Effects of a Nonionic Surfactant on Growth, Photosynthesis, and Transpiration of New Guinea Impatiens in the Greenhouse[1]

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Abstract

Production of quality greenhouse and nursery crops is dependent on high quality and quantities of water. At present, in some regions, insufficient water supply is a growing concern. This study was conducted to evaluate growth of New Guinea impatiens (*Impatiens hawkerii* 'Celebrate Salmon'), when watered with a polyoxyethylenesorbitan monolaurate (C58H114O26) solution commercially known as Tween 20, at differing irrigation levels compared with a conventional water regimen without the surfactant, and also to determine how Tween 20 would affect photosynthesis and transpiration. The treatment design was a 3 by 6 complete factorial design plus a control. The two factors were irrigation and Tween 20. Irrigation levels of 20%, 40%, or 60% of the full crop evapotranspiration (ET) requirements were used in combination with Tween 20 concentrations of either 0, 25, 50, 75, 100, or 125 $mg \cdot L^{-1}$ (0, 0.003338, 0.00668, 0.0100145, 0.01335, or 0.01669 oz per gallon). The control group was watered with tap water to container capacity with about 30% leachate. Evapotranspiration was determined as the difference of the applied water amount minus the leachate of the control. Plants irrigated with Tween 20 from 25 to 125 $mg \cdot L^{-1}$ (0.003338 to 0.01669 oz per gallon) at the 40% or 60% irrigation level had the same height and growth index as plants in the control after three months of growth. Plant fresh and dry weights were not different between the control and the treatments of Tween 20 from 50 to 125 $mg \cdot L^{-1}$ (0.00668 to 0.01669 oz per gallon) at the 60% irrigation level or the treatment of Tween 20 at 100 $mg \cdot L^{-1}$ (0.01335 oz per gallon) at the 40% irrigation level. Tween 20 had no effect on net photosynthetic rate. Tween 20 decreased the amount of transpired water of New Guinea impatiens 'Celebrate Salmon'. When the Tween 20 concentration increased from 0 to 100 $mg \cdot L^{-1}$ (0 to 0.01335 oz per gallon) at the 60% irrigation level, the transpiration rate and stomatal conductance decreased markedly by 43% and 47%, respectively, and water use efficiency was increased by 47%. Results from this study suggest that Tween 20 is able to increase plant water use efficiency through regulation of stomatal conductance or transpiration under deficit irrigation.

Index words: irrigation management, chemigation, source-sink physiology, deficit irrigation, wetting agent, Tween 20, adjuvant.

Species used in this study: New Guinea Impatiens (*Impatiens hawkerii* W. Bull. 'Celebrate Salmon').

Chemicals used in this study: Tween 20 (polyoxyethylenesorbitan monolaurate). (aka: polysorbate 20, polyoxyethylene (20) sorbitan monolaurate).

Significance to the Horticulture Industry

Adequate availability of high quality water for greenhouse plant production is an ever- present concern for growers. In a preliminary study, we first determined the daily amount of water required to grow a crop of New Guinea impatiens. Next, we conducted this study growing New Guinea with 40%, 60%, and 80% less water on a daily basis, with and without an additive of Tween 20 injected into the irrigation at various rates. This study is one of several (Beauchamp 2018, Greenwell 2017, Yang 2008) we have conducted demonstrating the same or increased growth for plants grown with less water when Tween 20 is added to the water supply compared with an ideal or deficit water supply. Results indicate that Tween 20 would make it possible to grow a crop with about half as much water as would be used when growing a crop with water alone.

Introduction

Greenhouse and nursery crops need significant amounts of water during establishment, growth, and flowering. Abundant water is not always available, which can lead to deficit irrigation (the application of water below full crop-water requirements) (Fereres and Soriano 2007). Deficit irrigation will severely decrease the size, beauty, and value of horticultural crops.

Plant water status is highly affected by substrate moisture and transpiration rate. Transpiration is the major force moving water in plants (Kramer and Boyer 1995). Transpiration from plant cells lowers the matric potential of cell walls due to evaporation, producing a water potential gradient causing water to move from the root to the cell surface of leaves. Plants transpire about 95% of the root-absorbed water into the air (Kramer and Boye 1995), with only a small percent of plant water involved in metabolic activities (Rosenberg et al. 1983). Transpiration can be decreased 50 to 75% without affecting plant growth (Tanner and Beevers 1990; 2001).

Surfactants are known to decrease surface tension. Surfactants have been reported in many studies to increase

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irrigation efficiency by increasing moisture retention in potting substrates (Bilderback and Lorscheider 1997, Guillen et al. 2005) and by decreasing water repellency of soil (Cisar et al. 2000, Snyder et al. 1984, Wallis et al. 1989). A few studies have reported decreased transpiration rate after spraying surfactants on leaves (Kubik and Michalczuk 1993) or the whole plants (Manthey and Dahleen 1998), or after keeping cut flowers in a surfactant solution (Ichimura, et al. 2005).

In the dynamic process of transpiration, the matric potential of cell walls due to evaporation is a decisive factor with which cell water potentials will tend to come to equilibrium and further drive sap up in the plant. Our hypothesis was that if a surfactant molecule is small enough to move through the xylem, then adding such a surfactant to irrigation water would decrease surface tension of leaf menisci, matric potential and the total water potential will increase, the driving force of transpiration will decrease, and as a result transpiration rate will decrease. Plants watered with the surfactant solution would have less water stress than those watered without surfactants. To some degree, plants watered with surfactant solutions at decreased irrigation levels would maintain similar growth as well-watered plants. Adding surfactant in irrigation systems should therefore provide a partial solution of water scarceness in horticulture.

To choose the right surfactant to decrease transpiration rate, some factors should be considered: (1) non-toxic to plant and environment, (2) small and water-soluble molecules, (3) nonionic, (4) relatively effective in deceasing surface tension at low concentration (Colwell and Rixon 1961). Even though both cationic and anionic surfactants are used with plants (Dobozy and Bartha 1976), use of nonionic surfactants is often critical since nonionic surfactants do not affect water hardness, nutrient balance, or enzyme activity and are compatible with most herbicides due to lack of ionization (Bayer and Foy 1982).

Polyoxyethylenesorbitan monolaurate ($C_{58}H_{114}O_{26}$), commercially known as Tween® 20, is one of the most frequently used safe, nonionic, biodegradable, low molecular weight (about 1227.54 $g{\cdot}mol^{-1}$) surfactants in a variety of industries, such as food (Bos and Van Vliet 2001), flavor and fragrance (Baydar and Baydar 2005), immunocytochemistry (Sato and Myoraku 2004), pharmacy (Chou et al. 2005) cosmetics (Jimenez 2001), and agriculture (Mitchell and Linder 1950, O'Sullivan and O'Donovan 1982). The hydrophilic-lipophilic balance (HLB) of Tween 20 is 16.7 (Avendano-Gomez et al. 2005), which indicates that it is a water soluble. Tween 20 approached the critical micelle concentration (CMC) at about 43 $mg{\cdot}L^{-1}$, 75 $mg{\cdot}L^{-1}$, and 98 $mg{\cdot}L^{-1}$ (0.0057, 0.0100145, 0.01309 oz per gallon) (Tran and Yu 2005) in water (difference due to different manufacturers), and above 100 $mg{\cdot}L^{-1}$ in the plant (Bernath and Vieth 1972).

In a preliminary study (Yang 2008, data not shown), using Tween 20 increased moisture retention of the substrate Fafard 3B (a blend of peat, perlite, vermiculite, and pine bark, Conrad Fafard, Inc., Agawam, Mass.) and decreased the amount of transpired water of New Guinea impatiens 'Celebrate Salmon'. Subsequently we set up a study to determine if growth of New Guinea impatiens in Fafard 3B, watered with a Tween 20 solution at differing irrigation levels, would be comparable to plants at conventional levels with no surfactant in the water supply. A second objective was to determine how Tween 20 would affect photosynthesis, stomatal conductance and transpiration.

Materials and Methods

In this study, rooted New Guinea impatiens cuttings were transplanted into 16.5-cm (6.5 in) diameter, 1.71 L (57.5 oz) azalea pots filled with Fafard 3B in mid-August. The substrate was amended with 6.6 $kg{\cdot}m^{-3}$ (13.3 lbs/yd) controlled-release fertilizer Polyon 18N–2.6P–9.9K (Pursell Technologies Inc., Sylacauga, Ala.) and 0.9 $kg{\cdot}m^{-3}$ (1.8 $lb{\cdot}yd^{-1}$) Micromax (The Scotts Co., Marysville, Ohio). Plants were hand-watered every other day and grown in a double layer polyethylene-covered greenhouse at the Paterson Greenhouse Complex, Auburn University, Ala. (32° 36′N × 85° 29′W, USDA Hardiness Zone 8a) for three months. Maximum photosynthetically active radiation in the greenhouse was 600 $\mu mol{\cdot}m^2{\cdot}s$ and daily maximum/minimum temperature in the greenhouse was 27 ± 6 C/18 ± 3 C. Based on an expectation that plants in the different treatments would have different flower numbers and flowers significantly affecting plant transpiration, all flower buds were removed whenever visible. Leachates were collected every two weeks using the nondestructive Virginia Tech Extraction Method (Bilderback 2001, Wright, 1986) and were analyzed for pH and electrical conductivity (EC) using a Model 63 pH and conductivity meter (YSI Incorporated, Yellow Springs, Ohio). The results of EC and pH of leachates revealed no difference between treatments, therefore no supplemental fertilizer was added during the study.

The treatment design was a 3 by 6 complete factorial design plus a control. The two factors were irrigation and Tween 20 (Monomer-Polymer & Dajac Labs, Inc., Feasterville, Pa.). Irrigation levels of 20%, 40%, or 60% of the full crop evapotranspiration (ET) requirements were used in combination with Tween 20 concentrations of either 0, 25, 50, 75, 100, or 125 $mg{\cdot}L^{-1}$ (0, 0.003338, 0.00668, 0.0100145, 0.01335, or 0.01669 oz per gallon). The control group was irrigated with tap water to container capacity with about 30% leachate (Bilderback and Loscheider 1997). The full crop ET requirement was determined as the difference of the applied water amount minus the leachate of the control (Allen et al. 1998). Because the main purpose of our study was to save water, we did not include treatments of the 100% irrigation level with 25 to 125 $mg{\cdot}L^{-1}$ Tween 20.

Photosynthetic parameters were measured with a portable photosynthesis system LICOR 6400 (LI-COR, Inc., Lincoln, NE.) between 1100 HR to 1300 HR on a sunny, cloudless day near the end of study. Measurements were made on a second fully expanded leaf from the top on each plant. Photosynthetically active radiation was set at 1,200 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$. The CO_2 flux was adjusted to maintain an inside chamber concentration of 350 $\mu mol{\cdot}mol^{-1}$. Relative humility was at 40 to 50% and air temperature was 28 °C

Table 1. **Statistical summary showing that the results of two-way 3 by 6 ANOVAs and post hoc tests for the effects of irrigation, Tween 20 ($mg \cdot L^{-1}$), and irrigation × Tween 20 interaction on height (cm), width (cm), growth index (GI, cm), shoot fresh and dry weight (g), net photosynthesis rate (P_n, $\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$), stomatal conductance ($g_{s\ H2O}$, $mmol\ CO_2 \cdot m^{-2} \cdot s^{-1}$), intercellular CO_2 concentration (C_i, $\mu mol\ CO_2 \cdot mol^{-1}$), stomatal limitation ($L_s$), vapor pressure deficit at the leaf surface (VPDL, kPa), foliar transpiration rate (E, $mmol \cdot m^{-2} \cdot s^{-1}$), and water use efficiency (WUE, $\mu mol\ CO_2 \cdot mmol^{-1}\ H_2O$) of *Impatiens hawkerii* 'Celebrate Salmon' after three-month growth in the greenhouse.**

Treatment	Ht	Width[x]	GI[w]	Fresh wt	Dry wt	P_n	$g_{s\ H2O}$	C_i	L_s	VPDL	E	WUE
Irrigation[z]												
60%	28.00a[y]	35.63a	33.08a	171.40a	16.31a	12.39a	165.71a	247.56a	37.42b[v]	2.30c	3.62a	3.63b
40%	27.36a	33.81a	31.66a	134.41b	13.04b	11.57ab	122.72b	209.23b	47.15a	2.36b	2.79b	4.54a
20%	25.47b	29.51b	28.17b	83.09c	7.70c	10.65b	97.83c	199.25b	49.77a	2.39a	2.29c	4.77a
Tween 20												
0	24.06b	29.72c	27.83c	102.23b	9.70c	10.55a	192.49a	282.97a	28.61c	2.30e	4.15a	2.70c
25	26.67a	30.86bc	29.46bc	106.50b	10.52bc	11.35a	131.16b	232.05b	41.41b	2.33d	2.94b	4.02b
50	27.06a	33.42abc	31.30ab	127.19ab	11.89abc	11.46a	123.94bc	215.56bc	45.50ab	2.35c	2.80bc	4.40ab
75	27.44a	34.17ab	31.93ab	136.37ab	12.91abc	11.79a	117.19cd	210.46bc	46.88ab	2.36bc	2.68bc	4.53ab
100	28.28a	35.31a	32.96a	156.83a	14.94a	12.10a	101.67d	181.06c	54.27a	2.40a	2.45c	5.22a
125	28.17a	34.42ab	32.22a	148.68a	14.14ab	11.97a	106.07cd	189.95c	51.98a	2.38ab	2.38c	5.02a
F												
Main effect												
Irrigation	13.73	22.33	27.71	40.99	41.89	6.20	83.74	14.98	15.41	150.55	77.93	12.33
Tween 20	9.50	5.48	8.21	5.08	4.60	1.28	38.87	15.27	15.26	40.33	36.26	14.04
Interaction	0.27	0.52	0.37	1.03	0.56	0.14	4.25	0.54	0.55	1.51	3.94	0.52
P value												
Main effect												
Irrigation	<.0001	<.0001	<.0001	<.0001	<.0001	0.0033	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Tween 20	<.0001	0.0002	<.0001	0.0004	0.0009	0.2833	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Interaction	0.9855	0.8736	0.9558	0.4261	0.8412	0.9991	0.0001	0.8530	0.8479	0.1524	0.0003	0.8734

[z]Three irrigation levels were 60%, 40%, and 20% of of the full crop evapotranspiration (ET) requirements. The full crop ET requirement was determined as the difference of the applied water amount minus the leachate of the control. The control group was watered with tap water to container capacity with about 30% leachate.

[y]Mean separation within columns by the post hoc Tukey's HSD test ($P \leq 0.05$).

[x]Width was calculated as the average of the widest plant width and plant width perpendicular to the widest width.

[w]Growth index (GI) was calculated as the average of plant height, the widest plant width, and plant width perpendicular to the widest width.

[v]Stomatal limitation (L_s) was calculated as (1 - C_i/C_a) × 100, where C_a was ambient CO_2 concentration.

during the measurements. Net photosynthetic rate, foliar transpiration rate, intercellular CO_2 concentration, vapor pressure deficit at the leaf surface and stomatal conductance were recorded automatically. The ratio of the photosynthetic rate to the transpiration rate gives the water use efficiency (Nobel 1983). Stomatal limitation was calculated as (1 - C_i/C_a) × 100, where C_a was ambient CO_2 concentration (Berry and Downton 1982).

At the beginning and the end of the growth period, plant height was measured from the base of the stem to the top of the plant. Width was the average of width at the widest point and width perpendicular to the widest point. Plant growth was determined using a growth index, calculated as the average of plant height plus widest plant width plus plant width perpendicular to widest width. At the end of study, plant shoots were harvested for determination of fresh and dry weights. Shoot fresh weights were measured immediately after harvest and dry weights were measured after oven-drying at 70 C for 72 hr.

Analysis of the data was best considered in three stages. At the start of the experiment, initial growth data was analyzed in a one-way analysis of variance (ANOVA) with the treatment as a main factor. At the end of the study, to examine the influence of irrigation, Tween 20, and the interaction between the two factors on growth and photosynthesis parameters of New Guinea impatiens, independence could not be determined if two-way ANOVA was performed on this unbalanced factorial design (Herr 1986). Therefore, final data analysis was first performed on a 3 by 6 factorial design without the control using a two-way ANOVA with irrigation and Tween 20 as main effects, with significant F-values determined with Tukey's honestly significantly different (HSD) post hoc tests. Data were then subjected to final baseline comparison using Student's *t* tests to find if the growth or photosynthetic parameter in 18 treatments was significantly different from that of the control, respectively. Any statistical test with $P \leq 0.05$ was considered significant and reported as such where appropriate. Data analyses were conducted using the GLM procedure of SAS for Windows v.9.1 (SAS Institute Inc., Cary, N.C.).

Results and Discussion

Plant growth. Plant height (overall mean ± SD = 20.01 ± 1.87 cm), width (20.63 ± 1.74 cm), and growth index (20.42 ± 1.49 cm) of New Guinea impatiens did not significantly differ among the treatments at planting in August (one-way ANOVA for plant height: F = 1.39, df = 18, 95, P = 0.1566; width: F = 0.33, df = 18, 95, P = 0.9948; growth index: F = 0.60, df = 18, 95, P = 0.8933). However, by November, a two-way ANOVA among the plant growth parameters (Table 1) revealed significant influence (Fig. 1) by the main factors (irrigation, Tween 20), but not by the interaction between irrigation and Tween 20. Therefore, the effect of the main factors on plant growth parameters can be presented and discussed

Fig. 1. Appearance of *Impatiens hawkerii* 'Celebrate Salmon' under 18 treatments and the control after three-month growth in the greenhouse. Treatments included 18 combinations of irrigation levels of 20%, 40%, or 60% of the full crop evapotranspiration (ET) requirements with Tween 20 levels of either 0, 25, 50, 75, 100, or 125 mg·L^{-1}. The control was watered with tap water to container capacity with about 30% leachate (The far left plant in A or D). The full crop ET requirement was determined as the difference of the applied water amount minus the leachate of the control. A) Plants were irrigated with tap water at 20%, 40%, 60% or 100% of the full crop ET requirements. B) Plants were irrigated with 0, 25, 50, 75, 100, or 125 mg·L^{-1} Tween 20 at 20% of full crop ET requirements. C) Plants were irrigated with 0, 25, 50, 75, 100, or 125 mg·L^{-1} Tween 20 at 40% of full crop ET requirements. D) The far left plant was from the control group and the other plants were irrigated with 0, 25, 50, 75, 100, or 125 mg·L^{-1} Tween 20 at 60% of full crop ET requirements.

independently. Post hoc tests revealed that, in most cases plants grown with 25, 50, 75, 100, or 125 mg·L^{-1} (0.003338, 0.00668, 0.0100145, 0.01335, or 0.01669 oz per gallon) Tween 20 had a significantly higher height, width, growth index, fresh and dry weight than those grown with 0 mg·L^{-1} after three months of growth, averaged over irrigation level (Table 1). As Tween 20 concentration increased from 0 to 100 mg·L^{-1} (0.01335 oz per gallon) in the irrigation solution, plant height, width, growth index, fresh and dry weight increased. As the irrigation applied decreased from 60% to 20%, plant growth decreased, especially fresh and dry weight which decreased 51.52% and 52.79%.

From paired comparison *t* tests, deficit irrigation of fresh water at the 20%, 40%, or 60% irrigation level significantly inhibited plant growth compared to the control (Table 2). High stomatal conductance values (260.38 mmol m^{-2} s^{-1} H_2O) in the control indicated that the plants of the control had adequate water supply and were not affected by water deficit (Fig. 2C). While plants grown with the 40% or 60%

Table 2. Statistical summary showing that the results of Student's *t* tests between 18 treatments and the control on height (cm), width (cm), growth index (GI, cm), shoot fresh and dry weight (g) of *Impatiens hawkerii* 'Celebrate Salmon' after three-month growth in the greenhouse, respectively.

Treatment[z]		Ht			Width[y]			GI[x]			Fresh wt			Dry wt		
Irrigation	Tween 20	Mean	Mean (± SD) difference	P	Mean	Mean (± SD) difference	P	Mean	Mean (± SD) difference	P	Mean	Mean (± SD) difference	P	Mean	Mean (± SD) difference	P
control																
100%	0	26.00			38.67			34.44			203.33			19.22		
Treatment																
60%	0	25.67	-0.33 ± 2.18	0.7961	31.58	-7.08 ± 4.35	0.0181	29.61	-4.83 ± 3.34	0.0311	119.74	-83.59 ± 21.85	0.0053	11.80	-7.43 ± 1.54	0.0046
	25	27.67	1.67 ± 2.03	0.1861	32.08	-6.58 ± 4.92	0.0431	30.61	-3.83 ± 3.77	0.1091	126.33	-77.01 ± 13.06	0.0113	13.01	-6.21 ± 1.60	0.0137
	50	27.83	1.83 ± 0.94	0.0070	35.25	-3.42 ± 3.81	0.1515	32.78	-1.67 ± 2.65	0.3018	178.66	-24.68 ± 18.58	0.3056	16.76	-2.47 ± 1.40	0.2388
	75	28.83	2.33 ± 1.77	0.0455	37.42	-1.25 ± 4.41	0.6337	34.39	-0.06 ± 3.28	0.9772	182.46	-20.87 ± 18.69	0.4658	16.99	-2.23 ± 1.28	0.3886
	100	29.33	3.33 ± 1.06	0.0003	39.00	0.33 ± 5.32	0.9157	35.78	1.33 ± 3.77	0.5541	212.66	9.33 ± 20.33	0.8315	19.74	0.52 ± 1.72	0.9002
	125	29.17	3.17 ± 1.37	0.0025	38.42	0.25 ± 3.94	0.9146	35.33	0.89 ± 2.84	0.6000	208.54	5.21 ± 16.48	0.8501	19.57	0.35 ± 1.60	0.8973
40%	0	23.5	-2.50 ± 1.16	0.0039	31.92	-6.75 ± 4.43	0.0391	29.11	-5.33 ± 1.26	<.0001	116.84	-86.50 ± 11.91	0.0162	11.12	-8.10 ± 1.44	0.0102
	25	27.00	1.00 ± 2.28	0.4650	32.92	-5.75 ± 4.20	0.0390	30.94	-3.50 ± 2.90	0.0633	119.22	-84.12 ± 14.92	0.0052	11.89	-7.33 ± 1.63	0.0058
	50	27.67	1.67 ± 2.71	0.3115	34.25	-4.42 ± 4.74	0.1376	32.06	-2.39 ± 3.25	0.2321	121.91	-81.42 ± 13.78	0.0362	12.24	-6.98 ± 1.22	<.0001
	75	28.83	2.33 ± 0.97	0.0019	34.17	-4.50 ± 4.56	0.1184	32.22	-2.22 ± 3.18	0.2546	135.96	-67.38 ± 13.19	0.0199	13.20	-6.02 ± 1.80	0.0208
	100	28.83	2.83 ± 1.64	0.0134	35.50	-3.17 ± 3.69	0.1675	33.28	-1.17 ± 2.48	0.4344	166.73	-33.61 ± 15.82	0.1809	15.83	-3.39 ± 1.82	0.1550
	125	28.83	2.83 ± 1.22	0.0024	34.08	-4.58 ± 3.51	0.0471	32.33	-2.11 ± 2.52	0.1773	145.78	-57.56 ± 13.43	0.0487	13.97	-5.25 ± 1.29	0.0199
20%	0	23.00	-3.00 ± 1.61	0.0143	25.67	-13.00 ± 3.99	0.0002	24.78	-9.67 ± 3.05	0.0003	70.10	-133.20 ± 14.75	0.0004	6.18	-13.04 ± 0.35	<.0001
	25	25.33	-0.67 ± 1.53	0.4671	27.58	-11.08 ± 4.24	0.0011	26.83	-7.61 ± 3.11	0.0017	73.96	-129.38 ± 12.44	0.0002	6.67	-12.55 ± 0.66	<.0001
	50	25.67	-0.33 ± 1.65	0.7342	30.75	-7.92 ± 3.44	0.0026	29.06	-5.39 ± 2.45	0.0034	81.00	-122.33 ± 17.79	0.0002	6.68	-12.54 ± 0.93	<.0001
	75	25.67	-0.33 ± 1.32	0.6703	30.92	-7.75 ± 3.65	0.0043	29.17	-5.28 ± 2.61	0.0056	90.69	-112.65 ± 8.77	0.0004	8.55	-10.67 ± 0.90	<.0001
	100	26.67	3.64 ± 2.31	0.6279	31.42	-7.25 ± 4.07	0.0116	29.83	-4.61 ± 2.92	0.0211	91.09	-112.25 ± 7.25	0.0004	9.24	-9.98 ± 0.62	0.0003
	125	26.50	2.00 ± 1.16	0.4732	30.75	-7.92 ± 3.51	0.0029	29.39	-5.11 ± 2.48	0.0051	91.72	-111.67 ± 40.70	0.0008	8.87	-10.35 ± 0.64	0.0006

[z]18 treatments were combinations of irrigation levels of 20%, 40%, or 60% of the full crop evapotranspiration (ET) requirements with Tween 20 levels of either 0, 25, 50, 75, 100, or 125 $mg \cdot L^{-1}$. The full crop ET requirement was determined as the difference of the applied water amount minus the leachate of the control. The control group was watered with tap water to container capacity with about 30% leachate.

[y]Width was calculated as the average of the widest plant width and plant width perpendicular to the widest width.

[x]Growth index (GI) was calculated as the average of plant height, the widest plant width, and plant width perpendicular to the widest width.

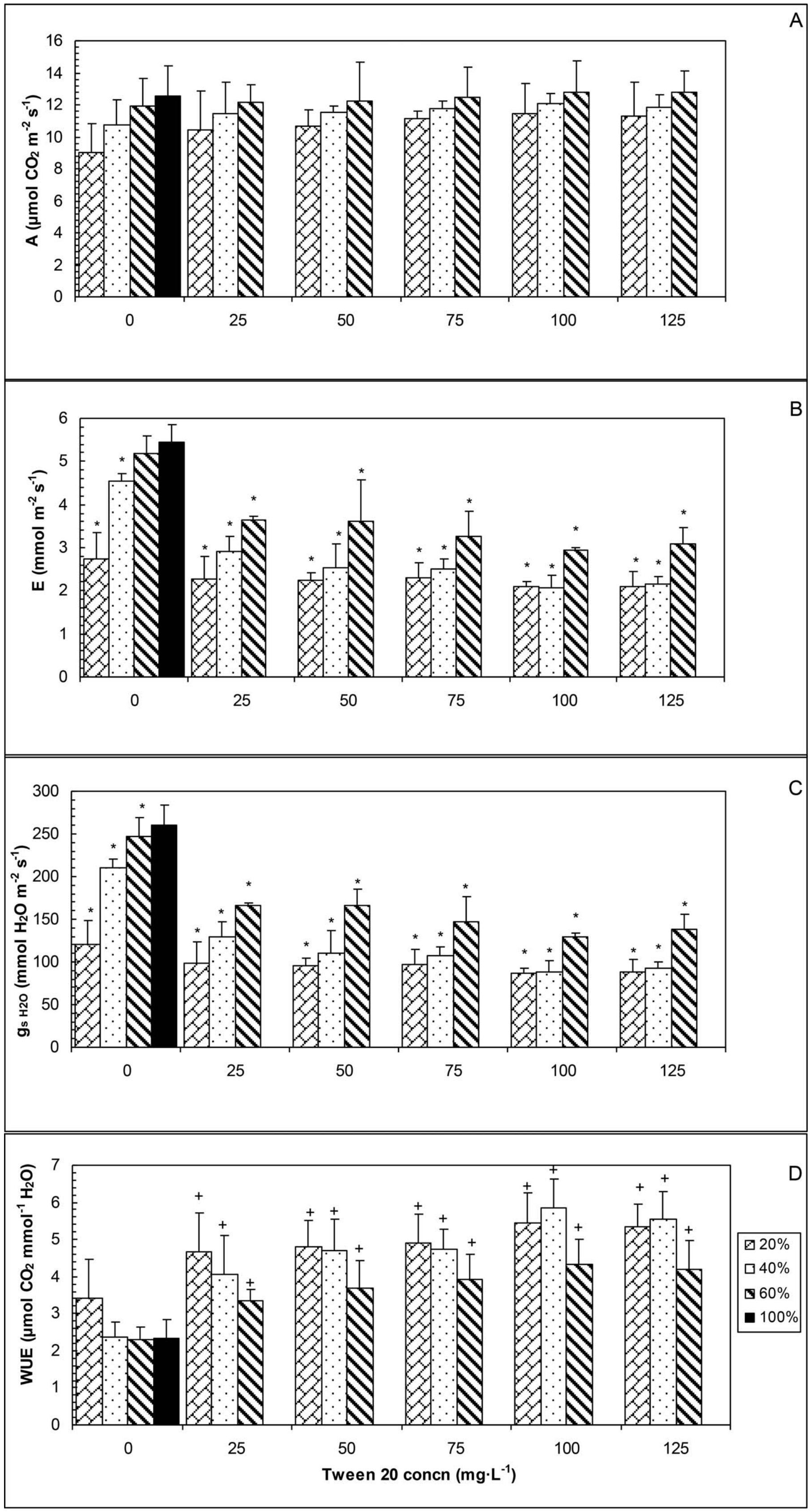

Fig. 2. **Statistical summary showing that the results of Student's *t* tests between 18 treatments and the control on net photosynthesis rate (P_n) (A), transpiration rate (E) (B), stomatal conductance ($g_{s\ H2O}$) (C), and water use efficiency (WUE) (D) of *Impatiens hawkerii* 'Celebrate Salmon' after three-month growth in the greenhouse, respectively. 18 treatments were combinations of irrigation levels of 20%, 40%, or 60% of the full crop evapotranspiration (ET) requirements with Tween 20 levels of either 0, 25, 50, 75, 100, or 125 mg·L^{-1}. The full crop ET requirement was determined as the difference of the applied water amount minus the leachate of the control. The control group (a black column) was watered with tap water to container capacity with about 30% leachate. Each bar is the mean ± SD of five replicates. An asterisk (*) or plus (+) denotes that the parameter of the treatment is significant lower or higher than the control according to the Student's *t* test, respectively. ($P \leq 0.05$).**

irrigation level combined with Tween 20 at 25, 50, 75, 100, or 125 $mg{\cdot}L^{-1}$ had the same height and growth index as plants in the control at the end of the study (Table 2). Plant fresh and dry weight in the treatment of the 60% irrigation level combined with either 50, 75, 100, or 125 $mg{\cdot}L^{-1}$ or in the treatment of the 40% irrigation level with 100 $mg{\cdot}L^{-1}$ were not different from those of the control.

Physiological characteristics. There were no significant differences between treatments in net photosynthetic rate due to the main effect Tween 20 (Two-way ANOVA with 18 treatments, $F = 1.28$, $df = 5, 72$, $P = 0.2833$). Net photosynthesis rate declined as the irrigation level decreased (Fig. 2A). Irrigation was the main factor to explain photosynthesis reduction ($F = 6.20$, $df = 2, 72$, $P = 0.0033$). As the irrigation decreased from the 60% to the 20% level, average stomatal conductance decreased significantly from 165.71 to 97.83 mmol $CO_2{\cdot}m^{-2}{\cdot}s^{-1}$, average intercellular CO_2 concentration decreased significantly from 247.56 to 199.25 μmol $CO_2{\cdot}mol^{-1}$ and stomatal limitation increased significantly from 37.42 to 49.77 (Table 1). The intercellular CO_2 concentration and stomatal conductance deceased with a Tween 20 concentration increase from 0 to 125 $mg{\cdot}L^{-1}$, (0 to 0.01669 oz per gallon) but the decrease in the intercellular CO_2 concentration by Tween 20 had no significant effect on photosynthesis. Reduction of net photosynthetic rate under water stress is considered a result of stomatal closure (Chaves 1991, Chen et al. 2006, Ramanjulu et al. 1998, Sharkey and Seemann 1989) and metabolic impairment (Calatayud et al. 2000). If there is a reduction in intercellular CO_2 concentration and an increase in stomatal limitation, the reduction of net photosynthesis rate is the result of decrease in stomatal conductance (Farquhar and Sharkey 1982, Xu 1997). On the other hand, if a net photosynthesis rate decrease accompanies an increase of intercellular CO_2 concentration and a reduction of stomatal limitation as well, the main constraint of photosynthesis is the result of the non-stomatal factors (Flexas and Medrano 2002). Hence, we attributed the main reasons for the reduction of net photosynthesis rate at lower irrigation levels in this study to the decrease of stomatal conductance. This suggests that stomatal control of water losses is an early response of New Guinea impatiens to water deficit, leading to a limitation of carbon uptake by the leaves.

Fig. 3A, B, C, and D shows that the relationship between irrigation and vapor pressure deficit, transpiration rate, stomatal conductance and water use efficiency at different Tween 20 concentration as the irrigation level decreased from 60% to 20%. As the irrigation level decreased, stomata gradually closed to avoid dehydration and vapor pressure deficit increased (Two-way ANOVA with 18 treatments, $F = 150.55$, $df = 2, 72$, $P < 0.001$). This increase was also significantly affected by Tween 20 ($F = 40.33$, $df = 5, 72$, $P < 0.001$). At the same irrigation level, as Tween 20 concentration increased from 0 to 100 $mg{\cdot}L^{-1}$, (0 to 0.01335 oz per gallon) vapor pressure deficit increased (Fig. 3A), and stomatal conductance and transpiration rate decreased (Fig. 3B, C). For example, at the 60% irrigation level, vapor pressure deficit increased slightly (Fig.3A), while the transpiration rate and stomatal conductance decreased markedly by 43% (Fig.3B) and 47% (Fig.3C), respectively. Our results are consistent with Kubik and Michalczuk (1993), who reported that Tween 20 accelerated the decrease in transpiration rate at 5.5 $mol{\cdot}m^{-3}$ when Tween 20 was applied in the leaf surface. The decrease of the transpiration rate we observed could be probably explained by the following reasons. The most effective concentration of Tween 20 to reduce transpiration was near 100 $mg{\cdot}L^{-1}$ (0.01335 oz per gallon) in this study (Fig. 3), which is around the CMC of Tween 20. In the CMC range, surface tension reaches the lowest levels (Greene and Bukovac 1974). Therefore, surface tension of leaf menisci could have sharply decreased, leading to further transpiration rate decrease. The result of the decrease of the transpiration rate partly confirmed our hypothesis, also seen in cut roses (Ichimura et al. 2005), demonstrating that a surfactant markedly decreased hydraulic conductance and transpiration rate. It is also important to note that the stomata of New Guinea impatiens are partly open during the night (Mankin et al., 1998), so transpiration remains important to water relations at night.

The result of the decrease of stomatal conductance by surfactants had been reported in a few studies (Kubik and Michalczuk 1993, Sanchez-Blanco et al. 2003), and the mechanism has been little understood. Potassium (K^+) flux out of the guard cell or K^+ accumulating in the epidermal cells is associated with stomatal closure (Penny and Bowling 1974). When membranes were modified with surfactants, stomatal closure was not related to electrical charge introduced to the membrane (Kubik and Michalczuk 1993), This response may indicate that the decrease of stomatal conductance is related to the modification of surfactant on membranes.

Water use efficiency of New Guinea impatiens treated with 100 $mg{\cdot}L^{-1}$ (0.01335 oz per gallon) Tween 20 at the 20%, 40% or 60% irrigation level was 38%, 59% or 47% higher than those with tap water at same irrigation level, respectively (Fig. 3D). The differences in water use efficiency between Tween 20 concentrations at the same irrigation levels were predominantly due to difference of the transpiration rate since an effect on photosynthesis rate was not significant, as discussed above (Fig. 2A).

Compared with the control, the transpiration rate of plants treated with 50 to 125 $mg{\cdot}L^{-1}$ (0.00668 to 0.01669 oz per gallon) Tween 20 at the 60% irrigation level or plants treated with 100 $mg{\cdot}L^{-1}$ (0.01335 oz per gallon) at a 40% irrigation level was lower (Fig. 2B) and water use efficiency was higher (Fig. 2D). Combined with the growth results, plants of those treatments had similar growth as the control. Therefore, New Guinea impatiens watered with 50 to 125 $mg{\cdot}L^{-1}$ (0.00668 to 0.01669 oz per gallon) Tween 20 at 60% of a full crop ET requirements or with 100 $mg{\cdot}L^{-1}$ (0.01335 oz per gallon) at 40% of full crop ET would be a reasonable irrigation level.

Results from this study suggest that the surfactant Tween 20 is able to increase plant water use efficiency through regulation of stomatal conduction or transpiration under deficit irrigation. Therefore, we speculate that other irrigation systems in the horticultural industry would

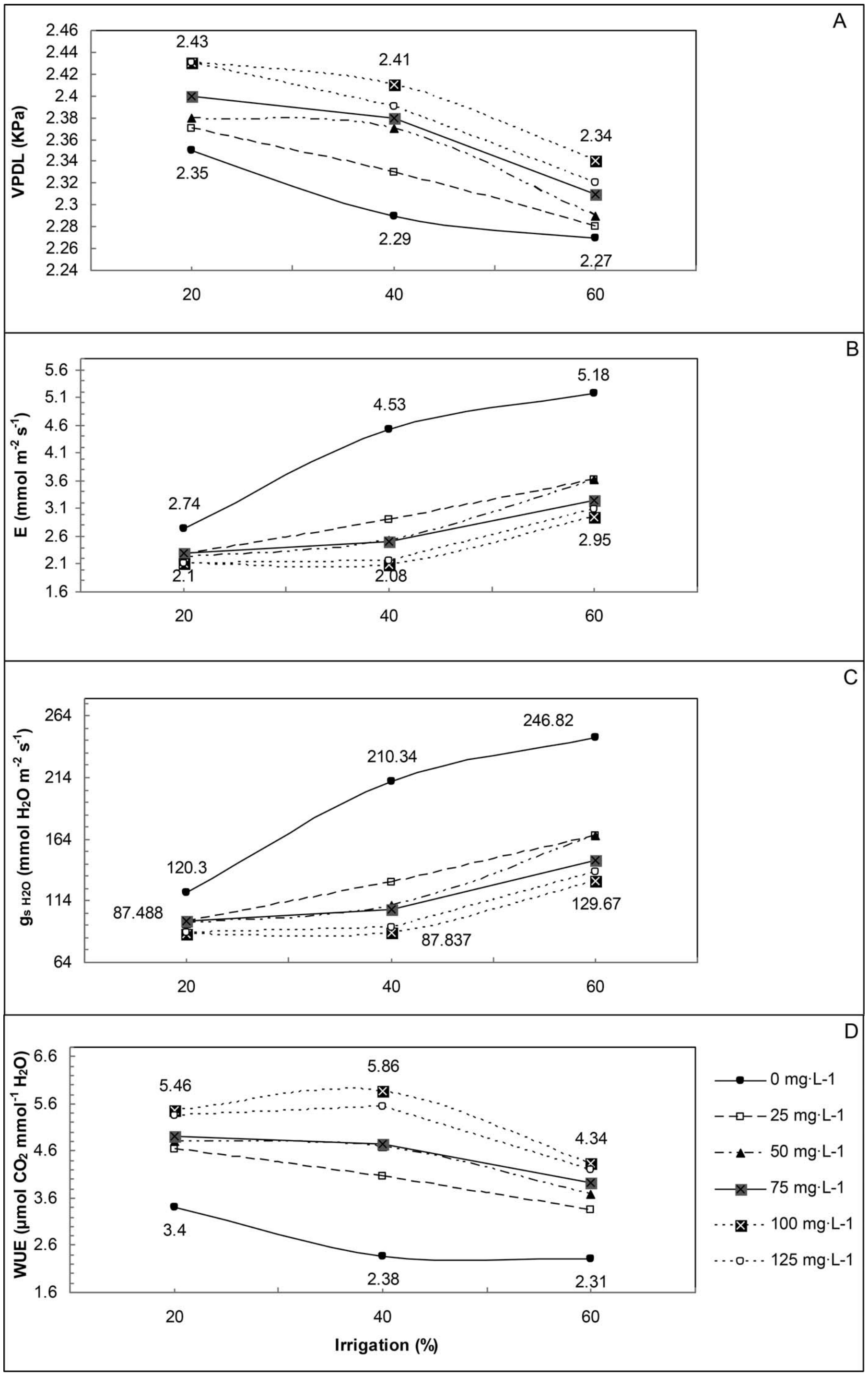

Fig. 3. Relationship between vapor pressure deficit (VPDL) and irrigation (A), transpiration rate (E) and irrigation (B), stomatal conductance ($g_{s\ H2O}$) and irrigation (C), and water use efficiency (WUE) and irrigation (D) at different Tween 20 concentrations for *Impatiens hawkerii* 'Celebrate Salmon' grown in the greenhouse for three months (two-way 3× 6 ANOVA, $P \leq 0.05$). Treatments were combinations of irrigation levels of 20%, 40%, or 60% of the full crop evapotranspiration (ET) requirements with Tween 20 levels of either 0, 25, 50, 75, 100, or 125 mg·L^{-1}. The full crop ET requirement was determined as the difference of the applied water amount minus the leachate of the control. The control group was watered with tap water to container capacity with about 30% leachate.

benefit from the use of such surfactants in times of drought. Further trials have been conducted (Greenwell 2017, Sibley et al. 2018, Yang 2008) or are underway (Beauchamp 2018) to investigate the use of Tween 20 to manage crop water stress in a broad line of species with different physiological characteristics.

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