Evaluation of a Commercial Extract of Giant Knotweed on Drought Tolerance of Impatiens

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Abstract. Regalia®, a commercial extract of giant knotweed [*Fallopia sachalinensis* F. Schmidt (synonyms: *Reynoutria sachalinensis* (F. Schmidt) Nakai, *Polygonum sachalinense* F. Schmidt, *Tiniaria sachalinensis* (F. Schmidt) Janch.)], was evaluated for its potential to enhance drought tolerance of container-grown impatiens (*Impatiens walleriana* Hook. f. ‘Super Elfin XP White’). In two separate experiments, Regalia® was foliar-applied once a week for 4 weeks at four different rates (0, 5, 10, or 15 mL·L⁻¹). In Expt. 1, Regalia® was applied to impatiens grown under three target substrate volumetric water contents (TVWCs): 85%, 55%, or 25%. In Expt. 2, Regalia® was applied to impatiens watered with 1, 3, or 6 days between waterings (DBW). In Expt. 1, root dry weight (RDW) of impatiens receiving applications of Regalia® at the 0.5× rate was greater compared with the 0.0× rate across all TVWCs. Additionally, soluble protein content was greater after Regalia® application at the 0.5×, 1.0×, or 1.5× rates compared with the 0.0× rate for plants grown at 55% TVWC. In Expt. 2, leaf greenness (SPAD) and leaf net photosynthetic rate (Pn) were greater with Regalia® applied at the 0.5× and 1.0× rates compared with the 0.0× rate, respectively. Soluble protein content was greater in impatiens treated with Regalia® at the 1.5× rate and 1 DBW and the 0.5× rate with 3 DBW compared with the 0.0× rate with 1 or 3 DBW. However, there was no indication that impatiens grown under different moisture levels had increased drought tolerance after application of Regalia®.

A topic gaining interest in agronomic and horticultural crop production is the promotion of plant health and stimulation of plant immunity (American Phytopathological Society, 2009). Strobilurin fungicides were among the first class of fungicides to show increased yields through direct effects on photosynthetic efficiency and transpiration rate (BASF, 2009). Moreover, multiple reports have indicated pyraclostrobin, a strobilurin fungicide, increased nitrate reductase activity, increased antioxidant enzymes, increased stress tolerance, reduced the amount

of CO₂ lost to the atmosphere (BASF, 2009; Köhle et al., 2002; Nason et al., 2007), and increased the overall green color of plants (Balba, 2007). Additionally, an extract of *Reynoutria sachalinensis* (giant knotweed) marketed as Regalia® by Marrone Bio Innovations (Davis, CA) as an organic fungicide with activity against powdery mildew, gray mold, and blights increased root growth of strawberry and tomato seedlings after application of Regalia® (Marrone Bio Innovations, 2011). The indirect mode of action of Regalia® is seen through the increased production of

phytoalexins, which strengthen the plant’s immune system (Konstantinidou-Doltsinis and Schmitt, 1998). After a plant has been affected by a biotic or abiotic agent, phytoalexins (antimicrobial compounds) are synthesized as a defense mechanism (Vasconsuelo and Boland, 2007). Some of these phytoalexins are lytic enzymes such as chitinases and glucanases, oxidizing agents, cell wall lignifications, pathogenesis-related proteins, and transcripts of unknown functions (Mert-Türk, 2002). Additionally, Regalia® has been reported to increase chlorophyll content and the activity of peroxidases, polyphenoloxidases, and Phe ammonia-lyase (Daayf et al., 1997). Peroxidases are involved in lignin polymerization, crosslinkage of cell wall constituents, catabolism of auxin, formation of reactive oxygen species (ROS), and defense against pathogenic organisms (Bakalovic et al., 2006). Lignin polymerization provides rigidity and structural support to cell walls (Kärkönen and Koutaniemi, 2010). Under water stress, plant species with more elastic cells have relatively no change in cell water potential as water is removed; however, the more rigid a cell wall is, the greater the change in water potential as water is removed. Therefore, a cell with a more rigid cell wall increases water uptake without severe dehydration (Mengel et al., 2001; Salleo, 1983; Verslues et al., 2006). Thus, if application of Regalia® increases peroxidase activity, it could result in heightened lignin polymerization and result in a more rigid cell wall preventing extreme cell dehydration.

With environmental stresses, governmental regulations, and increased human populations with limited water supplies (Burnett and van Iersel, 2008; Niu et al., 2008; Warsaw et al., 2009), plant producers and landscapers have had to follow stricter water use guidelines. Although it is known that some fungicides stimulate growth and may improve plant health (Balba, 2007), little research has evaluated these compounds for increasing ornamental plant tolerance to drought. Therefore, the objective of these experiments was to evaluate drought tolerance of impatiens treated with foliar applications of Regalia®.

Materials and Methods

Plant material and culture. On 24 June 2010, impatiens seedlings were potted individually from a 288-plug flat into 15.24-cm (1.8-L) containers with Sunshine Mix 1 [70:30 peat:perlite (v:v), nutrient charge, micronutrients, gypsum, calcium carbonate, wetting agent; SunGro Horticulture, Bellevue, WA] potting substrate. All containers were filled to the rim and lightly tapped twice on a hard surface to reduce air pockets. After potting, impatiens were watered, placed in a controlled environment greenhouse located on Mississippi State University’s main campus, and grown for 4 weeks under 65% shade and 21.1/18.3 °C (day/night) set point temperatures. On 23 July 2010 [Expts. 1 and 2 (Run 1)] and on 3 Sept. 2010 [Expts. 1 and 2 (Run 2)], impatiens were moved to an inflated, double-layer polyethylene Quonset greenhouse located on Mississippi

State University's R.R. Foil Plant Science Research Facility under 60% shade and 21.1/18.3 °C (day/night) set point temperatures. Experiments were repeated twice and conducted in a similar manner: 27 July 2010 to 24 Aug. 2010 [Expts. 1 and 2 (Run 1)] and 7 Sept. 2010 to 4 Oct. 2010 [Expts. 1 and 2 (Run 2)].

Determination of substrate volumetric water content. Physical properties were determined for Sunshine Mix 1 (Hidalgo, 2001): 90.9% total porosity, 28.3% air space, 62.6% water-holding capacity, and 0.11 g·cc⁻¹ bulk density. Substrate volumetric water content (VWC) was determined according to the instructions provided with a WaterScout SM100 Soil Moisture Sensor (Spectrum Technologies, Inc., Plainfield, IL) and fit to a regression model: $VWC = 0.00076503 \cdot MW - 0.79736$ ($P = 0.0001$; $R^2 = 0.99$; where MW = target mass wetness, defined as a percentage).

Water stress and fungicide management. At initiation of Expt. 1, containers were determined to be at 100% actual VWC (AVWC) 1 d before the first application of Regalia®. Based on AVWC, each container was hand-watered to the TVWC: 85% (control), 55%, or 25%. Containers maintained at 85% TVWC were not watered until 3 d after initial Regalia® application (DAIR), whereas containers maintained at 55% or 25% TVWC were not hand-watered until 8 or 15 DAIR, respectively (Fig. 1). Four rates of Regalia® were applied based on the recommended label rate of 0.48 g·L⁻¹ of active ingredient: 0.0× (water only), 0.5× (5 mL·L⁻¹), 1.0× (10 mL·L⁻¹), or 1.5× (15 mL·L⁻¹). Foliar applications of Regalia® were made once per week 3 h after watering containers to TVWC using a handheld sprayer (Model # 20010 with a 301120-4 brass nozzle; Chapin International, Inc., Batavia, NY). Fertilizer was applied to the substrate at 200 ppm nitrogen using Peter's Professional 20N-8.8P-16.6K Peat-Lite Special (Scotts-Sierra, Inc., Marysville, OH) at each watering. The experiment was conducted using a split plot design with the rate of Regalia® as the main plot factor in a randomized complete block design, TVWC as the subplot factor, with a three × four factorial treatment design and six single-pot replications for each treatment combination with two replications per block (total of three blocks). A single plant represented an experimental unit ($n = 72$).

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Fig. 1. Actual substrate volumetric water content (AVWC) after four weekly foliar applications of Regalia® to 'Super Elfin XP White' impatiens grown at three target substrate volumetric water contents (TVWCs). Data points represent daily averages pooled across experimental runs [27 July 2010 (Run 1) and 7 Sept. 2010 (Run 2)] and four rates [0.0×, 0.5×, 1.0× (1.0× = 10 mL·L⁻¹), and 1.5×] of Regalia® (Expt. 1; $n = 72$).

Table 1. Growth and water use efficiency of 'Super Elfin XP White' impatiens after four weekly foliar applications of Regalia® at four rates (1.0× = 10 mL·L⁻¹) to plants grown in containers maintained at three target substrate volumetric water contents (TVWCs) (Expt. 1; $n = 72$).

	FGI (cm) ^y	SDW (g) ^x	RDW (g) ^w	WUE (g·L ⁻¹) ^v	TWA (L) ^u
Rate					
0.0×	18.5	4.1	0.42 b ^t	5.7	1.2
0.5×	19.9	4.5	0.57 a	6.9	1.1
1.0×	19.9	4.5	0.52 ab	6.5	1.2
1.5×	17.2	3.6	0.41 b	5.4	1.1
TVWC					
85%	24.1 a	7.0 a	0.66 a	3.2 b	2.3 a
55%	19.9 b	4.1 b	0.48 b	4.3 b	1.0 b
25%	12.5 c	1.1 c	0.30 c	10.8 a	0.1 c
Effects					
Rate	0.1509 ^s	0.3437	0.0010	0.2484	0.7035
TVWC	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Rate × TVWC	0.8587	0.0552	0.8915	0.9937	0.8766

^aInitial application of Regalia® was made on 27 July 2010 (Run 1) or 7 Sept. 2010 (Run 2).

^yFGI = final growth indices [(height + width + perpendicular width) ÷ 3].

^xSDW = shoot dry weight, oven-dried for 72 h at 65 °C.

^wRDW = root dry weight, oven-dried for 72 h at 65 °C.

^vWUE = [(SDW + RDW) ÷ total water applied].

^uTWA = total mean water applied.

^tMeans (within a column) pooled across experimental runs with the same letters within rate or TVWC are not statistically different according to the Holm-Simulation method ($\alpha = 0.05$).

^sP value.

Expt. 2 was initiated similar to Expt. 1 and materials and methods were the same as in Expt. 1 except instead of maintaining daily TVWC, containers were watered with 1 (daily), 3, or 6 DBW. On the day of watering, containers were brought to 85% TVWC. Average AVWC during the duration of the experiment was 80% (1 DBW), 69% (3 DBW), or 61% (6 DBW) (Fig. 2). The experiment was conducted using a split plot design with rate of Regalia® as the main plot factor in a randomized complete block design, DBW as the subplot factor, with a three × four factorial treatment

design and six single-pot replications for each treatment combination with two replications per block (total of three blocks). A single plant represented an experimental unit ($n = 72$).

Growth index, biomass, and water use efficiency. At initiation of both experiments, initial growth index [IGI = (height + width + perpendicular width) ÷ 3] was determined for each plant. At the termination of Expt. 1 on 8 Aug. 2010 and Expt. 2 on 4 Oct. 2010, final growth index [FGI = (height + width + perpendicular width) ÷ 3], shoot dry weight (SDW), RDW, and total growth (TG = FGI - IGI)

were determined for each plant. Shoots were harvested by cutting the entire plant at the soil line, removing the entire upper portion of the plant. Roots were harvested by first soaking the whole container with the substrate and roots in a 17.7-L container of tap water for a minimum of 8 h and then washing the substrate from the roots over a screen to catch all fallen roots. Shoots and roots were dried in a forced-air dryer at 65 °C for 72 h before obtaining dry weights.

AVWC of each container was determined daily between 0600 and 0800 HR using a WaterScout SM100 Soil Moisture Sensor attached to a handheld FieldScout Soil Sensor Reader (Spectrum Technologies, Inc.). Daily readings were fit to the soil moisture curve and containers were hand watered to the TVWC. The amount of water applied per day per container was used to determine total water applied (TWA) over the 4-week period. Water use efficiency (WUE) was determined as described by Burnett and van Iersel (2008) with modifications using shoot and root dry weight [WUE = (SDW + RDW) ÷ total water applied].

Leaf greenness, photosynthesis, and midday stem water potential. Leaf greenness was measured using a handheld 502 SPAD chlorophyll meter (Konica Minolta, Osaka, Japan) at 2 and 4 weeks after initial application of Regalia® (WAIR). Leaf Pn was recorded at 2 and 4 WAIR using a CIRAS-2 portable photosynthesis system (PP Systems, Amesbury, MD) on the most recently matured leaf. Parameters were set at 2000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ photosynthetic photon flux density (using a tungsten halogen light attachment), ambient temperature, a relative humidity of 50% of ambient, and a CO₂ concentration of 350 $\mu\text{mol}\cdot\text{mol}^{-1}$ (per manufacturer's recommendation). Midday stem water potential (Ψ_{stem}) (–Mpa) was measured using a Scholander-type pressure chamber according to Kjellgren et al. (2009). Leaves were wrapped first in plastic wrap and then in aluminum foil for at least 1 h before measurement.

Antioxidant enzyme extractions and assays. To evaluate metabolic changes induced after application of Regalia®, leaf samples were collected at the end of each experiment, placed in Kraft #1 coin envelopes (Quality Park Products, Minneapolis, MN), frozen immediately with liquid nitrogen, and stored at –80 °C until analyzed for glutathione-S-transferase. Crude enzyme was extracted with 1 mL of a 50 mM sodium phosphate buffer (pH 7.5) as previously described by Venisse et al. (2001), then centrifuged at 14,000 g_n at 4 °C until plant tissue was clearly separated from the 1 mL of extraction buffer (20 to 40 min). Soluble protein (SP) content was determined for each sample according to Bradford (1976) using a Quick Start Bradford Protein Assay Kit 1 (Bio-Rad Laboratories, Hercules, CA).

Glutathione-S-transferase (GST) was assayed as previously described by Venisse et al. (2001) with some modifications. Samples (three replications from each rate × TVWC or rate × DBW combination) were analyzed in duplicates using an ELx808

Table 2. SPAD readings,^z photosynthetic rate^y (Pn), and stem water potential^x (Ψ_{stem}) of ‘Super Elfin XP White’ impatiens after four weekly foliar applications of Regalia® at four rates (1.0× = 10 mL·L^{–1})^w to plants grown in containers maintained at three target substrate volumetric water contents (TVWC) (Expt. 1; n = 72).

	SPAD		Pn (μmol·m ⁻² ·s ⁻¹)		Ψ _{stem} (–MPa)	
	Weeks after initial application of Regalia® ^{®w}					
	2	4	2	4	2	4
Rate						
0.0×	48.0 b ^v	48.0 ab	7.4	8.4	–0.079	–0.201
0.5×	52.2 a	50.7 a	11.9	9.0	–0.117	–0.052
1.0×	52.0 ab	47.3 ab	8.1	8.4	–0.093	–0.092
1.5×	48.5 ab	46.3 b	4.9	7.0	–0.070	–0.185
TVWC						
85%	54.5 a	55.2 a	12.0 a	11.7 a	–0.073 b	–0.061 b
55%	51.0 b	51.4 b	7.3 b	9.7 b	–0.120 ab	–0.078 b
25%	45.0 c	38.9 c	4.9 b	3.2 c	–0.081 a	–0.259 a
Effects						
Rate	0.0069 ^u	0.0495	0.4386	0.7771	0.8189	0.1771
TVWC	<0.0001	<0.0001	<0.0001	<0.0001	0.0410	0.0272
Rate × TVWC	0.3390	0.2693	0.9620	0.3245	0.4960	0.2104

^zSPAD = leaf greenness was determined using a handheld 502 SPAD meter (Konica Minolta Optics Inc., Minolta, Japan).

^yLeaf photosynthetic rate measured using a CIRAS-2 (PP Systems, Amesbury, MD).

^xStem water potential was measured by first wrapping the leaf in plastic film, then covering with aluminum foil, and held for at least 1 h before taking measurements.

^wInitial application of Regalia® was made on 27 July 2010 (Run 1) and 7 Sept. 2010 (Run 2).

^vMeans (within a column) pooled across experimental runs with the same letters within TVWC or rate are not statistically different according to the Holm-Simulation method ($\alpha = 0.05$).

^uP value.

Absorbance Microplate Reader with an ultra-violet filter (BioTek Instruments, Inc., Winooski, VT) at 340 nm for 10 min. Each well contained 20 μL of plant sample and 230 μL reaction buffer [0.1 M potassium phosphate buffer [pH 6.5], 3.6 mM reduced glutathione molecular weight (MW) 307.3, 100 mM 1-chloro-2,4-dinitrobenzene (CDNB; MW 202.6)]. Activity was determined by following the formation of the conjugate of 1 μmol of CDNB with reduced glutathione per minute at pH 6.5 at 25 °C (extinction coefficient of 9.6 $\text{mm}^{-1}\cdot\text{cm}^{-1}$). Specific activity of GST was expressed as $\mu\text{units}/\text{mg}$. Specific activity was achieved by reference to a standard curve developed from pure GST run on each plate.

Statistical analysis. Data were analyzed using linear mixed models with the GLIMMIX procedure of SAS (Version 9.2; SAS Institute Inc., Cary, NC) and with rate, TVWC, and DBW as fixed factors and block as a random factor. Data from each experimental run were pooled within an experiment when the run term was not significant at $\alpha = 0.05$. Pairwise treatment differences were obtained using the LSMEANS statement for main effects with mean separation according to the Holm-Simulation method ($\alpha = 0.05$). When there was a significant interaction (rate × TVWC or rate × DBW), the SLICEDIFF option was used to examine the pairwise comparisons and P values adjusted using the Holm-Simulation method.

Results

Growth index, biomass, and water use efficiency (Expt. 1). Final growth index and SDW did not differ significantly among the

Table 3. Glutathione-S-transferase (GST) activity and soluble protein (SP) content in leaves of ‘Super Elfin XP White’ impatiens after four weekly foliar applications of Regalia® at four rates (1.0× = 10 mL·L^{–1})^z to plants grown in containers maintained at three target substrate volumetric water contents (TVWCs) (Expt. 1; n = 36).

	GST ($\mu\text{units}/\text{mg}$)	SP ($\mu\text{g}\cdot\text{mL}^{-1}$)
Rate		
0.0×	92.4	1.2 b ^y
0.5×	65.5	2.2 a
1.0×	33.2	2.6 a
1.5×	80.2	2.5 a
TVWC		
85%	36.5 b	2.7 a
55%	50.5 b	2.7 a
25%	116.6 a	1.0 b
Effects		
Rate	0.0787 ^x	0.0044
TVWC	0.0007	<0.0001
Rate × TVWC	0.1606	0.0144

^zInitial application of Regalia® was made on 27 July 2010 (Run 2) and 7 Sept. 2010 (Run 2).

^yMeans (within a column) pooled across experimental runs with the same letters within rate or TVWC are not statistically different according to the Holm-Simulation method ($\alpha = 0.05$).

^xP value.

differing rates of Regalia® (Table 1). RDW was 36% greater after application of Regalia® at the 0.5× rate compared with the 0.0× rate; however, the 1.0× and 1.5× rates did not increase RDW. Additionally, differing levels of TVWC resulted in a significant effect on all parameters measured with decreased growth with decreasing TVWC. There were no significant interaction effects on growth

Fig. 2. Actual substrate volumetric water content (AVWC) after four weekly foliar applications of Regalia® to ‘Super Elfin XP White’ impatiens with 1 (daily), 3, or 6 d between waterings (DBW). On each day of watering, containers were watered to 85% target substrate volumetric water content. Data points represent daily averages pooled across all experimental runs [27 July 2010 (Run 1) and 7 Sept. 2010 (Run 2)] and four rates [0.0×, 0.5×, 1.0× (1.0× = 10 mL·L⁻¹), and 1.5×] of Regalia® (Expt. 2; n = 72).

Table 4. Growth and water use efficiency of ‘Super Elfin XP White’ impatiens after four weekly foliar applications of Regalia® at four rates (1.0× = 10 mL·L⁻¹)^a to plants grown in containers with 1 (daily), 3, or 6 d between waterings (DBW) (Expt. 2; n = 72).

	FGI (cm) ^y	SDW (g) ^x	RDW (g) ^w	WUE (g·L ⁻¹) ^v	TWA (L) ^u
Rate					
0.0×	20.9	5.0	0.36	2.8	2.13
0.5×	21.2	5.7	0.38	3.2	2.01
1.0×	22.3	5.9	0.40	3.5	1.96
1.5×	21.1	4.4	0.34	2.6	2.01
DBW					
1	23.4 a ^t	6.6 a	0.46 a	3.4 a	2.09 a
3	21.5 b	5.2 b	0.34 b	2.8 b	2.22 a
6	19.3 c	3.9 c	0.30 b	3.0 ab	1.77 b
Effects					
Rate	0.6735 ^s	0.918	0.7920	0.5427	0.6445
DBW	<0.0001	<0.0001	<0.0001	0.0044	0.0007
Rate × DBW	0.9610	0.8231	0.7685	0.7743	0.9690

^aInitial rate of Regalia® was applied on 27 July 2010 (Run 1) and 7 Sept. 2010 (Run 2).

^yFGI = final growth indices [(height + width + perpendicular width) ÷ 3].

^xSDW = shoot dry weight, oven-dried for 72 h at 65 °C.

^wRDW = root dry weight, oven-dried for 72 h at 65 °C.

^vWUE = [(SDW + RDW) ÷ total water applied].

^uTWA = total mean water applied.

^tMeans (within a column) pooled across experimental runs with the same letters within rate or TVWC are not statistically different according to the Holm-Simulation method ($\alpha = 0.05$).

^sP value.

index, biomass, WUE, or TWA. WUE was not significantly affected by rates of Regalia®; however, WUE decreased with increasing TVWC. TWA was similar among rates of Regalia® but increased with increasing TVWC.

Leaf greenness, photosynthesis, and midday stem water potential (Expt. 1). SPAD readings were greatest using the 0.5× rate at 2 WAIR compared with the 0.0× rate (Table 2). However, after 4 weeks, impatiens treated with the 0.5×, 1.0×, or 1.5× rates had similar

SPAD readings compared with the 0.0× rate. At 2 and 4 WAIR, SPAD readings increased with increasing TVWC. Pn and Ψ_{stem} were similar for impatiens treated with Regalia® at the 0.5×, 1.0×, and 1.5× rates compared with the 0.0× rate at 2 and 4 WAIR. Additionally, Pn at 2 and 4 WAIR was greater with increasing TVWC. Similarly, Ψ_{stem} at 4 WAIR was lower (more negative) in plants watered to 25% TVWC compared with plants watered to 85% or 55% TVWC.

Antioxidant enzyme extractions and assays (Expt. 1). GST activity in leaves of impatiens was similar after the application of Regalia® among all rates; however, GST did increase with decreasing TVWC (Table 3). There was no significant rate × TVWC effect on GST activity. Conversely, there was a significant rate × TVWC effect on SP content in impatiens leaves. SP content was greater in impatiens treated with the 1.5× rate compared with the 0.0× and 0.5× rates of Regalia® at 85% TVWC (Fig. 3). Additionally, impatiens treated with Regalia® at the 0.5×, 1.0×, or 1.5× rates and watered to 55% TVWC had greater SP content compared with plants treated with the 0.0× rate and watered to 55% TVWC.

Growth index, biomass, and water use efficiency (Expt. 2). Rate of Regalia® did not have a significant effect on FGI, SDW, or RDW (Table 4). However, FGI, SDW and RDW were less for plants watered with 3 and 6 DBW treatments compared with plants watered daily (1 DBW). There was no significant rate × DBW effect on FGI, SDW, or RDW. WUE was similar among all rates of Regalia®. However, impatiens watered with 1 DBW had greater WUE compared with plants watered with 3 DBW, yet TWA was similar between these two watering frequencies. WUE was similar between plants watered with 1 and 6 DBW, yet 18% more water was applied to plants watered with 1 DBW than plants watered with 6 DBW.

Leaf greenness, photosynthesis, and midday stem water potential (Expt. 2). There was no significant effect of Regalia® rate on SPAD readings at 2 WAIR; however, plants receiving the 0.5× rate of Regalia® had greater SPAD readings compared with the 0.0× and 1.5× rates at 4 WAIR (Table 5). Additionally, at 2 and 4 WAIR, plants watered with 1 and 3 DBW had greater SPAD readings compared with plants watered at 6 DBW. Pn was unaffected by rate of Regalia® at 2 WAIR; however, at 4 WAIR, impatiens treated with the 1.0× rate of Regalia® exhibited greater Pn compared with plants treated with the 0.0× rate. At 4 WAIR, plants watered with 1 and 3 DBW exhibited greater Pn compared with plants watered with 6 DBW. There was no significant rate × DBW effect on Pn. Impatiens treated with Regalia® at the 1.5× rate had 48% and 46% lower (more negative) Ψ_{stem} compared with the 0.0× and 0.5× rates at 2 WAIR, respectively. However, at 4 WAIR, Ψ_{stem} was similar among all rates.

Antioxidant enzyme extractions and assays (Expt. 2). GST activity was less in leaves of plants treated with Regalia® at the 0.5×, 1.0×, and 1.5× rates compared with the 0.0× rate (Table 6). Leaves of impatiens watered with 3 or 6 DBW had similar GST activity compared with plants watered with 1 DBW. There was no significant rate × DBW effect on GST. However, there was a significant rate × DBW effect on SP content. SP content was 86%, 65%, and 84% less in leaves of impatiens treated with

Table 5. SPAD readings^z (LCC), photosynthetic rate^y (Pn), and stem water potential^x (Ψ_{stem}) of 'Super Elfin XP White' impatiens after four weekly foliar applications of Regalia[®] at four rates (1.0 $\times$ = 10 mL·L⁻¹)^w to plants grown in containers watered with 1 (daily), 3, or 6 d between waterings (DBW) (Expt. 2; n = 72).

	SPAD		Pn ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)		Ψ_{stem} (-MPa)	
	Weeks after initial application of Regalia ^{®w}					
	2	4	2	4	2	4
Rate						
0.0 $\times$	53.5	54.2 b ^y	11.0	6.5 b	-0.065 b	-0.053
0.5 $\times$	53.5	57.5 a	13.6	9.1 ab	-0.068 b	-0.074
1.0 $\times$	54.5	55.5 ab	9.8	9.5 a	-0.084 ab	-0.095
1.5 $\times$	53.8	53.1 b	11.1	9.1 ab	-0.126 a	-0.130
DBW						
1	55.3 a	56.6 a	13.0 a	10.6 a	-0.069	-0.077
3	54.9 a	57.0 a	10.7 a	10.1 a	-0.090	-0.061
6	51.3 b	52.0 b	10.4 a	4.9 b	-0.097	-0.128
Effects						
Rate	0.7950 ^u	0.0045	0.9408	0.0365	0.0132	0.2716
DBW	<0.0001	<0.0001	0.0442	<0.0001	0.2788	0.0526
Rate $\times$ DBW	0.6583	0.2073	0.9475	0.3078	0.3496	0.8283

^zSPAD: Leaf greenness was determined using a handheld 502 SPAD meter (Konica Minolta Optics Inc., Minolta, Japan).

^yLeaf photosynthetic rate measured using a CIRAS-2 (PP Systems, Amesbury, MD).

^xStem water potential was measured by first wrapping the leaf in plastic film, then covering with aluminum foil, and held for at least 1 h before taking measurements.

^wInitial application of Regalia[®] was made on 27 July 2010 (Run 1) and Sept. 2010 (Run 2).

^uMeans (within a column) pooled across experimental runs with the same letters within TVWC or rate are not statistically different according to the Holm-Simulation method ($\alpha = 0.05$).

^vP value.

Table 6. Glutathione-S-transferase (GST) activity and soluble protein (SP) content in leaves of 'Super Elfin XP White' impatiens after four weekly foliar applications of Regalia[®] at four rates (1.0 $\times$ = 10 mL·L⁻¹)^z to plants grown in containers with 1 (daily), 3 or 6 d between watering (DBW) (Expt. 2; n = 36).

	GST ($\mu\text{units}\cdot\text{mg}^{-1}$)	SP ($\mu\text{g}\cdot\text{mL}^{-1}$)
Rate		
0.0 $\times$	62.4 a ^y	1.7 b
0.5 $\times$	18.9 b	4.1 a
1.0 $\times$	24.5 b	2.3 b
1.5 $\times$	23.6 b	4.5 a
DBW		
1	30.9	3.2
3	31.0	3.6
6	35.2	2.7
Effects		
Rate	<0.0001 ^x	0.0013
DBW	0.8234	0.3434
Rate $\times$ DBW	0.0717	0.0002

^zInitial application of Regalia[®] was made on 27 July 2010 (Run 1) and 7 Sept. 2010 (Run 2).

^yMeans (within a column) pooled across experimental runs with the same letters within rate or TVWC are not statistically different according to the Holm-Simulation method ($\alpha = 0.05$).

^xP value.

the 0.0 $\times$, 0.5 $\times$, and 1.0 $\times$ rates compared with the 1.5 $\times$ rate at 1 DBW, respectively (Fig. 4). Additionally, Regalia[®] applied at the 0.5 $\times$ rate increased SP content by 77% compared with the 0.0 $\times$ and 1.0 $\times$ rates in plants watered with 3 DBW; however, there were no significant differences in SP content among the 0.0 $\times$, 1.0 $\times$, and 1.5 $\times$ rates.

Discussion

Growth of impatiens was not enhanced by Regalia[®] application; however, increasing TVWC did increase growth. RDW increased after the application of Regalia[®] at the 0.5 $\times$ rate, which is consistent with a report from Marrone Bio Innovations (2011) that strawberry and tomato seedlings exhibited increased root growth after application of Regalia[®]. However, this increase in RDW does not suggest an increase in drought tolerance because overall growth of impatiens was not enhanced by Regalia[®] application. Results from these experiments were dependent on TVWC with growth increasing as TVWC increased. For plants maintained at 25% TVWC, there was substantially less TWA over the duration of the experiment, resulting in lower WUE ultimately affecting biomass production. WUE is greater with decreasing moisture content, resulting in less growth (Burnett and van Iersel, 2008); however, plants watered with 1 DBW had greater FGI and greater WUE compared with plants watered with 3 DBW with similar TWA between the two watering frequencies. Moreover, WUE was similar between plants watered with 1 and 6 DBW, yet TWA was 15% less for plants watered with 6 DBW compared with plants watered with 1 DBW. These results are likely the result of the AVWC remaining within a 20% range throughout the experiment, which could also explain the similarity in Ψ_{stem} among all TVWCs at 4 WAIR. Additionally, in Expt. 1, plants were watered daily based on TVWC and in Expt. 2, plants were watered based on DBW, which coincided with either the day of watering or the day after watering; therefore,

Ψ_{stem} recorded may not accurately reflect the degree of water stress. However, plants did show signs of wilting and reduced photosynthesis with decreasing TVWC. At the close of Expt. 1, impatiens maintained at 25% TVWC were showing signs of water stress injury and, as a result, were producing more GST than impatiens maintained at 85% or 55% TVWC (Gill and Tuteja, 2010). Unlike Expt. 1, the antioxidant enzyme GST in Expt. 2 was less in leaves treated with Regalia[®] at the 0.5 $\times$, 1.0 $\times$, and 1.5 $\times$ rates compared with plants treated at the 0.0 $\times$ rate. ROS are always present in the plant; however, only under elevated levels do they stimulate the production of antioxidant enzymes. Furthermore, several fungicides (carbendazim, tebuconazole, azoxystrobin, and JS399-19) have produced a decrease in O₂⁻ levels in flag leaves of winter wheat (Zhang et al., 2010). Therefore, Regalia[®] may have protected against the formation of ROS, reducing the need for antioxidant enzymes. Regalia[®] applied at the 1.5 $\times$ rate to plants maintained at 85% TVWC enhanced SP content compared with the 0.0 $\times$ rate at 85% TVWC and the 0.5 $\times$, 1.0 $\times$, or 1.5 $\times$ rates enhanced SP content compared with the 0.0 $\times$ rate at 55% TVWC, which is a typical response to application of Regalia[®] (Marrone Bio Innovations, 2012). Examination of main effects indicated that plants treated with Regalia[®] at the 1.0 $\times$ rate had a lower SP content than plants treated with the 0.5 $\times$ and 1.5 $\times$ rates, likely explained by the significant interaction between rate and TVWC. Plants treated with Regalia[®] at the 1.0 $\times$ rate and maintained at 55% TVWC had similar SP content to plants treated with the 0.5 $\times$ and 1.5 $\times$ rates and maintained at 55% TVWC with all three of the aforementioned Regalia[®] rates resulting in a higher SP content than using the 0.0 $\times$ rate. At 85% TVWC, plants treated with the 1.0 $\times$ rate of Regalia[®] had similar SP content to plants treated with the 0.0 $\times$ rate and the 1.5 $\times$ rate, suggesting decreased TVWC was the contributing factor (Rahman et al., 2004). Conversely, SP content was greater in leaves of plants treated with the 1.5 $\times$ rate in plants watered with 1 DBW compared with plants treated with the 0.0 $\times$, 0.5 $\times$, and 1.0 $\times$ rates and watered with 1 DBW. Additionally, Regalia[®] applied at the 0.5 $\times$ rate to plants watered with 3 DBW resulted in greater SP content compared with plants treated with the 0.0 $\times$ and 1.0 $\times$ rates and watered with 3 DBW.

In conclusion, the objective of these experiments was to evaluate the effects of Regalia[®] on drought tolerance of impatiens and, based on the data presented here, there was no indication that Regalia[®] increased drought tolerance. Generally, moderately stressed (55% TVWC) impatiens treated with Regalia[®] did have greener leaves (an indirect measurement of chlorophyll), a higher Pn, and greater soluble protein content, but this was not seen with plants maintained at 25% TVWC. Moreover, the contributing factor appeared to be VWC and not use of Regalia[®]. However, these results may not be seen with

Fig. 3. Soluble protein (SP) content in leaves of 'Super Elfin XP White' impatiens after four weekly foliar applications of Regalia® [0.0x, 0.5x, 1.0x (1.0x = 10 mL·L⁻¹), and 1.5x] to plants grown at three target substrate volumetric water contents (TVWCs). Means pooled across experimental runs [27 July 2010 (Run 1) and 7 Sept. 2010 (Run 2)] with the same letter are not significantly different according to the Holm-Simulation method ($\alpha = 0.05$) (Expt. 1; n = 36).

Fig. 4. Soluble protein (SP) content in leaves of 'Super Elfin XP White' impatiens after four weekly foliar applications of Regalia® [0.0x, 0.5x, 1.0x (1.0x = 10 mL·L⁻¹), and 1.5x] to plants grown in containers with 1 (daily), 3, or 6 d between waterings (DBW). Means pooled across experimental runs [27 July 2010 (Run 1) and 7 Sept. 2010 (Run 2)] with the same letter are not significantly different according to the Holm-Simulation method, $\alpha = 0.05$ (Expt. 2; n = 36).

every species or under every stress condition; therefore, individual assessment on plant stress tolerance or enhancements under stress should be determined before trying to use this product as a plant health protectant.

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