Post-transplant Water Utilization of Zinnia Seedlings Grown in Humectant-amended Substrate Maintained at Two Moisture Thresholds[1]

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Abstract

Humectant treatment and substrate moisture content (SMC) were studied to determine their impact on post-transplant water utilization in 'Thumbelina' zinnia (*Zinnia elegans* Jacq.). Four-week-old seedlings were transplanted from plug trays into pots containing soilless substrate amended with an aqueous solution of 1.6% Hydretain® or an equal volume of water (0% humectant). Seedlings were placed in an automated, sensor-controlled irrigation system and grown for 30 days at SMC levels of 0.45 or 0.25 $cm^3 \cdot cm^{-3}$ (0.06 or 0.03 fl $oz \cdot oz^{-1}$). Plant-water potential (Ψ_w) was significantly higher (less negative) in transplants grown in humectant-treated substrate at both SMC levels, and water-use efficiency (WUE) was 2X greater for seedlings grown in treated substrate maintained at 0.25 $cm^3 \cdot cm^{-3}$ than it was for transplants grown in untreated substrate at the same SMC level. No significant differences in height, stem diameter or shoot dry weight were observed when comparing plants grown in treated and untreated substrate. Root dry weight was significantly greater for seedlings grown in untreated substrate. Flowering was not affected by humectant treatment or by SMC. The results show that transplanted zinnia 'Thumbelina' seedlings require less irrigation when grown at a lower SMC threshold in soilless substrate amended with 1.6% Hydretain®.

Index words: automated irrigation control, cultural practices, plant-water relations, transplant establishment, water management, water-use efficiency.

Species used in this study: 'Thumbelina' zinnia (*Zinnia elegans* Jacq.).

Chemicals used in this study: Hydretain® - a proprietary blend of sugar alcohols, polysaccharides, and neutral salts of alphahydroxyproprionic acid.

Significance to the Horticultural Industry

The increasing scarcity, cost and regulation of groundwater sources for irrigation are issues that impact the long-term sustainability of horticultural crop production. To this end, providing water management strategies that improve substrate water retention and/or enhance water-use efficiency (WUE) is a topic of interest for greenhouse and nursery growers. In the current study, we examined changes in the water-holding capacity of a soilless substrate amended with humectant (1.6% Hydretain®), and investigated the impact of humectant treatment on plant-water relations, WUE, growth, and flowering of container-grown zinnia 'Thumbelina' transplants maintained at two substrate moisture content (SMC) set points. For transplants grown in humectant-amended substrate at a low SMC threshold (0.25 $cm^3 \cdot cm^{-3}$), irrigation volume and frequency were reduced and WUE substantially increased. Height, stem diameter, and shoot dry weight were unaffected by humectant application, but the same growth parameters were significantly greater for transplants grown at the higher SMC threshold (0.45 $cm^3 \cdot cm^{-3}$). Data from this study demonstrate that water utilization can be substantially improved when zinnia transplants are grown in humectant-amended substrate maintained at a low SMC threshold (0.25 $cm^3 \cdot cm^{-3}$). This treatment regime resulted in a substantial reduction in the total volume of irrigation required for successful crop production without affecting post-transplant growth or flowering.

Introduction

The future availability of groundwater sources for irrigation continues to be a concern for horticultural growers, especially those involved in nursery and greenhouse production, where the majority of plants are now grown in containers (U.S. Dept. Agric. 2016). It has been predicted that by the year 2025, greenhouses and nurseries will be identified as high water-use consumers and, as a consequence, future water consumption will be more closely monitored and controlled in an attempt to reduce water withdrawals from existing surface and groundwater supplies (Fulcher et al. 2016). With increasing population growth and the concomitant need for additional water resources, combined with increasingly strict governmental regulations on agricultural-use water consumption, horticultural crop growers must continue to look for innovative water management strategies that utilize existing groundwater supplies more effectively and efficiently.

Along with advances in irrigation scheduling (Nikolaou et al. 2019), the application of non-traditional soil amendments [defined here as non-fertilizer materials applied to a soil or substrate to improve production, vigor or growth (NCR-103 Committee Report 2004)] offers a management option that may help sustain production. The amendments studied include: humic acid-based root

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stimulants (Xu et al. 2014), composted waste products (Kandal et al. 2016), hydrophilic gels (Alkhasha et al. 2018) and modified organic materials such as biochar (Darling 2015). There is, however, another group of substances that possesses beneficial properties when used as soil or substrate amendments. These materials, referred to collectively as humectants, contain hydrophilic groups, often hydroxyl groups, which form hydrogen bonds with water. Aqueous solutions of these substances, when applied to growing media, attract water vapor from the rhizosphere where it condenses, thereby becoming available for absorption by plant roots. Humectants are generally under-investigated. An early study by Barrett (1991) reported that humectant-amended growing media improved drought resistance in container-grown geranium (*Pelargonium hortorum* L.H. Bailey), impatiens (*Impatiens walleriana* Hook.f.) and vinca (*Catharanthus roseus* L.). A later study by Roberts and Linder (2010) showed that humectant treatment was effective in delaying the onset of foliar wilt in container-grown seedlings of red maple (*Acer rubrum* L.), red oak (*Quercus rubra* L.) and yellow-poplar (*Liriodendron tulipifera* L.). Further investigation (Roberts et al. 2015) revealed the effectiveness of humectants in mitigating the impact of drought stress during bedding plant production. Based on these reports, and on the paucity of information that currently exists concerning the use of humectant technology in horticultural crop production, the present study was undertaken to assess the benefit of applying a humectant in conjunction with a sensor-based, on-demand irrigation system as a practical strategy for optimizing post-transplant plant-water relations, WUE, and growth in container-grown zinnia (*Zinnia elegans* Jacq. 'Thumbelina'), an economically important ornamental crop used in both bedding plant (Kessler 2004) and cut flower (Carter and Grieve 2010) production.

Materials and Methods

Single seeds of 'Thumbelina' zinnia (Ohio Heirloom Seeds, Columbus, OH) were sown in two 50-hole plug trays filled with Fafard®-15 (Sun Gro Horticulture, Agawan, MA), a commercial soilless substrate consisting of peat moss, perlite, dolomitic limestone and a wetting agent. After seeding, each plug tray was covered with plastic wrap and sub-irrigated with tap water to bring the substrate moisture content to field capacity. The seeded plug trays were placed under LED lights (156 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR; 12 h photoperiod) in a room where ambient temperature ranged from 20 to 23 C (68 to 73 F) and relative humidity from 45 to 55%. Four days after sowing, the plastic wrap was removed and, three days after that, both plug trays were sub-irrigated with a water-soluble fertilizer {Miracle Gro 24-8-16 [24N-3.5P-13.3K] (Scotts Miracle-Gro Products, Inc., Marysville, OH)} at a rate of 2.5 $mL \cdot L^{-1}$ (0.32 $oz \cdot gal^{-1}$) of water. The same fertilization regime was continued weekly for three weeks, at which time 50 uniformly-sized seedlings were selected and transplanted into individual SP#4 (0.26 gal) plastic pots filled with Fafard®-15. At the time of transplanting, a capacitance sensor (EC-5, Decagon Devices Inc., Pullman, WA) was inserted into each of four pots and positioned so its volume of influence was entirely within the substrate matrix (Hagen et al. 2015). All 50 transplants were then sub-irrigated with tap water and placed back beneath LED lights for an additional 10 days to allow time for root establishment and, in the case of the four pots containing EC-5 sensors, time for stabilization at the sensor-substrate interface. During this 10-day period, the seedlings were hand-watered daily and sub-irrigated once with the same water-soluble fertilizer previously mentioned.

On the first day of the study, 10 of the 50 transplants were harvested and measurements taken of plant height and stem diameter. After separating the shoots from the roots at the root collar, plant-water potential (Ψ_w) was determined for each shoot using the pressure chamber technique (Scholander et al. 1965). Each root system was then thoroughly washed and, along with the corresponding shoot tissue, separately bagged and oven-dried at 80 C for 48 h to obtain root and shoot dry weight. Height, stem diameter, and dry weight values for these 10 seedlings were averaged and subsequently used to represent the initial starting size and weight of the remaining 40 transplants.

Also on day one, each of the 40 remaining transplants was top-dressed with 1 g (0.04 oz) of Osmocote 14-14-14 (14.0N-6.1P-11.6K) controlled-release fertilizer (The Scotts Co., Marysville, OH). After fertilization, the substrate in 20 pots, including two with embedded sensors, was treated with humectant (Hydretain®, Ecologel Solutions LLC, Ocala, FL) by drenching the substrate with 125 mL (4.2 oz) of the product at a concentration of 1.6%, the manufacturer's recommended rate. Substrate in the remaining 20 pots, two of which also contained embedded sensors, was drenched with 125 mL of water (0% humectant). Pre-testing had shown that 125 mL of liquid, either the aqueous humectant solution or water, was sufficient to bring the SMC in each pot to field capacity without measurable leaching. The 40 transplanted seedlings were then divided into groups of 10, with plants within a group all receiving the same treatment. Each 10-plant grouping was transferred to a prototype sensor-controlled irrigation system {WaterMaster, [WM](Roberts et al. 2018)} located in the same room and under the same growing conditions previously described. The WM system consisted of four independent irrigation supply lines, each of which delivered water to 10 equally-spaced pressure-compensating emitters. Each emitter, in turn, was attached to a 10.2 cm (4 in.) diameter dribble ring placed on the substrate surface in each pot. A micro-irrigation valve positioned in the water line between each emitter and dribble ring allowed for precise equalization of water flow to each pot at 1.8 $mL \cdot s^{-1}$ (0.06 $oz \cdot s^{-1}$) with water pressure set at 137.9 kPa (20 psi). Each of the four irrigation supply lines was connected to a solenoid valve (75D V, Rainbird, Tucson, AZ) which, in turn, was linked via the EC-5 sensor to the WM microcontroller programmed to deliver irrigation water whenever SMC in the four sensor-containing pots fell below a preselected SMC set point. Two of the four irrigation lines were programmed to deliver water at 0.45 $\pm$0.04 $cm^3 \cdot cm^{-3}$, a SMC threshold at which substrate moisture is readily available for plant growth (Bayer et al. 2015). Of the two irrigation lines set at

Table 1. Number of irrigation events, total irrigation volume, average number of days between irrigation events, plant-water potential and water-use efficiency of container-grown zinnia transplants treated with humectant and grown for 30 days at two SMC set points[z].

Humectant concentration (%)	Substrate moisture content ($cm^3 \cdot cm^{-3}$)	No. of irrigation events	Total irrigation volume (L)	Avg. days between irrigation events	Plant-water potential (MPa)	Water-use efficiency[y] ($g \cdot L^{-1}$)
1.6	0.45	16	8.64	1.9	-0.44	6.29
	0.25	5	2.70	6.0	-0.59	19.73
0.0	0.45	17	9.18	1.8	-0.60	6.91
	0.25	10	5.40	3.0	-0.72	9.48
Significance:						
Humectant conc. (HC)		—[x]	—[x]	—[x]	*	***
Substrate moisture (SMC)		—	—	—	*	***
HC x SMC		—	—	—	NS	***

[z]Plants grown from seed in plug trays for 4 weeks prior to transplanting into SP#4 (0.26 gal) marketable pots filled with soilless substrate. Transplants were allowed to become established for 10 days before beginning the experiment. Each value represents the mean of 10 replications. *, ***, significant at $P \leq 0.05$ and $P \leq 0.001$, respectively. NS = nonsignificant.

[y]Water-use efficiency (WUE) was calculated for each seedling by dividing total dry biomass (g) by the corresponding total irrigation volume (L) applied over the 30 day experimental period.

[x]No statistical analyses possible on these data since only one sensor was used to control irrigation for each humectant x SMC treatment.

0.45 $cm^3 \cdot cm^{-3}$, one line delivered water to the substrate of 10 pots previously treated with humectant, while the other delivered water to 10 pots containing untreated substrate (0% humectant). The remaining two irrigation supply lines were programmed to deliver water at 0.25 ±0.05 $cm^3 \cdot cm^{-3}$, a SMC threshold at which plant growth can be negatively impacted because of marginal substrate moisture availability (van Iersel et al. 2010). As previously described, one of the two 0.25 $cm^3 \cdot cm^{-3}$ lines supplied irrigation water to 10 seedlings transplanted in humectant-treated substrate, while the other provided water to 10 transplants growing in untreated substrate. For each of the four irrigation supply lines, the EC-5 sensor controlling irrigation was located in the same pot position.

To improve the accuracy and reliability of substrate moisture readings, the output voltage of each EC-5 sensor, a measure of substrate dielectric permittivity, was converted to SMC using a substrate-specific calibration equation (Cobos and Chambers 2010). For Fafard®-15, the calibration equation was: SMC = [(output voltage X 1.8862) - 0.5624]. Thus, whenever the voltage in each irrigation supply line fell below the prescribed SMC set point (either 0.45 or 0.25 $cm^3 \cdot cm^{-3}$, as measured by the substrate-embedded sensor), the solenoid valve connected to the line was automatically activated for 30 s, delivering 54 mL of water to each of the 10 pots.

Starting on the first day of the experiment, and continuing for 30 days, the following measurements were recorded and stored every 90 min on the WM microcontroller SD card: current date, real clock time, duration of irrigation, irrigation cycle count, current SMC reading, and SMC set point reading. In addition, observations of flower and flower bud formation were manually recorded daily for each seedling. These daily observations included: date of first flower formed, number of flowers formed, and number of flower buds formed. At the end of 30 days the experiment was terminated and the plants harvested. Final measurements were taken of plant height, stem diameter, plant-water potential (Ψ_w), root dry weight, shoot dry weight, number of days from appearance of first flower until end of study, total number of flowers formed, and total number of flower buds formed. WUE was calculated as the increase in dry biomass (g) divided by the total volume of irrigation water applied (L). Using the average starting total dry weight (M_1) of the 10 "extra" seedlings harvested on day 1 (t_1), relative growth rate (RGR) for the 40 experimental plants was determined by subtracting M_1 from the final dry weight (M_2) of each seedling harvested on day 30 (t_2). RGR was calculated as: $RGR = (\ln M_2 - \ln M_1) \div (t_2 - t_1)$.

The experimental design for the study was a 2 by 2 factorial with two humectant treatments (1.6% and 0% Hydretain®) and two SMC threshold set points (0.45 and 0.25 $cm^3 \cdot cm^{-3}$). Prior to the start of the experiment each of the four irrigation lines was randomly assigned one of the four Hydretain®/SMC treatments. Upon completion of the investigation the data were subjected to an analysis of variance and analyzed using statistical software [*Statistix 10* (Analytical Software, Tallahassee, FL)]. Differences between treatment means were compared using Tukey's pairwise comparison test, $P \leq 0.05$.

Results and Discussion

Plant-water potential (Ψ_w). Ψ_w is frequently used as a measure of the overall status of water in plant systems (Ordog 2011). In the present study, the Ψ_w of zinnia transplants grown in substrate amended with humectant (1.6% Hydretain®) was significantly greater (less negative) than for similar transplants grown in untreated substrate (Table 1). This was especially true for zinnia transplants grown in humectant-treated substrate maintained at the higher SMC threshold (0.45 $cm^3 \cdot cm^{-3}$), where Ψ_w was 18% less negative than it was for comparable plants grown in untreated substrate at the same threshold (Table 1). Although statistical analysis of the data was not possible (only one sensor per irrigation line), data recorded and stored on the WM microcontroller SD card showed that the number of irrigation events and the total irrigation volume were both lower, while the average number of days between irrigation events was higher, in humectant-amended substrate (Table 1). These results indicate that even at a low SMC (0.25 $cm^3 \cdot cm^{-3}$), humectant-treated substrate provided sufficient moisture to support plant

Table 2. Growth and flowering of container-grown zinnia transplants treated with humectant and grown for 30 days at two substrate moisture thresholds[z].

Humectant concentration (%)	Substrate moisture content ($cm^3 \cdot cm^{-3}$)	Height (cm)	Stem diam. (mm)	Dry biomass (g)		Relative growth rate ($g \cdot g^{-1} \cdot d^{-1}$)	Flowering index[y]
				Shoot	Root		
1.6	0.45	27.1	5.3	2.82	0.49	0.081	4.7
	0.25	20.9	4.9	2.40	0.55	0.078	4.6
0.0	0.45	28.1	5.4	3.34	0.76	0.089	5.3
	0.25	22.8	4.6	2.34	0.50	0.076	4.9
Significance:							
Humectant conc. (HC)		NS	NS	NS	*	**	NS
Moisture content (SMC)		**	***	***	NS	NS	NS
HC x SMC		NS	NS	NS	**	*	NS

[z]Plants grown from seed in plug trays for 4 weeks prior to transplanting into SP#4 (1L) marketable pots filled with soilless substrate. Transplants allowed to become established for 10 days before beginning the experiment. Each value represents the mean of 10 replications. *, **, ***, significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively. NS, nonsignificant.

[y]Flowering index calculated by taking the average of the following three measurements: (1) number of days from appearance of first flower until end of study; (2) total number of flowers at end of study; (3) total number of flower buds at end of study.

growth. In comparing the Ψ_w of zinnia transplants grown at the two SMC levels used in this study, seedlings maintained at 0.45 $cm^3 \cdot cm^{-3}$ exhibited a greater (less negative) Ψ_w than similar seedlings grown at 0.25 $cm^3 \cdot cm^{-3}$ (Table 1). These results were expected based on previous studies showing that substrate moisture is readily available at SMC thresholds ranging from 0.40 to 0.45 $cm^3 \cdot cm^{-3}$ (van Iersel et al. 2010, Miralles-Crespo and van Iersel 2011).

During a typical dry-down cycle, water is absorbed first from larger pore spaces in the substrate and, eventually, from successively smaller spaces between and within the substrate matrix (Kramer and Boyer 1995). As dry-down continues, and hydrostatic pressure increases, substrate physical properties (e.g. bulk density, air space and total porosity) play an increasingly important role in governing the water-holding capacity of the growing medium (Taiz and Zeiger 2010). Plant-water potential data collected in the current study suggest that the hygroscopic properties attributable to Hydretain® helped ensure the availability of a more consistent source of substrate moisture over a wider range of SMC set points, while reducing the total volume of irrigation and increasing the average number of days between irrigation events (Table 1).

Water-use efficiency (WUE). WUE is defined here as the total plant dry matter (g) produced per unit volume of water (L) consumed. The greater the ratio, the higher the efficiency. In this study, at the end of the 30-day experimental period, the WUE of zinnia transplants grown in humectant-treated substrate was significantly greater than for similar transplants grown in untreated substrate and, likewise, the WUE of transplants grown at 0.25 $cm^3 \cdot cm^{-3}$ was greater than those grown at 0.45 $cm^3 \cdot cm^{-3}$ (Table 1). Most noteworthy, however, was the WUE interaction for transplants grown in humectant-treated substrate maintained at the lower SMC threshold (0.25 $cm^3 \cdot cm^{-3}$) where WUE reached 19.73 $g \cdot L^{-1}$ (Table 1). It was also interesting to observe, as mentioned above, that transplants grown at the lower SMC set point exhibited a higher WUE than similar seedlings grown at the higher SMC set point. These results, although unexpected, support earlier findings by Stoll et al. 2000, which suggested partial root zone drying may actually improve WUE by causing an increase in both xylem sap pH and abscisic acid concentration, which together resulted in a reduction in stomatal conductance and a subsequent decline in transpiration.

Since the physical properties of a growing medium largely determine how much water and oxygen are available for plant growth (Southern Nursery Assn. 2013), it is important to point out that the addition of an amendment may change the physical properties of the substrate, possibly impacting plant-water relationships such as WUE. While less likely to occur with liquid additives, incorporating solid amendments (e.g. biochar, manure, peat, or vermiculite) has been shown to alter substrate physical properties such as bulk density, air space and container capacity (Jacobs et al. 2003, Jahromi et al. 2018). Thus, any decision to utilize soil additives should take into account the potential impact of these amendments on the physical properties of an existing substrate.

Plant growth. At the end of this study (30 days after transplanting), no significant differences in height, stem diameter, or shoot dry weight were found between zinnia transplants grown in humectant-treated or in untreated substrate. However, there was a significant interaction noted for root dry weight. In this instance, the root dry weight of zinnia transplants grown in untreated substrate at 0.45 $cm^3 \cdot cm^{-3}$ was 55% greater than it was for similar transplants grown in humectant-treated substrate at the same SMC threshold (Table 2). These results suggest that for plants grown at a high SMC, moisture availability in humectant-treated substrate may initially be less than it is in untreated substrate. Since humectants such as Hydretain® form strong hydrogen bonds between hydrophilic components of the humectant and water molecules within the substrate matrix, it is possible that these bonds could limit the availability of moisture for absorption by plant roots until, presumably, plant-water potential decreases (becomes more negative), resulting in a directional shift in the water potential gradient. In a study using biochar as a substrate amendment, Jahromi et al. (2018) reported that the shoot dry weight of potted hydrangeas (*Hydrangea paniculata* Siebold) grown in 25% biochar-amended substrate declined 50% compared to the shoot dry weight of similar plants grown in non-amended substrate. The authors attribute this decrease to the ability of biochar to hold substrate water at a higher tension, thereby making it unavailable for absorption by plant roots. The same rationale could help explain the root dry weight data observed in the present investigation. Alternatively, the results might reflect enhanced root growth under mild water stress conditions (Roberts et al. 2017), a situation that would eventually be expected to improve in humectant-treated substrate. In addition to the significant interaction noted for root dry weight in the current investigation, there was also a significant RGR interaction (Table 2). Here, as with root dry weight, and probably for the same reason, the relative growth rate of zinnia transplants in untreated substrate (0% humectant) at a SMC threshold of 0.45 $cm^3 \cdot cm^{-3}$ was fastest (0.089 $g \cdot g^{-1} \cdot d^{-1}$) over the 30 day experimental period (Table 2).

Published studies on the growth response of humectant-treated plants are limited, especially investigations where humectants are used as post-transplant amendments. Ciardi et al. (1998) found that Hydretain® applied as a soil amendment was effective in improving post-transplant establishment rates in plug-grown tomato seedlings, and Arena (2001) reported an increase in the stem diameter of transplanted, container-grown live oaks treated with Hydretain®. Roberts et al. (2012) found that post-transplant treatment of drought-stressed red maple and yellow-poplar seedlings treated with either of two Hydretain® formulations did not significantly affect shoot growth, but may have indirectly impacted root growth by providing sufficient root zone water, thereby reducing the necessity for the roots in treated substrate to continually elongate in search of substrate moisture sources.

Flowering. There was no evidence in the current investigation to suggest that either humectant treatment or SMC threshold level had any significant effect on flowering or flower bud formation in transplanted zinnia 'Thumbelina' (Table 2). Although no published information could be found regarding the effects of humectant treatment on flowering, Bayer et al. (2015) reported that container-grown seedlings of *Gardenia jasminoides* J. Ellis 'Radicans' maintained at a SMC threshold of 0.40 $cm^3 \cdot cm^{-3}$ exhibited the highest number of flower buds, while poor flower bud development was found for similar plants grown at SMC thresholds between 0.20 to 0.30 $cm^3 \cdot cm^{-3}$. These findings, along with results from the current investigation, suggest that the flowering response to SMC is likely species specific, and that amending the substrate with 1.6% Hydretain® does not significantly impact flowering or flower bud formation.

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