

Effects of Water Stress during Growth on Xylem Anatomy, Xylem Functioning and Vase Life in Three *Zinnia elegans* Cultivars

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Abstract

In cut flowers, hydraulic properties and dimensions of xylem vessels in the stem directly influence vase-life and thus post-harvest quality. Xylem hydraulic conductance as well as recovery from air embolisms at the start of vase life strongly depends on number, diameter and length of xylem vessels in the base of the cut flower stems. In this research we employed different water availability levels (high and low water content) in the growing medium of *Zinnia elegans* plants of three cultivars ('Envy', 'Purple Prince' and 'Scarlet Flame') to modify xylem anatomy and post-harvest xylem functioning and vase life of cut flowers from these plants. Vase-life was longer among fresh-cut *Zinnia* flowers in all three cultivars grown under low water content in the root medium. *Zinnia* flowers of all cultivars grown at high water content were not able to sufficiently restore water uptake at the start of their vase life. Shoot hydraulic conductivity was lower in water-stressed plants but it was not different among the three *Zinnia* cultivars within the same treatment. Anatomical analysis showed smaller xylem vessel diameters but no differences in xylem number and length, with the exception that in cultivar Purple Prince vessels were longer in well-watered plants. We conclude from these results that within these three *Zinnia elegans* cultivars water stress conditions in the root environment significantly affected xylem anatomy and functioning which correlates well with a longer vase life. Differences in xylem properties between the three cultivars due to pre-harvest watering treatments were limited.

INTRODUCTION

Inadequate water uptake is one of the main reasons for inferior cut flower performance during vase life. Obstruction of xylem vessels during vase life due to microbial growth, formation of tyloses, deposition of materials in the lumen of xylem vessels, and the presence of air emboli in the vascular system may be reasons for restricted water uptake (van Doorn, 1997). Xylem anatomy (number, length and diameter of the vessels) determines the capacity for water transport (Nijssen et al., 2001a) as well as the vulnerability for cavitations (Tyree and Sperry, 1989). Possibility and duration of the repair process from air embolisms which are present in the base of cut flower stems after harvest in air (Nijssen, 2001b; van Ieperen et al., 2001), is strongly influenced by the dimensions of the xylem vessels (van Ieperen et al., 2002).

Xylem anatomy can vary with environmental factors during growth and genotype. Nijssen (2001b) reported large differences in xylem dimensions in stems of different *Chrysanthemum* cultivars grown under the same environmental conditions. Lovisolo and Schubert (1998) reported of reduced development of xylem vessels among plants grown at moderate water-stressed conditions with resultant low shoot hydraulic conductance led

to reduction in vulnerability of air embolism.

In present research project we aim to investigate the possibility to control the formation of xylem vessels and their dimensions in *Zinnia elegans* during growth to enhance post-harvest vase life. *Zinnia elegans* produces cut flowers with water balance related vase life problems and is of limited commercial value. However, *Zinnia elegans* also provides a well-established model system for fundamental research (in vitro) on xylem formation involving cell differentiation, elongation, fusions of vessel elements and programmed cell death (Fukuda et al., 1998). Research (in vivo and in vitro) will be carried out to investigate various sub processes of xylogenesis as influenced by genotype and environmental factors and their role in the establishing the xylem dimensions length and diameter. In this paper we present the first preliminary results of a search for *Zinnia elegans* cultivars with different responses (with respect to xylem dimensions, xylem functioning and vase life) on watering treatments during growth.

MATERIAL AND METHODS

Plant Material

Seeds of three *Zinnia elegans* cultivars (Envy, Purple Prince and Scarlet Flame) were sown in potting soil. After 10 days a homogeneous set of seedlings per cultivar was selected and transplanted to 10 liter pots filled with perlite (Agra-perlite: No 1; grading 0.6-1.5 mm). Plants were placed in a greenhouse at Wageningen University, The Netherlands and grown at 70% RH and 18/22 °C night/day temperature (setpoints). Two constant levels of volumetric water content (Θ ; v/v%) were continuously maintained in the pots during the whole growth period using ECH₂O probes (Model EC-20; Decagon Devices Inc, Pullman, Washington, USA) in combination with an automatic drip irrigation system. Half of the plants of each cultivar were grown at 70% (pF 0.5; well-watered) and half of the plants at 20% (pF 2; water-stressed). Each pot contained 3 plants of the same cultivar, 12 pots per cultivar x watering treatment combination. Part of the pots were treated as border plants and not used for further analysis.

Xylem Hydraulic Conductivity Measurement

Thirty-five days after transplanting, just before flowering, 3 pots per cultivar x watering combination were transported to the laboratory for hydraulic conductance measurements and vessel length determination (see below). Flower stems were harvest under water (to prevent air entrance) at the root-shoot junction and re-cut (under water) just above the first leaf-pair and 20-25 cm higher to get stem samples of sufficient length (longer than expected maximal vessel length). Hydraulic conductance of these stem segments was determined from pressure-flow relationships as described by van Ieperen et al. (2003). Hydraulic conductivity (K_h) was calculated from the measured hydraulic conductance and the length of each sample.

Xylem Anatomy (Number of Vessels, Diameter- and Length Distributions)

After measuring hydraulic conductivity a 2-3 cm long piece was cut from the middle of the lowest internode of each stem sample and stored in 75% ethanol for later anatomical analysis. 30 μ m thick stem cross-sections were made with microtome and digital images of the sections were made using a Nikon DXM-1200 camera on a Leica Aristoplan Microscope: whole cross-sections (magnification 10x) and all individual vascular bundles per cross-section (80x). Diameter analysis was done on images with individual vascular bundles (resolution \approx 1 μ m per pixel) and vessel counting was done on images of whole cross-sections using the free UTHSCSA ImageTool program (developed at the University of Texas Health Science Center at San Antonio, Texas). Vessel counting was done on all samples (n=3), diameter analysis on one representative sample per cultivar x watering-treatment combination (n=1; 1200-1400 vessels per sample).

Vessel length distribution was determined in all cultivar x watering treatment combinations (n=3) using the latex perfusion method (Zimmermann and Jeje, 1981) and

analysed according to Nijssen et al. (2001a).

Vase Life

After all plants started to flower (no difference in timing between cultivars and watering treatments) 4 pots with plants per cultivar x watering treatment combination were transported to the laboratory. Flower stems were harvested 1 cm above the root/shoot junction and re-cut by 0.5 cm using a sharp razor blade. Lower leaves were removed up to 10-15 cm from the stem base. To regain full turgidity and remove any embolisms in the vascular system of the flower stems, they were placed in buckets with ice/water mixture (3:1) and stored over night at 4 °C in darkness. After re-hydration the flower stems were re-cut by another 3 cm (razor blade) to remove any type of blockage from the base of the flower stems. Before starting the vase period all flowers were dehydrated to approximately 95% of their initial full turgor fresh weight. Dehydration was done by placing the flowers in a room at 20 °C, 60% RH and a light intensity of 14 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Philips, TLD 50W/84HF). Weight loss of individual flowers was monitored and the time to reach the 95% level of their initial weight (full turgor) recorded (no statistical significant differences between cultivars and watering treatments). After reaching 95% of their initial fresh weight the cut flowers were placed in vases (500 ml Erlenmeyer flasks) filled with tap water in the same room (Light period 12 h.). The base of the flower stem was free from the bottom of the flask and between 1-7 cm below the water surface (depth changed due to water uptake during vase period). Weight of all flowers was measured at 20, 44 and 70 h after the start of the dehydration period and expressed as percentage of the weight at full turgor. After each measurement the flasks were refilled with tap water. After finishing the vase period experiment, leaf area per cut flower was measured (LI-3100, LI-COR Inc, Lincoln, USA).

RESULTS

Water availability in the root environment during growth considerably influenced external characteristics, xylem properties and vase performance of *Zinnia* cut flowers.

General Characteristics

Water stress during growth clearly influenced the size of cut flowers in all three cultivars: leaf area, fresh weight (Table 1) and length (not shown) per cut flower stem were clearly decreased by the water-stressed treatment.

Xylem Functioning

Water stress during growth clearly reduced hydraulic conductivity (K_h) in the lower part of the cut flower stems in all three cultivars (Table 2). Irrespective of the watering treatment, K_h was largest in cultivar Purple Prince and lowest in cultivar Envy. Cultivar Scarlet Flame has intermediate values for K_h in both watering treatments.

Xylem Anatomy (Vascular Bundle Pattern, Number, Length and Diameter)

Xylem anatomy was clearly affected by the water-stressed treatments in all cultivars. Generally, xylem vessels were grouped within approximately 24 vascular bundles. The size of these vascular bundles and the number of vessels per vascular bundle were less regular in stems of well-watered plants in all three cultivars compared to water-stressed plants (Fig. 1). No statistical significant differences were found in the total number of xylem vessels per cross-section between all cultivars and watering treatments. Vessel length distribution was only influenced by the watering treatment in the cultivar Purple Prince (Table 3): maximal vessel length and half-length of the vessels were larger in the well-watered treatment in this cultivar.

Visual inspection of all images and quantitative analysis on images of representative cross-sections of each cultivar x watering treatment combination (based on K_h determination) suggests a clear effect of water stress during growth on the vessel diameter distribution: the diameter distribution in stems of plants grown at high water

content shifts towards the larger diameter classes (Fig. 2).

Vase Life

In general, well-watered *Zinnia elegans* plants produced cut flowers that did not sufficiently regain water uptake during the first phase of their vase life (Fig. 3). This resulted in severe leaf wilting and associated loss of ornamental value within 1-2 days. *Zinnia elegans* cut flowers of all three cultivars, which were grown at the water-stressed treatment, all regained water uptake and increased in fresh weight during vase-life. No statistical significant effects were measured between the three cultivars.

DISCUSSION

In general angiosperm plants respond to water stress by a variety of mechanisms ranging from physiological to molecular changes. Short-term response to water stress by higher plants involves stomatal closure. Some of the responses to water stress on the long term include decrease of conductivity to water flow along the “soil-plant-atmosphere continuum” (SPAC) also described as “bulk conductivity” by Moreshet et al. (1990). Such decrease in conductivity is determined by smaller xylem vessel diameter and length (Nijse, 2001b) and by smaller leaf surface area and reduced stomata density resulting in decreased stomatal conductivity and lower transpiration (Meinzer et al., 1996). Other responses to plant water stress include modification of other structures in root and shoot that will facilitate efficient storage of water in the plant. Long-term modifications of the shoot hydraulic conductivity by water stress during growth will inevitably influence the post-harvest water relations of cut flowers and therefore post-harvest quality.

Xylem hydraulic conductivity in the shoot is a very important parameter for determining effect of water stress on the plant. It decreases dramatically in plants subjected to water stress as a result of either modified xylem dimensions (number, length and diameter) or blockage of the vessels by air embolism (van Meeteren, 1992). Severe water stress may cause cavitations and air embolism (Schultz and Matthews, 1988) resulting in reduced or zero function of the xylem in transporting water. In cut flowers air embolisms are initially present in the basal part of the stems due to harvest in air. Removal of these air embolisms is essential for restoration of the water uptake during vase life and strongly depends on xylem anatomy near the cut surface. Larger xylem vessels embolize faster than smaller ones (van Ieperen et al., 2001) and repair much slower (van Ieperen, 2002). Therefore one will think that embolism should be minimal in water-stressed plants as they tend to develop smaller vessels. However, such water stress conditions results in lower pressure in these smaller vessels that eventually become embolized. The problem is usually countered by stomatal closure with additional advantage of lower transpiration rate (Sperry and Pockman, 1993; Sperry, 1986).

The stem xylem hydraulic conductance results (K_h) showed a significant difference between well-watered plants and water-stressed plants in all the three cultivars (Purple Prince, Envy and Scarlet flame). Among water-stressed plants, Envy had the least K_h value ($0.65 \text{ mgs}^{-1}\text{Pa}^{-1}\text{cm}$) while those of Purple Prince and Scarlet flame were not significantly different (Table 2). Similarly, among the well-watered plants, Envy was significantly different from the other cultivars with a lower K_h value ($1.27 \text{ mgs}^{-1}\text{Pa}^{-1}\text{cm}$) whilst the other two were not significantly different from each other (Table 2). In this experiment, water-stressed plants generally had lower shoot growth and stem cross-sectional area which all can affect xylem conductivity. The results on xylem number did not show any significant difference between the three cultivars and two watering treatments. The xylem vessel diameter distributions clearly differed across different water-stressed treatments (Fig. 2). Well-watered plants had greater proportion of bigger xylem vessels, which according to Poiseuille's Law (Ψ is proportional to R^4) will contribute greater to water transport than the smaller vessels, which dominate in water-stressed plants.

Zimmermann and Jeje (1981) reported a positive correlation between vessel diameter and vessel length in Angiosperms. However, according to our results, with the

exception of Purple Prince, there were no significant differences between the xylem length in well-watered and water-stressed plants (Table 3a). We can therefore not link with strong evidence the effect of xylem length on reduced hydraulic conductance in water-stressed plants of *Zinnia* cultivars. Lack of strong significant differences among the *Zinnia* cultivars makes it difficult now to relate genetic differences to effect of water stress on the *Zinnia* cultivars unless the experiment covers greater number of *Zinnia* cultivars. The most significant result however, is the fact that the reduced hydraulic conductivity among water-stressed *Zinnia* plants depends greatly on xylem vessel diameter rather than other dimensions (number and length). A strong correlation exists between vase life performance and watering treatment during growth.

Vase life of cut flowers varies greatly from species to species. However, in almost all species, vase life of cut flowers basically depends on the water balance of the cut flower. Therefore the vase life is greatly influenced by factors determining water uptake (such as xylem dimensions (number, length and diameter)) as well as transpiration (performance of the stomata). In this experiment we found a longer vase life and smaller diameter vessels for water-stressed plants in all three cultivars. Cut flowers, which did not recover from the 5% initial water loss during the first 24h of vase life (short vase life; Fig. 3), were generally harvested from the well-watered plants and contained vessels with larger diameters. The difference in the vase life may therefore be as a result of insufficient embolism repair (not measured) in well-watered plants as they contained xylem vessels with larger diameters (Fig. 2). This is in agreement with the findings in chrysanthemum (van Meeteren et al., 2005). However, since the well-watered plants also had greater leaf surface area (Table 1) and possibly also a higher stomatal density and “drought-untrained” stomata, transpiration rate might also have influenced the vase life results.

From functional and anatomical studies, we established that water stress during growth (a) positively influenced post-harvest vase life and (b) negatively influenced the hydraulic conductivity by (c) reducing xylem diameter in all three *Zinnia elegans* cultivars. The number of xylem vessels as well as xylem vessel length did not differ between watering treatments and cultivars. The only exception was the difference in xylem length among the two treatments of the Purple Prince cultivar.

Literature Cited

- Fukuda, H., Watanabe, Y., Kuriyama, H., Aoyagi, S., Sugiyama, M., Yamamoto, R., Demur, T. and Minami, A. 1998. Programming of Cell Death during Xylogenesis. *J. Plant Res.* 111:253-256.
- Lovisolo, C. and Schubert, A. 1998. Effect of water stress on vessel size and xylem hydraulic conductivity in *Vitis vinifera* L. *J. Exp. Bot.* 49:693-700.
- Meinzer, F.C., Fownes J.H. and Harrington, R.A. 1996. Growth indices and stomatal control of transpiration in *Acacia koa* stands planted at different densities. *Tree Physiol.* 16:607–615.
- Moreshet, S., Cohen, Y., Green, G.C. and Fuchs, M. 1990. The partitioning of hydraulic conductances within mature orange trees. *J. Exp. Bot.* 41: 833–839.
- Nijssse, J., van der Heijden, G.W.A.M., van Ieperen, W., Keijzer, C.J. and van Meeteren, U. 2001a. Xylem hydraulic conductivity related to conduit dimensions along chrysanthemum stems. *J. Exp. Bot.* 52:319-327.
- Nijssse, J. 2001b. Functional analysis of the water transport system in cut chrysanthemum. PhD thesis Wageningen University, ISBN 90-5808-449-3, June 2001.
- Schultz, H.R. and Matthews, M.A. 1988. Resistance to water transport in shoots of *Vitis vinifera* L. *Plant Physiol.* 88: 718–724.
- Sperry, J.S. 1986. Relationship of xylem embolism to xylem pressure potential, stomatal closure, and shoot morphology in the palm *Rhapis excelsa*. *Plant Physiol.* 80:110–116.
- Sperry, J.S. and Pockman, W.T. 1993. Limitation of transpiration by hydraulic conductance and xylem cavitation in *Betula occidentalis*. *Plant, Cell Environ.* 16: 279–287.
- Tyree, M.T. and Sperry, J.S. 1989. Vulnerability of xylem to cavitation and embolism.

- Annu. Rev. Plant Physiol. Plant Mol. Biol. 40:19–38.
- van Doorn, W.G. 1997. Water relations of cut flowers. Hort. Rev. 18:1-85.
- van Ieperen, W., Nijse, J., Keijzer, C.J. and van Meeteren, U. 2001. Induction of air embolism in xylem conduits of pre-defined diameter. J. Exp. Bot. 52:981-991.
- van Ieperen, W., van Meeteren, U. and Nijse, J. 2002. Embolism repair in cut flower stems: a physical approach. Postharvest Biol. Technol.:1-14.
- van Ieperen, W., Volkov, V.S. and van Meeteren, U. 2003. Distribution of xylem hydraulic resistance in fruiting truss of tomato influenced by water stress. J. Exp. Bot. 54:317-324.
- van Meeteren, U. 1992. Role of embolism and low water temperature in water balance of cut Chrysanthemum flowers. Sci. Hort. 51:275-284.
- van Meeteren, U. van Gelder A. and van Ieperen, W. Effect of Growth Conditions on Post Harvest Rehydration Ability of Cut Chrysanthemum Flowers. Acta Hort 669:287-295.
- Zimmermann, M.H. and Jeje, A.A. 1981. Vessel-length distribution in stems of some American woody plants. Can. J. Bot. 59:1882–1892.

Figures

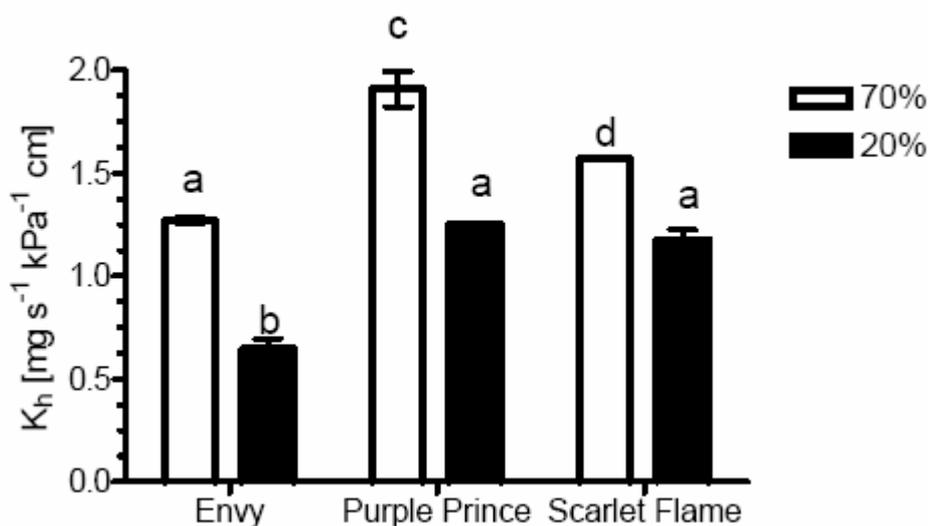


Fig. 1. Effect of volumetric water content in the root environment on hydraulic conductivity (K_h) in stems of three *Zinnia elegans* cultivars. Statistical significant differences are indicated by different letters ($P=0.05$, student t-test, $n=3$).

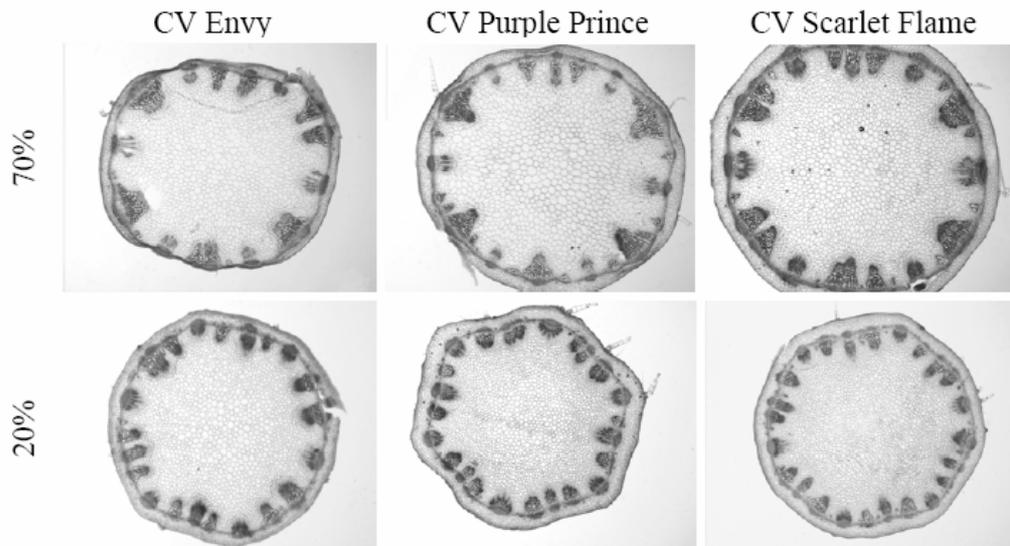


Fig. 2. Stem cross-sections of three *Zinnia elegans* cultivars grown at two levels of volumetric water content (Θ ; v/v %) in the root environment (70%, well-watered; 20%, water-stressed).

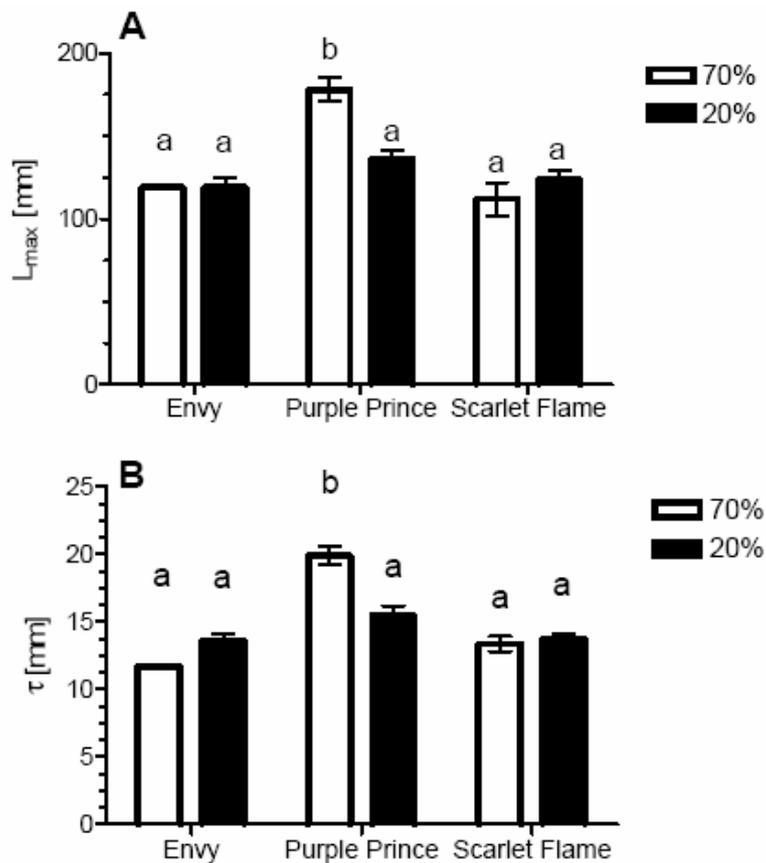


Fig. 3. Effect of volumetric water content in the root environment on (A) maximal length (L_{max}) and (B) half-length (τ ; 50% of the vessels are shorter than this length) in stems of three *Zinnia elegans* cultivars. Statistical significant differences are indicated by different letters ($P=0.05$, student t-test, $n=4$).

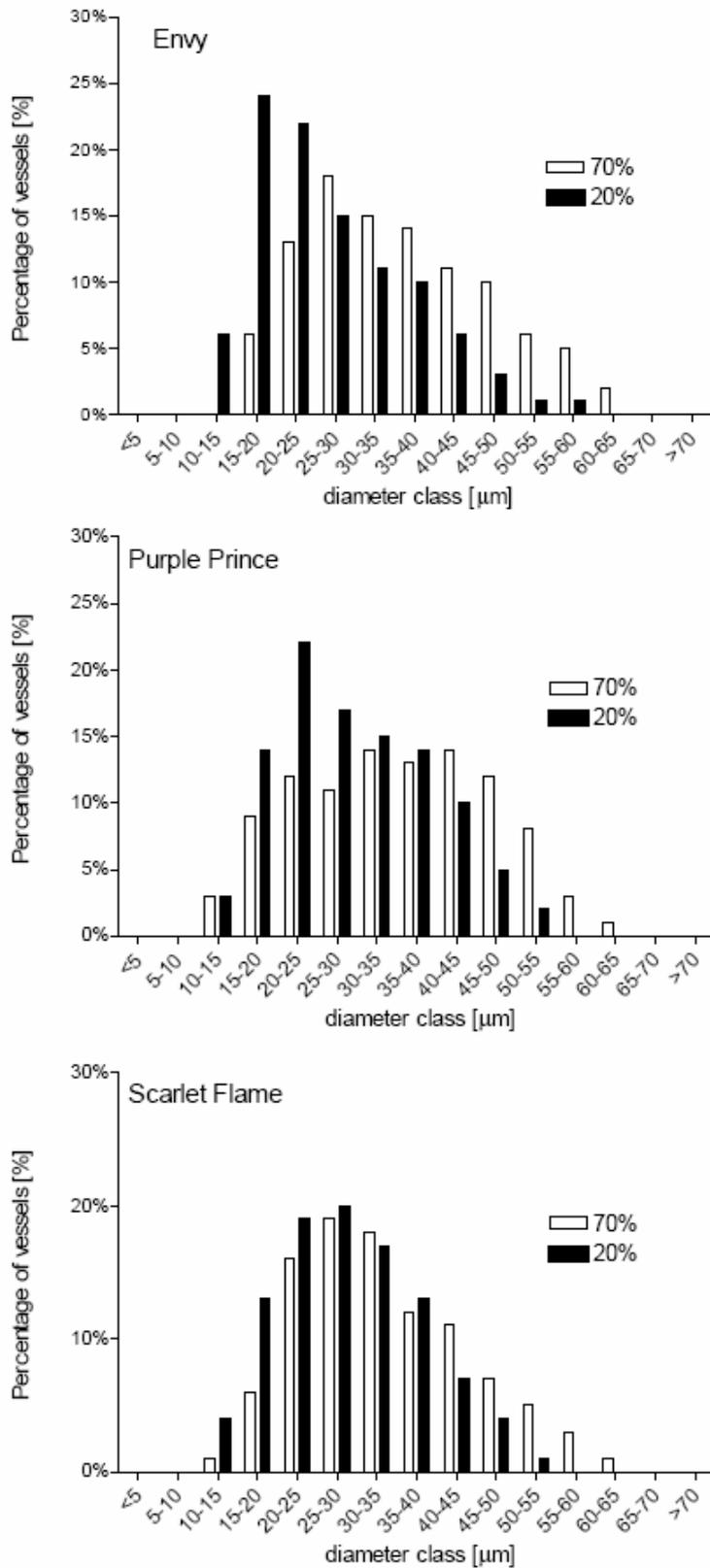


Fig. 4. Effect of volumetric water content in the root environment on xylem vessel diameter distribution in representative stems of three *Zinnia elegans* cultivars (one stem per cultivar x watering treatment).

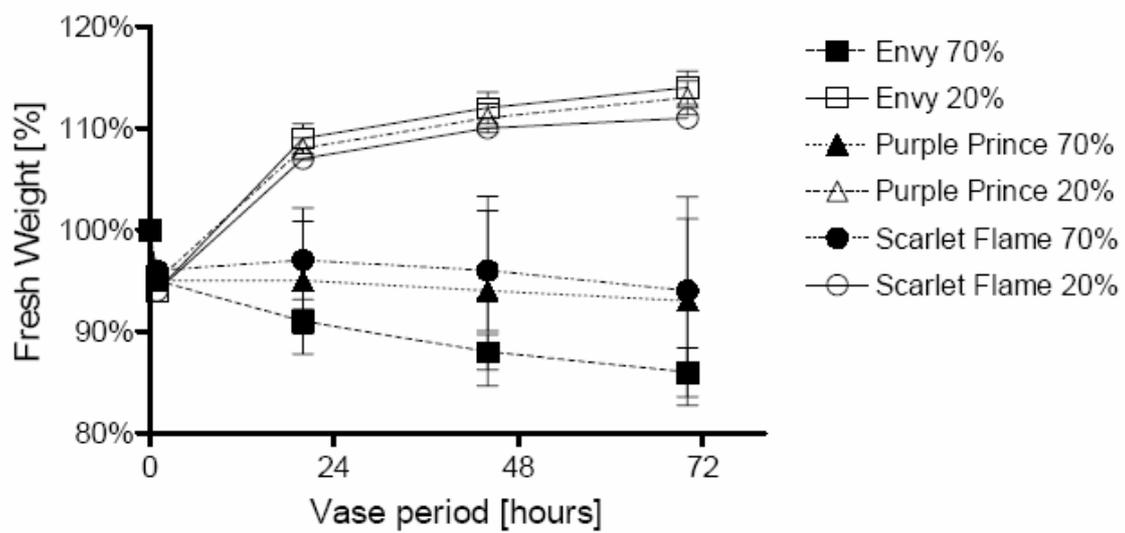


Fig. 5. Time course of fresh weight changes of *Zinnia elegans* cut flowers (three cultivars) during vase life after 1 h desicciation. Cut flowers were grown at low (open symbols) and high (closed symbols) volumetric water content in the root environment. (bars: SEM; n=4)

