

Reproductive water supply is prioritised during drought in tomato

Beatrice Harrison Day¹, Madeline Carins Murphy¹, and Timothy Brodribb¹

¹University of Tasmania

May 5, 2021

Abstract

Reproductive success largely defines the fitness of plant species. Understanding how heat and drought affect plant reproduction will thus be key to predicting future plant fitness under rising global temperatures. Recent work suggests reproductive tissues are highly vulnerable to water stress in perennial plants where reproductive sacrifice could preserve plant survival. However, most crop species are annuals where such a strategy would theoretically reduce fitness. We examined the reproductive strategy in tomato (*Solanum lycopersium*) to determine whether water supply to fruits was prioritized above vegetative tissues during drought. Using optical methods, we map xylem cavitation and tissue shrinkage in vegetative and reproductive organs during dehydration to determine the priority of water flow under acute imposed water stress. Stems and peduncles of tomato showed significantly greater resistance to xylem cavitation than vegetative tissues. This prioritization of reproductive water supply enabled tomato fruit to continue expanding during acute water stress, utilising xylem water made available by capacitance and cavitation of vegetative tissues. Here, tomato plants prioritize water supply to reproductive tissues, maintaining fruit development in drought conditions. These results emphasize the critical role of water transport in shaping tomato life history and suggest a broad relevance of hydraulic prioritization in plant ecology.



FACULTY OF SCIENCE,
ENGINEERING AND TECHNOLOGY

Dear Professor Amtmann.

We are excited to present this new paper entitled “Reproductive water supply is prioritised during drought in tomato”.

Previous research in perennial species has shown reproductive tissues to be readily sacrificed during drought to prolong the survival of the main plant, yet such a strategy would appear to be counterproductive in annual plants where rapid reproduction is a necessity. Using novel experimental techniques, we use the annual crop species tomato to examine the water supply to reproductive organs during drought.

We show here that the water supply to reproductive tissues is heavily prioritised over vegetative tissues under strong dehydration stress through hierarchical xylem resistance to cavitation. This strategy allows water to be preferentially supplied even in drought to support reproductive growth, an observation with important implications to ecology and crop production.

We believe this topical study has broad applications and will generate interest.



Best regards, Tim

Prof Tim Brodribb

School of Plant Science

University of Tasmania

Email- timothy.brodribb@utas.edu.au

Ph. +61 362261707

Suggested referees :

Antonio Diaz Espejo (editorial review board), Adam Roddy, Sean Gleason, Kathy Steppe, Robert Skelton.

Reproductive water supply is prioritised during drought in tomato.

Beatrice L. Harrison Day¹, Madeline R. Carins Murphy¹, Timothy J. Brodribb^{1*}.

¹School of Natural Sciences, University of Tasmania, Private Bag 55, Hobart, TAS 7001, Australia.

*Author for correspondence: Timothy Brodribb.

timothy.brodribb@utas.edu.au / +61 3 6226 1707

Funding : The project was funded by ARC Discovery grant/award no: DP190101552. The first author was also funded by an Australian Government Research Training Program (RTP) Scholarship.

Abstract:

Reproductive success largely defines the fitness of plant species. Understanding how heat and drought affect plant reproduction will thus be key to predicting future plant fitness under rising global temperatures. Recent work suggests reproductive tissues are highly vulnerable to water stress in perennial plants where reproductive sacrifice could preserve plant survival. However, most crop species are annuals where such a strategy would theoretically reduce fitness. We examined the reproductive strategy in tomato (*Solanum*

lycopersium) to determine whether water supply to fruits was prioritized above vegetative tissues during drought. Using optical methods, we map xylem cavitation and tissue shrinkage in vegetative and reproductive organs during dehydration to determine the priority of water flow under acute imposed water stress. Stems and peduncles of tomato showed significantly greater resistance to xylem cavitation than vegetative tissues. This prioritization of reproductive water supply enabled tomato fruit to continue expanding during acute water stress, utilising xylem water made available by capacitance and cavitation of vegetative tissues. Here, tomato plants prioritize water supply to reproductive tissues, maintaining fruit development in drought conditions. These results emphasize the critical role of water transport in shaping tomato life history and suggest a broad relevance of hydraulic prioritization in plant ecology.

Key words: Drought, *Solanum lycopersicum* (tomato), reproduction, xylem cavitation

Acknowledgements : This project was supported by the University of Tasmania discipline of biological sciences. Special thanks to the UTAS glasshouse staff, to Greg Jordan and to Christopher Lucani for technical and statistical support.

ORCID:

Beatrice L. Harrison Day: <https://orcid.org/0000-0003-3070-2890>

Madeline R. Carins-Murphy: <http://orcid.org/0000-0003-4370-9485>

Timothy J. Brodribb: <https://orcid.org/0000-0002-4964-6107>

Introduction:

Plant reproduction underpins global food production and is fundamental to plant genetic diversity and proliferation (Hedhly, Hormaza, & Herrero, 2009; Soltis & Soltis, 2014). As temperatures rise under the effects of climate change, causing soils to dry faster, the water available to plants is becoming increasingly limited (Brodribb et al. 2016). Water limitation and its damaging effect on the water transport capacity of plants has been linked with mass plant mortality around the globe (Adams et al., 2017; W. R. L. Anderegg et al., 2016; T. J. Brodribb, Powers, Cochard, & Choat, 2020; Choat et al., 2012). Despite the serious consequences of reproductive failure to agriculture and conservation, however, the effects of water stress on plant reproduction are not well resolved (Bourbia, Carins-Murphy, Gracie, & Brodribb, 2020; Roddy, 2019; Zhang, Zhang, Brodribb, & Hu, 2020). Hydraulic traits have been used to effectively model plant mortality in drought (W. R. Anderegg et al., 2015; Choat et al., 2018; Choat et al., 2012; Sperry & Love, 2015; Sperry & Tyree, 1988; Uri et al., 2013; Vilagrosa et al., 2012) yet such traits in reproductive and floral organs are not well integrated into whole plant hydraulic vulnerability models.

Plant tissue damage and mortality from drought has been closely linked with the cavitation of xylem cells, caused by the invasion of air into vascular conduits under tension (negative pressure $-MPa$) (Sperry & Tyree, 1988; Melvin T Tyree & Sperry, 1989). Decreases in soil water availability causes a drop in plant water potential, resulting in cavitation damage to the vascular system, progressively isolating plant tissues from their water source and ultimately resulting in the death of tissues (Savi et al., 2015; Brodribb et al., 2016). Xylem with greater resistance to cavitation maintains an intact water supply to tissues longer in water limiting conditions (Sperry & Tyree, 1988; Melvin T Tyree & Sperry, 1989). Relationships between xylem cavitation and plant damage have been widely characterized in woody tissues, but only recently have the implications of xylem cavitation been considered in the context of plant reproductive success (Bourbia et al., 2020; Roddy, Jiang, Cao, Simonin, & Brodersen, 2019; Zhang & Brodribb, 2017).

Reproduction depends on a supply of water for sustaining cell turgor and growth, delivered via xylem and phloem cells. The relative water contribution of these vascular cells types determines how tightly connected fruits and flowers are to dynamic changes in plant water deficit. Flowers which transpire rapidly must rely

on the xylem as the most efficient transporter of water, exposing them to the water stress experienced by the whole plant. Alternately, if flower water loss is negligible, then water may be supplied by the phloem, theoretically allowing the flower to be largely disconnected from the apoplastic water stress (Bourbia et al., 2020; Chapotin, Holbrook, Morse, & Gutiérrez, 2003; De La Barrera & Nobel, 2004). However, the estimated contribution of xylem supplied water to the water balance of fruit and flowers varies significantly. This is particularly apparent in tomato, with xylem contribution to total fruit water volume estimated to supply between 10% (L. C. Ho, Grange, & Picken, 1987) to 90% (Windt, Gerkema, & Van As, 2009) of tomato fruit water content (Hanssens, De Swaef, & Steppe, 2015; van Die & Willemse, 1980). In tomato, water accounts for more than 90% of ripe fruit mass, and fruit volumetric growth is primarily driven by water accumulation (Lim Chuen Ho, 1980; L. C. Ho et al., 1987; Matthews & Shackel, 2005), making the pathway of water crucial to understanding reproductive development (L. C. Ho et al., 1987; Li, Zhang, Hou, & Du, 2021; Windt et al., 2009). Recent MRI studies provide further support for the majority of water for reproduction being supplied by the xylem, with 90% of tomato water found to be xylem-delivered. Studies in other fruit (Hanssens et al., 2015; van Die & Willemse, 1980; Windt et al., 2009) also suggest most water is delivered to the fruit by the xylem rather than the phloem. Xylem-dominated hydration of reproductive tissue means that the vulnerability of this xylem to cavitation is highly relevant to understanding fruit -vulnerability to drought-damage, as well as the ecology and reproductive behaviour of plant species.

Evidence suggests that reproductive tissues are exposed to xylem cavitation in drought conditions, and water loss from flowering structures also contributes to whole plant water stress (Bourbia et al., 2020; Lambrecht, 2013; Roddy, 2019; Zhang, Carins Murphy, Cardoso, Jordan, & Brodribb, 2018). These observations highlight the need to include reproductive organs in hydraulic models to understand the broader patterns in xylem vulnerability throughout the plant (Bourbia et al., 2020). Based on the hydraulic vulnerability segmentation hypothesis, distal, short-lived organs requiring a low carbon investment should be more vulnerable to cavitation than non-redundant organs, helping to prologue plant survival in drought (Johnson et al., 2016; Melvin T. Tyree & Ewers, 1991; Zimmermann, 2013). By integrating reproductive tissues into whole plant hydraulic vulnerability studies, damage to reproductive organs in acute water stress may be compared with other organs based on their resistance to cavitation, to determine their hydraulic priority relative to other tissues (Bourbia et al., 2020; Zhang et al., 2020).

Recent data suggest that flowers are ranked low in the hierarchy of water supply priority during drought. This makes sense in the woody species examined where the high evaporative cost of flowering (Galen, Sherry, & Carroll, 1999; Lambrecht, 2013) makes flowers an avoidable liability under water stress. It has been shown that in woody and perennial species flowers cavitate prior to other tissues, and are shed, while long-lived important vegetative structures such as stems and leaves are maintained (Bourbia et al., 2020; Melvin T. Tyree & Ewers, 1991; Zhang et al., 2020). This order of tissue preservation was determined by different xylem vulnerability to cavitation, with peduncles found to be highly vulnerable, effectively isolating leaky inflorescences during water stress (Bourbia et al., 2020; Zhang et al., 2020). Applying the same adaptational logic to annual plants, it might be expected that the prioritization of reproductive tissues would be different given that these species only have a single opportunity to reproduce before death. We hypothesize that the reproductive tissues of annuals should be hydraulically prioritised through increased resistance to xylem cavitation relative to vegetative tissues. In this way water released from vegetative parts of the plant (as capacitance from shrinking tissue or through cavitation in vegetative structures (Hölttä, Cochard, Nikinmaa, & Mencuccini, 2009)) could be directed to reproductive tissues during acute water stress, enabling completion of reproduction even under conditions of acute water stress.

Here, we investigate the distribution of xylem vulnerability among reproductive and vegetative tissues in an herbaceous annual to determine the hydraulic priority of reproductive tissues relative to vegetative tissues in water-limiting conditions. Tomato was selected as a well-documented test species given its short, annual life history and large reproductive water requirements (Bertin, 2003; Bussi eres, 1994; Li et al., 2021; Shameer, Vallarino, Fernie, Ratcliffe, & Sweetlove, 2020; Van Ieperen, Volkov, & Van Meeteren, 2003). We hypothesise that fruit growth will be prioritized through increased resistance to cavitation in the xylem reproductive water supply (peduncles and stems) compared to vegetative supply (leaves and petioles); and that fruit

growth may be maintained even during water stress by drawing on the water provided by the capacitance and cavitation of less resistant tissues.

Materials and Methods:

Plant material and growing conditions.

Tomato plants (*Solanum lycopersicum* var. Rhinlands Rhune) were grown from seed in glasshouse facilities at the University of Tasmania, Australia. Plants experienced average day/night temperatures of 23/15 °C, an 18-hour photoperiod to ensure flowering, a relative humidity of approximately 40%, and were watered to field capacity daily to avoid drought stress. Seeds were germinated in punnets of fine pasteurised soil and then transferred to 2-l pots filled with potting mix (medium 7:4 mix of composted fine pine bark and coarse washed river sand). Plants were around 3 months old at commencement of experiments.

Plant preparation

Cavitation in the leaves, petioles, petals, and peduncles was monitored simultaneously in intact plants to determine the relative xylem vulnerability and order of cavitation among reproductive and vegetative organs within individual plants. Measurements of stem vulnerability were completed separately on replicate plants to avoid excessive manipulation (see below). Measurements were conducted on five replicate plants (3+ months old, ~1m tall) with fully expanded leaves and at least two flower clusters with separate peduncles. Well-watered plants were removed from pots and their roots were gently rinsed with water to remove most of the soil. This ensured complete cavitation of all tissues within 8 days. This duration ensured plant dehydration rates were fast enough to capture embolism formation but slow enough to maintain water potential equilibrium between slowly evaporating tissues (Bourbia et al., 2020).

Xylem vulnerability to cavitation

Water potential monitoring

Once prepared plants were transferred to the laboratory and allowed to dehydrate under artificial light at 22°C temperature. A stem psychrometer (ICT international, Armidale, NSW, Australia) was installed midway along the tomato stem above the first undamaged fully expanded leaves. A section of stem cuticle and epidermis 20-30mm was scraped off with fingernails to expose the xylem. The xylem was then rinsed with distilled water and gently dried to remove tissue debris and potential solute contaminants. The stem psychrometer was clamped onto the stem parallel to xylem. Parafilm (Bemis Co Inc, USA) was used to seal the psychrometer chamber to the stem and to cover any sections of stem with damaged cuticle. The psychrometer was set to log stem water potential (Ψ_s -MPa) every 20 min, with a Peltier cooling time of 10 seconds. The final cavitated water potential of tomato was not negative enough to require resetting of cooling time, with 10 seconds giving a stable reading of the wet-bulb temperature throughout drying. Ψ_s measurements made with the psychrometer were compared with measurements of leaf water potential made using detached leaves measured with a Scholander pressure chamber (PMS, Albany, OR, USA). Values found to be in agreement up until the point of leaf cavitation, at which point the pressure chamber readings became unreliable due to leaf damage. Due to the slow rate of plant dehydration in the laboratory (under low irradiance), water potential was assumed to be relatively homogeneous within the plant so psychrometer readings from the stem were applied to the whole plant.

Organ xylem vulnerability to cavitation.

The optical vulnerability method (OV) was used to quantify xylem vulnerability (Brodribb et al 2015). This technique is minimally invasive and allows simultaneous monitoring of xylem cavitation throughout the plant. Data produced using the OV technique has been shown to correspond very closely with vulnerability data collected using hydraulic and x-ray methods in Tomato (Skelton, Brodribb, & Choat, 2017) and many other species (Gauthey et al., 2020). A combination of custom-made “cavicams” (opensourceov.org) and a microscope were used simultaneously on each plant to monitor cavitation during dehydration.

Leaves : a cavicum was secured to a single fully expanded leaf per plant to view a region 5x5mm that included secondary and tertiary veins. Tissue was illuminated from underneath by LEDs (white-light emitting diodes). Images were acquired every 2 min for 8 days. Cameras were refocussed as necessary.

Petioles : a region of one petiole on an adjacent leaf per plant was selected close to the main stem. Using a sharp razor blade, a window of around 15 mm x 8 mm was carefully cut parallel to the surface to remove the epidermis and cuticle on one side of the petiole, exposing the xylem. The area was coated in hydrogel (Tensive Gel; Parker Laboratories Inc., Fairfield, NJ, USA) to improve light transmission and reduce evaporation from the surface. The section of petiole was then secured in a cavicum which acquired images as described above using reflected light.

Peduncles: a region of one peduncle supporting several open flowers, a variable number of buds and no fruit was selected per plant. A region of around 15 mm was carefully scraped with a fingernail to remove the epidermis and cuticle, exposing the xylem. No razor blade was used as the epidermal tissue was softer than the petiole equivalent and razor blades easily cut too deep into the vascular tissue. The area was coated in hydrogel then secured in a cavicum which acquired images using reflected light.

Petals: Images of one petal per plant from an intact apical inflorescence were captured with a Leica DFC450 digital camera, mounted on a Leica M205A stereomicroscope (Leica Microsystems, Wetzlar, Germany). The petals of a newly opened flower were taped to a glass plate to prevent movement/shrinkage during image capture. Light was transmitted through the petal illuminating the midrib xylem bundle and images captured as described above.

Stems

Due to the positioning of cameras, it was not possible to measure stem xylem vulnerability simultaneously with other tissues, so this was quantified on four individuals of the same age and size. One section of stem was selected per plant between the root collar and the first reproductive structures. Using a sharp razor blade, a window of around 20 mm was carefully cut to remove the epidermis and cuticle, exposing the xylem. Water potential was monitored with a stem psychrometer as above and image capture protocols were the same as described for the petiole and peduncle.

Image analysis

Image sequences were analysed using IMAGEJ (NIH), see <http://www.opensourceov.org/> for details. Briefly, the pixel values of each image were subtracted from the next in the sequence to quantify any differences between images. Pixel differences indicate a change in light transmission or reflection as water-filled vessels fill with air during cavitation events (Brodribb *et al.*, 2016). Due to the frequency of image capture, cavitation events can be separated from other “noise” due to movement, leaf shrinkage, or drying of hydrogel by filtering differences that are not fast changes confined to the xylem. Noise was removed using pixel thresholding leaving only pixels associated with cavitation events. The embolism area per image was calculated as the summed pixels and expressed as cumulative embolism, a percentage of the total cavitated area in pixels per sequence. Cumulative embolism was plotted against water potential to create vulnerability curves (Bourbia *et al.*, 2020; T. J. Brodribb, Carriqui, Delzon, & Lucani, 2017) and derive the water potential at which 50% of total xylem were cavitated (P50).

Tissue shrinkage/growth analyses.

To investigate the distribution of water between vegetative and reproductive tissues as plants dehydrated, we used cameras to simultaneously monitored the shrinkage dynamics of reproductive and vegetative organs, petioles, peduncles, and fruit within individual plants. 5 different plants were measured and exposed to the same dehydration and water potential monitoring as above. Intact proximal regions of *S. lycopersicum* petioles and peduncles were secured in cavicans as described above. Images were acquired using transmitted light to produce a silhouette every 10 minutes until the plant was fully desiccated (approximately 8 days). Cameras were refocussed every day as the tissue dried. Intact tomato fruit approximately 1cm diameter (older than 7 days) were photographed using a Leica DFC450 digital camera. Single fruit were secured to

the stage of a Leica M205A stereomicroscope (Leica Microsystems, Wetzlar, Germany) or a custom-built gantry holding a Nikon lens attached to a Raspberry PI camera and Images taken at intervals of 10 minutes until the plant was fully desiccated.

Images were processed in IMAGEJ (NIH). Image sequences were thresholded to isolate plant tissue from the background. The total thresholded area and fruit diameter were recorded every 50 images and this decrease in size, expressed as a percentage of the original area, was plotted against water potential.

To confirm that a vascular connection was required for fruit growth the growth of tomato fruit was also assessed once fruit were removed from the plant's vascular supply. A tomato fruit of ~1cm diameter was monitored as above, however once the plant water potential had reached -1MPa, after demonstrating initial positive expansion, the fruit was excised from the plant at the peduncle using a razor blade. Images were captured every 2 minutes until the rest of the plant was fully desiccated.

Phloem girdling

We compared the growth rate of fruit with intact xylem and phloem to that of fruit with only intact xylem to determine the relative importance of xylem and phloem water delivery for fruit expansion during water stress. Phloem tissue was selectively killed by heat girdling the pedicel, according to Guichard, Gary, Leonardi, and Bertin (2005) and Van De Wal, Windt, Leroux, and Steppe (2017). In brief, a length of insulated nichrome wire (length, 0.5 m; diameter, 0.25 mm) was coiled around the pedicel between the peduncle joint and the abscission zone, covering half of the total 8mm length and an electrical current was applied via a transformer to raise the temperature to 75°C for 1 min. Temperature was measured using a thermocouple in contact with the plant tissue under the heating coil (Van De Wal et al., 2017). Measurements were performed on three plants, using paired fruit supported by the same peduncle, with one fruit per pair heat girdled and the other maintained as an ungirdled control. Following girdling images were captured to monitor the projected area of both fruits as the plant dried. Plants were kept under laboratory light conditions with additional white light LED illumination, and water potential was recorded using an ICT psychrometer. Tomato fruit undergo a phase of cell division for 1-2 weeks post fertilisation, and then a period of cell expansion (2-6 weeks), driven by the filling of cells with osmolytes and water (Bertin, 2003; Gillaspy, Ben-David, & Gruissem, 1993; L. Ho, Sjut, & Hoad, 1982; Renaudin, Deluche, Cheniclet, Chevalier, & Frangne, 2017; Shameer et al., 2020), so larger fruits (2+cm diameter) were selected here to ensure fruit filling was responsible for growth rather than cell division.

Xylem diameter of petioles and peduncles

The xylem lumen diameter of three petioles and three peduncles from each of three additional plants was examined to test for any association between organ xylem vulnerability to cavitation and vessel size. Transverse sections of peduncles and petioles were made close to the base where they joined the main stem using a sliding microtome (Leica Microsystems, North Ryde, NSW, Australia) and a BFS-3MP Freezing Stage (Physitemp Instruments, Clifton, NJ, USA) at 30 µm thickness. Cross sections were stained with toluidine blue (5%) and mounted on glass slides with phenol glycerine jelly. Slides were photographed at 20x magnification using a Nikon Digital Sight DS-Fi2 (Tokyo, Japan) mounted on a compound microscope (DM1000; Leica Microsystems, Wetzlar, Germany). Vessel diameter was measured using ImageJ. Four representative fields of view (20x magnification) of entire xylem bundles were captured for each transverse section. Using IMAGEJ, a binary image was produced for each section, and vessel lumens were selected using the threshold function. An ellipse was automatically fitted to each xylem vessel lumen (fig. 1). The lumen area of each vessel was automatically measured using the 'analyse particles' function to calculate an average cross-section diameter for each vessel. Given that the flow rate of water through vascular tissue is directly proportional to the xylem diameter to the 4th power (Hagen-Poiseuille law), only the largest of xylem vessels contribute significantly to the water transport capacity of the vascular system (Petit, Anfodillo, & De Zan, 2009; Melvin T. Tyree & Ewers, 1991). Thus, for each section, xylem diameters were ranked and the mean diameter of the largest 5% of vessels measured. This allowed us to compare the theoretical water supply capacity and resilience to xylem collapse in vegetative and reproductive support structures.

Statistical analysis

The cavitation resistance of petioles, leaves, petals, and peduncles (all organs were measured on each individual) was compared using two-way ANOVA to test the effect of organ type and individual on $P50$ values in R. A log transformation was applied to fulfil assumptions of homoscedasticity and normality. Post hoc tests were computed using Tukey's HSD.

The effect of disabling phloem water delivery by heat girdling on fruit expansion was tested using a two-way repeat measures ANOVA. The interaction term was determined to be non-significant and was removed from the model. Assumptions were tested for normality and no transformations were applied. A qq plot could not be fitted given the small data set, however, the consistency of results indicated that model assumptions could be met.

The cavitation resistance (quantified as the $P50$ and $P88$) and mean diameter of the largest 5 % of vessels in peduncles and stems (measured on separate individuals) was compared using a student's T-test.

Results:

Shrinkage and growth under water stress

As whole plants dehydrated from 0 to -3MPa, peduncles were observed to shrink by a mean 20% ($\pm 4.99\%$) of their original hydrated area, and petioles by an average of 46% ($\pm 3.39\%$) (fig. 2). At the same time, fruit continued to grow, showing a mean increase from original size by 26% ($\pm 4.17\%$). This growth stopped immediately after the vascular connection with the stem was severed by cutting when stem water potential reached -1MPa.

The treatment of girdling pedicels was observed to have no effect ($P=0.711$) on fruit growth compared with the ungirdled control (fig. 3). No interaction was observed between treatment (girdled vs ungirdled) and water potential ($P=0.959$), indicating fruit of each treatment were growing in the same way under water stress, despite the phloem of the girdled fruit being fully disrupted. Both girdled and ungirdled fruit continued to grow significantly at decreasing water potentials ($4.26e-06^{***}$) with girdled fruit growing 4.25% ($\pm 0.4\%$) and ungirdled fruit growing 4.5% ($\pm 0.67\%$) over the 6 days of plant dehydration.

Xylem vulnerability of vegetative vs reproductive organs

The xylem supplying water to reproductive tissues was more resistant to cavitation than that supplying vegetative tissues (fig. 4). Peduncles were typically the last tissues to cavitate by $>50\%$ in each individual (fig. 4). Within individuals the peduncle $P50$ was typically lowest relative to that of leaves, petals, and petioles (fig. S1). In contrast the petiole $P50$ was highest in 4/5 plants (fig. S1). This pattern between organs was supported by the results of ANOVA in that organ type had a significant overall effect on $P50$ ($P=0.002^{**}$) (table 1). The mean $P50$ of peduncles ($-2.13\text{MPa} \pm 0.21$) was found to be significantly more negative than that of petioles ($-1.39\text{MPa} \pm 0.10$) leaves ($-1.53\text{MPa} \pm 0.13$) and petals ($-1.32\text{MPa} \pm 0.15$) (fig 5.). No differences were observed between the $P50$ of vegetative tissues and petals, but peduncles were consistently more resistant to cavitation, with lower $P50$ s than the other organs (fig. 5). The mean $P50$ for stems, however ($-1.91\text{MPa} \pm 0.28$, fig. 5) was similar to the $P50$ of the peduncles ($P=0.55$).

The mean $P50$ ($-1.91 \pm 0.28\text{MPa}$) and $P88$ ($-2.66 \pm 0.31\text{MPa}$) of stems was not significantly different ($P>0.05$ in both cases) to the $P50$ ($-2.13\text{MPa} \pm 0.21\text{MPa}$) and $P88$ (-2.24 ± 0.21) found in peduncles, showing substantial overlap in the cavitation resistance of these organs (fig. 4 and 5). The stem cavitation within individuals showed a pattern of cavitation that began comparatively early at hydrated water potentials, but continued cavitating until low water potentials in a similar way to peduncles (fig. S2).

Vascular anatomy

The xylem vessels within the petioles were found to be significantly larger in diameter than the vessels found in peduncles ($P=0.002$) (fig. S3). The mean diameter of the widest 5% of xylem in the peduncles was 11.6

μm , 30% smaller than the mean diameter of petioles ($16.5 \mu\text{m}$).

Discussion:

We demonstrate here that fruit development (and thus by extension, seed production) in an annual plant species is prioritised at the expense of vegetative tissues under water stress through relatively greater xylem resistance to cavitation in those tissues supplying water to fruit. We found that the xylem of vegetative tissues cavitated early during drought, while the loss of xylem function in the main stem and the peduncle occurred much later. These cavitation resistant tissues provided a xylem pathway that prioritized water supply to fruit from other plant parts even when the plant is fully disconnected from a soil water source. The prioritisation of water supply to reproduction in tomato means that unlike observations in perennial plants, the fruit of tomato behaves as a hydraulic parasite upon the vegetative body.

Fruit is hydraulically prioritised through cavitation resistance.

The advantages of prioritising the water requirements of reproduction over vegetative tissues observed here in tomato become particularly apparent when considering the ancestry and evolution of this species. Tomato belongs to the *Solanum* section *Lycopersicon*, in the *Solanaceae* that includes the cultivated tomato and 12 wild relatives (Bergougnoux, 2014; Peralta, Knapp, & Spooner, 2007). While the specifics of tomato domestication are uncertain, the wild species originate in Western South America, growing from Northern Chile to Ecuador and the Galapagos Islands, although domestication may have occurred in Mesoamerica (Bergougnoux, 2014; Blanca et al., 2012). These ancestral *Lycopersicon* occur in dry desert and pre-desert environments, including the Atacama Desert of Northern Chile where rainfall is highly sporadic, making the prioritisation of reproduction even under water stress a necessity for species persistence in the landscape (Bergougnoux, 2014; Chetelat, Pertuzé, Faúndez, Graham, & Jones, 2009; Fischer, Camus-Kulandaivelu, Allal, & Stephan, 2011; Knapp & Peralta, 2016). In the case of annual plants, it appears advantageous to ensure all available water is directed to reproduction, safeguarding the potential for future offspring above the survival of the parent plant. In an opportunistic annual species such as tomato where reproduction often takes place under conditions of ephemeral rain, there is good reason to expect that prioritization of reproduction would enhance fitness, as the need to set seed is paramount. We hypothesised that selection would favour individuals where xylem supply to fruiting tissue (in the stem and peduncle) was more resistant to cavitation than other organs, ensuring a water supply to fruit for as long as possible in drought conditions. The reasoning behind this prediction follows the hydraulic vulnerability segmentation hypothesis which considers the energetic or fitness cost of losing tissues to cavitation (Johnson et al., 2016; Melvin T. Tyree & Ewers, 1991; Zimmermann, 2013). Under the likely scenario that water becomes limited during reproduction (e.g. when growth was initiated after an ephemeral rain storm), the plant would be able to ensure reproductive completion, even if it required water to be extracted from the rest of the vegetative plant body.

Fruit water volume is supplied by xylem.

This idea of reproductive prioritisation depends on the assumption that the water reaching fruit is supplied by xylem and thus exposed to the tension and cavitation risk experienced by the broader plant in drought. The proportion of water proposed to be supplied to the tomato fruit by the xylem ranges from 10 to 90% (L. C. Ho et al., 1987; Windt et al., 2009). However, we found that phloem girdled fruit continued growing at the same rate as ungirdled fruit under water stress indicating that in this species fruit water is predominantly supplied by the xylem. This result is supported by previous studies in tomato (Van De Wal et al., 2017; Windt et al., 2009). By demonstrating that the xylem is the main source of water supplying reproductive tissues even during periods of water stress, these data emphasize the special relevance of the cavitation resistance of xylem in the peduncle for understanding fruit growth and survival in water stress. In this case, identifying xylem cavitation characteristics in reproductive tissues is highly relevant to understanding the ecology and reproductive behaviour of plant species.

We anticipated that the capacitance from shrinking tissue or early cavitation of vulnerable organs would make water stored in these tissues available to more resistant tissues, provided a xylem connection remains intact between them (Bourbia et al., 2020; Cochard & Tyree, 1990; Hölttä et al., 2009; Johnson et al., 2016).

Given the relative vulnerability of leaves compared with reproductive tissues, we argue that the collapse and early cavitation of non-prioritised leaves liberates water from these tissues, while a strong xylem connection remains intact under powerful dehydration stress allowing an intact passage of water from the vulnerable leaves to the hydraulically prioritised fruit (Hölttä et al., 2009). Organ hydraulic architecture may play a role in facilitating the variation in xylem vulnerability between reproductive and vegetative organs. Here, the small vessels of peduncles appeared more resistant to cavitation, while larger petiole vessels support delivery of the larger leaf water requirements for photosynthesis but cavitate more easily under drought stress. This observation supports literature indicating a relationship between vessel size and cavitation vulnerability (Cochard & Tyree, 1990; Gauthey et al., 2020; Hacke, Sperry, Pockman, Davis, & McCulloh, 2001; Jacobsen, Ewers, Pratt, Paddock, & Davis, 2005; Lens et al., 2011; Petit et al., 2009; Scoffoni et al., 2017).

Reproductive hydraulic vulnerability: a life history trait

Fruit prioritisation in this herbaceous annual differs significantly to the observations in woody and herbaceous species with a perennial life history. Evidence in *Pyrethrum*, a perennial daisy, suggests that the isolation of leaky flowers by early cavitation may delay dehydration damage to the vegetative tissue of plants (Bourbia et al., 2020). In the perennial case, the early cavitation of leaky petals and peduncles supports the survival of vegetative tissues (Bourbia et al., 2020). This perennial trait would allow the vegetative plant to persist through drought, enabling reproduction under more favourable future conditions. The susceptibility of fruit to water limitation has also been showed in woody perennial species such as olive (Fernandes, Cuevas, Diaz-Espejo, & Hernandez-Santana, 2018). These divergent patterns in annual and perennial species suggest strong hydraulic selection based on life history, with reproduction heavily prioritised in the annual plant, and readily sacrificed in perennial species. For annual plants in conditions where water is limiting, it appears advantageous to prioritise successful reproduction over the preservation of vegetative tissues. Alternately, for perennial plants, reproduction under drought conditions represents a water cost that can be deferred.

Like the perennial daisy, however, tomato may have mechanisms to isolate leaky flowers and fruit in immature developmental stages (Reichardt, Piepho, Stintzi, & Schaller, 2020). In these early stages, phytosulfokine (PFK) peptide hormone causes the early abortion of reproductive structures in response to drought induced environmental stress (Reichardt et al., 2020). This supports our observation that the tomato petals showed no greater cavitation resistance than vegetative tissues. Given these findings, established tomato fruit appear heavily hydraulically prioritised, while immature (and less reproductively viable) structures are sacrificed given their evaporative water costs, suggesting a transition point at which established fruit with a high chance of successful seed production become worthy of hydraulic investment.

Conclusions:

Including hydraulic vulnerability segmentation in the suite of life history traits that characterise annual and perennial plants provides a novel perspective on the variation in their ecological strategies. However, the xylem vulnerability of whole plants in drought, including their reproductive tissues, has yet to be fully examined across broader plant diversity. If these divergent patterns of reproductive vulnerability can be demonstrated to be typical of annual and perennial plants, then these hydraulic traits may significantly contribute to our understanding of plant behaviour in changing climates based on life history. In the annual species tomato we see fruit prioritisation driven through hierarchical xylem vulnerability, which ensures fruit survival and seed production through greater cavitation resistance in reproductive tissues relative to vegetative tissues. Our findings highlight the need to include reproductive tissues when examining whole plant water relations. These results are likely to be relevant to other crop species, particularly if prioritisation of reproduction through xylem resistance can be shown to be a common annual species trait. As temperatures and drought events increase globally, understanding the plant water transport traits that limit reproductive performance has direct applications for both crop production and conservation. Our results indicate xylem resistance to cavitation in the support structures of reproductive tissues as key to the success of fruit in water stress, a trait with potential implications for breeding resilient crops and climate provenanced species.

Author Contribution :

B.L.H.D., M.R.C-M. and T.J.M designed the experiment, B.L.H.D performed the experiment with assistance from M.R.C-M. and T.J.M. B.L.H.D. processed and interpreted the data with assistance from M.R.C-M. and T.J.M. B.L.H.D wrote the manuscript, and all authors reviewed and commented on the manuscript.

References:

- Adams, H. D., Zeppel, M. J. B., Anderegg, W. R. L., Hartmann, H., Landhäusser, S. M., Tissue, D. T., . . . Mcdowell, N. G. (2017). A multi-species synthesis of physiological mechanisms in drought-induced tree mortality. *Nature Ecology & Evolution*, 1 (9), 1285-1291. doi:10.1038/s41559-017-0248-x
- Anderegg, W. R., Flint, A., Huang, C.-y., Flint, L., Berry, J. A., Davis, F. W., . . . Field, C. B. (2015). Tree mortality predicted from drought-induced vascular damage. *Nature Geoscience*, 8 (5), 367-371.
- Anderegg, W. R. L., Martinez-Vilalta, J., Cailleret, M., Camarero, J. J., Ewers, B. E., Galbraith, D., . . . Trotsiuk, V. (2016). When a Tree Dies in the Forest: Scaling Climate-Driven Tree Mortality to Ecosystem Water and Carbon Fluxes. *Ecosystems*, 19 (6), 1133-1147. doi:10.1007/s10021-016-9982-1
- Bergougnoux, V. (2014). The history of tomato: From domestication to biopharming. *Biotechnology Advances*, 32 (1), 170-189. doi:10.1016/j.biotechadv.2013.11.003
- Bertin, N. (2003). A Model for an Early Stage of Tomato Fruit Development: Cell Multiplication and Cessation of the Cell Proliferative Activity. *Annals of botany*, 92 (1), 65-72. doi:10.1093/aob/mcg111
- Blanca, J., Cañizares, J., Cordero, L., Pascual, L., Diez, M. J., & Nuez, F. (2012). Variation Revealed by SNP Genotyping and Morphology Provides Insight into the Origin of the Tomato. *PLoS ONE*, 7 (10), e48198. doi:10.1371/journal.pone.0048198
- Bourbia, I., Carins-Murphy, M. R., Gracie, A., & Brodribb, T. J. (2020). Xylem cavitation isolates leaky flowers during water stress in pyrethrum. *New Phytologist*, 227 (1), 146-155. doi:10.1111/nph.16516
- Brodribb, T., Benaïme, D., & Marmottant, P. (2016). Revealing catastrophic failure of leaf networks under stress. *Proc Natl Acad Sci U S A*, 113 (17), 4865-4869. doi:10.1073/pnas.1522569113
- Brodribb, T. J., Carriqui, M., Delzon, S., & Lucani, C. (2017). Optical Measurement of Stem Xylem Vulnerability. *Plant Physiology*, 174 (4), 2054-2061. doi:10.1104/pp.17.00552
- Brodribb, T. J., Powers, J., Cochard, H., & Choat, B. (2020). Hanging by a thread? Forests and drought. *Science*, 368 (6488), 261-266. doi:10.1126/science.aat7631
- Brodribb, T. J., Skelton, R. P., McAdam, S. A., Benaïme, D., Lucani, C. J., & Marmottant, P. (2016). Visual quantification of embolism reveals leaf vulnerability to hydraulic failure. *New Phytol*, 209 (4), 1403-1409. doi:10.1111/nph.13846
- Bussieres, P. (1994). Water Import Rate in Tomato Fruit: A Resistance Model. 73 (1), 75-82. doi:10.1006/anbo.1994.1009
- Chapotin, S. M., Holbrook, N. M., Morse, S. R., & Gutierrez, M. V. (2003). Water relations of tropical dry forest flowers: pathways for water entry and the role of extracellular polysaccharides. *Plant, Cell & Environment*, 26 (4), 623-630. doi:10.1046/j.1365-3040.2003.00998.x
- Chetelat, R. T., Pertuze, R. A., Faundez, L., Graham, E. B., & Jones, C. M. (2009). Distribution, ecology and reproductive biology of wild tomatoes and related nightshades from the Atacama Desert region of northern Chile. *Euphytica*, 167 (1), 77-93. doi:10.1007/s10681-008-9863-6
- Choat, B., Brodribb, T. J., Brodersen, C. R., Duursma, R. A., Lopez, R., & Medlyn, B. E. (2018). Triggers of tree mortality under drought. *Nature*, 558 (7711), 531-539.

- Choat, B., Jansen, S., Brodribb, T. J., Cochard, H., Delzon, S., Bhaskar, R., . . . Zanne, A. E. (2012). Global convergence in the vulnerability of forests to drought. *Nature*, *491* (7426), 752-755. doi:10.1038/nature11688
- Cochard, H., & Tyree, M. T. (1990). Xylem dysfunction in *Quercus*: vessel sizes, tyloses, cavitation and seasonal changes in embolism. *Tree Physiology*, *6* (4), 393-407. doi:10.1093/treephys/6.4.393
- De La Barrera, E., & Nobel, P. S. (2004). Nectar: properties, floral aspects, and speculations on origin. *Trends in Plant Science*, *9* (2), 65-69. doi:10.1016/j.tplants.2003.12.003
- Fernandes, R. D. M., Cuevas, M. V., Diaz-Espejo, A., & Hernandez-Santana, V. (2018). Effects of water stress on fruit growth and water relations between fruits and leaves in a hedgerow olive orchard. *Agricultural water management*, *210*, 32-40.
- Fischer, I., Camus-Kulandaivelu, L., Allal, F., & Stephan, W. (2011). Adaptation to drought in two wild tomato species: the evolution of the *Asr* gene family. *New Phytologist*, *190* (4), 1032-1044. doi:10.1111/j.1469-8137.2011.03648.x
- Galen, C., Sherry, R. A., & Carroll, A. B. (1999). Are flowers physiological sinks or faucets? Costs and correlates of water use by flowers of *Polemonium viscosum*. *Oecologia*, *118* (4), 461-470. doi:10.1007/s004420050749
- Gauthey, A., Peters, J. M. R., Carins-Murphy, M. R., Rodriguez-Dominguez, C. M., Li, X., Delzon, S., . . . Choat, B. (2020). Visual and hydraulic techniques produce similar estimates of cavitation resistance in woody species. *New Phytologist*. doi:10.1111/nph.16746
- Gillaspy, G., Ben-David, H., & Gruissem, W. (1993). Fruits: a developmental perspective. *The Plant Cell*, *5* (10), 1439.
- Guichard, S., Gary, C., Leonardi, C., & Bertin, N. (2005). Analysis of Growth and Water Relations of Tomato Fruits in Relation to Air Vapor Pressure Deficit and Plant Fruit Load. *Journal of Plant Growth Regulation*, *24* (3), 201-213. doi:10.1007/s00344-005-0040-z
- Hacke, U. G., Sperry, J. S., Pockman, W. T., Davis, S. D., & McCulloh, K. A. (2001). Trends in wood density and structure are linked to prevention of xylem implosion by negative pressure. *Oecologia*, *126* (4), 457-461.
- Hanssens, J., De Swaef, T., & Steppe, K. (2015). High light decreases xylem contribution to fruit growth in tomato. *Plant, Cell & Environment*, *38* (3), 487-498. doi:10.1111/pce.12411
- Hedhly, A., Hormaza, J. I., & Herrero, M. (2009). Global warming and sexual plant reproduction. *Trends in Plant Science*, *14* (1), 30-36. doi:10.1016/j.tplants.2008.11.001
- Ho, L., Sjut, V., & Hoad, G. (1982). The effect of assimilate supply on fruit growth and hormone levels in tomato plants. *Plant Growth Regulation*, *1* (3), 155-171.
- Ho, L. C. (1980). Control of import into tomato fruits. *Berichte der Deutschen Botanischen Gesellschaft*, *93* (1), 315-325.
- Ho, L. C., Grange, R. I., & Picken, A. J. (1987). An analysis of the accumulation of water and dry matter in tomato fruit. *Plant, Cell and Environment*, *10* (2), 157-162. doi:10.1111/1365-3040.ep11602110
- Holttä, T., Cochard, H., Nikinmaa, E., & Mencuccini, M. (2009). Capacitive effect of cavitation in xylem conduits: results from a dynamic model. *Plant, Cell & Environment*, *32* (1), 10-21. doi:10.1111/j.1365-3040.2008.01894.x
- Jacobsen, A. L., Ewers, F. W., Pratt, R. B., Paddock, W. A., & Davis, S. D. (2005). Do Xylem Fibers Affect Vessel Cavitation Resistance? *Plant Physiology*, *139* (1), 546-556. doi:10.1104/pp.104.058404

- Johnson, D. M., Wortemann, R., Mcculloh, K. A., Jordan-Meille, L., Ward, E., Warren, J. M., . . . Domec, J.-C. (2016). A test of the hydraulic vulnerability segmentation hypothesis in angiosperm and conifer tree species. *Tree Physiology*, 36 (8), 983-993. doi:10.1093/treephys/tpw031
- Knapp, S., & Peralta, I. E. (2016). The Tomato (*Solanum lycopersicum* L., Solanaceae) and Its Botanical Relatives. In *Compendium of Plant Genomes* (pp. 7-21): Springer Berlin Heidelberg.
- Lambrecht, S. C. (2013). Floral Water Costs and Size Variation in the Highly Selfing (Polemoniaceae). *International journal of plant sciences*, 174 (1), 74-84. doi:10.1086/668230
- Lens, F., Sperry, J. S., Christman, M. A., Choat, B., Rabaey, D., & Jansen, S. (2011). Testing hypotheses that link wood anatomy to cavitation resistance and hydraulic conductivity in the genus *Acer*. *New Phytologist*, 190 (3), 709-723. doi:10.1111/j.1469-8137.2010.03518.x
- Li, H., Zhang, X., Hou, X., & Du, T. (2021). Developmental and water deficit-induced changes in hydraulic properties and xylem anatomy of tomato fruit and pedicel. *Journal of Experimental Botany*.
- Matthews, M. A., & Shackel, K. A. (2005). Growth and water transport in fleshy fruit. In *Vascular transport in plants* (pp. 181-197): Elsevier.
- Peralta, I., Knapp, S., & Spooner, D. (2007). The taxonomy of tomatoes: a revision of wild tomatoes (*Solanum* L. section *Lycopersicon* (Mill.) Wettst.) and their outgroup relatives (*Solanum* sections *Juglandifolium* (Rydb.) Child and *Lycopersicoides* (Child) Peralta). *Systematic Botany Monographs*, 84 , 1-186.
- Petit, G., Anfodillo, T., & De Zan, C. (2009). Degree of tapering of xylem conduits in stems and roots of small *Pinus cembra* and *Larix decidua* trees. *Botany*, 87 (5), 501-508. doi:10.1139/b09-025
- Reichardt, S., Piepho, H.-P., Stintzi, A., & Schaller, A. (2020). Peptide signaling for drought-induced tomato flower drop. *Science*, 367 (6485), 1482-1485. doi:10.1126/science.aaz5641
- Renaudin, J.-P., Deluche, C., Cheniclet, C., Chevalier, C., & Frangne, N. (2017). Cell layer-specific patterns of cell division and cell expansion during fruit set and fruit growth in tomato pericarp. *Journal of Experimental Botany*, 68 (7), 1613-1623. doi:10.1093/jxb/erx058
- Roddy, A. B. (2019). Energy Balance Implications of Floral Traits Involved in Pollinator Attraction and Water Balance. *International journal of plant sciences*, 180 (9), 944-953. doi:10.1086/705586
- Roddy, A. B., Jiang, G. F., Cao, K., Simonin, K. A., & Brodersen, C. R. (2019). Hydraulic traits are more diverse in flowers than in leaves. *New Phytologist*, 223 (1), 193-203. doi:10.1111/nph.15749
- Savi, T., Bertuzzi, S., Branca, S., Tretiach, M., & Nardini, A. (2015). Drought-induced xylem cavitation and hydraulic deterioration: risk factors for urban trees under climate change? *New Phytologist*, 205 (3), 1106-1116. doi:10.1111/nph.13112
- Scoffoni, C., Albuquerque, C., Brodersen, C. R., Townes, S. V., John, G. P., Cochard, H., . . . Sack, L. (2017). Leaf vein xylem conduit diameter influences susceptibility to embolism and hydraulic decline. *New Phytologist*, 213 (3), 1076-1092. doi:10.1111/nph.14256
- Shameer, S., Vallarino, J. G., Fernie, A. R., Ratcliffe, R. G., & Sweetlove, L. J. (2020). Flux balance analysis of metabolism during growth by osmotic cell expansion and its application to tomato fruits. *Plant Journal*, 103 (1).
- Skelton, R. P., Brodribb, T. J., & Choat, B. (2017). Casting light on xylem vulnerability in an herbaceous species reveals a lack of segmentation. *New Phytologist*, 214 (2), 561-569. doi:10.1111/nph.14450
- Soltis, P. S., & Soltis, D. E. (2014). Flower diversity and angiosperm diversification. *Flower development*, 85-102.
- Sperry, J. S., & Love, D. M. (2015). What plant hydraulics can tell us about responses to climate-change droughts. *New Phytologist*, 207 (1), 14-27. doi:10.1111/nph.13354

Sperry, J. S., & Tyree, M. T. (1988). Mechanism of Water Stress-Induced Xylem Embolism. *Plant Physiology*, 88 (3), 581-587. doi:10.1104/pp.88.3.581

Tyree, M. T., & Ewers, F. W. (1991). The hydraulic architecture of trees and other woody plants. *New Phytologist*, 119 (3), 345-360. doi:10.1111/j.1469-8137.1991.tb00035.x

Tyree, M. T., & Sperry, J. S. (1989). Vulnerability of xylem to cavitation and embolism. *Annual review of plant biology*, 40 (1), 19-36.

Urli, M., Porte, A. J., Cochard, H., Guengant, Y., Burlett, R., & Delzon, S. (2013). Xylem embolism threshold for catastrophic hydraulic failure in angiosperm trees. *Tree Physiology*, 33 (7), 672-683.

Van De Wal, B. A. E., Windt, C. W., Leroux, O., & Steppe, K. (2017). Heat girdling does not affect xylem integrity: an in vivo magnetic resonance imaging study in the tomato peduncle. *New Phytologist*, 215 (2), 558-568. doi:10.1111/nph.14610

van Die, J., & Willemse, N. C. (1980). The supply of water and solutes by phloem and xylem to growing fruits of *Yucca flaccida* Haw. *Berichte der Deutschen Botanischen Gesellschaft*, 93 (1), 327-337.

Van Ieperen, W., Volkov, V. S., & Van Meeteren, U. (2003). Distribution of xylem hydraulic resistance in fruiting truss of tomato influenced by water stress. *Journal of Experimental Botany*, 54 (381), 317-324. doi:10.1093/jxb/erg010

Vilagrosa, A., Chirino, E., Peguero-Pina, J. J., Barigah, T. S., Cochard, H., & Gil-Pelegrin, E. (2012). Xylem Cavitation and Embolism in Plants Living in Water-Limited Ecosystems. In *Plant Responses to Drought Stress* (pp. 63-109): Springer Berlin Heidelberg.

Windt, C. W., Gerkema, E., & Van As, H. (2009). Most Water in the Tomato Truss Is Imported through the Xylem, Not the Phloem: A Nuclear Magnetic Resonance Flow Imaging Study. *Plant Physiology*, 151 (2), 830-842. doi:10.1104/pp.109.141044

Zhang, F.-P., & Brodribb, T. J. (2017). Are flowers vulnerable to xylem cavitation during drought? *Proceedings of the Royal Society B: Biological Sciences*, 284 (1854), 20162642. doi:10.1098/rspb.2016.2642

Zhang, F.-P., Carins Murphy, M. R., Cardoso, A. A., Jordan, G. J., & Brodribb, T. J. (2018). Similar geometric rules govern the distribution of veins and stomata in petals, sepals and leaves. *New Phytologist*, 219 (4), 1224-1234. doi:10.1111/nph.15210

Zhang, F.-P., Zhang, J.-L., Brodribb, T. J., & Hu, H. (2020). Cavitation resistance of peduncle, petiole and stem is correlated with bordered pit dimensions in *Magnolia grandiflora*. *Plant Diversity*. doi:10.1016/j.pld.2020.11.007

Zimmermann, M. H. (2013). *Xylem structure and the ascent of sap* : Springer Science & Business Media.

Table 1: Results of ANOVA comparing the effect of organ and individual on the *P50* (water potential at which 50% of the xylem was cavitated) of petioles, peduncles, petals, and leaves of *S. lycopersium* (tomato)

Difference between factors

	Df	Sum Sq
organ	3	0.6891
individual	1	0.2179
residuals	15	0.4462
Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1	Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1	Signif. c

Figure captions :

Figure 1: a) Representative transverse section of a tomato peduncle, stained with 5% toluidine blue. b)

Xylem vessel lumens represented as ellipses to measure diameter. 20x magnification, bars 100 μm .

Figure 2: Changes in the size of *S. lycopersicum*(tomato) petioles, peduncles, and fruit with acute dehydration, expressed as a percentage of initial size which was considered 100%. Fruits that remained connected to the plant continued to grow while vegetative tissue shrank due to dehydration.

Figure 3: Box plots indicating the percentage increase in growth of tomato fruit connected to the plant by girdled and ungirdled phloem during dehydration (a) and over time (b). Solid lines indicate mean growth per treatment and dotted lines in (b) are individual treatments. There was no significant difference in fruit growth between phloem-girdled and ungirdled fruit suggesting that xylem was the primary source of water supply for fruit growth.

Figure 4. Vulnerability curves showing mean cumulative % xylem cavitation + s.e. with dehydration of peduncles, petals, petioles, leaves and stem of *S. lycopersium*.

Figure 5: Mean + s.e. *P50* values for *S. lycopersium* demonstrating no significant difference between leaves, petioles, and petals. Peduncles show significantly greater cavitation resistance than the other organs. Different letters indicate significant post-hoc differences ($p < 0.05$). *Stem *P50* calculated from separate data and not included in statistical tests.







