducing the separation of air from water ultimately creating gas bubbles in the plant conducting elements (Sperry & Tyree, 1988), ultimately blocking water movement and causing deleterious effects (Choat *et al.*, 2012). Some plants have repair mechanisms to embolisms achieved by refilling vessels to force air to dissolve in water, rerouting the water column through nearby xylem or create new xylem (Brodersen & McElrone, 2013). If the plant cannot escape from the drought, several symptoms will appear such as loss of leaf turgor, wilting, yellowing and premature leaf abscission, and under extreme drought, plant death (Salehi-Lisar & Bakhshayeshan-Agdam, 2016).

1.2.2.2. Signalling pathways involved in stomatal closure

The variation in the osmotic content of guard cells is induced by a chemical signalling pathway involving many players (Figure 2). Starting with ABA transported through the vascular system and/or *in situ* synthesised in the leaf, it can easily cross plasma membranes due to its weak acid and uncharged chemistry (Finkelstein & Rock, 2002). When in the guard cells, ABA can bind to the cytosolic PYRABACTIN RESISTANCE /PYR1-LIKE /REGULATORY COMPONENTS OF ABA RECEPTORS (PYR/PYL/RCAR) receptor in guard cells. This binding sequesters/deactivates class 2C protein phosphatases (PP2Cs, ABA insensitive 1 and 2), that releases the protein kinase Open Stomata 1 (OST1) from inhibition. OST1 then phosphorylates and stimulates SLow Anion Channel 1 (SLAC1) channels, leading to the efflux of anions, and depolarisation of the guard cell plasma membrane. As a result, the depolarisation-dependent K^+ channels are activated, leading to a net release of anions (Cl^-, $malate^{2-}$ and K^+. This reduction of osmotic pressure, or increase in osmotic potential, drives an efflux of water from the guard cells through aquaporins, reduced turgor and stomata aperture (Jezek & Blatt, 2017).

There are other components to this basic system. A study showed there is an alternative Ca^{2+}-dependent pathway which is common but not absolutely required for stomatal closure (Huang *et al.*, 2019). Another protein GHR1 (Guard cell Hydrogen peroxide- Resistant1) was recently proposed to act as a scaffold joining together various proteins needed for stomatal closure (Tee, 2018). ABA action can also down-regulate AQPs activity and thus inhibit the inner leaf water transport, this may result in a hydraulic signal to the stomata (Shatil-Cohen *et al.*, 2011), or on the contrary, ABA and OST1 can phosphorylate the Arabidopsis aquaporin AtPIP2:1 which increases water permeability in guard cells (Grondin *et al.*, 2015). This same aquaporin is known to be implicated in H_2O_2 (Rodrigues *et al.*, 2017) and CO_2 (Wang *et al.*, 2016) guard cell closure. Another intrinsic volatile involved in guard cell regulation which is nitric oxide (NO), can inactivate the inward

rectifier K^+ channel via a cGMP/cADPR-dependent increase of cytoplasmic Ca^{2+}, and induces the production of the lipid second messenger phosphatidic acid (Laxalt *et al.*, 2016). NO can also function as a blocker of the ABA-induced stomata closure, by post-translational modifications of key components of the cascade (Laxalt *et al.*, 2016). It has also been speculated upon to be transported by some animal aquaporins (Wang & Tajkhorshid, 2010). In conclusion, stomata require an intricate control that allow relatively quick opening and closing to respond to changing water availability, atmospheric conditions (humidity) and presence of pathogens.

1.2.2.3. Response of plants to increased evaporative demand

Focusing on soil water content represents a single dimension of how plants experience drought stress since changes in ambient humidity can also affect stomatal movements (Sussmilch & McAdam, 2017). As an indicator of the evaporative potential of the air, the vapour pressure deficit (VPD) represents the difference between the saturation vapour pressure and the actual vapour pressure at a given temperature (Monteith & Unsworth, 1990). As it takes account of both temperature and humidity, a decrease in air relative humidity leads to increased VPD and in response, the stomatal aperture decreases to restrict water loss and prevent desiccation (McAdam *et al.*, 2016; Novick *et al.*, 2016), even when the soil water content is not limiting (Sulman *et al.*, 2016). During the day, VPD naturally increases normally and can induce a transient stomatal closure associated with reduced net photosynthesis rate around midday (i.e. when VPD values are the largest) (Scoffoni *et al.*, 2017). However, how stomata sense changes in humidity is still based on assumptions. Thus, it is important to consider an altered vapour pressure deficit (VPD) induced by the closure of stomata in water stressed plants that could potentially have an impact on the stomatal regulations of nearby unstressed plants.

1.3. Plant volatiles

1.3.1. Plant volatile signalling

1.3.1.1. Biosynthesis

Plant volatile compounds are a large class of chemicals with about 1,700 organic substances discovered (Knudsen *et al.*, 2006). It is estimated that 1,000 Tg (teragram, 10^{12} gram) of volatile organic compounds per year are released (Junker, 2016). However, not every plant has the same profile, abundance or emission rate

(Vivaldo *et al.*, 2017). Thus, the most studied organic and inorganic volatiles for plant signalling will be described in this review (Figure 3).

Volatile organic compounds (VOCs) are low-molecular weight molecules, with a high vapour pressure at ambient temperature, that can represent about 10 % of the photosynthetic fixed carbon (Pickett & Khan, 2016). In flowers and roots, the site of biosynthesis is in epidermal cells (Bergougnoux *et al.*, 2007; Dudareva *et al.*, 1996; Huang *et al.*, 2012), and in vegetative organs, it takes place in secretory cells of glandular trichomes on the leaf surface as well as in mesophyll cells (Gang *et al.*, 2001; Turner *et al.*, 2000). The VOC classification is not officially established, but can be divided into five major classes, designated by their biosynthesis pathways with i) terpenoids, ii) fatty acid derivatives, iii) phenylpropanoids and benzenoids, iv) non-aromatic amino acid derivatives, and v) others.

1.3.1.1.1. Terpenoids

Terpenoids such as isoprene, monoterpenes and sesquiterpenes are a large and highly diverse class of VOCs and constitute more than half of the total emission by plants (Pichersky & Raguso, 2018). Two compartmentally separated pathways are involved in their biosynthesis which are the methylerythritol phosphate (MEP) pathway, considered exclusively plastidic (Hsieh *et al.*, 2008) and the mevalonic (MVA) pathway with a subcellular localisation distributed between the cytosol, the endoplasmic reticulum and the peroxisomes (Simkin *et al.*, 2011) (Figure 3). Both pathways use isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) deriving from pyruvate and acetyl-coA to synthesise all terpenoids. In the MEP pathway, seven enzymatic reactions lead to the formation of isoprene (C5), hemiterpenes (C5), monoterpenes (C10) and diterpenes (C20). In the MVA pathway, six enzymatic steps are responsible for the synthesis of sesquiterpenes (C15). There is a metabolic cross talk between the two compartments as IPP can be exported from plastids into the cytosol and then be used in the MVA pathway. The major enzymes involved in terpenoid formation are the terpene synthases (TPSs). They are able to synthesise multiple products from a single prenyl diphosphate substrate (Degenhardt *et al.*, 2009). The TPS gene family has more than 100 members from different plant species with a third expressed in flowers and fruits, and is divided into 7 subfamilies, from TPSa to TPSg (Aubourg *et al.*, 2002). For example, the most well-known terpenoid is isoprene, or 2-methyl-1,3-butadiene, representing 50 % of the world-wide total volatile organic compound emission and is synthesised by a TPS isoprene synthase (ISPS) that belongs to the subgroup b of the class 1 plant family. Overall, terpenoids are extensively used by plants in defence responses but are also utilised for pharmaceutical and food industrial applications (Abbas *et al.*, 2017) and studied in grape berries affected by water stress that can lead to flavour/aroma profile modifications in wine (Gambetta *et al.*, 2020).

1.3.1.1.2. Fatty acid derivatives

Fatty acid derivatives are also referred as lipoxygenase (LOX) products or C_6-compounds. Their biosynthesis takes place in the chloroplast and relies on the pool of acetyl-coA and C_{18} unsaturated fatty acids, such as linoleic or linolenic acids (Figure 3). It begins with the release of fatty acids from the chloroplast into the cytosol by lipoxygenases, followed by the stereospecific oxygenation of the unsaturated fatty acid to form 9-hydroperoxy and 13-hydroperoxy intermediates. In the next step, those products are metabolised via two distinct branches, the allene oxide synthase branch and the hydroperoxide lyase branch. The first one only uses 13-hydroperoxy intermediates to produce jasmonic acids, which in turn is converted into methyl jasmonate (MeJA). The second branch converts 9- and 13-hydroperoxy intermediates to C_6 and C_9 aldehydes, usually called green leaf volatiles (GLVs), which can be reduced to alcohols and esters. They are known to be implicated in herbivores interactions, defence priming, abiotic stress gene activation and have antimicrobial properties (Ameye *et al.*, 2018). For example, MeJA is often used to mimic a pathogen attack (Jiang *et al.*, 2017), and cis-Jasmone and (*Z*)-3-hexenol are also commonly studied as plant signals (Bruce *et al.*, 2008; Engelberth *et al.*, 2013; Farag *et al.*, 2005; Sugimoto *et al.*, 2014).

1.3.1.1.3. Phenylpropanoids and benzenoids

Phenylpropanoids and benzenoids are the second largest class of VOCs and function primarily for pollinator attraction (Schiestl, 2010). They all derive from L-Phenylalanine (L-Phe), which is synthesised in plastids through the Shikimate pathway (Figure 3). It involves seven enzymatic steps depending on the pool of phosphoenol pyruvate (PEP) and erythrose 4-phosphate (E4P). The volatile biosynthesis occurs in the cytosol by forming benzenoids (C_6-C_1), phenylpropenes (C_6-C_3) and phenylpropanoids-related compounds (C_6-C_2) (Vogt, 2010). Eugenol, chavicol or phenylacetaldehyde are common examples of studied plant signals (Cheng *et al.*, 2016; Gang *et al.*, 2001).

1.3.1.1.4. Non-aromatic amino acid derivatives

Non-aromatic amino acid derivatives abound in floral scent and fruit aromas. Their biosynthesis derives from amino acid containing nitrogen and sulphur, like alanine, valine, isoleucine and methionine. After their deamination or transamination into alpha-keto acids, they undergo different cleavage reactions, such as decarboxylation, reduction, oxidation or esterification, to finally form aldehydes, acids, alcohols and esters (Dudareva *et al.*, 2013) (Figure 3). For example, 2-methylpropyl acetate that originates from valine, and 3-methylthiopropionate and 3-(methylthio)propylacetate that derive from methionine can be found in the aroma volatiles of cucumber (Gonda *et al.*, 2010).

1.3.1.1.5. Others compounds (methanol, ethanol, acetaldehyde, ethylene, nitric oxide, ammonia)

Other important compounds that do not fit into the previous classes include methanol, ethanol, acetaldehyde, ethylene, nitric oxide and others (e.g. formaldehyde, acetone) (Figure 3). Pathways of synthesis of these compounds are well known but, in some cases, their function remains unclear.

Methanol (MeOH; CH_3OH) is a compound with a Henry's law constant of 0.46 $Pa.m^3.mol^{-1}$ at 25°C, making it highly soluble in water. It is emitted by plants with concentrations ranging up to several tens of ppb (parts per billion) (Jacob *et al.*, 2005) and is estimated to represent 0.11-0.16 % of photosynthetically fixed carbon (Macdonald & Fall, 1993). Methanol synthesis occurs in the degradation and formation of cell walls (Gaffe *et al.*, 1994) that forms a matrix composed of rhamnogalacturonan I, rhamnogalacturonan II and homogalacturonan (HG) (Figure 3). HG is a major pectic polymer composed of alpha-1,4-limked galacturonic acids, highly methyl-esterified and is selectively de-methyl-esterified by pectin methylesterases (PMEs) (Dorokhov *et al.*, 2015) which is encoded by a multigenic family (67 putative isoforms in *Arabidopsis thaliana*) (Wang *et al.*, 2013). The conversion of HG methoxyl groups into carboxyl groups results in methanol release and is triggered during changes in cell wall structures, seed maturation, fruit ripening, leaf expansion and mechanical or herbivore wounding (Dorokhov *et al.*, 2018).

Ethanol is synthesised in higher plants via alcoholic fermentation in the cytoplasm through the combined action of pyruvate decarboxylase and alcohol dehydrogenase (Kreuzwieser *et al.*, 1999). It was found to be emitted during flooding and during fast light-to-dark changes (Holzinger *et al.*, 2000).

Acetaldehyde derives from ethanol, which is oxidised to acetaldehyde by alcohol dehydrogenase (ADH) (Kreuzwieser *et al.*, 1999). However, only a small portion is emitted and the remainder is metabolised to acetate and acetyl-coA. Another pathway has been proposed involving the conversion of excess cytosolic pyruvate into acetaldehyde (Loreto & Schnitzler, 2010). Acetaldehyde has been shown to be emitted by plants in diverse conditions (Jud *et al.*, 2016; Rissanen *et al.*, 2020).

Ethylene is synthetised in all tissues by the conversion of S-adenosyl-L-methionine to 1-amino cyclopropane-a-carboxylic acid, and then converted to ethylene (Xu & Zhang, 2014). It is produced during germination, fruit ripening, senescence and is induced by drought, anoxia, mechanical and herbivory damage (Baldwin & Schultz, 1983; Broekgaarden *et al.*, 2015; Kazan, 2015).

Nitric oxide biosynthesis is known to be carried out through the conversion of nitrate/nitrite with different enzymes (e.g. nitrate reductase, nitrite:NO reductase, xanthine oxidase) present as cytosolic forms and as

plasma membrane-bound forms, as well as through non-enzymatic mechanisms (Procházková *et al.*, 2014). NO has been described as an endogenous signalling molecule linked to stomatal closure and abiotic stress (Laxalt *et al.*, 2016). The emission of NO was found in response to ozone, with a different profile from young and mature leaves (Bison *et al.*, 2017).

Ammonia (NH_3) formation in leaves is linked to four different processes. The first and largest source is photorespiration, which takes place in the mitochondria by releasing NH_3 during the decarboxylation of glycine (Keys *et al.*, 1978). The second is the nitrate/nitrite conversion liberating NH_3. The third is the lignin biosynthesis pathway happening in the apoplast (Nakashima *et al.*, 1997), and finally NH_3 is released in the cytosol during protein degradation and amino acid deamination (Olea *et al.*, 2004). Its emission was found to be correlated to leaf fall (Hansen *et al.*, 2013), photorespiration (Kumagai *et al.*, 2011) and seems to exponentially rise with an increase in temperature (Dusenge *et al.*, 2019; Husted & Schjoerring, 1996).

There are many more other volatile compounds emitted by plants but those will be further described in context with any literature and in the relevant Chapters.

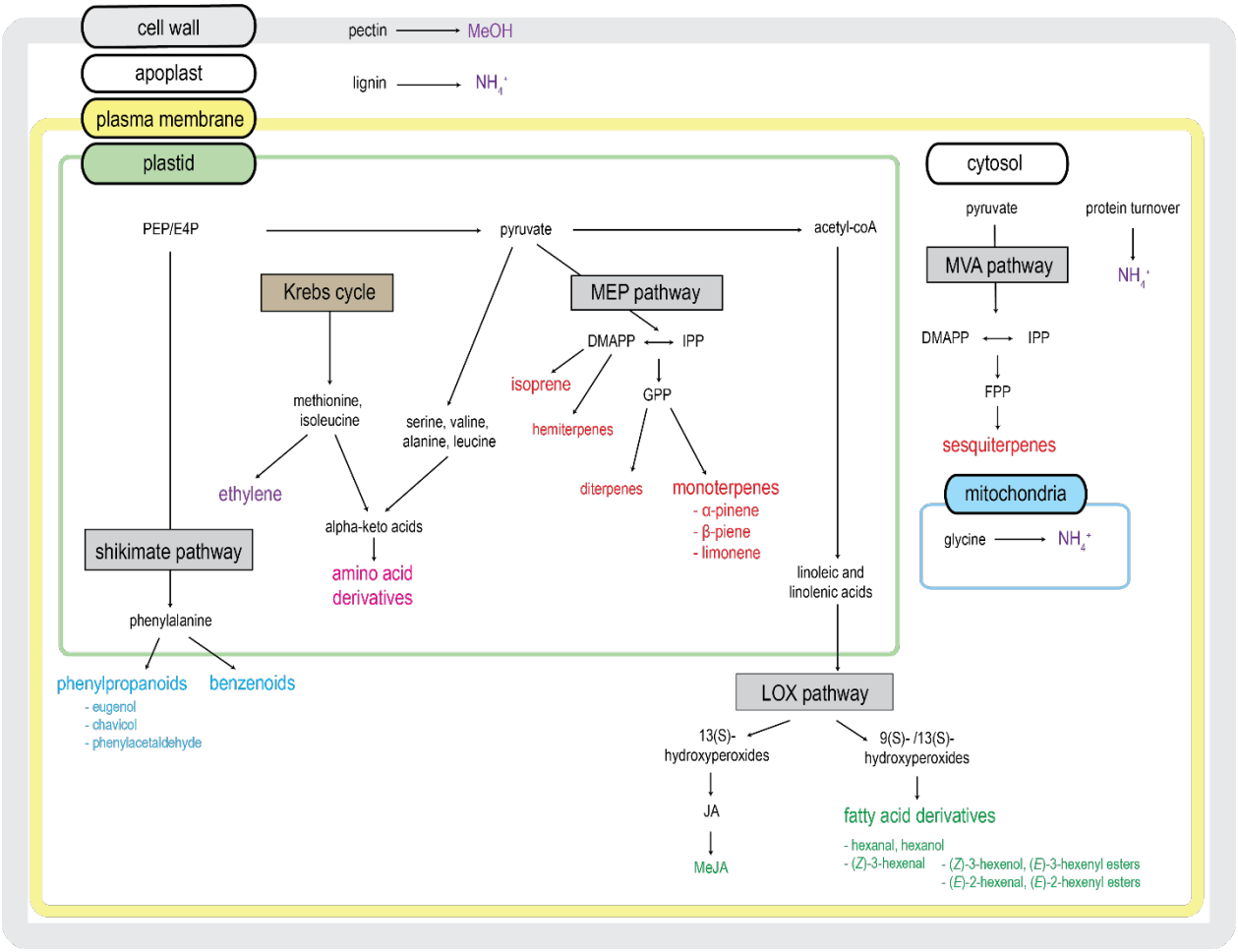


Figure 3. Biosynthesis of the major plant volatile compound families. Terpenoids (red) are synthesised in the plastid though the methylerythritol phosphate (MEP) pathway and in the cytosol through the mevalonic (MVA) pathway, fatty acid derivatives (green) in the cytosol through the lipoxygenase (LOX) pathway, phenylpropanoids (blue) in the cytosol through the shikimate pathway, and amino acid derivatives (pink) in the plastid (Dudareva, *et al.*, 2013; Hsieh *et al.*, 2008; Vogt, 2010). Abbreviations: DMAPP, dimethylallyl pyrophosphate; FPP, farnesyl pyrophosphate; GPP, geranyl pyrophosphate; IPP, isopentenyl pyrophosphate; JA, jasmonate; PEP/E4P, phosphoenolpyruvate/erythrose-4-phosphate;MeJA, methyl jasmonate; MeOH, methanol; NH_4^+, ammonia.

1.3.1.2. Storage

The main volatile storage structures in vascular plants are glandular trichomes, resin ducts and cavities, and vacuoles (Cna'ani *et al.*, 2017; Gershenzon *et al.*, 2000). Glandular trichomes are extra-cellular compartments present in Laminaceae, Asteraceae, Geraniaceae, Solanaceae and Cannabinaceae, on the surface of leaves, flowers and seeds. There are two types of trichomes, the capitate glandular trichomes that exude resinous material and the peltate glandular trichomes that produce and store volatile compounds, mainly terpenoids and phenylpropanoids. There are different types of peltate trichomes but they all are composed of a basal epidermal cell, short stalk cells, secretory cells and a storage space covered by a cuticle (Lange, 2015). Resin ducts and cavities are situated deep in plant tissues, in the intercellular spaces lined by an epithelium of secretory cells. Ducts are present in Pinaceae, Myrtaceae, Asteraceae, Umbelliferae and Leguminosae; and cavities in Rutacece, Clusiaceac, Myrtaceae (Gershenzon *et al.*, 2000). Glandular trichomes are known to be involved in plant pathogen defence since the content of the trichomes is exuded after contact with an insect (Lange, 2015), but recently those trichomes have been shown to also be a protective barrier against ozone stress linked with their density and the emission of LOX products (Li *et al.*, 2018). However, the mechanisms involved in the transport from biosynthesis sites and storage of volatile compounds in glandular trichomes and resin ducts are still unclear. Adebesin *et al.* (2017) showed that a plasma-membrane ATP-binding cassette (ABC) transporter in petunia flowers is involved in the floral volatile emission, but how those volatiles cross the cell wall and the cuticle are still unknown. Tissier *et al.* (2017) discussed the possible role of lipid transfer proteins (LTPs) to help cross the cell wall as LTP genes were shown to be highly expressed in glandular trichomes of tobacco (Harada *et al.*, 2010). However, the role of cuticle and cell wall in volatile emission remains unknown. Finally, plants can also temporarily store volatiles in vacuoles and in the leaf intercellular air space before emission and this will be discussed further in the next section.

1.3.1.3. Emission

Once the volatiles, sequestered in storage structures, reach the cuticle of the plant leaf, they are thought to diffuse and volatilise into the atmosphere (Tissier *et al.*, 2017). Indeed, any gas diffusing into or out of a leaf obeys Fick's first law, which means that the flux is proportional to the concentration difference between the leaf intercellular air space and the air outside the leaf boundary layer, and inversely proportional to the sum of the aggregate resistances between them (Harley, 2013). However, stomatal regulation seems to be involved in the emission of volatiles through passive diffusion (Widhalm *et al.*, 2015). Stomata are crucial for the uptake of CO_2 and the efflux of water vapour, and were found to play a role in the regulation of volatile

emission as well. Indeed, the change in stomata aperture, controlled by the guard cells and that occurs in response to environmental changes such as light or heat, showed changes in the rate of emission of some VOCs (Niinemets *et al.*, 2004; Niinemets *et al.*, 2002; Seidl-Adams *et al.*, 2014). Moreover, the emissions of methanol, acetone and acetaldehyde were shown to be correlated with transpiration and thus stomata regulation in *Pinus sylvestris* (Rissanen *et al.*, 2018). However, some VOC emissions, such as isoprene, α-pinene, linalool or 1,8-cineole were shown not to be controlled by stomata (Harley, 2013), even if the cuticular resistance is higher than stomatal resistance (Nobel, 2009). To explain this phenomenon, it was proposed that the susceptibility of a chemical to stomatal regulation was directly related to its Henry's law constant (*H*, $Pa.m^3.mol^{-1}$) (Niinemets *et al.*, 2002). This law determines the partitioning of a volatile between the liquid and the vapour phases. For example, isoprene is highly hydrophobic (*H* = 7,780 $Pa.m^3.mol^{-1}$) and thus, when newly produced, almost entirely partitions to the gas phase. On the contrary, methanol is very soluble (*H* = 0.461 $Pa.m^3.mol^{-1}$) and partitions strongly to the aqueous phase constituting temporary storage pools.

Direct emission of volatiles has been measured in many studies and varies under different biotic and abiotic conditions. In addition, VOC profiles are specific to species and development-stage. For example, the emission, composition and quantity may change during flowering (Pichersky & Gershenzon, 2002), fruit ripening (Taiti *et al.*, 2015) and leaf expansion (Portillo-Estrada *et al.*, 2017). There are two different kinds of volatile emissions, first, the constitutive emission and second, the stress-induced emission that can be stimulated, quenched or can induce *de novo* volatile synthesis. Also, plants can regulate their volatile emission based on a diurnal rhythm, as shown in fig species that are able to change the composition of the scent between sunrise and noon in order to attract distinct pollinator species (Conchou *et al.*, 2014). Or in cork oak trees, VOCs rapidly increase in the morning, associated with temperature and solar radiation, peak in the middle of the day and decrease during the afternoon and evening (Pio *et al.*, 2005). In normal conditions, a peak of methanol in the morning coincides with the increase in stomatal conductance, to then slowly decrease during the day (Hüve *et al.*, 2007). Moreover, several studies found that young and expending leaves seem to have higher emission rates than mature leaves (Galbally & Kirstine, 2002; Oikawa *et al.*, 2011). In terms of spatial distribution, few studies have investigated distances over which these cues are exchanged at effective concentrations (Huber & Bauerle, 2016). Lima bean emitter plants were found to induce a resistance against pathogen effect up to 50 cm (Heil & Adame-Álvarez, 2010) and sagebrush up to 60 cm (Karban *et al.*, 2006). Many gaps remain in understanding how volatiles are emitted from plants and more specific transporters are likely to be discovered as stomata seems to not be the only exit pathway.

1.3.1.4. Reception and signalling cascade

The way that plants sense volatiles remains an obscure part of the signalling pathway. As for the emission, how volatiles can cross the cuticle and cell walls to enter cells relies on open stomata and possible receptors. For example, ethylene is known to diffuse across membranes into nearby cells and tissues and has its receptor on the endoplasmic reticulum (ERT1-2, ERS1-2, EIN4 in *Arabidopsis thaliana*) (Bleecker *et al.*, 1988; Broekgaarden *et al.*, 2015). And the coronative-insensitive 1 (COI1) is known to be a jasmonate receptor (Dar, Uddin, Khan, Hakeem, & Jaleel, 2015).

After the reception, it has been shown that the direct action of volatiles on plants can induce early and late defence-associated responses. Rapid plasma membrane depolarisation and calcium fluxes were shown to be triggered by herbivore-induced GLVs (Zebelo *et al.*, 2012), as well as the activation of mitogen-activated protein kinases (MAPKs) (Dombrowski & Martin, 2018). These early signalling events are known to be involved in the activation of specific genes (Boller & Felix, 2009). For instance, several genes were induced by the GLV (*Z*)-3-hexenol in maize (Farag *et al.*, 2005) and by terpenoids in lima beans (Arimura *et al.*, 2000). Just as for the emission of volatiles, the reception cascade has missing links, and not only stomata are entry gates but receptors are also likely pathways.

1.3.1.5. Volatile analysis methods

Many analytical techniques to study plant volatile emission have been described in the literature (Tholl *et al.*, 2006). One of them, the dynamic head-space sampling method, has been applied to either the individual plant (Bourtsoukidis *et al.*, 2014; Ton *et al.*, 2007) or groups of plants by using separate greenhouses (Caparrotta *et al.*, 2018), as well as *in vitro* experiments (Algarra Alarcon *et al.*, 2015; Durenne *et al.*, 2018). It is also possible to sample enclosed parts of the plants to investigate leaf emission (Sharkey & Loreto, 1993) or branch emission (Saunier *et al.*, 2017).

Identification and quantification of emitted volatile also comprise various methods. Tholl *et al.* (2006) has reviewed the online analysis by proton transfer reaction-mass spectrometry (PTR-MS) and several types of volatile traps coupled with gas chromatography-mass spectrometry (GC-MS). Those methods can be used together, for instance, applying the PTR-MS online to measure methanol, acetaldehyde, ethanol, isoprene and in parallel employing the use of TenaxTA-filled thermal desorption tubes (TDS) with GC-MS which is highly efficient for terpenoids and C_6-coumpounds. The TDS tubes can trap volatiles from air flowing inside of them using suction pumps and then are desorbed into a thermal desorption unit and cryo-focussed onto a GC-MS system. For example, this method permitted the identification of cyclic terpene compounds such as

limonene, terpinolene and β-pinene and other compounds such as β-ocimene and β-caryophyllene in oak (Bourtsoukidis *et al.*, 2014). However, an increasing number of studies have adopted solid-phase micro-extraction (SPME) methods with fibres of different coatings offering alternative selectivity to sample volatile compounds (Vallarino *et al.*, 2018).

New methods are also emerging, as introduced in Table 1 with, for example, ion mobility spectrometry (IMS) coupled to gas chromatography for a continuous monitoring of plant volatile organic compounds (Vautz *et al.*, 2018); direct analysis in real time (DART) mass spectrometry (Maleknia *et al.*, 2009); direct contact sorptive extraction (DCSE) by using a polydimethylsiloxane (PDMS) coated magnetic stir bar (Twister) (Kfoury *et al.*, 2017); or molecularly imprinted sol gels (MISGs) – based localised surface plasmon resonance (LSPR) for the detection of cis-jasmone (Shang *et al.*, 2018).

Table 1. Review of current methods to sample and analyse plant-emitted volatile compounds.

Volatile sampling methods		**Compound detected**	**Plant species**	**References**
Proton Transfer – Mass Spectrometry (PTR-MS)		Isoprene	Poplar; Amazonian forest; oak	(Bracho-Nunez *et al.*, 2012; Fares *et al.*, 2010; Jud *et al.*, 2016; Saunier *et al.*, 2017)
			Poplar; Amazonian forest; oak	(Bracho-Nunez *et al.*, 2012; Fares *et al.*, 2010; Saunier *et al.*, 2017)
			Poplar	(Fares *et al.*, 2010)
			Amazonian forest	(Bracho-Nunez *et al.*, 2012)
			Amazonian forest; poplar	(Bracho-Nunez *et al.*, 2012; Jud *et al.*, 2016)
			Amazonian forest	(Bracho-Nunez *et al.*, 2012)
			Poplar	(Jud *et al.*, 2016)
		Methacrolein, methylvinylketone, isoprene hydroxy hydroperoxide	Oak	(Saunier *et al.*, 2017)
Photoionization detection (PID) system		Isoprene	Oak	(Geron *et al.*, 2016)
Adsorbent cartridge	Tenax (TA) Carbotrap Carbopack SuperQ Sulficarb Carbograph Carboxen	Monoterpenes	Beech; tomato; silver birch; Norway spruce; lima bean; alder; parsley; pine; oak; rosemary; aspen; croton; sweet chestnut; fava bean	(Bison *et al.*, 2017; Copolovici *et al.*, 2012; Copolovici *et al.*, 2014; Geron *et al.*, 2016; Hartikainen *et al.*, 2012; Kivimäenpää *et al.*, 2013; Llusia *et al.*, 2015; Lüpke *et al.*, 2017; Maja *et al.*, 2016; Nogués *et al.*, 2015; Salerno *et al.*, 2017; Šimpraga *et al.*, 2011; Soran *et al.*, 2014; Souza *et al.*, 2013; Tomescu *et al.*, 2017)
		Sesquiterpenes	Tomato; silver birch; Norway spruce; alder; pine; oak; rosemary; Brussel sprout; croton; fava bean	(Bison *et al.*, 2017; Copolovici *et al.*, 2012; Copolovici *et al.*, 2014; Hartikainen *et al.*, 2012; Kivimäenpää *et al.*, 2013; Llusia *et al.*, 2015; Nogués *et al.*, 2015; Salerno *et al.*, 2017; Weldegergis *et al.*, 2015)

		C_6 compounds / GLV / LOX products / fatty acid derivatives	Beech; tomato; silver birch; maize; Norway spruce; lima bean; alder; parsley; Brussels sprouts; aspen; fava bean; tomato	(Copolovici *et al.*, 2012; Copolovici *et al.*, 2014; Hartikainen *et al.*, 2012; Kivimäenpää *et al.*, 2013; Maja *et al.*, 2016; Salerno *et al.*, 2017; Šimpraga *et al.*, 2011; Soran *et al.*, 2014; Souza *et al.*, 2013; Tomescu *et al.*, 2017; Weldegergis *et al.*, 2015; Winter *et al.*, 2012)
		Methyl salicylate	Silver birch; Norway spruce	(Hartikainen *et al.*, 2012; Kivimäenpää *et al.*, 2013)
		Benzenoids	Croton	(Bison *et al.*, 2017)
		Nitriles	Brussels sprout	(Weldegergis *et al.*, 2015)
		Isoprene	Aspen; poplar	(Maja *et al.*, 2016; Yuan *et al.*, 2016)
Solid-phase micro-extraction (SPME)	Divinylbenzene/ carboxen/ Polydimethyl siloxane (DVB/CAR/PDMS)	Monoterpenes	Poplar; grapevine; *Helichrysum petiolare*; *Polygonum minus*; Douglas-fir tree	(Caser *et al.*, 2016; Gil *et al.*, 2013; Goh *et al.*, 2016; Junker *et al.*, 2017; Pellegrini *et al.*, 2012)
	Polydimethyl siloxane (PDMS)	Sesquiterpenes	Poplar	(Pellegrini *et al.*, 2012)
		C_9-C_{15} straight-chain aldehydes	Poplar	(Pellegrini *et al.*, 2012)
		C_{12}-C_{16} aliphatic hydrocarbons	Poplar	(Pellegrini *et al.*, 2012)
		C_6 compounds	Poplar; grapevine; *Helichrysum petiolare*; *Polygonum minus*	(Caser *et al.*, 2016; Goh *et al.*, 2016; Griesser *et al.*, 2015; Pellegrini *et al.*, 2012)
		Alcohol	Grapevine; fava bean	(Gil *et al.*, 2013; Salerno *et al.*, 2017)
		Ketone	Grapevine;	(Gil *et al.*, 2013)
		Phenylpropanoids	*Helichrysum petiolare*	(Caser *et al.*, 2016)
Transcriptomic and metabolomics profiling		Terpenoid	Grapevine	(Savoi *et al.*, 2016)
Ion mobility spectrometry (IMS) - GC		Terpene	Herbaceous plant species from Central Europe	(Vautz *et al.*, 2018)
Direct analysis in real time (DART) time-of-flight (TOF) mass spectrometry		Monoterpenes; sesquiterpenes; flavonoids; methanol; acetone	Eucalypts	(Maleknia *et al.*, 2009)
Direct contact sorptive extraction (DCSE)	Polydimethylsiloxane coated stir bars (Twisters)	Idem Tenax but more sensitivity	Tea plant	(Kfoury *et al.*, 2017)
Molecularly imprinted sol gels (MISGs) - based localised surface plasmon resonance (LSPR)		cis-Jasmone	-	(Shang *et al.*, 2018)

However, each method has its limitations, including the class and chain length of volatiles being able to be detected, the concentration at which they need to be measured as well as sensitivity and reliability issues. Solid-phase micro-extraction (SPME) is used to sample chemicals in food, beverages, flavours, forensics, and environmental volatiles, it allows compounds from C_3 to C_{20} to be detected and is highly effective for trapping terpenoids. SPME fibres are coated with an extraction phase comprising adsorptive particles embedded in a polymer. The advantages of SPME fibres are that they are solvent free, they can be automated, reusable, inexpensive and non-destructive to the sample. The selection of coatings is important to consider depending on the physical and chemical properties of the compounds to analyse, usually based on molecular weight (MW). For example, Carboxen/Polydimethylsiloxane (CAR/PDMS) fibres work well for low MW and highly volatile compounds. The macro- and mesoporous Divinylbenzene (DVB) is suited for higher MW compounds. The type of analyte includes gases and low MW amines, nitro-aromatic, polar semi-volatile, non-polar high MW, non-polar semi-volatile and alcohol compounds. After sampling, the analytes concentrated on the fibre are directly thermally desorbed in the GC-MS injector and transferred rapidly to the column. In comparison, PTR-MS allows the continuous detection of targeted compounds and adsorbent cartridges have been efficient to quantify volatile emissions. In conclusion, despite there being numerous techniques to trap and analyse volatiles described in the literature, to date there is not a perfect technique that covers the identification of the whole spectrum of volatiles and emission patterns of plants, thus multiple methods need to be applied or new methods must be developed and standardised (Lüpke *et al.*, 2017).

1.4. Plant volatile-induced responses

1.4.1. Effect of biotic and abiotic stresses on the emission of plant volatiles

Volatiles act as signal molecules for plants triggered by their environment stimuli (Frost *et al.*, 2008). Indeed, plants are confronted with a myriad of dangers including herbivore insects, bacteria or viruses, generally referred to as biotic stress (Verma *et al.*, 2016). Through evolutionary selection pressures, plants have developed various defence mechanisms including physical barriers (cell walls or cuticle) or chemical barriers (hydrolytic enzymes or antimicrobial compounds) (Boller & Felix, 2009). Plants can also emit specific volatiles to enhance the neighbours' level of resistance. For instance, mechanical wounding caused by herbivores was shown to induce VOC emission from the damaged plants, which attracted natural enemies of the herbivores (Yoneya & Takabayashi, 2014). When the cellular content from a cut becomes exposed to the atmosphere, broken cell walls, cytoplasmic and vacuolar contents are subject to air oxidation, triggering the action of

enzymes and, for example, subsequent emission of ethanol and LOX pathway products (Portillo-Estrada & Niinemets, 2018). In this same study, it was also shown that cuts through major veins lead to much greater release of volatiles than through intercostal areas. Many other examples of systems to study the emission of volatiles from biotic damage can be found in the literature, they showed the emission of methanol, LOX pathway volatiles, acetaldehyde or terpenes (Brilli et al., 2012; Mithöfer *et al.*, 2005; Rasulov *et al.*, 2019). Treatment with trans-2-pentenal showed significant results in the reduction of fungal disease severity and development (Lazazzara *et al.*, 2018). Priming of plant defence has also been shown to be mediated by volatiles (Engelberth *et al.*, 2004; Kessler *et al.*, 2006; Peng *et al.*, 2011; Ton *et al.*, 2007). For example, MeSA was shown to induce the systemic acquired resistance and priming of defence when applied repeatedly (Song & Ryu, 2018). And hexenol esters induced a closure of stomata preventing the propagation of *Pseudomonas synrigae* inside the leaves (López-Gresa *et al.*, 2018). It is important to note that one single volatile, as (*Z*)-3-hexenol, can induce defence responses (Sugimoto *et al.*, 2014), but sometimes a mixture of volatiles is required to have an effect (Pichersky & Raguso, 2018).

Volatiles may not just work as signals; indeed, under abiotic stress, i.e. stresses related to excessive heat, light, ozone or drought, they have been also described as self-protective (Loreto & Schnitzler, 2010). Indeed, because of their antioxidant attributes and by protecting plant membranes, volatiles can improve plant resistance. For instance, isoprene, the most widely studied VOC, can induce thermotolerance by stabilising chloroplastic membranes during heat stress (Possell & Loreto, 2013). Its presence was found in the structure organisation of plastid membranes in poplar (Velikova *et al.*, 2015) and it was also shown to maintain PSII stability by providing a more stable and homogeneous distribution of the light-absorbing centres and stabilise thylakoid membrane stiffness during heat stress (Pollastri *et al.*, 2019). However, some authors are refuting this idea since the normal concentration of isoprene would be too low to affect the membrane fluidity and isoprene is actually acting through changing the expression of many gene networks involved in stress response and plant growth (Harvey *et al.*, 2015; Zuo *et al.*, 2019). Also, volatiles like sesquiterpenes can scavenge reactive oxygen species to moderate oxidative stress independently of the type of abiotic stress (Vickers *et al.*, 2009). Interestingly, a study on salt stress showed the priming effects of stressed-plants when placed in the same environment with non-stressed plants, indeed, the non-stressed plants showed improved tolerance to salt stress, presumed to be via the exchange of airborne cues (Caparrotta *et al.*, 2018). Table 2 describes some of the main families of VOCs with specific compounds and the impact of several abiotic stresses on emissions compared with non-stressed plants. Although the chemical nature and quantity of emitted volatiles are plant- and stress-specific, it appears that flood, heat and cold generally tend to induce an increase of VOC emissions while drought stress leads to lower emissions.

Table 2. VOC emission variations induced by different abiotic stresses on different plant species. Arrows indicate if the emission increased (up) or decreased (down).

Family	VOC	Stress	Materials	VOC emission	References
Green leaf volatiles	acetaldehyde	Flood	*Quercus robur, Prunus serotina*	↗	(Bourtsoukidis *et al.*, 2014)
		Drought	*Quercus robur, Prunus serotina*	↘	(Bourtsoukidis *et al.*, 2014)
	(*Z*)-3-hexenol	Heat	*Solanum lycopersicum*	↗	(Copolovici *et al.*, 2012)
		Cold	*Solanum lycopersicum*	↗	(Copolovici *et al.*, 2012)
	methyl salicylate	Drought	*Quercus robur, Prunus serotina*	↗	(Bourtsoukidis *et al.*, 2014)
	isoprene	Light	*Pueraria lobata*	↗	(Sharkey & Loreto, 1993)
		Drought	*Pueraria lobata*	↘	(Sharkey & Loreto, 1993)
			Quercus pubescens	↘	(Saunier *et al.*, 2017)
			Populus alba	↘	(Brilli *et al.*, 2007)
			Quercus robur, Prunus serotina	↘	(Bourtsoukidis *et al.*, 2014)
		Heat	*Quercus rubra*	↘	(Singsaas & Sharkey, 2000)
Monoterpenes	linalool	Drought	*Nicotiana langsdorffii*	↘	(Di Carro, Ianni, & Magi, 2013)
	α-pinene	Drought	*Rosmarinus officinalis*	↘	(Nogués *et al.*, 2015)
		Drought	*Fagus sylvatica*	↗	(Šimpraga *et al.*, 2011)
	2-carene	Heat	*Solanum lycopersicum*	↗	(Copolovici *et al.*, 2012)
Sesquiterpenes	β-caryophyllene	Heat	*Solanum lycopersicum*	↗	(Copolovici *et al.*, 2012)
	α-farnesene	Ozone	*Picea abies*	↘	(Bourtsoukidis *et al.*, 2012)

To date, few studies have investigated VOC emissions under drought stress and most of these studies were conducted at the forest-scale (Possell & Loreto, 2013). However, a trend has been found showing a decrease in VOC emissions supposedly caused by two factors, stomatal closure and the reduction of photosynthesis altering *de novo* VOC synthesis (Bourtsoukidis *et al.*, 2014; Brilli *et al.*, 2007; Saunier *et al.*, 2017). In most of these studies, the VOCs investigated were limited to isoprene and terpenes from only a few plant species, therefore the results cannot be generalised to all plants and abiotic stresses. Moreover, drought stress is a complex parameter and includes different severity levels, which can lead to different responses. A severe stress seems to predominantly induce a decline of VOC emission rates, but a study on the effect of increasing drought on phenotypic plasticity of floral volatiles showed patterns of increase, decrease or both (Campbell *et al.*, 2018). In conclusion, as varied as the environmental stresses imposed to plants are, so are their volatile emission patterns and induced-responses too.

1.4.2. Hypothesis for plant communication via volatiles in drought/rehydration experiments

As it is clearly known that plants within the same environment, if attacked by predators, will emit volatiles to alert the surrounding plants, a similar phenomenon might be hypothesised for water deficit stress. Indeed, Table 3 reviews experiments in which stomatal conductance (g_s) was measured during drought and recovery of different plant species and showed that stomatal conductance of the well-watered controls often dropped

and recovered concomitantly with the water deficit treated plants, albeit less severely. For instance, this trend was observed in *Arabidopsis thaliana* (Scharwies, 2017) in Figure 4.

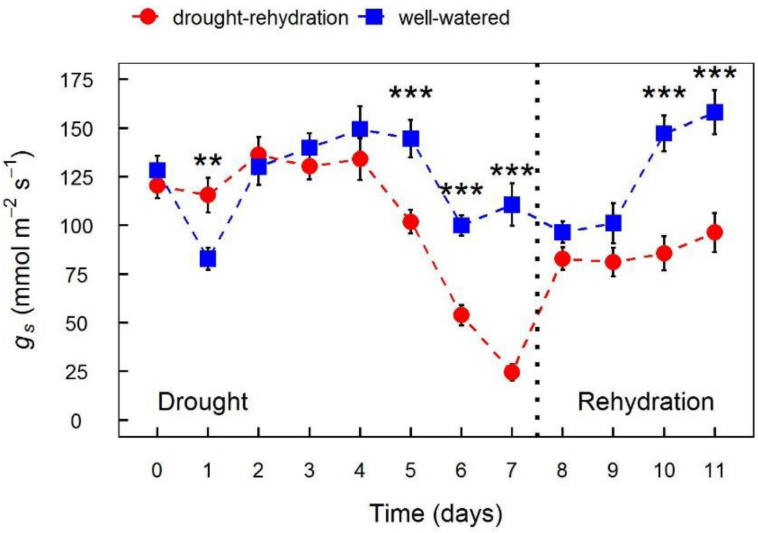


Figure 4. Stomatal conductance (g_s) in well-watered (blue squares) control group and drought-rehydration (red circles) groups during 7 days of stress and 4 days of re-watering (dashed line) in *Arabidopsis thaliana* Col0 leaves. Asterisks indicate significant differences within the treatment groups at P<0.001. Values are means of 5 leaves ± SE (Scharwies, 2017).

Many other examples can be found in the literature. A study conducted on potted grapevines in a glasshouse showed a decrease of stomatal conductance for the well-watered controls when placed in the same environment as the stressed plants (Dayer *et al.*, 2017). As expected, they observed a decrease of stomatal conductance for the stressed plants followed by an increase after re-watering. Meanwhile, the well-watered control plants also showed a reduction of g_s during the same period, followed by a recovery similar to the adjacent water-stressed grapevines. Using potted wheat in outdoor conditions, Zhou *et al.* (2015) found a significant drop of stomatal conductance in well-watered plants but the authors did not discuss this behaviour. Similarly, two other studies conducted on potted grapevines in a glasshouse also showed a decrease of g_s for the controls (Beis & Patakas, 2010; Martim *et al.*, 2009). Once again, the authors neglected to discuss this phenomenon in their results. Moreover, Sun *et al.* (2014) worked on potted olive plants in a glasshouse and the results showed that the g_s of the control plants varied over time between 100 mmol.m^{-2}.s^{-1} and 30 mmol.m^{-2}.s^{-1}, which is very low for well-watered plants and close to water-stressed g_s values, and then increased during recovery of the water-stressed plants. In particular, this concomitant increase of stomatal conductance in controls with the water deficit treated plants during the recovery period is intriguing (Cano *et al.*, 2014; Jackson *et al.*, 1995). While decreasing stomatal conductance of well-watered control plants during the

experiments could simply be an effect of aging, the simultaneous recovery indicates some form of common signal between control and treated plants. Normal changes in the environment, like variations in light, temperature, and humidity, could also be responsible for this behaviour. For example, fluctuation in stomatal conductance of well-watered control plants could be attributed to changes in light and temperature in the experiment by Zhang and Davies (1990) and to VPD in the experiment by Cai *et al.* (2015). However, many experiments showed the same trends and even experiments conducted outside with good air mixing showed a similar behaviour of stomatal reduction with subsequent recovery (Correia & Pereira, 1994; Zhou *et al.*, 2015). Thus, it is unlikely that a particular environment is needed to observe the behaviour.

Table 3. Overview of previously published studies in which stomatal conductance was measured in plants throughout drought and rehydration experiments.

References	Species	Environment	General observations
Correia and Pereira (1994)	*Lupinus albus* L.	Pots in field	Stomatal conductance of well-watered control plants showed significant reductions during the drought period of the experiments and recovery during the re-watering period (if applicable). Usually no environmental variables like light, temperature, and humidity were shown in the publication.
Jackson *et al.* (1995)	*Pinus sytvestris* L. and *Picea sitchensis* (Bong.) Carr.	Pots in greenhouse	
Martim *et al.* (2009)	*Vitis vinifera* L.	Pots in greenhouse	
Allario *et al.* (2013)	*Citrus sinensis* L. on *Citrus limonia* L. rootstock	Pots in greenhouse	
Cano *et al.* (2014)	*Eucalyptus dumosa* Cunn. Ex. Schauer. and *Eucalyptus pauciflora* Sieb. ex Spreng	Pots in greenhouse	
Zhou *et al.* (2015)	*Triticum aestivum* L.	Pots in field	
Dayer *et al.* (2017)	*Vitis vinifera* L.	Pots in greenhouse	
Wilson and Davies (1979)	*Sorghum bicolor* L.	Pots in growth chamber	Stomatal conductance of well-watered control plants declined only slightly during the drought period of the experiment and/or did not show a recovery during the re-watering period.
Galle *et al.* (2009)	*Nicotiana sylvestris* Speg. et Comes	Field and greenhouse	
Beis and Patakas (2010)	*Vitis vinifera* L.	Pots in rain shelter	

Taylor *et al.* (2011)	C3 and C4 grass species	Pots in growth chamber	
Zhang and Davies (1990)	*Zea mays* L.	Pots in greenhouse	Stomatal conductance of well-watered control plants responded mostly to changes in light and/or temperature and/or VPD.
Cai *et al.* (2015)	*Rhododendron delavayi* Franch.	Pots in greenhouse	
Zhang and Davies (1989)	*Zea mays* L.	Pots in greenhouse	Stomatal conductance of well-watered control plants did not show any change.
Hu *et al.* (2013)	*Poa pratensis* L.	Pots in growth chamber	

Together, these results indicate the potential of plants to exchange information about their water status to the surrounding plants to regulate stomata without direct contact. Plausible candidates of these information signals are volatile organic or inorganic compounds and constitute a promising hypothesis as airborne inter-plant signals related to drought stress.

1.5. Conclusion

This review of the literature on plant water regulation and plant volatile compounds reveals that there is a potential link between the emission of volatiles through stomata and drought stress. It is clear now that plant volatiles are key components of inter-plant signalling pathways and are significant actors in the plant defence system. In fact, volatiles can be compared to elicitors, also known as PAMPs (pathogen-associated molecular pattern) or DAMPs (damage-associated molecular pattern), but also priming molecules (Kessler *et al.*, 2006). Elicitors involve a direct recognition with contact of the pathogen, compared to VOC emission being an indirect recognition (Sharifi *et al.*, 2018).

Although there is evidence of a protective role of VOCs against herbivore attack, there is less evidence for protection against abiotic stress (Palmer-Young *et al.*, 2015). Moreover, the downstream signalling cascades from VOC detection require further investigation, as well as potentially more specific transporter and receptor proteins. Additionally, the timing of emission, and the spatial distribution are still unclear. Many questions are

still unanswered, with key ones like, how do plants perceive volatiles and via which mechanism is the signal transduced?

Even if there is little evidence of plant communication in the context of abiotic stress, there is a strong possibility that plants may exchange volatiles when they are under abiotic stress to "alert" surrounding plants. For example, some drought stress-related studies showed surprising results of control plants that change their physiology when they are in the same environment of stressed plants (reviewed above). Our hypothesis is that the plants sensing a water deficit will emit one or a blend of volatiles which are detected by nearby plants and triggering a closure of stomata. This hypothesis would open a new vision of plants that change their sessile condition to a highly dynamic system comprising a form of inter-signalling. Volatile compounds could thus be used to optimise agricultural practices. For example, they could be used to protect cropping systems (outdoor and glasshouse production (Jansen *et al.*, 2009)) by using sentinel plants (Pickett & Khan, 2016), or by using volatiles as markers of stress to diagnose the physiological state of a plant and increase the performance of treatments, fertilisation or irrigation (Niederbacher *et al.*, 2015).

1.6. Research questions and aims

From the literature review, our current knowledge about water stress coupled with volatiles (biosynthesis, emission, reception) is insufficient to explain the multiple observations of well-watered plants having the same physiological response as water stressed plants. Multiple plant species have indicated this signalling (Zhou *et al.*, 2015), including *Vitis vinifera* (Dayer *et al.*, 2020). Since it is an important horticultural plant to the Australian economy and because they have contrasting responses to water stress (Vandeleur *et al.*, 2009), Grenache, Chardonnay and Shiraz were selected. In addition, Arabidopsis was used as it has the advantage of being a model plant with ease of genetic transformation to probe the basis of volatile signalling.

The research aims are as follows:

- What are the volatile compounds emitted from grapevines under well-watered, compared to drought stress conditions, and what are their identities and emission rates under both conditions?
- Do well-watered grapevine and Arabidopsis plants perceive and respond to volatile signals from drought-stressed neighbours? Is the response affecting the regulation of stomata?
- Can a simple technique be developed to test the effect of potential volatile signals on leaf transpiration?

1.6.1. Plant volatile and physiology responses in drought/rehydration experiments in *Vitis vinifera*

Irregularities in drought/rehydration experiments showing a decrease of stomatal conductance in well-watered plants lead to the hypothesis of plant communication via volatiles. However, those studies were not sampling volatile compounds. Moreover, most studies investigating the effect of drought on volatile emissions were not separating the treatment groups and were not specifically looking for potential signals or other environmental cues that can influence both control and drought-stressed plants. Therefore, the aims are as follows:

- Adding volatile sampling and analysis methods to standard drought-rehydration experiments with measurements of plant physiological parameters
- Develop an experimental system to detect and quantify the emission of volatiles from individual potted grapevines either well-watered or water-stressed to determine their emission profiles while monitoring simultaneously leaf gas exchange variations

1.6.2. Plant volatile and physiology responses in drought stress experiments in *Arabidopsis thaliana*

Similar to the aims described in section 1.6.1. for *Vitis vinifera* and because *Arabidopsis thaliana* has different growing conditions, this study had the following aims:

- Adding volatile sampling and analysis methods to standard drought-rehydration experiments with measurements of plant physiological parameters
- Adding a separate control treatment by using individual growth chambers

1.6.3. Effect of volatiles on stomatal conductance

Studying whole plant response to volatiles has many difficulties and biases as current methods focus on building whole plant chambers or use photosynthetic leaf chamber to measure the emission of volatiles from a leaf while still attached to the plant. These create small artificial environments where slight changes in factors such as temperature or humidity would significantly affect physiological responses. Also, chambers used for photosynthesis measurements are connected to an array of tubing and valves that can potentially affect the measurement of volatile emissions. The following research goals therefore arise:

- Develop a single leaf measurement method to specifically study the effect of volatiles on stomatal conductance by using a liquid-flow meter connected to the petiole of a detached leaf (grapevine and Arabidopsis)

- Use simple inexpensive volatile gas sensors to assess volatile diffusion to or from a leaf

1.7. Significance of the research

This project has many potential outcomes and applications for plant biology and agriculture. It is expected to bring new knowledge about how plants regulate their responses to drought stress and provide new insights into plant communication under abiotic stresses. Potential demonstration of induced volatile emissions will allow agronomists to utilise these signals to improve the water use efficiency of crops and counteract the consequences of global warming on plant productivity, yield and quality. For example, in glasshouses, it appears that plants can tolerate a reduction in water and continue to produce crops with similar yields and even a higher quality leading to a double benefit (Caser *et al.*, 2016). Thus, some plants could be used as volatile "super-emitters" to elicit other plants to reduce their transpiration and hence reduce water use without reducing production. Similarly, the concept of "plant sentinel" is currently on trial by interplanting of crops because of their high sensitivity to predators and their ability to emit stress signals (Pickett & Khan, 2016). Hence, these "super-emitter' plants should be considered for tests in vineyards vulnerable to heat waves.

From a scientific perspective, plant water-stress measurements such as leaf pressure chamber or sap flow are destructive or invasive and may consequently induce stresses for the plant. Measurement of volatiles non-destructively could also be employed as markers of drought stress and be useful to understand water stress responses and be considered a target for crop breeders (Jansen *et al.*, 2009; Niederbacher *et al.*, 2015). This new area of research will bring new knowledge on plant water regulation as well as intra- and inter-plant communication under water stress. Furthermore, the findings may call into question the results and significance of many water stress experiments conducted in glasshouses hitherto where water stressed plants were co-located with well-watered plants. Overall, the biological and environmental aims of this project in the context of climate change and water availability will provide new ways of optimising water management and lead to additional benefits such as water savings and higher quality production.

1.8. Structure of the thesis

Chapter 1 describes the literature review of this study.

Chapter 2 describes the basic methods common to all the results in Chapters 3, 4 and 5.

Chapter 3 describes the results of the drought/rehydration experiments combined with volatile analysis on *Vitis vinifera* (cv. Chardonnay and Grenache).

Chapter 4 describes the results of the drought/rehydration experiments combined with volatile analysis on *Arabidopsis thaliana*.

Chapter 5 describes the method developed to study the effect of volatile on the water flow of single detached-leaf.

Chapter 6 describes the general conclusions of this study.

2. General methods

2.1. Plant growth

2.1.1. Arabidopsis thaliana

Source of seeds

Arabidopsis thaliana ecotype Col 0 wild type (WT) seeds were obtained from The Arabidopsis Information Resource (TAIR).

Seed sterilisation

Arabidopsis WT seeds were sterilised prior to germination using chlorine gas. Seeds in 1 mL open Eppendorf tubes were placed in a small desiccator containing a 100 mL beaker filled with 90 mL of bleach/sodium hypochlorite solution. Under a fume hood, 6 mL of concentrated HCl was added into the beaker until gas development was visible and the desiccator was then sealed for 2-3 h. The sterilised tubes were transferred to a clean bench for at least 3 h to allow chlorine gas to be expelled. Seeds were then stored in a dry cabinet at ambient laboratory temperature.

Seed germination and transfer to soil

Sterilised Arabidopsis seeds were plated on a solid culture medium in petri dishes under laminar flow. The medium consisted of 4.4 g Murashige and Skoog Basal Medium, 800 mL of purified water (Milli-Q Plus; Merck Millipore, USA), pH adjusted 5.6-5.8 with potassium hydroxide (KOH), Agar 20 $g.L^{-1}$, and autoclaved. The dishes with seeds were placed in a 4 °C dark cold room for 3 days, to overcome dormancy and synchronise germination. They were then placed in a small growth cabinet (1.2 m^3) under short-day conditions (10 h light at 21°C, 14 h dark at 17°C), photosynthetically active radiation (PAR) 100-150 $\mu mol.m^2.s^{-1}$, in a PC2 laboratory of the Plant Research Centre, Adelaide, Australia. After 7 days, the seedlings were transferred to plastic pots (170 cm^3), filled with a soil mixture (85 % Seedling Substrate Plus+ (Bord Na Móna), 15 % horticultural sand (Debco Pty Ltd) (v/v)) drenched with Confidor (Bayer) and placed again in the same growth cabinet. Watering was achieved by filling the bottom of the trays with reverse osmosis water every day for 30 min. The plants were grown for 5 weeks before conducting experiments.

2.1.2. Vitis vinifera

Source of plants

Vitis vinifera L. cultivars were obtained from different origins. Two year old Chardonnay vines in pots originated from cuttings taken from rooted vines (clone I10V1) in the Alverstoke vineyard of the University of Adelaide, Waite campus. Shiraz (GB02116, SARDI08) and Grenache (GB01491, 1889 Selection, Graetz) rootlings came from the Yalumba nursery, Barossa Valley, and were stored in a 4 °C dark cold room until being potted out. All vines were grown in 4.5 L pots, containing a mixture of UC soil mix (61.5 L sand, 38.5 L peat moss, 50 g calcium hydroxide, 90 g calcium carbonate and 100 g Nitrophoska© (12:5:1, N:P:K plus trace elements; Incitec Pivot Fertilisers, Southbank, Australia) per 100 L at pH 6.8) and coco peat (v/v). The pots were covered with a double layer of plastic mesh to let water through and reduce evaporation from the soil. They were placed in a temperature and humidity-controlled glasshouse in the Australian Plant Phenomics Facility (APPF), Waite campus, Adelaide, under natural light with approximately 23°C day, 17°C night and humidity 40 %. The vines were pruned to grow two to three shoots and oriented upright during their development using wooden stakes (up to 1.5 m), and were irrigated over two months by adding water until dripping from the bottom of pots every day. A soil fertiliser (Thrive 25:5:8.8 N:P:K plus trace elements; Yates, Australia) was applied once per week at a concentration of 2 $g.L^{-1}$ when 3-4 mature leaves had grown to bring the plants to approximately equal size.

2.2. Plant physiology

2.2.1. Stomatal conductance (g_s)

Leaf stomatal conductance (g_s) was measured using an AP4 Porometer (Delta-T Devices Ltd, UK) that measures the rate of change in humidity (non-steady state) within a small cup enclosing one side of a small area of a leaf (Monteith & Bull, 1970). The side of the leaf chosen to be measured had maximal conductance and high stomatal density, corresponding to the abaxial surface for both *Arabidopsis thaliana* and *Vitis vinifera*. The mid-point of the humidity range over which measurements were taken was determined from the ambient humidity ± 5 %. The instrument was calibrated according to the manufacturer instructions taking account of the barometric pressure (obtained from the Australian Bureau of Meteorology for the local area) and humidity range (generally 40 %). If temperature deviated from the calibration temperature by more than 1°C, this was indicated allowing a new calibration to be performed. The circular cup (6 mm diameter of enclosed leaf) was used on grapevines (average of 3 measurements per leaf avoiding the veins) and Arabidopsis (one measurement per leaf) to measure leaf stomatal conductance in $mmol.m^{-2}.s^{-1}$ (Figure 5).

Leaves selected were fully mature, of similar node positions and fully exposed to light, and measurements were conducted around midday (12:00 to 13:00, Australian central standard time).

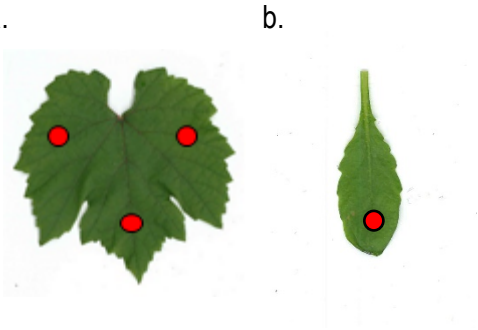


Figure 5. Measurements conducted with a porometer on *Vitis vinifera* and *Arabidopsis thaliana* leaves. On the abaxial (lower) leaf surfaces, an average of a) three readings for grapevine (red circles) and b) one reading for Arabidopsis (red circle) were taken to determine the leaf stomatal conductance ($mmol.m^{-2}.s^{-1}$).

2.2.2. Leaf and/or stem water potential (Ψ)

Leaf and/or stem water potential (Ψ) was measured using a Scholander pressure chamber (Model 600D, PMS Instrument Company, USA) (Scholander *et al.*, 1965). The midday stem water potential (Ψ_{stem}) was measured on *Vitis vinifera* plants by keeping selected leaves in the dark in opaque plastic/foil envelopes for an hour in order to stop transpiration so the leaf water potential and the stem water potential equilibrate. The petiole was rapidly snapped from the shoot, cut flat with a sharp razor blade near the original cut, and the leaf was placed inside the sealed chamber with at least 5 mm of the petiole exposed outside. While adding pressurised nitrogen gas, as soon as small drops were observed at the cut endpoint with a magnifying glass, pressure was recorded as the opposite of Ψ_{stem}. Measurements were conducted from about 13:00 to 15:00 (Australian central standard time) corresponding to the time when the weather conditions cause the maximum rate of water loss from the plant (midday). Selected leaves were fully mature and of similar node positions between treatment groups.

2.2.3. Leaf gas exchange

Net carbon assimilation rate (*NCAR*), transpiration (*E*) and stomatal conductance (g_s) of a *Vitis vinifera* leaf were measured either with a LCpro-SD Portable infrared gas analyser (ADC BioScientific Ltd., UK) with the broad leaf chamber (6.25 cm^2) (Vaast *et al.*, 2005), or a LI-6400XT (LI-COR, Biosciences Inc., USA) (Farquhar & Sharkey, 1982), from equations derived by von Caemmerer and Farquhar (1981). With both instruments, ambient carbon dioxide concentration and ambient water vapour concentration around the leaf within the

chamber were used, and measurements were recorded when stabilised (2-3 minutes). Specific parameters such as flow rate, PAR and how the measurements were conducted will be discussed further in the Chapters 3, 4 and 5. Selected leaves were fully mature, of similar node position and fully exposed to light. Both instruments were cross-checked and gave similar readings.

2.2.4. Temperature and humidity during plant growth and experiments

To continuously monitor temperature and relative humidity in the growth cabinets or glasshouses, wireless and waterproof data loggers with built-in sensors (Tinytag Plus 2, TGP-4500, Gemini Data Loggers Ltd, United Kingdom) were placed strategically to reflect the conditions experienced by the leaves in the plant growth environments. Vapour pressure deficit (VPD) was then calculated from Monteith and Unsworth (1990) as follow:

Eq. 1. 1

$$SVP\ [Pa] = 610.7 \times 10x^{7.5T/(237.3+T)}$$

Eq. 1. 2

$$VPD[Pa] = (\frac{100 - RH}{100}) \times SVP = (1 - \left(\frac{RH}{100}\right)) \times SVP$$

with saturation vapour pressure (SVP, Pa), vapour pressure deficit (VPD, Pa), temperature (T, °C) and relative humidity (RH, %). The sensors recorded with 10-min interval and VPD results were showed as the mean of the data from 12:00 to 13:00 (Australian central standard time), corresponding to the duration of most physiological measurements.

2.2.5. Projected leaf area

Projected leaf area was determined by scanning *Vitis vinifera* and *Arabidopsis thaliana* leave(s) (full colour, 300 DPI, *.jpg output format) placed inside a custom-made frame with known reference field and analysed by the image processing program 'ImageJ' with the Java plugin 'Leaf Area Macro v. 1.00'. The cardboard frame has a DIN A3 size with 1 cm border and a green reference square of 4 cm^2 printed on white paper and positioned to the left corner on the frame. Each scan is processed by converting to 8-bit, setting the threshold and converting to a mask. Then, the leaf and reference field are identified by the *Analyse Particles* function and the noise is reduced by the *Remove Outliers* function. The total area of each identified object in the image

was estimated with a relative error percentage. A *Batch Analysis* mode was used for whole plant projected leaf area estimation with the same calibration for all images.

2.2.6. Water field capacity (WFC)

Before each drought/rehydration experiments, a large amount of water was given to the *Vitis vinifera* pots from the top until starting dripping from the bottom. Two hours later, and being sure no water was dripping anymore, the pots were placed on a scale and the mass was recorded as the pot water field capacity (WFC). This method was repeated on two consecutive days and the average value was kept as reference for each pot throughout the experiments.

2.3. Chemical analysis methods

2.3.1. Solid-phase micro-extraction (SPME)

Solid-phase micro-extraction (SPME) was selected for the extraction of volatiles emitted by the plants. The fibre chosen was divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS), 2 cm, 50/30 µm, grey-notched, StableFlex core, needle gauge 23 Ga, Supelco (Sigma-Aldrich). The three phases offer a broader range of volatile selectivity and this length is double that of all the other phases available, hence increasing the surface area of which adsorption can occur. The handling and positioning of the fibres, as well as sampling method varied between experiments and the details are provided in the Chapters 3 and 4. Before every use, the SPME fibres were preconditioned (thermally cleaned) by manually exposing the fibre in a gas chromatogram (GC) injection port at 260 °C for 30 min with a constant flow of helium, and then, stored in a glass culture tube with polytetrafluoroethylene (PTFE) lined lid previously cleaned with ethanol (90%) and baked at 200°C overnight.

2.3.2. Gas chromatography-mass spectrometry (GC-MS) conditions and data analysis

The thermal desorption of the SPME fibres was done in the injection port of a 6890N gas chromatograph, coupled to a 5973N mass spectrometer (Agilent, USA). The gas chromatograph was fitted with a 60 m J&W DB-WAX UI fused silica capillary column (0.25 mm i.d., 0.25 µm film thickness) (Agilent, USA). The carrier gas was helium (ultrahigh purity, BOC Ltd., UK), and the flow rate was 1.5 $mL.min^{-1}$. The oven temperature program started at 40°C, held at this temperature for 3 min, then increased at 5°$C.min^{-1}$ to 240°C, and held at this temperature for 10 min. The injector was held at 240°C throughout the run with a borosilicate glass

SPME inlet liner (straight, SPME taper, 0.75 mm). Positive ion electron impact spectra at 70 eV were recorded in the range *m/z* 35-350 for scan runs. A sensitivity check was done before starting each experiment with a known chemical standard (e.g. 1-hexanol, 10 $mg.mL^{-1}$).

GC-MS chromatograms were analysed with the software ChemStation (Agilent, USA). Each peak was manually characterised by its retention time and compared to authentic mass spectral libraries (W11N17main, WILEY275) to determine the compounds identify. Relative content was estimated by peak area (in counts).

For the experiment in Chapter 3, section 3.5, the chromatogram peaks were compared with standard chemicals. Solutions were prepared by adding approximately 2 µL of each of the standards in 5 mL of milli-Q water in SPME vials and analysed by SPME-GC-MS (list in table 6, Chapter 3). Subsequent dilutions were made based on the peak intensity and resolution of the first dilution to determine the retention time of each compound. In addition, a series of alkanes (C_{10}-C_{25}) in solution was injected after the experiment in the GC-MS to determine the Kovats retention index (RI) with the equation as follow:

Eq. 2

$$RI = 100 * (n + (N - n) * \left(\frac{\log(RTunknown) - \log(RTlow)}{\log(RThigh) - \log(RTlow)}\right))$$

with n (number of carbon of lower alkane), N (number of carbon of higher alkane), RTunknown (retention time of compound of interest, in min), RTlow (retention time of lower alkane, in min), RThigh (retention time of higher alkane, in min).

2.4. Statistical and data analysis

Most physiological data was recorded and sorted with Microsoft Excel 2016 and most graphs were made with GraphPad Prism 9.

For the statistics, 2-way repeated-measures ANOVA with Bonferroni tests, t-tests (unpaired, two-tailed) and multi-linear regressions were conducted on the physiological data with GraphPad Prism 9 with guidance from Zar (2010). Data are presented as mean ± SD if not otherwise defined.

Pettitt homogeneity tests were conducted on the physiological data with XLSTAT 2019, providing p-values using Monte Carlo resamplings. This test can detect a change point in the mean value of observed series of

data (Kocsis *et al.*, 2020; Yozgatligil & Yazici, 2016). It is a rank-based method, non-parametric, that gives possible change point position and tests its statistical significance and generally used in climate studies.

Diagrams and pictures were created and/or modified with Adobe Illustrator 2021 and Microsoft PowerPoint 2016.

3. Volatile analysis during drought-rehydration experiments in *Vitis vinifera*

3.1. Introduction

When plants sense a deficit in water availability in the soil, the primary response is to close stomata to reduce transpiration and avoid desiccation, resulting in a decrease of stomatal conductance (g_s) (Osakabe *et al.*, 2014). In addition, the stem and leaf water potentials (Ψ_s , Ψ_l) are controlled within limits to reduce the possibility of catastrophic xylem embolism, but negative enough to allow continued water extraction from the soil (Santesteban *et al.*, 2019). Eventually, a limit is reached where Ψ_s and Ψ_l cannot decrease further and this is usually near -1.4 MPa for grapevine (Suter *et al.*, 2019).

The effect of water stress on plants is often examined on potted plants whereby they are subjected to a period of reduced irrigation, sometimes controlled to a specific lower soil water content or lower g_s (Allario *et al.*, 2013; Cano *et al.*, 2014), and then rehydrated over a period to examine recovery. Two treatments are usually selected, a well-watered group (control) and a water-stressed group. While the control group is adequately watered throughout the experiment, the water-stressed group has the irrigation cut off partially or totally for a defined duration depending of the intensity of the stress tested (low, mild or severe stress), and is rehydrated afterwards during the recovery phase generally until the measured parameters are back to initial values (Hu *et al.*, 2013; Dayer *et al.*, 2020).

In many studies, the two treatments are conducted on plants sharing the same environment (Correia & Pereira, 1994; Martim *et al.*, 2009; Zhou *et al.*, 2015, Dayer *et al.*, 2020). Since the discovery of leaf-emitted plant volatile compounds and their function in plant communication, this close proximity is likely to permit a cross-interaction between the experimental groups, as evidenced in biotic stress experiments (Šimpraga *et al.*, 2016; Yoneya & Takabayashi, 2014). Indeed, an intriguing phenomenon has been observed; while the water-stressed plants showed expected reduced stomatal conductance, the g_s of the well-watered group decreased as well in synchrony and then increased back to initial levels when the stressed group was rewatered (Dayer *et al.*, 2017; Scharwies, 2017). Even if the decrease in g_s of controls was to a lesser degree than for the stressed group, it was still noticeable, and as described in Table 3, Chapter 1, other plants showed a similar phenomenon during such experiments. However, this observation was not commented on by the authors and no follow-up experiments were published.

One hypothesis to explain this observation is inter-plant exchange of volatile compounds that causes stomatal closure. Indeed, it is well known that plants can emit and detect volatiles specifically in response to stresses (Dudareva *et al.*, 2013). This type of communication as well as some volatiles may protect against heat stress,

light and reactive oxygen species (Possell & Loreto, 2013). Also, many studies have investigated the effect of drought stress on the emission of volatiles (Bourtsoukidis *et al.*, 2014; Saunier *et al.*, 2017) but potential inter-plant communication has rarely been considered with only one reported case in the literature on salt stress (Caparrotta *et al.*, 2018). In general, results of those studies showed a decrease in volatile emission rate during drought due to the closure of stomata (Nogués *et al.*, 2015; Šimpraga *et al.*, 2011). However, the experimental protocol involved separation of the control group from the treatment group and did not include the recovery phase *(Lüpke et al.*, 2017). Conversely, the drought/rehydration studies described in Table 3, Chapter 1, did not conduct any volatile sampling and analysis.

In this study, drought-rehydration experiments were carried out on glasshouse-grown potted *Vitis vinifera* to study the stomatal responses while monitoring the emission of volatiles. Two types of experiments were performed. First, a standard experiment was undertaken in order to repeat the observations of Dayer *et al.* (2017) where vines under different treatments were co-located in the same glasshouse. Then, two similar experiments were conducted while also taking samples of volatiles. Second, clear plastic chambers were constructed to study the effect of drought on the emission of volatiles from individual isolated vines where control (well-watered) vines could be prevented from perceiving any volatiles emitted from water-stressed vines, and *vice versa*.

In the first series of experiments, it was expected to replicate the stomatal responses previously observed in control vines when co-located vines were water-stressed and recovered, and to identify key volatiles emitted during the different phases of the drought stress. From the second series of experiments, it was expected that volatile profiles and/or concentrations of volatiles from water-stressed vines would be different from the well-watered vines. Thus, candidate volatiles could be identified that may be responsible for the closure of stomata observed in the literature and matched the volatiles detected in the first series of experiments.

3.2. Drought/rehydration treatment with *Vitis vinifera* cv. Chardonnay vines in the same glasshouse

Drought-rehydration experiments were carried out on potted plants of *Vitis vinifera* cv. Chardonnay to study the stomatal responses. It was expected to replicate standard drought-rehydration experiments as in Dayer *et al.* (2017) where a decrease in g_s of well-watered plants occurred in synchrony with water-stressed plants. This would indicate possible inter-plant signalling.

3.2.1. Material and Methods

Plant and environmental conditions

Vitis vinifera cv. Chardonnay vines were potted and grown in glasshouse with natural light (spring August-September 2018) until reaching 1-2 shoots with approximately 10 leaves per shoot. The controlled environmental conditions were temperature 25°C day and 17°C night, humidity 40 % (details in section 2.1.2, Chapter 2).

Drought/rehydration experiment

Twenty vines were placed in two rows (30-50 cm inter-space) by alternating control well-watered (C) vines and water-stressed treated (WS) vines with the aim of mixing the vines so controls would be surrounded by water-stressed vines and increase the chance of exchange of emitted volatiles (Figure 6). Care was taken to avoid physical contact between vines. Two temperature and humidity sensors were placed among the vines and additional LED lamps were placed above the vines for supplemental PAR from 08:00 to 18:00 (Australian central standard time).

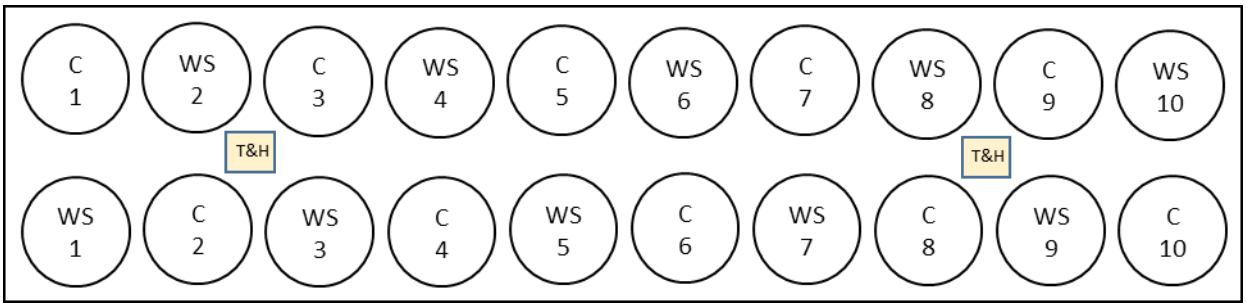


Figure 6. Diagram of the positioning of 10 control well-watered (C) vines and 10 water-stressed (WS) vines in the drought/rehydration experiment, with temperature and humidity sensors (T&H yellow squares).

The C vines were irrigated at water field capacity (WFC) every day at 17:00 by weighing the pots and replacing the mass of water used. For the WS vines, water was withheld from day 4 to day 11, until a defined value of leaf maximum daily stomatal conductance (g_s) of approximately 50 mmol H_20 $m^{-2}.s^{-1}$ was reached (Medrano, 2002). Then, water was resupplied to WFC until the end of the experiment.

From 12:00 to 13:00, g_s was measured with a porometer for all vines following a rotation order so as not to start with the same vine each day. Transpiration (*E*), net carbon assimilation rate (*NCAR*) and g_s were also measured with an infra-red gas analyser (IRGA; ADC LCpro-SD) every two days, with light set at fixed-PAR

1000 µmol.m^{-2}.s^{-1} (dose to saturation; Caravia *et al.*, 2016) using the LED light attachment, ambient CO_2 and water vapour concentrations, air flow at 300 mL.min^{-1}, and one measurement per leaf per vine (different leaf from porometer measurements). On day 3, day 9 and day 16, stem water potentials were determined on 5 vines per treatment and 2 leaves per vines. At the end of the experiment, projected leaf area was determined by harvesting and scanning all leaves, and analysed with ImageJ. All methods are described in Chapter 2.

3.2.2. Results

3.2.2.1. Stomatal conductance

Stomatal conductance averaged 205 ± 92 mmol.m^{-2}.s^{-1} (mean ± SD) for the C vines and 182 ± 69 mmol.m^{-2}.s^{-1} for the WS vines during the first 3 days of the experiment while all vines were watered daily to WFC (Figure 7a). When water was withheld for the WS vines, g_s quickly dropped over 3 days until reaching 47 ± 22 mmol.m^{-2}.s^{-1} on day 6, and was kept between 32 ± 27 and 98 ± 33 mmol.m^{-2}.s^{-1} until day 11 by adding only the mass of water that was used during the day. On day 12, watering to WFC was resumed and g_s of WS vines recovered to similar or even higher levels than at the start of the experiment by day 16. The g_s of the C group was unstable over time with lower values on day 6 and day 9, and higher values on day 7 and day 8 (Figure 7a). At the end of the experiment, on day 14, 15 and 16, g_s of the C vines remained stable, around 248 ± 73 mmol.m^{-2}.s^{-1} and 218 ± 110 mmol.m^{-2}.s^{-1} for the WS vines. Significant differences between the stomatal conductance of C and WS were found from day 5 to day 13 (two-way repeated-measures ANOVA with Bonferroni test, $p<0.05$, Supplementary Tables S1a and b).

The light incident on the leaf measured with the porometer (Figure 7b) did not stay constant over time but no difference was observed between the two treatments (two-way repeated-measures ANOVA with Bonferroni test, $p<0.05$, Supplementary Tables S2a and b).

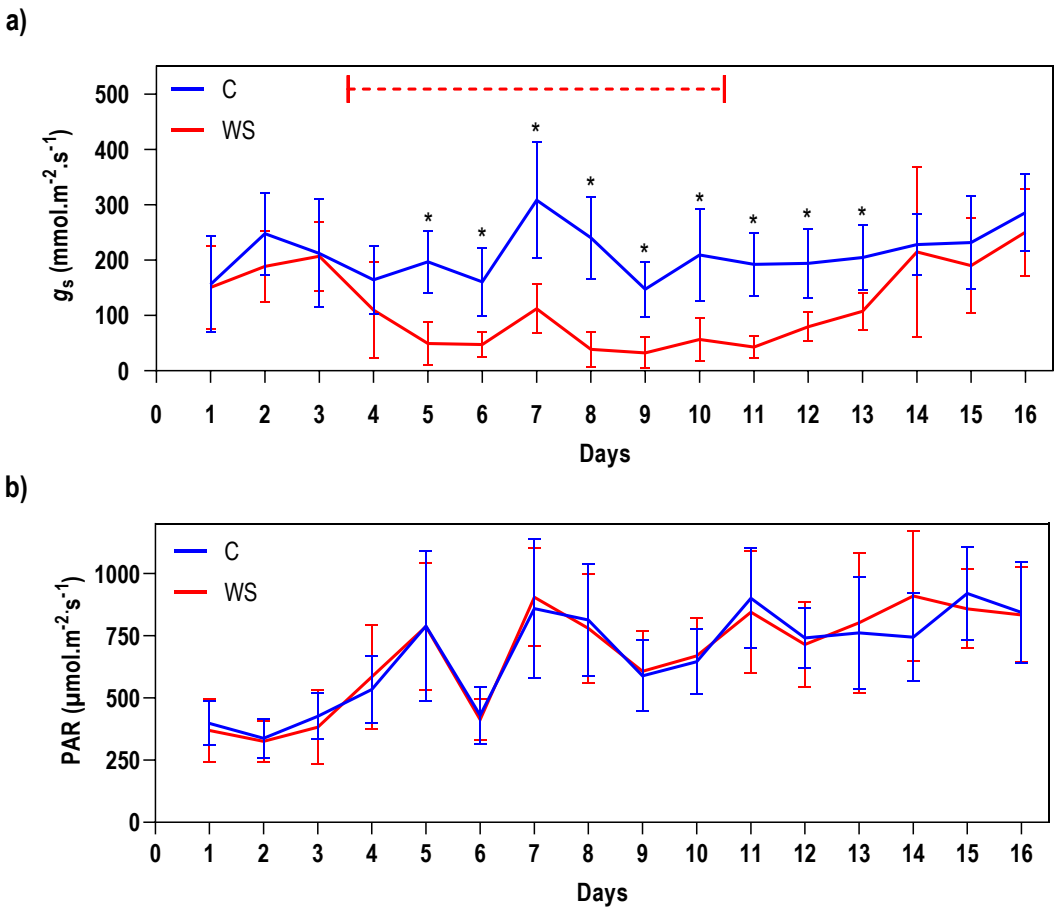


Figure 7. a) Stomatal conductance (g_s) measured on leaves of *Vitis vinifera* cv. Chardonnay used in a drought/rehydration experiment with vines organised in two rows with 30-50 cm interspace in the same glasshouse. Control vines (C, blue) were watered to field capacity every day and the dashed line represents the period during which the water-stressed vines (WS, red) stopped receiving water (mean ± SD, n=10). b) Leaf incident photosynthetically active radiation (PAR) was measured with the porometer light sensor simultaneously as g_s measurements. Significant differences between the control and treated groups by two-way repeated-measures ANOVA with Bonferroni post-tests are marked: * for $p<0.05$.

The Pettitt's homogeneity test was performed on the series of g_s data and detected no shift for either C or WS groups with or without the recovery phase included (Figure 8a and b).

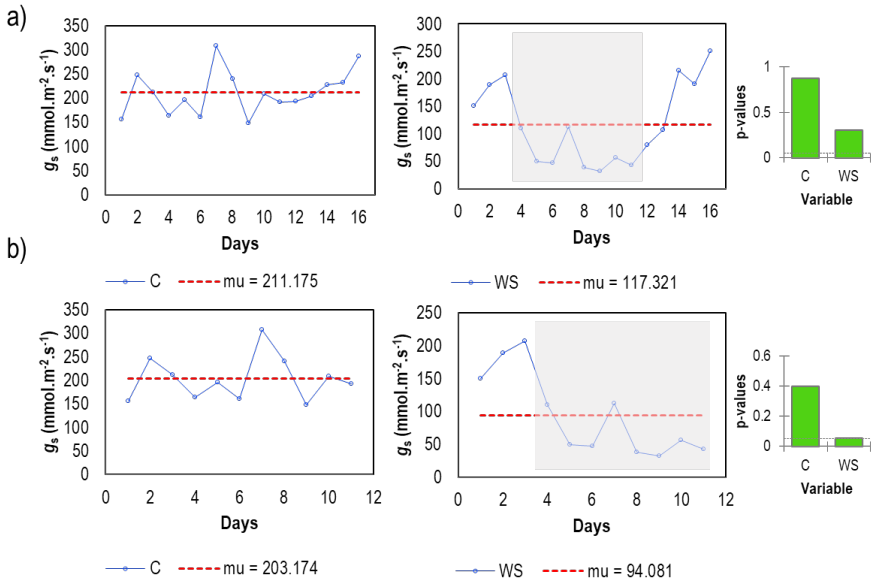


Figure 8. Pettitt homogeneity test on the g_s data measured with the porometer from well-watered vines (C) and water-stressed vines (WS) in a drought experiment (grey box). The test was performed on a) the whole series of data and b) without the recovery phase (mean, n=10), and the dotted lines represent the averaged value for the data series, with two mu values if a change point is detected ($p<0.05$).

3.2.2.2. Gas exchange

Transpiration (*E*), net carbon assimilation rate (*NCAR*) and g_s measured by the LCpro-SD IRGA showed the same trend as for porometer measurements of g_s for WS (Figure 9). However, for C vines, there was a decrease in *NCAR* and g_s on day 4 followed by an increase to similar values observed initially. Significant differences for *E* and *NCAR* between C and WS were found on day 6, day 8 and day 10, and for g_s on day 6, day 8, day 10 and day 12 (two-way repeated-measures ANOVA with Bonferroni post-tests, $p<0.05$, Supplementary Tables S3a and b, S4a and b, S5a and b, respectively).

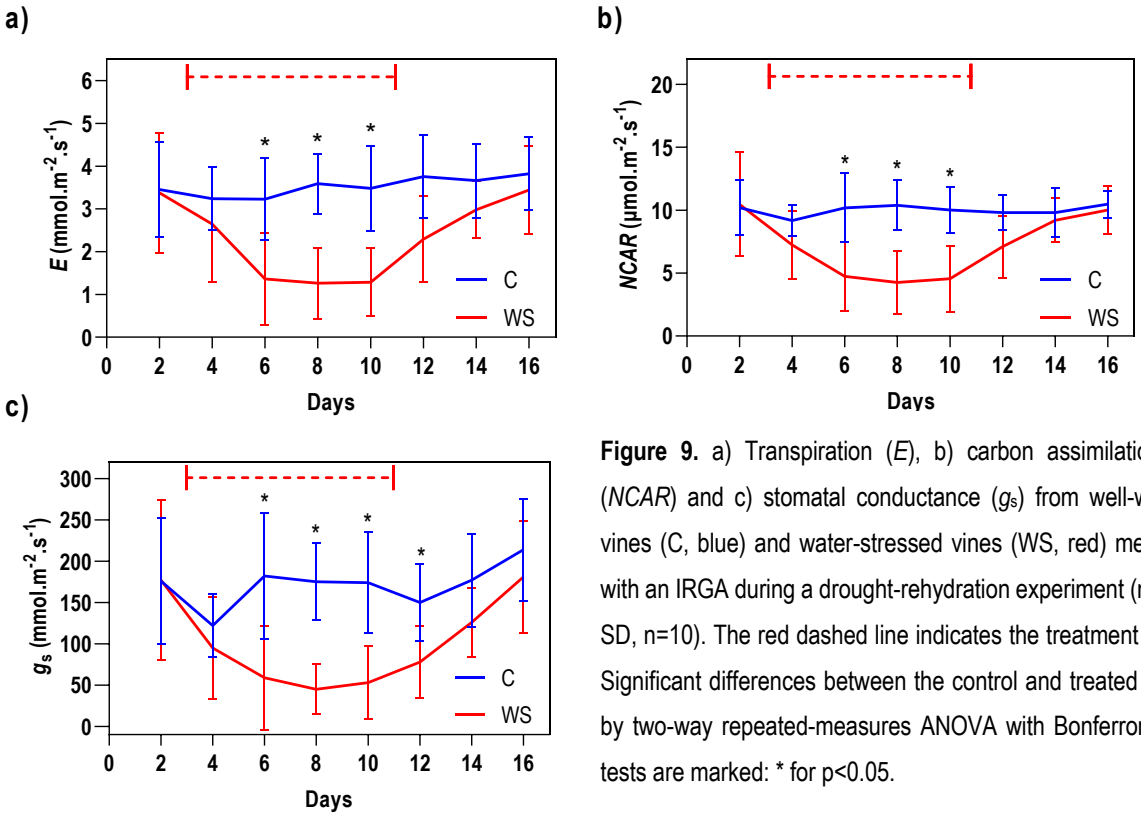


Figure 9. a) Transpiration (*E*), b) carbon assimilation rate (*NCAR*) and c) stomatal conductance (g_s) from well-watered vines (C, blue) and water-stressed vines (WS, red) measured with an IRGA during a drought-rehydration experiment (mean ± SD, n=10). The red dashed line indicates the treatment period. Significant differences between the control and treated groups by two-way repeated-measures ANOVA with Bonferroni post-tests are marked: * for $p<0.05$.

In the *E* series of data, the Pettitt homogeneity test revealed no shift for C and WS, but when without the recovery phase, a shift of increased values was detected for the C group on day 8 and a decrease of values for the WS group on day 6 ($p<0.05$, Figure 10b). Similar to *E* without the recovery phase, a decrease in the WS group was detected in *NCAR* and g_s data but no change for the C group (data not shown).

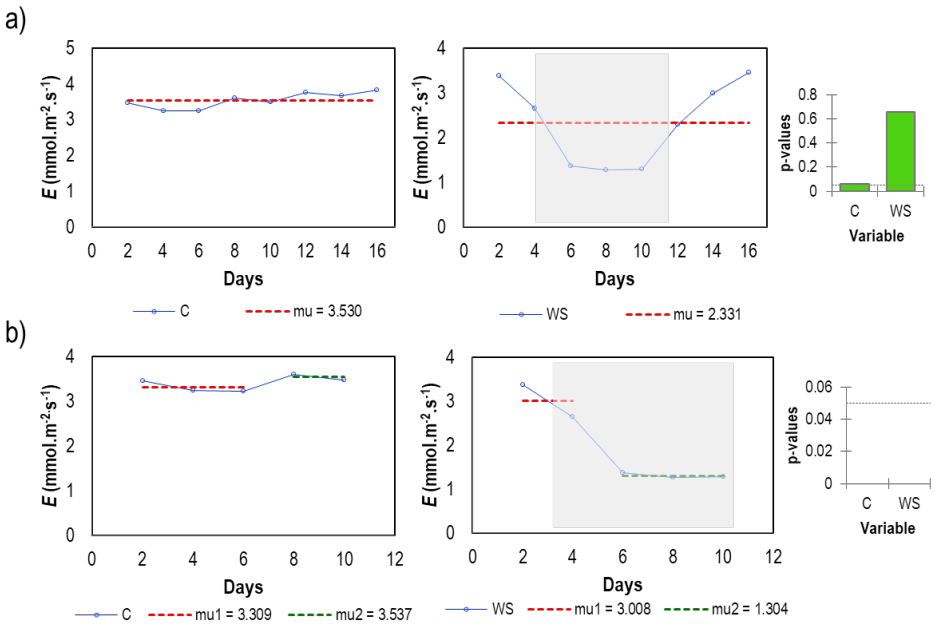


Figure 10. Pettitt homogeneity test on the *E* data measured with the IRGA at fixed PAR from well-watered vines (C) and water-stressed vines (WS) in a drought experiment (grey box). The test was performed on a) the whole series of data with and b) without the recovery phase (mean, n=10), and the dotted lines represent the averaged value for the data series with two mu values if a change point is detected ($p<0.05$).

3.2.2.3. Other parameters (VPD, Ψ_s, leaf area)

Vapour pressure deficit (VPD) reached its highest value of 2.7 ± 0.1 kPa on day 4 (Figure 11a). The stem water potential (Ψ_s) for C vines was constant over time with values never getting lower than the threshold that is considered to indicate water stress in grapevines (Suter *et al.*, 2019). In contrast, the WS vines reached an average Ψ_s of -1.0 MPa on day 10 and increased back to an average of -0.5 MPa, similar to the C vines on the last day of the recovery (Figure 11b). The Ψ_s of C and WS groups was significantly different on day 10 (two-way repeated-measures ANOVA with Bonferroni post-tests, $p<0.05$, Supplementary Tables S6a and b). The projected leaf area between the treatments C and WS (Figure 11c) was not significantly different (t test, $p<0.05$, Supplementary Table S25).

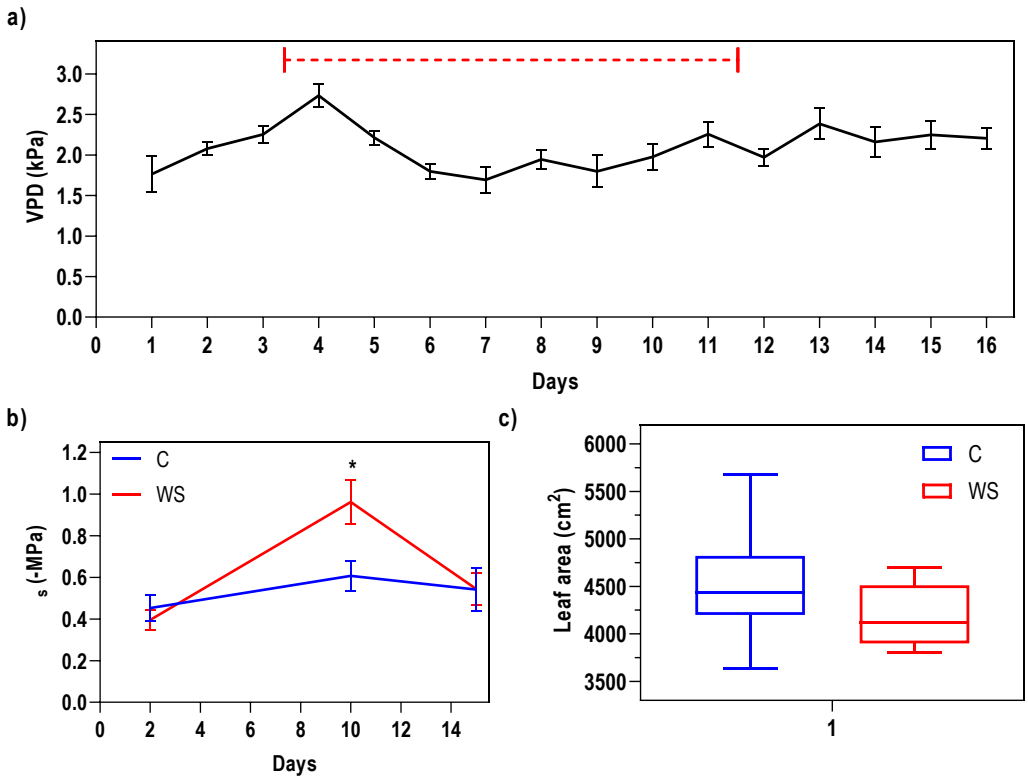
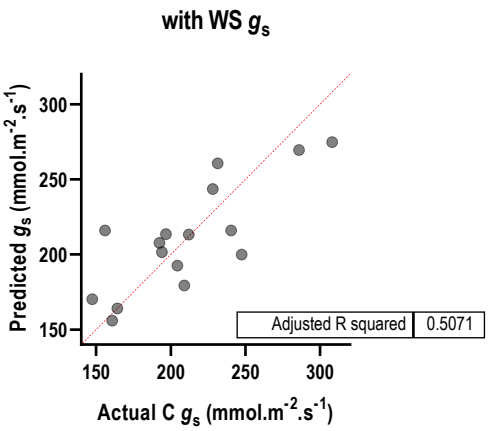


Figure 11. a) Vapour pressure deficit (VPD) calculated from temperature and relative humidity from sensors placed among the vines during the drought/rehydration experiment. The red dashed line indicates the treatment period. Each point represents the mean value from 11:00 to 13:00 (n=2). b) Stem water potential (Ψ_s) from well-watered treatment (C, blue) and water-stressed treatment (WS, red) during a drought/rehydration experiment (mean ± SD, n=5). c) Project leaf area (mean ± SD, n=10). Significant differences between the control and treated groups by two-way repeated-measures ANOVA with Bonferroni post-tests are marked: * for $p<0.05$.

3.2.2.4. Multi-variable analysis

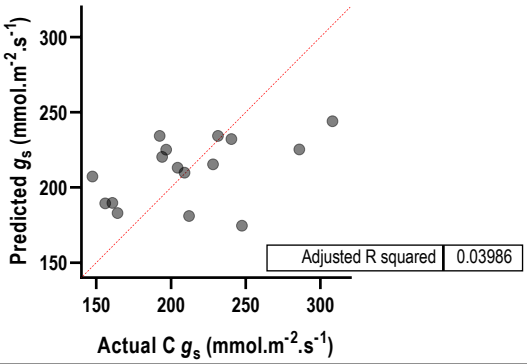
A multi-linear regression analysis was performed on C g_s with different parameters (C PAR, VPD, time, with or without WS g_s) showing that C g_s could be predicted from C PAR and WS g_s with an adjusted R^2 of 0.51 (Figure 12a, Supplementary Table S29). However, the prediction of C g_s was no longer significant without including WS g_s (Figure 12b, Supplementary Table S30).

a)



Parameter estimates	Variable	Estimate	Standard error	95% CI (asymptotic)	|t|	P value	P value summary
β0	Intercept	163.5	69.01	11.58 to 315.3	2.369	0.0372	*
β1	WS gs	0.4376	0.1244	0.1639 to 0.7114	3.518	0.0048	**
β2	C PAR	0.2077	0.06916	0.05546 to 0.3599	3.003	0.0120	*
β3	VPD	-52.13	31.84	-122.2 to 17.95	1.637	0.1299	ns
β4	Time	-3.989	2.813	-10.18 to 2.203	1.418	0.1839	ns

b)

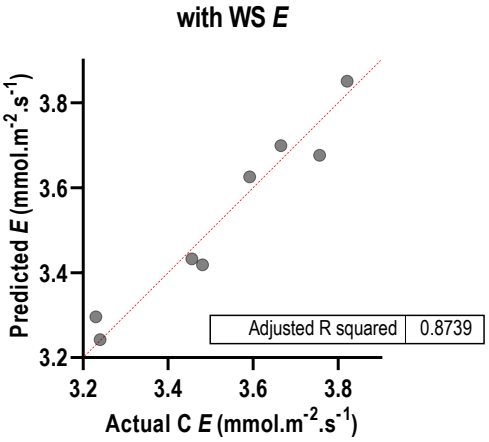


Parameter estimates	Variable	Estimate	Standard error	95% CI (asymptotic)	|t|	P value	P value summary
β0	Intercept	180.0	96.09	-29.31 to 389.4	1.874	0.0855	ns
β1	C PAR	0.1229	0.09048	-0.07422 to 0.3201	1.358	0.1993	ns
β2	VPD	-22.05	42.81	-115.3 to 71.23	0.5150	0.6159	ns
β3	Time	-0.6137	3.692	-8.657 to 7.429	0.1663	0.8707	ns

Figure 12. Multilinear regressions of control well-watered (C) g_s and other variables a) with or b) without water-stressed (WS) g_s from *Vitis vinifera* during a drought/rehydration experiment. C PAR, photosynthetic active radiation of the C group (µmol.m^{-2}.s^{-1}); VPD, vapour pressure deficit (kPa); time, days of the experiment.

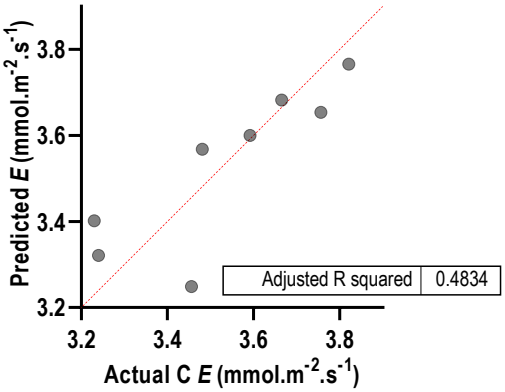
A similar result was obtained for transpiration rate (*E*) from the IRGA measurements with C *E* only significantly predicted by WS *E* (Figure 13, Supplementary Tables S31 and S32). No significant prediction was obtained with *NCAR* and g_s data (data not shown, Supplementary Tables S33 and S34, and S35 and S36, respectively). It is important to note that the IRGA was measuring with a fixed PAR and the analysis used the PAR measured by the porometer light sensor.

a)



Parameter estimates	Variable	Estimate	Standard error	95% CI (asymptotic)	|t|	P value	P value summary
β0	Intercept	3.390	0.2502	2.593 to 4.186	13.55	0.0009	***
β1	WS E	0.1630	0.04456	0.02119 to 0.3048	3.658	0.0353	*
β2	C PAR	0.001099	0.0003544	-2.877e-005 to 0.002227	3.101	0.0532	ns
β3	VPD	-0.4164	0.1400	-0.8620 to 0.02928	2.973	0.0589	ns
β4	Time	-0.006803	0.01402	-0.05141 to 0.03781	0.4854	0.6607	ns

b)



Parameter estimates	Variable	Estimate	Standard error	95% CI (asymptotic)	|t|	P value	P value summary
β0	Intercept	3.239	0.4994	1.852 to 4.625	6.485	0.0029	**
β1	C PAR	0.0004385	0.0006171	-0.001275 to 0.002152	0.7106	0.5166	ns
β2	VPD	-0.08752	0.2173	-0.6908 to 0.5157	0.4028	0.7077	ns
β3	Time	0.02187	0.02352	-0.04343 to 0.08716	0.9297	0.4051	ns

Figure 13. Multilinear regressions of control well-watered (C) *E* and other variables a) with or b) without water-stressed (WS) *E* from *Vitis vinifera* during a drought/rehydration experiment. C PAR, photosynthetic active radiation of the C group (µmol.m^{-2}.s^{-1}); VPD, vapour pressure deficit (kPa); time, days of the experiment.

3.2.2.5. Correlation analysis of the position of the replicates

A Pearson correlation analysis was performed on the stomatal conductance of the biological replicates to investigate the position effect of the experimental set-up. By analysing the correlations of the C and WS replicates (Figure 14), it can be observed that better correlations were found for the plants from C3 to C7 corresponding to replicates positioned in the middle of the experimental set-up, i.e. with more WS replicates surrounding them (see Figure 6).

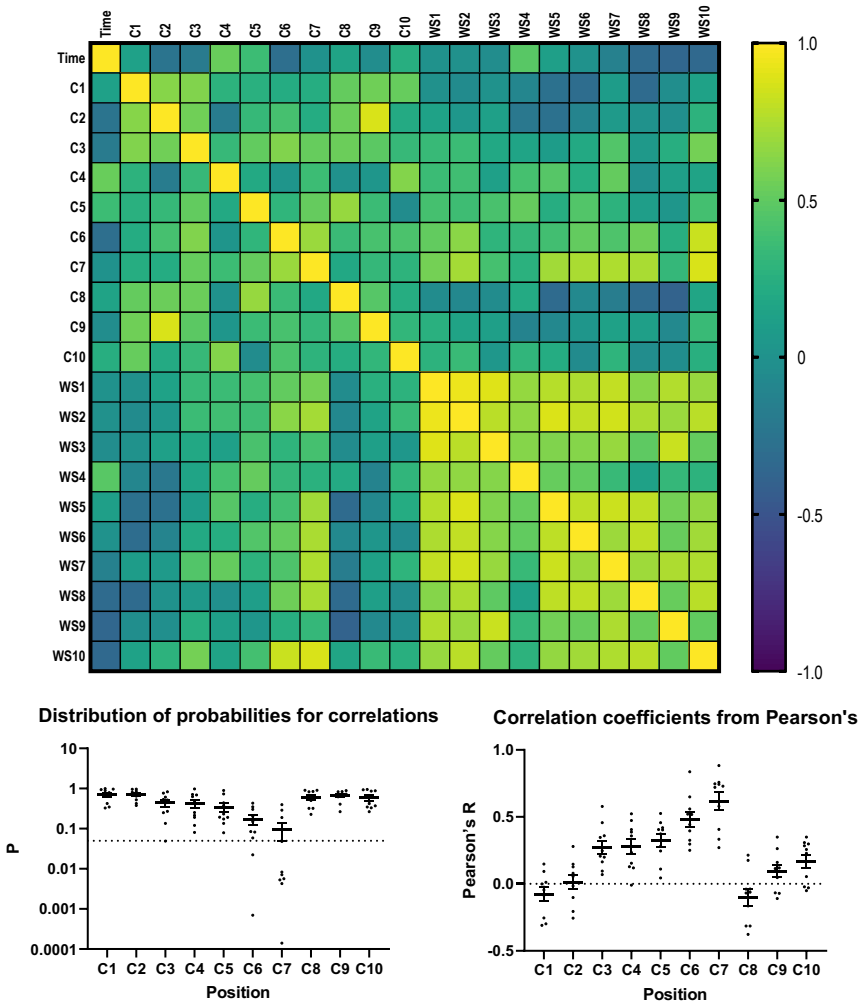


Figure 14. Pearson correlation matrix for control (C) and water-stressed (WS) replicate data of stomatal conductance, indicating the correlation differences based on the position of the replicate in the experimental set-up (see Figure 6), with the probabilities and coefficients of the correlations for the C replicates.

3.2.3. Discussion

Some drought/rehydration experiments reported in the literature have investigated drought-induced variations in stomatal conductance comparing well-watered vines, as the control group, and water-stressed vines, as the treatment group (Table 3, Chapter 1). In most studies, the g_s of vines experiencing a decrease in water availability was reduced relative to controls because stomata are closing. It is assumed, but not always shown, that control well-watered vines have relatively constant g_s over time. However, in some studies, g_s of control plants appeared synchronised with the stressed vines, both during the water stress phase and during recovery (Dayer *et al.*, 2017; Martim *et al.*, 2009; Zhou *et al.*, 2015). Those studies are often conducted with potted control and treatment plants in the same growing environment (indoor or outdoor similarly). Common environmental variables such as PAR and VPD may synchronise these fluctuations in g_s, but this is not always evident (Levin *et al.*, 2007; McAdam & Brodribb, 2015; Tardieu & Simonneau, 1998).

Here, a synchronised response of controls to water-stressed vines was not as clearly observed as in Dayer *et al.* (2017), but the g_s of C vines decreased on some days during the stress phase (day 4, day 6 and day 9, Figure 7a) and the multi-linear regressions revealed a correlation between C g_s and WS g_s, including PAR, and between C *E* and WS *E* (Figure 12). In addition, better correlations between C and WS g_s replicates were found for the vines positioned in the middle of the experimental set-up, which were more likely to receive signals from the stressed plants (Figure 14). However, the Pettitt homogeneity test did not reveal a shift in the g_s and E data for either groups (Figure 8). Overall, those results highlight an effect of the stressed vines on their surrounding control vines, thus the same experiment was repeated in the next section but with the same cultivar as in Dayer *et al.* (2017) (*Vitis vinifera* cv. Grenache) with the addition of volatile compounds sampling and analysis.

3.3. Drought/rehydration treatment with *Vitis vinifera* cv. Grenache vines in the same glasshouse and volatile emission analysis

The synchronisation of stomatal conductance between water-stressed and well-watered vines observed in Dayer *et al.* (2017) was observed in the Grenache cultivar and since it is considered as more isohydric compared to Chardonnay (Schultz, 2003; Soar *et al.*, 2006), the expected response might therefore be present at greater intensity.

The experiment was designed for the same goals as the previous experiment (section 3.2, Chapter 3), and in addition, volatile samples were also extraction during the experiment using SPME, potentially leading to the identification of active chemicals in such signalling.

3.3.1. Material and Methods

Plant and environmental conditions

Vitis vinifera cv. Grenache vines were potted and grown in glasshouse under natural light (summer February 2019) until reaching 1-2 shoots with approximately 10 leaves per shoot. The environmental conditions were temperature 25°C day and 17°C night, humidity 40 %.

Drought/rehydration treatment

Eighteen vines were placed in two rows (30-50 cm inter-space) by alternating well-watered control (C) and water-stressed treated (WS) vines with the aim of mixing the plants so control vines would be surrounded by water-stressed vines and increase the chance of exchange of emitted volatiles (Figure 15). Care was taken to avoid physical contact between vines. Two temperature and humidity sensors were placed among the plants and additional LED lamps were added for minimal light exposure from 08:00 to 18:00 (Australian central standard time).

The C vines were watered to water field capacity (WFC) every day at 17:00. For the WS vines, water was withheld from day 5 until a maximum daily water conductance (g_s) of approximately or below 50 mmol H_20 $m^{-2}.s^{-1}$ was reached (Medrano, 2002). Then, water was resupplied to WFC until the end of the experiment. From 12:00 to 13:00, g_s was measured with a porometer for all vines following a rotation order and not starting with the same vine each day. Transpiration (*E*), net carbon assimilation rate (*NCAR*) and g_s were also measured with an infra-red gas analyser (IRGA; LCpro-SD) every two days, with fixed-PAR set at 1000 $\mu mol.m^{-2}.s^{-1}$,

ambient CO_2 and water vapour concentrations, air flow at 300 $mL.min^{-1}$, one measurement per leaf per vine (different leaf from porometer).

Figure 15. Vine positioning for the drought/rehydration experiment in the clear glasshouse under additional LED lamps, with nine control well-watered (C) vines and nine water-stressed (WS) vines, and temperature and humidity sensors (T&H yellow squares). Selected leaves for stomatal measurements with the porometer were flagged in red and the selected leaf for gas exchange measurements with the IRGA was flagged in blue. SPME fibres (red dots) were placed among the vines at selected times.

For volatile sampling, SPME fibres (DVB/CAR/PDMS) were placed among the vines from 13:00 to 14:00 on day 2, day 6, day 8 and day 12. Their coating was manually exposed at middle height of the vines on custom-made stands. Then, the fibres were thermally desorbed on a GC-MS system on the day of collection, and then reconditioned for the next day. On day 3, day 9 and day 16, stem water potentials were determined on

5 vines per treatment and 2 leaves per vines (right after volatile sampling). At the end of the experiment, projected leaf area was determined by harvesting and scanning all leaves analysed with ImageJ. All methods are described in Chapter 2.

3.3.2. Results

3.3.2.1. Stomatal conductance

Stomatal conductance gave stable readings of 123 ± 5.2 mmol.m^{-2}.s^{-1} (mean ± SD) for the C treatment and 128 ± 1.7 mmol.m^{-2}.s^{-1} for the WS treatment during the first 4 days of the experiment while the vines were watered daily to WFC (Figure 16a). Once watering was withheld for the WS vines, g_s quickly dropped over 4 days until reaching an average of 9 ± 9 mmol.m^{-2}.s^{-1} on day 9. Water was then re-supplied and the WS g_s returned to 128 ± 24 mmol.m^{-2}.s^{-1} on day 13, and remained stable until the end of the experiment. The g_s of the C group was stable over time with slightly higher peaks on day 8 (135 mmol.m^{-2}.s^{-1}), day 9 (142 mmol.m^{-2}.s^{-1}) and day 13 (158 mmol.m^{-2}.s^{-1}). Significant differences between the stomatal conductance of C and WS were found from day 7 to day 12 (two-way repeated-measures ANOVA with Bonferroni test, $p<0.05$, Supplementary Tables S7a and b). No major variation of PAR was observed during the experiment (Figure 16b) with no significant difference between C and WS (two-way repeated-measures ANOVA with Bonferroni post-tests, $p<0.05$, Supplementary Tables S8a and b).

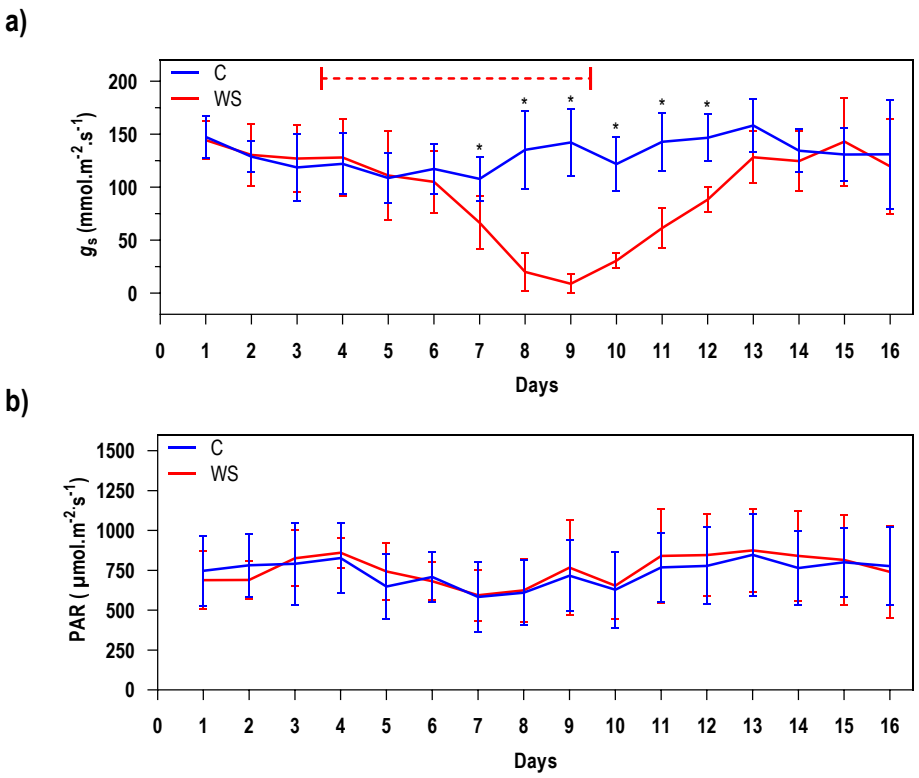


Figure 16. a) Stomatal conductance (g_s) measured on leaves of *Vitis vinifera* cv. Grenache used in a drought/rehydration experiment with vines placed in two rows with 30-50 cm interspace in the same glasshouse. Control vines (C, blue) were watered to field capacity every day and the dashed line represents the period during which the water-stressed vines (WS, red) stopped receiving water (mean ± SD, n=9). b) Leaf incident photosynthetically active radiation (PAR) was measured with the leaf porometer light sensor simultaneously as g_s measurements. Significant differences between the control and treated groups by two-way repeated-measures ANOVA with Bonferroni post-tests are marked: * for $p<0.05$.

The Pettitt homogeneity test did not detect changes in the series of the C data and only detected a shift on day 5 in the WS data when the recovery phase was not considered ($p<0.05$, Figure 17b).

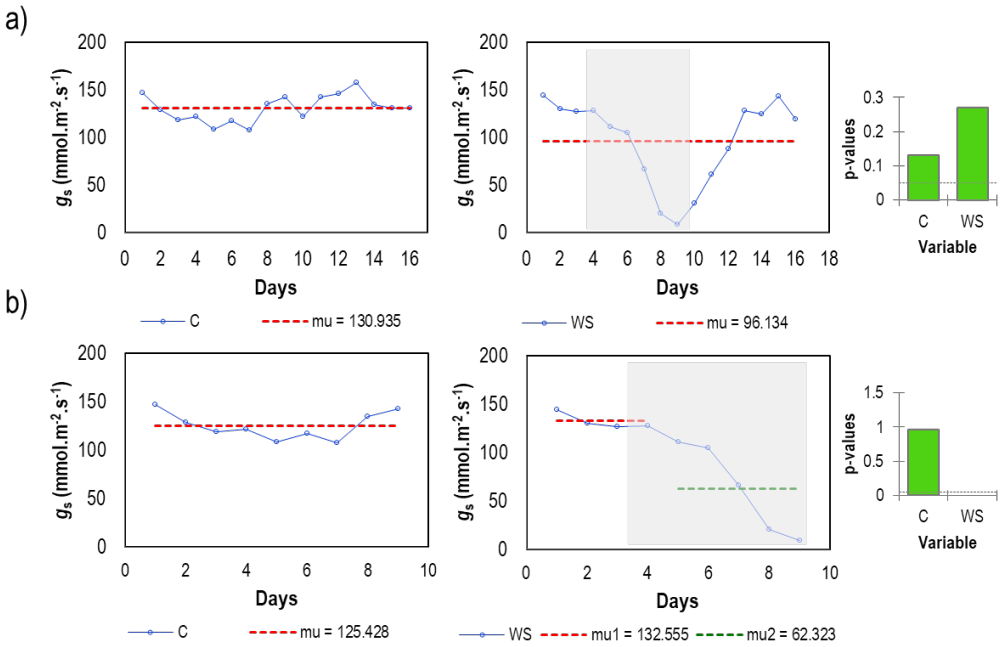


Figure 17. Pettitt homogeneity test on the stomatal conductance (g_s) data measured with the porometer from well-watered (C) and water-stressed (WS) vines in a drought experiment (grey box). The test was performed a) on the whole series of data and b) without the recovery phase (mean, n=9), and the dotted lines represent the averaged value for the data series with two mu values if a change point is detected ($p<0.05$).

3.3.2.2. Gas exchange

Transpiration (*E*), net carbon assimilation rate (*NCAR*) and stomatal conductance (g_s) measured by the IRGA showed the same trend as that of g_s (porometer) for the WS group (Figure 18). For the C vines, there was a slight decrease of *NCAR* and g_s on day 5 followed by an increase. Significant differences for *E*, *NCAR* and g_s between C and WS were found on day 7, day 9 and day 11 (two-way repeated-measures ANOVA with Bonferroni post-tests, $p<0.05$, Supplementary Tables S9a and b, S10a and b, S11a and b, respectively).

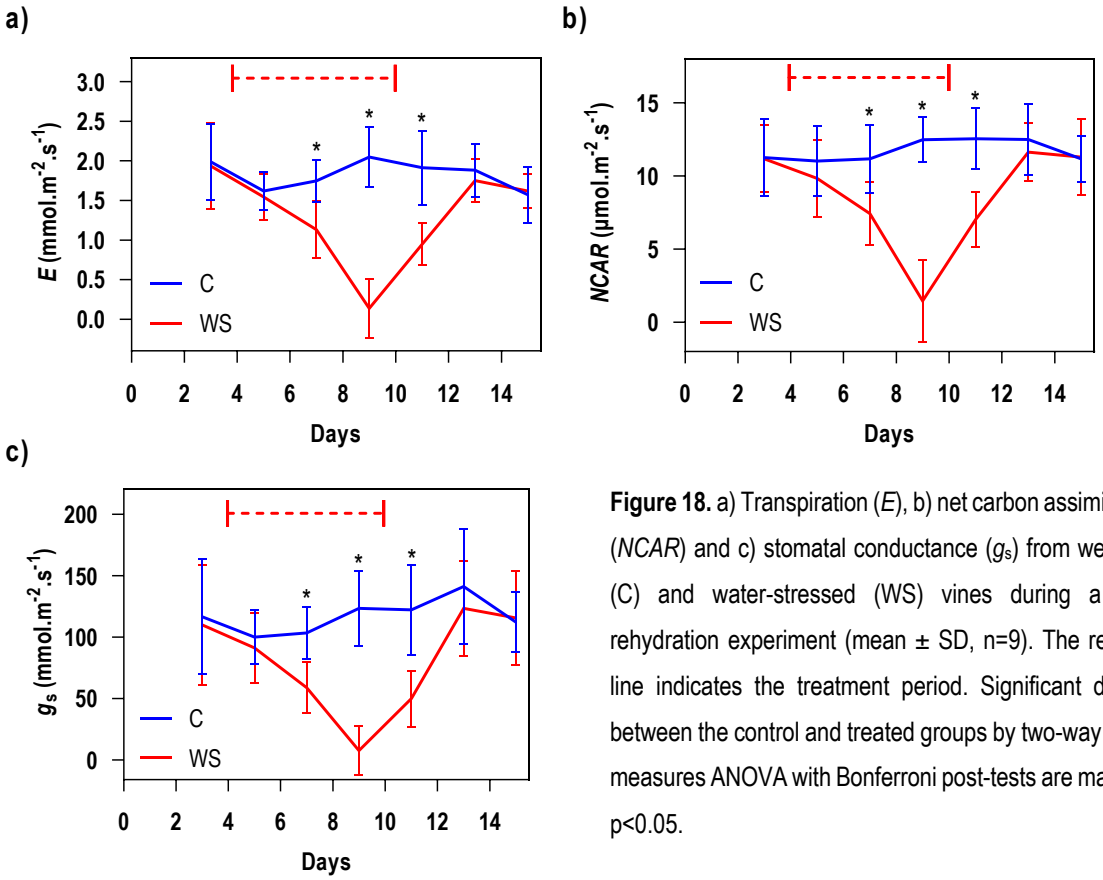


Figure 18. a) Transpiration (*E*), b) net carbon assimilation rate (*NCAR*) and c) stomatal conductance (g_s) from well-watered (C) and water-stressed (WS) vines during a drought-rehydration experiment (mean ± SD, n=9). The red dashed line indicates the treatment period. Significant differences between the control and treated groups by two-way repeated-measures ANOVA with Bonferroni post-tests are marked: * for $p<0.05$.

The Pettitt homogeneity test revealed the same trend for *E*, *NCAR* and g_s (IRGA) as for the g_s (porometer), with only a change-point for the WS data series without the recovery phase ($p<0.05$, data not shown).

3.3.2.3. Other parameters (VPD, Ψ_s)

VPD varied slightly over time but stayed lower than 2 kPa (Figure 19a). The stem water potential (Ψ_s) of the C vines was relatively constant over time around -0.4 MPa with values never getting lower than the threshold vines are considered stressed (Figure 19b), while Ψ_s of the WS vines reached -1 MPa on average on day 9, and increased back to -0.4 MPa like the C vines on the last day of the experiment. The Ψ_s of C and WS was significantly different on day 9 (two-way repeated-measures ANOVA with Bonferroni post-tests, $p<0.05$, Supplementary Tables S12a and b).

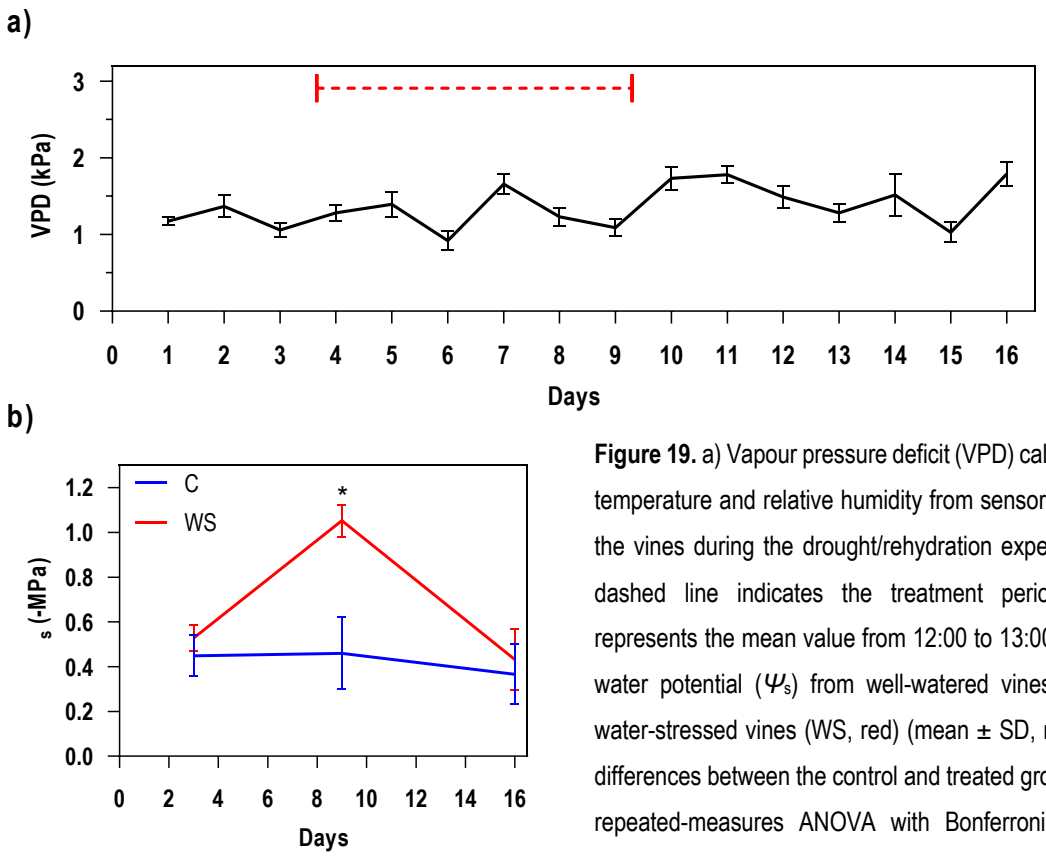
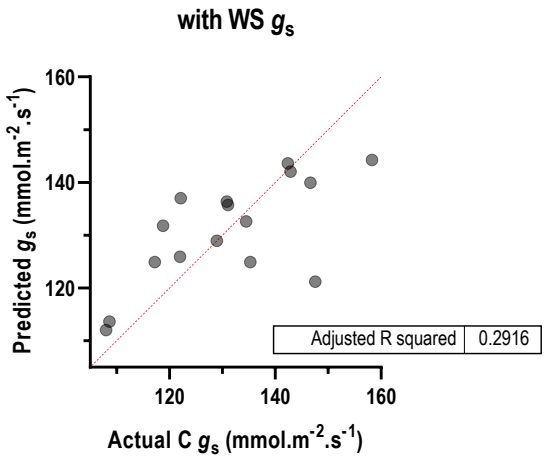


Figure 19. a) Vapour pressure deficit (VPD) calculated from the temperature and relative humidity from sensors placed among the vines during the drought/rehydration experiment. The red dashed line indicates the treatment period. Each point represents the mean value from 12:00 to 13:00 (n=2). b) Stem water potential (Ψ_s) from well-watered vines (C, blue) and water-stressed vines (WS, red) (mean ± SD, n=5). Significant differences between the control and treated groups by two-way repeated-measures ANOVA with Bonferroni post-tests are marked: * for p<0.05

3.3.2.4. Multi-variable analysis

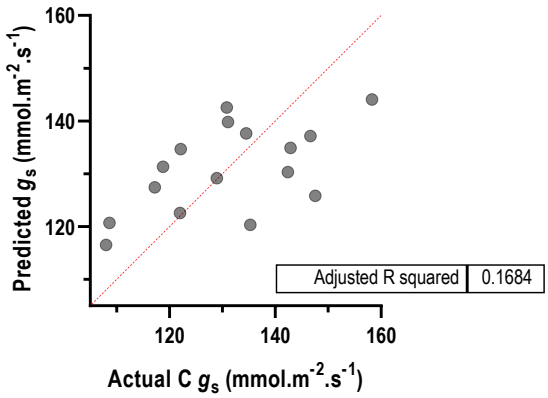
A multi-linear regression analysis was performed on C g_s with different parameters (C PAR, VPD, time, with or without WS g_s) and results showed that C g_s could be predicted by C PAR only when the WS g_s data was included (Figure 20a, Supplementary Table S37). The adjusted R^2 increased from 0.17 to 0.29 when with WS g_s suggesting that there may have been an influence on C g_s vine from the WS vines (Figure 20b, Supplementary Table S38). A similar analysis with the IRGA data revealed a not significant prediction for *E*, *NCAR* or g_s, but the R^2 increased with WS data included (data not shown, Supplementary Tables S39 and S40, and S41 and S42, and S43 and S44, respectively).

a)



Parameter estimates	Variable	Estimate	Standard error	95% CI (asymptotic)	|t|	P value	P value summary
β0	Intercept	35.99	41.34	-55.00 to 127.0	0.8706	0.4026	ns
β1	WS gs	-0.1697	0.09660	-0.3824 to 0.04286	1.757	0.1066	ns
β2	C PAR	0.1491	0.05657	0.02459 to 0.2736	2.636	0.0232	*
β3	VPD	-1.868	12.68	-29.78 to 26.05	0.1473	0.8855	ns
β4	Time	0.4744	0.7687	-1.218 to 2.166	0.6172	0.5497	ns

b)



Parameter estimates	Variable	Estimate	Standard error	95% CI (asymptotic)	|t|	P value	P value summary
β0	Intercept	65.89	40.82	-23.04 to 154.8	1.614	0.1324	ns
β1	C PAR	0.08225	0.04537	-0.01659 to 0.1811	1.813	0.0949	ns
β2	VPD	-2.064	13.74	-32.01 to 27.88	0.1502	0.8831	ns
β3	Time	0.8584	0.7986	-0.8815 to 2.598	1.075	0.3035	ns

Figure 20. Multilinear regressions of control well-watered (C) g_s and other variables a) with or b) without water-stressed (WS) g_s from *Vitis vinifera* during a drought/rehydration experiment. C PAR, photosynthetic active radiation of the C group (µmol.m^{-2}.s^{-1}); VPD, vapour pressure deficit (kPa); time, days of the experiment.

3.3.2.5. Correlation analysis of the position of the replicates

A Pearson correlation analysis was performed on the stomatal conductance of the biological replicates to investigate the position effect of the experimental set-up (Figure 21). It can be observed that the C3 and C4 replicates showed better positive correlations with all WS replicates, than the other C replicates. The C3 and C4 plants correspond to replicates positioned in the middle of the experimental set-up, i.e. with more WS replicates surrounding them (see Figure 15).

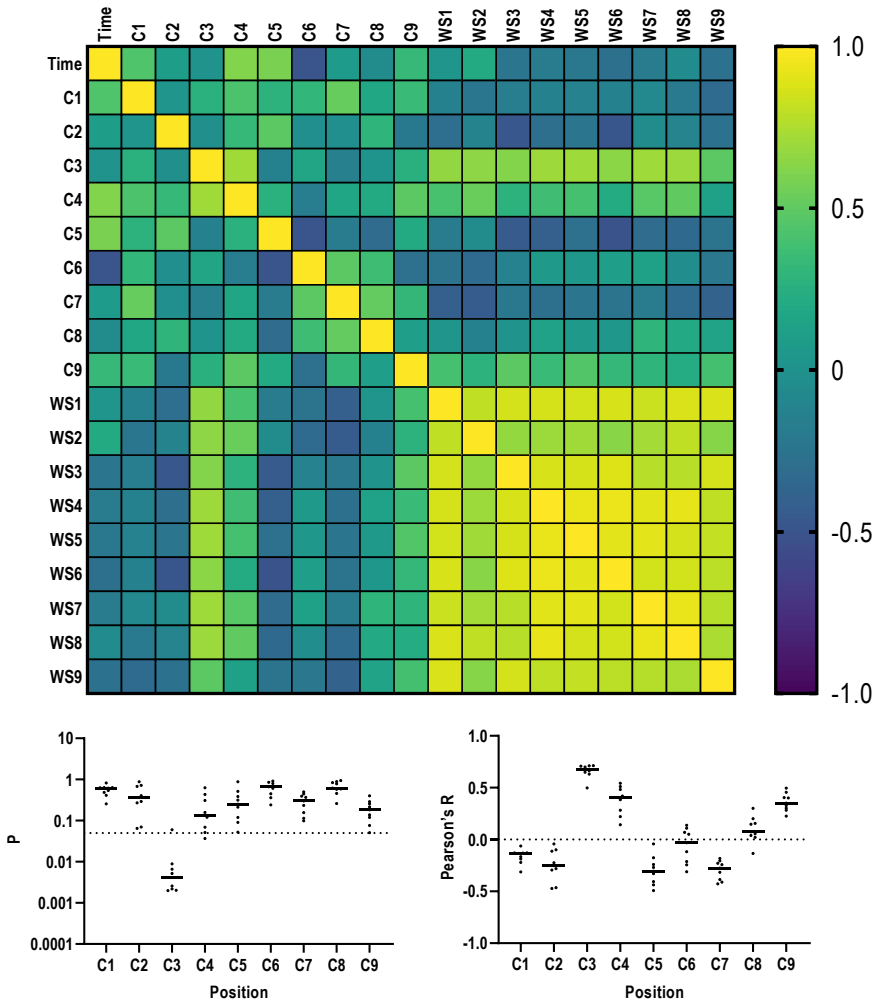


Figure 21. Pearson correlation matrix for control well-watered (C) and water-stressed (WS) biological replicate data of stomatal conductance, indicating the correlation differences based on the position of the replicate in the experimental set-up (see Figure 15), with the probabilities and coefficients of the correlations for the C replicates.

3.3.2.6. Volatile analysis

Four time points were selected for volatile sampling with the SPME (day 2, no stress; day 6, moderate stress, day 8, severe stress; and day 12, recovery). Data analysis of the GC-MS chromatograms revealed 20 peaks (compounds) that were common between the samples. The criteria for compound identity were that the library matches had to be greater than 50 % (match factor in Table 4) and was known to be emitted by plants (references in Table 4). However, no comparison with known standards or Kovats index calculations were conducted, and thus the compounds will be described by their # and library match names. It is important to note that it was not possible to differentiate the volatiles emitted by the control group from the water-stressed group since the SPME fibres were collecting volatiles from all the plants in the glasshouse. Hence, the focus of the data analysis is to compare between time points and the different phases of the stress.

Table 4. List of the compounds identified in *Vitis vinifera* cv. Grenache well-watered and drought-stressed plants, based on library match (%) and averaged retention time.

Peak #	Name from library match	Match factor (%)	Averaged retention time (min)	Reference
1	acetone	72	4.8	(Rissanen *et al.*, 2018)
2	2-butanone	72	6.1	(Souza *et al.*, 2013)
3	ethanol	78	6.8	(Holzinger *et al.*, 2000)
4	α-pinene	96	8.8	(Campbell *et al.*, 2018)
5	toluene	94	9.2	(Park *et al.*, 2009)
6	hexanal	87	10.3	(Ebel *et al.*, 1995)
7	β-pinene	90	10.9	(Campbell *et al.*, 2018)
8	butanol	80	12.3	(Maleknia *et al.*, 2007)
9	heptanal	89	13.3	(da Rocha *et al.*, 2017)
10	limonene	92	13.7	(Combariza *et al.*, 1994)
11	eucalyptol	98	14.1	(Niinemets *et al.*, 2002)
12	styrene	93	15.4	(Araya *et al.*, 2019)
13	1-methyl-2-(1-methylethyl)-benzene	95	15.8	(Dalai *et al.*, 2006)
14	octanal	95	16.4	(Hu *et al.*, 2009)
15	cyclohexanone	78	16.5	(Saunier *et al.*, 2020)
16	nonanal	91	19.3	(Hu *et al.*, 2009)
17	acetic acid	87	20.6	(Dewhirst *et al.*, 2020)
18	2-ethylhexanol	83	21.7	(Wei *et al.*, 2004)
19	benzaldehyde	94	22.7	(da Rocha *et al.*, 2017)
20	pivalic acid	68	22.9	(Park *et al.*, 2017)

Different classes of volatiles were identified such as alcohols (e.g. ethanol), aldehydes (e.g. hexanal), terpenoids (e.g. eucalyptol) and ketones (e.g. acetone) (Table 4), and the area of each peak was measured (in counts) and compared between samples. *Vitis vinifera* cv. Grenache vines were found to strongly emit 2-butanone, ethanol, nonanal, acetic acid and 2-ethyl hexanol on day 2 when all the plants were watered (Figure 22a). On days 6 and 8, when drought stress was imposed on half of the vines, the peak areas of the compounds drastically decreased (Figure 22c) and increased again on day 12 when all the plants were

watered. Some compounds exhibited a much larger peak on day 8 when the stress was severe, such as α-pinene, limonene or 1-methyl-2-(1-methylethyl)-benzene (Figure 22b).

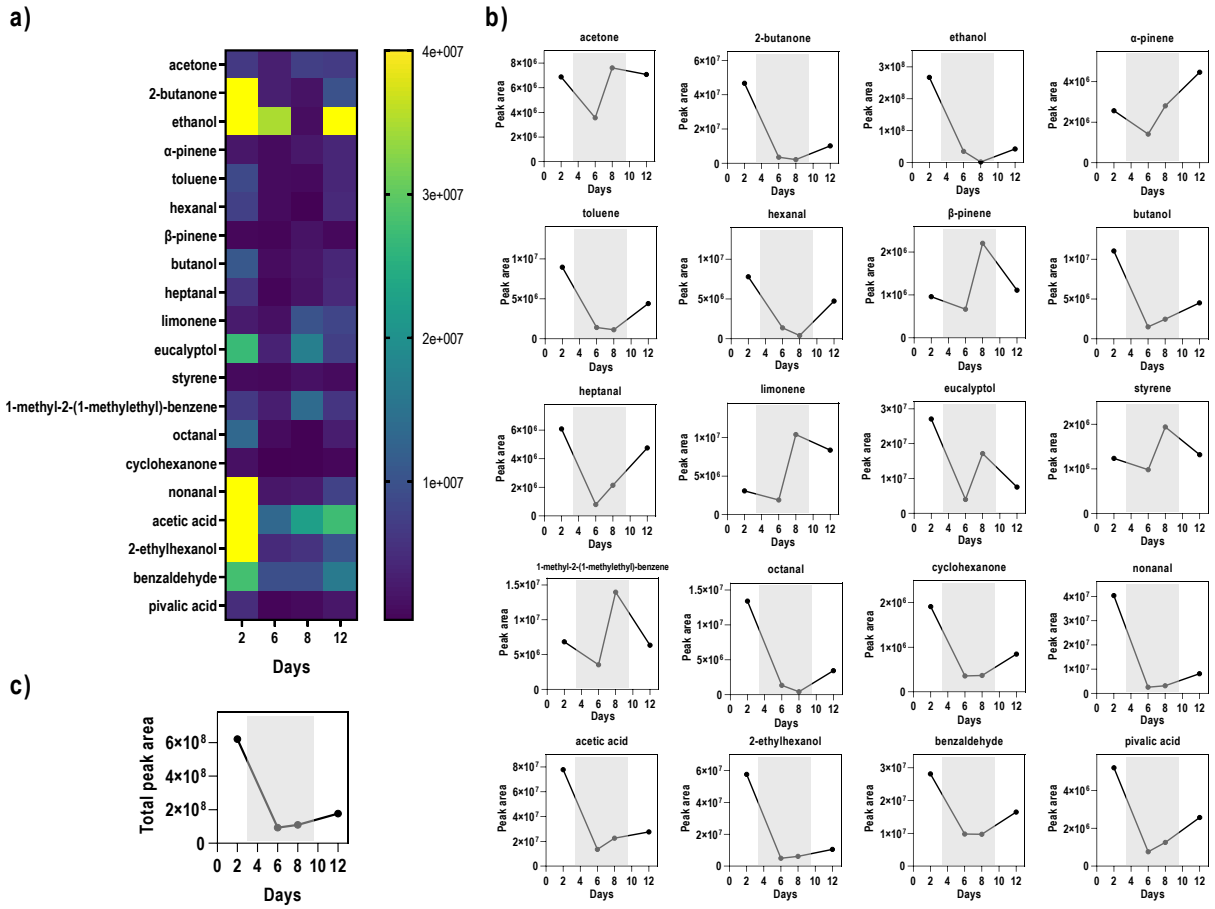


Figure 22. Overview of peak areas (counts) of total ion chromatograms from *Vitis vinifera* cv. Grenache during a drought-rehydration experiment (analysed with SPME-GC-MS). Plant-emitted volatile compound names were allocated from library matches (>50%) and literature references. a) Heatmap plots of chromatographic peak areas of the individual compounds found in the control well-watered and water-stressed vines. b) Peak area from individual volatile compounds over time. Watering was withheld from day 3 to day 9 (grey box). c) Total peak area over time.

3.3.2.7. Combined analysis of physiology and chemistry results

The volatile analysis results were compared with the previous results of stomatal conductance, C PAR and VPD. The Pearson correlations (Figure 23a) with respective p values (<0.05) (Figure 23b) between volatiles and g_s of both WS and C plants revealed positive correlations between several volatiles. For example, β-pinene, limonene, styrene and 1-methyl-2-(1-methylethyl)-benzene were negatively correlated with WS g_s

(not statistically significant) whereas α-pinene was strongly positively correlated with C g_s (statistically significant) (Figure 23c).

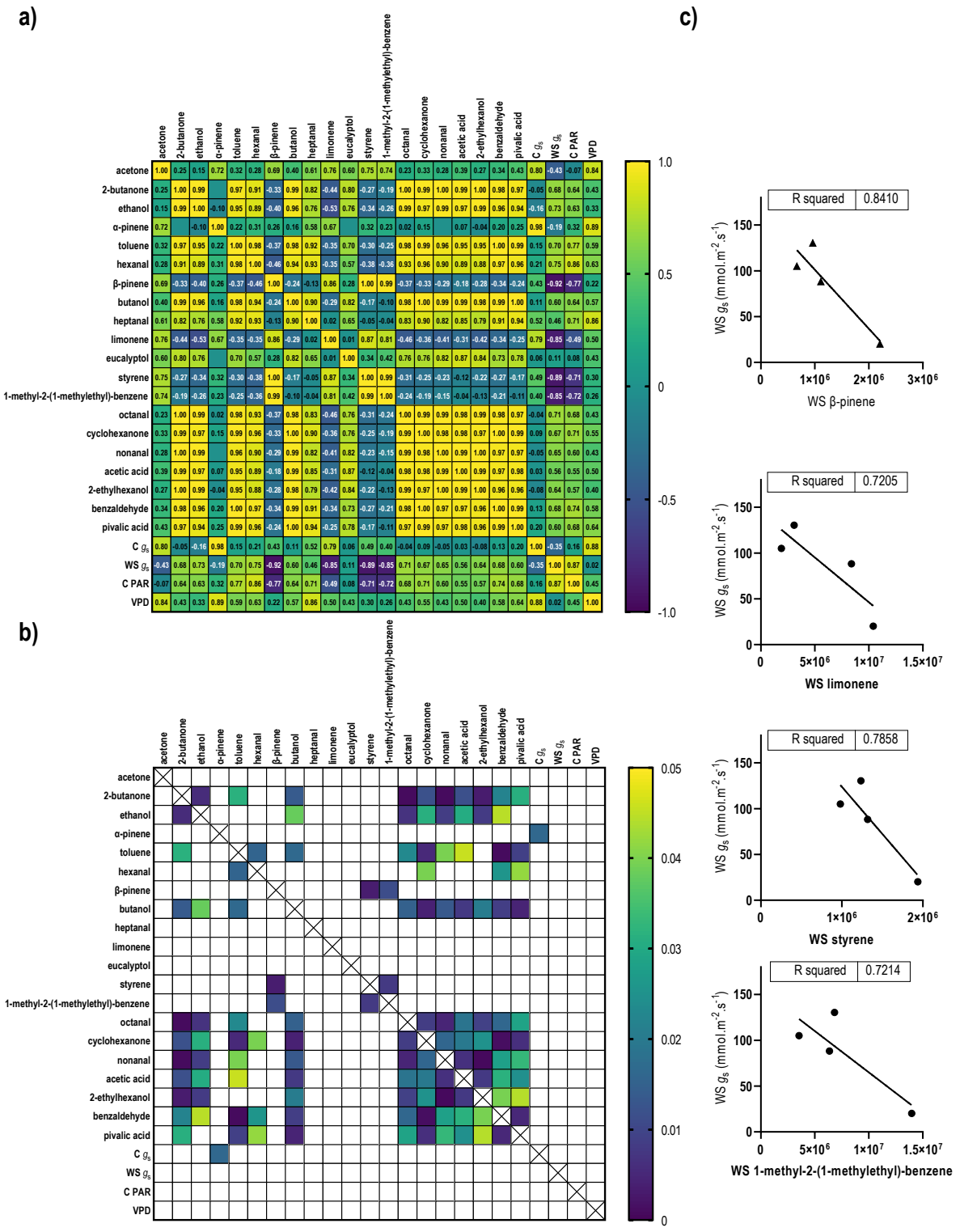


Figure 23. Pearson correlation matrices for control well-watered (C) and water-stressed (WS) plants indicating some differences in the correlation between volatiles, stomatal conductance (g_s), C photosynthetic active radiation (PAR) and vapour pressure deficit

(VPD). a) The correlation coefficients and b) p values (<0.05) are shown for each combination in the relevant square. c) Linear regressions of WS g_s with β-pinene, limonene, styrene and 1-methyl-2-(1-methylethyl)-benzene.

3.3.3. Discussion

Similar to the results for Chardonnay described in section 3.2, Chapter 3, a clear synchronisation between g_s of control and water stressed vines was not present. The Pettitt homogeneity test did not detect a shift in the C data series for the porometer and IRGA data (Figure 17). However, the regression analysis suggested that there was an effect of WS vines on the g_s of C vines based on the improvement in the predictability of C g_s when WS g_s data were included and accounting for changes in VPD and PAR (Figure 20). The same observation was obtained for the IRGA parameters. The analysis of the g_s of the individual C replicates also showed a greater positive correlation for the vines positioned in the middle of the experimental set-up, i.e. with more water-stressed plants surrounding them (Figure 21).

The chromatographic analysis obtained from the use of SPME-fibres placed for an hour among the vines revealed many single compound peaks. Twenty of them were selected among all samples and were identified from library matches only (>50% match) and listed in Table 4. The peak area (in counts) was used to compare the compounds relative content between days of sampling. The total peak area content showed a reduction of emission on the moderate and severe stress days. This is similar to the results found in the literature and described in Table 2, section 1.4.1, Chapter 1. On the contrary, the content of five of the volatiles (acetone, β-pinene, limonene, eucalyptol, styrene and 1-methyl-2-(1-methylethyl)-benzene) increased on the maximal stress day of the WS group and decreased after rewatering (Figure 22). It is known that β-pinene has been implicated in contributing to systemic resistance induction in the same and neighbouring plants of Arabidopsis when challenged with avirulent *Pseudomonas syringae* (Riedlmeier *et al.*, 2017). Some of the terpenes here identified have been linked to abiotic stress in plants previously (summarised in Boncan *et al.* (2020)), and heat stress in Chardonnay resulted in emission that differed between clones, one of which had a mutation in a MEP pathway enzyme (Bertamini *et al.*, 2019).

Overall, these results show a potential effect of the water-stressed vines on the stomatal regulations compared with well-watered vines and that some volatile compound emissions were disrupted by the water stress, by either an increase or a decrease. Thus, this experiment was repeated in the next section.

3.4. Drought/rehydration treatment with *Vitis vinifera* cv. Grenache vines in the same glasshouse while monitoring transpiration using the 'Droughtspotter' and volatile emission analysis

As described in Chapter 3.3, Grenache was chosen for this experiment since this cultivar was used by Dayer *et al.* (2017) where synchronisation was evident between control and water-stressed plants. Given the results of the multilinear regressions in previous sections that indicated an effect of the water-stressed on the control group stomatal conductance, it was considered that a further more controlled experiment using the Droughtspotter gravimetric platform (Phenospex, Netherlands) in the Australian Plant Phenomics Facility (APPF) (also used in Dayer *et al.* (2017)) may yield more conclusive results. Volatile samples were again taken during the experiment to confirm results obtained in previous sections.

The Droughtspotter platform was situated in a clear glasshouse with supplementary light and consisted of a precision irrigation system allowing accurate and reproducible water application for drought stress experiments (Cousins *et al.*, 2020). Based on a mass target, the platform can adjust the weight and watering at selected times with a precision of 1 g. Hence, it was possible to change the weight target during the course of the experiment to, first, reduce the watering and induce a progressive drought stress for half of the plants before completely stopping the watering. Plant transpiration rates were calculated with high temporal resolution by the loss of weight of the pots.

3.4.1. Material and methods

Plant and environmental conditions

Vitis vinifera cv. Grenache vines were potted and grown in glasshouse under natural light (Autumn, May 2019) until reaching 1-2 shoots with approximately 10 leaves per shoot.

Drought/rehydration experiment

The potted vines were moved from the glasshouse they grew in into the Droughtspotter platform with 25°C during the day, 17°C at night and 40 % humidity. This platform was equipped with additional LED lamps to assure a constant minimal light exposure above the vines from 8:00 to 18:00 (Australian central standard time), and temperature and humidity sensors were placed among the vines. Each pot was placed on an electronic balance for continuous weighing (every 10 min) and daily watering to replace the water lost by transpiration and evaporation at 6:00 and 18:00, for two weeks until the onset of the experiment.

The vines were divided in two groups, ten control well-watered (C) and then water-stressed (WS) vines were distributed on individual balances (50 cm between vines) with the aim of mixing the plants so control vines were surrounded by water-stressed vines to increase the chance of exchange of emitted volatiles (Figure 24). Care was taken to avoid physical contact between vines or with surrounding structures since this would interfere with the weight measurements. At the start, and for all vines, water field capacity (WFC) weight target values were kept for the automated irrigation during the whole experiment. The C vines were watered twice a day (6:00 and 18:00) over the period of the experiment. After 4 days of watering to WFC, the irrigation of the WS group was cut off for 7 days until a defined value of leaf maximum daily g_s of approximately 50 mmol H_20 $m^{-2}.s^{-1}$ was reached (Medrano, 2002), then the drought treatment was maintained constant but replacing the amount of water transpired daily for two days. After that, WS vines were rehydrated by irrigating the pots back to WFC until the end of the experiment. Daily and nightly water use was calculated from the continuous mass measurements of pots every 10 min.

Figure 24. Positioning of 10 control well-watered (C) and 10 water-stressed (WS) vines in the Droughtspotter precision irrigation system. Each vine was on an individual electronic balance (circle) monitoring the weight every 10 min to determine the loss of water during the day and watering the vines accordingly. Temperature and humidity sensors (T&H yellow squares) were placed among the vines, and solid-phase micro-extraction (SPME) fibres were manually exposed and hung between the vine leaves to extract volatile compounds for a period of one hour (12:00 to 13:00) at selected days.

SPME fibres were exposed between 12:00 to 13:00 to extract volatiles emitted from all vines by manually exposing the coating of the fibre and hanging it between the vines at the leaf level (Figure 24c). They were then desorbed on a GC-MS system on the day of collection and reconditioned for the next day of sampling. Stomatal conductance was measured on all vines (3 fully expanded flagged leaves each) with a porometer every day from 13:00 to 15:00 and following a rotation order starting with a different vine each day. Transpiration (*E*), net carbon assimilation rate (*NCAR*) and g_s were also measured with an infra-red gas analyser (IRGA; LCpro-SD) every two days at the same time as the porometer. Parameters on the IRGA were fixed-PAR set at 1000 µmol.m^{-2}.s^{-1}, ambient CO_2 and water vapour concentrations, air flow at 300 mL.min^{-1} and one measurement per flagged leaf per plant (different leaf from porometer). On day 11, stem water potentials were determined on 4 vines per treatment and 2 leaves per vine. Finally, projected leaf area was determined by scanning all leaves and analysed with ImageJ. All methods are described in Chapter 2.

3.4.2. Results

3.4.2.1. Stomatal conductance

During the first 4 days where both treatments were watered at WFC, measurements of the stomatal conductance showed g_s of 390 ± 48 mmol.m^{-2}.s^{-1} (mean ± SD) for the control (C) treatment and a higher g_s for the water-stressed (WS) treatment 486 ± 51 mmol.m^{-2}.s^{-1} (Figure 25a). Once water was reduced for the WS vines, their g_s matched the C g_s from day 5 to day 7, and quickly dropped until reaching 11 ± 4 mmol.m^{-2}.s^{-1} on day 9. This g_s was kept approximately constant until day 11 by adding only the mass of water that was used during the day, and then water was re-supplied and the WS g_s returned to the same level as the C treatment on day 14 and until day 19. The g_s of the C treatment linearly decreased over time from 440 ± 51 to 207 ± 34 mmol.m^{-2}.s^{-1} from day 1 to day 16, with a drop on day 6. At the end of the experiment, on day 17 and day 18, a drop of g_s for both treatments was observed and the watering was increased for all vines. No significant differences were found for g_s or PAR between C and WS (two-way repeated-measures ANOVA with Bonferroni post-tests, $p<0.05$, Supplementary Tables S13a and b, and S14a and b, respectively).

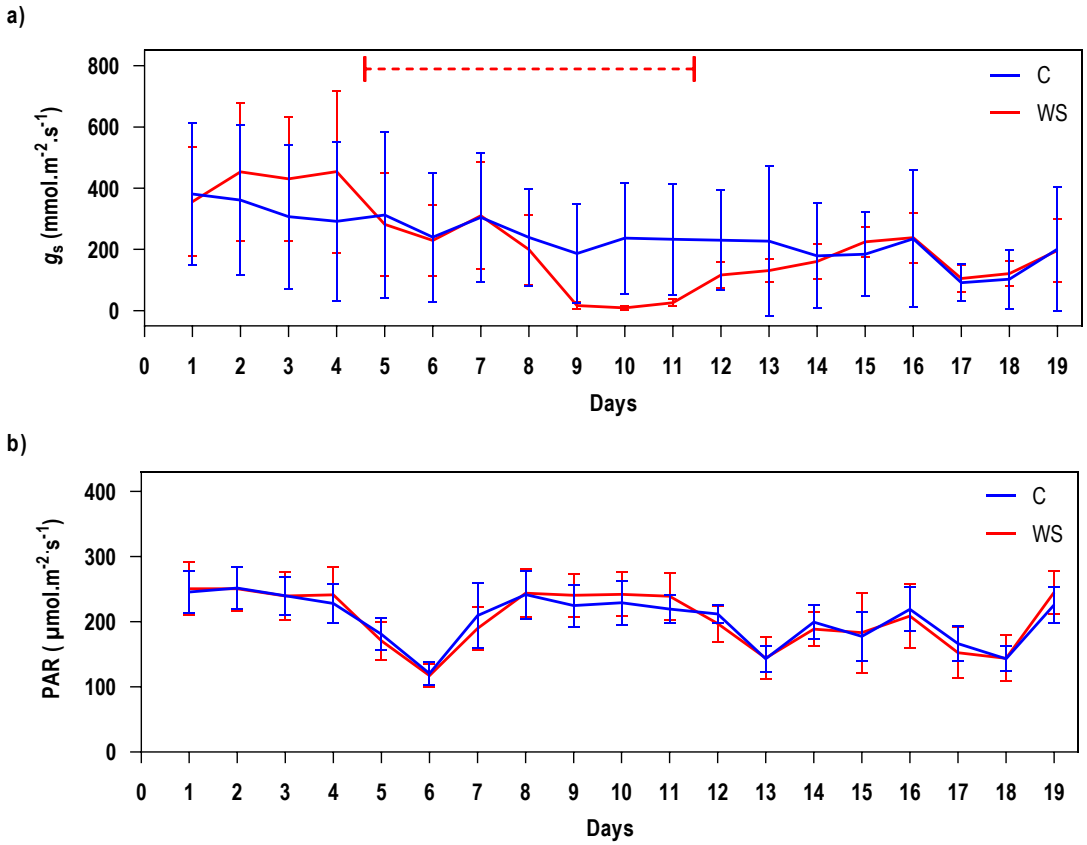


Figure 25. a) Stomatal conductance (g_s) measured on leaves of *Vitis vinifera* cv. Grenache used in a drought/rehydration experiment with vines arranged in two rows with 50 cm interspace in the same glasshouse with automated irrigation. Control (C, blue) vines were watered to field capacity every day of the experiment and water-stressed (WS, red) vines had water from day 1 to day 3. Then, watering was reduced from day 4 to day 9, after which watering was kept constant (red dashed line). On day 11, irrigation to field capacity was resumed (mean ± SD, n=10). b) Leaf incident photosynthetically active radiation (PAR) was measured with the porometer light sensor simultaneously as g_s measurements.

The Pettitt homogeneity tests revealed a shift in the data series of C on day 9 with a decrease of values on day 9 but not when the recovery was not considered, and a shift was detected for WS data series on day 8 in both analyses (Figure 26a and b).

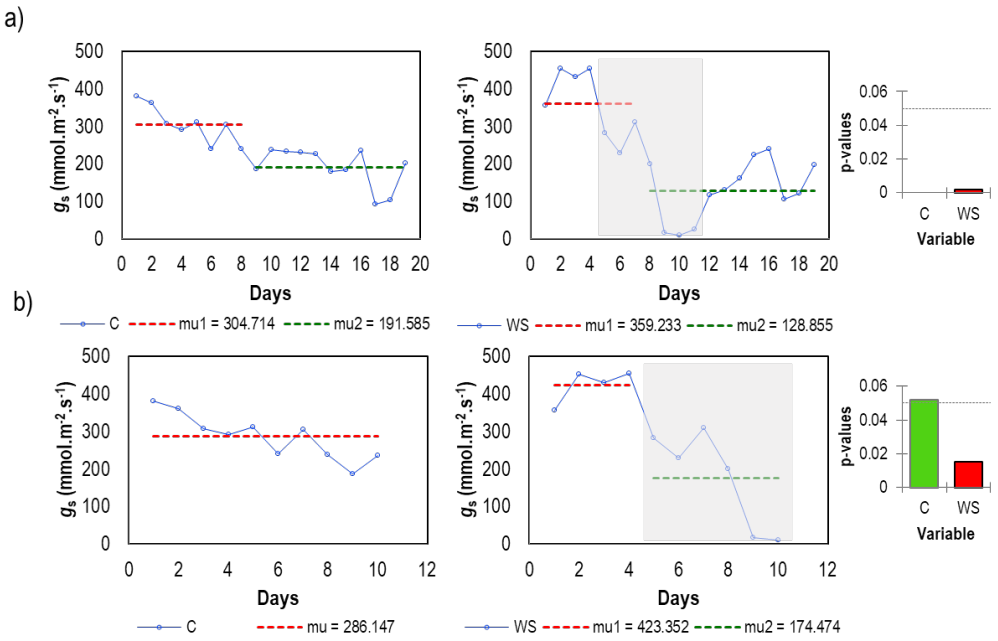


Figure 26. Pettitt homogeneity test on the stomatal conductance (g_s) data measured with the porometer from well-watered vines (C) and water-stressed vines (WS) in a drought experiment (grey box). The test was performed on a) the whole series of data and b) without the recovery (mean, n=10), and the dotted lines represent the averaged value for the data series with two mu values if a change point is detected ($p<0.05$).

3.4.2.2. Gas exchange

Transpiration (*E*), net carbon assimilation rate (*NCAR*) and stomatal conductance (g_s) measured by the IRGA showed the same trend as that for g_s measured by the porometer (Figure 27). However, the drop on day 9 for the C vines was not observed as no measurements were taken on that day. Significant differences for *E*, *NCAR* and g_s between C and WS were found on day 11 (two-way repeated-measures ANOVA with Bonferroni post-tests, $p<0.05$, Supplementary Tables S15a and b, S16a and b, S17a and b, respectively).

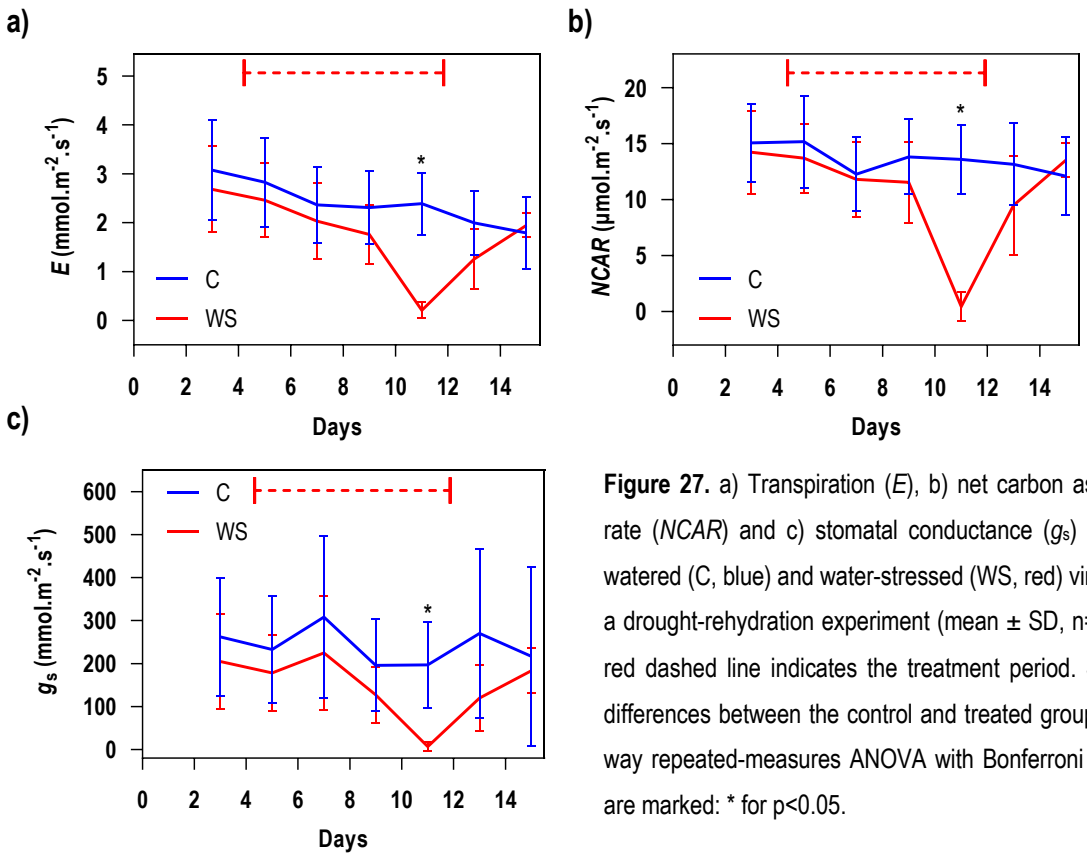


Figure 27. a) Transpiration (*E*), b) net carbon assimilation rate (*NCAR*) and c) stomatal conductance (g_s) from well-watered (C, blue) and water-stressed (WS, red) vines during a drought-rehydration experiment (mean ± SD, n=10). The red dashed line indicates the treatment period. Significant differences between the control and treated groups by two-way repeated-measures ANOVA with Bonferroni post-tests are marked: * for p<0.05.

Pettitt homogeneity test performed on *E* revealed the same results as for the g_s with the porometer, showing a shift in the data series of C and WS with decreased values on day 9 (p<0.05, Figure 28), but no shift was detected for *NCAR* and g_s (p<0.05, data not shown).

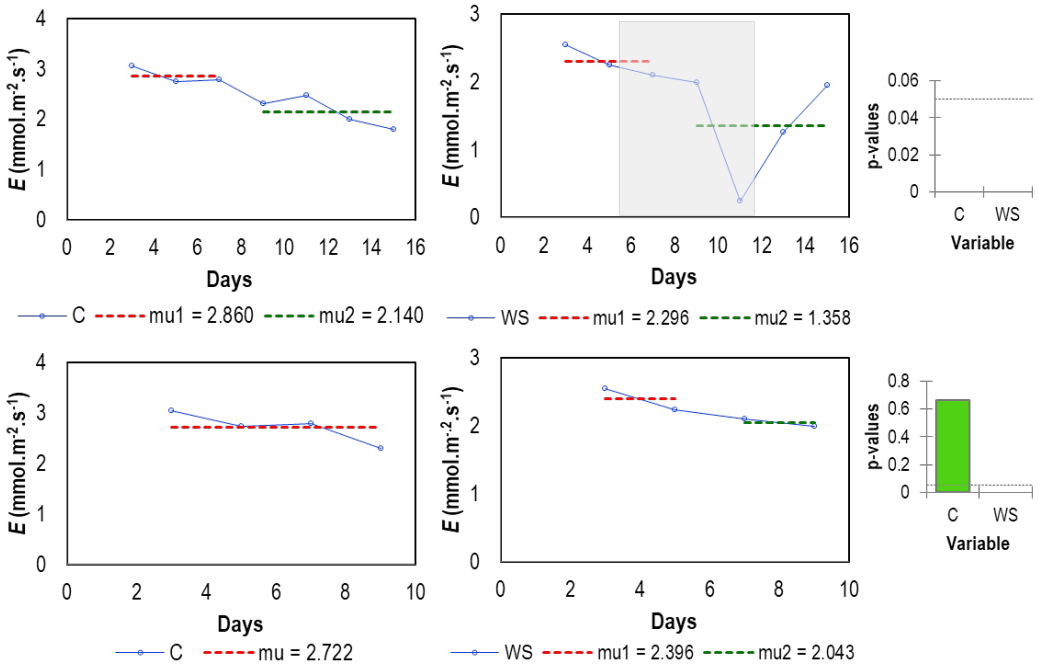


Figure 28. Pettitt homogeneity test on transpiration (*E*) data measured with the IRGA from control well-watered (C) and water-stressed (WS) vines in a drought experiment (grey box). The test was performed on a) the whole series of data and b) without the recovery phase (mean, n=10), and the dotted lines represent the averaged value for the data series with two mu values if a change point is detected ($p<0.05$).

3.4.2.3. Other parameters (VPD, Ψ_s, leaf area)

VPD was steady from day 1 to day 11 and was more varying for the rest of the experiment without going above 2 kPa (Figure 29a). The stem water potential was only measured on the last day of stress of the WS treatment (day 11) and the results showed that the C vines were not stressed with Ψ_s of -0.4 MPa and the WS vines were stressed with Ψ_s below -1 MPa (Figure 29b). The Ψ_s of C and WS was significantly different on day 11 (t test, $p<0.05$, Supplementary Table S26). The projected leaf area between the treatments C and WS (Figure 29c) was not significantly different (t test, $p<0.05$, Supplementary Table S27).

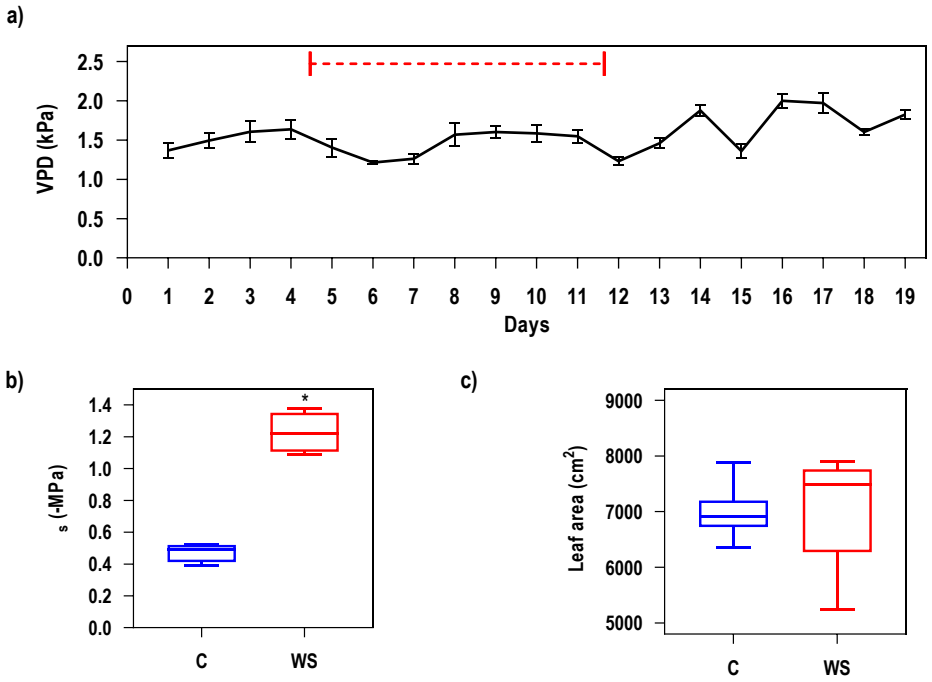


Figure 29. a) Vapour pressure deficit (VPD) calculated from the temperature and relative humidity from sensors placed among the well-watered vines during the drought/rehydration experiment from 12:00 to 13:00 (mean ± SD, n=2). The red dashed line indicates treatment period. b) Stem water potential (Ψ_s) from control well-watered (C, blue) and water-stressed (WS, red)) vines (mean ± SD, n=5). c) Project leaf area (mean ± SD, n=6). Significant differences between the control and treated groups by t test are marked: * for $p<0.05$.

3.4.2.4. Daily and nightly water use

Day and night water use (WU) was calculated from the pot weight every 10 min and showed that the well-watered vines (C) did not have a linear WU over time with decrease on day 6, day 12, day 15 and day 18 (Figure 30). Significant differences for day WU between C and WS were found on day 9, day 10 and day 11, and night consumption on day 8, day 9 and day 10 (two-way repeated-measures ANOVA with Bonferroni post-tests, $p<0.05$, Supplementary Tables S18a and b, and S19a and b, respectively). Interestingly, the drop in stomatal conductance detected by the porometer on day 9 for the C vines did not reflect a decrease in WU on the same day.

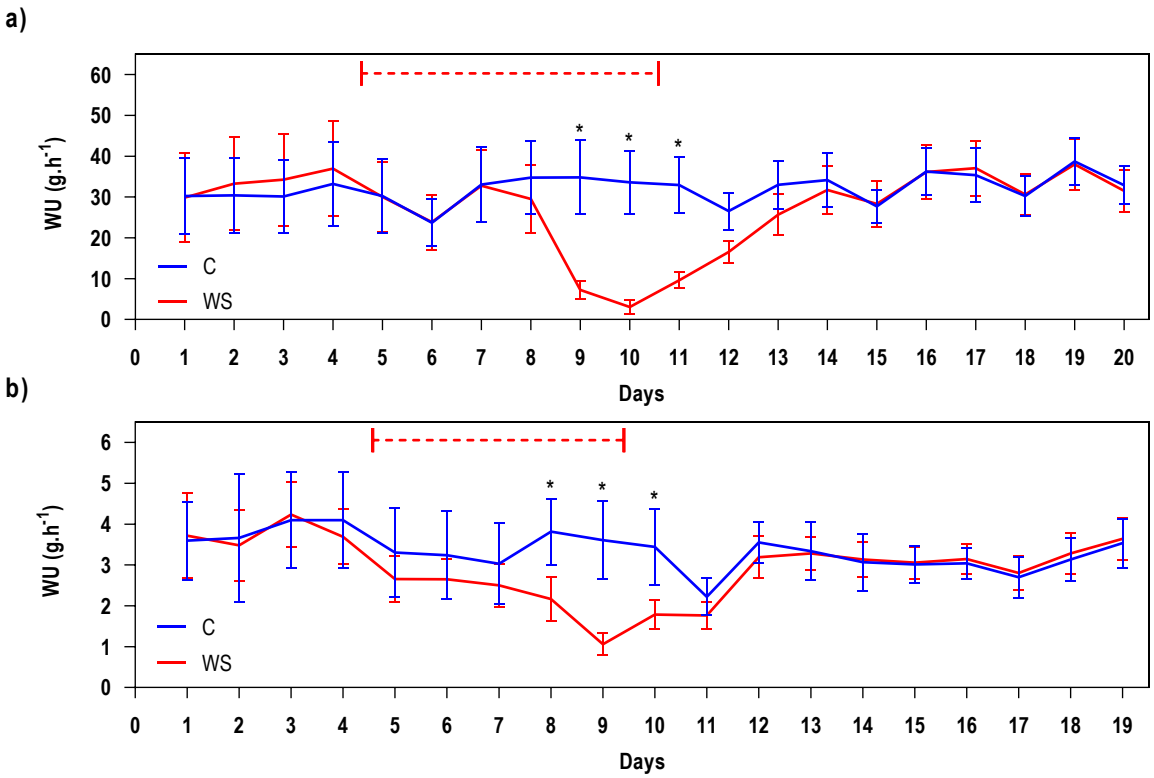
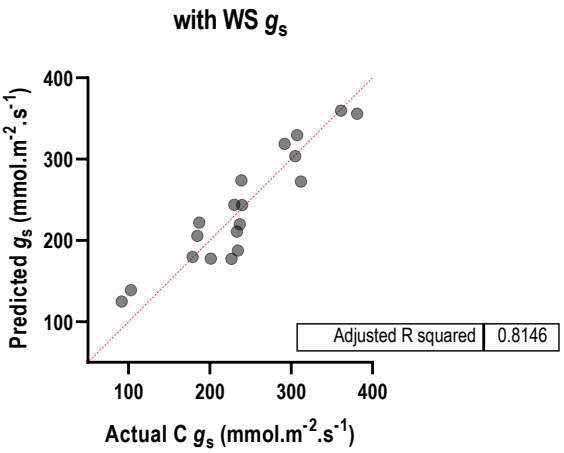


Figure 30. a) Day and b) night water use (WU) from control well-watered (C, blue) and water-stressed vines (WS, red) during a drought/rehydration experiment, calculated from continuous pot weight measurements (mean ± SD, n=5). Control vines (C, blue) were watered to field capacity every day and the dashed line represents the period during which the water-stressed vines (WS, red) stopped receiving water. Significant differences between the control and treated groups by two-way repeated-measures ANOVA with Bonferroni post-tests are marked: * for $p<0.05$.

3.4.2.5. Multi-variable analysis

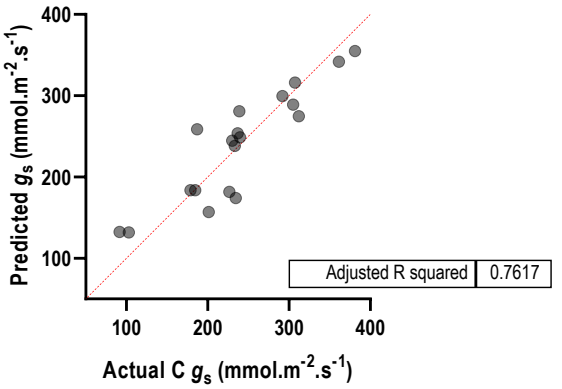
A multi-linear regression analysis was performed on C g_s with different parameters (C PAR, VPD, time, with or without WS g_s) and results showed a strong correlation between C g_s and both C PAR and WS g_s (Figure 31a, Supplementary Table S45). These correlations are no longer significant without WS g_s included in the analysis, as well as a reduced R^2 (Figure 31b, Supplementary Table S46). A similar analysis with the IRGA data revealed no significant prediction for *E*, *NCAR* or g_s (Supplementary Tables S47 and S48, and S49 and S50, and S51 and S52, respectively).

a)



Parameter estimates	Variable	Estimate	Standard error	95% CI (asymptotic)	|t|	P value	P value summary
β0	Intercept	278.8	60.10	149.9 to 407.8	4.640	0.0004	***
β1	WS gs	0.1680	0.07308	0.01124 to 0.3247	2.299	0.0374	*
β2	CPAR	0.5559	0.2194	0.08528 to 1.027	2.533	0.0239	*
β3	VPD	-89.19	46.64	-189.2 to 10.85	1.912	0.0765	ns
β4	Time	-5.336	2.464	-10.62 to -0.05111	2.166	0.0481	*

b)



Parameter estimates	Variable	Estimate	Standard error	95% CI (asymptotic)	|t|	P value	P value summary
β0	Intercept	315.2	65.75	175.1 to 455.3	4.794	0.0002	***
β1	CPAR	0.5142	0.2480	-0.01435 to 1.043	2.074	0.0558	ns
β2	VPD	-62.54	51.22	-171.7 to 46.64	1.221	0.2410	ns
β3	Time	-8.657	2.263	-13.48 to -3.835	3.826	0.0017	**

Figure 31. Multilinear regressions of control well-watered C g_s and other variables a) with or b) without water-stressed WS g_s from *Vitis vinifera* during a drought/rehydration experiment. C PAR, photosynthetic active radiation of the C group (µmol.m^{-2}.s^{-1}); VPD, vapour pressure deficit (kPa); time, days of the experiment.

3.4.2.6. Correlation analysis of the position of the replicates

A Pearson correlation analysis was performed on the stomatal conductance of the C and WS biological replicates and did not reveal differences based on their positions in the experimental set-up (Figure 32).

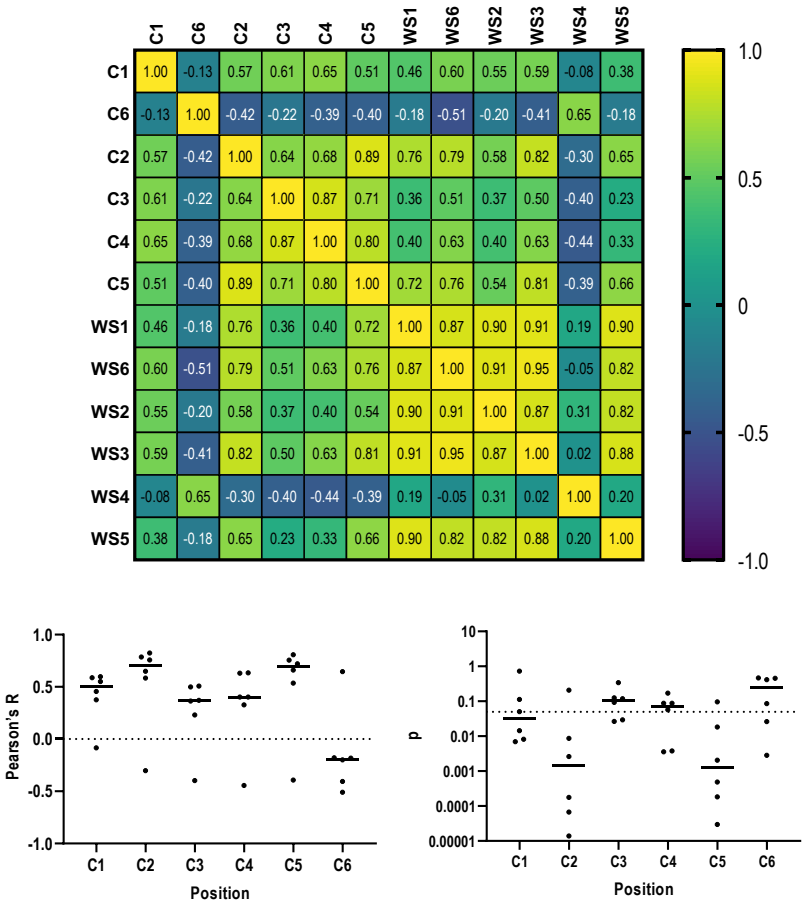


Figure 32. Pearson correlation matrix for control well-watered (C) and water-stressed (WS) biological replicate data of stomatal conductance, indicating the correlation differences based on the position of the replicate in the experimental set-up (see Figure 24a), with the probabilities and coefficients of the correlations for the C replicates.

The same correlation analysis was performed on the data series without the recovery phase since there were more replicates (i.e. 4 vines were harvested for stem water potential on day 10) with the replicate order arranged as in Figure 24a, but did not reveal noticeable difference based on the position (Figure 33).

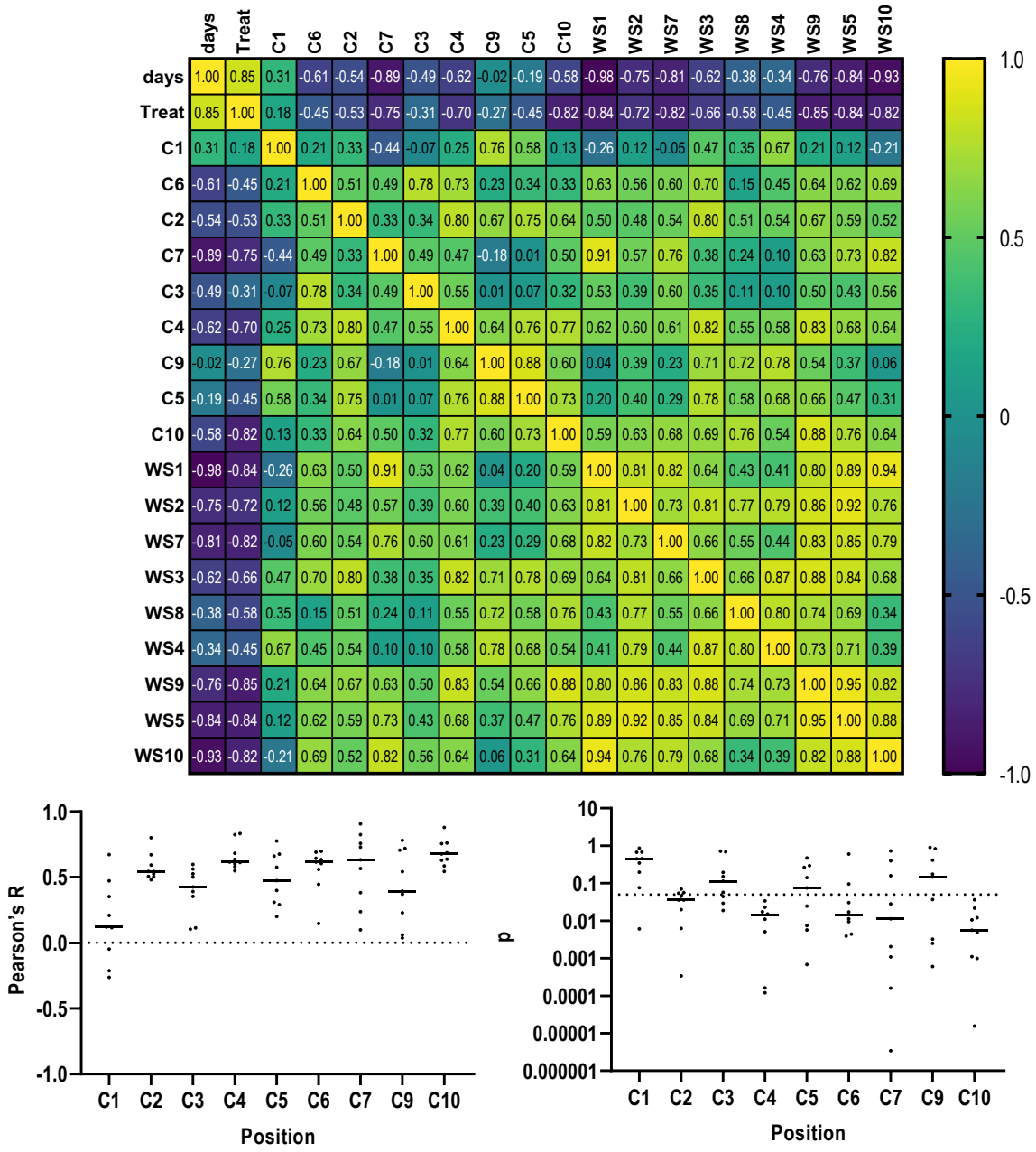


Figure 33. Pearson correlation matrix for C replicates and WS repetitions data of stomatal conductance until day 10 (without the recovery), indicating the correlation differences based on the position of the replicate in the experimental set-up (see Figure 24a), with the probabilities and coefficients of the correlations for the C replicates.

3.4.2.7. Volatile analysis

Four time points were selected for volatile sampling with the SPME (day 2, no stress; day 6, moderate stress, day 9, severe stress; and day 13, recovery). Data analysis of the GC-MS chromatogram followed the same methodology as in section 3.3.2.6, Chapter 3, and revealed 26 peaks, based on match factor and references (Table 5).

Table 5. List of the compounds identified in *Vitis vinifera* cv. Grenache well-watered plants and drought-stressed plants, based on library match (%) and averaged retention time.

Peak #	Name from library match	Match factor (%)	Averaged retention time (min)	Reference
1	acetone	64	4.7	(Rissanen *et al.*, 2018)
2	2-butanone	53	5.9	(Souza *et al.*, 2013)
3	ethanol	64	6.6	(Holzinger *et al.*, 2000)
4	benzene	90	6.8	(Araya *et al.*, 2019)
5	3-methylbutanal	53	7.5	(Mazza & Cottrell, 1999)
6	α-pinene	96	8.6	(Campbell *et al.*, 2018)
7	toluene	60	9.1	(Park *et al.*, 2009)
8	hexanal	58	10.2	(Ebel *et al.*, 1995)
9	β-pinene	83	10.8	(Campbell *et al.*, 2018)
10	ethylbenzene	93	11.4	(Araya *et al.*, 2019)
11	p-xylene	83	11.6	(Araya *et al.*, 2019)
12	1,3-dimethyl-benzene	94	11.8	(Bylka *et al.*, 2010)
13	1-butanol	58	12.1	(Maleknia *et al.*, 2007)
14	limonene	91	13.5	(Combariza *et al.*, 1994)
15	Eucalyptol (1,8-cineole)	98	13.9	(Niinemets *et al.*, 2002)
16	styrene	90	15.2	(Araya *et al.*, 2019)
17	m-cymene	94	15.6	(Geron *et al.*, 2016)
18	1,2,3-trimethylbenzene	95	15.9	(Ogunwande *et al.*, 2008)
19	octanal	89	16.2	(Hu *et al.*, 2009)
20	cyclohexanone	50	16.3	(Saunier *et al.*, 2020)
21	3-ethyl-o-xylene	60	17.2	(Ajayi *et al.*, 2015)
22	nonanal	90	19.1	(Hu *et al.*, 2009)
23	acetic acid	91	20.4	(Dewhirst *et al.*, 2020)
24	2-ethylhexanol	90	21.6	(Wei *et al.*, 2004)
25	pivalic acid	92	22.6	(Park *et al.*, 2017)
26	4-methyl-benzaldehyde	80	24.9	(Saucier *et al.*, 2014)

Different classes of volatiles were identified such as alcohols (e.g. ethanol), aldehydes (e.g. hexanal), terpenoids (e.g. eucalyptol) and ketones (e.g. acetone) and the area of each peak was calculated (in counts) to compare between samples (Figure 34a). The *Vitis vinifera* cv. Grenache vines were found to strongly emit acetic acid on day 2 when all plants were watered. On days 6 and 9, when drought stress was imposed on half of the vines, the detection of some of those compounds increased, and decreased again on day 12 when all the plants were watered (benzene, 3-methyl butanal, α-pinene, toluene, β-pinene, ethylbenzene, p-xylene, 1,3-dimethyl benzene, eucalyptol (1,8-cineole), 1,2,3-trimethylbenzene, cyclohexanone, nonanal, 2-ethyl hexanol, pivalic acid, 4-methyl-benzaldehyde) (Figure 34b).

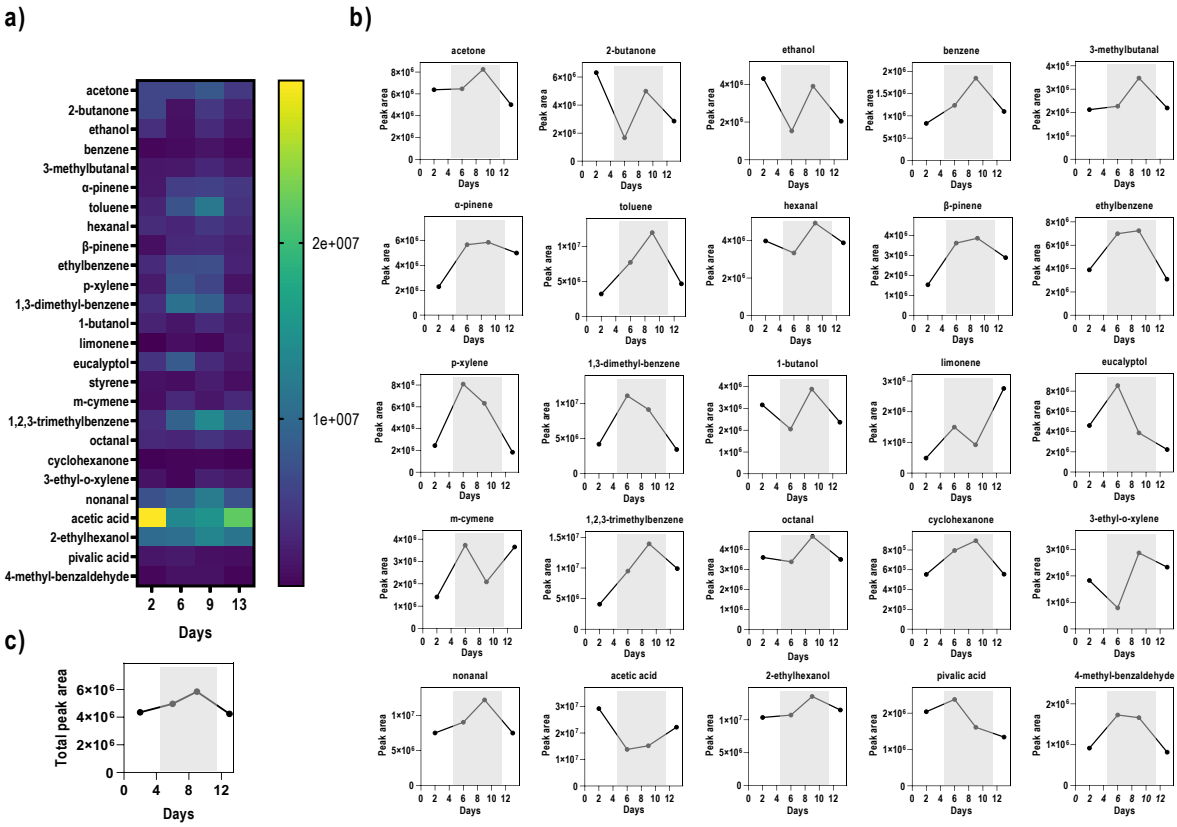


Figure 34. Overview of peak areas (counts) of total ion chromatograms from *Vitis vinifera* cv. Grenache during a drought-rehydration experiment (sampled with SPME fibres and analysed with GC-MS). Plant-emitted volatile compound names were allocated from library matches (>50%) and literature references. a) Heatmap plots of chromatographic peak areas of the individual compounds found in the control well-watered and water-stressed vines. b) Peak area from individual volatile compounds over time. Watering was withheld from day 5 to day 11 (grey box). c) Total peak area over time.

3.4.2.8. Combined analysis of physiology and chemistry results

The volatile results were compared with the previous results of g_s, WU, C PAR and VPD. In Figure 35a, the Pearson correlations with respective p value (<0.05, Figure 35b) between volatiles and g_s of both WS and C plants revealed negative correlations for α-pinene (only statistically significant for C g_s), β-pinene (not statistically significant) and 1,2,3-trimethylbenzene (statistically significant for both groups). 2-Butanone and ethanol were statistically significantly positively correlated with C PAR, as well as 1-butanol but not statistically significantly. m-Cymene was negatively correlated with C PAR and C night WU (statistically significant). Benzene, 3-methylbutanal, toluene and nonanal were negatively correlated with WS day and night WU (statistically significant). For each of these compounds, the linear regressions revealed R^2 above 0.9 (Figure 36).

Figure 35. Pearson correlation matrix for well-watered (C) and water-stressed (WS) plants indicating some differences in the correlation between volatiles, stomatal conductance (g_s), water use (WU), photosynthetic active radiation (PAR) and vapour pressure deficit (VPD). a) The correlation coefficients and b) p values (<0.05) are shown for each combination in the relevant square.

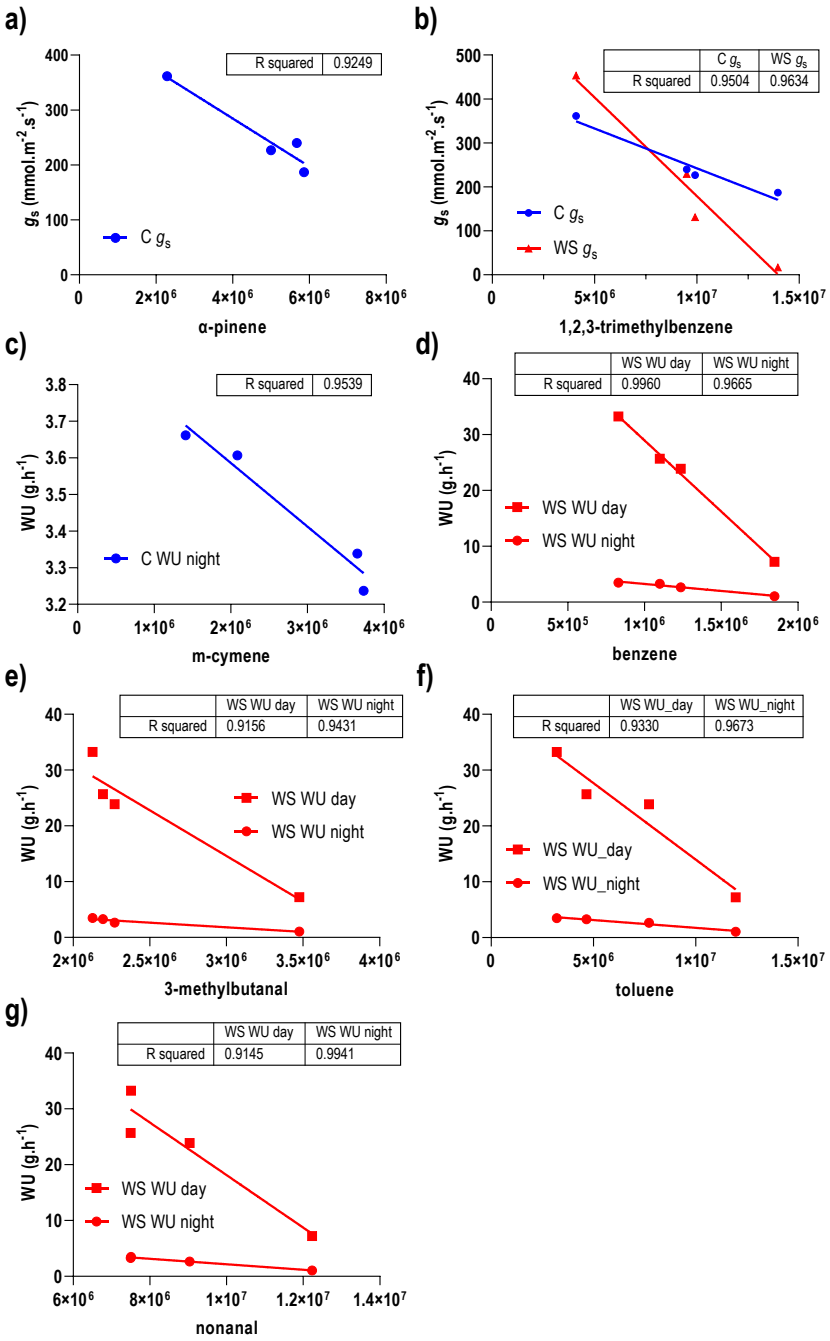


Figure 36. Linear regressions of a) α-pinene and control well-watered (C) stomatal conductance (g_s), b) 1.2.3-trimethylbenzene and C and water-stressed (WS) g_s, c) m-cymene and C night water use (WU), d) benzene and WS day and night WU, e) 3-methylbutanal and WS day and night WU, f) toluene and WS day and night WU, and g) nonanal and WS day and night WU.

3.4.3. Discussion

Similar to the previous experiments, the response of decrease of g_s for the well-watered vines in synchrony to the water-stressed vines was not as clear as previously observed in the literature. However, in this experiment, C g_s gradually decreased over time and a clear drop can be observed on day 9, also detected by the Pettitt homogeneity test as a decreasing shift in the series of data (Figure 26). Moreover, even if slight changes in PAR were observed (despite additional lightning above the plants), the multi-linear regression showed a better prediction of C g_s including the WS g_s data than without, as well as for C *E* and WS *E* (Figure 31).

The volatile analysis allowed the identification of 26 volatile compounds (Table 5). Comparing with the previous experiment with the same cultivar, 17 compounds had the same library match, 9 compounds were new (i.e. benzene, 3-methylbutanal, ethylbenzene, p-xylene, 1,3-dimethylbenzene, m-cymene, 1,2,3-trimethylbenzene, 3-ethyl-o-xylene and 4-methyl-benzaldehyde) and 3 were not detected (i.e. heptanal, 1-methyl-2-(1-methylethyle)-benzene and benzaldehyde). The total peak area stayed constant over time with a slight increase on day 9, which is different from the previous experiment (Figure 34). The combined effect of volatiles released from both C and WS may also complicate interpretation though it is interesting that 1,2,3-trimethylbenzene showed a significant negative correlation with both C and WS g_s (Figure 36b) and could constitute a promising candidate for the volatile communication hypothesis.

3.5. Individual flow-through chambers to study volatile emission during a drought/rehydration treatment

Separating individual vines with plant-size chambers to study the effect of a stress (e.g. herbivores, ozone, flood, or drought) on volatile emission has previously been done with different experimental set-ups (Ton *et al.*, 2007; Bourtsoukidis *et al.*, 2014; Lüpke *et al.*, 2017) but not for the drought stress effect on volatile emission from *Vitis vinifera*. Here, an experimental set up was designed to allow the investigation of up to eight potted Chardonnay vines in parallel and to prevent, as much as possible, the cross contamination of volatiles between control plants and water-stressed plants. The goal was to measure physiological parameters while sampling volatiles from plants that were watered every day and from plants that were deprived of water and rehydrated.

3.5.1. Material and methods

Plant and environmental conditions

Vitis vinifera cv. Chardonnay vines were potted and grown in a temperature-controlled glasshouse under natural light (Winter, June-July 2018) until reaching 1-2 shoots with approximately 10 leaves per shoot. The environmental conditions were temperature 25°C day and 17°C night, humidity 40 %.

Individual clear chambers and drought/rehydration treatment

A custom-made flow-through chamber system was built to allow a dynamic headspace sampling method for volatiles emitted by vines separated from each other (Figure 37 and 38). The chambers were cylindrical (height 100 cm, diameter 32 cm, volume 80 L) made of clear flexible polyethylene plastic sheets (thickness 0.7 mm), glued and fixed onto a wooden board with modelling clay for sealing and easy insertion of plants. An air pump with an electrical motor (18.69 $m^3.h^{-1}$ air flow, model 1550-600, GAST, USA) flushed air equally to all the chambers through clear vinyl tubing (i.d. 10 mm) and plastic fittings (connectors and Y-splits). A valve was added before each chamber to adjust the air flow rate to approximately 6.5 $L.min^{-1}$ at the outlet of the chamber, which was measured with a portable mass flow meter (range 0-10 $L.min^{-1}$; GFM17, Aalborg, USA). The air was scrubbed before entering each chamber with custom-made air filters built with polyvinyl chloride (PVC) pipes filled with layers of glass wool, activated charcoal foam and activated charcoal particles. In addition to the system, parallel tubing connections at the input and output ports of the chambers were added to connect the head of a infra-red gas analyser (IRGA; LI-6400XT) to measure the gas exchange of

the vine within the chamber. Some examples of similar systems can be found in the literature (Bourtsoukidis *et al.*, 2014; Lüpke *et al.*, 2017).

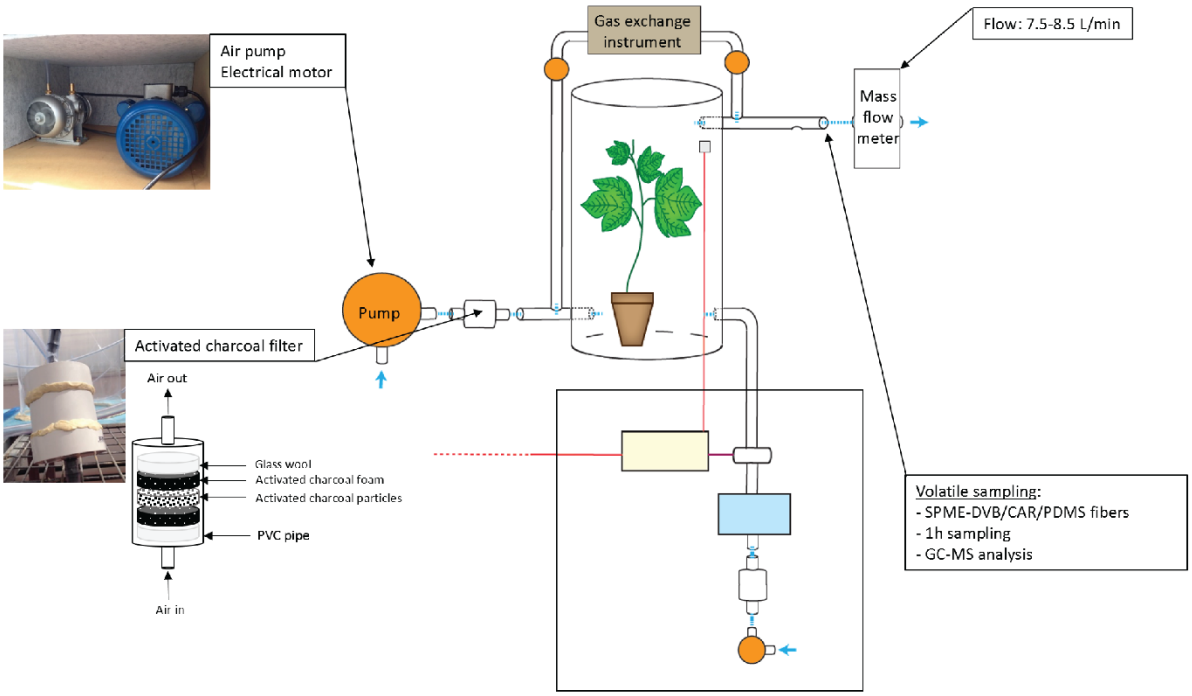


Figure 37. System schematic of the dynamic headspace sampling chamber allowing the extraction of volatile compounds from an individual potted grapevine while monitoring the whole plant gas exchange. It includes a pump pushing air through the chamber at a flow rate of approximately 6.5 L.min^{-1}, custom-made air filters with activated charcoal. The volatiles emitted by the vine were sampled at the outlet of the chamber with SPME fibres and analysed with GC-MS.

At the start of the experiment and after determining the pot water field capacity (WFC), the vines were transferred from the glasshouse to inside the chambers and divided into two groups. Four vines were watered every day to WFC (control treatment, C), and four vines were watered for 4 days, deprived of water for 5 days and re-watered for 5 days (water-stressed treatment, WS).

Water deficit was imposed by reducing the amount of irrigation until a defined value of leaf maximum daily stomatal conductance of approximately 50 mmol H_20 m^{-2}.s-1 was reached (Medrano, 2002). Then, each day, from 11:00 to 12:00, volatile samples were extracted on a solid-phase micro-extraction (SPME) fibre (DVB/CAR/PDMS), manually exposed and placed at the outlet of each chamber for an hour. In the meantime, the whole plant gas exchange was measured with the LI-6400XT IRGA, connected to a system of valves, tubing and micro-pumps (Parkers CTS Micro Diaphragm Pump E193, flow rate 2.5 L.min^{-1}, Parker Hannifin,

USA) connected to each chamber inlet and outlet. It was auto-programmed to take 1 sample every minute and do a match of the IRGAs every 15 min. Based on the length and diameter of the tubing, and the flow rate of the pumps, the first 5 measurements were discarded and the next 5 measurements of CO_2 and H_2O concentrations were averaged for analysis. These measurements followed a rotation order to start with a different vine every day. Thus, transpiration rate (*E*, mmol.m^{-2}.s^{-1}) and net carbon exchange rate (*NCAR*, µmol.m^{-2}.s^{-1}) were calculated from the IRGA parameters according to Pearcy *et al.* (2000) and Long *et al.* (1996). *E* calculation was as follow:

Eq. 1

$$E = \frac{Ue \times 1000 \times (Wo - We)}{TLA \times ((P \times 1013.25) - Wo))}$$

where *We* and *Wo* are the water vapour pressures (mbar) of air entering and leaving the chamber respectively, *P* (atmospheric pressure, atm) and TLA equals total projected leaf area (m^2).

And NCAR calculation was as follow:

Eq. 2.1

$$Ue = \frac{P \times F}{R \times (T_{air} + 273.15)}$$

where *Ue* is the total molar flow rate entering the chamber (mol.s^{-1}) for *F* (flow rate, cm^3.s^{-1}), *R* equals the gas constant (82.1 cm^3.atm.mol^{-1}.K^{-1}), T_{air} (air temperature, °C), and constants 1013.25 mbar.atm^{-1}, 273.15 °K, and:

Eq.2.2

$$NCAR = \frac{Ue \times (Ce - Co)}{TLA} - Co \times E/1000$$

where *Ce* and *Co* are mol fractions of CO_2 entering and leaving the chamber respectively.

From 13:00 to 15:00, one vine at a time starting from the control treatment was removed from a chamber to measure g_s and the photosynthetic active radiation (PAR) incident on the leaf with a porometer (AP4 leaf Porometer) (3 flagged leaves and 3 measurements per leaf). The vine was then placed on an electronic balance to replace the water consumed by weight difference from the WFC reference. It was decided to start with the control well-watered vines to limit the potential volatile contamination from the stressed vines. During the cessation of irrigation for the water-stress treatment, the vines were weighed to follow water consumption.

Whenever the vines were measured, watered and placed back in the chambers, the air flow rate from the outlet was checked.

All SPME fibres were desorbed on a GC-MS system on the day of collection and then reconditioned for the next day. On the last day, all leaves were harvested and scanned to determine the projected leaf area per plant with ImageJ, and additional volatile samples (blanks) were collected at the outlets of the empty chambers.

An additional part of the set up was built to regulate the humidity in the four chambers of the water-stressed treatment to match the humidity in the control treatment. One chamber for each treatment had a temperature and humidity sensor that was monitored by a 'vapour pressure deficit (VPD) controller' composed of a microcontroller (Arduino UNO), tubing, electronic valves, a pump and an air filter, connected to a plastic container filled with distilled water (black square in Figure 37). The microcontroller calculated VPD simultaneously in both chambers and if the VPD difference reached a threshold, the valves would open to increase the water vapour in the WS chamber and reduce VPD. Unfortunately, difficulties were encountered in the programming and recording of data, so this control system was not used.

Additional temperature and humidity sensors were placed inside one WS chamber and one C chamber and one outside the chambers, with measurements taken at 10-min intervals. Data was recovered at the end of the experiment and vapour pressure deficit (VPD) was calculated. All methods are described in Chapter 2.

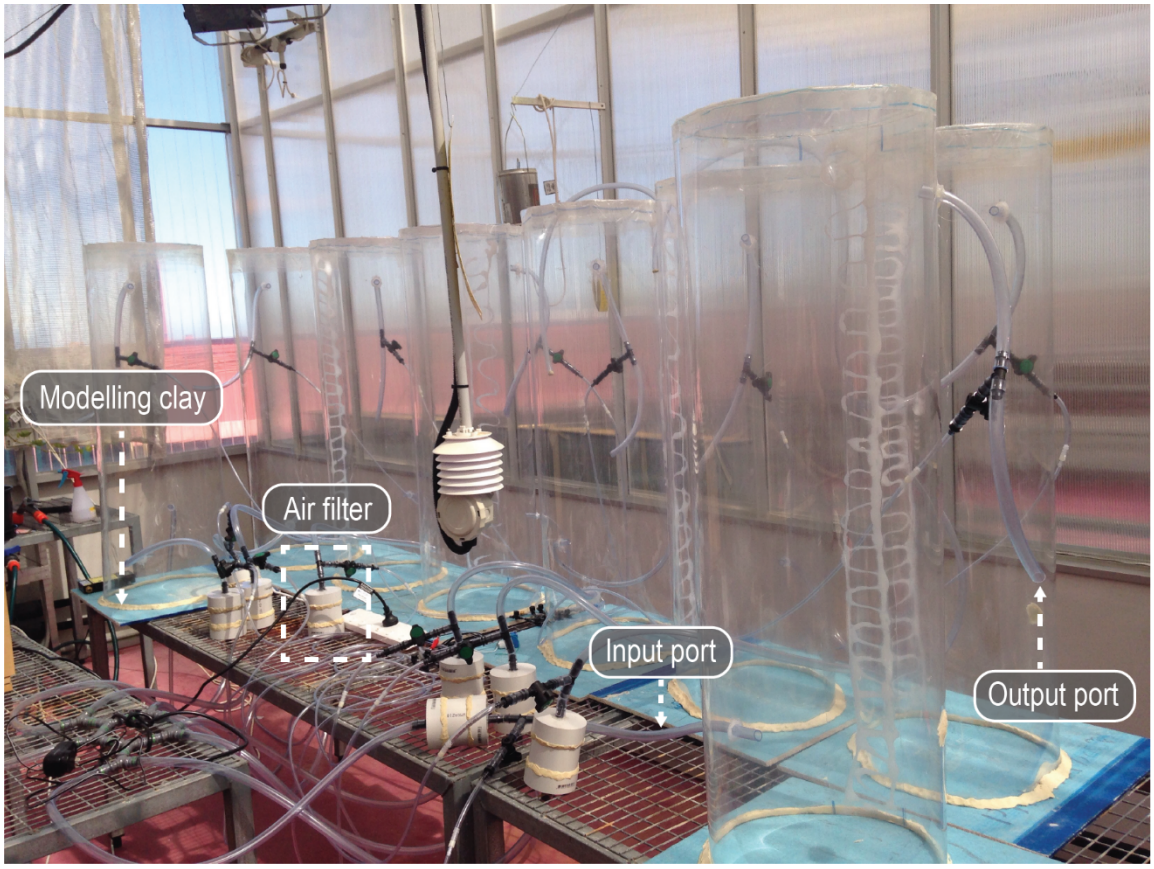


Figure 38. Custom-made dynamic headspace sampling system in the glasshouse. The clear plastic chambers allowed the sampling of volatile compounds from individual potted grapevines while monitoring the whole plant gas exchange with an infra-red gas analyser connected to the input and output ports. Modelling clay was used to seal the base to the chambers and to enable easy access to the vines. It includes a pump to provide an air flow rate of approximately 6.5 $L.min^{-1}$ per chamber and custom-made air filters were filled with activated charcoal to act as scrubbers of external volatiles.

GC-MS analysis

C_{10}-C_{25} saturated alkanes and numerous volatile compounds which were selected from the literature were analysed on the GC-MS system to determine their retention time and calculate their Kovats retention indices for greater accuracy in compound identification (see section 2.3.1, Chapter 2) (Table 6).

Table 6. List of volatile compounds analysed with retention time (RT), calculated retention index (RI), literature RI and their corresponding references found in the literature or the National Institute of Standards and Technology (NIST) database.

Standard compound	RT	RI	RI literature	References
C_{10}-C_{25} saturated alkanes				
n-decane (C_{10})	7.377			
n-undecane (C_{11})	9.676			
n-dodecane (C_{12})	12.611			
n-tridecane (C_{13})	15.803			

n-tetradecane (C_{14})	18.74			
n-pentadecane (C_{15})	21.322			
n-hexadecane (C_{16})	23.71			
n-heptadecane (C_{17})	25.981			
n-octadecane (C_{18})	28.129			
n-nonadecane (C_{19})	30.258			
n-icosane (C_{20})	32.29			
n-henicosane (C_{21})	34.245			
n-docosane (C_{22})	36.123			
n-tricosane (C_{23})	37.701			
n-tetracosane (C_{24})	39.669			
n-pentacosane (C_{25})	41.344			
α-pinene	7.798	1020.5	1027	(Högnadóttir & Rouseff, 2003)
hexanal	9.504	1093.4	1083	(Tatsuka *et al.*, 1990)
β-pinene	9.792	1104.5	1113	(Högnadóttir & Rouseff, 2003)
trans-2-pentenal	10.932	1146.1	1135	(Bianchi *et al.*, 2007)
1-penten-3-ol	11.779	1174.2	1165	(Tatsuka *et al.*, 1990)
4-methyl-2-pentanol	11.94	1179.4	1168	(Umano *et al.*, 1999)
2-ethyl hexanal	12.39	1193.3	1197	NIST
2-pentyl furan	13.545	1231.7	1231	(Umano & Shibamoto, 1987)
ocimene	13.638	1234.7	1245	(Choi, 2003)
γ-terpinene	13.916	1243.6	1262	(Choi, 2003)
p-cymene	14.782	1270.4	1277	(Högnadóttir & Rouseff, 2003)
octanal	15.363	1287.5	1300	(Culleré *et al.*, 2004)
1-octen-3-one	15.741	1298.3	1305	(Valim *et al.*, 2003)
trans-2-heptenal	16.472	1324.3	1318	(Umano & Shibamoto, 1987)
1- hexanol	17.207	1349.9	1356	(Tatsuka *et al.*, 1990)
2-nonanone	18.156	1381.4	1394	NIST
trans-2-hexen-1-ol	18.59	1395.3	1409	(Tatsuka *et al.*, 1990)
1-heptanol	19.88	1445.8	1461	(Tatsuka *et al.*, 1990)
1-octen-3-ol	19.655	1436.9	1438	(Valim *et al.*, 2003)
linalool oxide	20.315	1462.5	1453	(Ong & Acree, 1999)
2-ethyl-1-hexanol	20.813	1481.3	1484	(Cho *et al.*, 2008)
decanal	21.068	1490.7	1510	(Högnadóttir & Rouseff, 2003)
α-copaene	21.098	1491.8	1488	(Umano *et al.*, 1994)
α-cubebene	22.069	1532.4	1463	(Choi, 2003)
terpinen-4-ol	23.651	1597.7	1593	(Högnadóttir & Rouseff, 2003)
trans-caryophyllene	24.101	1617.9	1618	(Högnadóttir & Rouseff, 2003)
β-terpineol	24.239	1624.1	1625	NIST
trans-β-farnesene	24.375	1630.2	1674	(Choi, 2003)
phenylacetaldehyde	24.671	1643.4	1671	(Culleré *et al.*, 2004)
safranal	24.761	1647.4	-	-
α-gurjunene	24.974	1656.8	-	-
α-humulene	25.784	1691.7	1680	(Choi, 2003)
α-terpineol	25.799	1692.3	1688	(Lee & Noble, 2003)
γ-terpineol	25.848	1694.4	-	-
4-ethyl benzaldehyde	26.991	1748	1753	(Le Guen, Prost, & Demaimay, 2000)
α-farnesene	27.01	1748.9	1748	(Katumi Umano et al., 1994)
nerol	28.116	1799.4	1753	(Nishimura, 1995)
geranyl acetone	29.328	1857.2	-	-
nerolidol	32.194	1995.4	2010	(Choi, 2003)
nerolidol	32.992	2036.6	2054	(Choi, 2003)

3.5.2. Results

3.5.2.1. Stomatal conductance

Measurements of g_s showed relatively stable values varying between 344 ± 62 mmol.m^{-2}.s^{-1} (mean ± SD) to 425 ± 86 mmol.m^{-2}.s^{-1} for both C and WS groups during the first 4 days of the experiment while the vines were watered daily to WFC (Figure 39a). Once water was withheld for the WS vines, g_s quickly dropped until reaching 16 ± 10 mmol.m^{-2}.s^{-1} on day 8. Water was then re-supplied and the WS g_s recovered to 297 ± 116 mmol.m^{-2}.s^{-1} for the WS vines on day 11, similar to the C g_s that was at 235 ± 130 mmol.m^{-2}.s^{-1}.

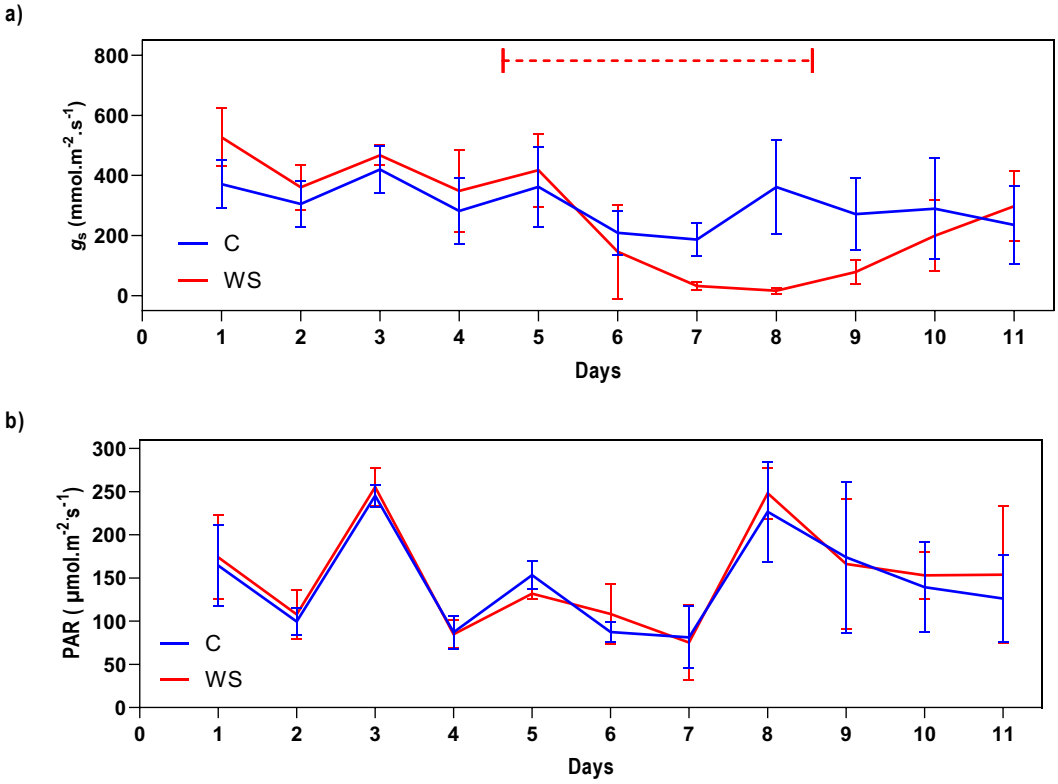


Figure 39. a) Stomatal conductance (g_s) measured on leaves of *Vitis vinifera* cv. Chardonnay used in a drought/rehydration experiment where all vines were placed inside individual clear plastic chambers. Control vines (C, blue) were watered to field capacity every day and the dashed line represents the period during which the water-stressed vines (WS, red) stopped receiving water (mean ± SD, n= 4). b) Leaf incident photosynthetically active radiation (PAR) was measured with the porometer light sensor simultaneously as g_s measurements.

The g_s of the C group was not as stable as expected as a decrease can be observed on day 6 and 7, recorded by the photosynthetically active radiation sensor of the porometer (Figure 39b), followed by a rapid increase on day 8. PAR varied between around 250 and 100 µmol.m^{-2}.s^{-1} over the course of the experiment since no

additional lighting were available. No significant differences were found between the g_s of C and WS and the PAR of C and WS (two-way repeated-measures ANOVA with Bonferroni post-tests, $p<0.05$, Supplementary Table S20a and b, and S21a and b, respectively).

The Pettitt homogeneity test detected a shift in the data series of the WS group on day 6 ($p<0.05$) but not for the C group (Figure 40a). No shift was detected for both groups when not considering the recovery phase (Figure 40b).

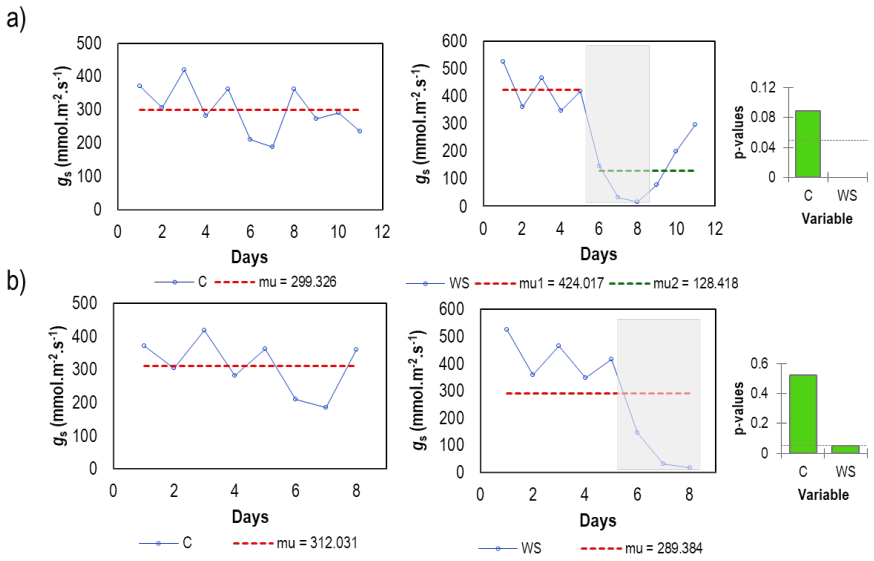


Figure 40. Pettitt homogeneity test on the stomatal conductance (g_s) data measured with the porometer from well-watered vines (C) and water-stressed vines (WS) in a drought experiment (grey box). The test was performed on a) the whole series of data and b) without the recovery phase (mean, n=9), and the dotted lines represent the averaged value for the data series with two mu values if a change point is detected ($p<0.05$).

3.5.2.2. Whole plant gas exchange

Whole plant gas exchange was determined from the difference of $[CO_2]$ and $[H_2O]$ going in and out of the chambers measured by infra-red gas analysers. Transpiration (*E*) and net carbon assimilation rate (*NCAR*) were stable from day 1 to day 4, for both groups, with *E* of 0.09 mmol.m^{-2}.s^{-1} for C and 0.12 mmol.m^{-2}.s^{-1} for WS, and *NCAR* of 0.7 µmol.m^{-2}.s^{-1} for C and 1.1 µmol.m^{-2}.s^{-1} for WS (Figure 41). Transpiration and *NCAR* of WS vines decreased when water was withheld reaching almost 0 in both cases. Both parameters increased back to initial values during the recovery. For the C treatment on day 7, a decrease of *E* and *NCAR* was observed and it increased back to the same previous values on day 8. *E* and *NCAR* of the treatments C and

WS were significantly different on day 8 (two-way repeated-measures ANOVA with Bonferroni post-tests, p< 0.05, Supplementary Tables S22a and b, and S23a and b, respectively).

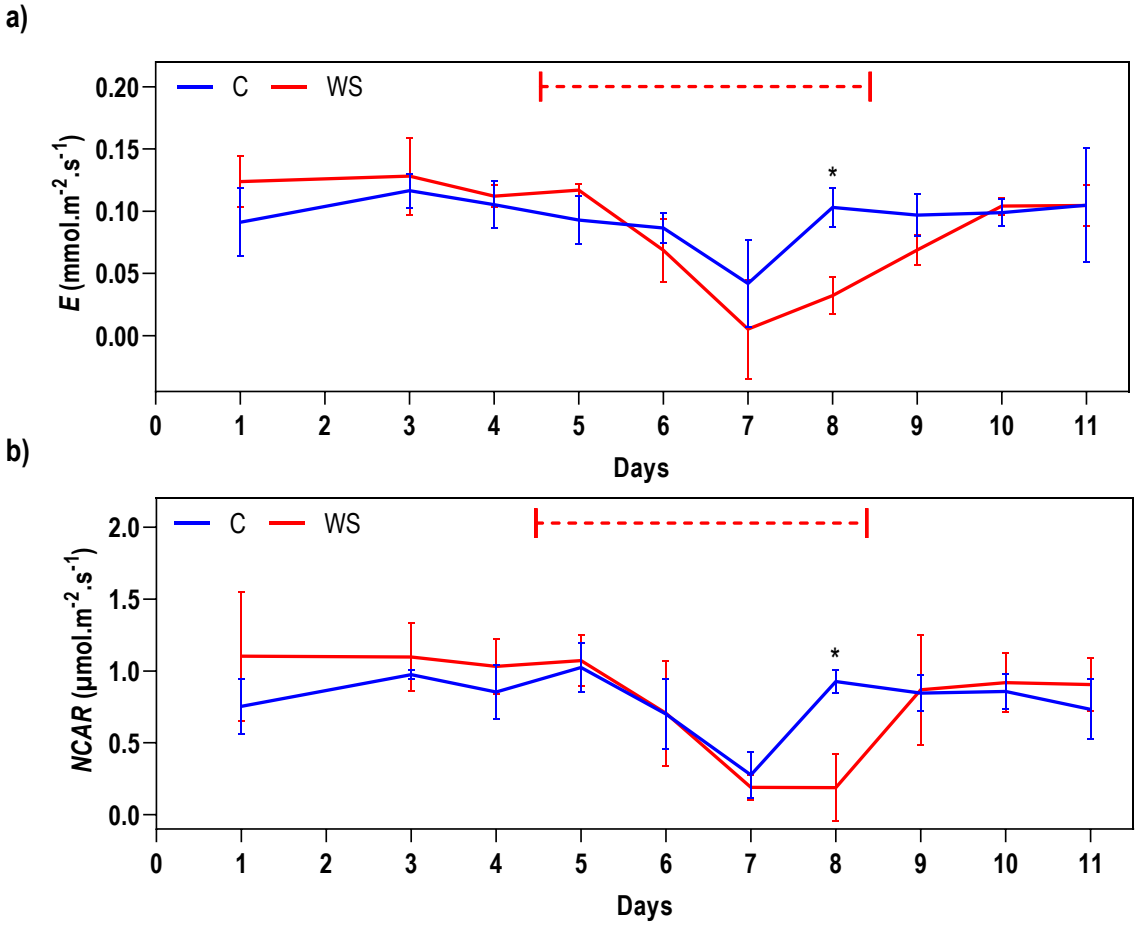


Figure 41. a) Transpiration (*E*) and b) net carbon assimilation rate (*NCAR*) calculated from the concentrations of CO_2 and H_2O in the air going inside and outside of plastic chambers containing individual *Vitis vinifera* cv. Chardonnay vines, used in a drought/rehydration experiment. Control vines (C, blue) were watered to field capacity every day and the dashed line represents the period during which the water-stressed vines (WS, red) stopped receiving water (mean ± SD, n=4). Significant differences between the control and treated groups by two-way repeated-measures ANOVA with Bonferroni post-tests are marked: * for p<0.05.

The Pettitt's test was also performed on *E* and *NCAR* data series and revealed a shift in the data series of WS for the whole experiment and without the recovery phase (p<0.05), but no shift was detected with the C data series (Figure 42).

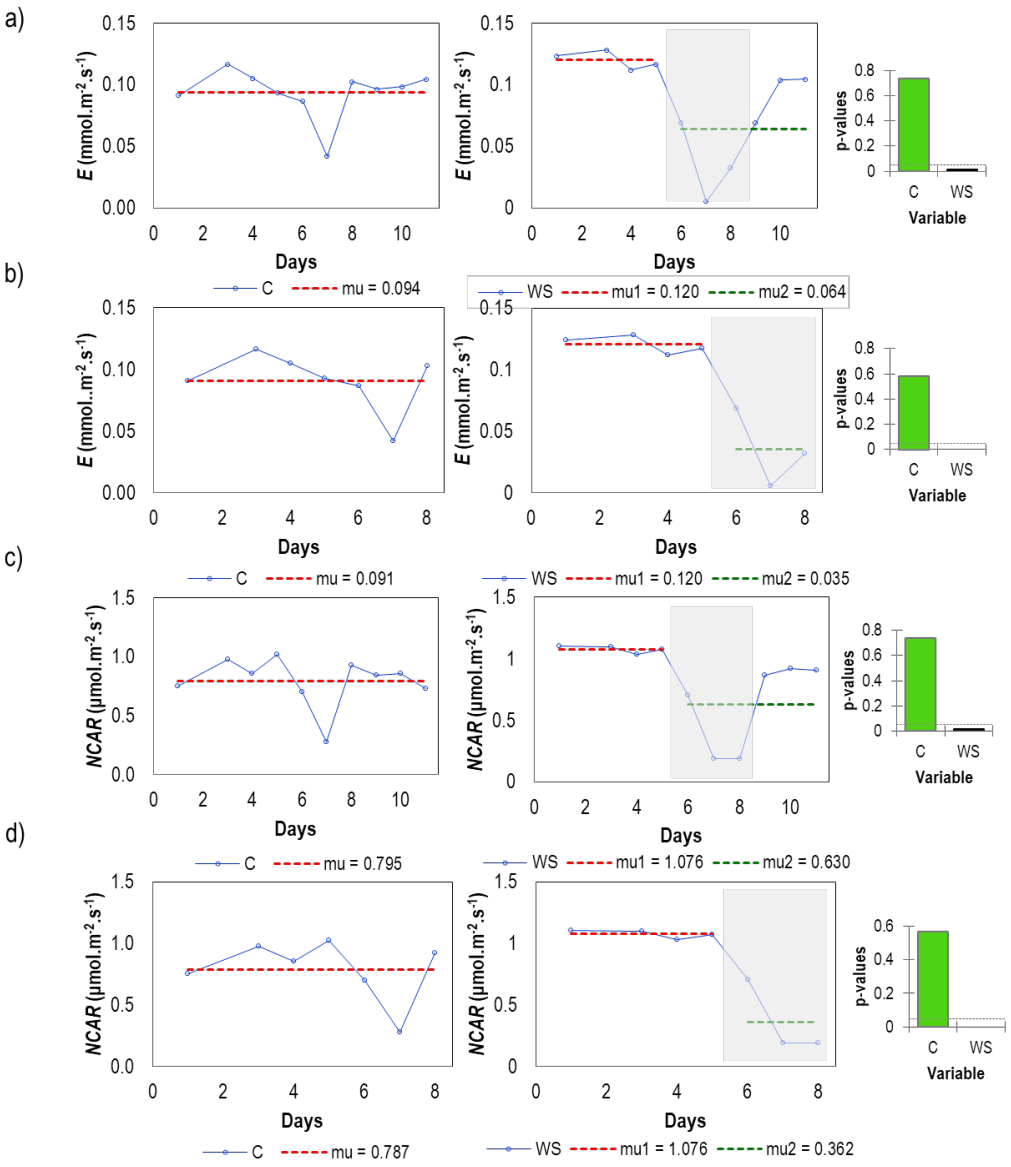


Figure 42. Pettitt homogeneity test on a) and b) transpiration (*E*), and c) and d) net carbon assimilation rate (*NCAR*) from well-watered (C) and water-stressed (WS) vines in a drought experiment (grey box). The test was performed on a) and c) the whole series of data, and b) and d) without the recovery phase (mean, n=9), and the dotted lines represent the averaged value for the data series with two mu values if a change point is detected ($p<0.05$).

3.5.2.3. Other parameters (VPD, pot and plant mass, leaf area)

The vapour pressure deficit (VPD) inside the WS chamber was lower than inside the C chamber initially but increased as expected form the start of the stress phase until re-watering (from day 4 to day 9, Figure 43a). Significant differences were found between C and WS on day 1, day 2, day 4 and day 6 (two-way repeated-

measures ANOVA with Bonferroni post-tests, p<0.05, Supplementary Tables S24a and b). The pot and plant mass measurements showed the expected decrease as soon as watering was stopped indicating reduced soil water content (Figure 43c). The control vines were using approximately the same amount of water every day. Although the projected leaf area of the WS vines was lower at the end of the experiment (Figure 43b), there was no significant difference between C and WS (t test, Supplementary Table S28).

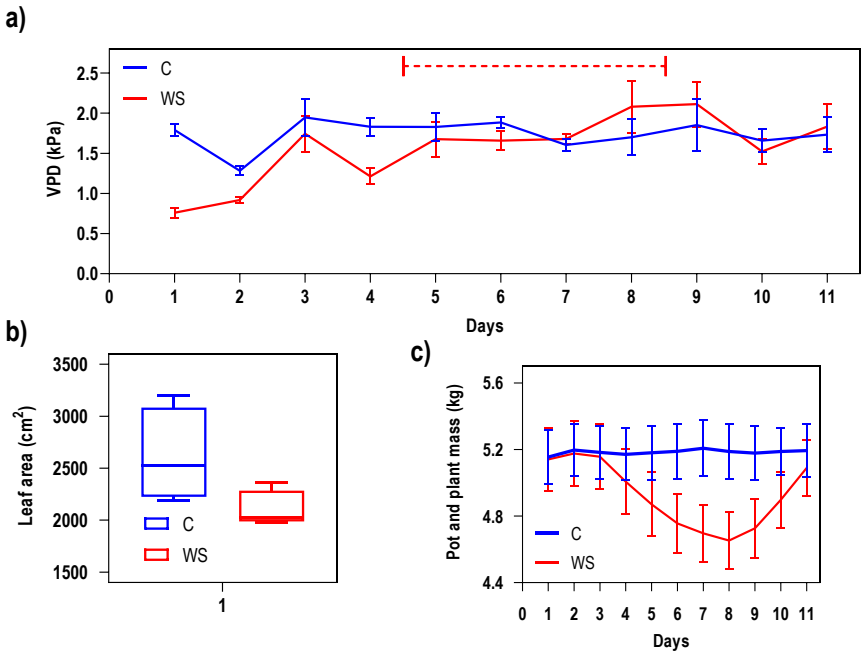


Figure 43. a) Vapour pressure deficit (VPD) calculated from the temperature and relative humidity from the plastic chambers of the well-watered vines (C, blue line), water-stressed vines (WS, red line) and outside the plastic chamber during the drought/rehydration experiment. The red dashed line indicates the treatment period. The red dashed line indicates the treatment period. The red dashed line indicates the treatment period. Each point represents the mean value ± SD of 8 logs from 11:00 to 12:45 (n=1). b) Project leaf area (n=4). c) The pot and plant mass measured daily before watering (n=4). Significant differences between the control and treated groups by two-way repeated-measures ANOVA with Bonferroni post-tests are marked: * for p<0.05.

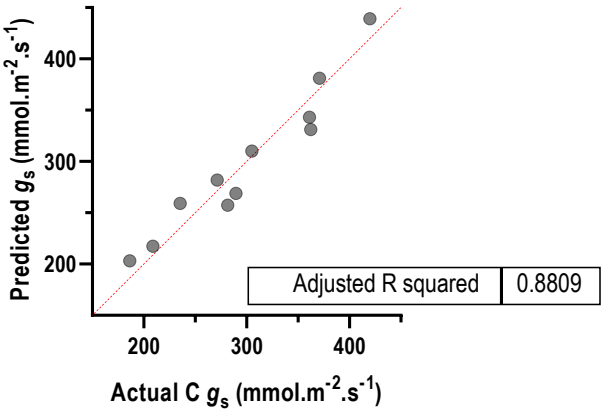
3.5.2.4. Multi-variable analysis

A multi-linear regression analysis was performed on C g_s with different parameters (C PAR, VPD, time, with or without WS g_s) and results showed a strong correlation between C g_s and C PAR with or without WS g_s included in the analysis (Figure 44), and no correlation between C g_s and WS g_s (Supplementary Tables S53 and S54, respectively). Similar analyses were conducted on *E* and *NCAR* and showed no significant

prediction of the C from WS or C PAR, but R^2 increased if WS *E* or *NCAR* were included (Supplementary Tables S55 and S56, and S57 and S58, respectively).

a)

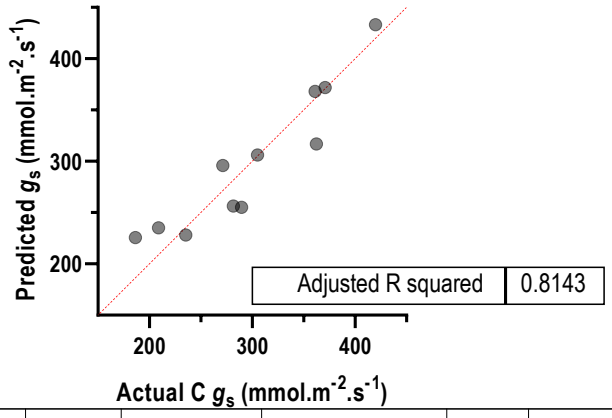
with WS g_s



Parameter estimates	Variable	Estimate	Standard error	95% CI (asymptotic)	|t|	P value	P value summary
β0	Intercept	229.5	77.17	40.69 to 418.3	2.974	0.0248	*
β1	WS gs	0.1393	0.06284	-0.01448 to 0.2931	2.216	0.0685	ns
β2	C PAR	1.059	0.1612	0.6643 to 1.453	6.567	0.0006	***
β3	VPD	-49.96	48.51	-168.6 to 68.73	1.030	0.3428	ns
β4	Time	-5.183	3.375	-13.44 to 3.075	1.536	0.1755	ns

b)

without WS g_s



Parameter estimates	Variable	Estimate	Standard error	95% CI (asymptotic)	|t|	P value	P value summary
β0	Intercept	256.1	95.18	31.08 to 481.2	2.691	0.0310	*
β1	C PAR	1.078	0.2010	0.6023 to 1.553	5.360	0.0011	**
β2	VPD	-28.20	59.31	-168.4 to 112.0	0.4755	0.6489	ns
β3	Time	-10.37	3.034	-17.55 to -3.201	3.420	0.0111	*

Figure 44. Multilinear regressions of control well-watered (C) g_s and other variables a) with or b) without water-stressed (WS) g_s from *Vitis vinifera* during a drought/rehydration experiment. C PAR, photosynthetic active radiation of the C group (µmol.m^{-2}.s^{-1}); VPD, vapour pressure deficit (kPa); time, days of the experiment.

3.5.2.5. Volatile analysis

The chromatograms obtained from the SPME-GC-MS method were analysed as described in Chapter 2, section 2.3. Twenty peaks of similar retention times (RT) were selected for comparison between the control C and treatment WS groups, for each time points. The compound identity was assigned utilising mass spectral library matches (>50%). The identification was also confirmed with calculations of Kovats retention indices (RI) and comparison with RI found in the literature, as well as RT of known standards where possible (Table 7). Different classes of volatiles were identified including alcohols (e.g. 2-ethyl hexanol), aldehydes (e.g. 2-ethyl hexanal), terpenoids (e.g. myrcene) and ketones (e.g. methyl vinyl ketone).

Table 7. List of volatiles identified and analysed from whole plants of *Vitis vinifera* cv. Chardonnay combining well-watered and drought-stressed plants separated with clear flow-through chambers. Identification was based on comparison of chromatographic retention time (RT), library match factor (%), RT of known standards (marked with *, see section 2.3, Chapter 2), Kovats retention indices (RI) found in the literature or in the National Institute of Standards and Technology (NIST) database.

Compound name	RT (min)	Library match factor (%)	RI	RI literature	Reference
methyl vinyl ketone	6.385	70	-	-	-
myrcene	11.817	94	1175	1176	(Högnadóttir & Rouseff, 2003)
2-ethylhexanal*	12.585	43	1199	1197	NIST
ocimene*	13.867	97	1242	1245	(Choi, 2003)
3-methyl-2-buten-1-ol	16.371	60	1320	1324	NIST
6-methyl-5-hepten-2-one	16.881	60	1338	1341	(Tatsuka *et al.*, 1990)
2,6-dimethyl-5-heptenal	17.309	68	1353	1358	NIST
allo-ocimene	17.83	97	1370	1396	(Combariza *et al.*, 1994)
cymenene	19.58	93	1433	-	-
2-ethylhexanol*	20.87	80	1483	1484	(Cho *et al.*, 2008)
linalool	22.216	94	1538	1548	(Ong & Acree, 1999)
β-caryophyllene*	23.651	97	1597	1618	(Högnadóttir & Rouseff, 2003)
trans-β-farnesene*	25.109	95	1662	1674	(Choi, 2003)
α-humulene*	25.368	91	1673	1680	(Choi, 2003)
trans-γ-bisabolene	25.713	70	1688	-	-
trans-α-bergamotene	26.493	72	1724	-	-
α-farnesene*	27.021	96	1749	1674	(Choi, 2003)
2-phenyl-2-propanol	27.239	64	1759	1776	NIST
trans-geraniol	29.173	50	1849	1865	NIST
α-patchoulene	30.17	42	1896.008	1888	(Osorio *et al.*, 2006)

In order to compare between the groups and between days, the volatile content was estimated from the total ion chromatographic peak areas of the individual compounds. The total peak area of water-stressed group showed an overall reduction of 45% compared to the control group. *Vitis vinifera* cv. Chardonnay was found

to be a strong emitter of 6-methyl-5-hepten-2-one, 2-ethyl-hexanol and α–farnesene (Figure 45a). For the WS group, some terpenes like ocimene, trans-γ-bisabolene, trans-α-bergamotene and trans-α-farnesene content increased during the drought stress and decreased during the recovery, while they kept increasing for the C group. Some other compounds like 2-ethyl-hexanol, α-humulene and 2-phenyl-2-propanol stayed constant over time.

Volatiles samples were taken a day after the experiment from emptied chambers to investigate whether volatiles could remain in the chambers. It revealed traces of volatiles still present and these consisted of 13 out of the 20 compounds that were previously identified from the plants (Figure 45a).

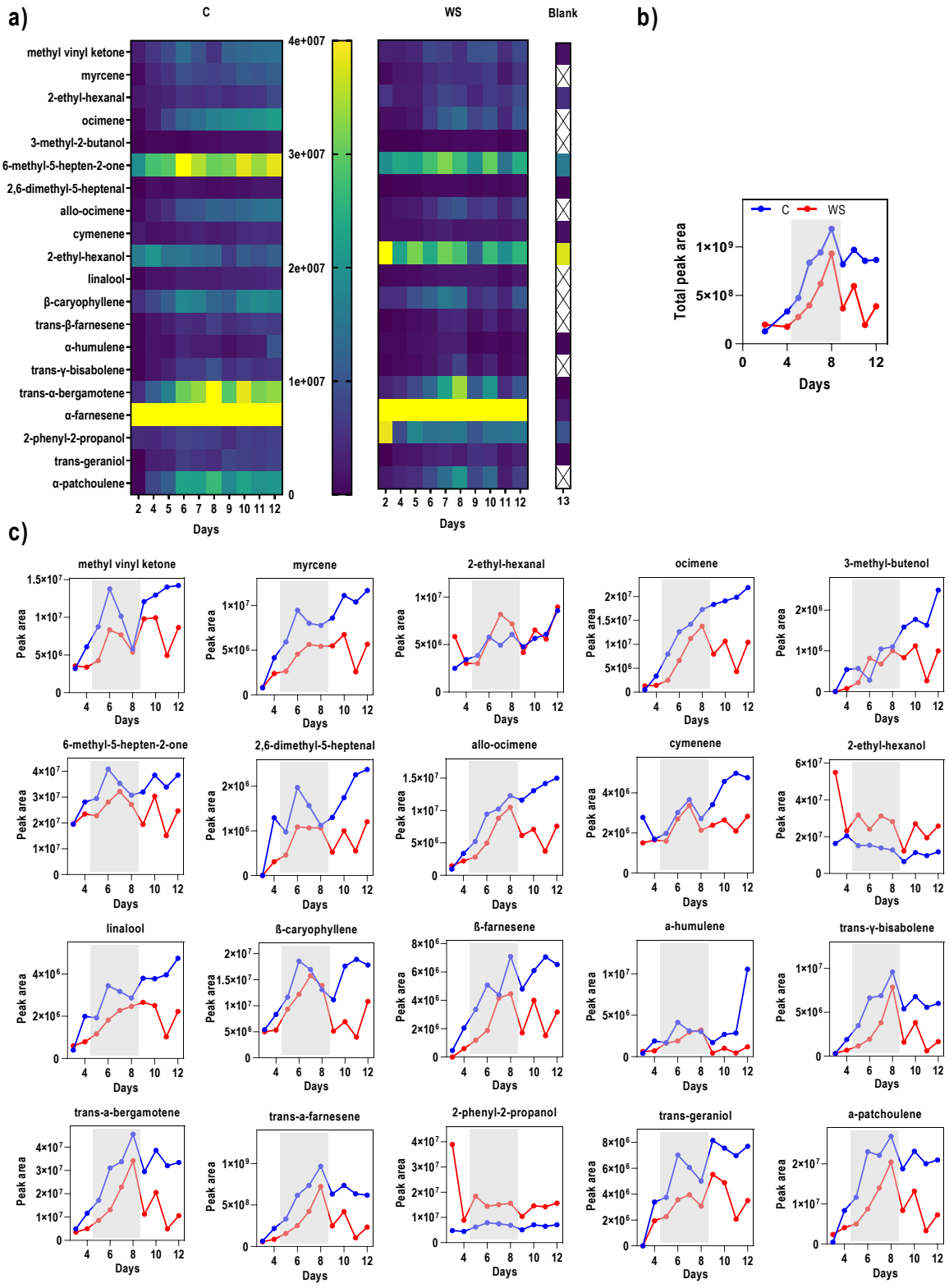


Figure 45. Overview of peak areas (counts) of total ion chromatograms from *Vitis vinifera* cv. Chardonnay during a drought-rehydration experiment, where vines were placed inside individual clear plastic chambers (sampled with SPME and analysed with

GC-MS) (mean ± SD, n=4). Plant-emitted volatile compound names were allocated from library matches (>50%), comparison with Kovats retention indices and some with retention times of known standards (see Table 7). a) Heatmap plots of differentially emitted volatile compounds of control well-watered vines (C, blue) and water-stressed vines (WS, red). b) Total peak area of volatile compounds over time. Watering was withheld from day 4 to day 8 (grey box). c) Peak area from individual volatile compounds over time.

3.5.2.6. Combined analysis of physiology and chemistry results

Correlations between identified volatiles, g_s, PAR and VPD of both WS and C vines revealed no strong correlation between the volatiles and g_s in the well-watered C group (Figure 46a), but for the water-stressed WS group, some correlation coefficients were high (R^2>0.8 and p<0.05) and negative for ocimene, allo-ocimene and linalool (Figure 46b).

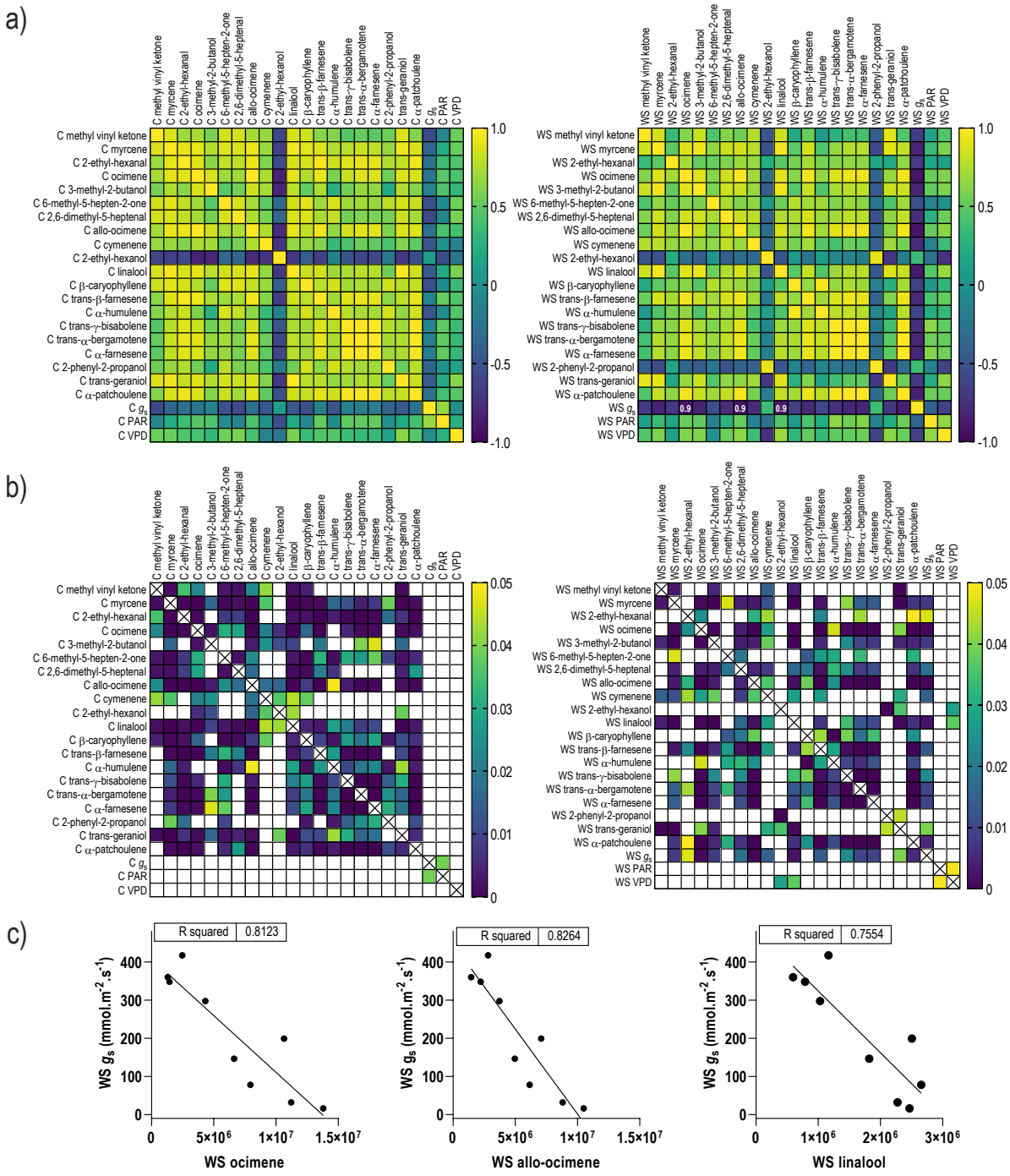


Figure 46. Pearson correlation matrices for a) well-watered (C) and b) water-stressed (WS) plants indicating some differences in the correlation between volatiles, stomatal conductance (g_s), photosynthetic active radiation (PAR) and vapour pressure deficit (VPD). The correlation coefficient is shown for each combination in the relevant square. c) Linear regressions of WS g_s and ocimene, allo-ocimene and linalool.

3.5.3. Discussion

The custom-made dynamic headspace sampling system that was built in this study and inspired by other experimental set-ups (Bourtsoukidis *et al.*, 2014; Lüpke *et al.*, 2017; Ton *et al.*, 2007) was found to be successful at simultaneously measuring physiological responses of potted vines to drought and sampling volatiles. To compare to Dayer *et al.* (2017), stomatal conductance was also measured with a porometer as the main physiological trait but this required removal of the plants from chambers during the measurements, as well as performing the watering immediately afterwards. Thus, every measurement of stomatal conductance was from the plants outside the chambers and even with careful manipulation and timing, such as starting with the well-watered (C) group at each time point, it is not possible to rule out a potential contamination of volatiles between WS and C treatments. Even if the multilinear regressions indicated that this did not happen, transpiration and net carbon assimilation rate measured by the IRGA connected to the input and output ports of the chambers was also able to follow the vine responses to drought and could be used as the main parameter for further experiments. In addition, activated charcoal, similar to the inside of the custom-made filters used in this study, has been found to fail to stop volatiles emitted by microorganisms and be the cause of observed plant responses (García-Gómez *et al.*, 2019).

Since the vines were grown under natural light in a large glasshouse, their stomata were affected by changes in light intensity (Inoue & Kinoshita, 2017; Shimazaki *et al.*, 2007), complicating the interpretation of data from the well-watered group regarding a possible influence from the WS group. Indeed, the multi-linear regression showed a highly significant positive correlation between C PAR and C g_s independent of the WS g_s (Figure 44). This result is interesting compared with previous results for experiments where vines were not isolated. The multilinear regression could predict stomatal conductance in the C vines based on PAR alone with an adjusted R^2 of 0.88.

Whole plant gas exchange measurements (Figure 41) revealed a strong reduction in transpiration (*E*) and net carbon assimilation (*NCAR*) during the water stress period for the WS treatment, presumed to be caused by stomatal closure induced by the water deficit on the WS plants. Interestingly, there was also a decrease observed in the control plants (at day 7) to a similar degree as WS plants. This corresponded to a period of a few days where the PAR was significantly reduced (Figure 39b) due to cloudy conditions. Based on the multilinear regression for C g_s, it is likely due to PAR alone rather than the volatiles emitted from the WS chambers.

The VPD measurements were unexpected (Figure 43a) in that the WS chambers sometimes had lower VPD compared to the C chambers more evident in the early stages of the experiment. It is considered that VPD

would gradually increase in the WS chambers as the soil of the WS vines dried out and the WS vines reduced transpiration. This increase in VPD could be observed during the period of the water stress compared to the relatively stable VPD in the C chambers. An explanation for the smaller than expected change in VPD in WS chambers is that the fast air flow into the chambers replaced the air sufficiently rapidly to result in a similar humidity as the C vine chambers. Moreover, only one data logger per treatment was available, which was placed at the bottom of the chamber, thus they may not have measured the conditions inside the canopy accurately and were dependent on the mixing dynamics within the chamber.

The volatile analysis showed the emission of many volatiles from the vines by placing SPME fibres with the coating exposed directly at the outlet of the chambers (Table 7). This method was selected because of its low price, its wide untargeted detection range and without the need to pre-concentrate the samples. It also allowed to use an air flow inside the chamber to be high enough to avoid humidity and condensation to build up. However, accurate quantification of each volatile with the use of internal standards was not possible, due to the concerns of possible contamination as the plastic (polyethylene) is a good adsorber of volatiles (Capone, 1999) and could potentially affect the plants. In fact, this occurred from the volatiles emitted by the plants themselves as the analysis from the empty chambers revealed the presence of volatiles that the plastic might have potentially retained. Nevertheless, the integration of the peak areas showed changes between the WS and C treatments with an overall reduction in the WS volatile relative content, particularly evident after day 8 (Figure 45), similar to what has been observed in the literature (Bourtsoukidis *et al.*, 2014; Brilli *et al.* 2007). Twenty volatile compounds were identified with seven of them being confirmed with comparison of the retention time and mass spectra of known standards that were also injected with each batch. Correlation analysis between the semi-quantitative analysis of volatiles and the physiological parameters in the WS treatment revealed significant correlations for ocimene, allo-ocimene and linalool. These compounds could be potential candidates for signalling of abiotic stress and some have previously been found to be implicated in signalling of biotic stress (Copolovici *et al.*, 2012; Farré-Armengol *et al.*, 2017; Zeng *et al.*, 2017). Monoterpenes were also found to be emitted by grapevine clones under heat stress in Bertamini *et al.* (2021).

In conclusion, the goals of this experiment were achieved with identification of particular volatiles (requiring further identification confirmation) that correlated with the g_s, of WS plants and showed differences to the C plants. Although there was the issue of removing the plants from the chambers during g_s measurements that might have contaminated the controls, this was not evident from the multilinear regression analysis (Figure 44) which showed no effect of WS g_s on C g_s, or no significant reduction in C g_s corresponding to the WS treatment period from the Pettitt homogeneity test (Figure 40). In this respect, it would appear that the

chambers successfully isolated the influence of the WS plants on the g_s of the C plants based on previous data for *Vitis vinifera* (Dayer *et al.*, 2017) and Arabidopsis (Scharwies, 2017). The experimental system could be improved with the addition of more diverse filters for the air entering the chambers (not just activated charcoal), using a more inert plastic (Teflon) or glass for the plant chambers and by supplying supplemental PAR to reduce the influence of external PAR fluctuations.

3.6. General discussion and conclusion

In this chapter, the aim was to replicate drought/rehydration experiments in the same conditions as previous studies that showed a reduction of stomatal conductance of well-watered plants in synchrony with water-stressed plants (references in Table 3, section 1.4.2, Chapter 1), as well as characterise the volatiles emitted by grapevines related to drought stress. The drought-induced reduction in stomatal conductance for all cultivars was gradual over days with maximal reduction on the last day of drought, and the increase back to normal upon rewatering was gradual as well. In all experiments, the three phases of drought stress were achieved with no stress, g_s > 150 mmol.m^{-2}.s^{-1}, moderate stress, 150 mmol.m^{-2}.s^{-1} < g_s > 50 mmol.m^{-2}.s^{-1}, and severe stress, g_s > 50 mmol.m^{-2}.s^{-1} (Medrano, 2002). Determining other indicators as gas exchange (*E*, *NCAR*) or stem water potentials confirmed that the plants were stressed, and showed similar results as stomatal conductance measured with the porometer.

Nevertheless, a clear continuous decrease of stomatal conductance of the well-watered vines in synchrony to the water-stressed vines was not clear. Some irregularities from the well-watered vines were detected by the two-way repeated-measures ANOVA where no significant differences were detected between the C and the WS groups. Indeed, it would be expected that g_s would be different during the stress phase if the C g_s remains stable and the WS decreased. In addition, as the Pettitt's test was able to detect a shift in the data series for most WS data, sometimes considering all the series and sometimes without the recovery, a decrease in the C g_s data series was revealed by this test in the section 3.4.2.2. In addition, the multi-linear regressions revealed the C g_s was best predicted by including WS g_s in the analysis.

Decreases of C g_s on single days were also measured in the experiments in sections 3.3 and 3.4 but were not continuous and unfortunately, since the plants were in a clear glasshouse, slight changes in light intensity were likely to affect the stomatal conductance of the plants and interfere with testing the hypothesis. Thus, it is not possible to rule out the light effect and positively affirm that the well-watered plants were modulating their stomatal movement only in accordance to the water-stressed plants, even though most multi-linear analyses revealed a stronger effect of WS g_s on C g_s than C PAR.

Over 300 volatile metabolites can be found in a single GC-MS chromatogram originating from *Vitis vinifera* leaves (Weingart *et al.*, 2012). For this volatile analysis, an untargeted approach was selected which enabled detection and putative identification. In this study, the comparative relative quantifications were based on peak areas of total ion chromatographic peak areas of the individual compounds, and a total of 47 volatiles were assigned according to mass spectral library matches. For Chardonnay, 20 volatiles were identified and verified using Kovats retention indexes and 7 volatiles were verified by injection of authentic standards. For Grenache, 28 compounds were identified with mass spectral library matches only. Some volatiles were similar to others observed on Pinot noir (Griesser *et al.*, 2015).

Different groups of compounds analysed were found to be influenced by drought stress. A general decrease of volatiles was observed in experiments in section 3.3 and 3.5 in Chapter 3 (similar to references in Table 2, section 1.4.1, Chapter 1) as well as no change of volatile contents for certain volatiles as seen in section 3.4, Chapter 3. Interestingly, some volatiles also increased during the severe stress phase, such as α- and β-pinene. Griesser *et al.* (2015) found both an increase and a decrease of overall volatile contents in drought stressed grapevine leaves. Ebel *et al.* (1995) also found a higher emission of C_6-alcohols, aldehydes and esters in apples trees. Similar to the biotic stress-induced volatile communication that usually involves a volatile to be synthesised or its concentration to be increased to be detected by neighbouring plants (Ueda *et al.*, 2012), the same mechanism could be involved in abiotic-stress conditions, and thus, the volatiles with increased content during the stress could constitute candidates for said communication.

However, these results must be taken with consideration as accurate quantification with an internal standard was not carried out and only the total ion chromatographic peak area counts were measured and compared between samples. This can pose issues as the amount of volatiles captured by the SPME fibres can depend on the duration of sampling (compounds becoming equilibrated onto the fibre), the air movement of the glasshouse and the closeness of the fibres to the plants. However, this was standardised to the best of my ability in these experiments.

The nature of volatiles was also different between experiments. In sections 3.3 and 3.4 where all the plants were located together, major known vine volatiles such as α-, β- pinene, limonene or eucalyptol (1,8-cineole) (Gil *et al.*, 2013) were found, but not in the experiment in section 3.5 where vines were in individual chambers. This could indicate that differences are likely to be observed between volatiles emitted by Grenache and Chardonnay under drought stress. Differences have been previously seen between volatile emissions of genotypes and accessions within grapevine cultivars (Rid *et al.*, 2019) and other species (Niederbacher *et al.*, 2015).

Another reason for the difference between experiments could come from the choice of plastic (polyethylene) used for the chambers that could have retained the volatiles. Indeed, it is known that some materials like polyethylene can scalp volatiles, and especially eucalyptol (Capone, 1999). Since the SPME fibres were placed at the exit of the chambers and not inside, perhaps some of the volatiles may not have been captured. To confirm this, blank samples from empty chambers after the experiment revealed that traces of some volatiles still remained. Thus, the protocol of placing the fibres freely between the plants revealed a different volatile profile from the chamber experiment. On the other hand, this same protocol could not discriminate between volatiles emitted from the control treatment from those emitted by the well-watered treatment. For instance, it is not possible to say if the increase of α-pinene observed in the experiment in section 3.4 originates from the control plants or the water-stressed plants, or both groups simultaneously.

For all of these reasons, it was not possible to confirm which compound or blend of compounds could be involved in a presumed plant communication, but these experiments revealed some potential candidates that should be tested in priming experiments (Erb *et al.*, 2015; Ton *et al.*, 2007).

4. Volatile analysis during drought-rehydration experiments in *Arabidopsis thaliana*

4.1. Introduction

As in Chapter 3, drought-rehydration experiments were conducted on *Arabidopsis thaliana* wild type plants to repeat the observations of Dayer *et al.* (2017) and Scharwies (2017) where plants under different treatments were co-located in the same glasshouse, with addition of taking samples of volatiles.

Arabidopsis is known to be a non-natural emitter of isoprene but is largely used for transgenic purposes (Loivamäki *et al.*, 2007), with investigation of specific roles of volatiles such as caryophyllene (Alquézar *et al.*, 2017) or isoprene and ocimene (Faralli *et al.*, 2020). The wild-type also was used to study the effect of bacteria (Hung *et al.*, 2013) and biotic stress (Body *et al.*, 2019) on volatile emission.

In this series of experiments, it was expected to replicate results from the literature (Table 3, section 1.4.2, Chapter 1) where the well-watered plants had changes in the stomatal responses when co-located with plants that were drought-stressed and rehydrated. The simultaneous monitoring of volatile emission should match the profiles of the series of experiments in Chapter 3 and potentially confirm the volatile candidates for the inter-plant signalling.

4.2. Drought/rehydration experiment with *Arabidopsis thaliana* Col0 in three growth cabinets with volatile emission analysis

4.2.1. Material and methods

Plant and environment conditions

Arabidopsis thaliana Col0 were potted and grown in a small growth cabinet with artificial light (PAR 100-150 $\mu mol.m^{-2}.s^{-1}$) for 5 weeks with short-day conditions (10h light, 21°C / 14h dark, 17°C; humidity 60 %) (details in section 2.1.1, Chapter 2).

Drought/rehydration treatment

Plants were distributed in three small growth cabinets (Figure 46). Sixteen control well-watered (CWW) plants were placed in a cabinet with 8 plants per tray, and were watered during the experiment by flooding the trays every day for 30 min at 17:00 (Australian central standard time). In a second cabinet, 16 control water-stressed (CWS) plants were placed and were watered for 2 days, then the irrigation was stopped until wilting, and resumed for 4 days. In a third cabinet, 16 treatment well-watered (TWW) and 16 treatment water-stressed (TWS) were placed, and he TWW group had the same watering protocol as the CWW group and TWS as

CWS. One temperature and humidity sensor was placed in each cabinet with continuous 10-min interval monitoring.

From 12:00 to 14:00, stomatal conductance (g_s) was measured with a porometer on 5 selected plants per treatment and on 4 flagged leaves per plant, starting with the CWW group, then the TWW and TWS, to finish with the CWS group, in order to limit possible volatile contamination between the growth cabinets. Water consumption was monitored by weighing the pots every day before watering.

At 14:00, SPME fibres (DVB/CAR/PDMS) were placed in each cabinet on selected days with the coating manually exposed on custom-made stands for 1h. Then, the fibres were thermally desorbed and analysed with GC-MS with the same conditions as detailed in section 2.3, Chapter 2.

Figure 46. *Arabidopsis thaliana* Col0 plant positioning in 3 growth cabinets with control well-watered (CWW) group, control water-stressed (CWS) group, treatment well-watered (TWW) group and treatment water-stressed (TWS) group for the drought/rehydration experiment, with respective light intensities.

4.2.2. Results

4.2.2.1. Stomatal conductance and VPD results

Measurements of the stomatal conductance showed a similar g_s for the control well-watered (248 ± 44 mmol.m^{-2}.s^{-1}, mean ± SD, CWW) group, the treatment well-watered (189 ± 39 mmol.m^{-2}.s^{-1}, TWW) group and the treatment water-stressed (200 ± 54 mmol.m^{-2}.s^{-1}, TWS) group for the first three days (Figure 47a). The CWS g_s was lower (131 ± 22 mmol.m^{-2}.s^{-1}) because of a lower light intensity (Figure 46). As irrigation was

stopped for CWS and TWS groups on day 4, a decrease of g_s can be observed on day 8, until reaching 32 ± 22 mmol.m^{-2}.s^{-1} for the CWS group and 33 ± 8 mmol.m^{-2}.s^{-1} for the TWS group on day 11 with the wilting of the leaves being observed.

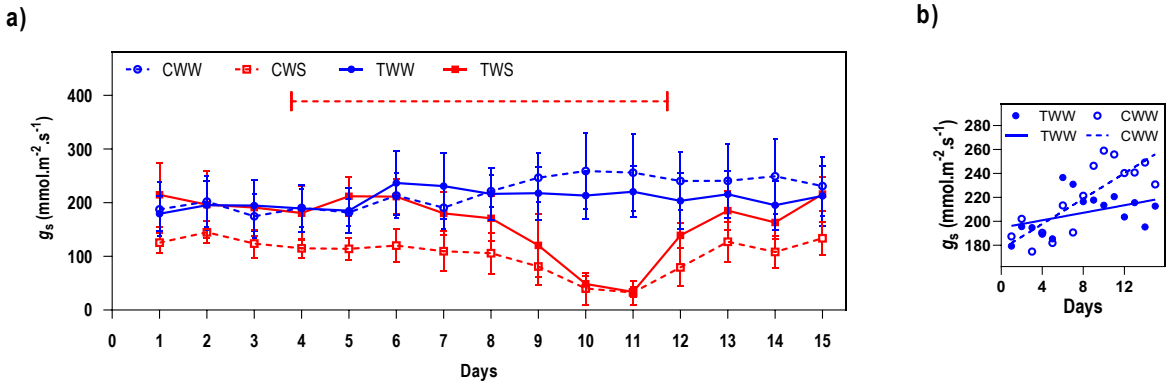


Figure 47. a) Stomatal conductance (g_s) measured on leaves of *Arabidopsis thaliana* Col0 used in a drought/rehydration experiment with three growth cabinets containing control well-watered (CWW, dotted blue line) plants that were watered every day, control water-stressed (CWS, dotted red line) plants that did not receive water during the red dashed line period, and both treatment well-watered (TWW, full blue line) and treatment water-stressed (TWS, full red line) plants (mean ± SD, n=5). b) Linear regression between stomatal conductance (g_s) and the time of the control well-watered (CWW) group and the treatment well-watered (TWW) which have been in the same growth cabinet than water-stressed plants.

The stomatal conductance of the TWW group stayed constant over time and the CWW group had similar values as the TWW group until day 8, then CWW g_s stayed higher for the rest of the experiment. The linear regressions showed that the slopes of CWW and TWW lines are not equal (Figure 47b, $p<0.05$). No significant differences were found between CWW and TWW (two-way repeated-measures ANOVA with Bonferroni post-tests, $p<0.05$, Supplementary Tables S59a and b).

At the end of the recovery, on day 15, CWW, TWW and TWS resumed to a similar g_s, with CWS g_s being lower for the same reason as mentioned above (lower light intensity) than at the beginning of the trial.

The Pettitt homogeneity tests revealed a shift in the data series of both CWW (day 7, $p<0.05$) and TWW (day 6, $p<0.05$) groups with an increase of g_s, and no shift for the CWS and TWS groups (Figure 48). When the test was performed on the data series without the recovery phase, it found the same results for the well-watered groups and found a decreasing shift in the series of data of CWS and TWS groups on day 7 ($p<0.05$, data not shown).

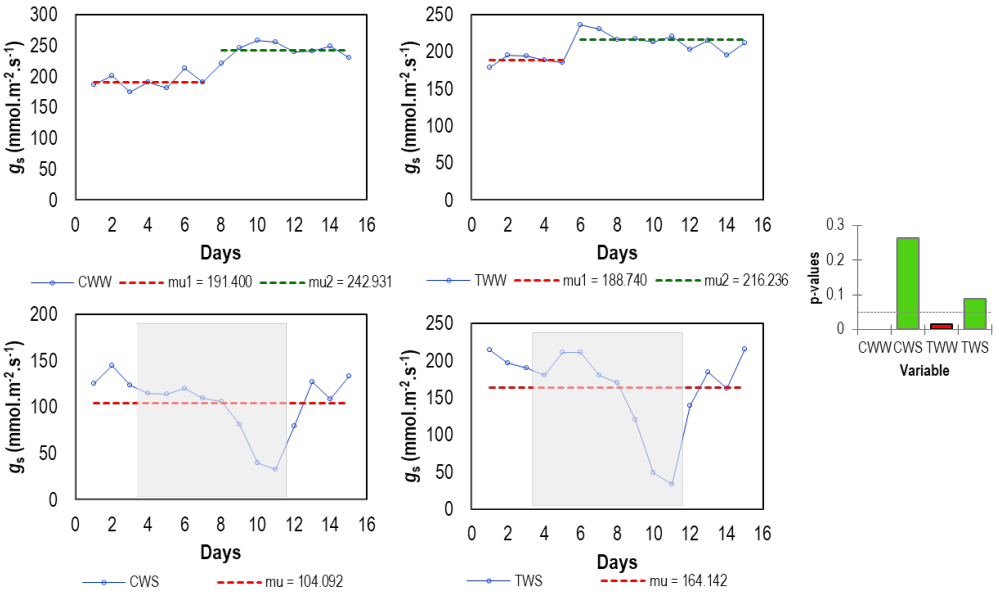


Figure 48. Pettitt homogeneity test on the stomatal conductance (g_s) data measured with the porometer from control well-watered (CWW) and treatment well-watered (TWW) plants that received water every day, and from control water-stressed (CWS) and treatment water-stressed (TWS) plants that did not receive water from day 4 to day 11 (grey box) (mean, n=5). The dotted lines represent the averaged value for the data series with two mu values if a change point is detected ($p<0.05$).

From temperature and relative humidity measurements (Figure 49), the VPD calculations revealed only one significant difference on day 5 between the cabinets, with the VPD staying the highest for the whole experiment in the control water-stressed (CWS) treatment cabinet but lower than 1.5 kPa (two-way repeated-measures ANOVA with Bonferroni tests, $p<0.05$, Supplementary Tables S60a and b). The multi-linear regression analysis did not show a significant effect of TWW_TWS VPD on TWW g_s (Supplementary Tables S61).

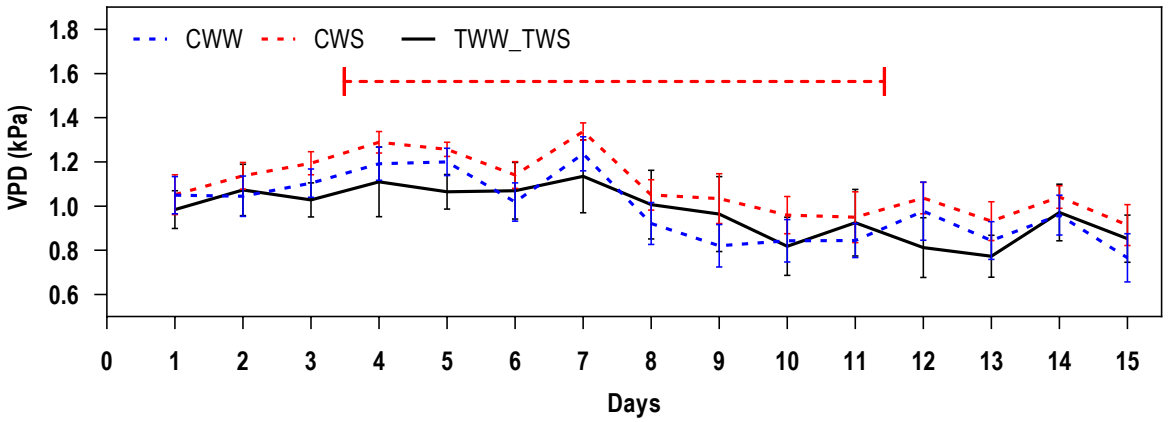


Figure 49. Vapour pressure deficit (VPD) calculated form the temperature and relative humidity from sensors placed in three growth cabinets, one with the control well-watered (CWW, dotted blue line) group which was watered every day, one with the control water-stressed (CWS, dotted red line) group which irrigation was cut off during the red dashed line period, and one with both treatment well-watered and treatment water-stressed (TWW_TWS, full black line) groups of *Arabidopsis thaliana* Col0. Each point represents the mean ± SD from 12:00 to 13:00 with 10-min interval (n=1).

4.2.2.2. Volatile analysis

Three time points were selected for volatile sampling with the SPME on day 2, day 9 and day 14 for technical reasons, so unfortunately not during the most severe drought stress phase (day 11). Data analysis of the GC-MS chromatograms revealed 28 peaks (compounds) that were common between all samples. The criteria for compound identity were that the library matches had to be greater than 50 % (match factor in Table 8) and was known to be emitted by plants (references in Table 8). However, no comparison with known standards or Kovats index calculations were conducted, and thus the compounds will be described by their # and library match names.

Table 8. List of the compounds identified in *Arabidopsis thaliana* Col0 well-watered and water-stressed plants, based on library match factor (%), averaged retention time and references found in the literature.

Peak #	Name from library match	Match factor (%)	Averaged retention time (min)	References
1	acetone	80	4.9	(Rissanen *et al.*, 2018)
2	2-butanone	80	6.1	(Souza *et al.*, 2013)
3	isopropyl alcohol	86	6.7	(Ebel *et al.*, 1995)
4	ethanol	78	6.9	(Holzinger *et al.*, 2000)
5	α-pinene	97	8.8	(Campbell *et al.*, 2018)
6	toluene	83	9.3	(Park *et al.*, 2009)
7	butyl acetate	80	10.2	(Scutareanu *et al.*, 1997)
8	hexanal	53	10.5	(Ebel *et al.*, 1995)
9	β-pinene	96	11.1	(Campbell *et al.*, 2018)
10	ethylbenzene	92	11.7	(Araya *et al.*, 2019)
11	p-xylene	94	12.1	(Araya *et al.*, 2019)
12	1-butanol	87	12.3	(Maleknia *et al.*, 2007)
13	3-heptanone	54	12.5	(Zhao *et al.*, 2016)
14	cumene	91	13.1	(Kegge *et al.*, 2013)
15	heptanal	95	13.4	(da Rocha *et al.*, 2017)
16	2-ethylhexanal	60	13.5	(Hung *et al.*, 2013)
17	limonene	99	13.8	(Combariza *et al.*, 1994)
18	eucalyptol	98	14.2	(Niinemets *et al.*, 2002)
19	ethyltoluene	53	14.6	(Scascighini *et al.*, 2005)
20	styrene	96	15.5	(Araya *et al.*, 2019)
21	m-cymene	95	15.9	(Geron *et al.*, 2016)
22	1,2,3-trimethylbenzene	95	16.2	(Ogunwande *et al.*, 2008)
23	octanal	87	16.4	(Hu *et al.*, 2009)
24	cyclohexanone	55	16.6	(Saunier *et al.*, 2020)
25	6-methyl-5-hepten-2-one	96	17.8	(Tatsuka *et al.*, 1990)
26	nonanal	96	19.3	(Hu *et al.*, 2009)
27	acetic acid	87	20.8	(Dewhirst *et al.*, 2020)
28	2-ethylhexanol	90	21.8	(Wei *et al.*, 2004)

Arabidopsis thaliana was found to be a strong emitter of isopropyl alcohol, ethanol, acetic acid and 2-ethylhexanol (Figure 50).

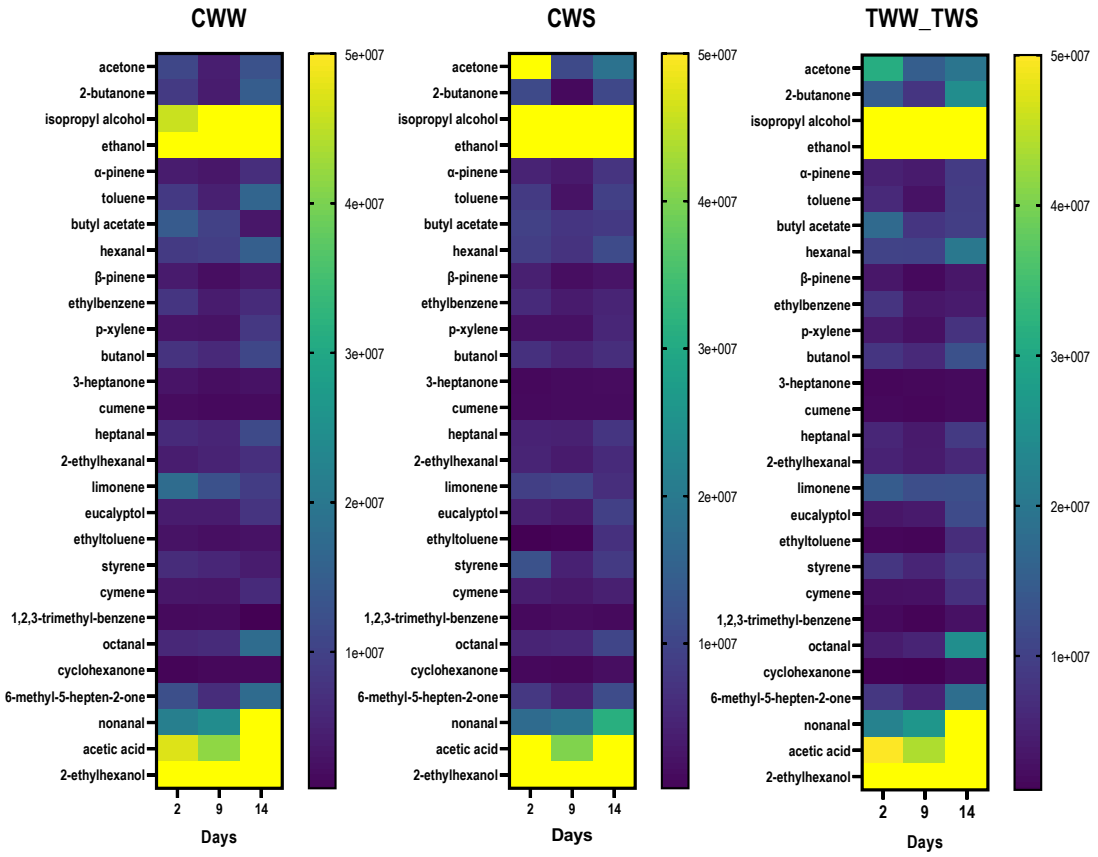


Figure 50. Heatmap plots of content and kinetics of volatile compounds analysed with SPME-GC-MS of *Arabidopsis thaliana* Col0 during a drought-rehydration experiment, with three growth cabinets containing control well-watered (CWW) plants that were watered every day, control water-stressed (CWS) plants that were water-stressed on day 9 and rehydrated on day 14, and both treatment well-watered (TWW) and treatment water-stressed (TWS) plants. Values represent chromatographic peak areas of the individual compounds.

The total chromatographic peak area of the individual compounds showed an increase of volatiles of the CWW group over time and a decrease for the CWS and combined TWW_TWS on day 9, followed by an increase on day 14 after rewatering. The TWW_TWS group had a higher volatile content at the start that could be explained by the fact that this growth cabinet contained double the number of plants than CWW and CWS cabinets (Figure 51).

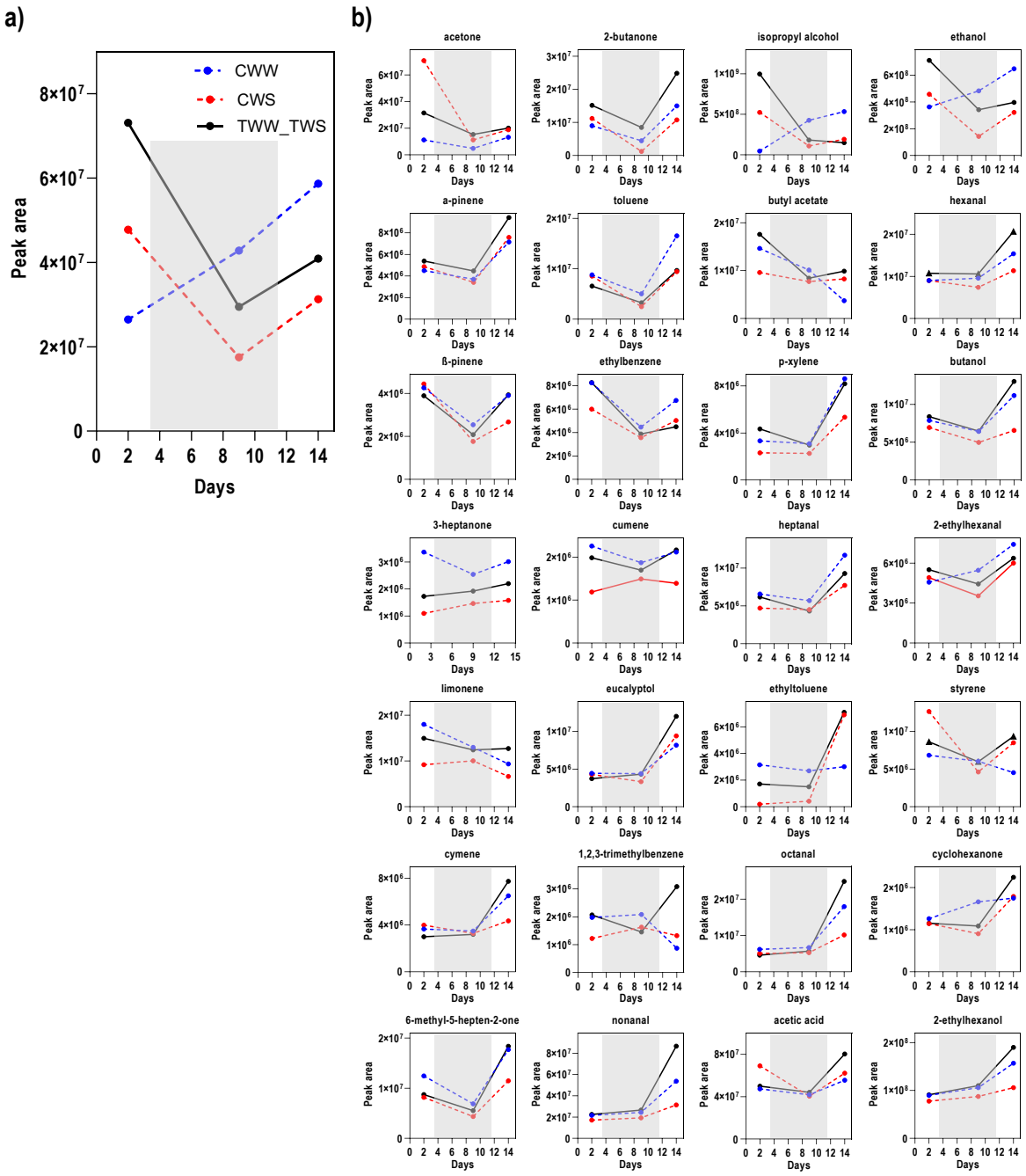


Figure 51. Content and kinetics of single volatile compounds analysed with SPME-GC-MS of *Arabidopsis thaliana* Col0 during a drought-rehydration experiment, with three growth cabinets containing control well-watered (CWW, blue dotted line) plants that were watered every day, control water-stressed (CWS, red dotted line) plants that were water-stressed (grey box), and both treatment well-watered and treatment water-stressed plants (TWW_TWS), with a) representation of the total chromatographic peak areas and b) single volatile compounds over time.

4.2.2.3. Combined analysis of physiological and chemical analyses

Correlations between identified volatiles, g_s and VPD of CWW (Figure 52), CWS (Figure 53) and combined TWW and TWS (TWW_TWS, Figure 54) groups were performed. Strong positive correlations (>0.9) were found between CWW g_s and isopropyl alcohol and cyclohexanone, and a negative correlation between g_s and limonene, but neither of these were statistically significant. For the CWS group, many compounds were positively correlated (acetone, isopropyl alcohol, ethanol, butyl acetate, β-pinene, ethylbenzene, butanol, styrene, acetic acid) and negatively correlated (cumene and 1,2,3-trimethylbenzene) with g_s, with only styrene being statistically significant. For the TWW group, β-pinene, cumene, 2-ethylhexanal and styrene showed a strong negative correlation with g_s, but only β-pinene was significant. For the TWS group, strong positive correlations were found between g_s and acetone, ethanol, butyl acetate and ethylbenzene, but neither of them were statistically significant.

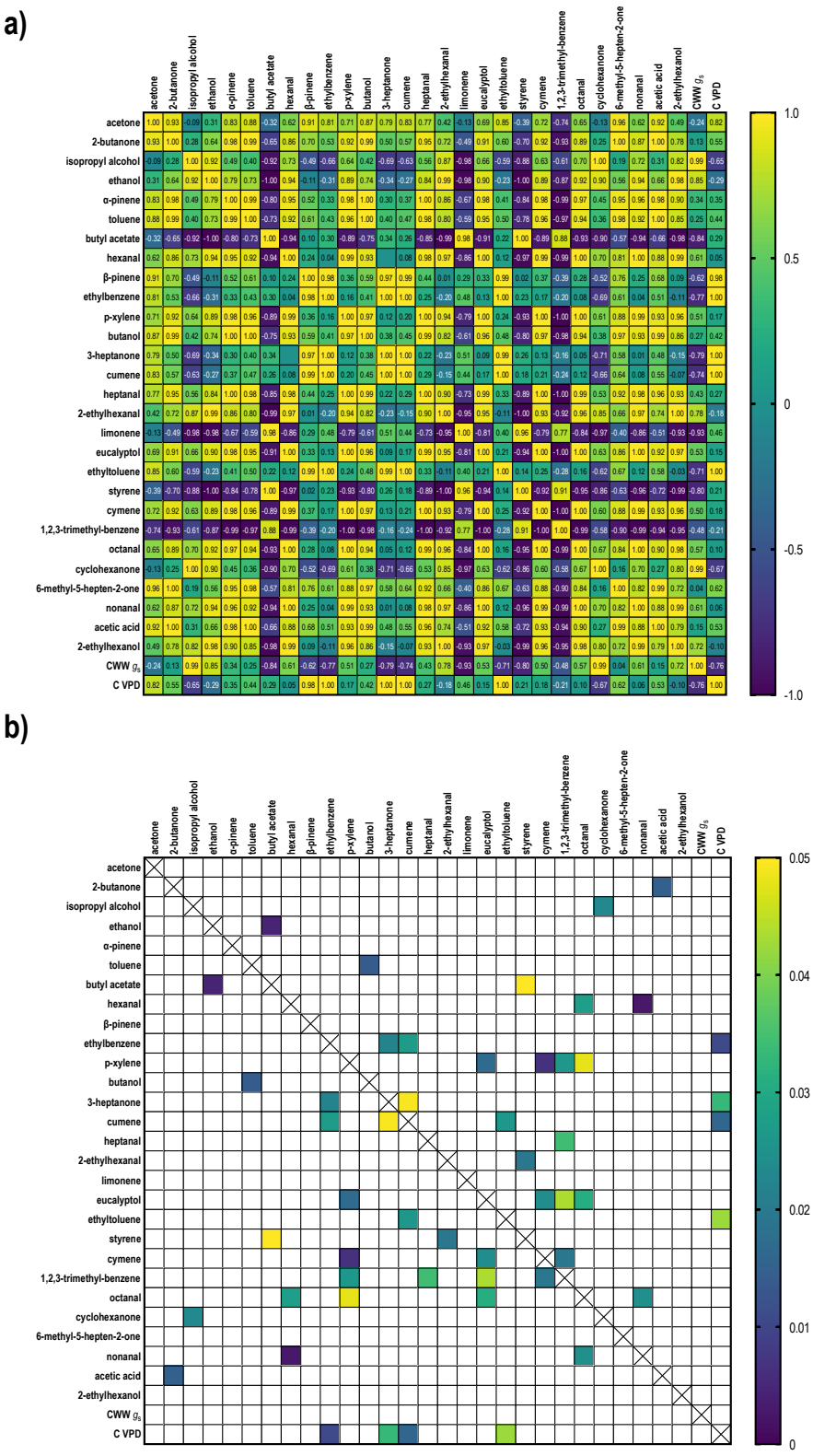


Figure 52. Pearson correlation matrices for control well-watered (CWW) plants between volatiles, stomatal conductance (g_s) and vapour pressure deficit (VPD). a) The correlation coefficient is shown for each combination in the relevant square and b) corresponding p-values (<0.05).

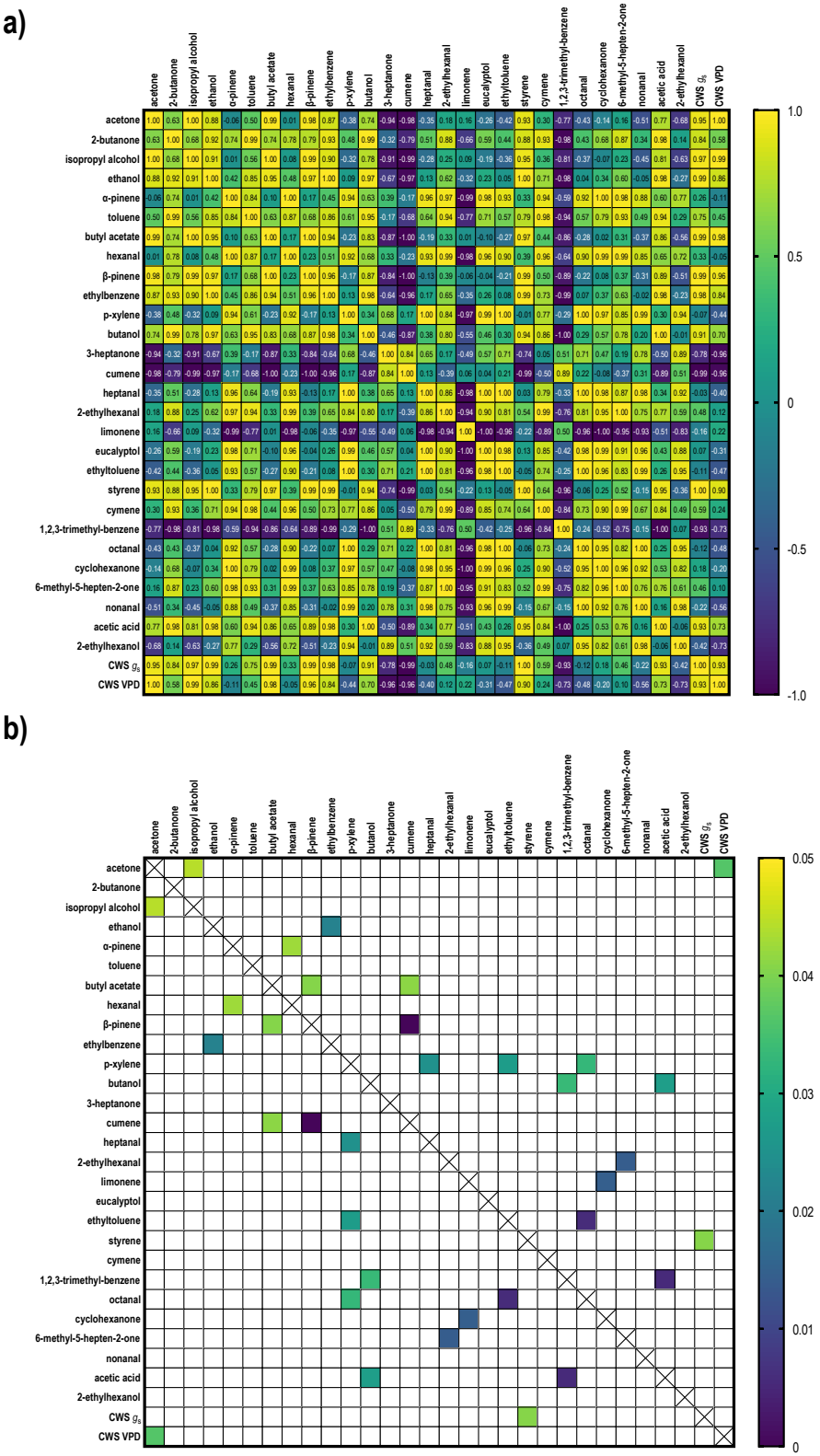


Figure 53. Pearson correlation matrices for control water-stressed (CWS) plants between volatiles, stomatal conductance (g_s) and vapour pressure deficit (VPD). a) The correlation coefficient is shown for each combination in the relevant square and b) corresponding p-values (<0.05).

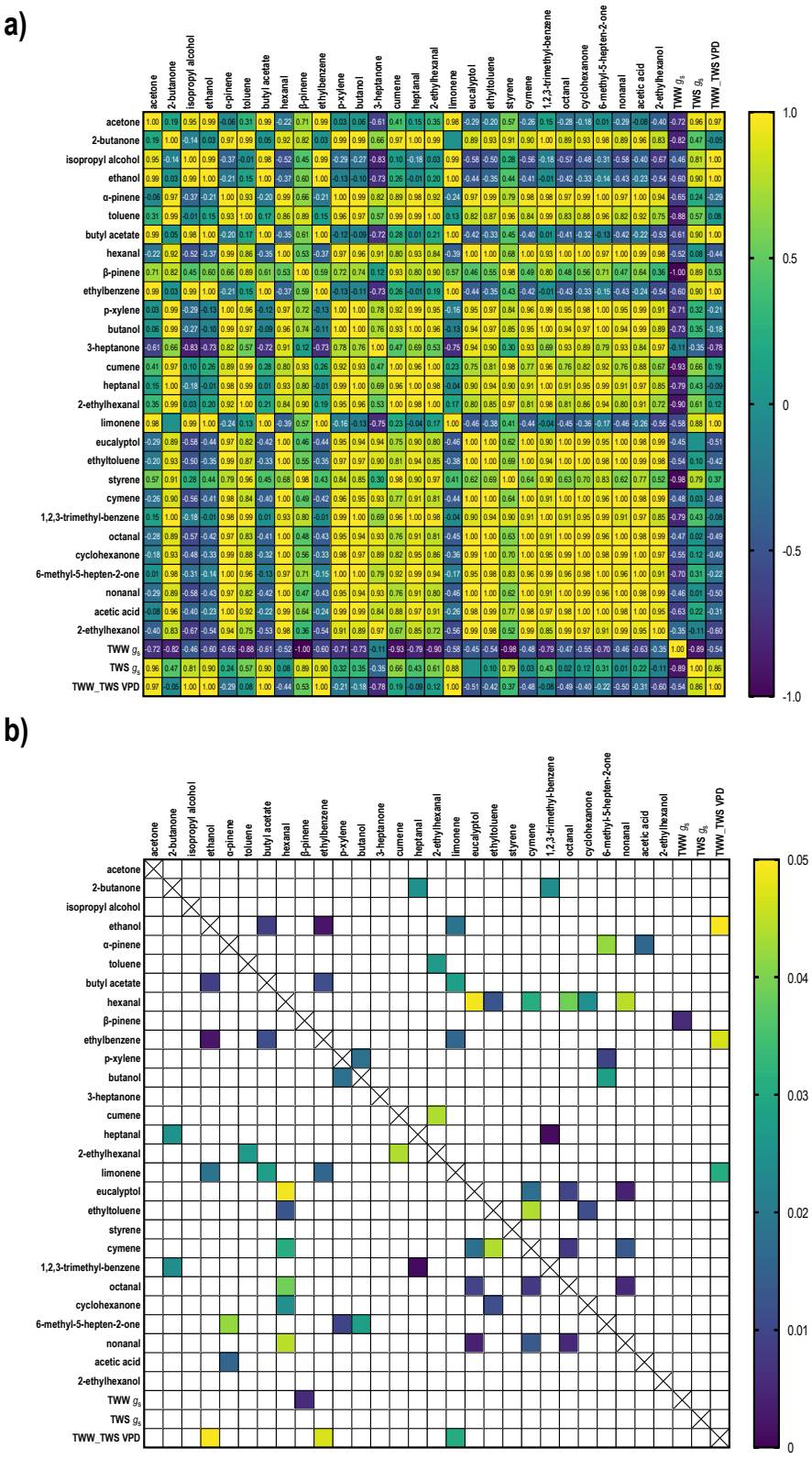


Figure 54. Pearson correlation matrices for treatment well-watered (TWW) and water-stressed (TWS) plants between volatiles, stomatal conductance (g_s) and vapour pressure deficit (VPD). a) The correlation coefficient is shown for each combination in the relevant square and b) corresponding p-values (<0.05).

4.2.3. Discussion

This experiment aimed to replicate drought/rehydration experiments from the literature showing well-watered plants mimicking the decrease of stomatal conductance from water-stressed plants (Scharwies, 2017). The results of this study showed that the treatment well-watered (TWW) plants that were in the same cabinet as the treatment water-stressed (TWS) plants had their g_s remaining stable or even increased over time, however not as much as the control well-watered (CWW) group in a separate growth cabinet (Figure 47).

The volatile analysis revealed that many compounds were detected around the plants (Table 8) and this is quite different from the literature since Arabidopsis is usually considered to be a low-emitter of volatiles and rarely used in volatile experiments (Vivaldo *et al.*, 2017). The well-watered plants showed an increase in total volatile emission, that supposedly could be explained by the new leaves growing and expanding (Hüve *et al.*, 2007). The drought stress, on the contrary, induced a general decrease of volatiles for the CWS group as well as in the TWW_TWS group where half of the plants were stressed (Figure 50). This is consistent with the studies described in Table 2, section 1.4.1, Chapter 1. Only styrene was found to significantly correlate with a stressed group g_s and constitutes a potential candidate for inter-plant signalling.

4.3. Drought/rehydration experiment on *Arabidopsis thaliana* in two growth cabinets and volatile emission analysis

4.3.1. Material and methods

Plant and environment conditions

Arabidopsis thaliana Col0 were potted and grown in a small growth cabinet with artificial light (PAR 150 $\mu mol.m^{-2}.s^{-1}$) for 5 weeks with short-day conditions (10 h light, 21°C / 14 h dark, 17°C; humidity 60 %).

Drought/rehydration treatment

Plants were distributed in two small growth cabinets with similar PAR (Figure 55). Sixteen control well-watered (C) plants were placed in a cabinet with 8 plants per tray and were watered during the experiment by flooding the trays every day for 30 min at 17:00. In the second growth cabinet, 16 treatment well-watered (WW) plants and 16 treatment water-stressed (WS) plants were placed. The WW group had the same watering protocol as the C group, and the WS group was watered for 3 days, then the irrigation was interrupted until wilting and resumed for 5 days. One temperature and humidity sensor was placed in each cabinet with 10-min interval measurements.

From 12:00 to 14:00, stomatal conductance (g_s) was measured with a porometer for 5 selected plants per treatment and on 4 flagged leaves per plants, starting with the C group, then the WW to finish with the WS group, in order to limit possible volatile contamination between the cabinets. Water consumption was monitored by weighing the pots every day before watering.

At 14:00, SPME fibres (DVB/CAR/PDMS) were placed in each cabinet during selected days with the coating manually exposed on custom-made stands for 1h and analysed by GC-MS (details in section 2.3, Chapter 2).

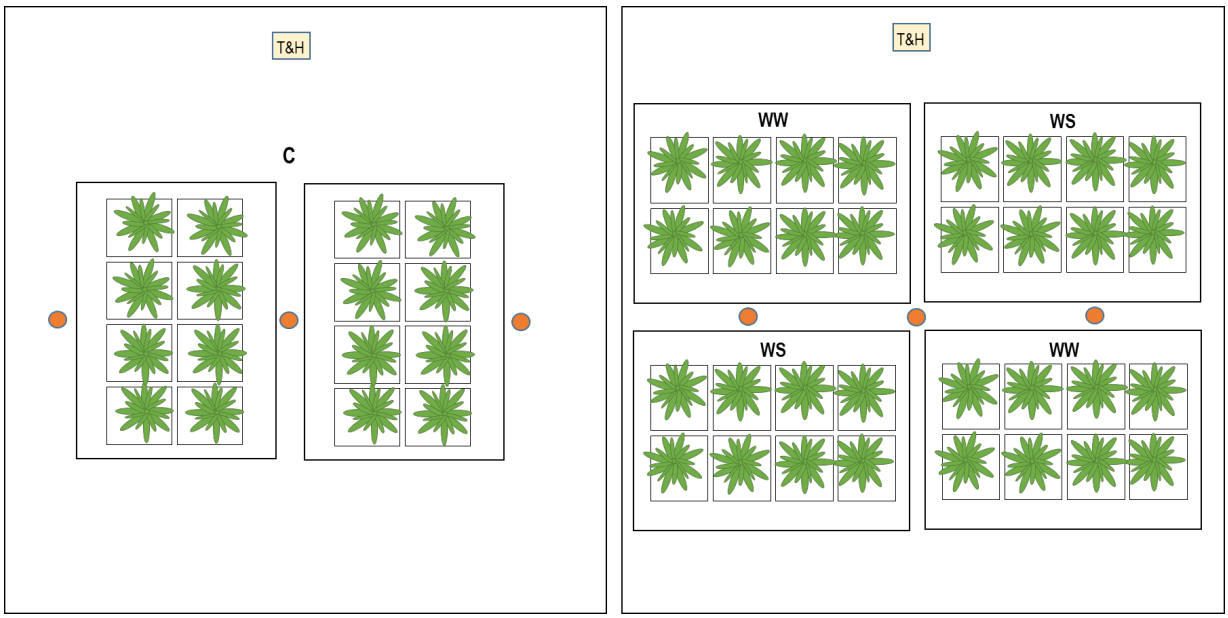


Figure 55. *Arabidopsis thaliana* Col0 plant distribution in 2 growth cabinets with the control well-watered (C) group, the treatment well-watered (WW) group and the treatment water-stressed (WS) group for the drought/rehydration experiment. The T&H yellow squares represent the position of the temperature and humidity sensors and the orange circles the SPME fibres during volatile sampling.

4.3.2. Results

4.3.2.1. Stomatal conductance and VPD results

Measurements of the stomatal conductance showed a similar g_s for the control well-watered (C) group, the treatment well-watered (WW) group and treatment water-stressed (WS) group for the first three days, of approximately 256 ± 46 mmol.m^{-2}.s^{-1} (mean ± SD) (Figure 56). As irrigation ceased for the WS group on day 4, a decrease in g_s can be observed on day 13, until reaching 50 mmol.m^{-2}.s^{-1} on day 17 and observation of wilting of the leaves. The stomatal conductance of the C group stayed constant over time and the WW g_s gradually decreased over time, as shown by the regression lines, and a slight decrease of g_s can be observed on day 16 and 17. The two-way repeated-measures ANOVA with Bonferroni post-tests revealed that WW g_s is significantly lower than WS g_s at the start of the experiment and from C g_s throughout the experiment ($p<0.05$, Supplementary Tables S62a and b). During the recovery phase, the WS plants recovered quickly and g_s increased again albeit not to the average g_s measured at the beginning of the experiment, most likely due to the flowering observed on day 20.

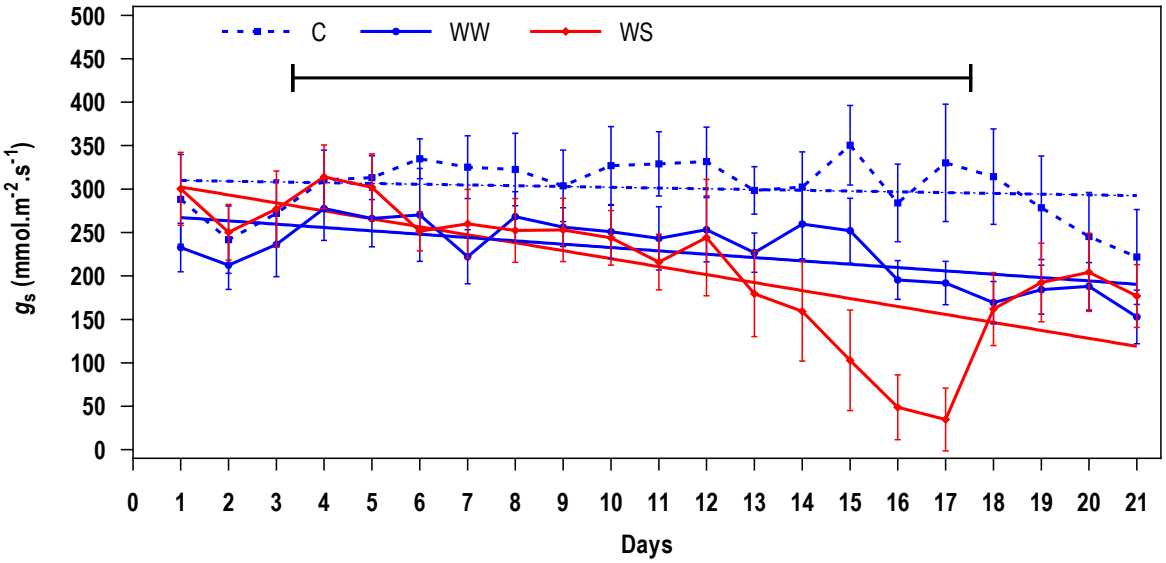


Figure 57. Stomatal conductance (g_s) measured on leaves of *Arabidopsis thaliana* Col0 used in a drought/rehydration experiment with two growth cabinets containing control well-watered (C, dotted blue line) plants that were watered every day, and both treatments well-watered (WW, full blue line) and water-stressed (WS, full red line) plants that did not receive water from day 4 to day 17 (black line) and then were irrigated (mean ±SD, n=5).

The Pettitt homogeneity test showed no significant shift in the series of data of the C group and a significant shift for the WS group on day 10 (Figure 58) when the stomatal conductance begins to decrease ($p<0.05$). Interestingly, the test also detected a shift for the WW group on day 16 with decreasing values ($p<0.05$). When the test was conducted on the data series without the recovery phase, no shift was detected for the C and WW groups, but was detected for the WS group on day 10 ($p<0.05$, data not shown).

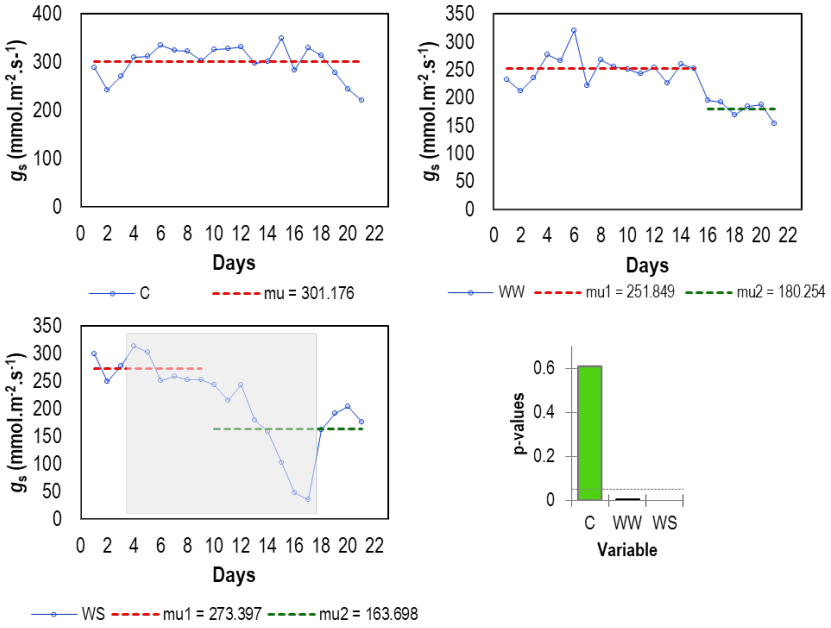


Figure 58. Pettitt homogeneity test on the stomatal conductance (g_s) data measured with the porometer from control well-watered (C) and treatment well-watered (WW) plants that received water every day, and from treatment water-stressed (WS) plants that did not receive water from day 4 to day 18 (grey box) (mean, n=5). The dotted lines represent the averaged value for the data series with two mu values if a change point is detected ($p<0.05$).

From temperature and humidity measurements (Figure 59), the VPD between the two cabinets were significantly different on day 8, day 14 and day 15, but values never exceeded 1.5 kPa (two-way repeated-measures ANOVA with Bonferroni post-tests, $p<0.05$, Supplementary Tables S63a and b). The multi-linear regression analysis did not show a significant effect of WW_WS VPD on WW g_s (Supplementary Tables S64).

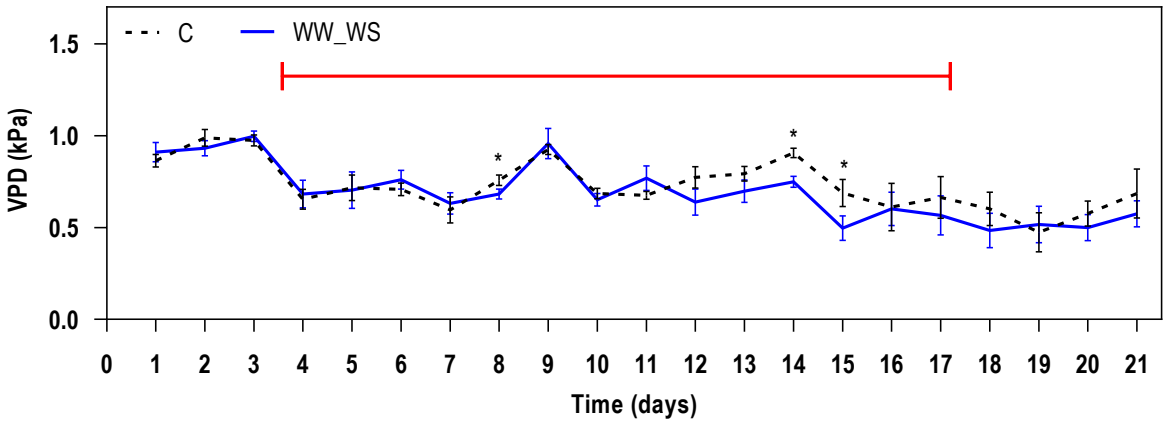


Figure 59. Vapour pressure deficit (VPD) calculated with the temperature and relative humidity from sensors placed in two growth cabinets with the *Arabidopsis thaliana* col0 plants, contained the control well-watered (C, dotted blue line) group which were watered

every day, and with both treatment well-watered and treatment water-stressed (WW_WS, full black line) which irrigation was cut off from day 4 to day 11 (red line). Each point represents the mean ± SD from 12:00 to 13:00 with 10-min interval (n=1). Significant differences by two-way repeated-measured ANOVA with Bonferroni post-tests are marked: * for $p<0.05$.

4.3.2.2. Volatile analysis

Three time points were selected for volatile sampling with the SPME on day 3, day 16 and day 18 (before the stress, severe stress and recovery day, respectively). Data analysis of the GC-MS chromatograms revealed 23 peaks (compounds) that were common between all samples and the criteria for compound identity was as described in section 4.2.2.2 with library match and references in Table 9.

Table 9. List of the compounds identified in *Arabidopsis thaliana* Col0 well-watered and water-stressed plants, based on library match factor (%), averaged retention time and references found in the literature.

Peak #	Name from library match	Match factor (%)	Averaged retention time (min)	References
1	acetone	72	4.7	(Rissanen *et al.*, 2018)
2	2-propanol	80	6.5	(Ebel *et al.*, 1995)
3	ethanol	78	6.8	(Holzinger *et al.*, 2000)
4	α-pinene	96	8.6	(Campbell *et al.*, 2018)
5	toluene	93	9.01	(Park *et al.*, 2009)
6	butyl acetate	64	9.9	(Scutareanu *et al.*, 1997)
7	hexanal	87	10.2	(Ebel *et al.*, 1995)
8	β-pinene	96	10.8	(Campbell *et al.*, 2018)
9	ethylbenzene	94	11.5	(Araya *et al.*, 2019)
10	m-xylene	83	11.7	(Idris *et al.*, 2019)
11	p-xylene	95	11.8	(Araya *et al.*, 2019)
12	1-butanol	80	12.1	(Maleknia *et al.*, 2007)
13	cumene	87	12.8	(Kegge *et al.*, 2013)
14	heptanal	95	13.2	(da Rocha *et al.*, 2017)
15	limonene	99	13.6	(Combariza *et al.*, 1994)
16	Eucalyptol (1,8-cineole)	99	14.0	(Niinemets *et al.*, 2002)
17	ethyltoluene	94	14.3	(Scascighini *et al.*, 2005)
18	m-cymene	97	15.7	(Geron *et al.*, 2016)
19	octanal	95	16.2	(Hu *et al.*, 2009)
20	6-methyl-5-hepten-2-one	96	17.5	(Tatsuka *et al.*, 1990)
21	nonanal	98	19.1	(Hu *et al.*, 2009)
22	acetic acid	86	20.5	(Dewhirst *et al.*, 2020)
23	2-ethylhexanol	90	21.6	(Wei *et al.*, 2004)

Arabidopsis thaliana was found to be a strong emitter of eucalyptol (1,8-cineole), nonanal, acid acetic and 2-ethyl-hexanol (Figure 60).

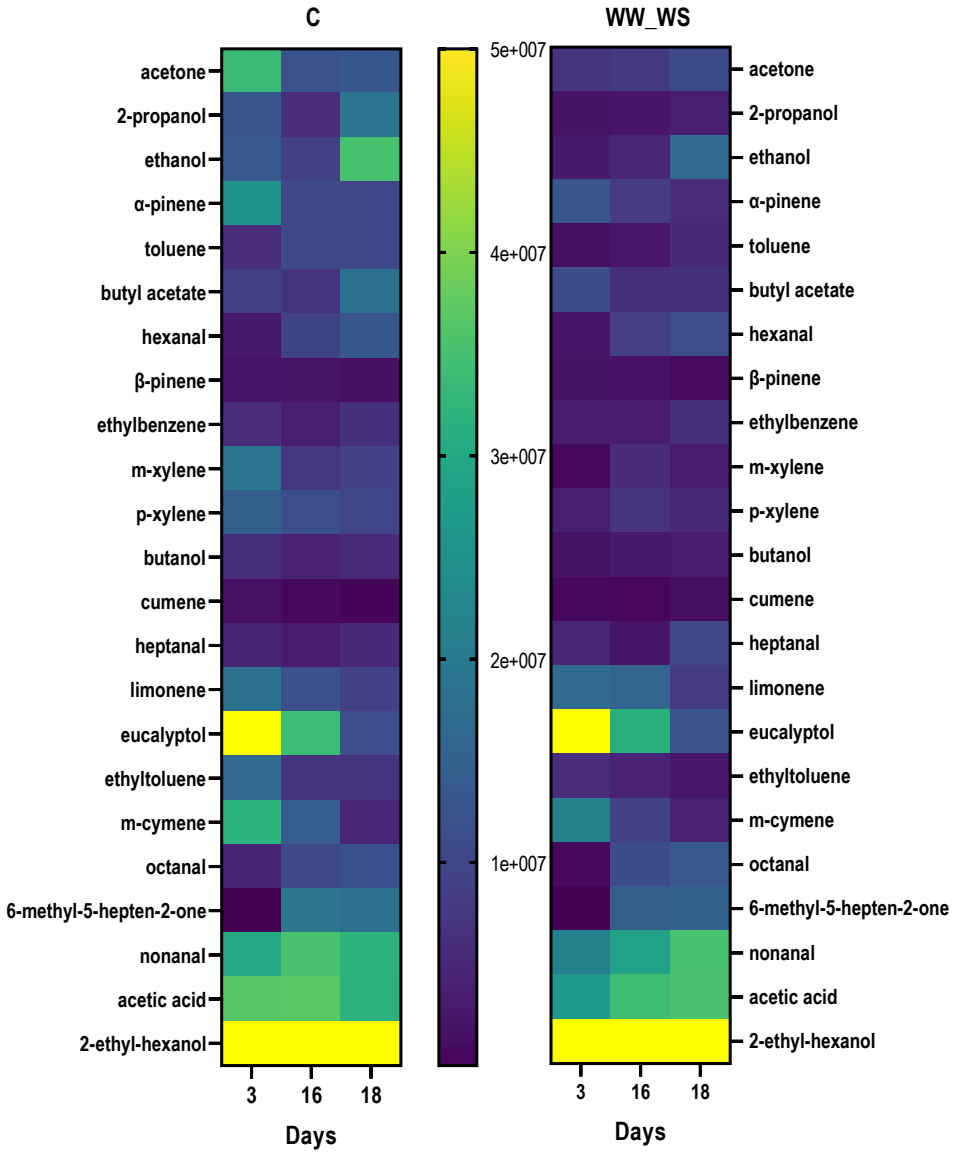


Figure 60. Heatmap plots of content and kinetics of volatile compounds analysed with SPME-GC-MS of *Arabidopsis thaliana* Col0 during a drought-rehydration experiment, with two growth cabinets containing control (C) plants that were watered every day, and both treatment well-watered and treatment water-stressed (WW_WS) plants that were water-stressed on day 6 and rehydrated on day 18. Values represent total chromatographic peak areas of the individual compounds.

The total chromatographic peak area showed an overall decrease of content for both C and WW_WS groups (Figure 61).

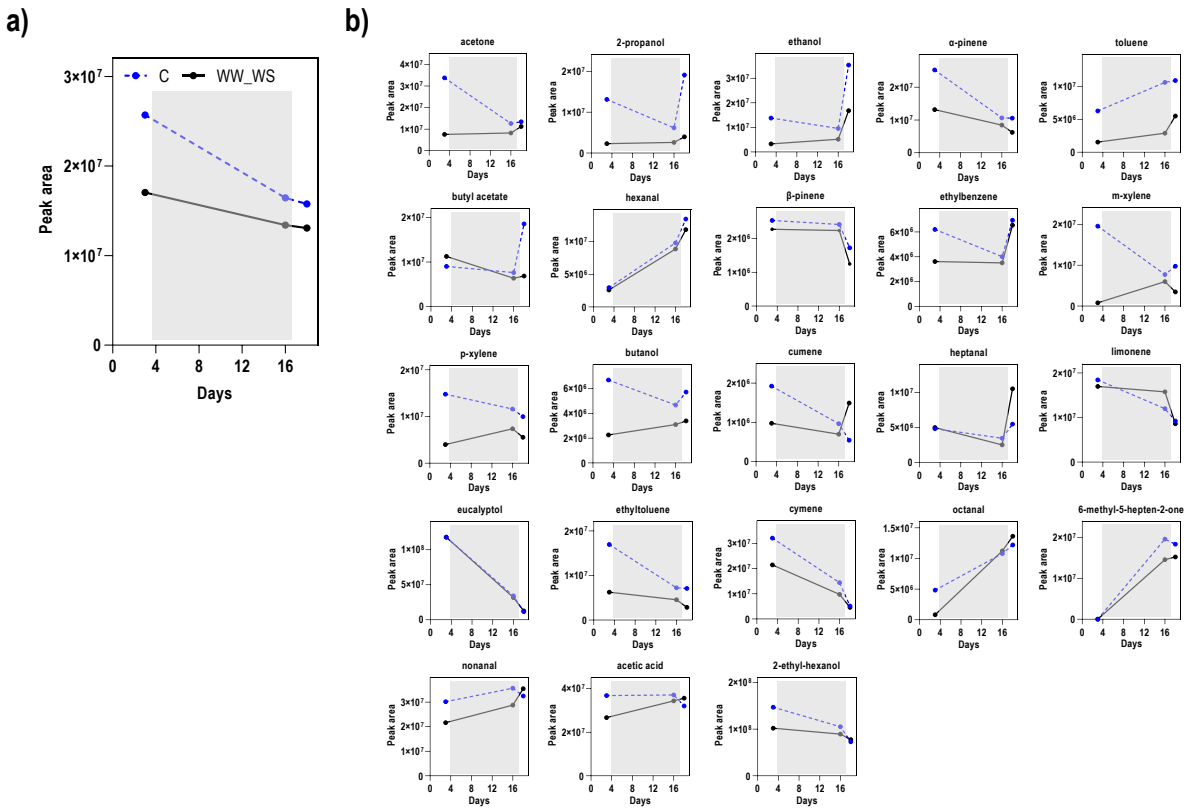


Figure 61. Content and kinetics of single volatile compounds analysed with SPME-GC-MS of *Arabidopsis thaliana* during a drought-rehydration experiment, with two growth cabinets containing control well-watered (C, blue dotted line) plants that were watered every day, and both treatment well-watered and treatment water-stressed plants (WW_WS) plants, with a) representation of the total chromatographic peak areas and b) single volatile compounds.

4.3.2.3. Combined analysis of physiological and volatile results

Correlations between identified volatiles, g_s and VPD of CWW (Figure 62) and combined WW and WS (Figure 63) groups were performed. Positive correlations (>0.9) were found between C g_s and ethanol, butyl acetate and hexanal, and negative correlations between g_s and β-pinene, p-xylene, m-cymene, acetic acid and 2-ethyl-hexanol, but neither of them were significant. For the WW group, β-pinene, eucalyptol, ethyltoluene, m-mycene and 2-ethyl-hexanol showed positive correlations, and toluene, hexanal, butanol, octanal, 6-methyl-5-hepten-2-one, nonanal and acetic acid showed negative correlations, but only hexanal was significant. And for the WS group, strong positive correlations were found between g_s and butyl hexanal (non-significant) and negative correlations with m-xylene and p-xylene which were significant.

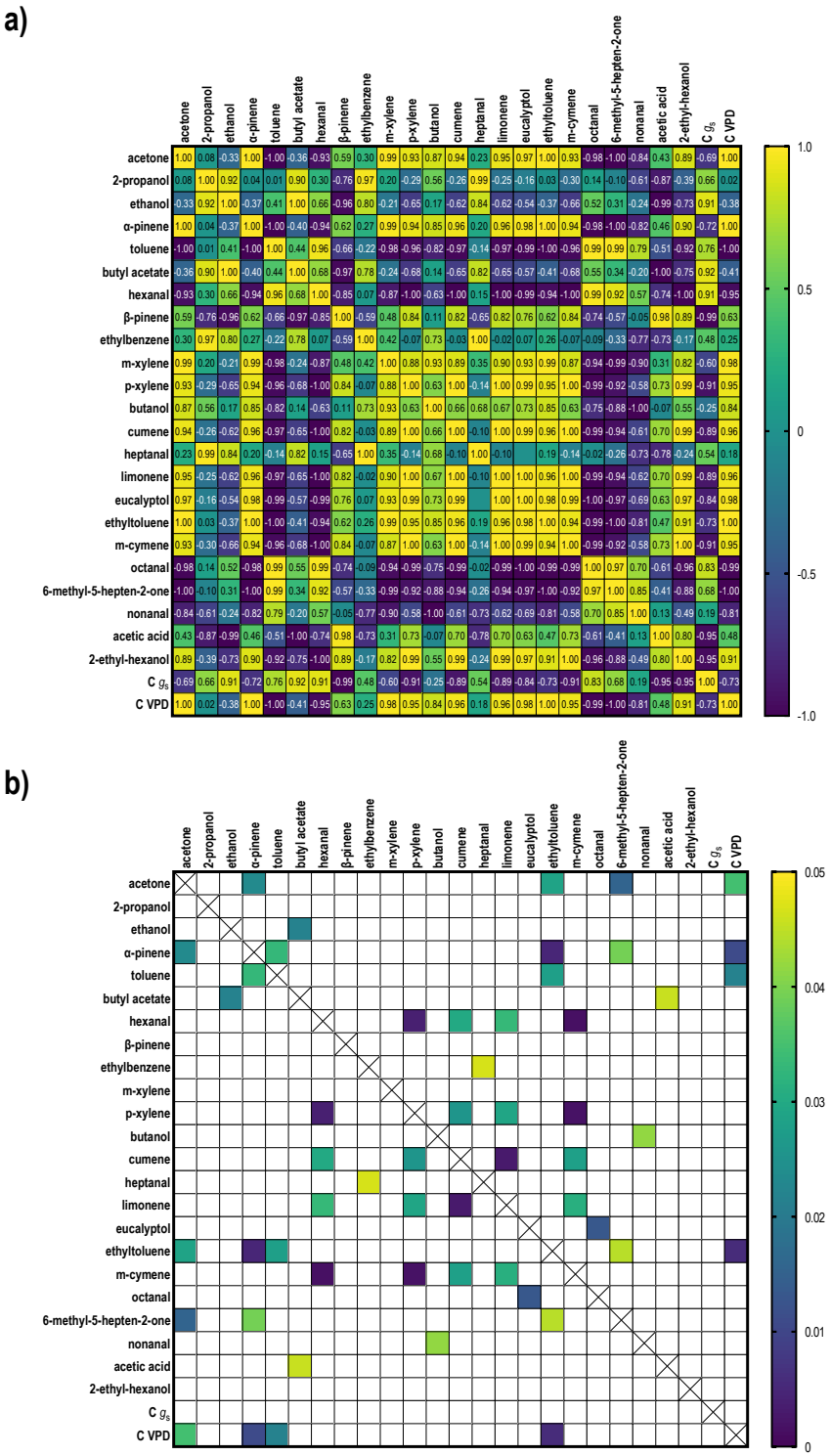


Figure 62. Pearson correlation matrices for control well-watered (C) plants between volatiles, stomatal conductance (g_s) and vapour pressure deficit (VPD). a) The correlation coefficient is shown for each combination in the relevant square and b) corresponding p-values (<0.05).

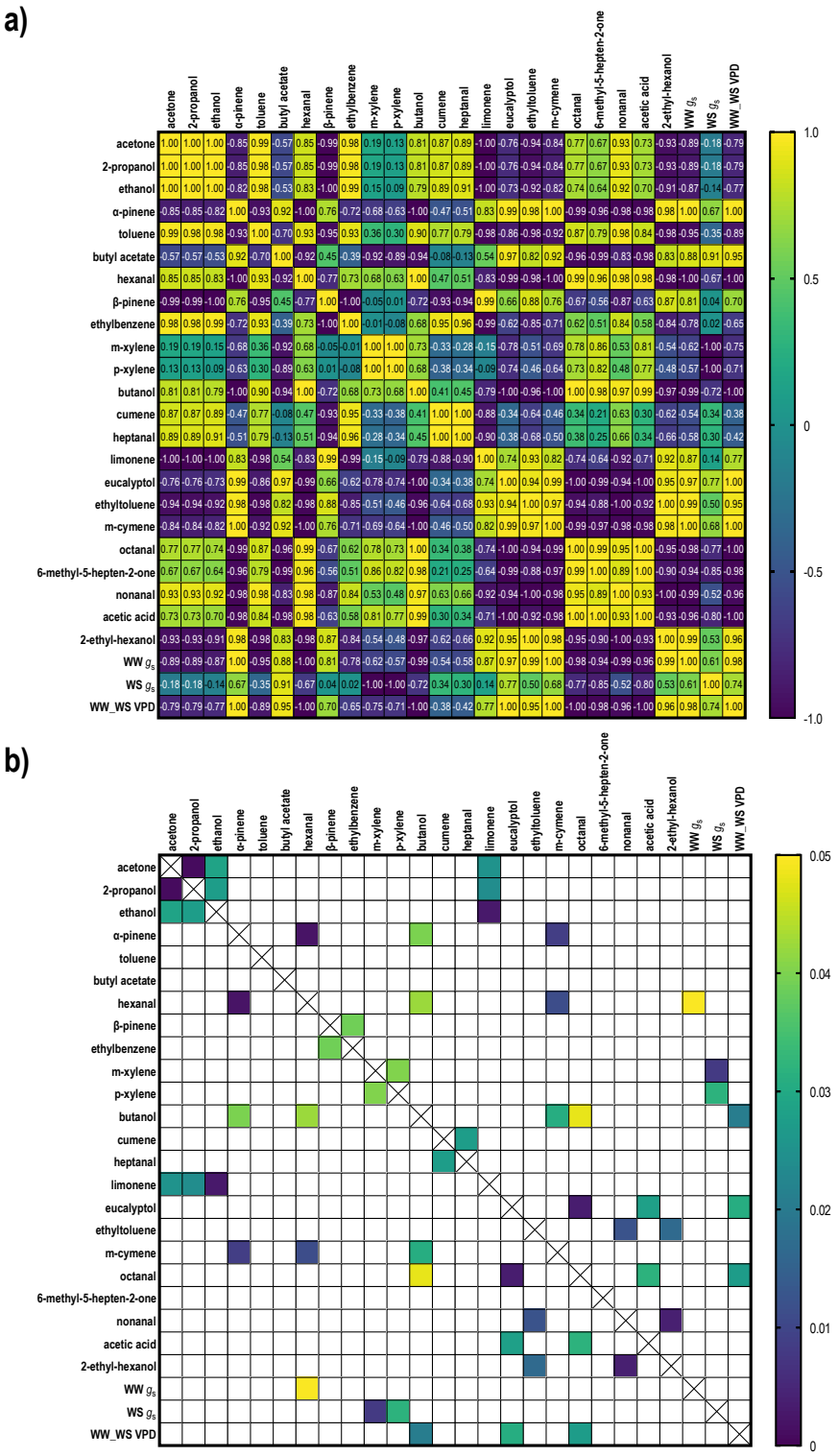


Figure 63. Pearson correlation matrices for treatment well-watered (WW) and treatment water-stressed (WS) plants between volatiles, stomatal conductance (g_s) and vapour pressure deficit (VPD). a) The correlation coefficient is shown for each combination in the relevant square and b) corresponding p-values (<0.05).

4.3.3. Discussion

This drought/rehydration experiment gave similar results to that seen in the literature (Table 3, section 1.4.2, Chapter 1) with a decrease of stomatal conductance of the treatment well-watered (WW) group during the severe stress phase of the treatment water-stressed (WS) group, also detected with the Pettitt homogeneity test as a decreasing shift in the data series (Figure 58). During the recovery phase, both groups WW and WS had their g_s increasing.

Compared to the first experiment in section 4.2, the chromatographic analysis revealed 22 similar peaks, 1 different peak (m-xylene), and 5 volatiles that were not detected (Table 9). Similar volatiles were found for Arabidopsis under heat stress (Truong *et al.*, 2014). Here, the overall volatile content showed a different trend from the first experiment with a decrease for the two treatments (Figure 61a). However, the days selected for the volatile samples were different since the first experiment in section 4.2 did not have a sampling day during the severe stress phase while this experiment did. Three volatiles showed statistically significant correlations to the WW and WS groups, which were hexanal, m-xylene and p-xylene, and could be added to the list of potential candidates for the inter-plant volatile signalling.

4.4. Drought/rehydration experiment on *Arabidopsis thaliana* in two growth cabinets

4.4.1. Material and methods

Plant and environment conditions

Arabidopsis thaliana Col0 were potted and grown in a small growth cabinet with artificial light (PAR 150 $\mu mol.m^{-2}.s^{-1}$) for 5 weeks with short-day conditions (10 h light, 21°C / 14 h dark, 17°C; humidity 60 %).

Drought/rehydration treatment

Plants were distributed in two small growth cabinets with similar light intensities (Figure 64). Sixteen control well-watered (C) plants were placed in a cabinet with 8 plants per tray and were watered during the experiment by flooding the trays every day for 30 min at 17:00. In the second growth cabinet, 16 treatment well-watered (WW) plants and 16 treatment water-stressed (WS) plants were placed, and the WW group had the same watering protocol as the C group, and the WS group was watered for 3 days, then the irrigation was interrupted until wilting was observed and resumed for a further 5 days. The trays position in the cabinet was rotating every two days after watering (to not have the same plants in the centre with maximal light intensity).

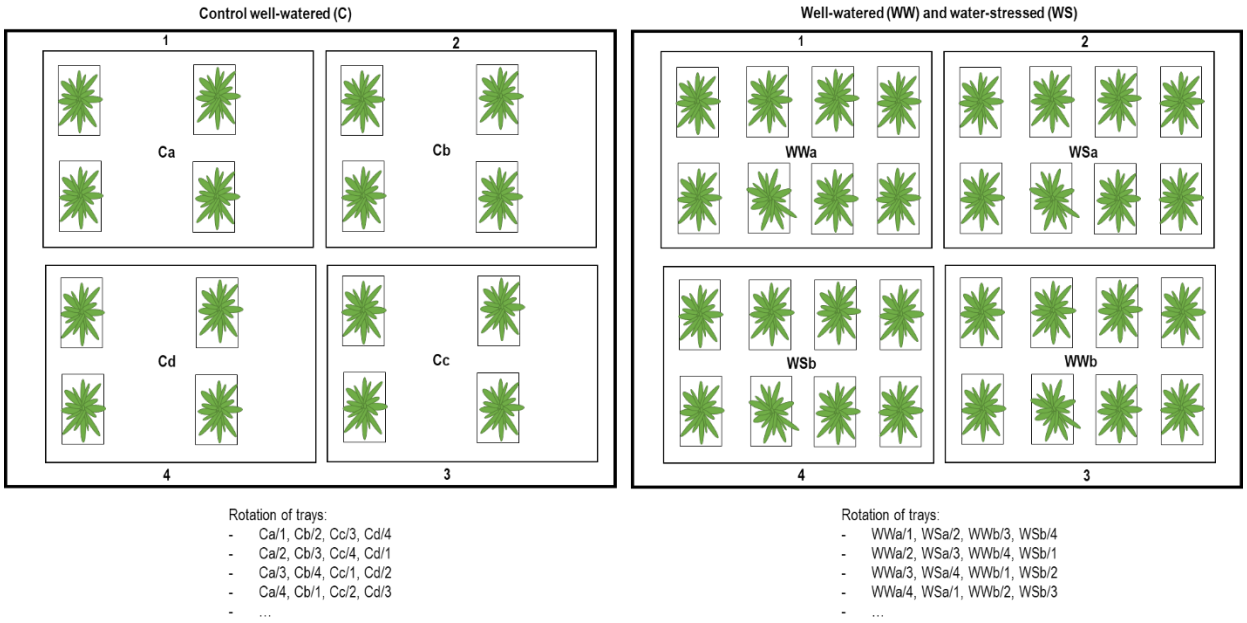


Figure 64. *Arabidopsis thaliana* Col0 plant distribution in two growth cabinets with the control well-watered (C) group, the treatment well-watered (WW) group and the treatment water-stressed (WS) group for the drought/rehydration experiment. The trays were rearranged every two days following a rotation order.

One temperature and humidity sensor was placed in each cabinet with 10-min interval measurements. At 12:00, stomatal conductance (g_s) was measured every two days with a porometer for all plants per treatment

and on 3 flagged leaves per plants, starting with the C group, then the WW group, to finish with the WS group, in order to limit possible volatile contamination between the cabinets.

4.4.2. Results

4.4.2.1. Stomatal conductance and VPD results

Measurements of the stomatal conductance showed a high g_s for the control well-watered (C) group, the treatment well-watered (WW) group and treatment water-stressed (WS) group for the first three days averaged 207 ± 9 mmol.m^{-2}.s^{-1} (mean ± SD). As irrigation was cut off for WS on day 4, a decrease of g_s can be observed on day 11, until reaching 9 ± 1 mmol.m^{-2}.s^{-1} on day 19 with the wilting of the leaves. The stomatal conductance of the C group and the WW group stayed similar over time with a slight decrease over time as shown by the regression lines (Figure 65). A significant difference was found between the C and WW groups only on day 1 (two-way repeated-measures ANOVA with Bonferroni tests, $p<0.05$, Supplementary Tables S65a and b).

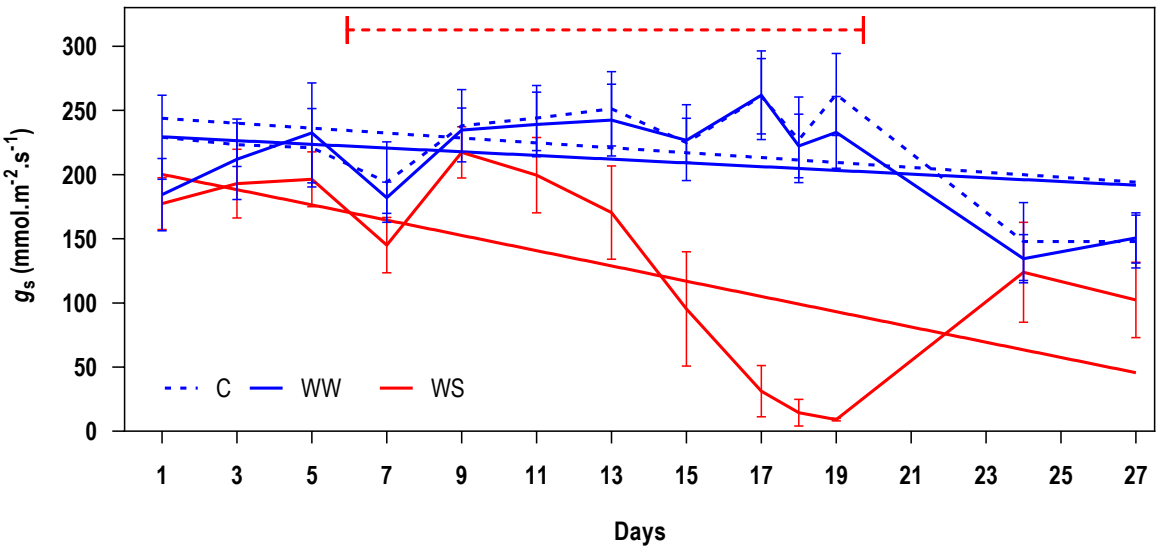


Figure 65. Stomatal conductance (g_s) measured on leaves of *Arabidopsis thaliana* Col0 used in a drought/rehydration experiment with two growth cabinets containing control well-watered (C, dotted blue line) plants that were watered every day, and with both treatment well-watered (TWW, full blue line) and treatment water-stressed (WS, full red line) plants that did not receive water (dashed red line) and then were re-irrigated (mean ± SD, n=16).

The Pettitt homogeneity test did not detect a shift in the series of data for the C and WW groups (Figure 66) and detected a shift for the WS group with decreasing values on day 8 ($p<0.05$).

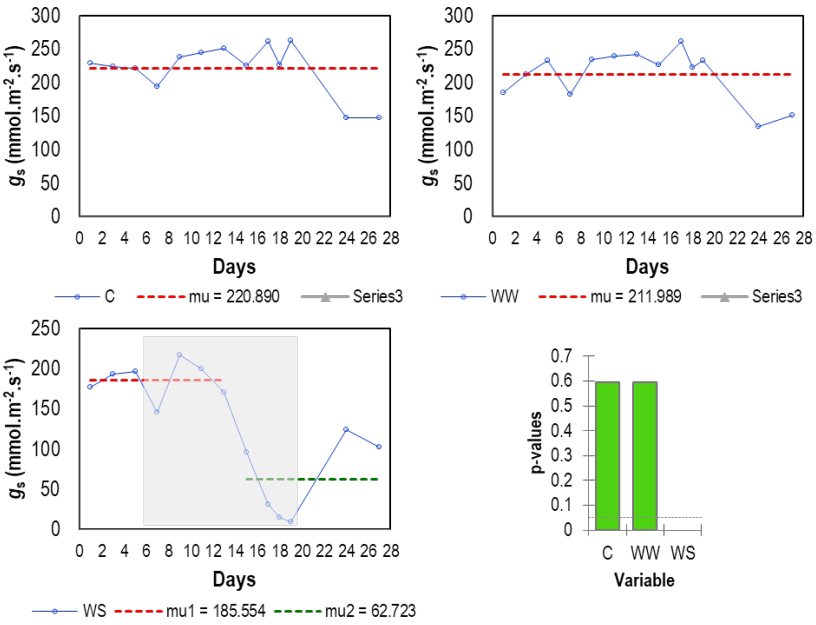


Figure 66. Pettitt homogeneity test on the stomatal conductance (g_s) data measured with the porometer from control well-watered (C) plants and treatment well-watered (WW) plants that received water every day, and from treatment water-stressed (WS) plants which irrigation was cut-off (grey box) and re-hydrated (mean, n=16). The dotted lines represent the averaged value for the data series with two mu values if a change point is detected ($p<0.05$).

From temperature and humidity measurements, VPD values between the two cabinets never exceeded 1 kPa (Figure 67) and were significantly different on day 7 (two-way repeated-measures ANOVA with Bonferroni post-tests, $p<0.05$, Supplementary tables S66a and b). The multi-linear regression analysis did not show a significant effect of WW_WS VPD on WW g_s (Supplementary Tables S67).

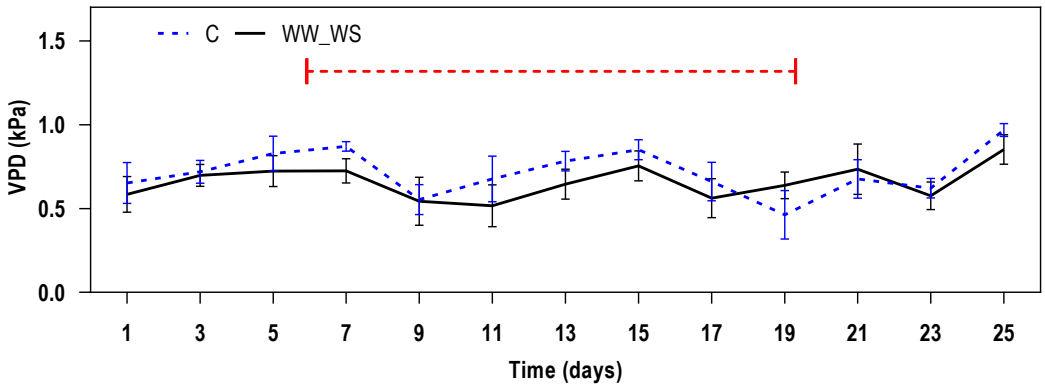


Figure 67. Vapour pressure deficit (VPD) calculated from the temperature and relative humidity data from sensors placed in the growth cabinets of *Arabidopsis thaliana* Col0 with the control well-watered (C, dotted blue line) group which were watered every

day, and with both treatment well-watered and treatment water-stressed (WW_WS, full black line) groups which irrigation was cut-off (red dashed line) and re-hydrated. Each point represents the mean ± SD from 12:00 to 13:00 with 10-min interval (n=1).

4.4.3. Discussion

This experiment aimed to replicate the results of the second experiment in section 4.3, but it was decided to take less porometer measurements to reduce the potential stressful effect of repeatedly clamping the head of the instrument on the leaf. It was actually observed that as the leaves were losing turgescence caused by the interruption of the irrigation, a round mark appeared from the pressure of the seals (Figure 68). However, the stomatal conductance of the WW group did not show a reduction of g_s during the drought-stress phase of the WS group, and gave similar results to that of the control C group (Figure 65).

Figure 68. Picture of an *Arabidopsis thaliana* Col0 plant from the water-stressed (WS) group on day 18 (second to last day of drought stress). The white dashed square shows where the porometer head was clamped on the leaf to take stomatal conductance measurements.

4.5. General conclusion

Compared to the results in Chapter 3, all three experiments had the same protocol and same Arabidopsis model (wild-type Col0), however, each result concerning stomatal conductance (g_s) was different. The first in section 4.2 did not show a decrease of well-watered (WW) plants g_s following the water-stressed (WS) plants g_s, but it was lower than the control (C) plants g_s once the stress phase started. In the second experiment in section 4.3, a decrease of WW g_s was observed and confirmed with the Pettitt's homogeneity test, and in the third experiment in section 4.4, WW and C g_s were not different.

The volatile analysis revealed 29 different volatile compounds between the two experiments in sections 4.2 and 4.3. The identification and quantity determination can be discussed the same way as in Chapter 3, section 3.6, with the *Vitis vinifera* samples since the methodology was the same. Those volatiles were common to others studies (Hung 2013, Body 2019), where styrene was positively correlated to WS g_s and m-xylene and p-xylene were negatively correlated to WS g_s ($p<0.05$), adding to the list of potential candidates for the inter-plant signalling.

In conclusion, more repetitions are needed to affirm if there is a decrease of g_s from well-watered plants when in the same environment as water-stressed plants (Scharwies 2017), or if there are other factors that were potentially not controlled that impacted on the experiments. One possibility could be that volatiles emitted from the soil are affecting stomatal conductance. Indeed, the soil used in the experiments with Arabidopsis was autoclaved, as opposed to the experiments with *Vitis vinifera*, and could potentially alter the composition of the volatiles coming from the root system (Gulati *et al.*, 2020) or soil-borne microorganisms (e.g. fungal volatiles increasing root formation (Moisan *et al.*, 2020)).

5. Stomatal responses to potential volatile signals in *Arabidopsis thaliana* and *Vitis vinifera* utilising a liquid flow meter to monitor single leaf transpiration

5.1. Abstract

Stomatal aperture adjustments can be observed with different methods but most often they are inferred using gas exchange systems or humidity monitoring that involve partial or total covering of the leaf with cuvettes. These methods deduce changes in stomatal aperture as changes in leaf conductance to water vapour leaving the leaf. Here, we present a method for measurement of water flow into a transpiring leaf, free of attached-cuvettes, to determine its water consumption and to monitor changes in leaf transpiration associated with changes in stomatal aperture. To examine responses in transpiration to potential volatile signal compounds, leaf cuvettes are not optimal since the volatiles may be scalped by the associated plastic tubing and filtration systems and may also potentially damage infra-red gas analysers and humidity sensors. The method we describe uses sensitive flow meters connected to detached-leaves of *Vitis vinifera* and *Arabidopsis thaliana* plants. Stable transpiration rates over several hours could be obtained that were similar to reported transpiration rates for these species. Transitions from light to dark and *vice-versa* showed rapid changes in transpiration rate as the stomata responded similarly to other studies. This test became a routine method to verify the fitness of the leaf connected to the flow meter. Comparison of simultaneous gas exchange with flow into the leaf showed some differences in rates that indicated non-linear capacitive effects in the leaf. Cuvettes applied to Arabidopsis leaves appeared to significantly restrict transpiration presumably due to pressure from the seals on the delicate leaf veins. In addition, some volatiles were tested and it was found that volatile methanol induced rapid closure of stomata similar to the dark response. This closure was also rapidly recoverable after the free volatile methanol diffused away as monitored by volatile gas sensors. Volatiles were also observed to be emitted from leaves corresponding to changes in flow rate and light to dark transition.

5.2. Introduction

Plant stomata are small pores composed of two guard cells on the surface of leaves. By regulating the aperture of stomata, plants adjust the loss of water from transpiration and the intake of CO_2 for photosynthesis when responding to various factors (Hetherington & Woodland, 2003) such as water availability (Buckley, 2019; Hernandez-Santana *et al.*, 2016; Tombesi *et al.*, 2015), temperature (Caemmerer & Evans, 2015; Urban *et al.*, 2017), light (Inoue & Kinoshita, 2017; Shimazaki *et al.*, 2007), CO_2 concentration (Engineer *et al.*, 2016; Israelsson *et al.*, 2006), biotic stress (Melotto *et al.*, 2006) and volatile compounds in the atmosphere (Jiang *et al.*, 2020; Niinemets & Reichstein, 2003). Hence, studying stomatal movement has been heavily scrutinised in order to understand how plants respond to their environment.

Stomatal variations can be monitored with various methods such as using stirred or unstirred chambers attached to the leaf (e.g. gas-exchange portable photosynthetic system (Ceciliato *et al.*, 2019; Jiang *et al.*, 2020; Rasulov *et al.*, 2019) and porometer (Toro *et al.*, 2019)), or with leaf isolated epidermal peels (Mott *et al.*, 2014). These methods have been extensively used to improve the understanding of how stomata are regulated but these require direct contact with the leaf and isolation of the atmosphere over the measurement area of the leaf making it difficult to study the impacts of volatile molecules exchanged between plants.

In the previous chapters, it was hypothesised that plants use volatiles to communicate during drought stress, by emitting compounds affecting stomata and to potentially induce their closure. However, most experiments were conducted in large spaces and on potted whole plants, making it difficult to control the flow of volatiles and target the stomatal responses. This study was conducted in order to experiment directly at the leaf level without enclosing it in a small environment and to examine the stomatal responses from the water flux travelling through the petiole. This alternative method allowed online monitoring of the transpiration rate of a detached leaf by measuring the continuous rate of water flow entering the leaf using sensitive liquid flow meters.

The method was applied to both *Vitis vinifera* and *Arabidopsis thaliana* to validate reproducible results of continuous and homogeneous water flow rates (Q) from single leaves over time. Different artificial sap solutions feeding the leaf were trialled to optimise Q measurements. Then, comparisons with another gas-exchange method (photosynthetic infra-red gas analyser (IRGA) system) were conducted simultaneously to assess the water transport entering and leaving the leaf, that is, the flow meter measured the flow rate into the leaf via the petiole, while the gas exchange system measured the water exiting the leaf as water vapour through stomata and the cuticle. Also, light to dark transitions were examined as a reproducible way to test the responsiveness of stomata and to compare with literature data (Elhaddad *et al.*, 2014; Jardine *et al.*,

2012). Responses to increased air movement around the leaf to decrease the leaf boundary layer resistance was also examined.

Second, possible plant volatile organic compounds that may act as signals for plants were tested for their effect on transpiration. For instance, López-Gresa *et al.* (2018) showed that four hexenyl esters ((*Z*)-3-hexenyl acetate, (*Z*)-3-hexenyl propionate, (*Z*)-3-hexenyl butyrate, and (*Z*)-3-hexenyl isobutyrate) were responsible for the closure of stomata in response to a pathogen attack. Other common plant-emitted compounds were tested such as ethanol (Jud *et al.*, 2016) and methanol which had large effects (Folkers *et al.*, 2008). In addition, the technique was improved by monitoring other factors (e.g. air flow surrounding the leaf) and adding gas sensors surrounding the leaf to monitor the flow of volatiles during treatments and/or the ones emitted by the leaf.

5.3. Material and methods

5.3.1. Plant and environmental conditions

Arabidopsis thaliana Col0 were potted and grown in a small growth cabinet with artificial light (PAR 100-150 $\mu mol.m^{-2}.s^{-1}$) for 5 weeks with short-day environmental conditions (10 h light, 21°C / 14 h dark, 17°C; humidity 60 %) (as described in section 2.1.1, Chapter 2).

Vitis vinifera L. cv. Shiraz (Syrah) vines were potted and grown in glasshouse under natural light until reaching 1-2 shoots with approximately 10 leaves per shoot. The environmental conditions were temperature 25°C day and 17°C night, humidity 40 % (as described in section 2.1.2, Chapter 2).

5.3.2. Flow meter parameters

Flow rate (*Q*, $mmol.m^{-2}.s^{-1}$) into single leaves was monitored using a modified XYL'EM embolism meter (Instrutec, France) with high precision liquid flow meters (LIQUI-FLOW, Bronkhorst High-Tech B.V., Netherlands) (Cochard, 2002; Cochard *et al.*, 2000; Cochard *et al.*, 2002), at different sensitivities depending on the species being measured (*V. vinifera*, 5 $g.h^{-1}$; Arabidopsis, 0.5 $g.h^{-1}$ maximum flow rate) (Figure 69). The instrument was filled with a solution of purified water (Milli-Q Plus; Merck Millipore, Billerica, MA, USA) and 10 mM KCl, which was degassed (1.0 x 5.5 Mini Module ™; Membrana GmbH, Germany) to avoid blockages and cavitation in the leaf xylem. A low-pressure tank was used to apply small pressure gradients to the flow and was connected to the leaf petiole with silicone tubing (Mastreflex L/S, Precision Pump Tubing, C-FLEX, L/S, Cole-Parmer, USA) which had the last 20 cm section filled with an artificial sap composed of

purified degassed water with MES (2-(N-Morpholino)-ethanesulfonic acid; 1 mM, pH 5.5), potassium nitrate (KNO_3; 10 mM), and filtered with a 0.2 µm syringe filtration unit (Filtropur S 0.2, Sarstedt, Germany). Through the dedicated software 'XYL_WIN', flow rate ($g.h^{-1}$), temperature and pressure were recorded at selected time intervals.

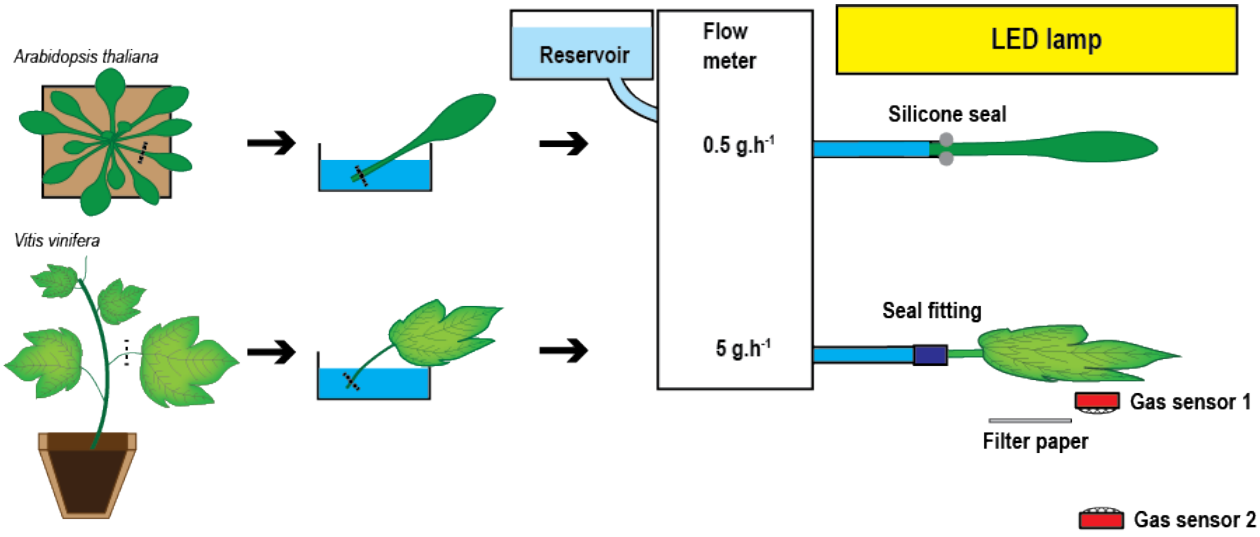


Figure 69. Depiction of the method used to perform whole-leaf real-time-resolved analyses of stomatal responses to volatile compounds in *Arabidopsis thaliana* and *Vitis vinifera*. Leaves were severed in an artificial sap (MES, 1 mM; KNO_3, 10 mM) and connected to a flow meter for continuous measurements of water flow (*Q*, $mmol.m^{-2}.s^{-1}$) under a LED lamp (PAR 150 $\mu mol.m^{-2}.s^{-1}$). Volatile compounds (e.g. ethanol, methanol, hexenyl esters) were added underneath the leaf on a filter paper and monitored with gas sensors to follow their effect on stomatal responses.

5.3.3. *Vitis vinifera* measurements

Fully expanded mature leaves were randomly selected from *V. vinifera* with similar growth stages (between nodes 3-6). They were severed from the shoots by cutting with a pair of sharp scissors (about 2-3 mm distance to the stem junction) and instantly immersed in a petri dish filled with the artificial sap solution (MES, 1 mM; KNO_3, 10 mM). The petiole was recut a second time with a razor blade to avoid risk of embolism. Within a minute, the petiole was then tightly sealed with plastic fittings to the tubing of the flow meter. Light was provided by a dedicated photosynthetic LED light (Mars Reflector 48, Mars Hydro, USA) approximately 30-40 cm over the leaf providing 150 $\mu mol.m^{-2}.s^{-1}$ PAR at leaf level. Experiments were carried out around midday for maximal transpiration and photosynthesis, and flow rates were recorded at 5-second intervals. Once the

measurements were complete, the projected leaf area was calculated from scans with ImageJ to determine the water flow rates (Q) in $mmol.m^{-2}.s^{-1}$ (as described in section 2.2.5, Chapter 2).

5.3.4. *Arabidopsis thaliana* measurements

The same protocol was utilised for *A. thaliana* leaves, but with slight modifications. The petiole was directly cut with a razor blade, and quickly immersed in a petri dish with artificial sap. The second cut was performed following the recommendations described in Ceciliato *et al.* (2019), i.e. by gently moving the razor blade back and forth and not pressing the blade against the petiole, potentially damaging the xylem conduits. The tubing was sealed with silicone paste (Xantopren L blue, Heraeus, Germany) since the petiole was not circular in cross section and could not sustain a pressure seal.

5.3.5. Dark transitions

Leaves of plants were exposed to saturating light (PAR 150 $\mu mol.m^{-2}.s^{-1}$), then exposed to darkness by switching off the LED lamp.

5.3.6. Simultaneous measurements with a gas exchange instrument

After monitoring a steady flow rate from a leaf connected to the flow meter, a LCpro-SD portable photosynthesis system (ADC BioScientific Ltd., UK) was added to enclose a portion of the leaf for *V. vinifera* and the whole leaf for *A. thaliana* (sealed at the petiole), supplied with ambient CO_2 concentration, temperature and humidity, air flow at 300 $mL.min^{-1}$ to measure transpiration rates (E). The LCpro-SD head was used with a clear top so the light was provided by the LED lamp over the leaf. Data acquisition was programmed with automatically timed-logging with 17-s intervals.

5.3.7. Volatile treatment application

A range of volatiles was tested to examine their impact on water flow rates whilst the leaf was connected to the flow meter. A piece of cellulose filter paper (Whatman plc, UK) was placed approximately 2 cm underneath the leaf and then, the volatile molecules were pipetted with different concentrations and volumes (Table 10). The concentrations of the esters were set according to López-Gresa *et al.* (2018).

Table 10. Volatile compounds selected, concentrations and volumes added on the filter papers.

Volatile compounds	Concentrations	Volumes added on the filter papers
ethanol (EtOH)	100 %	1 mL
methanol (MeOH)	100 %	200 µL, 100 µL, 50 µL
(*Z*)-3-hexenyl butyrate	5 µM in ethanol (100 %)	200 µL
(*Z*)-3-hexenyl propionate	5 µM in ethanol (100 %)	200 µL

(Z)-3-hexenyl isobutyrate	5 µM in ethanol (100 %)	200 µL
(Z)-3-hexenyl acetate	5 µM in ethanol (100 %)	200 µL

5.3.8. Sensors parameters

A temperature/humidity sensor (DHT22, Aosong (Guangzhou) ElectronicsCo.,Ltd, China) and two gas sensors (Grove-gas sensor MQ3, Seeed studio) were added to the system (Ionescu & Vancu, 1996). One gas sensor was placed directly underneath the leaf while another was placed 15 cm below to distinguish between the origins of the volatiles (e.g. emitted by the leaf or coming from the surrounding environment) (Figure 69). The MQ3 is composed of micro aluminium oxide (Al_20_3) ceramic tube, tin dioxide (SnO_2) sensitive layer, measuring electrode and heater, fixed into a small chamber and is highly sensitive to organic solvent vapours such as ethanol. The module was connected to an analog digital converter (10 bit) pin of an Arduino UNO microcontroller to record measurements (Supplementary Program 1). The DHT22 was connected to digital input pins and recorded simultaneously with the gas sensors (Supplementary Program 2).

5.3.9. Fan application

A cooling fan (Panaflo, model FBA08A24H, air volume 39.6 CFM, Panasonic, Japan) was added to the system to test the boundary layer resistance, potentially changing the water flow rate. The fan was connected to a 12 V battery (NP7-12, Yuasa Corporation, China) (Figure 70).

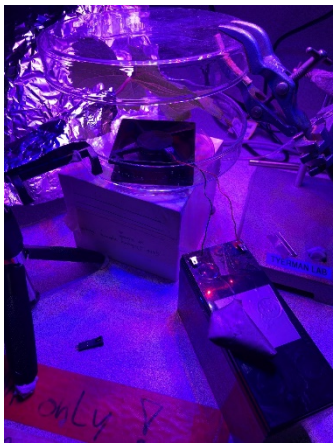


Figure 70. Installation of a fan under a *Vitis vinifera* leaf connected to a flow meter for water flow measurements.

5.3.10. Data analysis

The decrease of water flow and transpiration rates were analysed by fitting exponential one phase decay curves to determine the half life of the variations. Goodness of fit was determined by the regression coefficient (R^2) and the first derivative was calculated to obtain the maximum rate of change.

5.4. Results

5.4.1. Water flow into single leaves measured with the flow meter

After connecting a leaf to the flow meter, it took approximately 30-60 min for *V. vinifera* leaves and 20-30 min for Arabidopsis leaves to reach a plateau and stabilise with the flow rates averaging 1.94 ± 0.6 mmol.m^{-2}.s^{-1} (mean ± SD, n=47) for *V. vinifera* (Figure 71) and 1.05 ± 0.29 mmol.m^{-2}.s^{-1} (mean ± SD, n=9) for Arabidopsis leaves. For some leaves, measurements were stable over time (Figure 71b), while for others, the first two hours were stable after which flow slightly decreased for the next 3-4 hours (Figure 71a).

Two artificial saps were tested in the flow meter system (i.e. a solution of KCl and a solution of MES/KNO_3). High and similar flow rates were obtained with both sap solutions (Figure 71) and the MES/KNO_3 artificial sap solution was kept for the rest of the experiments.

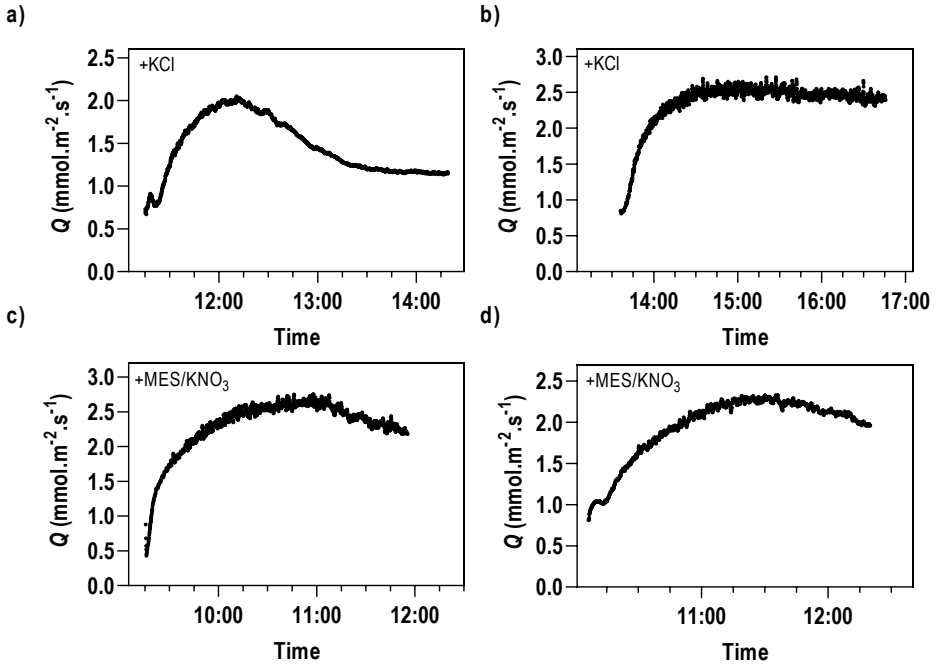


Figure 71. Flow rate (Q) measured into *V. vinifera* (cv. Shiraz) leaves connected to a liquid flow meter. a) and b) leaves had an artificial sap composed of KCl (10 mM), and c) and d) an artificial sap of MES (1 mM, pH 5.5) and KNO_3 (10 mM). Data shown is representative of one leaf result, and a), b), c) and d) leaf samples were taken from different plants of similar node position and age.

To test the durability of the system, one leaf stayed connected to the flow meter from 14:00 until midnight with the LED lamp on (Figure 72). The flow rate reached a peak after 30 min of 1.2 mmol.m^{-2}.s^{-1} and slightly

decreased after 2h and stayed steady until the end. Oscillations can be observed in this example and in other replications.

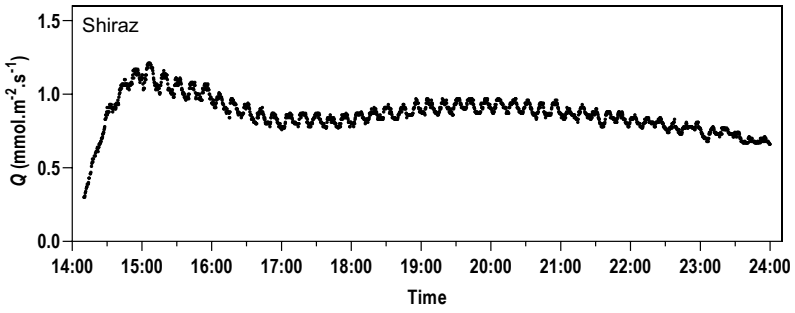


Figure 72. Flow rate (Q) measured into a *V. vinifera* (cv. Shiraz) leaf connected to a flow meter. Continuous measurements were taken from 14:00 to 24:00 with a LED lamp turned on and delivering a 150 µmol.m^{-2}.s^{-1} PAR.

5.4.2. Effects of dark-light transitions on flow rates

To examine the reactivity of the leaf, dark-light transitions were conducted by turning the LED lamp positioned above the leaf off and on. For Arabidopsis (Figure 73), the decrease of flow was almost instant and reached approximately 0 with a half life of 1020 ± 777 s with a maximum rate of change of -0.00219 ± 0.0006 mmol.m^{-2}.s^{-2} (n=3). For *V. vinifera*, the half life was 309 ± 56 s with a maximum rate of change of -0.00132 ± 0.0004 mmol.m^{-2}.s^{-2} (n=3). When the LED lamp was turned back on the flow rate increased to reach a similar level. This dark transition test was used throughout the remaining experiments to assess the responsiveness of the stomata while being connected to the flow meter.

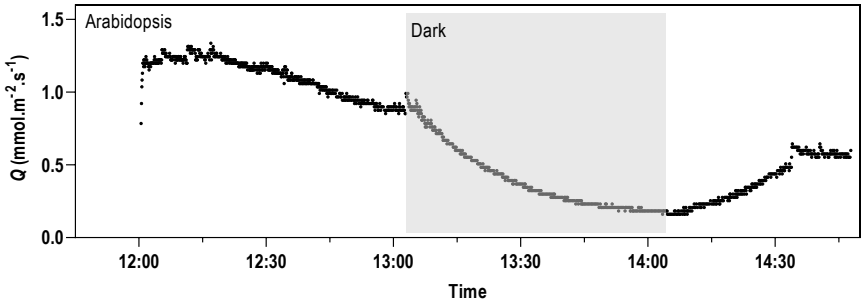


Figure 73. Flow into an *Arabidopsis thaliana* leaf using the liquid flow meter. Dark transition (grey box) was achieved by turning off the LED lamp over the leaf. Data shown is representative of a single leaf replicate out of repetitions with similar results (n=2).

5.4.3. Comparison of flow rates compared with transpiration rates utilising a gas-exchange system during light to dark transitions

As the flow meter is measuring the water entering the leaf via the xylem, the water that is transpired through stomata was measured by connecting a gas-exchange photosynthetic system (LCpro-SD) for comparison. For both species, the instrument was connected to the leaf for approximately one hour after a steady rate of *Q* was measured with the flow meter.

For Arabidopsis, as soon as the LCpro-SD head was clamped on the leaf, *Q* measured with the flow meter was disrupted and displayed fast oscillations around 0 $mmol.m^{-2}.s^{-1}$, but returned to initial values once the head was removed (Figure 74a). The LCpro-SD recorded similar transpiration rates as the flow meter measured prior to the attachment of the head, starting at 0.5 $mmol.m^{-2}.s^{-1}$. Then, the dark transition induced a decrease of transpiration measured with the LCpro-SD and an increase was observed once the light was turned on again.

For *V. vinifera*, clamping the LCpro-SD head on the leaf disrupted *Q* measured with the flow meter but not as much as for Arabidopsis (Figure 74b). *E* measured with the LCpro-SD reached a twofold higher rate than *Q* after 1 h. The dark transition induced a decrease of *E* and *Q*, but with different rates (-0.0016 flow rate per second with half life of 909 s, and -0.0027 flow rate per second and half life 428 s, respectively). For both species, a drop of *E* from the LCpro-SD can be observed in the first 30 min after positioning the head on the leaf and this trend was also observed in other repetitions (n=3).

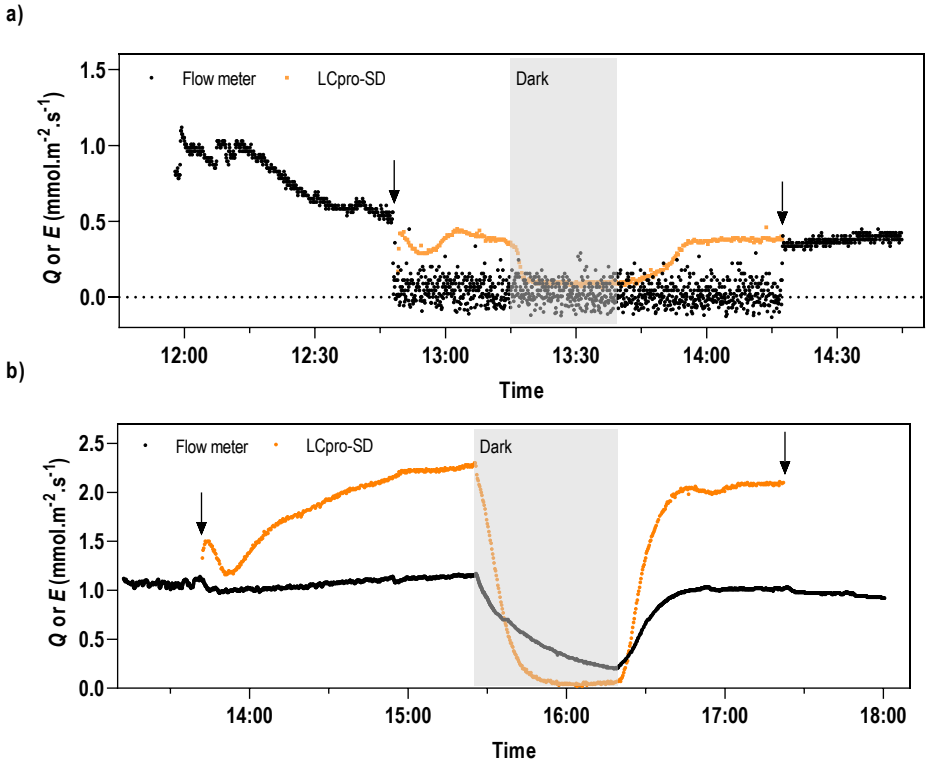


Figure 74. Measurements of flow rate (*Q*) and transpiration rate (*E*) (mmol.m^{-2}.s^{-1}) for a) *Arabidopsis thaliana* Col0 and b) *V. vinifera* (cv Shiraz) leaves with a liquid flow meter connected to the petiole (black dots) and with a gas exchange photosynthetic system (LCpro-SD, orange dots) connected to the leaf. The arrows indicate when the gas analyser was attached and removed from the leaf. Dark transitions (grey box) were achieved by turning off the LED lamp over the leaf. For both species, data shown are from one representative experiment out of repetitions with similar results (n=3).

5.4.4. Effect of stirred air on water flow rates using a fan

In order to optimise the experimental set-up, a fan was installed under the leaf to test if the potential increase of the air movement around the leaf decreases the leaf boundary layer resistance. The fan was turned on after obtaining a steady flow rate. This caused a rapid increase in flow followed by a slower decay back toward the initial rate (Figure 75). This response was observed several times with variable decay rates (n=6), and it was decided that all remaining experiments would be conducted without the fan in order to record diffusive responses with the gas sensors.

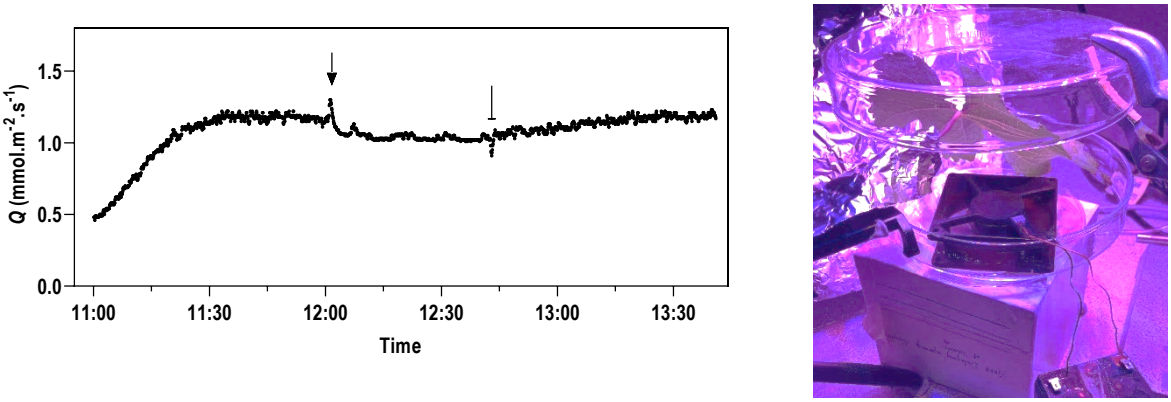


Figure 75. Flow rate (*Q*) measured for a *V. vinifera* (cv. Shiraz) leaf connected to a flow meter. A fan was turned on (↓) and off (⊥) under the leaf. Data shown are from one representative experiment out of repetitions with similar results (n=6).

5.4.5. Effect of volatile compounds on water flow rates

5.4.5.1. Ethanol and hexenyl esters

Ethanol was used as the solvent to dilute the hexenyl esters, thus it was examined for *V. vinifera* leaves connected to the flow meter to determine whether the flow rates are altered (Figure 76). A series of experiments showed inconsistent results where sometimes a small decrease of *Q* was induced and sometimes no effect (i.e. 2 out of 18 replications showed a response).

The four hexenyl esters in ethanol ((*Z*)-3-hexenyl butyrate, (*Z*)-3-hexenyl isobutyrate, (*Z*)-3-hexenyl propionate, (*Z*)-hexenyl acetate) were also tested in duplicate at the same concentration as detailed in López-Gresa *et al.* (2018) but they did not induce a reduction of *Q* of the leaves (Figure 76).

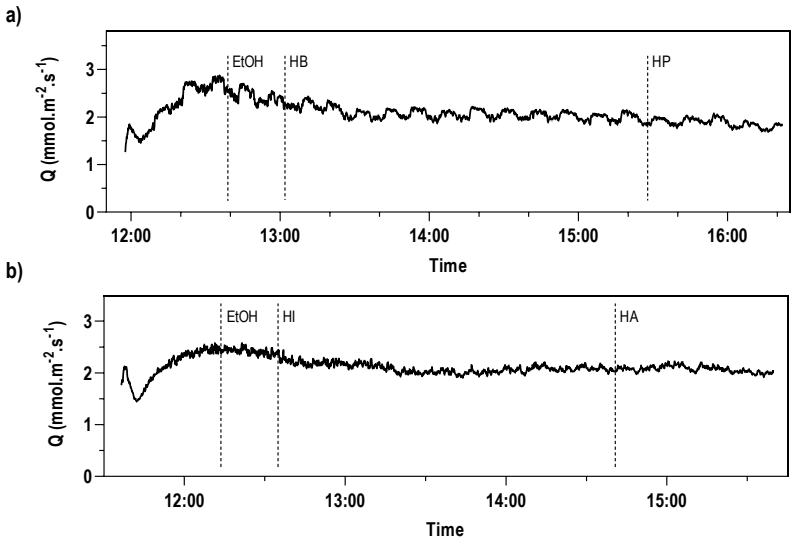


Figure 76. Measurements of flow rate (*Q*, mmol.m^{-2}.s^{-1}) of *V. vinifera* (cv. Shiraz) leaves connected to the flow meter. a) Ethanol (EtOH, 100 % purity), hexenyl butyrate (HB, 5 µM (in ethanol)) and hexenyl propionate (HP, 5 µM (in ethanol)) were added on a filter paper placed underneath the leaf. b) Ethanol, hexenyl isobutyrate (HI, 5 µM (in ethanol)) and hexenyl acetate (HA, 5 µM (in ethanol)) were added on a filter paper underneath the leaf. Data shown are from one representative experiment out of repetitions with similar results (n=2).

5.4.5.2. Methanol

Methanol was checked for suitability as a solvent for compound dilution. Different volumes of pure methanol (200 µL, 100 µL and 50 µL) were added onto a filter paper which was placed approximately 2 cm under *V. vinifera* leaves that were connected to the flow meter. As a result, after a delay, the flow rate started to drastically decrease and then increase again until reaching a similar rate to the initial conditions prior to the addition (Figure 77). On average, the max rate of change in flow rate for the decrease was -0.003023 ± 0.0007 mmol.m^{-2}.s^{-2} (mean ± SD, n=4), which is twice as high than the maximum rate during the dark transition. This effect was also observed on leaves that displayed oscillating flow rates (Figure 77b). Lower volumes of methanol (100 µL and 50 µL) were applied under the same leaf and 50 µL exhibited a similar reduced span of flow rate compared to 100 µL (Figure 7b). The methanol treatments were monitored with gas sensors placed close to the leaf and the filter paper, and 15 cm under the leaf (gas sensor 1 and gas sensor 2 in Figure 69). In every trial, the gas sensor 1 sensed more methanol and before the gas sensor 2, and methanol stopped being detected after an average of 15 min application. As for the other experiments, a dark transition was conducted at the end of each trial to assess the reactiveness of the stomata.

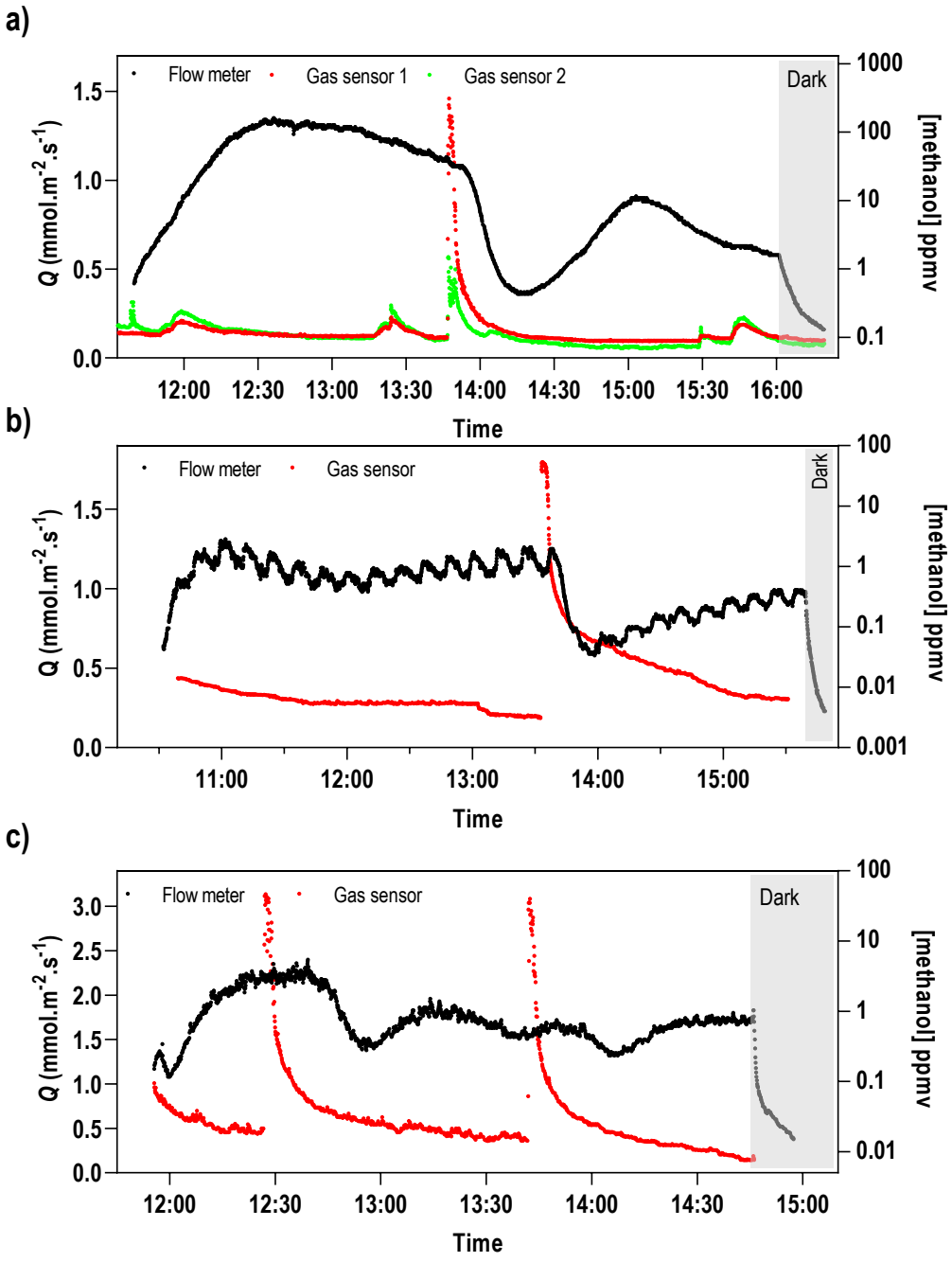


Figure 77. Measurements of flow rate (Q, mmol.m^{-2}.s^{-1}) of *V. vinifera* (cv. Shiraz) leaves connected to the flow meter (black line, left y-axis). Different doses of pure methanol were added on a filter paper with a) 100 µL, b) 200 µL and c) 100 µL and 50 µL in chronological order, under the leaf and were simultaneously monitored with gas sensors (right y-axis) placed directly under the leaf (red) and 20 cm under the leaf (green). Dark transitions (grey box) were achieved by turning off the LED lamp over the leaf. Data shown are from one representative experiment out of repetitions with similar results (n=14).

5.4.6. Leaf-emitted volatiles during stomatal oscillations and dark transition

Repeated large oscillations were observed on a *V. vinifera* leaf connected to the flow meter, after 1.5 hour in one experiment (Figure 78). These oscillations were longer and wider than those observed in Figure 72 or Figure 76. As the gas sensors were placed under the leaf, the gas sensor 1 detected volatiles during the upper period of the oscillations but the gas sensor 2 which is positioned 15 cm below the leaf did not. This likely indicates that the volatiles (presumably some sort of alcohol) were emitted from the leaf when stomata were open. Additionally, a dark transition was performed at the end of the experiment and as the flow rate decreased, the gas sensor 1 (close to the leaf) detected volatiles but the gas sensor 2 did not. This was observed multiple times (n=3).

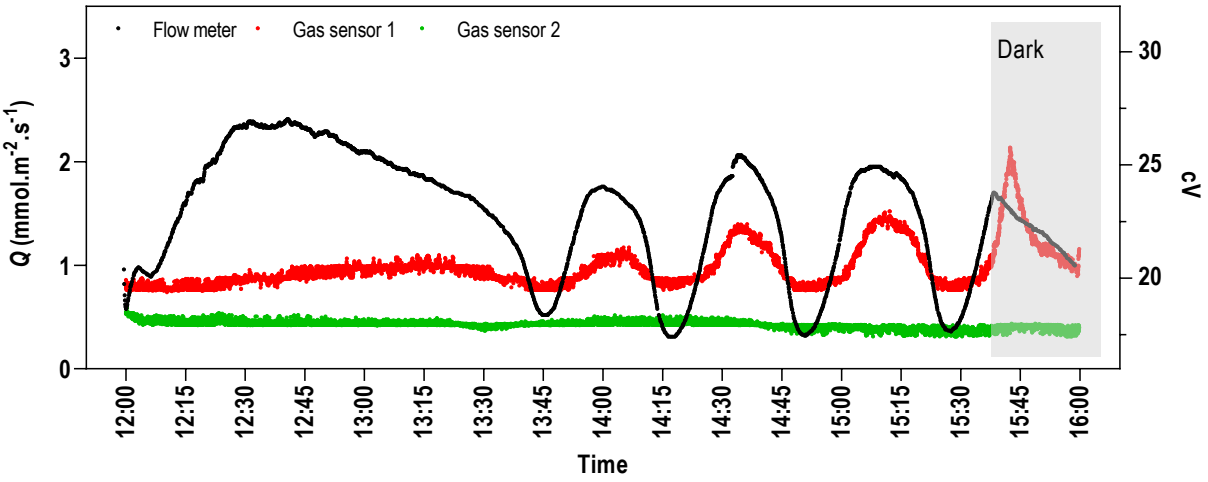


Figure 78. Flow rate (*Q*) measured for a *V. vinifera* (cv. Shiraz) leaf connected to a flow meter where large oscillations were recorded corresponding to oscillations in volatile emissions from the leaf (right y-axis). Gas sensors were placed directly under the leaf (red) and 20 cm under the leaf (green). Dark transitions (grey box) were achieved by turning off the LED lamp over the leaf. Data shown are from one replicate (n=1).

5.4.7. General results

Table 11 provides a summary of all the tests performed on *V. vinifera* and *Arabidopsis thaliana* leaves connected to the flow meter including the number of replicates and frequency of responses.

Table 11. Overview of the tests performed on *Vitis vinifera* and *Arabidopsis thaliana* leaves connected to the flow meter.

Species	Test	Result	Number of repetitions	Frequency of response
Vitis vinifera cv. Shiraz	Flow rate (Q, mmol.m^{-2}.s^{-1})	1.85 ± 0.57 (mean ± SD)	51	-
	Dark-light transition	Decrease and increase of flow	12	100%
	Fan	Rapid increase and return to initial levels	6	100%
	Oscillations	-	51	23%
	LCpro-SD (E, mmol.m^{-2}.$^{s-1}$)	2.98± 0.54 (mean ± SD)	9	-
	methanol	Decrease and increase of flow	14	80%
	ethanol	Change of flow	18	11%
	hexenyl butyrate	Change of flow	5	0%
	hexenyl propionate	Change of flow	1	0%
	hexenyl isobutyrate	Change of flow	1	0%
	hexenyl acetate	Change of flow	2	0%
Arabidopsis thaliana Col0	Flow rate (Q, mmol.m^{-2}.s^{-1})	1.055 ±.029 (mean ± SD)	9	100%
	LCpro-SD (E, mmol.m^{-2}.$^{s-1}$)	0.52 ± 0.17 (mean ± SD)	5	-
	Dark-light transition	Decrease and increase of flow	8	100%

5.5. Discussion

5.5.1. Leaf water flow monitored with a liquid flow meter

Quantifying stomatal responses is important to understand how plants respond to environmental stimuli. In this study, the liquid flow meter connected to single leaves provided a robust method for measuring the changes in flow rates and presumed stomatal movements to different factors such as light variation or air movement. Here, *Arabidopsis thaliana* and *Vitis vinifera* leaf petioles were connected to a liquid flow meter filled with artificial sap and as water was moved into the leaf, the flow rate was calculated in mmol.m^{-2}.s^{-1} and monitored over 5-s intervals. Continuous measurements were conducted with steady flow rates that were recorded for up to 10 hours for *V. vinifera* and 5 hours for Arabidopsis, confirming that the cut of the petiole in the solution posed little effect when following the protocol described in Ceciliato *et al.* (2019). Oscillations in Q were also observed and are relatively common when monitoring stomatal conductance (Yang *et al.*, 2005, Ballard *et al.*, 2019). Various tests were conducted to validate and improve this method. For instance, a fan was added to increase the velocity of air movement and potentially diminish the boundary layer resistance that would hypothetically increase the transpiration rate. However, the increased air current did

little to no effect since the leaf returned to its original flow rate after a couple minutes. Thus, all experiments proceeding this were conducted without the additional of a fan.

As stomata are strongly affected by light variation (Inoue & Kinoshita, 2017), dark transitions were conducted on the leaves and this resulted in a rapid decrease in flow rate, generally to zero flow, and instantly increasing once the light was turned on. This demonstrated that stomata were still strongly responsive to an environmental variation even when detached from the shoot, and this test became a control test for the remaining experiments.

5.5.2. Comparison with a leaf gas-exchange system

Transpiration rates are generally determined from the water vapour released from open stomata on a leaf. However, the flow method described here provides measurements of the water entering the leaf through the petiole xylem vessels. In order to evaluate these flows as a measure of transpiration, a gas exchange system was simultaneously attached to the leaf. The parallel measurements revealed that the two systems were successful in being able to measure the variations in stomatal movements caused by the light changes.

Flow into the leaf may not necessarily correspond to leaf transpiration if the leaf is re-hydrating, growing or dehydrating. That is, the water volume of the leaf may not be constant in time during these experiments and therefore the flow rate may not correspond exactly to the transpiration rate. This was evident in several experiments were both transpiration and flow were monitored on the same leaf. Rarely was there an exact match between flow and transpiration, as it was never the case for *V. vinifera* leaves with the LCpro-SD providing measurements twofold higher than the flow meter, but was more often for Arabidopsis leaves. Interestingly the transitions from light to dark also revealed differences between flow and transpiration (e.g. Figure 74) where transpiration reached zero well before flow into the leaf was zero. This would indicate a capacitance effect in the leaf and in this case a rehydration of the leaf when the stomata have closed. Other causes for disparity between flow into the leaf and transpiration rate measured over a smaller fraction of the leaf surface area under the IRGA cuvette is stomatal patchiness (Düring & Stoll, 1996). It is known that *V. vinifera* leaves display this phenomenon as a heterobaric leaf type (Düring, 1992) and it is likely that different regions of the leaf surface can have very different transpiration rates resulting in the disparity between IRGA measurements of transpiration over a small portion of leaf area compared to flow into the whole leaf. Another reason for differences can be the effect of the IRGA seal pressure on the leaf surface causing a disruption in vascular continuity in the leaf. This was very clearly shown for Arabidopsis where as soon as the LCpro-SD head was clamped on the leaf, readings from the flow meter were disrupted, probably because of the xylem

conduits were being squeezed by the cuvette seals. For *V. vinifera*, disruption of the flow meter measurements was also observed but were less severe.

5.5.3. Methanol-induced stomatal closure

Some volatiles are known to affect stomata, for examples, gaseous hydrogen sulphide (H_2S) was recently found to mediate stomatal movements (Du *et al.*, 2019) and some green leaf volatiles were shown to induce the closure of stomata for pathogen defence responses (López-Gresa *et al.*, 2018). These GLVs were tested here at the same concentrations by adding certain volumes on a filter paper close to the leaf on *V. vinifera* but showed no effect on flow rates. As solvents, ethanol and methanol were both tested to determine their suitability, and while ethanol did not provide consistent results, pure methanol induced a large and rapid decrease in transpiration with dose-dependency. Indeed, methanol (CH_3OH) is a highly water-soluble volatile that is known to be emitted by plants (Jacob *et al.*, 2005). MeOH emission is triggered during changes in cell wall structures, as seed maturation, fruit ripening, leaf expansion and biotic stress (Dorokhov *et al.*, 2018). A morning peak of methanol has be shown in normal conditions coinciding with an increase in stomatal conductance, followed by a slow and gradual decrease during the day (Hüve *et al.*, 2007). Moreover, methanol has been found to induce defence reactions in intact leaves from the same and neighbouring plants, and to activate resistance genes (Dorokhov *et al.*, 2012). Spraying leaves with methanol showed a stimulation of photosynthesis activity and productivity in C3 plants (Nonomura & Benson, 1992), and the regulation of genes involved in signalling, defence and metabolism in *Arabidopsis thaliana* (Downie *et al.*, 2004). A rise in temperature also induced an increase in MeOH emission by up to 12% per degree, and a dark to light transition increased the MeOH emission by twofold (Folkers *et al.*, 2008). Pathogen interactions induce the emission of MeOH, for example, during *Manduca sexta* caterpillar attack in *Nicotiana attenuata* (Von Dahl *et al.*, 2006), and during feeding of *Euphydryas aurinia* caterpillars on *Succisa pratensis* (Peñuelas *et al.*, 2005). This emission of methanol seems to be regulated by stomata and has been found to induce defence reactions in intact leaves from the same and neighbouring plants (Tran *et al.*, 2018) and to activate resistance genes (Dorokhov *et al.*, 2012). However, there is no evidence of direct application of methanol inducing a quick change in stomatal aperture.

5.5.4. Leaf-emitted volatiles

Gas sensors were added to the experimental system to monitor diffusion of volatiles to estimate when the leaf would detect the volatiles and when the volatiles would eventually disperse in the air. Nevertheless, the particular sensors used were also able to detect volatiles emitted by the leaf. They were detected during the

dark transition as observed in other studies (Graus *et al.*, 2004; Jud *et al.*, 2016) and during the oscillations of transpiration rates measured by the flow meter observed on one occasion (Figure 78). The inexpensive MQ3 gas sensor used here is not specific for ethanol and can also detect other alcohols including methanol, which was used for calibration. The gas sensors closest to the leaf detected volatiles during the peak of the oscillation that then disappeared during the lower part of the oscillations or became too low in concentrations for the threshold sensitivity of the sensors. Thus, it strongly suggests that the volatiles were emitted by the leaf since the second gas sensor under from the leaf did not detect any. Thus, this result shows a potential feedback and role of volatiles in stomatal regulation.

5.5.5. Conclusion and future directions

In conclusion, the method described here for monitoring flow into an intact leaf free of a cuvette attached to the surface was advantageous for monitoring the responses in transpiration to externally applied volatiles. These volatiles would normally not be used in conjunction with infra-red gas analysers (IRGAs) since they could damage the sensors and/or be absorbed by the plastics in the system. Here, it is possible to treat a single leaf with volatile compounds to follow their effect on transpiration rates, as well as measure the volatiles emitted by the leaf potentially released through stomata.

In addition, the method allowed to investigate how leaves are actually responding to being connected to IRGAs. Adding the leaf chamber to Arabidopsis leaves showed a drastic reduction and erratic flow rate measured with the flow meter. It seems that the transpiration through stomata measured by the IRGA was still possible and gave similar values, and the stomata were still responsive to a dark transition, but it would appear that the section of the leaf within the IRGA may have been partially compromised in connections to the xylem in the petiole. After the IRGA was detached from the leaf, the flow rate returned to the levels before attachment. This suggests that the leaf-seals of the IRGA might squeeze veins and xylem conduits blocking water flow in the leaf. Another interesting observation is the correlation between flow and transpiration when the IRGA is attached. To date, this is the first time this configuration has been done and further work could potentially indicate capacitive effects within the leaf to changes in environmental variables. For example, the response to increased air movement was interesting in this respect, and indicating a rapid control on transpiration due to changes in boundary layer resistance (Figure 75).

The disadvantage of the technique is that it involves the cutting of leaves from the plant, potentially inducing a wounding response on the plant and the leaf. There is also a chance of inducing embolisms during cutting the petiole and connecting to the flow meter. In addition, the artificial sap used in these experiments was likely

to be far removed from being similar to the composition of natural xylem sap. However, it would be possible to introduce a system to change the sap and monitor the changes in flow over time to test the impact of different xylem mobile molecules, as well as testing other chemicals (e.g. ABA) that are known to be found in the xylem (Coupel-Ledru *et al.*, 2017).

6. General discussion, limitations, and future directions

6.1. General discussion

6.1.1. Introduction

The role of emitted-volatiles in between-plant interactions was described as active for plant defence against pathogens (Bouwmeester *et al.*, 2019; Brilli *et al.*, 2019; Lazazzara *et al.*, 2018) and for protection against abiotic stress (Cofer *et al.*, 2018; Fini *et al.*, 2017). Stomata are central players in these interactions as they are considered as entry and exit gates of volatiles (Jiang *et al.*, 2020; Niinemets *et al.*, 2002; Rissanen *et al.*, 2018). In parallel, stomata are also key players in water regulations in case of drought stress (Osakabe *et al.*, 2014), thus it would not be surprising if stomata also play a role in a volatile signalling during drought stress. To support this hypothesis, multiple studies reported a drought-like stomatal response of well-watered plants when in the same environment as stressed plants (Dayer *et al.*, 2017; Scharwies, 2017). While the stomatal conductance (g_s) of plants suffering from a deficit in water availability decreased to reduce transpiration, the stomatal conductance of the well-watered plants in the same environment decreased as well, although not with the same amplitude. As the plants were singly potted and no root interaction was possible (Falik *et al.*, 2012), airborne volatile signalling became the most plausible explanation.

6.1.2. *Vitis vinifera* and *Arabidopsis thaliana* drought/rehydration experiments showed a significant effect of water-stressed stomatal conductance on well-watered stomatal conductance

At first, it was decided to reproduce the experiments available in the literature multiple times and to add a gas sampling method to the protocol to analyse the volatile emitted in this specific configuration. Indeed, there is already numerous studies about the effect of drought stress on plant volatiles but those experiments were separating the treatment groups (Campbell *et al.*, 2018; Jud *et al.*, 2016; Scott *et al.*, 2019) and not looking at potential inter-plant signalling. In Chapter 3, for *V. vinifera*, the observations of the g_s graphs showed singular decreases for the well-watered (WW) plants in the first two experiments in sections 3.2 and 3.3, and a constant decrease in the third experiment in section 3.4. Even if variations in light intensity over the course of the experiments were likely to impact the results (Dayer *et al.*, 2017), the multilinear regressions showed a stronger significant effect of the water-stressed (WS) g_s on WW g_s than PAR. For the third experiment, the Pettitt homogeneity test also detected a shift in the control (C) data as a decrease in the mean values.

In Chapter 4, for *A. thaliana*, where PAR was fixed and a third C treatment in a separate growth cabinet was possible, the first two experiments in section 4.2 and 4.3 showed a difference between the g_s of the C plants and the g_s of the WW co-located with the stressed plants, but this did not occur in the third experiment in section 4.4. Overall, there is accumulating evidence from the literature and from this study that a type of inter-plant interaction exists between water-stressed plants signalling to the well-watered plants, and as a result, a change in the physiology of the well-watered plants. The pathway is still unclear but in Scharwies (2017), an overexpression line of AtTIP2;1 showed less variation in g_s compared to the wild-type control plants, leading to a possible role of aquaporins in such mechanism (Ding & Chaumont, 2020).

6.1.3. *Vitis vinifera* and *Arabidopsis thaliana* volatile response to drought stress reveals candidates for inter-plant signalling

Vitis vinifera and *Arabidopsis thaliana* are not the main species of interest in global volatile research. For example, Vivaldo *et al.* (2017) conducted a study on 109 species and revealed *V. vinifera* as a species that 'don't share any VOC with the other species' or 'do not emit VOCs', and Arabidopsis was not even considered. In other studies, even if grape bunches and wine volatiles are usually of more agronomical and industrial value (Gil *et al.*, 2013; Kalua & Boss, 2010; Savoi *et al.*, 2016; Wang *et al.*, 2019), vine leaf emission was observed in non-stressed conditions (Giacomuzzi *et al.*, 2017), in biotic stress (Algarra Alarcon *et al.*, 2015; Chalal *et al.*, 2015; Ricciardi *et al.*, 2021), heat (Bertamini *et al.*, 2021) and drought (Griesser *et al.*, 2015) revealing that 46 out of 95 volatiles were affected by the drought stress.

In Chapter 3 on *V. vinifera*, the volatiles most affected by drought stress when all plants were co-located were α-pinene, β-pinene, limonene, styrene, 1,2,3-trimethylbenzene and 1-methyl-2-(1-methylethyl)-benzene, and from the chambers experiment, ocimene, allo-ocimene and linalool were strongly negatively correlated with WS g_s. Similarly, in Chapter 4, the experiments on Arabidopsis revealed β-pinene, hexanal, m-xylene and p-xylene to be affected during the drought stress phase. Although not all significant, those volatiles should be tested in the potential induction of closure of stomata, as well as understanding the underlying mechanisms. It is known that small volatiles can be transported through stomata or diffuse freely through membranes, based on their size, permeability, volatility and depending on environmental factors (Niinemets *et al.*, 2004). Bigger compounds are likely to move through other pathways and potentially protein channels (Abedesin *et al.*, 2017).

The implications of this inter-plant signalling is also important to consider on a large scale and in intensive protected horticulture in glasshouse, where the proximity and enclosed space could favour volatile

accumulation and exchange. In addition, for example, 1,2,3-trimethylbenzene which was identified in this study, has been found to be synthesised in plants (Ogunwande *et al.*, 2008) but also to be emitted by motor vehicle exhaust (Luo *et al.*, 2019), thus this particular volatile could have implications in highly polluted area for plant productions. Lastly, this project also proved that experimental protocols should be elaborated taking into account whether to separate the treatment from the control groups from any studied stresses (biotic or abiotic), as it has been previously evidenced in a salinity stress study showing an effect of the stressed plants on the non-stressed plants via airborne signals (Caparotta *et al.*, 2018).

6.1.4. Single leaf experiment to study the effect of volatiles on water flow

With the inconsistency of the results of the whole plant experiments in large greenhouses and growth cabinets and the environmental conditions (light, VPD), a new method was considered to study leaf water flow responses to volatile application. This method was designed to work on both studied species (*V. vinifera* and Arabidopsis) on single detached-leaves and the results showed a good reproducibility of water flow measurements. Thus, a comparison with other methods was conducted and selected volatiles were applied on leaves. Methanol seemed to induce the strongest effect on stomatal closure and thus could be a candidate for the drought-like response on well-watered plants (Figure 77, section 5.4.5.2, Chapter 5). Unfortunately, this compound was not used as an internal standard for calibration and was not detected with the SPME-GC-MS method used in the previous experiments described in Chapter 3 and 4 on whole plants.

6.2. Limitations

Several limitations can be considered in this research. One of them is that plants are highly susceptible to leaf damage and are known to emit volatiles in response (Li *et al.*, 2018; Portillo-Estrada & Niinemets, 2018). Thus, it can be hypothesised that the instruments used in the drought/rehydration experiments could have triggered a volatile emission, contaminating the drought-stress induced volatiles. Indeed, it was observed that the seals of the porometer and IRGA were leaving a mark on the leaves after measurements. The pressure bomb and the flow meter required a leaf to be cut from the plant to be either inserted in the pressure chamber or connected to the tubing. These manipulations could potentially break the trichomes at the surface of the leaf (Tissier *et al.*, 2017) and cause a biotic-like stress to the plants.

Another limitation is in regard of not being able to differentiate the volatiles coming from the well-watered plants from the water-stressed plants in the drought/rehydration experiments since all plants were present in the same environment. This is why a platform with individual chambers was created to circumvent this issue by comparing the profile of volatiles obtained and help to select candidates. At the same time, this system

enabled the measurement of whole plant carbon assimilation. However, the volatile results showed different volatile blends among experiments that could be explained by the use of different grapevine cultivars (Rid *et al.*, 2019) and also because of the plastic components. Moreover, the chambers being relatively small enclosures might have caused a stress to the plants (Brilli *et al.*, 2007) and removal of the plants during the experiment could have increased the risk of cross-contamination between the treatments. There are, in the literature, more suited set-ups with inert components, complex regulators for air flow and sampling methods (Lüpke *et al.*, 2017).

6.3. Future directions

To date, the hypothesis of plant exchange of volatiles leading to the closure of stomata has not been mentioned in the literature. With the evidence of the decrease of stomatal conductance from well-watered plants and the interesting results of this study, much more can be done to investigate this phenomenon. The volatile candidates highlighted in the study should be tested for triggering stomatal closure, after confirming their identity by repeating these experiments. Additionally, their effects on whole plants could be trialled and field tests in the vineyard performed to study potential beneficial effects during heat waves with less drought-linked damage on vines.

7. Appendices

7.1. Supplementary information for Chapter 3

Supplementary table S1a. Two-way repeated-measures analysis of variance (ANOVA) of stomatal conductance (g_s) from control (C) well-watered vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.2.2.1, Chapter 3.

Table Analysed	g_s (porometer)				
Two-way repeated-measures ANOVA	Matching: Stacked				
Assume sphericity?	No				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	Geisser-Greenhouse's epsilon
Time x Treatment	10.01	<0.0001	****	Yes	
Time	24.82	<0.0001	****	Yes	0.3898
Treatment	22.07	<0.0001	****	Yes	
Subject	15.06	<0.0001	****	Yes	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Time x Factor	319657	15	21310	F (15, 270) = 6.430	P<0.0001
Time	792430	15	52829	F (5.847, 105.2) = 15.94	P<0.0001
Treatment	704677	1	704677	F (1, 18) = 26.37	P<0.0001
Subject	480948	18	26719	F (18, 270) = 8.061	P<0.0001
Residual	894908	270	3314		
Difference between treatment means					
Mean of C	211.2				
Mean of WS	117.3				
Difference between means	93.85				
SE of difference	18.28				
95% CI of difference	55.46 to 132.2				
Data summary					
Number of columns (Treatment)	2				
Number of rows (Time)	16				
Number of subjects (Subject)	20				
Number of missing values	0				

Supplementary table S1b. Multiple comparison with Bonferroni tests of stomatal conductance (g_s) from control well-watered (C) vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.2.2.1, Chapter 3.

Compare each cell mean with the other cell mean in that row								
Number of families	1							

Number of comparisons per family	16							
Alpha	0.05							
Bonferroni's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value			
C - WS								
Day 1	5.616	-118.3 to 129.5	No	ns	>0.9999			
Day 2	58.89	-47.36 to 165.1	No	ns	>0.9999			
Day 3	4.984	-123.5 to 133.5	No	ns	>0.9999			
Day 4	54.34	-62.87 to 171.5	No	ns	>0.9999			
Day 5	147.4	72.00 to 222.9	Yes	****	<0.0001			
Day 6	113.4	36.62 to 190.2	Yes	**	0.0026			
Day 7	196.1	64.34 to 327.9	Yes	**	0.0022			
Day 8	201.6	108.4 to 294.9	Yes	****	<0.0001			
Day 9	115.2	51.23 to 179.1	Yes	***	0.0003			
Day 10	152.7	46.44 to 258.9	Yes	**	0.0028			
Day 11	149.7	77.67 to 221.8	Yes	***	0.0001			
Day 12	114.6	34.98 to 194.2	Yes	**	0.0030			
Day 13	97.02	21.63 to 172.4	Yes	**	0.0067			
Day 14	13.38	-179.8 to 206.5	No	ns	>0.9999			
Day 15	41.40	-88.60 to 171.4	No	ns	>0.9999			
Day 16	35.27	-78.10 to 148.6	No	ns	>0.9999			
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
C - WS								
Day 1	156.0	150.4	5.616	36.24	10	10	0.1550	17.64
Day 2	247.5	188.6	58.89	31.05	10	10	1.896	17.59
Day 3	212.1	207.1	4.984	36.71	10	10	0.1358	15.32
Day 4	164.2	109.9	54.34	33.75	10	10	1.610	16.05
Day 5	196.7	49.26	147.4	21.68	10	10	6.801	15.86
Day 6	160.8	47.35	113.4	20.59	10	10	5.508	11.42
Day 7	308.1	112.0	196.1	35.86	10	10	5.469	12.13
Day 8	240.4	38.80	201.6	25.47	10	10	7.917	12.32
Day 9	147.5	32.31	115.2	17.98	10	10	6.405	14.12
Day 10	209.1	56.43	152.7	29.31	10	10	5.210	12.88

Day 11	192.4	42.67	149.7	19.27	10	10	7.768	11.32
Day 12	194.0	79.43	114.6	21.60	10	10	5.305	11.99
Day 13	204.5	107.4	97.02	21.25	10	10	4.566	14.29
Day 14	228.1	214.7	13.38	51.67	10	10	0.2590	11.31
Day 15	231.5	190.1	41.40	38.13	10	10	1.086	17.99
Day 16	285.9	250.6	35.27	33.16	10	10	1.063	17.69

Supplementary table S2a. Two-way repeated-measures analysis of variance (ANOVA) of photosynthetically active radiation (PAR) from control (C) well-watered vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.2.2.1, Chapter 3.

Table Analysed	PAR (porometer)				
Two-way repeated-measures ANOVA	Matching: Stacked				
Assume sphericity?	No				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	Geisser-Greenhouse's epsilon
Time x Treatment	1.061	0.7759	ns	No	
Time	52.93	<0.0001	****	Yes	0.3355
Treatment	0.003811	0.9528	ns	No	
Subject	19.03	<0.0001	****	Yes	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Time x Treatment	232151	15	15477	F (15, 270) = 0.7081	P=0.7759
Time	11579004	15	771934	F (5.033, 90.59) = 35.32	P<0.0001
Treatment	833.8	1	833.8	F (1, 18) = 0.003604	P=0.9528
Subject	4164352	18	231353	F (18, 270) = 10.58	P<0.0001
Residual	5901716	270	21858		
Difference between column means					
Mean of C	671.0				
Mean of WS	674.2				
Difference between means	-3.228				
SE of difference	53.78				
95% CI of difference	-116.2 to 109.8				
Data summary					
Number of columns (Treatment)	2				
Number of rows (Time)	16				
Number of subjects (Subject)	20				
Number of missing values	0				

Supplementary table S2b. Multiple comparison with Bonferroni tests of photosynthetically active radiation (PAR) from control well-watered (C) vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.2.2.1, Chapter 3.

Compare each cell mean with the other cell mean in that row								
Number of families	1							
Number of comparisons per family	16							
Alpha	0.05							
Bonferroni's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value			
C - WS								
Day 1	28.31	-139.5 to 196.2	No	ns	>0.9999			
Day 2	12.40	-109.8 to 134.6	No	ns	>0.9999			
Day 3	44.04	-151.6 to 239.7	No	ns	>0.9999			
Day 4	-51.31	-327.2 to 224.6	No	ns	>0.9999			
Day 5	3.033	-423.6 to 429.7	No	ns	>0.9999			
Day 6	17.27	-136.6 to 171.1	No	ns	>0.9999			
Day 7	-45.47	-421.9 to 331.0	No	ns	>0.9999			
Day 8	33.22	-305.2 to 371.7	No	ns	>0.9999			
Day 9	-18.27	-250.5 to 214.0	No	ns	>0.9999			
Day 10	-22.72	-240.0 to 194.6	No	ns	>0.9999			
Day 11	55.22	-288.3 to 398.7	No	ns	>0.9999			
Day 12	26.67	-204.4 to 257.8	No	ns	>0.9999			
Day 13	-39.93	-430.1 to 350.2	No	ns	>0.9999			
Day 14	-165.8	-512.7 to 181.0	No	ns	>0.9999			
Day 15	61.44	-203.8 to 326.7	No	ns	>0.9999			
Day 16	10.28	-290.9 to 311.5	No	ns	>0.9999			
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
C - WS								
Day 1	397.9	369.6	28.31	48.43	10	10	0.5845	16.26
Day 2	338.3	325.9	12.40	35.83	10	10	0.3460	17.96
Day 3	426.5	382.4	44.04	55.68	10	10	0.7910	15.02
Day 4	533.7	585.1	-51.31	78.93	10	10	0.6501	15.48

Day 5	789.5	786.5	3.033	124.7	10	10	0.02433	17.57
Day 6	430.4	413.2	17.27	44.45	10	10	0.3884	16.38
Day 7	859.3	904.7	-45.47	108.5	10	10	0.4190	16.14
Day 8	813.4	780.2	33.22	99.26	10	10	0.3347	17.99
Day 9	588.6	606.8	-18.27	67.96	10	10	0.2688	17.72
Day 10	646.1	668.8	-22.72	63.52	10	10	0.3577	17.63
Day 11	900.5	845.3	55.22	100.1	10	10	0.5514	17.31
Day 12	741.4	714.7	26.67	66.64	10	10	0.4002	16.18
Day 13	761.8	801.8	-39.93	113.6	10	10	0.3515	17.18
Day 14	744.3	910.1	-165.8	99.55	10	10	1.666	15.76
Day 15	919.9	858.4	61.44	77.51	10	10	0.7927	17.58
Day 16	843.8	833.6	10.28	88.30	10	10	0.1164	17.94

Supplementary table S3a. Two-way repeated-measures analysis of variance (ANOVA) of transpiration (*E*) from control (C) well-watered vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.2.2.2, Chapter 3.

Table Analysed	E (IRGA)				
Two-way repeated-measures ANOVA	Matching: Stacked				
Assume sphericity?	No				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	Geisser-Greenhouse's epsilon
Time x Treatment	10.24	<0.0001	****	Yes	
Time	14.17	<0.0001	****	Yes	0.7098
Treatment	22.14	<0.0001	****	Yes	
Subject	15.22	0.0004	***	Yes	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Time x Treatment	26.63	7	3.804	F (7, 126) = 4.822	P<0.0001
Time	36.84	7	5.262	F (4.969, 89.43) = 6.671	P<0.0001
Treatment	57.56	1	57.56	F (1, 18) = 26.18	P<0.0001
Subject	39.57	18	2.198	F (18, 126) = 2.787	P=0.0004
Residual	99.40	126	0.7889		
Difference between column means					
Mean of C	3.530				
Mean of WS	2.331				
Difference between means	1.200				
SE of difference	0.2344				
95% CI of difference	0.7071 to 1.692				
Data summary					
Number of columns (Treatment)	2				
Number of rows (Time)	8				
Number of subjects (Subject)	20				

Number of missing values	0				

Supplementary table S3b. Multiple comparison with Bonferroni tests of transpiration (*E*) from control well-watered (C) vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.2.2.2, Chapter 3.

Compare each cell mean with the other cell mean in that row								
Number of families	1							
Number of comparisons per family	8							
Alpha	0.05							
Bonferroni's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value			
C - WS								
Day 2	0.08200	-1.688 to 1.852	No	ns	>0.9999			
Day 4	0.5980	-0.9637 to 2.160	No	ns	>0.9999			
Day 6	1.867	0.4615 to 3.273	Yes	**	0.0053			
Day 8	2.327	1.260 to 3.394	Yes	****	<0.0001			
Day 10	2.196	0.9376 to 3.454	Yes	***	0.0003			
Day 12	1.462	0.09200 to 2.832	Yes	*	0.0317			
Day 14	0.6850	-0.3960 to 1.766	No	ns	0.5166			
Day 16	0.3800	-0.9490 to 1.709	No	ns	>0.9999			
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
C - WS								
Day 2	3.456	3.374	0.08200	0.5675	10	10	0.1445	17.01
Day 4	3.240	2.642	0.5980	0.4862	10	10	1.230	14.05
Day 6	3.230	1.363	1.867	0.4535	10	10	4.117	17.80
Day 8	3.592	1.265	2.327	0.3434	10	10	6.776	17.48
Day 10	3.481	1.285	2.196	0.4041	10	10	5.434	17.19
Day 12	3.756	2.294	1.462	0.4426	10	10	3.303	17.99
Day 14	3.665	2.980	0.6850	0.3466	10	10	1.976	16.99
Day 16	3.821	3.441	0.3800	0.4274	10	10	0.8891	17.37

Supplementary table S4a. Two-way repeated-measures analysis of variance (ANOVA) of net carbon assimilation rate (*NCAR*) from control (C) well-watered vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.2.2.2, Chapter 3.

Table Analysed	*NCAR* (IRGA)				
Two-way repeated-measures ANOVA	Matching: Stacked				
Assume sphericity?	No				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	Geisser-Greenhouse's epsilon
Time x Treatment	14.71	<0.0001	****	Yes	
Time	14.68	0.0001	***	Yes	0.5688
Treatment	20.44	<0.0001	****	Yes	
Subject	10.67	0.0221	*	Yes	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Time x Treatment	226.8	7	32.40	F (7, 126) = 6.703	P<0.0001
Time	226.4	7	32.34	F (3.981, 71.66) = 6.692	P=0.0001
Treatment	315.1	1	315.1	F (1, 18) = 34.47	P<0.0001
Subject	164.5	18	9.141	F (18, 126) = 1.891	P=0.0221
Residual	609.0	126	4.833		
Difference between column means					
Mean of C	10.00				
Mean of WS	7.198				
Difference between means	2.807				
SE of difference	0.4780				
95% CI of difference	1.802 to 3.811				
Data summary					
Number of columns (Treatment)	2				
Number of rows (Time)	8				
Number of subjects (Subject)	20				
Number of missing values	0				

Supplementary table S4b. Multiple comparison with Bonferroni tests of net carbon assimilation rate (*NCAR*) from control well-watered (C) vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.2.2.2, Chapter 3.

Compare each cell mean with the other cell mean in that row								
Number of families	1							
Number of comparisons per family	8							
Alpha	0.05							
Bonferroni's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value			

C - WS								
Day 2	-0.2680	-5.039 to 4.503	No	ns	>0.9999			
Day 4	1.948	-1.130 to 5.026	No	ns	0.4749			
Day 6	5.430	1.630 to 9.230	Yes	**	0.0026			
Day 8	6.115	2.979 to 9.251	Yes	****	<0.0001			
Day 10	5.456	2.272 to 8.640	Yes	***	0.0005			
Day 12	2.696	-0.1769 to 5.569	No	ns	0.0742			
Day 14	0.6150	-1.934 to 3.164	No	ns	>0.9999			
Day 16	0.4610	-1.801 to 2.723	No	ns	>0.9999			
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
C - WS								
Day 2	10.19	10.46	-0.2680	1.479	10	10	0.1812	13.69
Day 4	9.178	7.230	1.948	0.9404	10	10	2.071	12.65
Day 6	10.18	4.750	5.430	1.228	10	10	4.423	18.00
Day 8	10.38	4.261	6.115	1.008	10	10	6.068	17.25
Day 10	10.02	4.563	5.456	1.012	10	10	5.392	15.97
Day 12	9.811	7.115	2.696	0.8959	10	10	3.009	14.17
Day 14	9.804	9.189	0.6150	0.8228	10	10	0.7474	17.87
Day 16	10.48	10.02	0.4610	0.7045	10	10	0.6544	14.07

Supplementary table S5a. Two-way repeated-measures analysis of variance (ANOVA) of stomatal conductance (g_s) from control (C) well-watered vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.2.2.2, Chapter 3.

Table Analysed	g_s (IRGA)				
Two-way repeated-measures ANOVA	Matching: Stacked				
Assume sphericity?	No				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	Geisser-Greenhouse's epsilon
Time x Treatment	9.221	0.0004	***	Yes	
Time	17.41	<0.0001	****	Yes	0.5989
Treatment	20.21	<0.0001	****	Yes	
Subject	12.88	0.0051	**	Yes	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Time x Treatment	0.08816	7	0.01259	F (7, 126) = 4.122	P=0.0004
Time	0.1665	7	0.02378	F (4.192, 75.46) = 7.783	P<0.0001
Treatment	0.1932	1	0.1932	F (1, 18) = 28.23	P<0.0001
Subject	0.1232	18	0.006843	F (18, 126) = 2.240	P=0.0051
Residual	0.3850	126	0.003056		

Difference between column means					
Mean of C	0.1713				
Mean of WS	0.1018				
Difference between means	0.06950				
SE of difference	0.01308				
95% CI of difference	0.04202 to 0.09698				
Data summary					
Number of columns (Column Factor)	2				
Number of rows (Time)	8				
Number of subjects (Subject)	20				
Number of missing values	0				

Supplementary table S5b. Multiple comparison with Bonferroni tests of stomatal conductance (g_s) from control well-watered (C) vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.2.2.2, Chapter 3.

Compare each cell mean with the other cell mean in that row								
Number of families	1							
Number of comparisons per family	8							
Alpha	0.05							
Bonferroni's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value			
C - WS								
Day 2	-0.001000	-0.1225 to 0.1205	No	ns	>0.9999			
Day 4	0.02700	-0.04565 to 0.09965	No	ns	>0.9999			
Day 6	0.1230	0.02508 to 0.2209	Yes	**	0.0088			
Day 8	0.1300	0.07431 to 0.1857	Yes	****	<0.0001			
Day 10	0.1210	0.04625 to 0.1958	Yes	***	0.0008			
Day 12	0.07200	0.009759 to 0.1342	Yes	*	0.0171			
Day 14	0.05100	-0.01845 to 0.1204	No	ns	0.2784			
Day 16	0.03300	-0.05650 to 0.1225	No	ns	>0.9999			
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
C - WS								

Day 2	0.1760	0.1770	-0.001000	0.03899	10	10	0.02564	17.07
Day 4	0.1220	0.09500	0.02700	0.02290	10	10	1.179	15.15
Day 6	0.1820	0.05900	0.1230	0.03149	10	10	3.906	17.36
Day 8	0.1750	0.04500	0.1300	0.01761	10	10	7.384	15.42
Day 10	0.1740	0.05300	0.1210	0.02386	10	10	5.071	16.47
Day 12	0.1500	0.07800	0.07200	0.02010	10	10	3.582	17.92
Day 14	0.1770	0.1260	0.05100	0.02217	10	10	2.300	16.49
Day 16	0.2140	0.1810	0.03300	0.02889	10	10	1.142	17.88

Supplementary table S6a. Two-way repeated-measures analysis of variance (ANOVA) of stem water potential (Ψ_s) from control (C) well-watered vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.2.2.3, Chapter 3.

Table Analysed	Ψ_s (pressure bomb)				
Two-way repeated-measures ANOVA	Matching: Stacked				
Assume sphericity?	No				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	Geisser-Greenhouse's epsilon
Time x Treatment	21.46	0.0001	***	Yes	
Time	58.52	<0.0001	****	Yes	0.9694
Treatment	6.523	0.0048	**	Yes	
Subject	3.508	0.6856	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Time x Treatment	0.2471	2	0.1236	F (2, 16) = 17.19	P=0.0001
Time	0.6737	2	0.3368	F (1.939, 15.51) = 46.87	P<0.0001
Treatment	0.07510	1	0.07510	F (1, 8) = 14.87	P=0.0048
Subject	0.04039	8	0.005049	F (8, 16) = 0.7025	P=0.6856
Residual	0.1150	16	0.007187		
Difference between column means					
Mean of C	0.5337				
Mean of WS	0.6337				
Difference between means	-0.1001				
SE of difference	0.02595				
95% CI of difference	-0.1599 to -0.04024				
Data summary					
Number of columns (Treatment)	2				
Number of rows (Time)	3				
Number of subjects (Subject)	10				
Number of missing values	0				

Supplementary table S6b. Multiple comparison with Bonferroni tests of stem water potential (Ψ_s) from control well-watered (C) vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.2.2.3, Chapter 3.

Compare each cell mean with the other cell mean in that row								
Number of families	1							
Number of comparisons per family	3							
Alpha	0.05							
Bonferroni's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value			
C - WS								
Day 2	0.05720	-0.04878 to 0.1632	No	ns	0.4202			
Day 10	-0.3544	-0.5305 to -0.1783	Yes	**	0.0011			
Day 15	-0.003000	-0.1813 to 0.1753	No	ns	>0.9999			
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
C - WS								
Day 2	0.4526	0.3954	0.05720	0.03475	5	5	1.646	7.663
Day 10	0.6070	0.9614	-0.3544	0.05680	5	5	6.239	7.212
Day 15	0.5414	0.5444	-0.003000	0.05775	5	5	0.05195	7.329

Supplementary table S7a. Two-way repeated-measures analysis of variance (ANOVA) of stomatal conductance (g_s) from control (C) well-watered vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.3.2.1, Chapter 3.

Table Analysed	g_s (porometer)				
Two-way repeated-measures ANOVA	Matching: Stacked				
Assume sphericity?	No				
Alpha	0.05				
Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)	Geisser-Greenhouse's epsilon
Time	<0.0001	****	Yes	F (5.662, 89.83) = 15.89	0.3774
Treatment	0.0001	***	Yes	F (1, 16) = 25.21	
Time x Treatment	<0.0001	****	Yes	F (15, 238) = 16.09	
Random effects	SD	Variance			
Subject	13.24	175.2			
Residual	24.95	622.3			
Was the matching effective?					
Chi-square, df	35.93, 1				
P value	<0.0001				
P value summary	****				

Is there significant matching (P < 0.05)?	Yes				
Difference between column means					
Predicted mean of C	130.9				
Predicted mean of WS	96.21				
Difference between predicted means	34.66				
SE of difference	6.902				
95% CI of difference	20.03 to 49.29				
Data summary					
Number of columns (Treatment)	2				
Number of rows (Time)	16				
Number of subjects (Subject)	18				
Number of missing values	2				

Supplementary table S7b. Multiple comparison with Bonferroni tests of stomatal conductance (g_s) from control well-watered (C) vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.3.2.1, Chapter 3.

Compare each cell mean with the other cell mean in that row								
Number of families	1							
Number of comparisons per family	16							
Alpha	0.05							
Bonferroni's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value			
C - WS								
Day 1	3.062	-30.13 to 36.25	No	ns	>0.9999			
Day 2	-1.488	-41.67 to 38.69	No	ns	>0.9999			
Day 3	-8.415	-60.10 to 43.27	No	ns	>0.9999			
Day 4	-6.030	-59.94 to 47.88	No	ns	>0.9999			
Day 5	-2.517	-61.34 to 56.31	No	ns	>0.9999			
Day 6	12.13	-32.01 to 56.27	No	ns	>0.9999			
Day 7	41.58	3.948 to 79.22	Yes	*	0.0234			
Day 8	115.2	64.69 to 165.6	Yes	****	<0.0001			

Day 9	133.5	90.50 to 176.6	Yes	****	<0.0001			
Day 10	91.25	56.06 to 126.4	Yes	****	<0.0001			
Day 11	81.44	41.66 to 121.2	Yes	****	<0.0001			
Day 12	58.38	27.64 to 89.12	Yes	***	0.0002			
Day 13	30.00	-11.01 to 71.01	No	ns	0.3478			
Day 14	9.829	-34.44 to 54.10	No	ns	>0.9999			
Day 15	-12.35	-71.06 to 46.35	No	ns	>0.9999			
Day 16	11.26	-68.10 to 90.62	No	ns	>0.9999			
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
C - WS								
Day 1	147.6	144.5	3.062	9.351	8	9	0.3274	14.25
Day 2	128.9	130.4	-1.488	10.85	9	9	0.1371	11.74
Day 3	118.8	127.2	-8.415	14.87	9	9	0.5657	16.00
Day 4	122.1	128.1	-6.030	15.38	9	9	0.3920	15.24
Day 5	108.6	111.1	-2.517	16.19	9	9	0.1555	12.74
Day 6	117.2	105.1	12.13	12.62	9	9	0.9612	15.41
Day 7	108.0	66.40	41.58	10.76	9	9	3.865	15.41
Day 8	135.3	20.13	115.2	13.62	9	9	8.454	11.72
Day 9	142.4	8.844	133.5	10.86	9	9	12.29	9.244
Day 10	122.0	30.73	91.25	8.848	9	9	10.31	9.139
Day 11	142.9	61.44	81.44	11.21	9	9	7.263	14.29
Day 12	146.6	88.25	58.38	8.392	9	9	6.957	12.30
Day 13	158.3	128.3	30.00	11.80	9	9	2.543	15.98
Day 14	134.5	124.7	9.829	12.14	9	8	0.8098	12.53
Day 15	130.8	143.2	-12.35	16.28	9	9	0.7586	13.20
Day 16	131.0	119.8	11.26	22.78	9	9	0.4943	15.76

Supplementary table S8a. Two-way repeated-measures analysis of variance (ANOVA) of photosynthetically active radiation (PAR) from control (C) well-watered vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.3.2.1, Chapter 3.

Table Analysed	PAR (porometer)				
Two-way repeated-measures ANOVA	Matching: Stacked				
Assume sphericity?	No				
Alpha	0.05				
Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)	Geisser-Greenhouse's epsilon
Time	<0.0001	****	Yes	F (5.714, 90.67) = 7.737	0.3809
Treatment	0.8348	ns	No	F (1, 16) = 0.04495	

Time x Treatment	0.7627	ns	No	F (15, 238) = 0.7206	
Random effects	SD	Variance			
Subject	184.8	34165			
Residual	124.0	15366			
Was the matching effective?					
Chi-square, df	240.8, 1				
P value	<0.0001				
P value summary	****				
Is there significant matching (P < 0.05)?	Yes				
Difference between column means					
Predicted mean of C	736.0				
Predicted mean of WS	754.8				
Difference between predicted means	-18.73				
SE of difference	88.36				
95% CI of difference	-206.0 to 168.6				
Data summary					
Number of columns (Treatment)	2				
Number of rows (Time)	16				
Number of subjects (Subject)	18				
Number of missing values	2				

Supplementary table S8b. Multiple comparison with Bonferroni tests of photosynthetically active radiation (PAR) from control well-watered (C) vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.3.2.1, Chapter 3.

Compare each cell mean with the other cell mean in that row								
Number of families	1							
Number of comparisons per family	15							
Alpha	0.05							
Bonferroni's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value			
C - WS								
Day 1	-1.488	-41.28 to 38.31	No	ns	>0.9999			
Day 2	-8.415	-59.64 to 42.82	No	ns	>0.9999			
Day 3	-6.030	-59.46 to 47.40	No	ns	>0.9999			

Day 4	-2.517	-60.79 to 55.76	No	ns	>0.9999			
Day 5	12.13	-31.62 to 55.88	No	ns	>0.9999			
Day 6	41.58	4.283 to 78.88	Yes	*	0.0219			
Day 7	115.2	65.18 to 165.1	Yes	****	<0.0001			
Day 8	133.5	90.95 to 176.1	Yes	****	<0.0001			
Day 9	91.25	56.43 to 126.1	Yes	****	<0.0001			
Day 10	81.44	42.02 to 120.9	Yes	****	<0.0001			
Day 11	58.38	27.93 to 88.83	Yes	***	0.0002			
Day 12	30.00	-10.65 to 70.65	No	ns	0.3260			
Day 13	9.829	-34.02 to 53.68	No	ns	>0.9999			
Day 14	-12.35	-70.51 to 45.81	No	ns	>0.9999			
Day 15	11.26	-67.40 to 89.92	No	ns	>0.9999			
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
C - WS								
Day 1	128.9	130.4	-1.488	10.85	9	9	0.1371	11.74
Day 2	118.8	127.2	-8.415	14.87	9	9	0.5657	16.00
Day 3	122.1	128.1	-6.030	15.38	9	9	0.3920	15.24
Day 4	108.6	111.1	-2.517	16.19	9	9	0.1555	12.74
Day 5	117.2	105.1	12.13	12.62	9	9	0.9612	15.41
Day 6	108.0	66.40	41.58	10.76	9	9	3.865	15.41
Day 7	135.3	20.13	115.2	13.62	9	9	8.454	11.72
Day 8	142.4	8.844	133.5	10.86	9	9	12.29	9.244
Day 9	122.0	30.73	91.25	8.848	9	9	10.31	9.139
Day 10	142.9	61.44	81.44	11.21	9	9	7.263	14.29
Day 11	146.6	88.25	58.38	8.392	9	9	6.957	12.30
Day 12	158.3	128.3	30.00	11.80	9	9	2.543	15.98
Day 13	134.5	124.7	9.829	12.14	9	8	0.8098	12.53
Day 14	130.8	143.2	-12.35	16.28	9	9	0.7586	13.20
Day 15	131.0	119.8	11.26	22.78	9	9	0.4943	15.76

Supplementary table S9a. Two-way repeated-measures analysis of variance (ANOVA) of transpiration (*E*) from control (C) well-watered vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.3.2.2, Chapter 3.

Table Analysed	*E* (IRGA)				
Two-way repeated-measures ANOVA	Matching: Stacked				
Assume sphericity?	No				
Alpha	0.05				

Source of Variation	% of total variation	P value	P value summary	Significant?	Geisser-Greenhouse's epsilon
Time x Treatment	29.98	<0.0001	****	Yes	
Time	18.98	<0.0001	****	Yes	0.6641
Treatment	19.36	<0.0001	****	Yes	
Subject	7.541	0.0322	*	Yes	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Time x Treatment	13.64	6	2.274	F (6, 96) = 19.87	P<0.0001
Time	8.636	6	1.439	F (3.984, 63.75) = 12.58	P<0.0001
Treatment	8.811	1	8.811	F (1, 16) = 41.08	P<0.0001
Subject	3.432	16	0.2145	F (16, 96) = 1.874	P=0.0322
Residual	10.99	96	0.1144		
Difference between column means					
Mean of C	1.824				
Mean of WS	1.295				
Difference between means	0.5289				
SE of difference	0.08251				
95% CI of difference	0.3540 to 0.7038				
Data summary					
Number of columns (Treatment)	2				
Number of rows (Time)	7				
Number of subjects (Subject)	18				
Number of missing values	0				

Supplementary table S9b. Multiple comparison with Bonferroni tests of transpiration (*E*) from control well-watered (C) vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.3.2.2, Chapter 3.

Compare each cell mean with the other cell mean in that row								
Number of families	1							
Number of comparisons per family	7							
Alpha	0.05							
Bonferroni's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value			
C - WS								
Day 3	0.05111	-0.6977 to 0.7999	No	ns	>0.9999			
Day 5	0.07556	-0.3109 to 0.4620	No	ns	>0.9999			

Day 7	0.6156	0.1516 to 1.080	Yes	**	0.0063			
Day 9	1.911	1.368 to 2.454	Yes	****	<0.0001			
Day 11	0.9644	0.3877 to 1.541	Yes	**	0.0010			
Day 13	0.1322	-0.3170 to 0.5815	No	ns	>0.9999			
Day 15	-0.04778	-0.4890 to 0.3935	No	ns	>0.9999			
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
C - WS								
Day 3	1.988	1.937	0.05111	0.2425	9	9	0.2108	15.79
Day 5	1.620	1.544	0.07556	0.1246	9	9	0.6062	15.36
Day 7	1.747	1.131	0.6156	0.1488	9	9	4.137	14.79
Day 9	2.048	0.1367	1.911	0.1762	9	9	10.84	15.99
Day 11	1.913	0.9489	0.9644	0.1800	9	9	5.359	12.62
Day 13	1.883	1.751	0.1322	0.1450	9	9	0.9118	15.44
Day 15	1.571	1.619	-0.04778	0.1389	9	9	0.3439	13.24

Supplementary table S10a. Two-way repeated-measures analysis of variance (ANOVA) of net carbon assimilation rate (*NCAR*) from control (C) well-watered vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.3.2.2, Chapter 3.

Table Analysed	*NCAR* (IRGA)				
Two-way repeated-measures ANOVA	Matching: Stacked				
Assume sphericity?	No				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	Geisser-Greenhouse's epsilon
Time x Treatment	26.61	<0.0001	****	Yes	
Time	18.61	<0.0001	****	Yes	0.6862
Treatment	19.48	<0.0001	****	Yes	
Subject	8.683	0.0239	*	Yes	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Time x Treatment	436.5	6	72.75	F (6, 96) = 15.99	P<0.0001
Time	305.3	6	50.89	F (4.117, 65.87) = 11.19	P<0.0001
Treatment	319.5	1	319.5	F (1, 16) = 35.89	P<0.0001
Subject	142.4	16	8.902	F (16, 96) = 1.957	P=0.0239
Residual	436.7	96	4.549		
Difference between column means					
Mean of C	11.73				
Mean of WS	8.544				
Difference between means	3.185				
SE of difference	0.5316				

95% CI of difference	2.058 to 4.312				
Data summary					
Number of columns (Treatment)	2				
Number of rows (Time)	7				
Number of subjects (Subject)	18				
Number of missing values	0				

Supplementary table S10b. Multiple comparison with Bonferroni tests of net carbon assimilation rate (*NCAR*) from control well-watered (C) vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.3.2.2, Chapter 3.

Compare each cell mean with the other cell mean in that row								
Number of families	1							
Number of comparisons per family	7							
Alpha	0.05							
Bonferroni's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value			
C - WS								
Day 3	0.08778	-3.512 to 3.688	No	ns	>0.9999			
Day 5	1.177	-2.485 to 4.838	No	ns	>0.9999			
Day 7	3.739	0.4467 to 7.031	Yes	*	0.0208			
Day 9	11.02	7.576 to 14.46	Yes	****	<0.0001			
Day 11	5.523	2.648 to 8.398	Yes	***	0.0002			
Day 13	0.8633	-2.384 to 4.111	No	ns	>0.9999			
Day 15	-0.1133	-3.340 to 3.114	No	ns	>0.9999			
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
C - WS								
Day 3	11.26	11.17	0.08778	1.165	9	9	0.07536	15.70
Day 5	11.01	9.834	1.177	1.186	9	9	0.9919	15.85
Day 7	11.16	7.426	3.739	1.067	9	9	3.503	15.91
Day 9	12.48	1.462	11.02	1.070	9	9	10.29	12.42
Day 11	12.54	7.012	5.523	0.9312	9	9	5.931	15.82
Day 13	12.48	11.62	0.8633	1.047	9	9	0.8242	15.38
Day 15	11.17	11.28	-0.1133	1.015	9	9	0.1117	13.17

Supplementary table S11a. Two-way repeated-measures analysis of variance (ANOVA) of stomatal conductance (g_s) from control (C) well-watered vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.3.2.2, Chapter 3.

Table Analysed	g_s (IRGA)				
Two-way repeated-measures ANOVA	Matching: Stacked				
Assume sphericity?	No				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	Geisser-Greenhouse's epsilon
Time x Treatment	18.17	<0.0001	****	Yes	
Time	20.46	<0.0001	****	Yes	0.5755
Treatment	15.98	0.0002	***	Yes	
Subject	10.55	0.0395	*	Yes	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Time x Treatment	0.05027	6	0.008379	F (6, 96) = 8.347	P<0.0001
Time	0.05658	6	0.009430	F (3.453, 55.24) = 9.394	P<0.0001
Treatment	0.04420	1	0.04420	F (1, 16) = 24.23	P=0.0002
Subject	0.02918	16	0.001824	F (16, 96) = 1.817	P=0.0395
Residual	0.09637	96	0.001004		
Difference between column means					
Mean of C	0.1170				
Mean of WS	0.07952				
Difference between means	0.03746				
SE of difference	0.007610				
95% CI of difference	0.02133 to 0.05359				
Data summary					
Number of columns (Treatment)	2				
Number of rows (Time)	7				
Number of subjects (Subject)	18				

Supplementary table S11b. Multiple comparison with Bonferroni tests of stomatal conductance (g_s) from control well-watered (C) vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.3.2.2, Chapter 3.

Compare each cell mean with the other cell mean in that row								
Number of families	1							
Number of comparisons per family	7							
Alpha	0.05							
Bonferroni's multiple	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value			

comparisons test								
C - WS								
Day 3	0.006667	-0.06264 to 0.07598	No	ns	>0.9999			
Day 5	0.008889	-0.02872 to 0.04650	No	ns	>0.9999			
Day 7	0.04444	0.01386 to 0.07503	Yes	**	0.0027			
Day 9	0.1156	0.07735 to 0.1538	Yes	****	<0.0001			
Day 11	0.07222	0.02655 to 0.1179	Yes	**	0.0015			
Day 13	0.01778	-0.04491 to 0.08046	No	ns	>0.9999			
Day 15	-0.003333	-0.05135 to 0.04468	No	ns	>0.9999			
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
C - WS								
Day 3	0.1167	0.1100	0.006667	0.02248	9	9	0.2965	15.98
Day 5	0.1000	0.09111	0.008889	0.01207	9	9	0.7365	14.87
Day 7	0.1033	0.05889	0.04444	0.009923	9	9	4.479	16.00
Day 9	0.1233	0.007778	0.1156	0.01211	9	9	9.544	13.77
Day 11	0.1222	0.05000	0.07222	0.01441	9	9	5.011	13.42
Day 13	0.1411	0.1233	0.01778	0.02024	9	9	0.8784	15.46
Day 15	0.1122	0.1156	-0.003333	0.01517	9	9	0.2197	13.54

Supplementary table S12a. Two-way repeated-measures analysis of variance (ANOVA) of stem water potential (Ψ_s) from control (C) well-watered vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.3.2.3, Chapter 3.

Table Analysed	Ψ_s (pressure bomb)				
Two-way repeated-measures ANOVA	Matching: Stacked				
Assume sphericity?	No				
Alpha	0.05				
Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)	Geisser-Greenhouse's epsilon
Time	0.0022	**	Yes	F (1.211, 6.659) = 21.44	0.6054
Treatment	0.0034	**	Yes	F (1, 6) = 21.98	
Time x Treatment	0.0010	**	Yes	F (2, 11) = 13.77	
Random effects	SD	Variance			
Subject	0.03107	0.0009652			
Residual	0.1131	0.01280			
Was the matching effective?					
Chi-square, df	0.07982, 1				
P value	0.7775				
P value summary	ns				

Is there significant matching (P < 0.05)?	No					
Difference between column means						
Predicted mean of C	0.4248					
Predicted mean of WS	0.6703					
Difference between predicted means	-0.2455					
SE of difference	0.05236					
95% CI of difference	-0.3736 to -0.1173					
Data summary						
Number of columns (Treatment)	2					
Number of rows (Time)	3					
Number of subjects (Subject)	8					
Number of missing values	1					

Supplementary table S12b. Multiple comparison with Bonferroni tests of stem water potential (Ψ_s) from control well-watered (C) vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.3.2.3, Chapter 3.

Compare each cell mean with the other cell mean in that row								
Number of families	1							
Number of comparisons per family	3							
Alpha	0.05							
Bonferroni's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value			
C - WS								
Day 3	-0.08008	-0.2835 to 0.1233	No	ns	0.6669			
Day 9	-0.5925	-0.9345 to -0.2505	Yes	**	0.0067			
Day 16	-0.06625	-0.3786 to 0.2461	No	ns	>0.9999			
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
C - WS								
Day 3	0.4483	0.5283	-0.08008	0.05744	4	3	1.394	4.979
Day 9	0.4600	1.053	-0.5925	0.08821	4	4	6.717	4.151
Day 16	0.3663	0.4325	-0.06625	0.09502	4	4	0.6972	6.000

Supplementary table S13a. Two-way repeated-measures analysis of variance (ANOVA) of stomatal conductance (g_s) from control (C) well-watered vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.4.2.1, Chapter 3.

Table Analysed	g_s (porometer)				
Two-way repeated-measures ANOVA	Matching: Stacked				
Assume sphericity?	No				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	Geisser-Greenhouse's epsilon
Time x Treatment	6.997	<0.0001	****	Yes	
Time	26.78	0.0008	***	Yes	0.08745
Treatment	0.4563	0.7578	ns	No	
Subject	45.43	<0.0001	****	Yes	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Time x Treatment	567763	18	31542	F (18, 180) = 3.440	P<0.0001
Time	2172826	18	120713	F (1.574, 15.74) = 13.16	P=0.0008
Treatment	37027	1	37027	F (1, 10) = 0.1004	P=0.7578
Subject	3686382	10	368638	F (10, 180) = 40.20	P<0.0001
Residual	1650564	180	9170		
Difference between column means					
Mean of C	239.2				
Mean of WS	213.7				
Difference between means	25.49				
SE of difference	80.42				
95% CI of difference	-153.7 to 204.7				
Data summary					
Number of columns (Treatment)	2				
Number of rows (Time)	19				
Number of subjects (Subject)	12				
Number of missing values	0				

Supplementary table S13b. Multiple comparison with Bonferroni tests of stomatal conductance (g_s) from control well-watered (C) vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.4.2.1, Chapter 3.

Compare each cell mean with the other cell mean in that row							
Number of families	1						
Number of comparisons per family	19						
Alpha	0.05						

Bonferroni's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value		
C - WS							
Day 1	25.70	-458.5 to 509.9	No	ns	>0.9999		
Day 2	-91.58	-634.7 to 451.6	No	ns	>0.9999		
Day 3	-123.3	-629.4 to 382.9	No	ns	>0.9999		
Day 4	-162.5	-765.0 to 440.0	No	ns	>0.9999		
Day 5	30.22	-521.3 to 581.8	No	ns	>0.9999		
Day 6	10.79	-417.3 to 438.9	No	ns	>0.9999		
Day 7	-5.236	-454.5 to 444.0	No	ns	>0.9999		
Day 8	39.22	-288.6 to 367.0	No	ns	>0.9999		
Day 9	170.3	-197.8 to 538.4	No	ns	0.9728		
Day 10	227.6	-181.5 to 636.6	No	ns	0.5225		
Day 11	207.5	-200.5 to 615.6	No	ns	0.7145		
Day 12	113.6	-238.4 to 465.5	No	ns	>0.9999		
Day 13	95.53	-449.5 to 640.5	No	ns	>0.9999		
Day 14	17.65	-342.3 to 377.6	No	ns	>0.9999		
Day 15	-40.42	-330.3 to 249.4	No	ns	>0.9999		
Day 16	-4.000	-468.8 to 460.8	No	ns	>0.9999		
Day 17	-13.49	-138.5 to 111.6	No	ns	>0.9999		
Day 18	-17.90	-217.8 to 182.0	No	ns	>0.9999		
Day 19	4.528	-404.1 to 413.1	No	ns	>0.9999		
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t
C - WS							
Day 1	381.2	355.5	25.70	119.3	6	6	0.2153
Day 2	361.4	453.0	-91.58	136.5	6	6	0.6710
Day 3	307.3	430.6	-123.3	126.5	6	6	0.9742
Day 4	291.8	454.3	-162.5	151.6	6	6	1.071
Day 5	312.2	282.0	30.22	130.6	6	6	0.2313
Day 6	239.8	229.1	10.79	98.49	6	6	0.1096
Day 7	305.0	310.2	-5.236	112.0	6	6	0.04677
Day 8	238.9	199.7	39.22	80.02	6	6	0.4901
Day 9	186.8	16.56	170.3	66.97	6	6	2.543
Day 10	236.9	9.365	227.6	73.97	6	6	3.076
Day 11	233.3	25.79	207.5	74.09	6	6	2.801
Day 12	230.1	116.5	113.6	69.30	6	6	1.639
Day 13	226.7	131.2	95.53	101.7	6	6	0.9397
Day 14	178.7	161.0	17.65	73.99	6	6	0.2386
Day 15	184.6	225.0	-40.42	59.82	6	6	0.6756
Day 16	234.6	238.6	-4.000	97.04	6	6	0.04122
Day 17	91.54	105.0	-13.49	30.63	6	6	0.4403
Day 18	103.1	121.0	-17.90	43.21	6	6	0.4142
Day 19	201.1	196.6	4.528	91.98	6	6	0.04922

Supplementary table S14a. Two-way repeated-measures analysis of variance (ANOVA) of photosynthetically active radiation (PAR) from control (C) well-watered vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.4.2.1, Chapter 3.

Table Analysed	PAR (porometer)				
Two-way repeated-measures ANOVA	Matching: Stacked				
Assume sphericity?	No				
Alpha	0.05				
Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)	Geisser-Greenhouse's epsilon
Row Factor	<0.0001	****	Yes	F (2.982, 41.25) = 41.77	0.1657
Column Factor	0.8666	ns	No	F (1, 18) = 0.02905	
Row Factor x Column Factor	0.7136	ns	No	F (18, 249) = 0.7881	
Random effects	SD	Variance			
Subject	22.90	524.4			
Residual	25.14	632.0			
Was the matching effective?					
Chi-square, df	108.5, 1				
P value	<0.0001				
P value summary	****				
Is there significant matching (P < 0.05)?	Yes				
Difference between column means					
Predicted mean of C	203.0				
Predicted mean of WS	204.8				
Difference between predicted means	-1.832				
SE of difference	10.75				
95% CI of difference	-24.41 to 20.75				
Data summary					
Number of columns (Treatment)	2				
Number of rows (Time)	19				
Number of subjects (Subject)	20				
Number of missing values	75				

Supplementary table S14b. Multiple comparison with Bonferroni tests of photosynthetically active radiation (PAR) from control well-watered (C) vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.4.2.1, Chapter 3.

Compare each cell mean with the other cell mean in that row							
Number of families	1						
Number of comparisons per family	19						

Alpha	0.05						
Bonferroni's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value		
C - WS							
Day 1	-5.017	-62.21 to 52.18	No	ns	>0.9999		
Day 2	1.400	-50.38 to 53.18	No	ns	>0.9999		
Day 3	0.4367	-52.42 to 53.30	No	ns	>0.9999		
Day 4	-13.45	-71.98 to 45.08	No	ns	>0.9999		
Day 5	10.15	-31.78 to 52.08	No	ns	>0.9999		
Day 6	3.500	-24.83 to 31.83	No	ns	>0.9999		
Day 7	19.68	-48.16 to 87.53	No	ns	>0.9999		
Day 8	-2.083	-59.97 to 55.80	No	ns	>0.9999		
Day 9	-15.72	-66.88 to 35.45	No	ns	>0.9999		
Day 10	-12.77	-65.61 to 40.08	No	ns	>0.9999		
Day 11	-19.69	-92.68 to 53.29	No	ns	>0.9999		
Day 12	14.81	-41.46 to 71.08	No	ns	>0.9999		
Day 13	-1.333	-66.26 to 63.60	No	ns	>0.9999		
Day 14	10.89	-49.23 to 71.01	No	ns	>0.9999		
Day 15	-5.806	-130.4 to 118.8	No	ns	>0.9999		
Day 16	10.36	-90.12 to 110.8	No	ns	>0.9999		
Day 17	13.90	-80.70 to 108.5	No	ns	>0.9999		
Day 18	-0.5722	-88.92 to 87.78	No	ns	>0.9999		
Day 19	-19.18	-99.81 to 61.44	No	ns	>0.9999		
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t
C - WS							
Day 1	245.5	250.5	-5.017	16.25	10	10	0.3088
Day 2	251.9	250.5	1.400	14.84	10	10	0.09435
Day 3	239.9	239.5	0.4367	15.02	10	10	0.02908
Day 4	228.0	241.5	-13.45	16.47	10	10	0.8165
Day 5	180.8	170.6	10.15	11.96	10	10	0.8484
Day 6	120.8	117.3	3.500	8.125	10	10	0.4308
Day 7	209.6	189.9	19.68	18.96	10	10	1.038
Day 8	241.8	243.9	-2.083	16.60	10	10	0.1255
Day 9	224.7	240.5	-15.72	14.67	10	10	1.071
Day 10	229.1	241.9	-12.77	15.16	10	10	0.8424
Day 11	219.4	239.1	-19.69	17.16	6	6	1.148
Day 12	212.1	197.3	14.81	12.78	6	6	1.159
Day 13	143.1	144.4	-1.333	15.43	6	6	0.08644
Day 14	199.4	188.5	10.89	15.13	6	6	0.7196
Day 15	177.6	183.4	-5.806	29.40	6	6	0.1975
Day 16	219.1	208.8	10.36	24.33	6	6	0.4258
Day 17	166.5	152.6	13.90	20.74	6	5	0.6704
Day 18	143.2	143.8	-0.5722	17.85	6	5	0.03206
Day 19	225.9	245.1	-19.18	18.64	6	5	1.029

Supplementary table S15a. Two-way repeated-measures analysis of variance (ANOVA) of transpiration (*E*) from control (C) well-watered vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.4.2.2, Chapter 3.

Table Analysed	*E* (IRGA)				
Two-way repeated-measures ANOVA	Matching: Stacked				
Assume sphericity?	No				
Alpha	0.05				
Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)	Geisser-Greenhouse's epsilon
Time	<0.0001	****	Yes	F (2.860, 43.85) = 15.71	0.4767
Treatment	0.0112	*	Yes	F (1, 18) = 7.994	
Time x Treatment	<0.0001	****	Yes	F (6, 92) = 6.862	
Random effects	SD	Variance			
Subject	0.4118	0.1696			
Residual	0.6063	0.3676			
Was the matching effective?					
Chi-square, df	18.74, 1				
P value	<0.0001				
P value summary	****				
Is there significant matching (P < 0.05)?	Yes				
Difference between column means					
Predicted mean of C	2.377				
Predicted mean of WS	1.764				
Difference between predicted means	0.6128				
SE of difference	0.2167				
95% CI of difference	0.1575 to 1.068				
Data summary					
Number of columns (Treatment)	2				
Number of rows (Time)	7				
Number of subjects (Subject)	20				
Number of missing values	16				

Supplementary table S15b. Multiple comparison with Bonferroni tests of transpiration (*E*) from control well-watered (C) vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.4.2.2, Chapter 3.

Compare each cell mean with the other cell mean in that row							
Number of families	1						
Number of comparisons per family	7						
Alpha	0.05						

Bonferroni's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value		
C - WS							
Day 3	0.3920	-0.9138 to 1.698	No	ns	>0.9999		
Day 5	0.3700	-0.7757 to 1.516	No	ns	>0.9999		
Day 7	0.3320	-0.7275 to 1.391	No	ns	>0.9999		
Day 9	0.5450	-0.3814 to 1.471	No	ns	0.6324		
Day 11	2.181	1.483 to 2.879	Yes	****	<0.0001		
Day 13	0.7417	-0.4955 to 1.979	No	ns	0.4967		
Day 15	-0.1567	-1.428 to 1.114	No	ns	>0.9999		
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t
C - WS							
Day 3	3.075	2.683	0.3920	0.4292	10	10	0.9133
Day 5	2.829	2.459	0.3700	0.3761	10	10	0.9837
Day 7	2.365	2.033	0.3320	0.3492	10	10	0.9506
Day 9	2.307	1.762	0.5450	0.3038	10	10	1.794
Day 11	2.387	0.2060	2.181	0.2085	10	10	10.46
Day 13	1.995	1.253	0.7417	0.3670	6	6	2.021
Day 15	1.790	1.947	-0.1567	0.3202	6	6	0.4893

Supplementary table S16a. Two-way repeated-measures analysis of variance (ANOVA) of net carbon assimilation rate (*NCAR*) from control (C) well-watered vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.4.2.2, Chapter 3.

Table Analysed	*NCAR* (IRGA)				
Two-way repeated-measures ANOVA	Matching: Stacked				
Assume sphericity?	No				
Alpha	0.05				
Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)	Geisser-Greenhouse's epsilon
Time	<0.0001	****	Yes	F (2.886, 44.24) = 17.92	0.4809
Treatment	0.0125	*	Yes	F (1, 18) = 7.689	
Time x Treatment	<0.0001	****	Yes	F (6, 92) = 15.06	
Random effects	SD	Variance			
Subject	1.949	3.798			
Residual	2.683	7.201			
Was the matching effective?					
Chi-square, df	22.34, 1				
P value	<0.0001				

P value summary	****				
Is there significant matching (P < 0.05)?	Yes				
Difference between column means					
Predicted mean of C	13.52				
Predicted mean of WS	10.72				
Difference between predicted means	2.795				
SE of difference	1.008				
95% CI of difference	0.6773 to 4.912				
Data summary					
Number of columns (Treatment)	2				
Number of rows (Time)	7				
Number of subjects (Subject)	20				
Number of missing values	16				

Supplementary table S16b. Multiple comparison with Bonferroni tests of net carbon assimilation rate (*NCAR*) from control well-watered (C) vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.4.2.2, Chapter 3.

Compare each cell mean with the other cell mean in that row							
Number of families	1						
Number of comparisons per family	7						
Alpha	0.05						
Bonferroni's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value		
C - WS							
Day 3	0.8330	-4.048 to 5.714	No	ns	>0.9999		
Day 5	1.463	-3.520 to 6.446	No	ns	>0.9999		
Day 7	0.4660	-4.039 to 4.971	No	ns	>0.9999		
Day 9	2.278	-2.459 to 7.015	No	ns	>0.9999		
Day 11	13.14	9.726 to 16.55	Yes	****	<0.0001		
Day 13	3.663	-4.318 to 11.65	No	ns	>0.9999		
Day 15	-1.422	-7.327 to 4.483	No	ns	>0.9999		
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t
C - WS							
Day 3	15.06	14.23	0.8330	1.608	10	10	0.5181
Day 5	15.17	13.71	1.463	1.626	10	10	0.8997
Day 7	12.26	11.80	0.4660	1.485	10	10	0.3138
Day 9	13.81	11.54	2.278	1.560	10	10	1.460
Day 11	13.59	0.4550	13.14	1.057	10	10	12.43

Day 13	13.15	9.485	3.663	2.348	6	6	1.560
Day 15	12.09	13.51	-1.422	1.558	6	6	0.9124

Supplementary table S17a. Two-way repeated-measures analysis of variance (ANOVA) of stomatal conductance (g_s) from control (C) well-watered vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.4.2.2, Chapter 3.

Table Analysed	g_s (IRGA)				
Two-way repeated-measures ANOVA	Matching: Stacked				
Assume sphericity?	No				
Alpha	0.05				
Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)	Geisser-Greenhouse's epsilon
Time	0.0012	**	Yes	F (2.552, 39.12) = 7.046	0.4253
Treatment	0.0474	*	Yes	F (1, 18) = 4.530	
Time x Treatment	0.1051	ns	No	F (6, 92) = 1.813	
Random effects	SD	Variance			
Subject	0.08216	0.006750			
Residual	0.08875	0.007876			
Was the matching effective?					
Chi-square, df	38.39, 1				
P value	<0.0001				
P value summary	****				
Is there significant matching (P < 0.05)?	Yes				
Difference between column means					
Predicted mean of C	0.2328				
Predicted mean of WS	0.1468				
Difference between predicted means	0.08598				
SE of difference	0.04040				
95% CI of difference	0.001113 to 0.1709				
Data summary					
Number of columns (Treatment)	2				
Number of rows (Time)	7				
Number of subjects (Subject)	20				
Number of missing values	16				

Supplementary table S17b. Multiple comparison with Bonferroni tests of stomatal conductance (g_s) from control well-watered (C) vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.4.2.2, Chapter 3.

Compare each cell mean with the other cell mean in that row							
Number of families	1						
Number of comparisons per family	7						
Alpha	0.05						
Bonferroni's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value		
C - WS							
Day 3	0.05700	-0.1123 to 0.2263	No	ns	>0.9999		
Day 5	0.05500	-0.09419 to 0.2042	No	ns	>0.9999		
Day 7	0.08300	-0.1417 to 0.3077	No	ns	>0.9999		
Day 9	0.06900	-0.05437 to 0.1924	No	ns	0.7131		
Day 11	0.1900	0.08094 to 0.2991	Yes	**	0.0013		
Day 13	0.1500	-0.1833 to 0.4833	No	ns	0.9051		
Day 15	0.03333	-0.3289 to 0.3956	No	ns	>0.9999		
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t
C - WS							
Day 3	0.2620	0.2050	0.05700	0.05546	10	10	1.028
Day 5	0.2330	0.1780	0.05500	0.04848	10	10	1.134
Day 7	0.3080	0.2250	0.08300	0.07298	10	10	1.137
Day 9	0.1960	0.1270	0.06900	0.03956	10	10	1.744
Day 11	0.1970	0.007000	0.1900	0.03166	10	10	6.001
Day 13	0.2700	0.1200	0.1500	0.08641	6	6	1.736
Day 15	0.2167	0.1833	0.03333	0.08788	6	6	0.3793

Supplementary table S18a. Two-way repeated-measures analysis of variance (ANOVA) of daily water consumption from control (C) well-watered vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.4.2.4, Chapter 3.

Table Analysed	Daily water consumption				
Two-way repeated measures ANOVA	Matching: Stacked				
Assume sphericity?	Yes				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Time x Treatment	23.52	<0.0001	****	Yes	
Time	28.66	<0.0001	****	Yes	
Treatment	5.374	0.2267	ns	No	
Subject	32.38	<0.0001	****	Yes	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Time x Treatment	6036	19	317.7	F (19, 190) = 23.35	P<0.0001

Time	7356	19	387.2	F (19, 190) = 28.45	P<0.0001
Treatment	1380	1	1380	F (1, 10) = 1.660	P=0.2267
Subject	8312	10	831.2	F (10, 190) = 61.08	P<0.0001
Residual	2586	190	13.61		
Difference between column means					
Mean of C	32.10				
Mean of WS	27.30				
Difference between means	4.795				
SE of difference	3.722				
95% CI of difference	-3.498 to 13.09				
Data summary					
Number of columns (Treatment)	2				
Number of rows (Time)	20				
Number of subjects (Subject)	12				
Number of missing values	0				

Supplementary table S18b. Multiple comparison with Bonferroni tests of daily water consumption from control well-watered (C) vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.4.2.4, Chapter 3.

Compare each cell mean with the other cell mean in that row								
Number of families	1							
Number of comparisons per family	20							
Alpha	0.05							
Bonferroni's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value			
C - WS								
Day 1	0.3728	-12.68 to 13.42	No	ns	>0.9999			
Day 2	-2.819	-15.87 to 10.23	No	ns	>0.9999			
Day 3	-4.133	-17.18 to 8.917	No	ns	>0.9999			
Day 4	-3.729	-16.78 to 9.321	No	ns	>0.9999			
Day 5	0.2185	-12.83 to 13.27	No	ns	>0.9999			
Day 6	-0.2114	-13.26 to 12.84	No	ns	>0.9999			
Day 7	0.2842	-12.77 to 13.33	No	ns	>0.9999			

Day 8	5.227	-7.823 to 18.28	No	ns	>0.9999			
Day 9	27.61	14.56 to 40.66	Yes	****	<0.0001			
Day 10	30.55	17.50 to 43.60	Yes	****	<0.0001			
Day 11	23.36	10.31 to 36.41	Yes	****	<0.0001			
Day 12	9.974	-3.076 to 23.02	No	ns	0.4050			
Day 13	7.271	-5.779 to 20.32	No	ns	>0.9999			
Day 14	2.434	-10.62 to 15.48	No	ns	>0.9999			
Day 15	-0.6241	-13.67 to 12.43	No	ns	>0.9999			
Day 16	0.1257	-12.92 to 13.18	No	ns	>0.9999			
Day 17	-1.700	-14.75 to 11.35	No	ns	>0.9999			
Day 18	-0.3856	-13.44 to 12.66	No	ns	>0.9999			
Day 19	0.7326	-12.32 to 13.78	No	ns	>0.9999			
Day 20	1.341	-11.71 to 14.39	No	ns	>0.9999			
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
C - WS								
Day 1	30.22	29.85	0.3728	4.262	6	6	0.08746	200.0
Day 2	30.43	33.25	-2.819	4.262	6	6	0.6615	200.0
Day 3	30.13	34.26	-4.133	4.262	6	6	0.9698	200.0
Day 4	33.21	36.94	-3.729	4.262	6	6	0.8750	200.0
Day 5	30.27	30.05	0.2185	4.262	6	6	0.05127	200.0
Day 6	23.67	23.88	-0.2114	4.262	6	6	0.04960	200.0
Day 7	33.05	32.77	0.2842	4.262	6	6	0.06669	200.0
Day 8	34.78	29.55	5.227	4.262	6	6	1.226	200.0
Day 9	34.83	7.219	27.61	4.262	6	6	6.478	200.0
Day 10	33.60	3.051	30.55	4.262	6	6	7.168	200.0
Day 11	32.96	9.599	23.36	4.262	6	6	5.482	200.0
Day 12	26.53	16.56	9.974	4.262	6	6	2.340	200.0
Day 13	32.97	25.70	7.271	4.262	6	6	1.706	200.0
Day 14	34.16	31.73	2.434	4.262	6	6	0.5710	200.0
Day 15	27.72	28.34	-0.6241	4.262	6	6	0.1464	200.0
Day 16	36.23	36.11	0.1257	4.262	6	6	0.02949	200.0
Day 17	35.36	37.06	-1.700	4.262	6	6	0.3988	200.0
Day 18	30.22	30.61	-0.3856	4.262	6	6	0.09048	200.0
Day 19	38.73	38.00	0.7326	4.262	6	6	0.1719	200.0
Day 20	32.86	31.52	1.341	4.262	6	6	0.3147	200.0

Supplementary table S19a. Two-way repeated-measures analysis of variance (ANOVA) of nightly water consumption from control (C) well-watered vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.4.2.4, Chapter 3.

Table Analysed	Nightly water consumption				
Two-way repeated-measures ANOVA	Matching: Stacked				
Assume sphericity?	Yes				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Time x Treatment	14.79	<0.0001	****	Yes	
Time	29.47	<0.0001	****	Yes	
Treatment	5.296	0.2352	ns	No	
Subject	33.20	<0.0001	****	Yes	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Time x Treatment	30.14	18	1.674	F (18, 180) = 8.570	P<0.0001
Time	60.06	18	3.336	F (18, 180) = 17.08	P<0.0001
Treatment	10.79	1	10.79	F (1, 10) = 1.595	P=0.2352
Subject	67.67	10	6.767	F (10, 180) = 34.64	P<0.0001
Residual	35.16	180	0.1954		
Difference between column means					
Mean of C	3.342				
Mean of WS	2.907				
Difference between means	0.4352				
SE of difference	0.3445				
95% CI of difference	-0.3325 to 1.203				
Data summary					
Number of columns (Treatment)	2				
Number of rows (Time)	19				
Number of subjects (Subject)	12				
Number of missing values	0				

Supplementary table S19b. Multiple comparison with Bonferroni tests of nightly water consumption from control well-watered (C) vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.4.2.4, Chapter 3.

Compare each cell mean with the other cell mean in that row								
Number of families	1							
Number of comparisons per family	19							
Alpha	0.05							
Bonferroni's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value			
C - WS								

Day 1	-0.1183	-1.413 to 1.176	No	ns	>0.9999			
Day 2	0.1800	-1.115 to 1.475	No	ns	>0.9999			
Day 3	-0.1333	-1.428 to 1.161	No	ns	>0.9999			
Day 4	0.4117	-0.8830 to 1.706	No	ns	>0.9999			
Day 5	0.6467	-0.6480 to 1.941	No	ns	>0.9999			
Day 6	0.5867	-0.7080 to 1.881	No	ns	>0.9999			
Day 7	0.5317	-0.7630 to 1.826	No	ns	>0.9999			
Day 8	1.653	0.3587 to 2.948	Yes	**	0.0026			
Day 9	2.547	1.252 to 3.841	Yes	****	<0.0001			
Day 10	1.655	0.3604 to 2.950	Yes	**	0.0026			
Day 11	0.4567	-0.8380 to 1.751	No	ns	>0.9999			
Day 12	0.3667	-0.9280 to 1.661	No	ns	>0.9999			
Day 13	0.05667	-1.238 to 1.351	No	ns	>0.9999			
Day 14	-0.06500	-1.360 to 1.230	No	ns	>0.9999			
Day 15	-0.04667	-1.341 to 1.248	No	ns	>0.9999			
Day 16	-0.1000	-1.395 to 1.195	No	ns	>0.9999			
Day 17	-0.1067	-1.401 to 1.188	No	ns	>0.9999			
Day 18	-0.1483	-1.443 to 1.146	No	ns	>0.9999			
Day 19	-0.1050	-1.400 to 1.190	No	ns	>0.9999			
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
C - WS								
Day 1	3.598	3.717	-0.1183	0.4247	6	6	0.2786	190.0
Day 2	3.662	3.482	0.1800	0.4247	6	6	0.4238	190.0
Day 3	4.100	4.233	-0.1333	0.4247	6	6	0.3139	190.0
Day 4	4.100	3.688	0.4117	0.4247	6	6	0.9692	190.0
Day 5	3.303	2.657	0.6467	0.4247	6	6	1.523	190.0
Day 6	3.237	2.650	0.5867	0.4247	6	6	1.381	190.0
Day 7	3.032	2.500	0.5317	0.4247	6	6	1.252	190.0
Day 8	3.818	2.165	1.653	0.4247	6	6	3.893	190.0
Day 9	3.607	1.060	2.547	0.4247	6	6	5.996	190.0
Day 10	3.442	1.787	1.655	0.4247	6	6	3.897	190.0
Day 11	2.218	1.762	0.4567	0.4247	6	6	1.075	190.0
Day 12	3.555	3.188	0.3667	0.4247	6	6	0.8633	190.0
Day 13	3.338	3.282	0.05667	0.4247	6	6	0.1334	190.0

Day 14	3.068	3.133	-0.06500	0.4247	6	6	0.1530	190.0
Day 15	3.012	3.058	-0.04667	0.4247	6	6	0.1099	190.0
Day 16	3.043	3.143	-0.1000	0.4247	6	6	0.2354	190.0
Day 17	2.698	2.805	-0.1067	0.4247	6	6	0.2511	190.0
Day 18	3.135	3.283	-0.1483	0.4247	6	6	0.3492	190.0
Day 19	3.538	3.643	-0.1050	0.4247	6	6	0.2472	190.0

Supplementary table S20a. Two-way repeated-measures analysis of variance (ANOVA) of stomatal conductance (g_s) from control (C) well-watered vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.5.2.1, Chapter 3.

Table Analysed	g_s (Porometer)				
Two-way repeated-measures ANOVA	Matching: Stacked				
Assume sphericity?	No				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	Geisser-Greenhouse's epsilon
Time x Treatment	19.38	<0.0001	****	Yes	
Time	46.82	<0.0001	****	Yes	0.3723
Treatment	1.305	0.5348	ns	No	
Subject	18.07	<0.0001	****	Yes	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Time x Treatment	436313	10	43631	F (10, 60) = 8.061	P<0.0001
Time	1054006	10	105401	F (3.723, 22.34) = 19.47	P<0.0001
Treatment	29381	1	29381	F (1, 6) = 0.4334	P=0.5348
Subject	406763	6	67794	F (6, 60) = 12.52	P<0.0001
Residual	324773	60	5413		
Difference between column means					
Mean of C	299.3				
Mean of WS	262.8				
Difference between means	36.54				
SE of difference	55.51				
95% CI of difference	-99.29 to 172.4				
Data summary					
Number of columns (Treatment)	2				
Number of rows (Time)	11				
Number of subjects (Subject)	8				
Number of missing values	0				

Supplementary table S20b. Multiple comparison with Bonferroni tests of stomatal conductance (g_s) from control well-watered (C) vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.5.2.1, Chapter 3.

Compare each cell mean with the other cell mean in that row								
Number of families	1							
Number of comparisons per family	11							
Alpha	0.05							
Bonferroni's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value			
C - WS								
Day 1	-155.9	-437.5 to 125.6	No	ns	0.5366			
Day 2	-55.21	-293.1 to 182.7	No	ns	>0.9999			
Day 3	-47.29	-291.5 to 196.9	No	ns	>0.9999			
Day 4	-66.79	-461.8 to 328.2	No	ns	>0.9999			
Day 5	-55.08	-451.9 to 341.7	No	ns	>0.9999			
Day 6	62.45	-409.3 to 534.2	No	ns	>0.9999			
Day 7	154.4	-43.24 to 352.0	No	ns	0.1074			
Day 8	344.7	-252.7 to 942.1	No	ns	0.2357			
Day 9	193.0	-197.6 to 583.6	No	ns	0.4624			
Day 10	90.04	-390.3 to 570.4	No	ns	>0.9999			
Day 11	-62.25	-449.2 to 324.7	No	ns	>0.9999			
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
C - WS								
Day 1	371.0	526.9	-155.9	62.62	4	4	2.490	5.766
Day 2	305.2	360.4	-55.21	53.99	4	4	1.023	5.994
Day 3	419.8	467.1	-47.29	42.78	4	4	1.105	4.032
Day 4	281.5	348.3	-66.79	87.61	4	4	0.7624	5.734
Day 5	362.3	417.4	-55.08	89.80	4	4	0.6134	5.963
Day 6	208.9	146.5	62.45	86.10	4	4	0.7253	4.243
Day 7	186.5	32.09	154.4	28.82	4	4	5.356	3.322
Day 8	361.1	16.43	344.7	78.46	4	4	4.393	3.029
Day 9	271.3	78.31	193.0	63.04	4	4	3.062	3.675
Day 10	289.5	199.5	90.04	102.8	4	4	0.8761	5.374
Day 11	235.5	297.7	-62.25	87.30	4	4	0.7131	5.926

Supplementary table S21a. Two-way repeated-measures analysis of variance (ANOVA) of photosynthetically active radiation (PAR) from control (C) well-watered vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.5.2.1, Chapter 3.

Table Analysed	PAR (Porometer)				
Two-way repeated-measures ANOVA	Matching: Stacked				
Assume sphericity?	Yes				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Time x Treatment	1.108	0.9910	ns	No	
Time	66.77	<0.0001	****	Yes	
Treatment	0.2569	0.5570	ns	No	
Subject	3.988	0.2180	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Time x Treatment	4338	10	433.8	F (10, 60) = 0.2385	P=0.9910
Time	261348	10	26135	F (10, 60) = 14.37	P<0.0001
Treatment	1006	1	1006	F (1, 6) = 0.3866	P=0.5570
Subject	15610	6	2602	F (6, 60) = 1.431	P=0.2180
Residual	109118	60	1819		
Difference between column means					
Mean of C	144.1				
Mean of WS	150.8				
Difference between means	-6.761				
SE of difference	10.87				
95% CI of difference	-33.37 to 19.85				
Data summary					
Number of columns (Treatment)	2				
Number of rows (Time)	11				
Number of subjects (Subject)	8				
Number of missing values	0				

Supplementary table S21b. Multiple comparison with Bonferroni tests of photosynthetically active radiation (PAR) from control well-watered (C) vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.5.2.1, Chapter 3.

Compare each cell mean with the other cell mean in that row								
Number of families	1							
Number of comparisons per family	11							
Alpha	0.05							
Bonferroni's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value			
C - WS								
Day 1	-9.375	-99.69 to 80.94	No	ns	>0.9999			

Day 2	-8.333	-98.65 to 81.98	No	ns	>0.9999			
Day 3	-10.08	-100.4 to 80.23	No	ns	>0.9999			
Day 4	2.292	-88.02 to 92.61	No	ns	>0.9999			
Day 5	21.46	-68.86 to 111.8	No	ns	>0.9999			
Day 6	-20.83	-111.1 to 69.48	No	ns	>0.9999			
Day 7	5.833	-84.48 to 96.15	No	ns	>0.9999			
Day 8	-21.50	-111.8 to 68.81	No	ns	>0.9999			
Day 9	7.667	-82.65 to 97.98	No	ns	>0.9999			
Day 10	-13.79	-104.1 to 76.52	No	ns	>0.9999			
Day 11	-27.71	-118.0 to 62.61	No	ns	>0.9999			
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
C - WS								
Day 1	164.5	173.9	-9.375	30.74	4	4	0.3050	66.00
Day 2	99.58	107.9	-8.333	30.74	4	4	0.2711	66.00
Day 3	245.3	255.4	-10.08	30.74	4	4	0.3280	66.00
Day 4	87.08	84.79	2.292	30.74	4	4	0.07455	66.00
Day 5	153.3	131.9	21.46	30.74	4	4	0.6981	66.00
Day 6	87.50	108.3	-20.83	30.74	4	4	0.6777	66.00
Day 7	81.25	75.42	5.833	30.74	4	4	0.1898	66.00
Day 8	226.6	248.1	-21.50	30.74	4	4	0.6994	66.00
Day 9	173.9	166.2	7.667	30.74	4	4	0.2494	66.00
Day 10	139.3	153.1	-13.79	30.74	4	4	0.4487	66.00
Day 11	126.2	153.9	-27.71	30.74	4	4	0.9014	66.00

Supplementary table S22a. Two-way repeated-measures analysis of variance (ANOVA) of transpiration (*E*) from control (C) well-watered vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.5.2.2, Chapter 3.

Table Analysed	*E* (IRGA)				
Two-way repeated-measures ANOVA	Matching: Stacked				
Assume sphericity?	No				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	Geisser-Greenhouse's epsilon
Time x Treatment	16.25	0.0008	***	Yes	
Time	54.52	0.0001	***	Yes	0.3281
Treatment	0.9844	0.2053	ns	No	
Subject	2.928	0.4095	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Time x Treatment	73.95	9	8.217	F (9, 54) = 3.851	P=0.0008

Time	248.1	9	27.57	F (2.953, 17.72) = 12.92	P=0.0001
Treatment	4.480	1	4.480	F (1, 6) = 2.017	P=0.2053
Subject	13.33	6	2.221	F (6, 54) = 1.041	P=0.4095
Residual	115.2	54	2.134		
Difference between column means					
Mean of C	6.078				
Mean of WS	5.604				
Difference between means	0.4733				
SE of difference	0.3332				
95% CI of difference	-0.3421 to 1.289				
Data summary					
Number of columns (Treatment)	2				
Number of rows (Time)	10				
Number of subjects (Subject)	8				
Number of missing values	0				

Supplementary table S22b. Multiple comparison with Bonferroni tests of transpiration (*E*) from control well-watered (C) vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.5.2.2, Chapter 3.

Compare each cell mean with the other cell mean in that row								
Number of families	1							
Number of comparisons per family	10							
Alpha	0.05							
Bonferroni's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value			
C - WS								
Day 1	-2.125	-7.071 to 2.820	No	ns	>0.9999			
Day 3	-0.7540	-6.714 to 5.206	No	ns	>0.9999			
Day 4	-0.4483	-4.071 to 3.175	No	ns	>0.9999			
Day 5	-1.558	-5.746 to 2.631	No	ns	0.8390			
Day 6	1.162	-3.671 to 5.995	No	ns	>0.9999			
Day 7	2.377	-5.130 to 9.884	No	ns	>0.9999			

Day 8	4.593	1.527 to 7.659	Yes	**	0.0065			
Day 9	1.813	-1.236 to 4.863	No	ns	0.3883			
Day 10	-0.3409	-2.293 to 1.611	No	ns	>0.9999			
Day 11	0.01337	-9.352 to 9.379	No	ns	>0.9999			
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
C - WS								
Day 1	5.902	8.027	-2.125	1.104	4	4	1.926	5.580
Day 3	7.556	8.310	-0.7540	1.098	4	4	0.6869	4.155
Day 4	6.815	7.264	-0.4483	0.6774	4	4	0.6617	4.237
Day 5	6.021	7.578	-1.558	0.6419	4	4	2.426	3.384
Day 6	5.607	4.445	1.162	0.9116	4	4	1.274	4.287
Day 7	2.722	0.3448	2.377	1.727	4	4	1.376	5.918
Day 8	6.674	2.080	4.593	0.7094	4	4	6.475	5.987
Day 9	6.279	4.466	1.813	0.6676	4	4	2.716	5.389
Day 10	6.411	6.752	-0.3409	0.4156	4	4	0.8202	5.136
Day 11	6.789	6.776	0.01337	1.576	4	4	0.008482	3.731

Supplementary table S23a. Two-way repeated-measures analysis of variance (ANOVA) of net carbon assimilation rate (*NCAR*) from control (C) well-watered vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.5.2.2, Chapter 3.

Table Analysed	*NCAR* (IRGA)				
Two-way repeated-measures ANOVA	Matching: Stacked				
Assume sphericity?	No				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	Geisser-Greenhouse's epsilon
Time x Treatment	16.82	0.0009	***	Yes	
Time	49.96	0.0003	***	Yes	0.3232
Treatment	0.02184	0.8934	ns	No	
Subject	6.710	0.0493	*	Yes	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Time x Treatment	39447	9	4383	F (9, 54) = 3.808	P=0.0009
Time	117190	9	13021	F (2.908, 17.45) = 11.31	P=0.0003
Treatment	51.24	1	51.24	F (1, 6) = 0.01953	P=0.8934
Subject	15741	6	2624	F (6, 54) = 2.279	P=0.0493
Residual	62157	54	1151		
Difference between column means					
Mean of C	129.8				
Mean of WS	131.4				
Difference between means	-1.601				

SE of difference	11.45				
95% CI of difference	-29.63 to 26.42				
Data summary					
Number of columns (Treatment)	2				
Number of rows (Time)	10				
Number of subjects (Subject)	8				
Number of missing values	0				

Supplementary table S23b. Multiple comparison with Bonferroni tests of net carbon assimilation rate (*NCAR*) from control well-watered (C) vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.5.2.2, Chapter 3.

Compare each cell mean with the other cell mean in that row								
Number of families	1							
Number of comparisons per family	10							
Alpha	0.05							
Bonferroni's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value			
C - WS								
Day 1	-56.06	-269.4 to 157.3	No	ns	>0.9999			
Day 3	-19.41	-157.4 to 118.5	No	ns	>0.9999			
Day 4	-28.09	-120.7 to 64.47	No	ns	>0.9999			
Day 5	-8.435	-93.86 to 76.99	No	ns	>0.9999			
Day 6	0.04900	-163.3 to 163.4	No	ns	>0.9999			
Day 7	15.55	-63.48 to 94.59	No	ns	>0.9999			
Day 8	119.6	1.859 to 237.3	Yes	*	0.0474			
Day 9	-2.512	-196.8 to 191.7	No	ns	>0.9999			
Day 10	-9.604	-101.6 to 82.44	No	ns	>0.9999			
Day 11	-27.06	-127.0 to 72.83	No	ns	>0.9999			
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
C - WS								
Day 1	123.3	179.4	-56.06	38.89	4	4	1.442	4.101
Day 3	159.0	178.4	-19.41	19.34	4	4	1.004	3.115

Day 4	139.6	167.7	-28.09	21.44	4	4	1.311	5.997
Day 5	166.0	174.5	-8.435	19.78	4	4	0.4264	5.996
Day 6	114.7	114.7	0.04900	35.07	4	4	0.001397	5.207
Day 7	45.88	30.32	15.55	15.00	4	4	1.037	4.320
Day 8	150.9	31.31	119.6	19.81	4	4	6.036	3.731
Day 9	137.9	140.5	-2.512	32.03	4	4	0.07844	3.648
Day 10	140.0	149.6	-9.604	18.92	4	4	0.5076	4.851
Day 11	120.6	147.7	-27.06	22.89	4	4	1.182	5.870

Supplementary table S24a. Two-way repeated-measures analysis of variance (ANOVA) of vapour pressure deficit (VPD) from control (C) well-watered vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.5.2.3, Chapter 3.

Table Analysed	VPD				
Two-way repeated-measures ANOVA	Matching: Stacked				
Assume sphericity?	No				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	Geisser-Greenhouse's epsilon
Time x Treatment	26.26	<0.0001	****	Yes	
Time	46.43	<0.0001	****	Yes	0.1516
Treatment	5.422	0.0425	*	Yes	
Subject	15.24	<0.0001	****	Yes	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Time x Treatment	6.449	10	0.6449	F (10, 140) = 55.37	P<0.0001
Time	11.40	10	1.140	F (1.516, 21.22) = 97.89	P<0.0001
Treatment	1.331	1	1.331	F (1, 14) = 4.981	P=0.0425
Subject	3.742	14	0.2673	F (14, 140) = 22.95	P<0.0001
Residual	1.631	140	0.01165		
Difference between column means					
Mean of C	1.737				
Mean of WS	1.563				
Difference between means	0.1739				
SE of difference	0.07794				
95% CI of difference	0.006781 to 0.3411				
Data summary					
Number of columns (Treatment)	2				
Number of rows (Time)	11				
Number of subjects (Subject)	16				
Number of missing values	0				

Supplementary table S24b. Multiple comparison with Bonferroni tests of vapour pressure deficit (VPD) from control well-watered (C) vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.5.2.3, Chapter 3.

Compare each cell mean with the other cell mean in that row								
Number of families	1							
Number of comparisons per family	11							
Alpha	0.05							
Bonferroni's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value			
C - WS								
Day 1	1.032	0.9155 to 1.149	Yes	****	<0.0001			
Day 2	0.3642	0.2801 to 0.4484	Yes	****	<0.0001			
Day 3	0.2034	-0.1819 to 0.5887	No	ns	>0.9999			
Day 4	0.6200	0.4385 to 0.8016	Yes	****	<0.0001			
Day 5	0.1505	-0.1870 to 0.4880	No	ns	>0.9999			
Day 6	0.2265	0.04920 to 0.4039	Yes	**	0.0092			
Day 7	-0.07551	-0.1922 to 0.04119	No	ns	0.5118			
Day 8	-0.3801	-0.8656 to 0.1053	No	ns	0.2047			
Day 9	-0.2616	-0.7798 to 0.2565	No	ns	>0.9999			
Day 10	0.1361	-0.1131 to 0.3853	No	ns	0.9514			
Day 11	-0.1022	-0.5361 to 0.3317	No	ns	>0.9999			
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
C - WS								
Day 1	1.791	0.7587	1.032	0.03451	8	8	29.90	13.95
Day 2	1.283	0.9184	0.3642	0.02441	8	8	14.92	12.52
Day 3	1.948	1.745	0.2034	0.1142	8	8	1.781	14.00
Day 4	1.831	1.211	0.6200	0.05366	8	8	11.56	13.79
Day 5	1.828	1.677	0.1505	0.09895	8	8	1.521	13.22
Day 6	1.885	1.658	0.2265	0.05029	8	8	4.505	11.32
Day 7	1.603	1.679	-0.07551	0.03457	8	8	2.184	13.96
Day 8	1.699	2.080	-0.3801	0.1404	8	8	2.707	12.38
Day 9	1.852	2.114	-0.2616	0.1529	8	8	1.711	13.68
Day 10	1.658	1.522	0.1361	0.07377	8	8	1.845	13.90
Day 11	1.731	1.834	-0.1022	0.1271	8	8	0.8037	13.18

Supplementary table S25. Unpaired t-test of projected leaf area from control (C) well-watered vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.2.2.3, Chapter 3.

Table Analysed	Projected leaf area
Column B	WS
vs.	vs.
Column A	C
Unpaired t test	
P value	0.1281
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=1.595, df=18
How big is the difference?	
Mean of column A	4545
Mean of column B	4197
Difference between means (B - A) ± SEM	-348.8 ± 218.7
95% confidence interval	-808.3 to 110.6
R squared (eta squared)	0.1239
F test to compare variances	
F, DFn, Dfd	3.398, 9, 9
P value	0.0828
P value summary	ns
Significantly different (P < 0.05)?	No
Data analysed	
Sample size, column A	10
Sample size, column B	10

Supplementary table S26. Unpaired t-test of stem water potential (Ψ_s) from control (C) well-watered vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.4.2.3, Chapter 3.

Table Analysed	Ψ (pressure bomb)
Column B	WS
vs.	vs.
Column A	C
Unpaired t test	
P value	<0.0001
P value summary	****
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=10.66, df=6
How big is the difference?	
Mean of column A	0.4751
Mean of column B	1.225
Difference between means (B - A) ± SEM	0.7499 ± 0.07031
95% confidence interval	0.5778 to 0.9219
R squared (eta squared)	0.9499
F test to compare variances	
F, DFn, Dfd	4.555, 3, 3

P value	0.2448
P value summary	ns
Significantly different (P < 0.05)?	No
Data analysed	
Sample size, column A	4
Sample size, column B	4

Supplementary table S27. Unpaired t-test of projected leaf area from control (C) well-watered vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.4.2.3, Chapter 3.

Table Analysed	Projected leaf area
Column B	WS
vs.	vs.
Column A	C
Unpaired t test	
P value	0.8354
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=0.2133, df=10
How big is the difference?	
Mean of column A	6979
Mean of column B	7076
Difference between means (B - A) ± SEM	97.34 ± 456.4
95% confidence interval	-919.5 to 1114
R squared (eta squared)	0.004529
F test to compare variances	
F, DFn, Dfd	4.109, 5, 5
P value	0.1471
P value summary	ns
Significantly different (P < 0.05)?	No
Data analysed	
Sample size, column A	6
Sample size, column B	6

Supplementary table S28. Unpaired t-test of projected leaf area from control (C) well-watered vines and water-stressed (WS) vines during the drought/rehydration experiment, section 3.5.2.3, Chapter 3.

Table Analysed	Projected leaf area
Column B	WS
vs.	vs.
Column A	C
Unpaired t test	
P value	0.0803
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed

t, df	t=2.102, df=6
How big is the difference?	
Mean of column A	2613
Mean of column B	2101
Difference between means (B - A) ± SEM	-512.1 ± 243.7
95% confidence interval	-1108 to 84.05
R squared (eta squared)	0.4241
F test to compare variances	
F, DFn, Dfd	6.525, 3, 3
P value	0.1578
P value summary	ns
Significantly different (P < 0.05)?	No
Data analysed	
Sample size, column A	4
Sample size, column B	4

Supplementary table S29. Multi-linear regression of C g_s (porometer) and parameters (C PAR, VPD, time) with WS g_s of the drought experiment, section 3.2.2.4, Chapter 3.

Dependent variable	C g_s (porometer						
Regression type	Least squares						
Model							
Analysis of Variance	SS	DF	MS	F (DFn, DFd)	P value		
Regression	19544	4	4886	F (4, 11) = 4.859	P=0.0167		
WS g_s	12447	1	12447	F (1, 11) = 12.38	P=0.0048		
C PAR	9068	1	9068	F (1, 11) = 9.017	P=0.0120		
VPD	2695	1	2695	F (1, 11) = 2.680	P=0.1299		
Time	2021	1	2021	F (1, 11) = 2.010	P=0.1839		
Residual	11062	11	1006				
Total	30605	15					
Parameter estimates	Variable	Estimate	Standard error	95% CI (asymptotic)	|t|	P value	P value summary
β0	Intercept	163.5	69.01	11.58 to 315.3	2.369	0.0372	*
β1	WS g_s	0.4376	0.1244	0.1639 to 0.7114	3.518	0.0048	**
β2	C PAR	0.2077	0.06916	0.05546 to 0.3599	3.003	0.0120	*
β3	VPD	-52.13	31.84	-122.2 to 17.95	1.637	0.1299	ns
β4	Time	-3.989	2.813	-10.18 to 2.203	1.418	0.1839	ns
Goodness of Fit							
Degrees of Freedom	11						

R squared	0.6386						
Adjusted R squared	0.5071						
Multi-collinearity	Variable	VIF	R2 with other variables				
β0	Intercept						
β1	WS g_s	1.240	0.1938				
β2	C PAR	2.673	0.6259				
β3	VPD	1.105	0.09466				
β4	Time	2.676	0.6264				
Normality of Residuals	Statistics	P value	Passed normality test (alpha=0.05)?	P value summary			
Anderson-Darling (A2*)	0.1559	0.9423	Yes	ns			
D'Agostino-Pearson omnibus (K2)	0.4971	0.7799	Yes	ns			
Shapiro-Wilk (W)	0.9824	0.9799	Yes	ns			
Kolmogorov-Smirnov (distance)	0.08821	>0.1000	Yes	ns			
Data summary							
Rows in table	16						
Rows skipped (missing data)	0						
Rows analysed (# cases)	16						
Number of parameter estimates	5						
#cases/#parameters	3.2						

Supplementary table S30. Multi-linear regression of C g_s (porometer) and parameters (C PAR, VPD, time) without WS g_s of the drought experiment, section 3.2.2.4, Chapter 3.

Dependent variable	C g_s (porometer)						
Regression type	Least squares						
Model							
Analysis of Variance	SS	DF	MS	F (DFn, DFd)	P value		
Regression	7097	3	2366	F (3, 12) = 1.208	P=0.3490		
C PAR	3615	1	3615	F (1, 12) = 1.846	P=0.1993		
VPD	519.6	1	519.6	F (1, 12) = 0.2653	P=0.6159		

Time	54.15	1	54.15	F (1, 12) = 0.02764	P=0.8707		
Residual	23508	12	1959				
Total	30605	15					
Parameter estimates	Variable	Estimate	Standard error	95% CI (asymptotic)	|t|	P value	P value summary
β0	Intercept	180.0	96.09	-29.31 to 389.4	1.874	0.0855	ns
β1	C PAR	0.1229	0.09048	-0.07422 to 0.3201	1.358	0.1993	ns
β2	VPD	-22.05	42.81	-115.3 to 71.23	0.5150	0.6159	ns
β3	Time	-0.6137	3.692	-8.657 to 7.429	0.1663	0.8707	ns
Goodness of Fit							
Degrees of Freedom	12						
R squared	0.2319						
Adjusted R squared	0.03986						
Multi-collinearity	Variable	VIF	R2 with other variables				
β0	Intercept						
β1	C PAR	2.349	0.5743				
β2	VPD	1.025	0.02431				
β3	Time	2.365	0.5772				
Normality of Residuals	Statistics	P value	Passed normality test (alpha=0.05)?	P value summary			
Anderson-Darling (A2*)	0.4630	0.2224	Yes	ns			
D'Agostino-Pearson omnibus (K2)	1.442	0.4864	Yes	ns			
Shapiro-Wilk (W)	0.9305	0.2482	Yes	ns			
Kolmogorov-Smirnov (distance)	0.1315	>0.1000	Yes	ns			
Data summary							
Rows in table	16						
Rows skipped (missing data)	0						
Rows analysed (# cases)	16						
Number of parameter estimates	4						
#cases/#parameters	4.0						

Supplementary table S31. Multi-linear regression of C *E* and parameters (C PAR, VPD, time) with WS *E* of the drought experiment, section 3.2.2.4, Chapter 3.

Dependent variable	C *E*						
Regression type	Least squares						
Model							
Analysis of Variance	SS	DF	MS	F (DFn, DFd)	P value		
Regression	0.3214	4	0.08036	F (4, 3) = 13.12	P=0.0304		
WS *E*	0.08193	1	0.08193	F (1, 3) = 13.38	P=0.0353		
C PAR	0.05889	1	0.05889	F (1, 3) = 9.618	P=0.0532		
VPD	0.05413	1	0.05413	F (1, 3) = 8.841	P=0.0589		
Time	0.001442	1	0.001442	F (1, 3) = 0.2356	P=0.6607		
Residual	0.01837	3	0.006123				
Total	0.3398	7					
Parameter estimates	Variable	Estimate	Standard error	95% CI (asymptotic)	|t|	P value	P value summary
β0	Intercept	3.390	0.2502	2.593 to 4.186	13.55	0.0009	***
β1	WS *E*	0.1630	0.04456	0.02119 to 0.3048	3.658	0.0353	*
β2	C PAR	0.001099	0.0003544	-2.877e-005 to 0.002227	3.101	0.0532	ns
β3	VPD	-0.4164	0.1400	-0.8620 to 0.02928	2.973	0.0589	ns
β4	Time	-0.006803	0.01402	-0.05141 to 0.03781	0.4854	0.6607	ns
Goodness of Fit							
Degrees of Freedom	3						
R squared	0.9459						
Adjusted R squared	0.8739						
Multi-collinearity	Variable	VIF	R2 with other variables				
β0	Intercept						
β1	WS *E*	1.948	0.4866				
β2	C PAR	4.901	0.7960				
β3	VPD	1.799	0.4441				
β4	Time	5.391	0.8145				

Normality of Residuals	Statistics	P value	Passed normality test (alpha=0.05)?	P value summary			
Anderson-Darling (A2*)	0.3369	0.4027	Yes	ns			
D'Agostino-Pearson omnibus (K2)	0.8954	0.6391	Yes	ns			
Shapiro-Wilk (W)	0.9248	0.4702	Yes	ns			
Kolmogorov-Smirnov (distance)	0.2200	>0.1000	Yes	ns			
Data summary							
Rows in table	8						
Rows skipped (missing data)	0						
Rows analysed (# cases)	8						
Number of parameter estimates	5						
#cases/#parameters	1.6						

Supplementary table S32. Multi-linear regression of C *E* and parameters (C PAR, VPD, time) without WS *E* of the drought experiment, section 3.2.2.4, Chapter 3.

Dependent variable	C *E*						
Regression type	Least squares						
Model							
Analysis of Variance	SS	DF	MS	F (DFn, DFd)	P value		
Regression	0.2395	3	0.07983	F (3, 4) = 3.184	P=0.1463		
C PAR	0.01266	1	0.01266	F (1, 4) = 0.5050	P=0.5166		
VPD	0.004069	1	0.004069	F (1, 4) = 0.1623	P=0.7077		
Time	0.02168	1	0.02168	F (1, 4) = 0.8644	P=0.4051		
Residual	0.1003	4	0.02508				
Total	0.3398	7					
Parameter estimates	Variable	Estimate	Standard error	95% CI (asymptotic)	|t|	P value	P value summary

β0	Intercept	3.239	0.4994	1.852 to 4.625	6.485	0.0029	**
β1	C PAR	0.0004385	0.0006171	-0.001275 to 0.002152	0.7106	0.5166	ns
β2	VPD	-0.08752	0.2173	-0.6908 to 0.5157	0.4028	0.7077	ns
β3	Time	0.02187	0.02352	-0.04343 to 0.08716	0.9297	0.4051	ns
Goodness of Fit							
Degrees of Freedom	4						
R squared	0.7048						
Adjusted R squared	0.4834						
Multi-collinearity	Variable	VIF	R2 with other variables				
β0	Intercept						
β1	C PAR	3.629	0.7244				
β2	VPD	1.058	0.05438				
β3	Time	3.706	0.7301				
Normality of Residuals	Statistics	P value	Passed normality test (alpha=0.05)?	P value summary			
Anderson-Darling (A2*)	0.1697	0.8968	Yes	ns			
D'Agostino-Pearson omnibus (K2)	0.3650	0.8332	Yes	ns			
Shapiro-Wilk (W)	0.9785	0.9552	Yes	ns			
Kolmogorov-Smirnov (distance)	0.1519	>0.1000	Yes	ns			
Data summary							
Rows in table	8						
Rows skipped (missing data)	0						
Rows analysed (# cases)	8						
Number of parameter estimates	4						
#cases/#parameters	2.0						

Supplementary table S33. Multi-linear regression of C *NCAR* and parameters (C PAR, VPD, time) with WS *NCAR* of the drought experiment, section 3.2.2.4, Chapter 3.

Dependent variable	C *NCAR*						
Regression type	Least squares						
Model							
Analysis of Variance	SS	DF	MS	F (DFn, DFd)	P value		
Regression	0.8044	4	0.2011	F (4, 3) = 1.569	P=0.3706		
WS *NCAR*	0.1690	1	0.1690	F (1, 3) = 1.319	P=0.3341		
C PAR	0.1064	1	0.1064	F (1, 3) = 0.8304	P=0.4293		
VPD	0.7219	1	0.7219	F (1, 3) = 5.633	P=0.0982		
Time	0.06142	1	0.06142	F (1, 3) = 0.4792	P=0.5386		
Residual	0.3845	3	0.1282				
Total	1.189	7					
Parameter estimates	Variable	Estimate	Standard error	95% CI (asymptotic)	|t|	P value	P value summary
β0	Intercept	11.79	1.131	8.191 to 15.39	10.42	0.0019	**
β1	WS *NCAR*	0.08115	0.07067	-0.1438 to 0.3061	1.148	0.3341	ns
β2	C PAR	0.001496	0.001642	-0.003729 to 0.006721	0.9113	0.4293	ns
β3	VPD	-1.386	0.5839	-3.244 to 0.4724	2.373	0.0982	ns
β4	Time	-0.04452	0.06431	-0.2492 to 0.1601	0.6922	0.5386	ns
Goodness of Fit							
Degrees of Freedom	3						
R squared	0.6766						
Adjusted R squared	0.2454						
Multicollinearity	Variable	VIF	R2 with other variables				
β0	Intercept						
β1	WS *NCAR*	1.720	0.4185				
β2	C PAR	5.025	0.8010				

β3	VPD	1.494	0.3307				
β4	Time	5.421	0.8155				
Normality of Residuals	Statistics	P value	Passed normality test (alpha=0.05)?	P value summary			
Anderson-Darling (A2*)	0.3199	0.4459	Yes	ns			
D'Agostino-Pearson omnibus (K2)	0.5899	0.7446	Yes	ns			
Shapiro-Wilk (W)	0.9505	0.7166	Yes	ns			
Kolmogorov-Smirnov (distance)	0.1919	>0.1000	Yes	ns			
Data summary							
Rows in table	8						
Rows skipped (missing data)	0						
Rows analysed (# cases)	8						
Number of parameter estimates	5						
#cases/#parameters	1.6						

Supplementary table S34. Multi-linear regression of C *NCAR* and parameters (C PAR, VPD, time) without WS *NCAR* of the drought experiment, section 3.2.2.4, Chapter 3.

Dependent variable	C *NCAR*						
Regression type	Least squares						
Model							
Analysis of Variance	SS	DF	MS	F (DFn, DFd)	P value		
Regression	0.6354	3	0.2118	F (3, 4) = 1.531	P=0.3364		
C PAR	0.01663	1	0.01663	F (1, 4) = 0.1202	P=0.7463		
VPD	0.5562	1	0.5562	F (1, 4) = 4.020	P=0.1155		
Time	0.0004017	1	0.0004017	F (1, 4) = 0.002903	P=0.9596		
Residual	0.5535	4	0.1384				
Total	1.189	7					

Parameter estimates	Variable	Estimate	Standard error	95% CI (asymptotic)	|t|	P value	P value summary
β0	Intercept	11.87	1.173	8.612 to 15.13	10.12	0.0005	***
β1	C PAR	0.0005025	0.001450	-0.003522 to 0.004527	0.3466	0.7463	ns
β2	VPD	-1.023	0.5104	-2.440 to 0.3938	2.005	0.1155	ns
β3	Time	-0.002977	0.05525	-0.1564 to 0.1504	0.05388	0.9596	ns
Goodness of Fit							
Degrees of Freedom	4						
R squared	0.5345						
Adjusted R squared	0.1853						
Multi-collinearity	Variable	VIF	R2 with other variables				
β0	Intercept						
β1	C PAR	3.629	0.7244				
β2	VPD	1.058	0.05438				
β3	Time	3.706	0.7301				
Normality of Residuals	Statistics	P value	Passed normality test (alpha=0.05)?	P value summary			
Anderson-Darling (A2*)	0.2683	0.5744	Yes	ns			
D'Agostino-Pearson omnibus (K2)	0.7656	0.6819	Yes	ns			
Shapiro-Wilk (W)	0.9513	0.7240	Yes	ns			
Kolmogorov-Smirnov (distance)	0.1945	>0.1000	Yes	ns			
Data summary							
Rows in table	8						
Rows skipped (missing data)	0						
Rows analysed (# cases)	8						
Number of parameter estimates	4						

#cases/#parameters	2.0						

Supplementary table S35. Multi-linear regression of C g_s (IRGA) and parameters (C PAR, VPD, time) with WS g_s of the drought experiment, section 3.2.2.4, Chapter 3.

Dependent variable	C g_s (IRGA)						
Regression type	Least squares						
Model							
Analysis of Variance	SS	DF	MS	F (DFn, DFd)	P value		
Regression	3610	4	902.4	F (4, 3) = 2.102	P=0.2840		
WS g_s	1227	1	1227	F (1, 3) = 2.858	P=0.1895		
C PAR	45.37	1	45.37	F (1, 3) = 0.1057	P=0.7665		
VPD	1810	1	1810	F (1, 3) = 4.215	P=0.1324		
Time	14.56	1	14.56	F (1, 3) = 0.03392	P=0.8656		
Residual	1288	3	429.3				
Total	4898	7					
Parameter estimates	Variable	Estimate	Standard error	95% CI (asymptotic)	|t|	P value	P value summary
β0	Intercept	254.3	65.35	46.37 to 462.3	3.892	0.0301	*
β1	WS g_s	0.3044	0.1801	-0.2687 to 0.8776	1.690	0.1895	ns
β2	C PAR	0.03037	0.09343	-0.2670 to 0.3277	0.3251	0.7665	ns
β3	VPD	-66.12	32.20	-168.6 to 36.37	2.053	0.1324	ns
β4	Time	0.6653	3.613	-10.83 to 12.16	0.1842	0.8656	ns
Goodness of Fit							
Degrees of Freedom	3						
R squared	0.7370						
Adjusted R squared	0.3864						
Multi-collinearity	Variable	VIF	R2 with other variables				

β0	Intercept						
β1	WS g_s	1.554	0.3563				
β2	C PAR	4.858	0.7942				
β3	VPD	1.357	0.2630				
β4	Time	5.107	0.8042				
Normality of Residuals	Statistics	P value	Passed normality test (alpha=0.05)?	P value summary			
Anderson-Darling (A2*)	0.3990	0.2754	Yes	ns			
D'Agostino-Pearson omnibus (K2)	5.386	0.0677	Yes	ns			
Shapiro-Wilk (W)	0.8872	0.2203	Yes	ns			
Kolmogorov-Smirnov (distance)	0.1943	>0.1000	Yes	ns			
Data summary							
Rows in table	8						
Rows skipped (missing data)	0						
Rows analysed (# cases)	8						
Number of parameter estimates	5						
#cases/#parameters	1.6						

Supplementary table S36. Multi-linear regression of C g_s (IRGA) and parameters (C PAR, VPD, time) without WS g_s of the drought experiment, section 3.2.2.4, Chapter 3.

Dependent variable	C g_s (IRGA)						
Regression type	Least squares						
Model							
Analysis of Variance	SS	DF	MS	F (DFn, DFd)	P value		
Regression	2383	3	794.2	F (3, 4) = 1.263	P=0.3993		
C PAR	158.6	1	158.6	F (1, 4) = 0.2523	P=0.6419		
VPD	873.3	1	873.3	F (1, 4) = 1.389	P=0.3039		

Time	677.1	1	677.1	F (1, 4) = 1.077	P=0.3580		
Residual	2515	4	628.7				
Total	4898	7					
Parameter estimates	Variable	Estimate	Standard error	95% CI (asymptotic)	|t|	P value	P value summary
β0	Intercept	253.2	79.08	33.63 to 472.7	3.202	0.0328	*
β1	C PAR	-0.04908	0.09772	-0.3204 to 0.2222	0.5023	0.6419	ns
β2	VPD	-40.55	34.40	-136.1 to 54.97	1.179	0.3039	ns
β3	Time	3.865	3.724	-6.475 to 14.20	1.038	0.3580	ns
Goodness of Fit							
Degrees of Freedom	4						
R squared	0.4865						
Adjusted R squared	0.1014						
Multi-collinearity	Variable	VIF	R2 with other variables				
β0	Intercept						
β1	C PAR	3.629	0.7244				
β2	VPD	1.058	0.05438				
β3	Time	3.706	0.7301				
Normality of Residuals	Statistics	P value	Passed normality test (alpha=0.05)?	P value summary			
Anderson-Darling (A2*)	0.2271	0.7235	Yes	ns			
D'Agostino-Pearson omnibus (K2)	0.4478	0.7994	Yes	ns			
Shapiro-Wilk (W)	0.9704	0.9009	Yes	ns			
Kolmogorov-Smirnov (distance)	0.1798	>0.1000	Yes	ns			
Data summary							
Rows in table	8						
Rows skipped (missing data)	0						

Rows analysed (# cases)	8						
Number of parameter estimates	4						
#cases/#parameters	2.0						

Supplementary table S37. Multi-linear regression of C g_s (porometer) and parameters (C PAR, VPD, time) with WS g_s of the drought experiment, section 3.3.2.4, Chapter 3.

Dependent variable	C g_s (porometer)						
Regression type	Least squares						
Model							
Analysis of Variance	SS	DF	MS	F (DFn, DFd)	P value		
Regression	1489	4	372.3	F (4, 11) = 2.544	P=0.0993		
WS g_s	451.9	1	451.9	F (1, 11) = 3.088	P=0.1066		
C PAR	1017	1	1017	F (1, 11) = 6.947	P=0.0232		
VPD	3.176	1	3.176	F (1, 11) = 0.02170	P=0.8855		
Time	55.74	1	55.74	F (1, 11) = 0.3809	P=0.5497		
Residual	1610	11	146.3				
Total	3099	15					
Parameter estimates	Variable	Estimate	Standard error	95% CI (asymptotic)	|t|	P value	P value summary
β0	Intercept	35.99	41.34	-55.00 to 127.0	0.8706	0.4026	ns
β1	WS g_s	-0.1697	0.09660	-0.3824 to 0.04286	1.757	0.1066	ns
β2	C PAR	0.1491	0.05657	0.02459 to 0.2736	2.636	0.0232	*
β3	VPD	-1.868	12.68	-29.78 to 26.05	0.1473	0.8855	ns
β4	Time	0.4744	0.7687	-1.218 to 2.166	0.6172	0.5497	ns
Goodness of Fit							
Degrees of Freedom	11						
R squared	0.4805						
Adjusted R squared	0.2916						
Multi-collinearity	Variable	VIF	R2 with other variables				
β0	Intercept						
β1	WS g_s	1.929	0.4816				
β2	C PAR	2.076	0.5182				
β3	VPD	1.279	0.2183				

β4	Time	1.373	0.2717				
Normality of Residuals	Statistics	P value	Passed normality test (alpha=0.05)?	P value summary			
Anderson-Darling (A2*)	0.5155	0.1621	Yes	ns			
D'Agostino-Pearson omnibus (K2)	5.744	0.0566	Yes	ns			
Shapiro-Wilk (W)	0.9222	0.1830	Yes	ns			
Kolmogorov-Smirnov (distance)	0.1786	>0.1000	Yes	ns			
Data summary							
Rows in table	16						
Rows skipped (missing data)	0						
Rows analysed (# cases)	16						
Number of parameter estimates	5						
#cases/#parameters	3.2						

Supplementary table S38. Multi-linear regression of C g_s (porometer) and parameters (C PAR, VPD, time) without WS g_s of the drought experiment, section 3.3.2.4, Chapter 3.

Dependent variable	C g_s (porometer)						
Regression type	Least squares						
Model							
Analysis of Variance	SS	DF	MS	F (DFn, DFd)	P value		
Regression	1037	3	345.7	F (3, 12) = 2.012	P=0.1660		
C PAR	564.8	1	564.8	F (1, 12) = 3.287	P=0.0949		
VPD	3.877	1	3.877	F (1, 12) = 0.02257	P=0.8831		
Time	198.5	1	198.5	F (1, 12) = 1.155	P=0.3035		
Residual	2062	12	171.8				
Total	3099	15					
Parameter estimates	Variable	Estimate	Standard error	95% CI (asymptotic)	|t|	P value	P value summary
β0	Intercept	65.89	40.82	-23.04 to 154.8	1.614	0.1324	ns
β1	C PAR	0.08225	0.04537	-0.01659 to 0.1811	1.813	0.0949	ns
β2	VPD	-2.064	13.74	-32.01 to 27.88	0.1502	0.8831	ns

β3	Time	0.8584	0.7986	-0.8815 to 2.598	1.075	0.3035	ns
Goodness of Fit							
Degrees of Freedom	12						
R squared	0.3347						
Adjusted R squared	0.1684						
Multi-collinearity	Variable	VIF	R2 with other variables				
β0	Intercept						
β1	C PAR	1.137	0.1204				
β2	VPD	1.279	0.2183				
β3	Time	1.262	0.2076				
Normality of Residuals	Statistics	P value	Passed normality test (alpha=0.05)?	P value summary			
Anderson-Darling (A2*)	0.7019	0.0536	Yes	ns			
D'Agostino-Pearson omnibus (K2)	3.322	0.1900	Yes	ns			
Shapiro-Wilk (W)	0.8855	0.0474	No	*			
Kolmogorov-Smirnov (distance)	0.2040	0.0738	Yes	ns			
Data summary							
Rows in table	16						
Rows skipped (missing data)	0						
Rows analysed (# cases)	16						
Number of parameter estimates	4						
#cases/#parameters	4.0						

Supplementary table S39. Multi-linear regression of C *E* and parameters (C PAR, VPD, time) with WS *E* of the drought experiment, section 3.3.2.4, Chapter 3.

Dependent variable	C *E*						
Regression type	Least squares						
Model							
Analysis of Variance	SS	DF	MS	F (DFn, DFd)	P value		

Regression	0.1655	4	0.04137	F (4, 2) = 2.345	P=0.3206		
WS *E*	0.1090	1	0.1090	F (1, 2) = 6.180	P=0.1308		
C PAR	0.1091	1	0.1091	F (1, 2) = 6.183	P=0.1307		
VPD	0.007452	1	0.007452	F (1, 2) = 0.4225	P=0.5824		
Time	0.09363	1	0.09363	F (1, 2) = 5.308	P=0.1478		
Residual	0.03528	2	0.01764				
Total	0.2008	6					
Parameter estimates	Variable	Estimate	Standard error	95% CI (asymptotic)	|t|	P value	P value summary
β0	Intercept	0.7033	0.7075	-2.341 to 3.747	0.9941	0.4249	ns
β1	WS *E*	-0.2492	0.1003	-0.6806 to 0.1821	2.486	0.1308	ns
β2	C PAR	0.002164	0.0008702	-0.001580 to 0.005908	2.487	0.1307	ns
β3	VPD	0.1356	0.2086	-0.7618 to 1.033	0.6500	0.5824	ns
β4	Time	-0.03672	0.01594	-0.1053 to 0.03186	2.304	0.1478	ns
Goodness of Fit							
Degrees of Freedom	2						
R squared	0.8243						
Adjusted R squared	0.4728						
Multi-collinearity	Variable	VIF	R2 with other variables				
β0	Intercept						
β1	WS *E*	1.293	0.2266				
β2	C PAR	2.218	0.5491				
β3	VPD	1.340	0.2536				
β4	Time	1.613	0.3800				
Normality of Residuals	Statistics	P value	Passed normality test (alpha=0.05)?	P value summary			
Anderson-Darling (A2*)	N too small						

D'Agostino-Pearson omnibus (K2)	N too small						
Shapiro-Wilk (W)	0.9431	0.6671	Yes	ns			
Kolmogorov-Smirnov (distance)	0.1799	>0.1000	Yes	ns			
Data summary							
Rows in table	7						
Rows skipped (missing data)	0						
Rows analysed (# cases)	7						
Number of parameter estimates	5						

Supplementary table S40. Multi-linear regression of C *E* and parameters (C PAR, VPD, time) without WS *E* of the drought experiment, section 3.3.2.4, Chapter 3.

Dependent variable	C *E*						
Regression type	Least squares						
Model							
Analysis of Variance	SS	DF	MS	F (DFn, DFd)	P value		
Regression	0.05648	3	0.01883	F (3, 3) = 0.3914	P=0.7693		
C PAR	0.04297	1	0.04297	F (1, 3) = 0.8934	P=0.4143		
VPD	0.004917	1	0.004917	F (1, 3) = 0.1022	P=0.7701		
Time	0.04266	1	0.04266	F (1, 3) = 0.8870	P=0.4158		
Residual	0.1443	3	0.04810				
Total	0.2008	6					
Parameter estimates	Variable	Estimate	Standard error	95% CI (asymptotic)	|t|	P value	P value summary
β0	Intercept	0.9871	1.153	-2.682 to 4.656	0.8561	0.4549	ns
β1	C PAR	0.001223	0.001294	-0.002895 to 0.005341	0.9452	0.4143	ns

β2	VPD	0.1100	0.3440	-0.9847 to 1.205	0.3197	0.7701	ns
β3	Time	-0.02333	0.02477	-0.1022 to 0.05550	0.9418	0.4158	ns
Goodness of Fit							
Degrees of Freedom	3						
R squared	0.2813						
Adjusted R squared	-0.4374						
Multi-collinearity	Variable	VIF	R2 with other variables				
β0	Intercept						
β1	C PAR	1.798	0.4439				
β2	VPD	1.336	0.2518				
β3	Time	1.429	0.3000				
Normality of Residuals	Statistics	P value	Passed normality test (alpha=0.05)?	P value summary			
Anderson-Darling (A2*)	N too small						
D'Agostino-Pearson omnibus (K2)	N too small						
Shapiro-Wilk (W)	0.9113	0.4052	Yes	ns			
Kolmogorov-Smirnov (distance)	0.2474	>0.1000	Yes	ns			
Data summary							
Rows in table	7						
Rows skipped (missing data)	0						
Rows analysed (# cases)	7						
Number of parameter estimates	4						
#cases/#parameters	1.8						

Supplementary table S41. Multi-linear regression of C *NCAR* and parameters (C PAR, VPD, time) with WS *NCAR* of the drought experiment, section 3.3.2.4, Chapter 3.

Dependent variable	C *NCAR*						
Regression type	Least squares						
Model							
Analysis of Variance	SS	DF	MS	F (DFn, DFd)	P value		
Regression	2.808	4	0.7020	F (4, 2) = 4.000	P=0.2099		
WS *NCAR*	1.566	1	1.566	F (1, 2) = 8.921	P=0.0962		
C PAR	1.407	1	1.407	F (1, 2) = 8.018	P=0.1054		
VPD	0.5743	1	0.5743	F (1, 2) = 3.272	P=0.2122		
Time	0.0005074	1	0.0005074	F (1, 2) = 0.002891	P=0.9620		
Residual	0.3510	2	0.1755				
Total	3.159	6					
Parameter estimates	Variable	Estimate	Standard error	95% CI (asymptotic)	|t|	P value	P value summary
β0	Intercept	5.808	2.232	-3.795 to 15.41	2.602	0.1214	ns
β1	WS *NCAR*	-0.1558	0.05217	-0.3803 to 0.06865	2.987	0.0962	ns
β2	C PAR	0.007660	0.002705	-0.003979 to 0.01930	2.832	0.1054	ns
β3	VPD	1.193	0.6594	-1.644 to 4.030	1.809	0.2122	ns
β4	Time	0.002585	0.04808	-0.2043 to 0.2095	0.05377	0.9620	ns
Goodness of Fit							
Degrees of Freedom	2						
R squared	0.8889						
Adjusted R squared	0.6667						
Multi-collinearity	Variable	VIF	R2 with other variables				
β0	Intercept						
β1	WS *NCAR*	1.229	0.1866				
β2	C PAR	2.154	0.5357				
β3	VPD	1.346	0.2572				

β4	Time	1.475	0.3222				
Normality of Residuals	Statistics	P value	Passed normality test (alpha=0.05)?	P value summary			
Anderson-Darling (A2*)	N too small						
D'Agostino-Pearson omnibus (K2)	N too small						
Shapiro-Wilk (W)	0.9263	0.5197	Yes	ns			
Kolmogorov-Smirnov (distance)	0.2200	>0.1000	Yes	ns			
Data summary							
Rows in table	7						
Rows skipped (missing data)	0						
Rows analysed (# cases)	7						
Number of parameter estimates	5						
#cases/#parameters	1.4						

Supplementary table S42. Multi-linear regression of C *NCAR* and parameters (C PAR, VPD, time) without WS *NCAR* of the drought experiment, section 3.3.2.4, Chapter 3.

Dependent variable	C *NCAR*						
Regression type	Least squares						
Model							
Analysis of Variance	SS	DF	MS	F (DFn, DFd)	P value		
Regression	1.242	3	0.4141	F (3, 3) = 0.6483	P=0.6348		
C PAR	0.5502	1	0.5502	F (1, 3) = 0.8612	P=0.4218		
VPD	0.4267	1	0.4267	F (1, 3) = 0.6680	P=0.4736		
Time	0.06211	1	0.06211	F (1, 3) = 0.09722	P=0.7756		
Residual	1.917	3	0.6388				
Total	3.159	6					

Parameter estimates	Variable	Estimate	Standard error	95% CI (asymptotic)	|t|	P value	P value summary
β0	Intercept	6.890	4.202	-6.482 to 20.26	1.640	0.1996	ns
β1	C PAR	0.004376	0.004716	-0.01063 to 0.01938	0.9280	0.4218	ns
β2	VPD	1.025	1.254	-2.965 to 5.014	0.8173	0.4736	ns
β3	Time	0.02815	0.09027	-0.2591 to 0.3154	0.3118	0.7756	ns
Goodness of Fit							
Degrees of Freedom	3						
R squared	0.3933						
Adjusted R squared	-0.2134						
Multi-collinearity	Variable	VIF	R2 with other variables				
β0	Intercept						
β1	C PAR	1.798	0.4439				
β2	VPD	1.336	0.2518				
β3	Time	1.429	0.3000				
Normality of Residuals	Statistics	P value	Passed normality test (alpha=0.05)?	P value summary			
Anderson-Darling (A2*)	N too small						
D'Agostino-Pearson omnibus (K2)	N too small						
Shapiro-Wilk (W)	0.9099	0.3954	Yes	ns			
Kolmogorov-Smirnov (distance)	0.2156	>0.1000	Yes	ns			
Data summary							
Rows in table	7						
Rows skipped (missing data)	0						
Rows analysed (# cases)	7						
Number of parameter estimates	4						
#cases/#parameters	1.8						

Supplementary table S43. Multi-linear regression of C g_s (IRGA) and parameters (C PAR, VPD, time) with WS g_s of the drought experiment, section 3.3.2.4, Chapter 3.

Dependent variable	C g_s (IRGA)						
Regression type	Least squares						
Model							
Analysis of Variance	SS	DF	MS	F (DFn, DFd)	P value		
Regression	829.4	4	207.4	F (4, 2) = 1.304	P=0.4775		
WS g_s	94.76	1	94.76	F (1, 2) = 0.5960	P=0.5209		
C PAR	527.7	1	527.7	F (1, 2) = 3.319	P=0.2101		
VPD	26.75	1	26.75	F (1, 2) = 0.1683	P=0.7214		
Time	1.003	1	1.003	F (1, 2) = 0.006305	P=0.9439		
Residual	318.0	2	159.0				
Total	1147	6					
Parameter estimates	Variable	Estimate	Standard error	95% CI (asymptotic)	|t|	P value	P value summary
β0	Intercept	4.381	66.99	-283.8 to 292.6	0.06539	0.9538	ns
β1	WS g_s	-0.1090	0.1412	-0.7167 to 0.4987	0.7720	0.5209	ns
β2	C PAR	0.1485	0.08154	-0.2023 to 0.4994	1.822	0.2101	ns
β3	VPD	8.136	19.84	-77.21 to 93.48	0.4102	0.7214	ns
β4	Time	0.1141	1.436	-6.066 to 6.295	0.07941	0.9439	ns
Goodness of Fit							
Degrees of Freedom	2						
R squared	0.7229						
Adjusted R squared	0.1686						
Multi-collinearity	Variable	VIF	R2 with other variables				
β0	Intercept						

β1	WS g_s	1.346	0.2568				
β2	C PAR	2.160	0.5370				
β3	VPD	1.344	0.2562				
β4	Time	1.453	0.3120				
Normality of Residuals	Statistics	P value	Passed normality test (alpha=0.05)?	P value summary			
Anderson-Darling (A2*)	N too small						
D'Agostino-Pearson omnibus (K2)	N too small						
Shapiro-Wilk (W)	0.9498	0.7283	Yes	ns			
Kolmogorov-Smirnov (distance)	0.1874	>0.1000	Yes	ns			
Data summary							
Rows in table	7						
Rows skipped (missing data)	0						
Rows analysed (# cases)	7						
Number of parameter estimates	5						
#cases/#parameters	1.4						

Supplementary table S44. Multi-linear regression of C g_s (IRGA) and parameters (C PAR, VPD, time) without WS g_s of the drought experiment, section 3.3.2.4, Chapter 3.

Dependent variable	C g_s (IRGA)						
Regression type	Least squares						
Model							
Analysis of Variance	SS	DF	MS	F (DFn, DFd)	P value		
Regression	734.7	3	244.9	F (3, 3) = 1.780	P=0.3238		
C PAR	433.0	1	433.0	F (1, 3) = 3.147	P=0.1741		
VPD	35.27	1	35.27	F (1, 3) = 0.2563	P=0.6475		
Time	5.259	1	5.259	F (1, 3) = 0.03822	P=0.8575		

Residual	412.8	3	137.6				
Total	1147	6					
Parameter estimates	Variable	Estimate	Standard error	95% CI (asymptotic)	|t|	P value	P value summary
β0	Intercept	11.82	61.67	-184.4 to 208.1	0.1917	0.8602	ns
β1	C PAR	0.1228	0.06921	-0.09747 to 0.3430	1.774	0.1741	ns
β2	VPD	9.314	18.40	-49.23 to 67.86	0.5063	0.6475	ns
β3	Time	0.2590	1.325	-3.957 to 4.475	0.1955	0.8575	ns
Goodness of Fit							
Degrees of Freedom	3						
R squared	0.6403						
Adjusted R squared	0.2805						
Multi-collinearity	Variable	VIF	R2 with other variables				
β0	Intercept						
β1	C PAR	1.798	0.4439				
β2	VPD	1.336	0.2518				
β3	Time	1.429	0.3000				
Normality of Residuals	Statistics	P value	Passed normality test (alpha=0.05)?	P value summary			
Anderson-Darling (A2*)	N too small						
D'Agostino-Pearson omnibus (K2)	N too small						
Shapiro-Wilk (W)	0.9332	0.5780	Yes	ns			
Kolmogorov-Smirnov (distance)	0.2077	>0.1000	Yes	ns			
Data summary							
Rows in table	7						
Rows skipped (missing data)	0						
Rows analysed (# cases)	7						

Number of parameter estimates	4						
#cases/#parameters	1.8						

Supplementary table S45. Multi-linear regression of C g_s (porometer) and parameters (C PAR, VPD, time) with WS g_s of the drought experiment, section 3.4.2.5, Chapter 3.

Dependent variable	C g_s (porometer)						
Regression type	Least squares						
Model							
Analysis of Variance	SS	DF	MS	F (DFn, DFd)	P value		
Regression	88698	4	22174	F (4, 14) = 20.77	P<0.0001		
WS g_s	5640	1	5640	F (1, 14) = 5.284	P=0.0374		
C PAR	6851	1	6851	F (1, 14) = 6.418	P=0.0239		
VPD	3903	1	3903	F (1, 14) = 3.657	P=0.0765		
Time	5006	1	5006	F (1, 14) = 4.690	P=0.0481		
Residual	14944	14	1067				
Total	103642	18					
Parameter estimates	Variable	Estimate	Standard error	95% CI (asymptotic)	|t|	P value	P value summary
β0	Intercept	278.8	60.10	149.9 to 407.8	4.640	0.0004	***
β1	WS g_s	0.1680	0.07308	0.01124 to 0.3247	2.299	0.0374	*
β2	C PAR	0.5559	0.2194	0.08528 to 1.027	2.533	0.0239	*
β3	VPD	-89.19	46.64	-189.2 to 10.85	1.912	0.0765	ns
β4	Time	-5.336	2.464	-10.62 to -0.05111	2.166	0.0481	*
Goodness of Fit							
Degrees of Freedom	14						
Multiple R	0.9251						
R squared	0.8558						
Adjusted R squared	0.8146						
Multi-collinearity	Variable	VIF	R2 with other variables				
β0	Intercept						
β1	WS g_s	1.767	0.4339				
β2	C PAR	1.662	0.3983				
β3	VPD	1.991	0.4978				
β4	Time	3.242	0.6915				

Normality of Residuals	Statistics	P value	Passed normality test (alpha=0.05)?	P value summary			
Anderson-Darling (A2*)	0.4558	0.2381	Yes	ns			
D'Agostino-Pearson omnibus (K2)	2.887	0.2360	Yes	ns			
Shapiro-Wilk (W)	0.9216	0.1212	Yes	ns			
Kolmogorov-Smirnov (distance)	0.1346	>0.1000	Yes	ns			
Data summary							
Rows in table	19						
Rows skipped (missing data)	0						
Rows analysed (# cases)	19						
Number of parameter estimates	5						
#cases/#parameters	3.8						

Supplementary table S46. Multi-linear regression of C g_s (porometer) and parameters (C PAR, VPD, time) without WS g_s of the drought experiment, section 3.4.2.5, Chapter 3.

Dependent variable	C g_s (porometer)						
Regression type	Least squares						
Model							
Analysis of Variance	SS	DF	MS	F (DFn, DFd)	P value		
Regression	83058	3	27686	F (3, 15) = 20.18	P<0.0001		
C PAR	5900	1	5900	F (1, 15) = 4.300	P=0.0558		
VPD	2045	1	2045	F (1, 15) = 1.490	P=0.2410		
Time	20091	1	20091	F (1, 15) = 14.64	P=0.0017		
Residual	20584	15	1372				
Total	103642	18					
Parameter estimates	Variable	Estimate	Standard error	95% CI (asymptotic)	|t|	P value	P value summary
β0	Intercept	315.2	65.75	175.1 to 455.3	4.794	0.0002	***
β1	C PAR	0.5142	0.2480	-0.01435 to 1.043	2.074	0.0558	ns
β2	VPD	-62.54	51.22	-171.7 to 46.64	1.221	0.2410	ns
β3	Time	-8.657	2.263	-13.48 to -3.835	3.826	0.0017	**

Goodness of Fit							
Degrees of Freedom	15						
R squared	0.8014						
Adjusted R squared	0.7617						
Multi-collinearity	Variable	VIF	R2 with other variables				
β0	Intercept						
β1	CPAR	1.651	0.3941				
β2	VPD	1.868	0.4647				
β3	Time	2.126	0.5297				
Normality of Residuals	Statistics	P value	Passed normality test (alpha=0.05)?	P value summary			
Anderson-Darling (A2*)	0.2603	0.6704	Yes	ns			
D'Agostino-Pearson omnibus (K2)	0.04239	0.9790	Yes	ns			
Shapiro-Wilk (W)	0.9748	0.8664	Yes	ns			
Kolmogorov-Smirnov (distance)	0.1369	>0.1000	Yes	ns			
Data summary							
Rows in table	19						
Rows skipped (missing data)	0						
Rows analysed (# cases)	19						
Number of parameter estimates	4						
#cases/#parameters	4.8						

Supplementary table S47. Multi-linear regression of C *E* and parameters (C PAR, VPD, time) with WS *E* of the drought experiment, section 3.4.2.5, Chapter 3.

Dependent variable	C *E*						
Regression type	Least squares						
Model							
Analysis of Variance	SS	DF	MS	F (DFn, DFd)	P value		

Regression	1.138	4	0.2846	F (4, 2) = 12.12	P=0.0777		
WS *E*	0.01203	1	0.01203	F (1, 2) = 0.5123	P=0.5484		
CPAR	0.01235	1	0.01235	F (1, 2) = 0.5257	P=0.5438		
VPD	0.03270	1	0.03270	F (1, 2) = 1.392	P=0.3594		
Time	0.4694	1	0.4694	F (1, 2) = 19.98	P=0.0466		
Residual	0.04698	2	0.02349				
Total	1.185	6					
Parameter estimates	Variable	Estimate	Standard error	95% CI (asymptotic)	|t|	P value	P value summary
β0	Intercept	2.891	1.026	-1.525 to 7.306	2.817	0.1063	ns
β1	WS *E*	-0.07668	0.1071	-0.5377 to 0.3843	0.7157	0.5484	ns
β2	CPAR	-0.001596	0.002202	-0.01107 to 0.007876	0.7250	0.5438	ns
β3	VPD	0.6623	0.5613	-1.753 to 3.078	1.180	0.3594	ns
β4	Time	-0.1125	0.02517	-0.2208 to -0.004214	4.470	0.0466	*
Goodness of Fit							
Degrees of Freedom	2						
R squared	0.9604						
Adjusted R squared	0.8811						
Multi-collinearity	Variable	VIF	R2 with other variables				
β0	Intercept						
β1	WS *E*	2.014	0.5034				
β2	CPAR	2.189	0.5431				
β3	VPD	1.376	0.2733				
β4	Time	3.020	0.6688				
Normality of Residuals	Statistics	P value	Passed normality test (alpha=0.05)?	P value summary			
Anderson-Darling (A2*)	N too small						

D'Agostino-Pearson omnibus (K2)	N too small						
Shapiro-Wilk (W)	0.8725	0.1953	Yes	ns			
Kolmogorov-Smirnov (distance)	0.2455	>0.1000	Yes	ns			
Data summary							
Rows in table	7						
Rows skipped (missing data)	0						
Rows analysed (# cases)	7						
Number of parameter estimates	5						
#cases/#parameters	1.4						

Supplementary table S48. Multi-linear regression of C *E* and parameters (C PAR, VPD, time) without WS *E* of the drought experiment, section 3.4.2.5, Chapter 3.

Dependent variable	C *E*						
Regression type	Least squares						
Model							
Analysis of Variance	SS	DF	MS	F (DFn, DFd)	P value		
Regression	1.126	3	0.3755	F (3, 3) = 19.09	P=0.0186		
CPAR	0.006669	1	0.006669	F (1, 3) = 0.3390	P=0.6013		
VPD	0.04548	1	0.04548	F (1, 3) = 2.312	P=0.2257		
Time	0.6947	1	0.6947	F (1, 3) = 35.31	P=0.0095		
Residual	0.05902	3	0.01967				
Total	1.185	6					
Parameter estimates	Variable	Estimate	Standard error	95% CI (asymptotic)	|t|	P value	P value summary
β0	Intercept	2.409	0.7083	0.1542 to 4.663	3.400	0.0425	*
β1	CPAR	-0.001118	0.001919	-0.007226 to 0.004991	0.5823	0.6013	ns
β2	VPD	0.7584	0.4988	-0.8290 to 2.346	1.520	0.2257	ns

β3	Time	-0.1002	0.01687	-0.1539 to -0.04656	5.943	0.0095	**
Goodness of Fit							
Degrees of Freedom	3						
R squared	0.9502						
Adjusted R squared	0.9004						
Multi-collinearity	Variable	VIF	R2 with other variables				
β0	Intercept						
β1	CPAR	1.987	0.4967				
β2	VPD	1.297	0.2292				
β3	Time	1.620	0.3826				
Normality of Residuals	Statistics	P value	Passed normality test (alpha=0.05)?	P value summary			
Anderson-Darling (A2*)	N too small						
D'Agostino-Pearson omnibus (K2)	N too small						
Shapiro-Wilk (W)	0.9746	0.9296	Yes	ns			
Kolmogorov-Smirnov (distance)	0.1733	>0.1000	Yes	ns			
Data summary							
Rows in table	7						
Rows skipped (missing data)	0						
Rows analysed (# cases)	7						
Number of parameter estimates	4						
#cases/#parameters	1.8						

Supplementary table S49. Multi-linear regression of C *NCAR* and parameters (C PAR, VPD, time) with WS *NCAR* of the drought experiment, section 3.4.2.5, Chapter 3.

Dependent variable	C *NCAR*						

Regression type	Least squares						
Model							
Analysis of Variance	SS	DF	MS	F (DFn, DFd)	P value		
Regression	8.312	4	2.078	F (4, 2) = 6.857	P=0.1313		
WS *NCAR*	0.08788	1	0.08788	F (1, 2) = 0.2900	P=0.6441		
CPAR	1.557	1	1.557	F (1, 2) = 5.137	P=0.1516		
VPD	2.841	1	2.841	F (1, 2) = 9.373	P=0.0922		
Time	4.237	1	4.237	F (1, 2) = 13.98	P=0.0647		
Residual	0.6061	2	0.3031				
Total	8.918	6					
Parameter estimates	Variable	Estimate	Standard error	95% CI (asymptotic)	|t|	P value	P value summary
β0	Intercept	11.19	3.469	-3.731 to 26.12	3.227	0.0841	ns
β1	WS *NCAR*	-0.03054	0.05670	-0.2745 to 0.2134	0.5385	0.6441	ns
β2	CPAR	-0.01807	0.007974	-0.05238 to 0.01624	2.266	0.1516	ns
β3	VPD	6.116	1.998	-2.479 to 14.71	3.062	0.0922	ns
β4	Time	-0.2896	0.07745	-0.6228 to 0.04365	3.739	0.0647	ns
Goodness of Fit							
Degrees of Freedom	2						
R squared	0.9320						
Adjusted R squared	0.7961						
Multi-collinearity	Variable	VIF	R2 with other variables				
β0	Intercept						
β1	WS *NCAR*	1.461	0.3156				
β2	CPAR	2.226	0.5507				
β3	VPD	1.351	0.2599				
β4	Time	2.217	0.5489				

Normality of Residuals	Statistics	P value	Passed normality test (alpha=0.05)?	P value summary			
Anderson-Darling (A2*)	N too small						
D'Agostino-Pearson omnibus (K2)	N too small						
Shapiro-Wilk (W)	0.9740	0.9255	Yes	ns			
Kolmogorov-Smirnov (distance)	0.1392	>0.1000	Yes	ns			
Data summary							
Rows in table	7						
Rows skipped (missing data)	0						
Rows analysed (# cases)	7						
Number of parameter estimates	5						
#cases/#parameters	1.4						

Supplementary table S50. Multi-linear regression of C *NCAR* and parameters (C PAR, VPD, time) without WS *NCAR* of the drought experiment, section 3.4.2.5, Chapter 3.

Dependent variable	C *NCAR*						
Regression type	Least squares						
Model							
Analysis of Variance	SS	DF	MS	F (DFn, DFd)	P value		
Regression	8.224	3	2.741	F (3, 3) = 11.85	P=0.0360		
CPAR	1.483	1	1.483	F (1, 3) = 6.411	P=0.0853		
VPD	3.169	1	3.169	F (1, 3) = 13.70	P=0.0342		
Time	4.964	1	4.964	F (1, 3) = 21.46	P=0.0189		
Residual	0.6940	3	0.2313				
Total	8.918	6					
Parameter estimates	Variable	Estimate	Standard error	95% CI (asymptotic)	|t|	P value	P value summary

β0	Intercept	10.08	2.429	2.346 to 17.81	4.148	0.0255	*
β1	CPAR	-0.01667	0.006582	-0.03761 to 0.004282	2.532	0.0853	ns
β2	VPD	6.331	1.710	0.8875 to 11.77	3.701	0.0342	*
β3	Time	-0.2679	0.05784	-0.4520 to -0.08386	4.632	0.0189	*
Goodness of Fit							
Degrees of Freedom	3						
R squared	0.9222						
Adjusted R squared	0.8444						
Multicollinearity	Variable	VIF	R2 with other variables				
β0	Intercept						
β1	CPAR	1.987	0.4967				
β2	VPD	1.297	0.2292				
β3	Time	1.620	0.3826				
Normality of Residuals	Statistics	P value	Passed normality test (alpha=0.05)?	P value summary			
Anderson-Darling (A2*)	N too small						
D'Agostino-Pearson omnibus (K2)	N too small						
Shapiro-Wilk (W)	0.9253	0.5113	Yes	ns			
Kolmogorov-Smirnov (distance)	0.1995	>0.1000	Yes	ns			
Data summary							
Rows in table	7						
Rows skipped (missing data)	0						
Rows analysed (# cases)	7						
Number of parameter estimates	4						
#cases/#parameters	1.8						

Supplementary table S51. Multi-linear regression of C g_s (IRGA) and parameters (C PAR, VPD, time) with WS g_s of the drought experiment, section 3.4.2.5, Chapter 3.

Dependent variable	C g_s (IRGA)						
Regression type	Least squares						
Model							
Analysis of Variance	SS	DF	MS	F (DFn, DFd)	P value		
Regression	0.005562	4	0.001390	F (4, 2) = 0.5766	P=0.7132		
WS g_s	0.0003556	1	0.0003556	F (1, 2) = 0.1475	P=0.7380		
CPAR	8.163e-005	1	8.163e-005	F (1, 2) = 0.03385	P=0.8710		
VPD	0.001006	1	0.001006	F (1, 2) = 0.4173	P=0.5845		
Time	0.0006103	1	0.0006103	F (1, 2) = 0.2531	P=0.6649		
Residual	0.004823	2	0.002412				
Total	0.01039	6					
Parameter estimates	Variable	Estimate	Standard error	95% CI (asymptotic)	|t|	P value	P value summary
β0	Intercept	0.4763	0.3807	-1.162 to 2.114	1.251	0.3375	ns
β1	WS g_s	0.1509	0.3929	-1.540 to 1.841	0.3840	0.7380	ns
β2	CPAR	-0.0001246	0.0006771	-0.003038 to 0.002789	0.1840	0.8710	ns
β3	VPD	-0.1373	0.2126	-1.052 to 0.7772	0.6460	0.5845	ns
β4	Time	-0.003597	0.007151	-0.03436 to 0.02717	0.5030	0.6649	ns
Goodness of Fit							
Degrees of Freedom	2						
R squared	0.5356						
Adjusted R squared	-0.3933						
Multi-collinearity	Variable	VIF	R2 with other variables				
β0	Intercept						
β1	WS g_s	2.075	0.5181				

β2	CPAR	2.017	0.5041				
β3	VPD	1.922	0.4797				
β4	Time	2.375	0.5789				
Normality of Residuals	Statistics	P value	Passed normality test (alpha=0.05)?	P value summary			
Anderson-Darling (A2*)	N too small						
D'Agostino-Pearson omnibus (K2)	N too small						
Shapiro-Wilk (W)	0.9600	0.8183	Yes	ns			
Kolmogorov-Smirnov (distance)	0.1584	>0.1000	Yes	ns			
Data summary							
Rows in table	7						
Rows skipped (missing data)	0						
Rows analysed (# cases)	7						
Number of parameter estimates	5						
#cases/#parameters	1.4						

Supplementary table S52. Multi-linear regression of C g_s (IRGA) and parameters (C PAR, VPD, time) without WS g_s of the drought experiment, section 3.4.2.5, Chapter 3.

Dependent variable	C g_s (IRGA)						
Regression type	Least squares						
Model							
Analysis of Variance	SS	DF	MS	F (DFn, DFd)	P value		
Regression	0.005206	3	0.001735	F (3, 3) = 1.005	P=0.4983		
CPAR	0.0001302	1	0.0001302	F (1, 3) = 0.07543	P=0.8014		
VPD	0.002672	1	0.002672	F (1, 3) = 1.548	P=0.3018		
Time	0.001831	1	0.001831	F (1, 3) = 1.060	P=0.3789		
Residual	0.005179	3	0.001726				

Total	0.01039	6					
Parameter estimates	Variable	Estimate	Standard error	95% CI (asymptotic)	|t|	P value	P value summary
β0	Intercept	0.5872	0.2098	-0.08061 to 1.255	2.798	0.0679	ns
β1	CPAR	-0.0001562	0.0005686	-0.001966 to 0.001653	0.2746	0.8014	ns
β2	VPD	-0.1838	0.1478	-0.6541 to 0.2864	1.244	0.3018	ns
β3	Time	-0.005145	0.004997	-0.02105 to 0.01076	1.030	0.3789	ns
Goodness of Fit							
Degrees of Freedom	3						
R squared	0.5013						
Adjusted R squared	0.002622						
Multi-collinearity	Variable	VIF	R2 with other variables				
β0	Intercept						
β1	CPAR	1.987	0.4967				
β2	VPD	1.297	0.2292				
β3	Time	1.620	0.3826				
Normality of Residuals	Statistics	P value	Passed normality test (alpha=0.05)?	P value summary			
Anderson-Darling (A2*)	N too small						
D'Agostino-Pearson omnibus (K2)	N too small						
Shapiro-Wilk (W)	0.9229	0.4920	Yes	ns			
Kolmogorov-Smirnov (distance)	0.2435	>0.1000	Yes	ns			
Data summary							
Rows in table	7						
Rows skipped (missing data)	0						
Rows analysed (# cases)	7						

Number of parameter estimates	4						
#cases/#parameters	1.8						

Supplementary table S53. Multi-linear regression of C g_s and parameters (C PAR, VPD, time) with WS g_s of the drought experiment, section 3.5.2.4, Chapter 3.

Dependent variable	C g_s						
Regression type	Least squares						
Model							
Analysis of Variance	SS	DF	MS	F (DFn, DFd)	P value		
Regression	49815	4	12454	F (4, 6) = 19.49	P=0.0014		
WS	3139	1	3139	F (1, 6) = 4.913	P=0.0685		
CPAR	27553	1	27553	F (1, 6) = 43.12	P=0.0006		
VPD	677.8	1	677.8	F (1, 6) = 1.061	P=0.3428		
Time	1507	1	1507	F (1, 6) = 2.359	P=0.1755		
Residual	3834	6	638.9				
Total	53649	10					
Parameter estimates	Variable	Estimate	Standard error	95% CI (asymptotic)	|t|	P value	P value summary
β0	Intercept	229.5	77.17	40.69 to 418.3	2.974	0.0248	*
β1	WS g_s	0.1393	0.06284	-0.01448 to 0.2931	2.216	0.0685	ns
β2	C PAR	1.059	0.1612	0.6643 to 1.453	6.567	0.0006	***
β3	VPD	-49.96	48.51	-168.6 to 68.73	1.030	0.3428	ns
β4	Time	-5.183	3.375	-13.44 to 3.075	1.536	0.1755	ns
Goodness of Fit							
Degrees of Freedom	6						
R squared	0.9285						
Adjusted R squared	0.8809						
Multi-collinearity	Variable	VIF	R2 with other variables				
β0	Intercept						
β1	WS	1.971	0.4927				
β2	CPAR	1.268	0.2117				
β3	VPD	1.332	0.2494				
β4	Time	1.961	0.4900				

Normality of Residuals	Statistics	P value	Passed normality test (alpha=0.05)?	P value summary			
Anderson-Darling (A2*)	0.6306	0.0735	Yes	ns			
D'Agostino-Pearson omnibus (K2)	2.884	0.2364	Yes	ns			
Shapiro-Wilk (W)	0.8798	0.1033	Yes	ns			
Kolmogorov-Smirnov (distance)	0.2366	0.0858	Yes	ns			
Data summary							
Rows in table	11						
Rows skipped (missing data)	0						
Rows analysed (# cases)	11						
Number of parameter estimates	5						
#cases/#parameters	2.2						

Supplementary table S54. Multi-linear regression of C g_s and parameters (C PAR, VPD, time) without WS g_s of the drought experiment, section 3.5.2.4, Chapter 3.

Dependent variable	C g_s						
Regression type	Least squares						
Model							
Analysis of Variance	SS	DF	MS	F (DFn, DFd)	P value		
Regression	46676	3	15559	F (3, 7) = 15.62	P=0.0017		
C PAR	28622	1	28622	F (1, 7) = 28.73	P=0.0011		
VPD	225.2	1	225.2	F (1, 7) = 0.2261	P=0.6489		
Time	11649	1	11649	F (1, 7) = 11.69	P=0.0111		
Residual	6973	7	996.1				
Total	53649	10					
Parameter estimates	Variable	Estimate	Standard error	95% CI (asymptotic)	|t|	P value	P value summary
β0	Intercept	256.1	95.18	31.08 to 481.2	2.691	0.0310	*
β1	C PAR	1.078	0.2010	0.6023 to 1.553	5.360	0.0011	**
β2	VPD	-28.20	59.31	-168.4 to 112.0	0.4755	0.6489	ns
β3	Time	-10.37	3.034	-17.55 to -3.201	3.420	0.0111	*
Goodness of Fit							
Degrees of Freedom	7						

R squared	0.8700						
Adjusted R squared	0.8143						
Multi-collinearity	Variable	VIF	R2 with other variables				
β0	Intercept						
β1	C PAR	1.265	0.2095				
β2	VPD	1.278	0.2173				
β3	Time	1.016	0.01615				
Normality of Residuals	Statistics	P value	Passed normality test (alpha=0.05)?	P value summary			
Anderson-Darling (A2*)	0.2094	0.8141	Yes	ns			
D'Agostino-Pearson omnibus (K2)	0.5235	0.7697	Yes	ns			
Shapiro-Wilk (W)	0.9642	0.8227	Yes	ns			
Kolmogorov-Smirnov (distance)	0.1496	>0.1000	Yes	ns			
Data summary							
Rows in table	11						
Rows skipped (missing data)	0						
Rows analysed (# cases)	11						
Number of parameter estimates	4						
#cases/#parameters	2.8						

Supplementary table S55. Multi-linear regression of C *E* and parameters (C PAR, VPD, time) with WS *E* of the drought experiment, section 3.5.2.4, Chapter 3.

Dependent variable	C *E*						
Regression type	Least squares						
Model							
Analysis of Variance	SS	DF	MS	F (DFn, DFd)	P value		
Regression	0.002884	4	0.0007209	F (4, 5) = 4.802	P=0.0579		
WS *E*	0.0008565	1	0.0008565	F (1, 5) = 5.705	P=0.0625		
C PAR	0.0004151	1	0.0004151	F (1, 5) = 2.765	P=0.1572		

VPD	0.0001730	1	0.0001730	F (1, 5) = 1.152	P=0.3322		
Time	0.0003959	1	0.0003959	F (1, 5) = 2.637	P=0.1653		
Residual	0.0007507	5	0.0001501				
Total	0.003634	9					
Parameter estimates	Variable	Estimate	Standard error	95% CI (asymptotic)	|t|	P value	P value summary
β0	Intercept	-0.06040	0.08368	-0.2755 to 0.1547	0.7218	0.5028	ns
β1	WS *E*	0.2881	0.1206	-0.02197 to 0.5982	2.388	0.0625	ns
β2	C PAR	0.0001327	7.980e-005	-7.244e-005 to 0.0003378	1.663	0.1572	ns
β3	VPD	0.05212	0.04856	-0.07270 to 0.1769	1.073	0.3322	ns
β4	Time	0.002354	0.001450	-0.001372 to 0.006081	1.624	0.1653	ns
Goodness of Fit							
Degrees of Freedom	5						
R squared	0.7934						
Adjusted R squared	0.6282						
Multi-collinearity	Variable	VIF	R2 with other variables				
β0	Intercept						
β1	WS *E*	1.506	0.3361				
β2	C PAR	1.230	0.1871				
β3	VPD	1.833	0.4544				
β4	Time	1.294	0.2270				
Normality of Residuals	Statistics	P value	Passed normality test (alpha=0.05)?	P value summary			
Anderson-Darling (A2*)	0.4284	0.2467	Yes	ns			
D'Agostino-Pearson omnibus (K2)	1.634	0.4417	Yes	ns			
Shapiro-Wilk (W)	0.8991	0.2142	Yes	ns			
Kolmogorov-Smirnov (distance)	0.1900	>0.1000	Yes	ns			

Data summary							
Rows in table	10						
Rows skipped (missing data)	0						
Rows analysed (# cases)	10						
Number of parameter estimates	5						
#cases/#parameters	2.0						

Supplementary table S56. Multi-linear regression of C *E* and parameters (C PAR, VPD, time) without WS *E* of the drought experiment, section 3.5.2.4, Chapter 3.

Dependent variable	C *E*						
Regression type	Least squares						
Model							
Analysis of Variance	SS	DF	MS	F (DFn, DFd)	P value		
Regression	0.002027	3	0.0006757	F (3, 6) = 2.523	P=0.1544		
C PAR	0.0003884	1	0.0003884	F (1, 6) = 1.450	P=0.2739		
VPD	0.0008238	1	0.0008238	F (1, 6) = 3.075	P=0.1300		
Time	0.0002011	1	0.0002011	F (1, 6) = 0.7509	P=0.4195		
Residual	0.001607	6	0.0002679				
Total	0.003634	9					
Parameter estimates	Variable	Estimate	Standard error	95% CI (asymptotic)	|t|	P value	P value summary
β0	Intercept	-0.1216	0.1064	-0.3819 to 0.1388	1.142	0.2969	ns
β1	C PAR	0.0001283	0.0001066	-0.0001324 to 0.0003891	1.204	0.2739	ns
β2	VPD	0.1025	0.05843	-0.04050 to 0.2454	1.754	0.1300	ns
β3	Time	0.001642	0.001895	-0.002995 to 0.006279	0.8665	0.4195	ns
Goodness of Fit							
Degrees of Freedom	6						

R squared	0.5578						
Adjusted R squared	0.3367						
Multi-collinearity	Variable	VIF	R2 with other variables				
β0	Intercept						
β1	C PAR	1.229	0.1866				
β2	VPD	1.487	0.3277				
β3	Time	1.239	0.1929				
Normality of Residuals	Statistics	P value	Passed normality test (alpha=0.05)?	P value summary			
Anderson-Darling (A2*)	0.1599	0.9240	Yes	ns			
D'Agostino-Pearson omnibus (K2)	0.2299	0.8914	Yes	ns			
Shapiro-Wilk (W)	0.9782	0.9548	Yes	ns			
Kolmogorov-Smirnov (distance)	0.1329	>0.1000	Yes	ns			
Data summary							
Rows in table	10						
Rows skipped (missing data)	0						
Rows analysed (# cases)	10						
Number of parameter estimates	4						
#cases/#parameters	2.5						

Supplementary table S57. Multi-linear regression of C *NCAR* and parameters (C PAR, VPD, time) with WS *NCAR* of the drought experiment, section 3.5.2.4, Chapter 3.

Dependent variable	C *NCAR*						
Regression type	Least squares						
Model							
Analysis of Variance	SS	DF	MS	F (DFn, DFd)	P value		
Regression	0.2687	4	0.06717	F (4, 5) = 2.667	P=0.1555		

WS *NCAR*	0.04733	1	0.04733	F (1, 5) = 1.879	P=0.2288		
C PAR	0.08344	1	0.08344	F (1, 5) = 3.312	P=0.1284		
VPD	0.007406	1	0.007406	F (1, 5) = 0.2940	P=0.6109		
Time	0.01199	1	0.01199	F (1, 5) = 0.4762	P=0.5209		
Residual	0.1259	5	0.02519				
Total	0.3946	9					
Parameter estimates	Variable	Estimate	Standard error	95% CI (asymptotic)	|t|	P value	P value summary
β0	Intercept	-0.4717	1.145	-3.414 to 2.470	0.4121	0.6973	ns
β1	WS *NCAR*	0.2736	0.1996	-0.2395 to 0.7867	1.371	0.2288	ns
β2	C PAR	0.001929	0.001060	-0.0007954 to 0.004653	1.820	0.1284	ns
β3	VPD	0.3733	0.6884	-1.396 to 2.143	0.5422	0.6109	ns
β4	Time	0.01282	0.01858	-0.03494 to 0.06058	0.6901	0.5209	ns
Goodness of Fit							
Degrees of Freedom	5						
R squared	0.6808						
Multi-collinearity	Variable	VIF	R2 with other variables				
β0	Intercept						
β1	WS *NCAR*	1.726	0.4207				
β2	C PAR	1.293	0.2267				
β3	VPD	2.196	0.5445				
β4	Time	1.266	0.2103				
Normality of Residuals	Statistics	P value	Passed normality test (alpha=0.05)?	P value summary			
Anderson-Darling (A2*)	0.5732	0.1012	Yes	ns			
D'Agostino-Pearson omnibus (K2)	3.692	0.1579	Yes	ns			
Shapiro-Wilk (W)	0.8716	0.1043	Yes	ns			

Kolmogorov-Smirnov (distance)	0.2598	0.0542	Yes	ns			
Data summary							
Rows in table	10						
Rows skipped (missing data)	0						
Rows analysed (# cases)	10						
Number of parameter estimates	5						
#cases/#parameters	2.0						

Supplementary table S58. Multi-linear regression of C *NCAR* and parameters (C PAR, VPD, time) without WS *NCAR* of the drought experiment, section 3.5.2.4, Chapter 3.

Dependent variable	C *NCAR*						
Regression type	Least squares						
Model							
Analysis of Variance	SS	DF	MS	F (DFn, DFd)	P value		
Regression	0.2214	3	0.07378	F (3, 6) = 2.555	P=0.1514		
C PAR	0.06088	1	0.06088	F (1, 6) = 2.108	P=0.1967		
VPD	0.06485	1	0.06485	F (1, 6) = 2.246	P=0.1847		
Time	0.006147	1	0.006147	F (1, 6) = 0.2129	P=0.6608		
Residual	0.1733	6	0.02888				
Total	0.3946	9					
Parameter estimates	Variable	Estimate	Standard error	95% CI (asymptotic)	|t|	P value	P value summary
β0	Intercept	-1.150	1.105	-3.854 to 1.553	1.041	0.3380	ns
β1	C PAR	0.001606	0.001106	-0.001101 to 0.004314	1.452	0.1967	ns
β2	VPD	0.9091	0.6067	-0.5754 to 2.394	1.499	0.1847	ns
β3	Time	0.009079	0.01968	-0.03907 to 0.05723	0.4614	0.6608	ns

Goodness of Fit							
Degrees of Freedom	6						
R squared	0.5609						
Multi-collinearity	Variable	VIF	R2 with other variables				
β0	Intercept						
β1	C PAR	1.229	0.1866				
β2	VPD	1.487	0.3277				
β3	Time	1.239	0.1929				
Normality of Residuals	Statistics	P value	Passed normality test (alpha=0.05)?	P value summary			
Anderson-Darling (A2*)	0.2754	0.5761	Yes	ns			
D'Agostino-Pearson omnibus (K2)	0.3363	0.8452	Yes	ns			
Shapiro-Wilk (W)	0.9449	0.6086	Yes	ns			
Kolmogorov-Smirnov (distance)	0.1631	>0.1000	Yes	ns			
Data summary							
Rows in table	10						
Rows skipped (missing data)	0						
Rows analysed (# cases)	10						
Number of parameter estimates	4						
#cases/#parameters	2.5						

7.2. Supplementary information for Chapter 4

Supplementary table S59a. Two-way repeated-measures analysis of variance (ANOVA) of stomatal conductance (g_s) from control well-watered (CWW), control water-stressed (CWS), treatment well-watered (TWW) and treatment water-stressed (TWS) plants during the drought/rehydration experiment, section 4.2.2.1, Chapter 4.

Table Analysed	g_s (porometer)				
Two-way repeated-measures ANOVA	Matching: Stacked				

Assume sphericity?	No				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	Geisser-Greenhouse's epsilon
Time x Treatment	20.35	<0.0001	****	Yes	
Time	5.975	<0.0001	****	Yes	0.4254
Treatment	41.98	0.0003	***	Yes	
Subject	19.44	<0.0001	****	Yes	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Time x Treatment	294138	42	7003	F (42, 224) = 8.852	P<0.0001
Time	86375	14	6170	F (5.955, 95.28) = 7.798	P<0.0001
Treatment	606874	3	202291	F (3, 16) = 11.51	P=0.0003
Subject	281100	16	17569	F (16, 224) = 22.21	P<0.0001
Residual	177221	224	791.2		
Data summary					
Number of columns (Treatment)	4				
Number of rows (Time)	15				
Number of subjects (Subject)	20				
Number of missing values	0				

Supplementary table S59b. Multiple comparison with Bonferroni tests of stomatal conductance (g_s) from control well-watered (C), control water-stressed (CWS), treatment well-watered (TWW) and water-stressed (TWS) plants during the drought/rehydration experiment, section 4.2.2.1, Chapter 4.

Within each row, compare columns (simple effects within rows)								
Number of families	15							
Number of comparisons per family	6							
Alpha	0.05							
Bonferroni's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value			
Day 1								
CWW vs. CWS	61.85	-39.16 to 162.9	No	ns	0.2989			
CWW vs. TWW	8.150	-90.71 to 107.0	No	ns	>0.9999			
CWW vs. TWS	-27.20	-149.4 to 94.98	No	ns	>0.9999			
CWS vs. TWW	-53.70	-117.6 to 10.24	No	ns	0.1076			

CWS vs. TWS	-89.05	-209.1 to 31.02	No	ns	0.1523			
TWW vs. TWS	-35.35	-150.5 to 79.76	No	ns	>0.9999			
Day 2								
CWW vs. CWS	57.25	-35.80 to 150.3	No	ns	0.3099			
CWW vs. TWW	6.600	-95.61 to 108.8	No	ns	>0.9999			
CWW vs. TWS	5.600	-118.4 to 129.6	No	ns	>0.9999			
CWS vs. TWW	-50.65	-139.9 to 38.64	No	ns	0.4042			
CWS vs. TWS	-51.65	-176.8 to 73.49	No	ns	0.8343			
TWW vs. TWS	-1.000	-124.1 to 122.1	No	ns	>0.9999			
Day 3								
CWW vs. CWS	50.65	-29.86 to 131.2	No	ns	0.3303			
CWW vs. TWW	-20.05	-117.5 to 77.38	No	ns	>0.9999			
CWW vs. TWS	-16.20	-121.7 to 89.26	No	ns	>0.9999			
CWS vs. TWW	-70.70	-161.4 to 19.99	No	ns	0.1451			
CWS vs. TWS	-66.85	-168.8 to 35.10	No	ns	0.2642			
TWW vs. TWS	3.850	-105.7 to 113.4	No	ns	>0.9999			
Day 4								
CWW vs. CWS	75.35	7.017 to 143.7	Yes	*	0.0321			
CWW vs. TWW	1.100	-87.03 to 89.23	No	ns	>0.9999			
CWW vs. TWS	9.725	-90.97 to 110.4	No	ns	>0.9999			
CWS vs. TWW	-74.25	-160.3 to 11.83	No	ns	0.0910			
CWS vs. TWS	-65.63	-168.8 to 37.52	No	ns	0.2588			
TWW vs. TWS	8.625	-96.87 to 114.1	No	ns	>0.9999			
Day 5								
CWW vs. CWS	67.73	17.10 to 118.3	Yes	*	0.0101			
CWW vs. TWW	-3.450	-85.92 to 79.02	No	ns	>0.9999			
CWW vs. TWS	-30.00	-101.5 to 41.46	No	ns	>0.9999			
CWS vs. TWW	-71.18	-154.0 to 11.67	No	ns	0.0952			

CWS vs. TWS	-97.73	-168.5 to -26.97	Yes	*	0.0101			
TWW vs. TWS	-26.55	-114.2 to 61.11	No	ns	>0.9999			
Day 6								
CWW vs. CWS	93.05	9.936 to 176.2	Yes	*	0.0282			
CWW vs. TWW	-23.40	-139.9 to 93.06	No	ns	>0.9999			
CWW vs. TWS	2.100	-82.14 to 86.34	No	ns	>0.9999			
CWS vs. TWW	-116.5	-231.3 to -1.649	Yes	*	0.0469			
CWS vs. TWS	-90.95	-160.3 to -21.61	Yes	*	0.0111			
TWW vs. TWS	25.50	-89.10 to 140.1	No	ns	>0.9999			
Day 7								
CWW vs. CWS	80.98	-4.353 to 166.3	No	ns	0.0650			
CWW vs. TWW	-40.45	-159.6 to 78.75	No	ns	>0.9999			
CWW vs. TWS	10.40	-77.61 to 98.41	No	ns	>0.9999			
CWS vs. TWW	-121.4	-240.1 to -2.727	Yes	*	0.0449			
CWS vs. TWS	-70.58	-156.1 to 14.97	No	ns	0.1248			
TWW vs. TWS	50.85	-68.39 to 170.1	No	ns	0.9830			
Day 8								
CWW vs. CWS	115.6	38.85 to 192.3	Yes	**	0.0051			
CWW vs. TWW	4.950	-88.20 to 98.10	No	ns	>0.9999			
CWW vs. TWS	50.55	-33.17 to 134.3	No	ns	0.3882			
CWS vs. TWW	-110.6	-207.5 to -13.80	Yes	*	0.0249			
CWS vs. TWS	-65.04	-154.5 to 24.44	No	ns	0.2110			
TWW vs. TWS	45.60	-54.52 to 145.7	No	ns	0.9048			
Day 9								
CWW vs. CWS	165.3	73.96 to 256.7	Yes	**	0.0016			
CWW vs. TWW	28.65	-76.21 to 133.5	No	ns	>0.9999			
CWW vs. TWS	126.0	8.961 to 242.9	Yes	*	0.0343			
CWS vs. TWW	-136.7	-233.7 to -39.62	Yes	**	0.0080			

CWS vs. TWS	-39.36	-152.2 to 73.46	No	ns	>0.9999			
TWW vs. TWS	97.30	-22.14 to 216.7	No	ns	0.1310			
Day 10								
CWW vs. CWS	219.1	77.90 to 360.2	Yes	**	0.0068			
CWW vs. TWW	45.70	-93.37 to 184.8	No	ns	>0.9999			
CWW vs. TWS	210.4	61.07 to 359.6	Yes	*	0.0132			
CWS vs. TWW	-173.4	-258.6 to -88.13	Yes	***	0.0009			
CWS vs. TWS	-8.710	-66.70 to 49.28	No	ns	>0.9999			
TWW vs. TWS	164.7	77.83 to 251.5	Yes	**	0.0029			
Day 11								
CWW vs. CWS	223.1	76.75 to 369.4	Yes	**	0.0086			
CWW vs. TWW	35.35	-105.5 to 176.2	No	ns	>0.9999			
CWW vs. TWS	222.1	67.57 to 376.6	Yes	*	0.0130			
CWS vs. TWW	-187.7	-279.8 to -95.71	Yes	**	0.0015			
CWS vs. TWS	-0.9950	-45.72 to 43.73	No	ns	>0.9999			
TWW vs. TWS	186.7	87.29 to 286.2	Yes	**	0.0043			
Day 12								
CWW vs. CWS	160.8	55.09 to 266.6	Yes	**	0.0054			
CWW vs. TWW	36.75	-80.76 to 154.3	No	ns	>0.9999			
CWW vs. TWS	101.2	-5.253 to 207.6	No	ns	0.0619			
CWS vs. TWW	-124.1	-227.1 to -21.00	Yes	*	0.0195			
CWS vs. TWS	-59.67	-128.7 to 9.409	No	ns	0.0982			
TWW vs. TWS	64.40	-38.82 to 167.6	No	ns	0.3002			
Day 13								
CWW vs. CWS	113.4	-21.08 to 247.8	No	ns	0.1045			
CWW vs. TWW	25.15	-109.6 to 159.9	No	ns	>0.9999			
CWW vs. TWS	55.55	-78.94 to 190.0	No	ns	0.9755			
CWS vs. TWW	-88.20	-177.9 to 1.458	No	ns	0.0543			

CWS vs. TWS	-57.80	-138.4 to 22.80	No	ns	0.2234			
TWW vs. TWS	30.40	-58.82 to 119.6	No	ns	>0.9999			
Day 14								
CWW vs. CWS	140.8	4.176 to 277.3	Yes	*	0.0440			
CWW vs. TWW	53.95	-81.75 to 189.7	No	ns	>0.9999			
CWW vs. TWS	86.15	-49.98 to 222.3	No	ns	0.2863			
CWS vs. TWW	-86.80	-176.3 to 2.685	No	ns	0.0578			
CWS vs. TWS	-54.60	-122.0 to 12.79	No	ns	0.1352			
TWW vs. TWS	32.20	-57.66 to 122.1	No	ns	>0.9999			
Day 15								
CWW vs. CWS	97.05	-9.795 to 203.9	No	ns	0.0768			
CWW vs. TWW	18.20	-103.7 to 140.1	No	ns	>0.9999			
CWW vs. TWS	14.90	-91.94 to 121.7	No	ns	>0.9999			
CWS vs. TWW	-78.86	-187.2 to 29.48	No	ns	0.1877			
CWS vs. TWS	-82.15	-150.2 to -14.09	Yes	*	0.0180			
TWW vs. TWS	-3.295	-111.6 to 105.0	No	ns	>0.9999			
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
Day 1								
CWW vs. CWS	187.4	125.5	61.85	24.15	5	5	2.561	5.082
CWW vs. TWW	187.4	179.2	8.150	27.01	5	5	0.3018	6.870
CWW vs. TWS	187.4	214.6	-27.20	34.86	5	5	0.7803	7.809
CWS vs. TWW	125.5	179.2	-53.70	16.95	5	5	3.167	6.356
CWS vs. TWS	125.5	214.6	-89.05	27.81	5	5	3.202	4.796
TWW vs. TWS	179.2	214.6	-35.35	30.32	5	5	1.166	6.254
Day 2								
CWW vs. CWS	202.1	144.8	57.25	23.07	5	5	2.481	5.461
CWW vs. TWW	202.1	195.5	6.600	29.37	5	5	0.2248	7.988
CWW vs. TWS	202.1	196.5	5.600	34.89	5	5	0.1605	7.477
CWS vs. TWW	144.8	195.5	-50.65	22.35	5	5	2.267	5.568

CWS vs. TWS	144.8	196.5	-51.65	29.23	5	5	1.767	4.869
TWW vs. TWS	195.5	196.5	-1.000	34.42	5	5	0.02906	7.340
Day 3								
CWW vs. CWS	174.6	124.0	50.65	21.89	5	5	2.313	6.786
CWW vs. TWW	174.6	194.7	-20.05	27.88	5	5	0.7192	7.882
CWW vs. TWS	174.6	190.8	-16.20	29.82	5	5	0.5433	7.589
CWS vs. TWW	124.0	194.7	-70.70	23.97	5	5	2.950	6.305
CWS vs. TWS	124.0	190.8	-66.85	26.20	5	5	2.552	5.898
TWW vs. TWS	194.7	190.8	3.850	31.37	5	5	0.1227	7.898
Day 4								
CWW vs. CWS	190.3	115.0	75.35	17.55	5	5	4.294	5.890
CWW vs. TWW	190.3	189.2	1.100	24.99	5	5	0.04402	7.657
CWW vs. TWS	190.3	180.6	9.725	27.79	5	5	0.3499	7.070
CWS vs. TWW	115.0	189.2	-74.25	20.98	5	5	3.539	5.273
CWS vs. TWS	115.0	180.6	-65.63	24.25	5	5	2.706	4.927
TWW vs. TWS	189.2	180.6	8.625	30.08	5	5	0.2868	7.791
Day 5								
CWW vs. CWS	181.8	114.0	67.73	14.41	5	5	4.700	7.752
CWW vs. TWW	181.8	185.2	-3.450	21.98	5	5	0.1570	6.435
CWW vs. TWS	181.8	211.8	-30.00	19.70	5	5	1.523	7.041
CWS vs. TWW	114.0	185.2	-71.18	21.11	5	5	3.371	5.792
CWS vs. TWS	114.0	211.8	-97.73	18.73	5	5	5.218	6.329
TWW vs. TWS	185.2	211.8	-26.55	25.02	5	5	1.061	7.820
Day 6								
CWW vs. CWS	213.2	120.2	93.05	23.18	5	5	4.014	7.287
CWW vs. TWW	213.2	236.6	-23.40	32.39	5	5	0.7225	7.225
CWW vs. TWS	213.2	211.1	2.100	23.76	5	5	0.08840	7.528
CWS vs. TWW	120.2	236.6	-116.5	29.68	5	5	3.924	5.982

CWS vs. TWS	120.2	211.1	-90.95	19.90	5	5	4.570	7.963
TWW vs. TWS	236.6	211.1	25.50	30.13	5	5	0.8464	6.225
Day 7								
CWW vs. CWS	190.6	109.6	80.98	24.50	5	5	3.305	7.970
CWW vs. TWW	190.6	231.0	-40.45	32.60	5	5	1.241	6.894
CWW vs. TWS	190.6	180.2	10.40	25.30	5	5	0.4111	8.000
CWS vs. TWW	109.6	231.0	-121.4	32.03	5	5	3.791	6.648
CWS vs. TWS	109.6	180.2	-70.58	24.56	5	5	2.874	7.966
TWW vs. TWS	231.0	180.2	50.85	32.65	5	5	1.558	6.912
Day 8								
CWW vs. CWS	221.4	105.8	115.6	21.60	5	5	5.350	7.493
CWW vs. TWW	221.4	216.4	4.950	25.12	5	5	0.1970	6.638
CWW vs. TWS	221.4	170.8	50.55	23.15	5	5	2.184	7.103
CWS vs. TWW	105.8	216.4	-110.6	27.43	5	5	4.033	7.636
CWS vs. TWS	105.8	170.8	-65.04	25.63	5	5	2.537	7.913
TWW vs. TWS	216.4	170.8	45.60	28.66	5	5	1.591	7.893
Day 9								
CWW vs. CWS	246.4	81.05	165.3	25.65	5	5	6.445	7.431
CWW vs. TWW	246.4	217.7	28.65	30.09	5	5	0.9520	7.958
CWW vs. TWS	246.4	120.4	126.0	33.08	5	5	3.808	7.590
CWS vs. TWW	81.05	217.7	-136.7	26.90	5	5	5.080	7.160
CWS vs. TWS	81.05	120.4	-39.36	30.20	5	5	1.303	6.511
TWW vs. TWS	217.7	120.4	97.30	34.06	5	5	2.857	7.794
Day 10								
CWW vs. CWS	259.1	39.99	219.1	34.65	5	5	6.322	5.350
CWW vs. TWW	259.1	213.4	45.70	37.41	5	5	1.222	6.594
CWW vs. TWS	259.1	48.70	210.4	32.74	5	5	6.425	4.383
CWS vs. TWW	39.99	213.4	-173.4	23.55	5	5	7.362	7.089

CWS vs. TWS	39.99	48.70	-8.710	15.06	5	5	0.5782	6.050
TWW vs. TWS	213.4	48.70	164.7	20.63	5	5	7.981	5.024
Day 11								
CWW vs. CWS	255.9	32.77	223.1	33.78	5	5	6.605	4.768
CWW vs. TWW	255.9	220.5	35.35	38.52	5	5	0.9177	6.888
CWW vs. TWS	255.9	33.76	222.1	32.47	5	5	6.840	4.110
CWS vs. TWW	32.77	220.5	-187.7	23.34	5	5	8.044	5.728
CWS vs. TWS	32.77	33.76	-0.9950	10.73	5	5	0.09273	5.115
TWW vs. TWS	220.5	33.76	186.7	21.40	5	5	8.724	4.258
Day 12								
CWW vs. CWS	240.3	79.43	160.8	28.89	5	5	5.566	6.875
CWW vs. TWW	240.3	203.5	36.75	33.77	5	5	1.088	7.994
CWW vs. TWS	240.3	139.1	101.2	26.44	5	5	3.825	5.486
CWS vs. TWW	79.43	203.5	-124.1	28.33	5	5	4.379	6.987
CWS vs. TWS	79.43	139.1	-59.67	19.01	5	5	3.139	7.012
TWW vs. TWS	203.5	139.1	64.40	25.83	5	5	2.494	5.565
Day 13								
CWW vs. CWS	240.7	127.4	113.4	35.06	5	5	3.233	6.108
CWW vs. TWW	240.7	215.6	25.15	36.57	5	5	0.6877	6.747
CWW vs. TWS	240.7	185.2	55.55	34.94	5	5	1.590	6.052
CWS vs. TWW	127.4	215.6	-88.20	25.56	5	5	3.451	7.787
CWS vs. TWS	127.4	185.2	-57.80	23.17	5	5	2.495	7.998
TWW vs. TWS	215.6	185.2	30.40	25.39	5	5	1.197	7.747
Day 14								
CWW vs. CWS	249.1	108.4	140.8	33.81	5	5	4.163	5.444
CWW vs. TWW	249.1	195.2	53.95	37.19	5	5	1.451	6.928
CWW vs. TWS	249.1	163.0	86.15	34.04	5	5	2.531	5.556
CWS vs. TWW	108.4	195.2	-86.80	24.48	5	5	3.545	6.898

CWS vs. TWS	108.4	163.0	-54.60	19.36	5	5	2.820	7.987
TWW vs. TWS	195.2	163.0	32.20	24.79	5	5	1.299	7.058
Day 15								
CWW vs. CWS	230.8	133.8	97.05	28.20	5	5	3.442	6.284
CWW vs. TWW	230.8	212.6	18.20	35.04	5	5	0.5193	7.998
CWW vs. TWS	230.8	215.9	14.90	28.25	5	5	0.5273	6.314
CWS vs. TWW	133.8	212.6	-78.86	28.50	5	5	2.767	6.232
CWS vs. TWS	133.8	215.9	-82.15	19.56	5	5	4.199	7.999
TWW vs. TWS	212.6	215.9	-3.295	28.55	5	5	0.1154	6.263

Supplementary table S60a. Two-way repeated-measures analysis of variance (ANOVA) of vapour pressure deficit (VPD) from control well-watered (CWW), treatment well-watered and treatment water-stressed (TWW_TWS) plants during the drought/rehydration experiment, section 4.2.2.1, Chapter 4.

Table Analysed	VPD				
Two-way repeated-measures ANOVA	Matching: Stacked				
Assume sphericity?	No				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	Geisser-Greenhouse's epsilon
Time x Treatment	7.157	<0.0001	****	Yes	
Time	52.10	<0.0001	****	Yes	0.4140
Treatment	0.2144	0.3785	ns	No	
Subject	5.839	0.0007	***	Yes	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Time x Treatment	0.7212	14	0.05151	F (14, 308) = 4.539	P<0.0001
Time	5.250	14	0.3750	F (5.796, 127.5) = 33.04	P<0.0001
Treatment	0.02161	1	0.02161	F (1, 22) = 0.8079	P=0.3785
Subject	0.5884	22	0.02675	F (22, 308) = 2.356	P=0.0007
Residual	3.496	308	0.01135		
Difference between column means					
Mean of CWW	0.9881				
Mean of TWW_TWS	0.9726				
Difference between means	0.01550				
SE of difference	0.01724				
95% CI of difference	-0.02026 to 0.05125				
Data summary					

Number of columns (Treatment)	2				
Number of rows (Time)	15				
Number of subjects (Subject)	24				
Number of missing values	0				

Supplementary table S60b. Multiple comparison with Bonferroni tests of vapour pressure deficit (VPD) from control well-watered (CWW), and treatment well-watered and water-stressed (TWW_TWS) plants during the drought/rehydration experiment, section 4.2.2.1, Chapter 4.

Compare each cell mean with the other cell mean in that row								
Number of families	1							
Number of comparisons per family	15							
Alpha	0.05							
Bonferroni's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value			
CWW - TWW_TWS								
Day 1	0.06519	-0.04858 to 0.1790	No	ns	>0.9999			
Day 2	-0.02759	-0.1700 to 0.1148	No	ns	>0.9999			
Day 3	0.07679	-0.01952 to 0.1731	No	ns	0.2309			
Day 4	0.08149	-0.09301 to 0.2560	No	ns	>0.9999			
Day 5	0.1350	0.03903 to 0.2309	Yes	**	0.0021			
Day 6	-0.05067	-0.2003 to 0.09891	No	ns	>0.9999			
Day 7	0.1009	-0.08125 to 0.2831	No	ns	>0.9999			
Day 8	-0.08610	-0.2636 to 0.09134	No	ns	>0.9999			
Day 9	-0.1432	-0.3346 to 0.04816	No	ns	0.3119			
Day 10	0.02390	-0.1329 to 0.1807	No	ns	>0.9999			
Day 11	-0.08064	-0.2481 to 0.08687	No	ns	>0.9999			
Day 12	0.1654	-0.01407 to 0.3449	No	ns	0.0916			

Day 13	0.07149	-0.05052 to 0.1935	No	ns	0.9993			
Day 14	-0.01205	-0.1624 to 0.1383	No	ns	>0.9999			
Day 15	-0.08748	-0.2330 to 0.05805	No	ns	0.9085			
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
CWW - TWW_TWS								
Day 1	1.049	0.9843	0.06519	0.03457	12	12	1.886	21.99
Day 2	1.045	1.073	-0.02759	0.04300	12	12	0.6417	20.85
Day 3	1.104	1.028	0.07679	0.02915	12	12	2.634	21.32
Day 4	1.191	1.110	0.08149	0.05050	12	12	1.614	15.70
Day 5	1.200	1.065	0.1350	0.02894	12	12	4.663	20.75
Day 6	1.018	1.069	-0.05067	0.04472	12	12	1.133	19.39
Day 7	1.236	1.135	0.1009	0.05266	12	12	1.916	15.61
Day 8	0.9210	1.007	-0.08610	0.05260	12	12	1.637	18.24
Day 9	0.8212	0.9644	-0.1432	0.05637	12	12	2.541	17.48
Day 10	0.8426	0.8187	0.02390	0.04713	12	12	0.5072	20.21
Day 11	0.8446	0.9252	-0.08064	0.04885	12	12	1.651	16.44
Day 12	0.9777	0.8123	0.1654	0.05454	12	12	3.033	21.99
Day 13	0.8443	0.7728	0.07149	0.03702	12	12	1.931	21.73
Day 14	0.9594	0.9714	-0.01205	0.04508	12	12	0.2673	19.76
Day 15	0.7657	0.8532	-0.08748	0.04422	12	12	1.978	21.99

Supplementary table S61. Multilinear regressions of treatment well-watered stomatal conductance (TWW g_s) and other variables from *Vitis vinifera* during a drought/rehydration experiment, section 4.2.2.1, Chapter 4. TWS; treatment water-stressed, VPD, vapour pressure deficit.

Dependent variable	TWW g_s						
Regression type	Least squares						
Model							
Analysis of Variance	SS	DF	MS	F (DFn, DFd)	P value		
Regression	1152	3	383.9	F (3, 11) = 1.490	P=0.2712		
TWS g_s	192.2	1	192.2	F (1, 11) = 0.7460	P=0.4062		
TWW_TWS VPD	365.0	1	365.0	F (1, 11) = 1.417	P=0.2590		
Time	800.6	1	800.6	F (1, 11) = 3.108	P=0.1057		
Residual	2834	11	257.6				
Total	3986	14					
Parameter estimates	Variable	Estimate	Standard error	95% CI (asymptotic)	|t|	P value	P value summary
β0	Intercept	136.4	60.12	4.044 to 268.7	2.268	0.0444	*
β1	TWS g_s	-0.07185	0.08318	-0.2549 to 0.1112	0.8637	0.4062	ns
β2	TWW_TWS VPD	64.96	54.58	-55.16 to 185.1	1.190	0.2590	ns
β3	Time	2.415	1.370	-0.6002 to 5.430	1.763	0.1057	ns
Goodness of Fit							
Degrees of Freedom	11						
R squared	0.2890						
Adjusted R squared	0.09505						

Multi-collinearity	Variable	VIF	R2 with other variables				
β0	Intercept						
β1	TWS g_s	1.217	0.1786				
β2	TWW_TWS VPD	2.120	0.5282				
β3	Time	2.039	0.5097				
Normality of Residuals	Statistics	P value	Passed normality test (alpha=0.05)?	P value summary			
Anderson-Darling (A2*)	0.2313	0.7604	Yes	ns			
D'Agostino-Pearson omnibus (K2)	1.113	0.5732	Yes	ns			
Shapiro-Wilk (W)	0.9771	0.9458	Yes	ns			
Kolmogorov-Smirnov (distance)	0.1105	>0.1000	Yes	ns			
Data summary							
Rows in table	15						
Rows skipped (missing data)	0						
Rows analysed (# cases)	15						
Number of parameter estimates	4						
#cases/#parameters	3.8						

Supplementary table S62a. Two-way repeated-measures analysis of variance (ANOVA) of stomatal conductance (g_s) from control well-watered (C), treatment well-watered (WW) and treatment water-stressed (WS) plants during the drought/rehydration experiment, section 4.3.2.1, Chapter 4.

Table Analysed	g_s (porometer)				
Two-way repeated-measures ANOVA	Matching: Stacked				
Assume sphericity?	No				
Alpha	0.05				
Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)	Geisser-Greenhouse's epsilon
Time	<0.0001	****	Yes	F (8.779, 499.1) = 81.68	0.4390
Treatment	<0.0001	****	Yes	F (2, 57) = 73.61	
Time x Treatment	<0.0001	****	Yes	F (40, 1137) = 41.73	
Random effects	SD	Variance			
Subject	23.93	572.9			
Residual	31.93	1020			
Was the matching effective?					
Chi-square, df	388.1, 1				
P value	<0.0001				
P value summary	****				

Is there significant matching (P < 0.05)?	Yes				
Data summary					
Number of columns (Treatment)	3				
Number of rows (Time)	21				
Number of subjects (Subject)	60				
Number of missing values	3				

Supplementary table S62b. Multiple comparison with Bonferroni tests of stomatal conductance (g_s) from control well-watered (C), treatment well-watered (WW) and water-stressed (WS) plants during the drought/rehydration experiment, section 4.3.2.1, Chapter 4.

Within each row, compare columns (simple effects within rows)								
Number of families	21							
Number of comparisons per family	3							
Alpha	0.05							
Bonferroni's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value			
Day 1								
C vs. WW	55.50	22.15 to 88.85	Yes	***	0.0006			
C vs. WS	-12.06	-49.38 to 25.26	No	ns	>0.9999			
WW vs. WS	-67.56	-96.03 to -39.10	Yes	****	<0.0001			
Day 2								
C vs. WW	29.25	2.311 to 56.19	Yes	*	0.0295			
C vs. WS	-8.425	-36.67 to 19.82	No	ns	>0.9999			
WW vs. WS	-37.68	-61.56 to -13.79	Yes	***	0.0010			
Day 3								
C vs. WW	35.93	5.933 to 65.92	Yes	*	0.0142			
C vs. WS	-5.100	-37.82 to 27.62	No	ns	>0.9999			
WW vs. WS	-41.03	-73.27 to -8.783	Yes	**	0.0087			
Day 4								
C vs. WW	33.09	4.891 to 61.28	Yes	*	0.0167			

C vs. WS	-3.400	-31.56 to 24.76	No	ns	>0.9999			
WW vs. WS	-36.49	-65.58 to -7.400	Yes	**	0.0097			
Day 5								
C vs. WW	46.96	23.85 to 70.07	Yes	****	<0.0001			
C vs. WS	11.01	-14.94 to 36.96	No	ns	0.8762			
WW vs. WS	-35.95	-64.21 to -7.690	Yes	**	0.0087			
Day 6								
C vs. WW	64.55	28.18 to 100.9	Yes	***	0.0004			
C vs. WS	83.19	65.13 to 101.2	Yes	****	<0.0001			
WW vs. WS	18.64	-17.70 to 54.98	No	ns	0.5885			
Day 7								
C vs. WW	103.2	76.41 to 130.0	Yes	****	<0.0001			
C vs. WS	65.38	35.21 to 95.54	Yes	****	<0.0001			
WW vs. WS	-37.84	-66.22 to -9.454	Yes	**	0.0058			
Day 8								
C vs. WW	54.31	25.71 to 82.92	Yes	****	<0.0001			
C vs. WS	70.04	38.82 to 101.3	Yes	****	<0.0001			
WW vs. WS	15.73	-10.51 to 41.96	No	ns	0.4232			
Day 9								
C vs. WW	47.64	20.95 to 74.32	Yes	***	0.0003			
C vs. WS	50.88	19.93 to 81.82	Yes	***	0.0006			
WW vs. WS	3.238	-20.99 to 27.46	No	ns	>0.9999			
Day 10								
C vs. WW	76.04	45.41 to 106.7	Yes	****	<0.0001			
C vs. WS	83.16	52.24 to 114.1	Yes	****	<0.0001			
WW vs. WS	7.125	-17.44 to 31.69	No	ns	>0.9999			
Day 11								
C vs. WW	85.71	56.57 to 114.9	Yes	****	<0.0001			
C vs. WS	113.1	85.63 to 140.5	Yes	****	<0.0001			
WW vs. WS	27.34	0.08391 to 54.59	Yes	*	0.0491			

Day 12								
C vs. WW	78.17	47.73 to 108.6	Yes	****	<0.0001			
C vs. WS	87.41	43.35 to 131.5	Yes	****	<0.0001			
WW vs. WS	9.246	-34.19 to 52.68	No	ns	>0.9999			
Day 13								
C vs. WW	71.79	51.89 to 91.68	Yes	****	<0.0001			
C vs. WS	119.1	87.07 to 151.0	Yes	****	<0.0001			
WW vs. WS	47.26	16.26 to 78.26	Yes	**	0.0018			
Day 14								
C vs. WW	42.71	10.90 to 74.53	Yes	**	0.0053			
C vs. WS	143.2	103.6 to 182.8	Yes	****	<0.0001			
WW vs. WS	100.5	61.05 to 139.9	Yes	****	<0.0001			
Day 15								
C vs. WW	98.36	65.22 to 131.5	Yes	****	<0.0001			
C vs. WS	247.7	206.2 to 289.2	Yes	****	<0.0001			
WW vs. WS	149.4	110.4 to 188.3	Yes	****	<0.0001			
Day 16								
C vs. WW	88.54	60.13 to 116.9	Yes	****	<0.0001			
C vs. WS	235.1	202.4 to 267.8	Yes	****	<0.0001			
WW vs. WS	146.6	121.9 to 171.2	Yes	****	<0.0001			
Day 17								
C vs. WW	138.6	97.02 to 180.1	Yes	****	<0.0001			
C vs. WS	295.4	251.8 to 339.0	Yes	****	<0.0001			
WW vs. WS	156.8	132.0 to 181.6	Yes	****	<0.0001			
Day 18								
C vs. WW	144.9	110.6 to 179.3	Yes	****	<0.0001			
C vs. WS	152.4	113.6 to 191.1	Yes	****	<0.0001			
WW vs. WS	7.463	-20.03 to 34.96	No	ns	>0.9999			
Day 19								
C vs. WW	94.16	56.58 to 131.7	Yes	****	<0.0001			

C vs. WS	85.89	43.87 to 127.9	Yes	****	<0.0001			
WW vs. WS	-8.275	-38.37 to 21.82	No	ns	>0.9999			
Day 20								
C vs. WW	57.25	24.38 to 90.12	Yes	***	0.0004			
C vs. WS	40.93	2.839 to 79.01	Yes	*	0.0316			
WW vs. WS	-16.33	-46.13 to 13.48	No	ns	0.5272			
Day 21								
C vs. WW	68.98	33.37 to 104.6	Yes	****	<0.0001			
C vs. WS	44.99	8.030 to 81.94	Yes	*	0.0128			
WW vs. WS	-23.99	-50.63 to 2.655	No	ns	0.0898			
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
Day 1								
C vs. WW	288.2	232.7	55.50	13.13	20	20	4.226	29.34
C vs. WS	288.2	300.3	-12.06	14.87	20	20	0.8111	36.49
WW vs. WS	232.7	300.3	-67.56	11.29	20	20	5.985	33.16
Day 2								
C vs. WW	241.8	212.6	29.25	10.71	20	20	2.732	34.60
C vs. WS	241.8	250.2	-8.425	11.26	20	20	0.7484	36.70
WW vs. WS	212.6	250.2	-37.68	9.530	20	20	3.953	37.34
Day 3								
C vs. WW	272.0	236.1	35.93	11.97	20	20	3.000	37.95
C vs. WS	272.0	277.1	-5.100	13.05	20	20	0.3907	37.38
WW vs. WS	236.1	277.1	-41.03	12.86	20	20	3.191	37.01
Day 4								
C vs. WW	310.6	277.5	33.09	11.26	20	20	2.940	37.83
C vs. WS	310.6	314.0	-3.400	11.24	20	20	0.3024	37.84
WW vs. WS	277.5	314.0	-36.49	11.61	20	20	3.142	38.00
Day 5								
C vs. WW	313.1	266.2	46.96	9.199	20	20	5.105	35.75
C vs. WS	313.1	302.1	11.01	10.28	20	20	1.071	32.75
WW vs. WS	266.2	302.1	-35.95	11.27	20	20	3.190	36.98
Day 6								
C vs. WW	334.7	270.2	64.55	13.97	20	17	4.619	20.92
C vs. WS	334.7	251.6	83.19	7.208	20	20	11.54	38.00
WW vs. WS	270.2	251.6	18.64	13.96	17	20	1.335	20.84
Day 7								
C vs. WW	325.2	222.0	103.2	10.69	20	20	9.655	37.16
C vs. WS	325.2	259.9	65.38	12.04	20	20	5.431	37.67
WW vs. WS	222.0	259.9	-37.84	11.30	20	20	3.348	35.93
Day 8								
C vs. WW	322.5	268.2	54.31	11.35	20	20	4.783	33.72
C vs. WS	322.5	252.5	70.04	12.45	20	20	5.623	37.39
WW vs. WS	268.2	252.5	15.73	10.45	20	20	1.505	35.93

Day 9								
C vs. WW	303.9	256.2	47.64	10.51	20	20	4.534	29.18
C vs. WS	303.9	253.0	50.88	12.35	20	20	4.121	37.44
WW vs. WS	256.2	253.0	3.238	9.579	20	20	0.3380	31.40
Day 10								
C vs. WW	326.9	250.8	76.04	12.15	20	20	6.257	33.44
C vs. WS	326.9	243.7	83.16	12.28	20	20	6.773	34.03
WW vs. WS	250.8	243.7	7.125	9.809	20	20	0.7264	37.96
Day 11								
C vs. WW	329.1	243.4	85.71	11.64	20	20	7.366	38.00
C vs. WS	329.1	216.0	113.1	10.94	20	20	10.34	37.23
WW vs. WS	243.4	216.0	27.34	10.87	20	20	2.514	37.34
Day 12								
C vs. WW	331.5	253.3	78.17	12.15	20	20	6.433	37.83
C vs. WS	331.5	244.1	87.41	17.40	20	20	5.022	30.89
WW vs. WS	253.3	244.1	9.246	17.12	20	20	0.5402	29.67
Day 13								
C vs. WW	298.5	226.7	71.79	7.932	20	20	9.051	36.77
C vs. WS	298.5	179.4	119.1	12.60	20	20	9.447	29.63
WW vs. WS	226.7	179.4	47.26	12.14	20	20	3.894	26.69
Day 14								
C vs. WW	302.3	259.6	42.71	12.70	20	20	3.363	37.99
C vs. WS	302.3	159.1	143.2	15.72	20	20	9.106	34.09
WW vs. WS	259.6	159.1	100.5	15.65	20	20	6.419	33.84
Day 15								
C vs. WW	350.5	252.1	98.36	13.21	20	20	7.447	36.52
C vs. WS	350.5	102.8	247.7	16.53	20	20	14.99	36.05
WW vs. WS	252.1	102.8	149.4	15.43	20	20	9.680	32.43
Day 16								
C vs. WW	283.9	195.4	88.54	11.15	20	20	7.939	27.90
C vs. WS	283.9	48.83	235.1	13.02	20	20	18.05	36.87
WW vs. WS	195.4	48.83	146.6	9.732	20	20	15.06	30.95
Day 17								
C vs. WW	330.3	191.8	138.6	16.15	20	20	8.581	24.15
C vs. WS	330.3	34.91	295.4	17.16	20	20	17.22	29.03
WW vs. WS	191.8	34.91	156.8	9.848	20	20	15.93	33.90
Day 18								
C vs. WW	314.3	169.4	144.9	13.43	20	20	10.79	26.36
C vs. WS	314.3	161.9	152.4	15.42	20	20	9.880	35.54
WW vs. WS	169.4	161.9	7.463	10.86	20	20	0.6872	30.73
Day 19								
C vs. WW	278.4	184.2	94.16	14.73	20	20	6.395	27.06
C vs. WS	278.4	192.5	85.89	16.72	20	20	5.136	35.45
WW vs. WS	184.2	192.5	-8.275	11.91	20	20	0.6950	31.77
Day 20								
C vs. WW	245.2	188.0	57.25	12.94	20	20	4.424	29.19
C vs. WS	245.2	204.3	40.93	15.20	20	20	2.693	37.43
WW vs. WS	188.0	204.3	-16.33	11.78	20	20	1.385	31.44
Day 21								
C vs. WW	221.8	152.8	68.98	14.04	20	20	4.912	30.01

C vs. WS	221.8	176.8	44.99	14.65	20	20	3.071	32.92
WW vs. WS	152.8	176.8	-23.99	10.63	20	20	2.257	37.12

Supplementary table S63a. Two-way repeated-measures analysis of variance (ANOVA) of vapour pressure deficit (VPD) from control well-watered (C), and both treatment well-watered and treatment water-stressed (WW_WS) plants during the drought/rehydration experiment, section 4.3.2.1, Chapter 4.

Table Analysed	VPD				
Two-way repeated-measures ANOVA	Matching: Stacked				
Assume sphericity?	No				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	Geisser-Greenhouse's epsilon
Time x Treatment	6.104	<0.0001	****	Yes	
Time	75.83	<0.0001	****	Yes	0.1768
Treatment	1.514	0.0084	**	Yes	
Subject	1.414	0.0514	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Time x Treatment	0.3815	20	0.01907	F (20, 200) = 4.032	P<0.0001
Time	4.739	20	0.2370	F (3.535, 35.35) = 50.09	P<0.0001
Treatment	0.09464	1	0.09464	F (1, 10) = 10.71	P=0.0084
Subject	0.08841	10	0.008841	F (10, 200) = 1.869	P=0.0514
Residual	0.9462	200	0.004731		
Difference between column means					
Mean of C	0.7291				
Mean of WW_WS	0.6903				
Difference between means	0.03876				
SE of difference	0.01185				
95% CI of difference	0.01236 to 0.06515				
Data summary					
Number of columns (Treatment)	2				
Number of rows (Time)	21				
Number of subjects (Subject)	12				
Number of missing values	0				

Supplementary table S63b. Multiple comparison with Bonferroni tests of vapour pressure deficit (VPD) from control well-watered (C) and both treatment well-watered and water-stressed (WW_WS) plants during the drought/rehydration experiment, section 4.3.2.1, Chapter 4.

Compare each cell mean with the other cell mean in that row								
Number of families	1							
Number of comparisons per family	21							
Alpha	0.05							
Bonferroni's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value			
C - WW_WS								
Day 1	-0.04628	-0.1550 to 0.06248	No	ns	>0.9999			
Day 2	0.05630	-0.04703 to 0.1596	No	ns	>0.9999			
Day 3	-0.02280	-0.09067 to 0.04507	No	ns	>0.9999			
Day 4	-0.02756	-0.1845 to 0.1294	No	ns	>0.9999			
Day 5	0.01354	-0.1946 to 0.2217	No	ns	>0.9999			
Day 6	-0.05301	-0.1611 to 0.05513	No	ns	>0.9999			
Day 7	-0.03464	-0.1889 to 0.1196	No	ns	>0.9999			
Day 8	0.07477	0.01007 to 0.1395	Yes	*	0.0187			
Row 9	-0.03157	-0.2098 to 0.1467	No	ns	>0.9999			
Day 10	0.03336	-0.04024 to 0.1070	No	ns	>0.9999			
Day 11	-0.09465	-0.2388 to 0.04945	No	ns	0.3425			
Day 12	0.1342	-0.01948 to 0.2879	No	ns	0.1142			
Day 13	0.09574	-0.03194 to 0.2234	No	ns	0.2473			
Day 14	0.1570	0.09222 to 0.2217	Yes	****	<0.0001			
Day 15	0.1904	0.02470 to 0.3561	Yes	*	0.0196			
Day 16	0.009548	-0.2606 to 0.2797	No	ns	>0.9999			
Day 17	0.09850	-0.1592 to 0.3562	No	ns	>0.9999			
Day 18	0.1173	-0.09748 to 0.3321	No	ns	>0.9999			
Day 19	-0.04223	-0.2814 to 0.1969	No	ns	>0.9999			
Day 20	0.07631	-0.08729 to 0.2399	No	ns	>0.9999			
Day 21	0.1098	-0.1643 to 0.3838	No	ns	>0.9999			

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
C - WW_WS								
Day 1	0.8634	0.9097	-0.04628	0.02558	6	6	1.809	8.581
Day 2	0.9875	0.9312	0.05630	0.02553	6	6	2.205	9.899
Day 3	0.9743	0.9971	-0.02280	0.01680	6	6	1.357	9.967
Day 4	0.6539	0.6815	-0.02756	0.03761	6	6	0.7330	9.036
Day 5	0.7166	0.7030	0.01354	0.04970	6	6	0.2724	8.944
Day 6	0.7077	0.7607	-0.05301	0.02547	6	6	2.081	8.614
Day 7	0.5963	0.6309	-0.03464	0.03776	6	6	0.9172	9.626
Day 8	0.7573	0.6825	0.07477	0.01604	6	6	4.663	10.00
Day 9	0.9247	0.9563	-0.03157	0.03569	6	6	0.8846	6.076
Day 10	0.6850	0.6517	0.03336	0.01802	6	6	1.851	9.628
Day 11	0.6746	0.7692	-0.09465	0.02863	6	6	3.307	5.991
Day 12	0.7723	0.6381	0.1342	0.03760	6	6	3.569	9.603
Day 13	0.7931	0.6973	0.09574	0.03011	6	6	3.179	8.646
Day 14	0.9056	0.7487	0.1570	0.01598	6	6	9.822	9.872
Day 15	0.6876	0.4972	0.1904	0.04095	6	6	4.650	9.909
Day 16	0.6108	0.6012	0.009548	0.06463	6	6	0.1477	8.994
Day 17	0.6641	0.5656	0.09850	0.06379	6	6	1.544	9.961
Day 18	0.6014	0.4841	0.1173	0.05322	6	6	2.204	9.993
Day 19	0.4740	0.5162	-0.04223	0.05919	6	6	0.7135	9.951
Day 20	0.5762	0.4999	0.07631	0.04052	6	6	1.883	9.976
Day 21	0.6848	0.5750	0.1098	0.06154	6	6	1.784	7.649

Supplementary table S64. Multilinear regressions of treatment well-watered stomatal conductance (WW g_s) and other variables from *Vitis vinifera* during a drought/rehydration experiment, section 4.3.2.1, Chapter 4. WS; water-stressed, VPD, vapour pressure deficit.

Dependent variable	WW g_s						
Regression type	Least squares						
Model							
Analysis of Variance	SS	DF	MS	F (DFn, DFd)	P value		
Regression	11842	3	3947	F (3, 17) = 4.608	P=0.0155		
WS g_s	31.03	1	31.03	F (1, 17) = 0.03622	P=0.8513		
VPD	308.7	1	308.7	F (1, 17) = 0.3604	P=0.5562		
Time	3845	1	3845	F (1, 17) = 4.488	P=0.0492		
Residual	14564	17	856.7				
Total	26406	20					
Parameter estimates	Variable	Estimate	Standard error	95% CI (asymptotic)	|t|	P value	P value summary
β0	Intercept	300.9	76.13	140.3 to 461.5	3.952	0.0010	**
β1	WS g_s	0.02394	0.1258	-0.2414 to 0.2893	0.1903	0.8513	ns
β2	VPD	-40.75	67.89	-184.0 to 102.5	0.6003	0.5562	ns
β3	Time	-4.434	2.093	-8.850 to -0.01808	2.118	0.0492	*
Goodness of Fit							
Degrees of Freedom	17						
R squared	0.4485						
Adjusted R squared	0.3511						
Multicollinearity	Variable	VIF	R2 with other variables				
β0	Intercept						

β1	WS g_s	2.200	0.5455				
β2	VPD	2.565	0.6102				
β3	Time	3.938	0.7460				
Normality of Residuals	Statistics	P value	Passed normality test (alpha=0.05)?	P value summary			
Anderson-Darling (A2*)	0.3886	0.3538	Yes	ns			
D'Agostino-Pearson omnibus (K2)	2.279	0.3200	Yes	ns			
Shapiro-Wilk (W)	0.9569	0.4564	Yes	ns			
Kolmogorov-Smirnov (distance)	0.1345	>0.1000	Yes	ns			
Data summary							
Rows in table	21						
Rows skipped (missing data)	0						
Rows analysed (# cases)	21						
Number of parameter estimates	4						
#cases/#parameters	5.3						

Supplementary table S65a. Two-way repeated-measures analysis of variance (ANOVA) of stomatal conductance (g_s) from control well-watered (C) and treatment well-watered (WW) plants during the drought/rehydration experiment, section 4.4.2.1, Chapter 4.

Table Analysed	g_s (Porometer)				
Two-way repeated-measures ANOVA	Matching: Stacked				
Assume sphericity?	No				
Alpha	0.05				
Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)	Geisser-Greenhouse's epsilon
Time	<0.0001	****	Yes	F (5.261, 113.1) = 41.50	0.4384
Treatment	0.0448	*	Yes	F (1, 30) = 4.387	
Time x Treatment	0.0170	*	Yes	F (12, 258) = 2.106	
Random effects	SD	Variance			
Subject	8.076	65.22			
Residual	26.30	691.5			
Was the matching effective?					
Chi-square, df	7.523, 1				
P value	0.0061				
P value summary	**				
Is there significant matching (P < 0.05)?	Yes				
Difference between column means					
Predicted mean of C	221.1				

Predicted mean of WW	212.0				
Difference between predicted means	9.101				
SE of difference	4.345				
95% CI of difference	0.2271 to 17.98				
Data summary					
Number of columns (Treatment)	2				
Number of rows (Time)	13				
Number of subjects (Subject)	32				
Number of missing values	102				

Supplementary table S65b. Multiple comparison with Bonferroni tests of stomatal conductance (g_s) from control well-watered (C), and treatment well-watered (WW) plants during the drought/rehydration experiment, section 4.4.2.1, Chapter 4.

Compare each cell mean with the other cell mean in that row								
Number of families	1							
Number of comparisons per family	13							
Alpha	0.05							
Bonferroni's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value			
C - WW								
Day 1	44.69	10.80 to 78.58	Yes	**	0.0035			
Day 2	11.28	-21.41 to 43.97	No	ns	>0.9999			
Day 3	-11.56	-55.83 to 32.70	No	ns	>0.9999			
Day 4	12.15	-19.64 to 43.95	No	ns	>0.9999			
Day 5	3.308	-26.71 to 33.32	No	ns	>0.9999			
Day 6	4.923	-26.82 to 36.66	No	ns	>0.9999			
Day 7	8.949	-26.87 to 44.76	No	ns	>0.9999			
Day 8	-1.949	-33.12 to 29.23	No	ns	>0.9999			
Day 9	-0.9000	-48.67 to 46.87	No	ns	>0.9999			
Day 10	4.800	-39.48 to 49.08	No	ns	>0.9999			

Day 11	29.40	-15.36 to 74.16	No	ns	0.5552			
Day 12	13.50	-25.09 to 52.09	No	ns	>0.9999			
Day 13	-2.883	-32.65 to 26.89	No	ns	>0.9999			
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
C - WW								
Day 1	229.1	184.4	44.69	10.80	16	16	4.139	29.34
Day 2	223.3	212.0	11.28	9.901	13	13	1.139	18.54
Day 3	220.8	232.4	-11.56	13.76	13	13	0.8407	22.76
Day 4	194.2	182.1	12.15	9.368	13	13	1.297	15.53
Day 5	238.1	234.7	3.308	9.176	13	13	0.3605	19.86
Day 6	244.0	239.1	4.923	9.921	13	13	0.4962	24.00
Day 7	251.3	242.4	8.949	11.19	13	13	0.7995	23.96
Day 8	224.8	226.8	-1.949	9.493	13	13	0.2053	19.28
Day 9	261.0	261.9	-0.9000	14.35	10	10	0.06271	17.53
Day 10	227.0	222.2	4.800	13.19	10	10	0.3638	16.62
Day 11	262.3	232.9	29.40	13.46	10	10	2.184	17.67
Day 12	147.9	134.4	13.50	11.31	10	10	1.194	15.03
Day 13	147.8	150.6	-2.883	8.975	10	10	0.3213	17.95

Supplementary table S66a. Two-way repeated-measures analysis of variance (ANOVA) of vapour pressure deficit (VPD) from control well-watered (C), and both treatment well-watered and treatment water-stressed (WW_WS) plants during the drought/rehydration experiment, section 4.4.2.1, Chapter 4.

Table Analysed	VPD				
Two-way repeated measures ANOVA	Matching: Stacked				
Assume sphericity?	No				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	Geisser-Greenhouse's epsilon
Time x Treatment	8.970	0.0030	**	Yes	
Time	50.74	<0.0001	****	Yes	0.3518
Treatment	3.908	0.0050	**	Yes	
Subject	3.057	0.3670	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Time x Treatment	0.3162	12	0.02635	F (12, 120) = 2.692	P=0.0030
Time	1.789	12	0.1491	F (4.221, 42.21) = 15.23	P<0.0001
Treatment	0.1378	1	0.1378	F (1, 10) = 12.78	P=0.0050
Subject	0.1078	10	0.01078	F (10, 120) = 1.101	P=0.3670
Residual	1.175	120	0.009788		
Difference between column means					
Mean of C	0.7170				
Mean of WW_WS	0.6576				

Difference between means	0.05943				
SE of difference	0.01662				
95% CI of difference	0.02240 to 0.09647				
Data summary					
Number of columns (Treatment)	2				
Number of rows (Time)	13				
Number of subjects (Subject)	12				
Number of missing values	0				

Supplementary table S66b. Multiple comparison with Bonferroni tests of vapour pressure deficit (VPD) from control well-watered (C) and both treatment well-watered and water-stressed (WW_WS) plants during the drought/rehydration experiment, section 4.4.2.1, Chapter 4.

Compare each cell mean with the other cell mean in that row								
Number of families	1							
Number of comparisons per family	13							
Alpha	0.05							
Bonferroni's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value			
C - WW_WS								
Day 1	0.06856	-0.1803 to 0.3174	No	ns	>0.9999			
Day 2	0.02260	-0.1223 to 0.1675	No	ns	>0.9999			
Day 3	0.1045	-0.1086 to 0.3176	No	ns	>0.9999			
Day 4	0.1460	0.005789 to 0.2863	Yes	*	0.0407			
Day 5	0.01064	-0.2628 to 0.2841	No	ns	>0.9999			
Day 6	0.1603	-0.1243 to 0.4448	No	ns	0.7955			
Day 7	0.1378	-0.03345 to 0.3090	No	ns	0.1606			
Day 8	0.09604	-0.07442 to 0.2665	No	ns	0.7403			
Day 9	0.09836	-0.1524 to 0.3491	No	ns	>0.9999			
Day 10	-0.1761	-0.4503 to 0.09812	No	ns	0.4139			

Day 11	-0.05858	-0.3531 to 0.2360	No	ns	>0.9999			
Day 12	0.04568	-0.1128 to 0.2042	No	ns	>0.9999			
Day 13	0.1168	-0.05315 to 0.2867	No	ns	0.2860			
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
C - WW_WS								
Day 1	0.6527	0.5842	0.06856	0.06617	6	6	1.036	9.810
Day 2	0.7196	0.6970	0.02260	0.03874	6	6	0.5834	9.992
Day 3	0.8281	0.7235	0.1045	0.05680	6	6	1.841	9.891
Day 4	0.8700	0.7240	0.1460	0.03191	6	6	4.576	6.464
Day 5	0.5533	0.5427	0.01064	0.06914	6	6	0.1539	8.370
Day 6	0.6760	0.5158	0.1603	0.07594	6	6	2.110	9.930
Day 7	0.7827	0.6450	0.1378	0.04366	6	6	3.155	8.577
Day 8	0.8502	0.7541	0.09604	0.04388	6	6	2.189	8.824
Day 9	0.6606	0.5622	0.09836	0.06704	6	6	1.467	9.999
Day 10	0.4620	0.6381	-0.1761	0.06737	6	6	2.614	7.734
Day 11	0.6758	0.7344	-0.05858	0.07724	6	6	0.7584	9.359
Day 12	0.6216	0.5759	0.04568	0.04105	6	6	1.113	8.996
Day 13	0.9684	0.8516	0.1168	0.03945	6	6	2.961	6.748

Supplementary table S67. Multilinear regressions of treatment well-watered stomatal conductance (WW g_s) and other variables from *Vitis vinifera* during a drought/rehydration experiment, section 4.4.2.1, Chapter 4. WS; water-stressed, VPD, vapour pressure deficit.

Dependent variable	WW g_s						
Regression type	Least squares						
Model							
Analysis of Variance	SS	DF	MS	F (DFn, DFd)	P value		
Regression	4312	3	1437	F (3, 9) = 0.9970	P=0.4375		
WS g_s	2339	1	2339	F (1, 9) = 1.622	P=0.2347		
WW_WS VPD	1384	1	1384	F (1, 9) = 0.9599	P=0.3528		
Time	2532	1	2532	F (1, 9) = 1.756	P=0.2178		
Residual	12975	9	1442				
Total	17287	12					
Parameter estimates	Variable	Estimate	Standard error	95% CI (asymptotic)	|t|	P value	P value summary
β0	Intercept	357.3	92.38	148.3 to 566.3	3.868	0.0038	**
β1	WS g_s	-0.2699	0.2119	-0.7492 to 0.2094	1.274	0.2347	ns
β2	WW_WS VPD	-112.1	114.4	-370.9 to 146.7	0.9797	0.3528	ns
β3	Time	-5.264	3.972	-14.25 to 3.722	1.325	0.2178	ns
Goodness of Fit							
Degrees of Freedom	9						
R squared	0.2494						
Adjusted R squared	-0.0007519						
Multicollinearity	Variable	VIF	R2 with other variables				
β0	Intercept						
β1	WS g_s	2.013	0.5032				
β2	WW_WS VPD	1.073	0.06807				

β3	Time	1.992	0.4979				
Normality of Residuals	Statistics	P value	Passed normality test (alpha=0.05)?	P value summary			
Anderson-Darling (A2*)	0.7284	0.0429	No	*			
D'Agostino-Pearson omnibus (K2)	1.993	0.3691	Yes	ns			
Shapiro-Wilk (W)	0.8747	0.0604	Yes	ns			
Kolmogorov-Smirnov (distance)	0.2491	0.0266	No	*			
Data summary							
Rows in table	13						
Rows skipped (missing data)	0						
Rows analysed (# cases)	13						
Number of parameter estimates	4						
#cases/#parameters	3.3						

7.3. Supplementary information for Chapter 5

Supplementary Program 1. Arduino sensors program

```
/*
SD card datalogger
This example shows how to log data from three analog sensors
to an SD card using the SD library.
The circuit:
SD card attached to SPI bus as follows:
** UNO:  MOSI - pin 11, MISO - pin 12, CLK - pin 13, CS - pin 4 (CS pin can be changed)
and pin #10 (SS) must be an output
** Mega:  MOSI - pin 51, MISO - pin 50, CLK - pin 52, CS - pin 4 (CS pin can be changed)
and pin #52 (SS) must be an output
** Leonardo: Connect to hardware SPI via the ICSP header
Pin 4 used here for consistency with other Arduino examples
created 24 Nov 2010
modified 9 Apr 2012 by Tom Igoe
This example code is in the public domain.
*/
#define WAIT_TO_START    1 // Wait for serial input in setup()
#include "DHT.h"
#include <SD.h>
```

```
// Date and time functions using a DS1307 RTC connected via I2C and Wire lib
#include <Wire.h>
#include "RTClib.h"
// for 3xDHT22,
//      VCC: 5V or 3V
//      GND: GND
//      DATA: 8,9,10
#define DHT1PIN 7     // what pin we're connected to
//#define DHT2PIN 6
//#define DHT3PIN 5
// Uncomment whatever type you're using!
#define DHT2TYPE DHT22   // DHT 22  (AM2302)
DHT dht1(DHT1PIN, DHT2TYPE);
//DHT dht2(DHT2PIN, DHT2TYPE);
//DHT dht3(DHT3PIN, DHT2TYPE);
RTC_DS1307 rtc;
// On the Ethernet Shield, CS is pin 4. Note that even if it's not
// used as the CS pin, the hardware CS pin (10 on most Arduino boards,
// 53 on the Mega) must be left as an output or the SD library
// functions will not work.
const int chipSelect = 4;
File dataFile;
// the logging file
char timestamp[30];
//------------------------------------------------------------------------------
// call back for file timestamps
void dateTime(uint16_t* date, uint16_t* time) {
DateTime now = rtc.now();
sprintf(timestamp, "%02d:%02d:%02d  %2d/%2d/%2d  \n", now.hour(), now.minute(), now.second(), now.month(), now.day(),
now.year() - 2000);
//Serial.println("yy");
//Serial.println(timestamp);
// return date using FAT_DATE macro to format fields
*date = FAT_DATE(now.year(), now.month(), now.day());
// return time using FAT_TIME macro to format fields
*time = FAT_TIME(now.hour(), now.minute(), now.second());
}
//------------------------------
```

```
float sensor_volt0;
float sensorValue0;
float sensor_volt1;
float sensorValue1;
//float sensor_volt2;
//float sensorValue2;
float calvolts = 5.0 / 1024; //to convert analog read to volts with 5V reference
int   numreads = 20;      //number of reads for AD conversion
const long eventTime = 5000; //in ms
unsigned long previousTime = 0;
void setup() {
Serial.begin(9600);
//analogReference(EXTERNAL); // use AREF for reference voltage currently set at 5V from board
dht1.begin();
//dht2.begin();
//dht3.begin();
Wire.begin();
rtc.begin();
SdFile::dateTimeCallback(dateTime);
DateTime now = rtc.now();
sprintf(timestamp,  "%02d:%02d:%02d  %2d/%2d/%2d  \n",  now.hour(),  now.minute(),  now.second(),  now.month(),  now.day(),
now.year() - 2000);
//Serial.println("xx");
Serial.println(timestamp);
//Serial.print("Initializing SD card...");
// make sure that the default chip select pin is set to
// output, even if you don't use it:
pinMode(SS, OUTPUT);
// see if the card is present and can be initialized:
if (!SD.begin(chipSelect)) {
Serial.println("Card failed, or not present");
// don't do anything more:
while (1) ;
}
//Serial.println("card initialized.");
#if WAIT_TO_START
Serial.println("Type any character to start saving and another to finish after plotting");
while (!Serial.available());
```

```
#endif //WAIT_TO_START
char input = Serial.read(); //read buffer to clear it
char filename[] = "LOGGER00.CSV";
for (uint8_t i = 0; i < 100; i++) {
filename[6] = i / 10 + '0';
filename[7] = i % 10 + '0';
if (! SD.exists(filename)) {
// only open a new file if it doesn't exist
dataFile = SD.open(filename, FILE_WRITE);
break;  // leave the loop!
}
}
//Serial.print("Logging to: ");
Serial.println(filename);
//Set up header in csv file
// get time
// DateTime now = rtc.now();
char buf2[] = "YYMMDD-hh:mm:ss";
dataFile.println(now.toString(buf2));
dataFile.println("Unix_T,Eth_V1,Eth_V2,T1,H1");
}
void loop() {
unsigned long currentTime = millis();
/* This is my event_1 */
if ( currentTime - previousTime >= eventTime) {
previousTime = currentTime;
float h1 = dht1.readHumidity();
float t1 = dht1.readTemperature();
//float h2 = dht2.readHumidity();
//float t2 = dht2.readTemperature();
//float h3 = dht3.readHumidity();
//float t3 = dht3.readTemperature();
// check if returns are valid, if they are NaN (not a number) then something went wrong!
if (isnan(t1) || isnan(h1)) {
Serial.println("Failed to read from DHT #1");
} else {
//Serial.print("Humidity 1: ");
Serial.print(h1);
```

```
//Serial.print(" %\t");
Serial.print(",");
//Serial.print("Temperature 1: ");
Serial.print(t1);
//Serial.println(" *C");
Serial.print(",");
}
//if (isnan(t2) || isnan(h2)) {
//Serial.println("Failed to read from DHT #2");
//} else {
//Serial.print("Humidity 2: ");
//Serial.print(h2);
//Serial.print(" %\t");
//Serial.print(",");
//Serial.print("Temperature 2: ");
//Serial.print(t2);
//Serial.println(" *C");
//Serial.print(",");
//}
//if (isnan(t3) || isnan(h3)) {
// Serial.println("Failed to read from DHT #3");
//} else {
//Serial.print("Humidity 3: ");
//Serial.print(h3);
//Serial.print(" %\t");
//Serial.print(",");
//Serial.print("Temperature 3: ");
//Serial.print(t3);
//Serial.println(" *C");
//Serial.print(",");
sensorValue0 = 0;
sensorValue1 = 0;
//sensorValue2 = 0;
//Read AD gas sensors
for (int i = 0; i <= numreads; i++) {
sensorValue0 += analogRead(A0);
sensorValue1 += analogRead(A1);
//sensorValue2 += analogRead(A2);
```

```
//
delay(5);
}
sensor_volt0 = 100 * sensorValue0 * calvolts / numreads;
sensor_volt1 = 100 * sensorValue1 * calvolts / numreads;
//sensor_volt2 = 100 * sensorValue2 * calvolts / numreads;
//Serial.print("sensor_volt = ");
Serial.print(sensor_volt0); Serial.print(",");
Serial.println(sensor_volt1); //Serial.print(",");
//Serial.println(sensor_volt2);
//Serial.println("V");
//delay(2000);
//write to SD card
// get time
DateTime now = rtc.now();
dataFile.print(now.unixtime()); dataFile.print(",");
dataFile.print(sensor_volt0); dataFile.print(",");
dataFile.print(sensor_volt1); dataFile.print(",");
//dataFile.print(sensor_volt2); dataFile.print(",");
dataFile.print(t1); dataFile.print(",");
//dataFile.print(t2); dataFile.print(",");
//dataFile.print(t3); dataFile.print(",");
dataFile.println(h1); //dataFile.print(",");
//dataFile.print(h2); dataFile.print(",");
//dataFile.println(h3);
}
if (Serial.available()) {
// Close file and stop.
char input = Serial.read();
dataFile.close();
Serial.println(F("Done"));
while (1);
}
}
```

Supplementary Program 2. Arduino time program

```
lem // Date and time functions using a DS1307 RTC connected via I2C and Wire lib
#include "RTClib.h"
```

```
RTC_DS1307 rtc;
char daysOfTheWeek[7][12] = "Sunday", "Monday", "Tuesday", "Wednesday", "Thursday", "Friday", "Saturday";
void setup () {
while (!Serial); // for Leonardo/Micro/Zero
Serial.begin(9600);
if (! rtc.begin()) {
Serial.println("Couldn't find RTC");
while (1);
}
if (! rtc.isrunning(Serial.println("RTC is NOT running!");
 // following line sets the RTC to the date & time this sketch was compiled
 rtc.adjust(DateTime(F(__DATE__), F(__TIME__)));
// This line sets the RTC with an explicit date & time, for example to set
// January 21, 2014 at 3am you would call:
// rtc.adjust(DateTime(2014, 1, 21, 3, 0, 0));
}
}

void loop () {
DateTime now = rtc.now();
Serial.print(now.year(), DEC);
Serial.print('/');
Serial.print(now.month(), DEC);
Serial.print('/');
Serial.print(now.day(), DEC);
Serial.print(" (");
Serial.print(daysOfTheWeek[now.dayOfTheWeek()]);
Serial.print(") ");
Serial.print(now.hour(), DEC);
Serial.print(':');
Serial.print(now.minute(), DEC);
Serial.print(':');
Serial.print(now.second(), DEC);
Serial.println();
Serial.print(" since midnight 1/1/1970 = ");
Serial.print(now.unixtime());
Serial.print("s = ");
Serial.print(now.unixtime() / 86400L);
```

```
Serial.println("d");
// calculate a date which is 7 days, 12 hours, 30 minutes, and 6 seconds into the future
DateTime future (now + TimeSpan(7,12,30,6));
Serial.print(" now + 7d + 12h + 30m + 6s: ");
Serial.print(future.year(), DEC);
Serial.print('/');
Serial.print(future.month(), DEC);
Serial.print('/');
Serial.print(future.day(), DEC);
Serial.print(' ');
Serial.print(future.hour(), DEC);
Serial.print(':');
Serial.print(future.minute(), DEC);
Serial.print(':');
Serial.print(future.second(), DEC);
Serial.println();
Serial.println();
delay(3000);
}
```

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