

EFFECTS OF A NONIONIC SURFACTANT ON  
PLANT GROWTH AND PHYSIOLOGY

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EFFECTS OF A NONIONIC SURFACTANT ON  
PLANT GROWTH AND PHYSIOLOGY

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EFFECTS OF A NONIONIC SURFACTANT ON  
PLANT GROWTH AND PHYSIOLOGY

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## VITA

Xiaomei Yang, daughter of Chun Yang and Xiaoyun Wu, was born January 12, 1972, in Xuzhou, China. Being influenced by father, she dreamed to be a teacher and graduated from Xuzhou Normal University with a Bachelor of Science degree in Biology Education in July, 1994. She entered Graduate School of Nanjing University and graduated with a Master's degree in Botany in July, 1997. After working as a pharmaceutical engineer for several years in Nanjing Jinling Pharmaceutical Company, she entered the Graduate School of Auburn University as a Graduate Research Assistant on a Presidential fellowship in 2004 to pursue a Ph. D. in horticulture. During the Ph. D. study, she also got a Master of Probability and Statistics from the Department of Mathematics and Statistics in 2006 to help her research. In August 2008, she completed the Ph. D. in horticulture. Xiaomei is married to Wenliang Lu with son James.

DISSERTATION ABSTRACT  
EFFECTS OF A NONIONIC SURFACTANT ON  
PLANT GROWTH AND PHYSIOLOGY

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With a continuous declining trend in the availability of groundwater or public surface water to greenhouse and container nurseries, nursery growers and researchers are searching for effective ways to reduce water use and maintain optimal plant growth at the same time. In this project, we studied the effect of a low concentration of surfactant Tween 20 (polysorbate 20) on plant growth and physiology of two nursery crops.

In laboratory soil columns, the initial wetting of a commercial substrate Fafard 3B was accelerated and the moisture retention of the substrate treated with  $100 \text{ mg}\cdot\text{L}^{-1}$  Tween 20 solution increased 40.5% compared to that of water treatment at initial wetting.

In an outdoor container study, growth of New Guinea impatiens (*Impatiens hawkerii* 'Celebrate Salmon') in the treatment of  $100 \text{ mg}\cdot\text{L}^{-1}$  Tween 20 at the 60% of full

crop evapotranspiration (ET) irrigation level was increased 11% compared to the control (0 mg·L<sup>-1</sup> Tween 20 at 100% of ET) after three months. Growth of impatiens in treatment combinations of 50, 75, 100, or 125 mg·L<sup>-1</sup> Tween 20 at the 20% or 40% irrigation level was similar to that in the control throughout the experiment.

Under a 3 × 6 complete factorial design plus a control, irrigation levels of 20%, 40%, 60% of the full crop ET requirements were used in combination with Tween 20 concentrations of 0, 25, 50, 75, 100, and 125 mg·L<sup>-1</sup>. The control was tap watered to container capacity with 30% leachate. Plants mostly grew equally at the 40% or 60% irrigation levels when combined with 100 mg·L<sup>-1</sup> or higher concentrations of Tween 20. When Tween 20 concentration increased from 0 to 100 mg·L<sup>-1</sup> at the 60% of ET irrigation level, the transpiration rate and stomatal conductance decreased 43% and 47%, respectively, and water use efficiency was increased 47%.

In hydroponic studies containing 0 (control) to 125 mg·L<sup>-1</sup> Tween 20 modified Hoagland solutions, fresh and dry weight of peace lily (*Spathiphyllum floribundum* ‘Viscount’) and impatiens increased 17% to 33%, and 7% to 18% respectively, in the solutions with 50 to 125 mg·L<sup>-1</sup> Tween 20 compared with the control. Water use efficiency of peace lily and impatiens in 100 ppm Tween 20 solution was increased 166% and 221% compared to control, respectively.

In a greenhouse study, peace lily grew equally with at 60% of ET compared to 100% irrigation when the reduced water regime included Tween 20 at 75 mg·L<sup>-1</sup> or higher concentrations. Cost estimation indicated the minimal increase in cost from Tween 20 was offset by decreased water costs based on current municipal water costs.

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## **I. INTRODUCTION AND LITERATURE REVIEW**

Water resources in North America have generally been regarded as plentiful or taken for granted. However, in drought seasons, abundant water may not be available, or only available at a higher price. In the future, water withdrawal from underground or rivers is likely to be restrained or highly regulated. Insufficient water supply for irrigation will be normal in the future. To cope with scarce supplies, deficit irrigation, defined as the application of water below full crop-water requirements, is an important tool to achieve the goal of reducing irrigation water use (Feres and Soriano, 2007). Deficit irrigation is carried out aiming at maximizing crop productivity, but in ornamental nurseries, it will severely decrease crop size, beauty, and value. It is important to develop strategies now to address emerging concerns regarding water supplies.

Efficient management of substrate moisture and reducing plant production periods are important for nursery production in the light of scarce water resources. Adjuvants, wetting agents, or soil conditioners, have been commonly added to the commercial substrates to increase water penetration and moisture retention in order to reduce the risk of plant failure during establishment and reduce temporary drought stress. Most surfactants are generally considered to be inert, non-phytotoxic, or phytotoxic with no effect on overall growth, or, as concentrations increase, growth is inhibited. Tween 20 is one of a few adjuvants that are recognized as promoting plant growth per se, but is seldom used in potting substrates.

The objective of the investigations presented in this dissertation was to evaluate the possibility of Tween 20 as an irrigation additive to decrease plant water use while maintaining or promoting plant growth in the nursery. The hypothesis was that if a surfactant molecule is small enough to go into the xylem with the sap, then surfactant could be added to irrigation water to decrease surface tension of leaf menisci, and further to decrease transpiration rate. Plants watered with the surfactant solution will have less water stress than those watered without surfactants at decreased irrigation levels. Or, plants watered with surfactant solutions at decreased irrigation levels would maintain similar growth as well-watered plants.

### **Water resource and horticulture**

*Fresh water resources: precise and limited.* Fresh water is water with less than 0.5 parts per thousand dissolved salts (The Groundwater Foundation, 2005) and may be found in lakes, rivers, and bodies of groundwater. Seventy percent of the surface area of the Earth is covered by water, but only 3% is fresh water, of which a large amount is frozen in glaciers and polar ice caps, or deep in the ground, with only 0.3% accessible for use. The primary source of fresh water is rain from the hydrologic cycle. Although the hydrologic cycle does not lose water, fresh water supply is continuously decreasing by increasing population, massive damming, and depleted aquifers (Prathapar, 2000).

Over the last 70 years, the Earth's population has tripled, along with increased demand for food in conjunction with a fixed water supply, while water demand has increased six-fold. Some of the world's largest rivers no longer reach the sea for part of the year; more than 1.2 billion people lack access to fresh water, and more than 5 million

people die every year from contaminated water or water-related diseases (Cain and Gleick, 2005).

The global water crisis is a serious threat, not only to those who suffer, get sick, and die, but decreased water supplies also lead to ecological damage, reduces food production, undercuts economic growth, worsens conflicts over resources, and even affects global security (Coles, 2005). The UN-sponsored “Human and Environment” conference warned that water shortage would become a social crisis similar to the oil crisis in 1972. The UN’s Comprehensive Assessment of the Freshwater Resources of the World (W.M.O., 1997) estimated that about one-third of the world’s population lives in countries that were experiencing moderate-to-high water stress. Based on current practices, by the year 2025, about 18% of the world’s population will not meet daily water needs (Wallace, 2000), and as much as two-thirds of the world’s population may experience some water stress (W.M.O., 1997). Unless radical steps are taken to alter the way water is used and managed, the world could face a severe water shortage (Clarke and King, 2004).

*Water supply in the United States.* The United States is relatively well endowed with water. Annual precipitation averages nearly 30 inches or 4,200 billion gallons per day throughout the contiguous forty-eight states (Frederick, 1995). Much larger quantities of freshwater are stored in the nation's surface and groundwater reservoirs. Despite the apparent national abundance and the renewability of the resource, water availability has emerged as one of the nation's primary resource issues. Erratic temperatures, pollution, high costs of water supply equipment, and vicissitudes of the hydrologic cycle influence

the availability of freshwater to meet the local or regional demands in time. Abundant supplies in one area are of no help to water-deficits in other areas unless there are facilities to transport supplies among regions. Water flows naturally within hydrologic basins and can be moved between basins where transfer facilities have been constructed. However, water transport over long distances out of these existing channels in response to climate-induced changes in supply or demand is generally cost prohibitive. Thus, large seasonal, annual, and regional variations in precipitation and runoff pose major challenges in horticulture and down-to-earth risks for growers.

*Nursery irrigation in the United States.* Irrigation is the most significant use of water, accounting for over 90 percent of freshwater withdrawals consumed in the Western States (including Hawaii, Alaska and the seventeen contiguous western states) and roughly 65 percent nationwide. Improvement in water management has been one of the primary agricultural policy objectives since 1990 (U.S.D.A., 1994). Conservation and allocation of limited water supplies is central to irrigation management decisions.

Drought has resulted in extreme losses to agriculture in the United States recently. Drought has hampered Texas agricultural production since 1996 (Fannin, 2000). The 2000 and 2005 drought has led to over \$1 billion agricultural losses, while the 1996 and 1998 drought resulted in over \$2 billion in agricultural losses. Boyer (1982) surveyed the causes and costs of crop losses in USA from 1939 to 1978 (Table 1 and 2). Crop losses are generally due to drought rather than poor plant nutrition, plant health, or other causes (Passioura, 2002). Lack of irrigation water is a major limiting factor throughout agriculture and specifically horticulture.

More than 50% of production nurseries are located near cities in the U.S. (Beeson et al., 2004). Expansion of urban areas increases competition of water. Without protective legislation, container nurseries are among the first targets as a means to decrease non-human water use. Even with such legislation, amounts permitted are declining as has already occurred in Florida.

For some areas, groundwater is not available or has poor quality. Production costs for nursery crops are directly related to the costs of water, whether from wells, municipal, reclaimed or other sources. Recent droughts have led to local governments limiting water consumption of nursery growers in some states, such as California, Florida, North Carolina, Texas and Oregon (Beeson et al., 2004). Nutrient management laws such as in Maryland, Delaware and California also impact water use by restricting nutrient applications and nutrient release in nurseries. Such laws and regulations are expected to become more wide spread and stricter.

Overhead irrigation is the primary irrigation system for container production for the past 50 years (Lu and Sibley, 2006). Low irrigation efficiency and low water productivity is often a big problem. More and more nurseries are using or considering using micro-irrigation and cyclic irrigation for larger containers or increasing use of retention ponds to improve water availability. Growers are continuously improving irrigation efficiency of overhead irrigation for small containers, such as improving system uniformity, minimizing distance between containers, grouping plants of same water need, considering water time, cyclic irrigation and building windbreaks. More approaches are being investigated, such as soil wetting agents, container substrate moisture sensors,

irrigation models, container color and plant demand management. Although those methods are efficient, improving crop water use efficiency and promoting plant growth by adding irrigation additive has not been carefully investigated.

### **The use of surfactants in horticulture**

*Property of surfactants.* Surfactants are the most widely used adjuvant in agriculture (Miller and Westra, 1998). Surfactants are formulated to facilitate or improve the emulsifying, dispersing, spreading, sticking or penetrating of liquids. Surfactants were initially used to enhance the penetration and effectiveness of foliar-applied herbicides, defoliant, and insecticides by decreasing surface tension of aqueous systems. Now, surfactants have broader and more intensive use in immunoassays, biosynthesis of nucleic acids, floral induction, soil wetting, fruit thinning, hormone interactions, and photoperiodicity (Myers, 2006).

Surfactants work by altering the energy relationships at interfaces and lower surface tension and interfacial tension (Miller and Westra, 1998). Surfactant molecules are amphiphilic molecules, which contain both a hydrophobic and hydrophilic group. Amphiphilic molecules form micelles (globular aggregates whose hydrocarbon groups are out of contact with water) in aqueous solutions. Dilute aqueous solution of amphiphiles do not form micelles until their concentration surpasses a certain critical micelles concentration (CMC) (Colwell and Rixon, 1961). Above the CMC, almost all the added amphiphile aggregates forms micelles. CMC value depends on the identity of the amphiphile and the solution conditions. Many properties of surfactants undergo abrupt change as the CMC is reached. Surface and interfacial tension reach the lowest

levels while detergency and osmotic pressure of a surfactant reach the maximum values in the CMC range (Fig. 1).

*Surfactant application to increase irrigation efficiency in horticulture.* Applying surfactants in water-repellent soil (DeBano, 2000; Letey et al., 1961, 1962ab, 1963; Watson et al., 1969) and highly managed golf courses (Cisar et al., 2000) are common practices to increase irrigation efficiency. Penetration in water-repellent soils has been well explored on measurement of soil water-repellency (Letey et al., 1962b; Letey, 1969), techniques for using surfactants (Letey et al., 1963), including factors for increasing the effectiveness (Mustafa and Letey, 1971), longevity (Osborn, et al., 1969), movement and leaching (Miller, et al., 1975), and effect on soil aggregation (Mustafa and Letey, 1969), soil erosion (Osborn, et al., 1964) and pesticide (Huggenberger, et al., 1973).

Compared to field-grown plants, container plants are grown in substrates with a limited amount of water, nutrients, and root space. Desirable physical characteristics of container substrates and irrigation preciseness are critical for growing a quality plant (Yeager et al., 2007). Available water is defined as the amount of water held between container capacity, i.e. the total amount of water present in the substrate after saturated and drained, and unavailable water, i.e. the amount of water of the substrate at plant permanent wilting point when plant cannot extract water. Available water or unavailable water retained in substrate is recommended in the range of 25% to 35%, with container capacity in 45% to 65% (by volume). Most of commercial potting substrates are peat or bark-based. Iron humates and strongly adsorbed air film on the surface of peat, waxes and resins on the bark, and hot-air drying of peat and bark during processing for horticultural

use causes substrates to be difficult to be wet initially and rewet after they have dried-out in the container (Airhart, et al., 1978a; Bunt, 1988). Moisture content of peat in bales is usually between 45% and 65% (by weight; Bunt, 1988). When moisture content of peat is around 70% to 75%, the water is easily absorbed. Container capacity (often referred to as water holding capacity) of aged pine bark is approximately 61–70% (by volume), but only approximately 26–37% of the container capacity will be available for plant use (Bilderback, et al., 2005). For fresh pine bark, container capacity and the available water is much lower, 49% and 9.8% (by volume), respectively. If the moisture content of bark is less than 35% (by weight), very little water applied is retained by the bark (Airhart, et al., 1978a). As the moisture content increases from 35% to 50%, bark is easier to wet. It took 10 days to water air dry milled pine bark to achieve 20% saturation and 48 days to achieve 58–70% saturation (Airhart, et al., 1978b).

The convenient practice to deal with dry potting substrates is watering substrates during the mixing, or before potting. However, if powdered fertilizers are added to wet substrates, their uneven distribution will result in large variations in plant growth.

Comparing the wealth of data regarding surfactants on water-repellent soil, there is much less information focused on effects of surfactants on physiological characteristics of potting substrates and plant growth, although pine bark, sphagnum peat moss and commercial peat or bark-based mixes commonly contain surfactants to improve wetting and uniformity (Bilderback and Lorscheider, 1997).

Burge et al. (1998) reported that 24-h plus treatment with Agral LN or Tween 20 at 0.1 or 1 ml·L<sup>-1</sup> reduced water uptake of harvested inflorescences of *Limonium* ‘Chorus



Magenta' placed in a water or biocide solution and extended flowering time.

Nonylphenol polyethyleneglycol increased drainage, reduced water uptake, and did not affect EC and pH of leachates of coco fiber in drip irrigation (Guillen et al., 2005).

SaturAid granular soil wetter had little effect on moisture and air characteristics of pine bark, no effect on leachates and foliar nutrients, and increased plant growth when irrigation volume was decreased 15 or 30% (Bilderback and Lorscheider, 1997).

A few studies report the effects of alkylphenol ethoxylate (APE)-based Aqua-Gro products, the most common surfactants in the market, added in the substrates to increase water holding capacity and penetration (Williams and Nelson, 1992; Wang, 1994; Conrad Fafard, Inc., 2008). Blodgett et al. (1993) examined the effects of AquaGro-G on water absorption, retention, and evaporation of two potting mixes consisted of 2 peat : 1 perlite : 1 vermiculite and 1 bark : 1 peat : 1 perlite (by volume) through the subirrigation system. AquaGro-G was incorporated into potting mixes at  $830 \text{ g}\cdot\text{m}^{-3}$ . AquaGro-G increased water absorption of both mixes and did not affect mix evaporation. Elliott (1992) reported that AquaGro-G increased available water but had no effect on container capacity of peat-rockwool mix.

AquaGro-G did not promote plant growth and severely hampered shoot and root growth of New Guinea impatiens and poinsettia at higher concentration ( $\geq 0.94 \text{ kg}\cdot\text{m}^{-3}$ ) (Bhat et al., 1992). Chrysanthemum and geranium did not exhibit sensitivity to AquaGro-G. APE-based AquaGro-G has long-term accumulation effect in waterways and APE breakdown products will cause endocrine disorder (Moore and Moore, 2005).

## Effects of Tween 20 on plants

*Molecular formula and property of Tween 20.* The Tween series is comprised of Tween 20, 40, 60 and 80, with the difference in the length of the fatty acid chains. Tween 20 (polyoxyethylene sorbitan monolaurate,  $C_{58}H_{114}O_{26}$ , Fig. 2) with estimated low molecular weight of 1227.54 (Sigma-Aldrich, Inc., 2006), is one of the most frequently used, safe, nonionic surfactants in a variety of industries (Sigma-Aldrich, Inc., 2008), 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 (Sigma-Aldrich, Inc., 2006), which indicates that it is a water soluble and easily diluted in organic solvents. As previously studies reported Tween 20 approached the critical micelle concentration (CMC) of  $3.5 \times 10^{-5}$  M ( $43 \text{ mg} \cdot \text{L}^{-1}$ ),  $60 \text{ mg} \cdot \text{L}^{-1}$ ,  $0.059 \text{ mmol} \cdot \text{L}^{-1}$  ( $75 \text{ mg} \cdot \text{L}^{-1}$ ), or  $0.08 \text{ mmol} \cdot \text{L}^{-1}$  ( $98 \text{ mg} \cdot \text{L}^{-1}$ ) (Sigma-Aldrich, Inc., 2006; Tran and Yu, 2005) in the water (range attributed to different manufacturer), and above  $100 \text{ mg} \cdot \text{L}^{-1}$  in the plant (Bernath and Vieth, 1972). Tween 20 has a boiling point higher than  $100^\circ\text{C}$  and the pH of a 1% aqueous solution of is 5–7. It is very inexpensive ( $\$53.74/4\text{L}$ ) and biodegradable. All these properties of Tween 20, inexpensive, nontoxic, chemically inert, pH neutral, water and organic soluble, environmental friendly, and highly efficient decrease surface tension at low concentrations, make it be a potential ideal irrigation adjuvant (Colwell and Rixon, 1961).

*Effects of Tween 20 on plant cell membranes.* One effect of surfactants on plant tissue is through a direct action on membranes (Norris, 1982). Surfactant at low concentration may modify properties of the cell membrane, stimulating herbicide or nutrient absorption and increasing secondary metabolite production. At high concentration, surfactants may affect native structure of membrane-bound proteins or stability of lipid bilayers and lead to loss of membrane integrity.

Root or leaf discs protoplasts (St. John, et al., 1974), isolated protoplasts (Watson, et al., 1980) and artificial lipid micelles (Miller and St. John, 1974) have been used to study the action of surfactants on protoplast membrane structure and permeability. Tween 20 increased efflux only at higher concentration. Tween 20 at 0.001% (v/v) had no effect on isolated protoplast of oat (*Avena sativa*) membranes (Watson, et al., 1980), but 0.01% (v/v) Tween 20 caused an abrupt leakage of <sup>14</sup>C-labeled protoplast of wild onion cells and decreased photosynthetic rate, but did not affect soybean cells. Similar results were reported with Tween 80 causing membranes of oat coleoptile protoplasts to roughen, and eventually the protoplasts lyse (Ruesink, 1971).

In spite of the large number of studies on surfactant-induced liposome permeability and content release, the mechanism of the process is still far from being understood. In all cases reported so far, lipid membrane permeability plays a key role in controlling the overall behavior of the membrane system. Permeability changes induced by surfactants are generally described by means of a three-step model (Paternostre, et al., 1988; Inoue, 1996; Annesini, et al., 2000). First, surfactant molecules are incorporated with the lipid membrane and form nonspecific ionophores (Ziegler, et. al., 1985), whose

action is independent of the lipid composition of the membrane and facilitates ion transport through membranes (Bohmova, et al., 1991). Then, when the surfactant concentration exceeds a critical concentration,  $C^{\text{sat}}$ , phospholipids are solubilized into mixed micelles that coexist with surfactant-saturated vesicles. Finally, when the surfactant concentration reaches a second critical concentration,  $C^{\text{sol}}$ , complete rupture of the vesicles occurs and only mixed micelles are present (Fig. 3). The higher the concentration of surfactant, the higher the permeability values, leading to higher rupture of contents (Table 3). Due to redistribution of the surfactant through the bilayer, some surfactants may reseal the “hole” with membrane permeability thereby reduced. The permeability curves display a complex trend, with initial high values followed by a time-decaying trend. From a mathematical point of view, the permeability curves can be represented as a delta function followed by an exponentially decaying profile. However, no clear trend has been identified for Tween 20 permeability curves (Annesini, et al., 2000).

At sublytic surfactant concentrations, surfactant concentration,  $C < C^{\text{sat}}$ , the vesicle structure is still preserved and phospholipids retain the architecture of a closed bilayer. As a result of surfactant insertion, however, the membrane becomes more permeable and more nutrient molecules can be absorbed by roots. Therefore, we can hypothesize that with application of Tween 20, nutrients leaving the soil profile will be entrapped in the aqueous solution. It is worth noting that permeabilization of the lipid bilayer is important for root and leaf systems to enhance the absorption of enzyme-related

nutrients when they are used as membrane micro-reactors, or make it possible to load nutrient molecules into cell from the outside (Annesini, et al., 2000).

Attention also should be focused on the influence of Tween 20 and/or different physical-chemical parameters (temperature, physical state of lipid membrane, pH, etc.) on membrane permeability (Disalvo and Simon, 1995) if further intensive membrane studies are carried out.

*Effect of Tween 20 on plant growth.* The first refereed papers of Tween 20 on plant growth appeared in the early 1950s. Beal, et al. (1954) carried out a study to determine the effect of three chemicals on the alkaloidal yield of *Datura tatula* under field conditions. Tween 20 was used as a spreading and penetrating agent to increase hormone activity when applied to soil or foliage. They discovered by chance that 1% Tween 20 applied to the soil increased dry weight of the upper-ground portion of plants by 40%.

Stowe (1958, 1959, 1960, 1961), Stowe and Obreiter (1962) and Stowe and Dotts (1971) investigated several fatty acid esters, including Tween 20 and Tween 80, on the growth of pea epicotyl elongation. The fatty acid esters evaluated enhanced the activity of indoleacetic acid and gibberellic acid in promoting the growth of pea-stem sections and  $30 \text{ mg}\cdot\text{L}^{-1}$  Tween 20 stimulated the hypocotyl growth by about 90% (Stowe, 1958). They hypothesized that these fatty acid esters are neutral nonpolar and are hydrocarbons with a chain of at least 12 carbon atoms, i.e. length of their molecules between 20 to 30 Å. It has been postulated that lipids of 20 to 30 Å length can force apart lecithin molecules to cavities with radiation of 20 Å in the bilayer membrane. These lipids could alter the

charge distribution of chelating properties of membrane. Work by Stowe and others may not hold true, but the results suggest a close relationship between plant hormone activity and lipid metabolism, in accord with the work of Christiansen and Thimann (1950) and Westwood and Batjer (1960), who observed that lipid utilization paralleled auxin-induced growth in pea-stem sections. Similar results were also reported by Méndez and his coworkers (1967). They showed that 0.01% Tween 20 and IAA increased coleoptile elongation more than IAA alone and concluded that a low concentration of Tween 20 is capable of accentuating the growth-promoting activity of IAA.

Since it is possible that Tween 20 stimulation of plant growth could be a benefit from its components, fatty acids, oleate and laurate, which could be released by lipase (Nishida, 1957), MacDowall (1963) investigated effects of Tween 20, oleate-laurate and Trixon-100 on growth of tobacco. In isolated root cultured in White's medium,  $10 \text{ mg}\cdot\text{L}^{-1}$  Tween 20 and the combination solution of  $0.08 \text{ mg}\cdot\text{L}^{-1}$  oleate and  $0.09 \text{ mg}\cdot\text{L}^{-1}$  laurate did not stimulate growth and nicotine synthesis, but both increased respiration rates. It is possible that Tween 20 concentrations were too low to be effective, therefore, further study of concentration series of Tween 20 and oleate-laurate on plant growth is merited. Trixon-100 increased growth and nicotine synthesis indicating a beneficial effect of surface activity. MacDowall (1963) also reported that 0.001% Tween 20 stimulated growth and nicotine synthesis of solution-cultured tobacco, but did not report the effect of oleate-laurate.

All these data strongly suggest that the mode of action of Tween 20 in plants is possibly the combination of biochemical reactions and physical changes, but this is still not clear.

Inhibitory effects of high concentrations of Tweens were also reported. High concentrations of Tween 20 cause phytotoxic damages and decreased transpiration and photosynthesis. Kohlrabi treated with Tween 20 at 2% showed chlorotic and necrotic lesions on leaf surfaces and marked reduction in assimilation and transpiration rate after short-term increase of photosynthesis and transpiration (Noga, et al., 1986). Tween 20 (at 0.2%) was used as chemical inducers of transcription of the genes *OPR1* and *OPR2* in *Arabidopsis thaliana* and was toxic to plants (Biesgen and Weiler, 1999; Hunzicker, 2006).

*Effect of Tween 20 on enzymes.* Tween 20 has been used as a means of extracting enzymes from plant tissue. Tween 20 had no effect on proteases from various sources (Sugiura, et al., 1964; Norris, 1982), but stimulated N-demethylase activity in cotton hypocotyls (Frear, et al., 1969). Petrenko (1971) showed Tween 80 inhibited the respiratory chain enzymes of *Rhizobium meliloti*, including NADH oxidase, succinate oxidase, NADH dehydrogenase, and succinate dehydrogenase.

*Effect of Tween 20 on plant water use.* Very few reported in the literature about Tween 20 on plant water use. Atlox 209-FG, which is polysorbate surfactant, in the same group of Tween 20, at 0.1%, reduced water loss of bean by dipping leaves for 30 seconds (Coats and Foy, 1974). In this experiment, polysorbate oil, acting as an antitranspirant, was used to cover the leaf surface with a thin layer of oil and therefore reduce transpiration. These results were consistent with Kelley (1930), who reported stomatal closure after oil treatment.

## **Plant water use status**

Plant water status is highly affected by the transpiration rate. Transpiration is the major force moving water into plants (Kramer and Boyer, 1995). Transpiration from plant cells lowers the matric potential of evaporating cell walls, producing a water potential gradient causing water movement from the root to the evaporating cell surface. Plants transpire about 95% of the root-absorbed water into the air (Kramer and Boyer, 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 and without affecting long-distance transport of mineral nutrients (Tanner and Beevers, 1990, 2001). Pedersen and Sand-Jensen (1997) also reported that the lack of transpiration from leaf surfaces is not likely to constrain the growth and nutrient transfer of herbaceous plants. Smith (1991) demonstrated that the increase of the osmotic pressure of the xylem sap at low transpiration rates help maintain the acropetal ion flux.

## **Statement of Research Objectives**

The objective of this dissertation is to investigate whether it is possible to use the Tween 20 as an irrigation additive to decrease plant water use while maintaining or promoting plant growth in the nursery. The hypothesis was that if a surfactant molecule is small enough to go into the xylem with the sap, then surfactant could be added to irrigation water to decrease surface tension of leaf menisci, matric potential will increase, the total water potential will increase and the driving force of transpiration will decrease, and as a result transpiration rate will decrease. Plants watered with the surfactant solution will have less water stress than those watered without surfactants at decreased irrigation



levels. In some degree, plants watered with surfactant solutions at reduced irrigation levels would maintain similar growth as well-watered plants. Adding surfactant in irrigation systems would therefore provide a partial solution to limited water supplies.

While the outcome of the studies presented in this dissertation does not answer all possible questions regarding the application of Tween 20 in the irrigation system, it is hoped that the information will induce irrigation scientists to investigate surfactant irrigation additives of real worth to help nursery growers to save water and reduce nursery production periods.

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Table 1. Area of United States with land subject to environmental limitation (Boyer, 1982).

Environmental limitation	Area of U.S. Soil affected (%)
Drought	25.3
Shallowness	19.6
Cold	16.5
Wet	15.7
Alkaline salts	2.9
Saline or no soil	4.5
Other	3.4
None	12.1

Table 2. Distribution of insurance indemnities for crop losses in the USA from 1939 to 1978 (Boyer, 1982).

Cause of crop loss	Proportion of payments (%)
Drought	40.8
Excess water	16.4
Cold	13.8
Hail	11.3
Wind	7.0
Insect	4.5
Disease	2.7
Flood	2.1
Other	1.5

Table 3. Characteristic parameters of surfactant-liposome interaction (Annesini, et al., 2000).

	Triton X-100	Tween 20	Tween 60
CMC, mM	0.24	0.04	0.025
$C_w^{sat}$ , mM	0.218	0.101	0.151
$R^{sat}$	0.597	0.082	0.036
$K$ , mM <sup>-1</sup>	1.71	0.77	0.23
$x_s^{sat}$	0.374	0.077	0.035

CMC: surfactant critical micelle concentration;

$C_w^{sat}$  : the free surfactant concentration in the saturated bilayer;

$R^{sat}$  : surfactant-lipid molar ratio in the lipid bilayer at vesicle saturation;

$K$ : surfactant partition coefficient;

$x_s^{sat}$  : surfactant molar fraction in the lipid bilayer at vesicle saturation.

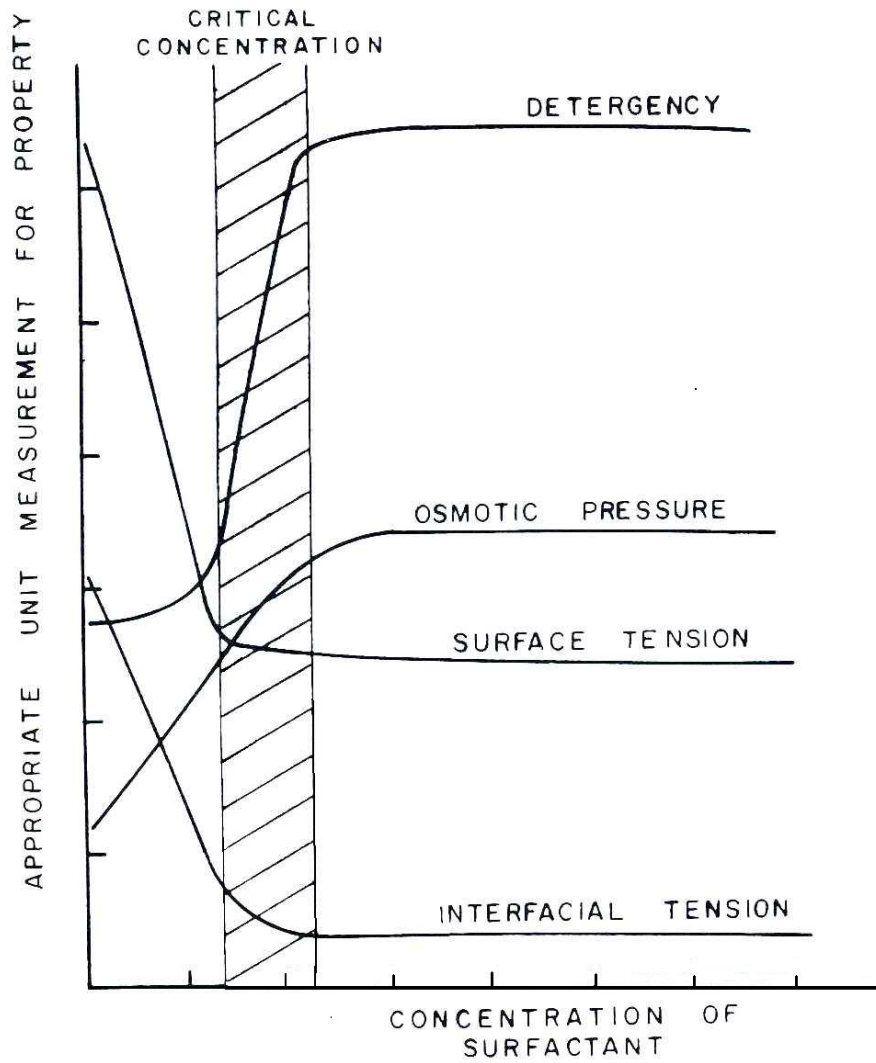


Fig. 1. Property changes of aqueous solution at the critical micelles concentration (Colwell and Rixon, 1961).

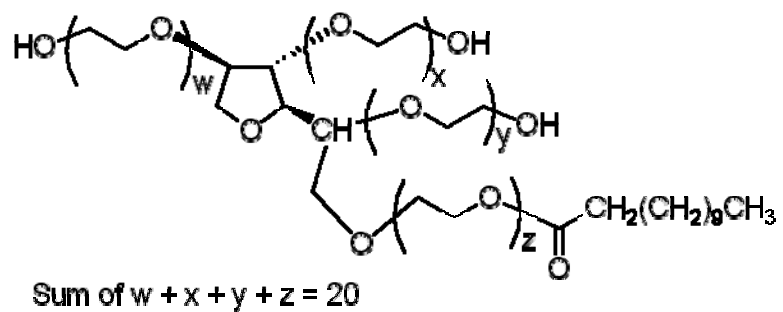


Fig. 2. Tween 20 structure. Tween 20 is a polyoxyethylene sorbitol ester with 20 ethylene oxide units ( $w + x + y + z = 20$ ) (Sigma-Aldrich, Inc., 2007).



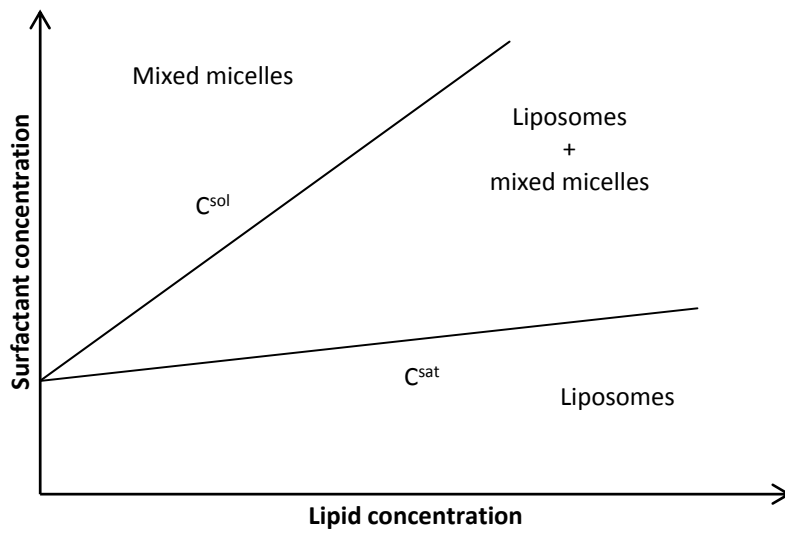


Fig. 3. Phase diagram of the lipid/surfactant/water system (Annesini, et al., 2000).

## II. SURFACTANT AFFECTS SUBSTRATE HYDROLOGICAL CHARACTERISTICS AND GROWTH OF NEW GUINEA IMPATIENS

*Additional index words.* polysorbate, wetting agents, moisture retention, greenhouse container production, *Impatiens hawkerii* 'Celebrate Salmon'.

*Abstract.* Surfactants are mainly used to manage water repellency in soils and increase moisture retention in potting substrates to increase irrigation efficiency. However, there are no reports of surfactants increasing plant growth while increasing moisture retention. The objectives of this research were to evaluate the effects of a common nonionic surfactant, Tween 20, on: 1) leaching and moisture retention of Fafard 3B in laboratory soil columns; 2) growth of New Guinea impatiens (*Impatiens hawkerii* 'Celebrate Salmon'); and 3) water requirements for plant growth. Tween 20 accelerated initial wetting of Fafard 3B in laboratory soil columns. Moisture retention of Fafard 3B treated with 100 mg·L<sup>-1</sup> Tween 20 solution increased 40.5% compared to that of the control at initial wetting. In an outdoor container study evaluating the effect of Tween 20 on the growth of New Guinea impatiens, 18 treatments were arranged in a complete 3 × 6 factorial design with one additional group of pots receiving plain water with about 30% leachate serving as a control. Eighteen treatments were irrigation levels of 20%, 40%, or 60% of the full crop evapotranspiration (ET) requirements in combination with Tween 20 concentrations of either 0, 25, 50, 75, 100, or 125 mg·L<sup>-1</sup>. The full crop ET requirement was determined as the difference of the applied water amount minus the leachate of the

control. Growth index of impatiens in the treatment of  $100 \text{ mg}\cdot\text{L}^{-1}$  Tween 20 at the 60% irrigation level was increased 11% compared to the control for the same time period. Growth of impatiens in treatment combinations of 50, 75, 100, or  $125 \text{ mg}\cdot\text{L}^{-1}$  Tween 20 at the 20% or 40% irrigation level was similar to the control throughout the experiment. Using Tween 20 as an irrigation additive in the irrigation water saved up to 80% of the irrigation water normally used. Our study demonstrated that Tween 20 is a unique irrigation additive with three-fold effects of increasing water retention of substrate and decreasing the amount of water required while simultaneously increasing plant growth when compared to the control. Since Tween 20 is a low-cost and safe nonionic surfactant, it is possible to use it in nurseries to reduce irrigation during production.

Chemical name used: polyoxyethylene sorbitan monolaurate (Tween 20);

Although there is an abundance of water resources in the United States, water availability has emerged as a major resource issue because of environmental pollution, high costs of water supply equipment, and vicissitudes of the hydrologic cycle, which can greatly impact the availability of fresh water to meet local or regional demands in time (Frederick, 1995). More than 50% of commercial nurseries are located near cities, and expansion of urban areas increases competition for water resources (Beeson et al., 2004). Container nurseries are frequently among the first targets for regulated decreases of water supply in urban areas. In recent years, many local governments have limited water consumption of nursery growers with laws and regulations. Environmental laws also restrict nutrient release from commercial nurseries. Large seasonal, annual, and regional

variations in rainfall and runoff pose major challenges to the horticultural industry and down-to-earth risks for nursery growers.

Surfactants are commonly used as adjuvants in agriculture to improve the emulsifying, dispersing, spreading, sticking or penetrating of pesticides (Miller and Westra, 1998). Surfactants are also common additives to pine bark and sphagnum peat moss-based mixes (Bilderback and Lorscheider, 1997). A considerable amount of agricultural research has been focused on the use of surfactants to increase moisture retention in water-repellent soil (DeBano, 2000; Letey et al., 1961, 1962ab, 1963; Watson et al., 1969) and the basic function of surfactants on soils, such as factors responsible for increasing the effectiveness (Mustafa and Letey, 1971), longevity (Osborn et al., 1969), movement and leaching (Miller et al., 1975), effects on soil aggregation (Mustafa and Letey, 1969) and pesticide mobility (Huggenberger et al., 1973). Very few studies go beyond the traditional manner for use of surfactants (Blodgett et al., 1993; Kostka, 2000; Kostka et al., 1997; Leinauer, 2002; Guillen et al., 2005). Because of the wide variety and utilization of surfactants in horticulture, studies on effects of surfactants on horticultural substrates and crops are merited and also critical to improve water use efficiency and irrigation management.

In this study, the nonionic commercial surfactant Tween<sup>®</sup> 20 (polyoxyethylene sorbitan monolaurate, C<sub>58</sub>H<sub>114</sub>O<sub>26</sub>, MW = 1227.54, Monomer-Polymer & Dajac Labs, Inc., Featerville, Pa.) was selected to examine its effects on: 1) leaching and moisture retention of Fafard 3B (a blend of peat, perlite, vermiculite, and pine bark, Conrad

Fafard, Inc., Agawam, Mass.); 2) growth of New Guinea impatiens (*Impatiens hawkerii* 'Celebrate Salmon'); and 3) water requirements for plant growth.

## **Materials and Methods**

*Expt. 1.* Environmental variables and plant influence important in leaching and water retention are difficult to control in both greenhouse and field studies. Therefore, we evaluated the influence of Tween 20 on leaching and moisture retention of substrate Fafard 3B (a blend of peat, perlite, vermiculite, and pine bark, Conrad Fafard, Inc., Agawam, Mass.) under controlled conditions (25 °C and 50% relative humidity). The procedure was conducted with substrate filled column cylinders (25.5 cm length × 10.5 cm i.d.) constructed from clear PVC pipe. Each PVC cylinder base plate had one drain hole in the center.

On 7 Sept. 2006, 12 substrate columns were loosely filled with thoroughly-mixed Fafard 3B to the height of 16 cm with a weight of  $352 \pm 0.05$  g (55.7% water) substrate for each column. Each cylinder was incrementally and gently tapped to settle the substrate and remove voids; then a minimal pressure was applied carefully by hand on the substrate surface with a flat stopper to level the surface within each cylinder. On the 1<sup>st</sup> day, 750 mL of Tween 20 solution at either 0 (control), 50, or 100 mg·L<sup>-1</sup> was evenly added onto one column surface, respectively. There were four replications for each concentration. From the 3<sup>rd</sup> to 17<sup>th</sup> day, 100 mL of each solution was added every other day. Loss of water due to evaporation was obtained by weighing the columns before watering. Leachate was collected and measured for each substrate column after watering. After 17 days, the final substrate height was 15.4 cm. Average evaporation volume of

Fafard 3B treated with Tween 20 solution at 0, 1, 50, or 100 mg·L<sup>-1</sup> were 9, 9.5, and 10.5 mL per day, respectively. Moisture retention was the total water contained in the substrate and was calculated using the following formula:

moisture retention = total applied volume of solution + initial water volume of substrate – total leachate volume – evaporation volume.

Tween 20 concentrations evaluated were based on previously reported critical micelle concentration (CMC) of  $3.5 \times 10^{-5}$  M (43 mg·L<sup>-1</sup>), 60 mg·L<sup>-1</sup>, 0.059 mmol·L<sup>-1</sup> (75 mg·L<sup>-1</sup>), or 0.08 mmol·L<sup>-1</sup> (98 mg·L<sup>-1</sup>) (Sigma-Aldrich, Inc., 2006; Tran and Yu, 2005) in the water (range attributed to different manufacturer), and above 100 ppm in the plant (Bernath and Vieth, 1972).

*Expt. 2.* In April, 2006, stem cuttings of *Impatiens hawkerii* 'Celebrate Salmon' were prepared as 2.5 cm long, single-node terminal cuttings with 2 fully-expanded leaves, 3-4 immature leaves and 1.2 cm of stem below the basal node. Cuttings were inserted into 36-cell trays filled with Fafard 3B. All cuttings were placed under a greenhouse mist system at the Paterson Greenhouse Complex at Auburn University, Ala. (32°36'N, 85°29'W, USDA Hardiness Zone 8a), providing overhead mist with tap water for 6 s every 16 min during daylight hours for a rooting period of 14 days. Maximum photosynthetically active radiation in the greenhouse was 600 μmol·m<sup>2</sup>·s and daily maximum/minimum temperature in the greenhouse was 27 ± 6 °C/18 ± 3 °C. After early root formation, plants were moved from the intermittent mist bed to a bench and watered by hand daily and fertilized twice per week using Pro-Sol 20-20-20 water-soluble fertilizer (Pro-Sol, Ozark, Ala.) applied as a drench at a rate of 100 ppm N for 45 days.

After establishment, On 24 July 2006, rooted New Guinea impatiens cuttings were transplanted into 15.24-cm azalea pots filled with Fafard 3B mix. The substrate was amended with  $6.6 \text{ kg}\cdot\text{m}^{-3}$  controlled-release fertilizer Polyon 18N–2.6P–9.9K and  $0.9 \text{ kg}\cdot\text{m}^{-3}$  Micromax. Plants were hand-watered every other day and grown outdoors in a 60% shade house for three months.

Treatment design was a  $3 \times 6$  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  $125 \text{ mg}\cdot\text{L}^{-1}$ . The control group was irrigated with tap water to container capacity with about 30% leachate (Bilderback, 2001). 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) with pots in 18 treatments irrigated to have no leachate. Because the main purpose of our study was to save water, we did not include treatments of 25, 50, 75, 100, or  $125 \text{ mg}\cdot\text{L}^{-1}$  Tween 20 at the 100% irrigation level.

The first month of the experiment was sunny and dry. Average daily temperatures were  $\approx 32 \text{ }^\circ\text{C}$  day/ $21 \text{ }^\circ\text{C}$  night and the second and third months with daily air temperatures around  $23$  to  $25 \text{ }^\circ\text{C}$  day/ $20 \text{ }^\circ\text{C}$  night. Hand irrigation was withheld any time that received more than 1 cm rainwater. After three months of growth, plants were measured to determine height, width (average of width at the widest point and width perpendicular to width at the widest point) and growth index (GI) [(height + width at the widest point + width perpendicular to width at the widest point)/3]. Leachates were collected every two weeks using the nondestructive Virginia Tech Extraction Method

(Wright, 1986) and were analyzed for pH and electrical conductivity (EC) using a Model 63 pH and conductivity meter (YSI Incorporated, Yellow Springs, Ohio). Results of EC and pH of leachates revealed no difference between treatments.

*Statistics.* Data analyses were conducted using SAS for Windows v.9.1 (SAS Institute Inc., Cary, N.C.) and GLM, REG and TTEST procedures were used according to experimental designs. Any statistical test with  $p\text{-value} \leq 0.05$  was considered significant and reported where appropriate.

In Expt. 2, the independence would disappear if two-way ANOVA was performed on the whole data set of the unbalanced factorial design (Herr, 1986). Therefore, data of Expt. 2 was best analyzed on a  $3 \times 6$  factorial design without the control using a two-way ANOVA with irrigation and Tween 20 as main effects following up the significant F-values with Tukey's honestly significantly different (HSD) post hoc tests, to examine the influence of irrigation, Tween 20 and the interaction between the two factors on growth parameters of New Guinea impatiens. Data were then subjected to final baseline comparison using Student's  $t$  tests to find if the growth parameter in 18 treatments was significantly different from that of the control, respectively.

## **Results and Discussion**

*Expt. 1.* At initial wetting, Tween 20-treated Fafard 3B absorbed more solution than the water-only treatment (Table 1). Moisture retention of Fafard 3B treated with  $100 \text{ mg}\cdot\text{L}^{-1}$  Tween 20 was 793.9 mL, an increase of 40.5% compared to that of the control, 565.2 mL (Table 2). Fafard 3B treated with  $50 \text{ mg}\cdot\text{L}^{-1}$  Tween 20 had higher moisture retention than non-treated Fafard 3B only in the first 7 days. Regression models and



regression lines of the substrate moisture retention between Tween 20 at 0, 50, and 100  $\text{mg}\cdot\text{L}^{-1}$  with days of treatment, respectively, showed that Tween 20 increased moisture retention only at the initial wetting based on intercepts, slopes and shapes of the 3 regression lines (Fig. 1).

The primary effect of surfactants on increasing moisture retention of horticultural substrates is through reducing surface tension of the air-water interface, modifying the solid-liquid contact angle, and affecting capillarity in unsaturated porous media (Henry and Smith, 2003). Because the capillary pressure is a function of surface tension, surface tension reductions can cause proportional decreases in capillary pressure (the negative soil water pressure). Decreases in capillary pressure caused by Tween 20 also lead to a smaller capillary height in the Tween 20-treated substrate than in the water-only substrate. Therefore, during the initial wetting of substrates, Tween 20-treated water could go to the pore spaces that were not accessible for water without Tween 20, allowing the substrate to quickly hold more water.

In contrast with increasing moisture retention at initial wetting, there was a stark decrease in leachate amount collected from substrates treated with Tween 20 on the 1<sup>st</sup> day. From the 5<sup>th</sup> to 11<sup>th</sup> day, a similar amount of solution passed through the Fafard 3B treated with Tween 20 compared the control. After the 13<sup>th</sup> day to the end of the experiment, more leachate volume was collected from the 100  $\text{mg}\cdot\text{L}^{-1}$  Tween 20 treated substrate than the control.

The increase in leaching from Tween 20 after initial treatments could be explained by increased soil water pressure (Henry and Smith, 2003). After initial

treatments the soil water pressure in porous substrates containing surfactant (higher pressure, lower surface tension) caused more leaching than was found in porous substrates without surfactants (lower pressure, higher surface tension).

These results suggest that Tween 20 as an irrigation adjuvant may allow sustained or greater growth of plants requiring high moisture and well-drained substrates at planting compared to plants grown with conventional irrigation. Also, with the addition of Tween 20 to the substrates, less irrigation amount or frequency could maintain the same container moisture retention.

*Expt. 2.* From a complete  $3 \times 6$  factorial data analysis, both irrigation and Tween 20 affected the growth of impatiens but there was no interaction between irrigation and Tween 20 (Table 2). Post hoc tests on Tween 20 showed that plants receiving 50, 75, 100, and  $125 \text{ mg}\cdot\text{L}^{-1}$  Tween 20 solution had greater height, width and GI than those without Tween 20. As the irrigation decreased from the 60% to 20% level, plant height, width and GI were significantly decreased. Plants in treatments of 100 or  $125 \text{ mg}\cdot\text{L}^{-1}$  Tween 20 at the 60% irrigation level grew best, with a 10% increase in GI in comparison to plants in control treatment from Student's *t* tests (Fig. 2). Growth of impatiens in the treatment combinations of 50, 75, 100, or  $125 \text{ mg}\cdot\text{L}^{-1}$  Tween 20 at the 20% or 40% irrigation levels were similar to those of the control over the course of the experiment. The saving of up to 80% irrigation water with Tween 20 could be partly attributed to moisture change in the substrates. Studies have also reported that Tween 20 stimulated plant growth (MacDowall, 1963) which has been attributed to growth-hormone activity (Méndez et al., 1967; Stowe, 1958; Stowe and Dotts, 1971; Nishida, 1957) or by alterations to the charge

distribution of chelating properties of membranes (Stowe, 1958, 1959, 1960; Stowe and Obreiter, 1962; Stowe and Dotts, 1971). Results of these earlier studies suggest that part of the plant response to Tween 20 in our study could be due to plant biochemical reactions continuing at reduced water levels when Tween 20 was added to irrigation.

This study indicated that Tween 20 is a unique irrigation additive with three-fold effects of increasing substrate water retention, promoting plant growth, and reducing water needed to maintain similar growth to plants with larger water use in the absence of Tween 20. Tween 20 is a low-cost, safe (Sigma-Aldrich, Inc., 2008) surfactant with potential use in commercial nurseries to reduce irrigation frequency and reduce production period. Further research examining application rates on more species in different irrigation systems, possible runoff problems, interaction of surfactant with fertilizers, and compatibility with herbicides is warranted.

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Table 1. Effects of Tween 20 solution on leachate volume and moisture retention on Fafard 3B in constructed laboratory columns. 750 mL of Tween 20 solution was applied on the 1st day and 100 mL was added every other day with four replications.

Tween 20 concn (mg·L <sup>-1</sup> )	1 <sup>st</sup> d	3 <sup>rd</sup> d	5 <sup>th</sup> d	7 <sup>th</sup> d	9 <sup>th</sup> d	11 <sup>th</sup> d	13 <sup>th</sup> d	15 <sup>th</sup> d	17 <sup>th</sup> d
	----- Leachate vol (mL) -----								
0 (control)	323.3 a <sup>z</sup>	35.7 ab	61.3 a	50.7 a	61.3 b	59.0 a	58.0 b	67.0 b	70.0 b
50	277.5 b	34.0 b	61.6 a	57.5 a	67.0 ab	64.0 a	65.5 ab	66.5 b	72.0 b
100	90.0 c	54.6 a	69.0 a	65.7 a	70.6 a	73.0 a	74.7 a	77.3 a	78.0 a
	----- Moisture retention <sup>y</sup> (mL) -----								
0 (control)	565.2 c	629.6 c	650.9 c	674.2 c	695.6 b	716.6 b	738.6 b	753.5 b	765.9 b
50	610.4 b	676.4 b	696.8 b	721.3 b	736.3 b	754.3 b	770.8 b	786.3 b	796.3 b
100	793.9 a	838.9 a	847.9 a	860.2 a	869.2 a	878.5 a	883.9 a	886.5 a	888.5 a

<sup>z</sup>Mean separation in columns by a different letter are different according to the post hoc Tukey's studentized range (HSD) test ( $p$ -value  $\leq 0.05$ ).

<sup>y</sup>Moisture retention was the total water contained in the substrate and was calculated as: moisture retention = total applied volume of solution + initial water volume of substrate – total leachate volume – evaporation volume.



Table 2. Effect of Tween 20 and irrigation on growth index (GI)<sup>z</sup>, height, and width of *Impatiens hawkerii* 'Celebrate Salmon' in a 3 × 6 factorial design grown in a 60% shade house for three months.

Treatment	GI <sup>z</sup> (cm)	Height (cm)	Width (cm)
<i>Irrigation (%)</i> <sup>x</sup>			
20	30.4 b <sup>w</sup>	28.2 b	31.6 b
40	32.6 a	28.7 ab	34.6 a
60	33.9 a	29.2 a	36.3 a
<i>Tween 20 (mg·L<sup>-1</sup>)</i>			
0	29.5 b	27.1 c	30.7 c
25	30.1 b	27.8 bc	31.3 bc
50	33.0 a	29.1 ab	34.9 ab
75	33.3 a	29.7 a	35.1 a
100	34.3 a	29.4 ab	36.7 a
125	33.8 a	29.1 ab	36.1 a
<i>P value</i>			
Main effect			
Irrigation amount	<.0001	0.1154	<.0001
Tween 20	<.0001	0.0024	<.0001
Interaction	0.9998	0.1017	0.9776

<sup>z</sup>GI was calculated as the average of plant height, the widest plant width, and plant width perpendicular to the widest width.

<sup>y</sup>Width was calculated as the average of the widest plant width and plant width perpendicular to the widest width.

<sup>x</sup>Three irrigation levels were 20%, 40%, or 60% of the full crop evapotranspiration (ET) requirements. 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.

<sup>w</sup>Means within columns for same factor followed by different letters are significantly different according to the post hoc Tukey's studentized range (HSD) test ( $p$ -value  $\leq$  0.05).

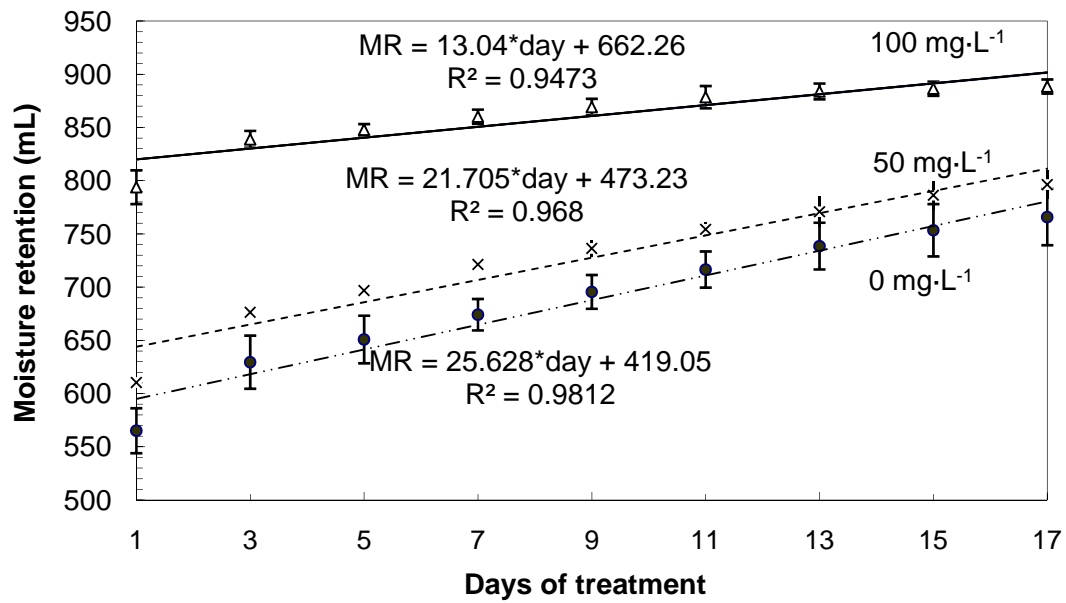


Fig. 1. Relationships between moisture retention of Fafard 3B and days of treatment subjected to 0, 50, and 100 mg·L<sup>-1</sup> Tween 20 solution added to constructed laboratory columns. On the 1<sup>st</sup> day, 750 mL of Tween 20 solution at either 0 (control), 50, or 100 mg·L<sup>-1</sup> was evenly added onto one column surface, respectively. There were four replications for each concentration. From the 3<sup>rd</sup> to 17<sup>th</sup> day, 100 mL of each solution was added every other day. Moisture retention was the total water contained in the substrate and was calculated as: moisture retention = total applied volume of solution + initial water volume of substrate – total leachate volume – evaporation volume. ( $p$ -value  $\leq 0.05$ , ANOVA).

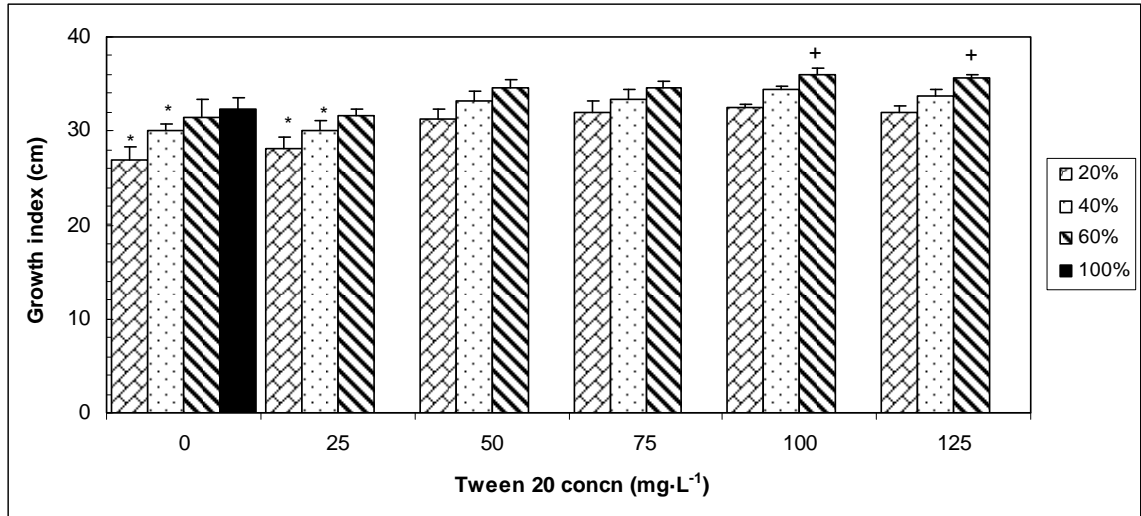


Fig. 2. Statistical summary showing that the results of Student's *t* tests between 18 treatments and the control on growth index of *Impatiens hawkerii* 'Celebrate Salmon' grown outdoors in a 60% shade house for three months and hand-irrigated daily. 18 treatments were combinations of irrigation levels 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<sup>-1</sup>. 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 six 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 ( $p$ -value  $\leq 0.05$ ).

**III. EFFECTS OF A NONIONIC SURFACTANT ON GROWTH,  
PHOTOSYNTHESIS, AND TRANSPIRATION OF NEW GUINEA IMPATIENS  
IN THE GREENHOUSE**

*Additional index words.* wetting agents, polysorbate, deficit irrigation, greenhouse container production, *Impatiens hawkerii* 'Celebrate Salmon'.

*Abstract.* Production of quality greenhouse and nursery crops is dependent on high quality and quantities of water. At present, insufficient water supply is a growing concern. In a preliminary study, we found that Tween 20 increased moisture retention of the substrate of Fafard 3B and decreased the amount of transpired water of New Guinea impatiens (*Impatiens hawkerii* 'Celebrate Salmon') in a hydroponic system. This study was to determine impact on growth of New Guinea impatiens, when watered with a Tween 20 solution 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  $3 \times 6$  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·L<sup>-1</sup>. The control group was watered with tap water to container capacity with about 30% leachate. ET 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·L<sup>-1</sup> 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 had no differences between the control and the treatments of Tween 20 from 50 to 125 mg·L<sup>-1</sup> at the 60% irrigation level or the treatment of Tween 20 at 100 mg·L<sup>-1</sup> at the 40% irrigation level. Tween 20 had no effect on net photosynthetic rate. With Tween 20 concentration increased from 0 to 100 mg·L<sup>-1</sup> 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.

Chemical name used: polyoxyethylene sorbitan monolaurate (Tween 20).

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 ornamental 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 evaporating cell walls, producing a water potential gradient causing water move from the root to the evaporating cell surface. Plants transpire about 95% of the root-absorbed water into the air (Kramer and Boyer, 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 Beever, 1990, 2001).

Surfactants are known to decrease surface tension. Surfactants have been reported in many studies to increase irrigation efficiency by increasing moisture retention in potting substrates (Bilderback and Lorscheider, 1997; Guillen, et al., 2005) and by decreasing water repellency of soil (Snyder, et al., 1984; Wallis, et al., 1989; Cisar et. al., 2000). 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 evaporating cell walls 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 go into the xylem with the sap, then surfactant could be added to irrigation water to decrease surface tension of leaf menisci, matric potential will increase, the total water potential will increase and the driving force of transpiration will decrease, and as a result transpiration rate will decrease. Plants watered with the surfactant solution will have less water stress than those watered without surfactants at decreased irrigation levels. In some degree, plants watered with surfactant solutions at decreased irrigation levels would maintain similar growth as well-watered plants. Adding surfactant in irrigation systems would therefore provide a partial solution of water scarcity 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 decreasing 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, enzyme activity and are compatible with most herbicides due to lack of ionization (Bayer and Foy, 1982).

Polyoxyethylene sorbitan monolaurate ( $C_{58}H_{114}O_{26}$ ), commercially known as Tween<sup>®</sup> 20, is one of the most frequently used safe, nonionic, biodegradable, low molecular weight (about 1227.54) 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 (Sigma-Aldrich, Inc., 2006), which indicates that it is a water soluble. Tween 20 approached the critical micelle concentration (CMC) at about  $43 \text{ mg}\cdot\text{L}^{-1}$ ,  $75 \text{ mg}\cdot\text{L}^{-1}$ ,  $98 \text{ mg}\cdot\text{L}^{-1}$  (Tran and Yu, 2005) in the water (difference due to different manufacturers), and above  $100 \text{ mg}\cdot\text{L}^{-1}$  in the plant (Bernath and Vieth, 1972).

In a preliminary study (data not shown), we found that Tween 20 increased moisture retention of the substrate Fafard 3B and also promoted the growth of New Guinea impatiens (*Impatiens hawkerii* 'Celebrate Salmon') grown in the containers filled with Fafard 3B under extreme low irrigation level (20% of full crop evapotranspiration requirement) in the field. 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 in the greenhouse. A second objective was to determine how Tween 20 would affect photosynthesis, stomatal conductance and transpiration.

## **Materials and Methods**

On 14 Aug. 2006, rooted New Guinea impatiens cuttings were transplanted into 16.5-cm diameter, 1.71 L azalea pots filled with Fafard 3B mix (a blend of peat, perlite, vermiculite, and pine bark, Conrad Fafard, Inc., Agawam, Mass.). The substrate was amended with  $6.6 \text{ kg}\cdot\text{m}^{-3}$  controlled-release fertilizer Polyon 18N–2.6P–9.9K (Pursell Technologies Inc., Sylacauga, Ala.) and  $0.9 \text{ kg}\cdot\text{m}^{-3}$  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. ( $32^{\circ} 36'N \times 85^{\circ} 29'W$ , USDA Hardiness Zone 8a) for three months. Maximum photosynthetically active radiation in the greenhouse was  $600 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}$  and daily maximum/minimum temperature in the greenhouse was  $27 \pm 6^{\circ}\text{C}/18 \pm 3^{\circ}\text{C}$ . Based on an expectation that plants in the different treatments would have different flower numbers and flowers significantly affect plant transpiration, all flower buds were removed whenever visible. Leachates were collected every two weeks using the nondestructive Virginia Tech Extraction Method (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  $3 \times 6$  complete factorial design plus a control. The two factors were irrigation and Tween 20 (Monomer-Polymer & Dajac Labs, Inc., Featerville, 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  $125 \text{ mg}\cdot\text{L}^{-1}$ . The control group was irrigated with tap water to container capacity with about 30% leachate (Bilderback, 2001). 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·L<sup>-1</sup> Tween 20.

Photosynthetic parameters were measured with a portable photosynthesis system LICOR 6400 (LI-COR, Inc., Lincoln, Nebr.) 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 of each plant. Photosynthetically active radiation was set at 1200 μmol·m<sup>-2</sup>·s<sup>-1</sup>. The CO<sub>2</sub> flux was adjusted to maintain an inside chamber concentration of 350 μmol·mol<sup>-1</sup>. Relative humidity was at 40–50% and air temperature was 28 °C during the measurements. Net photosynthetic rate, foliar transpiration rate, intercellular CO<sub>2</sub> 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  $(1 - C_i/C_a) \times 100$ , where C<sub>a</sub> was ambient CO<sub>2</sub> 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, the shoots of impatiens 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 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, the independence would disappear if two-way ANOVA was performed on this unbalanced factorial design (Herr, 1986). Therefore, final data analysis was first performed on a  $3 \times 6$  factorial design without the control using a two-way ANOVA with irrigation and Tween 20 as main effects, following up the significant F-values 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  $\pm$  SD =  $20.01 \pm 1.87$  cm), width ( $20.63 \pm 1.74$  cm), and growth index ( $20.42 \pm 1.49$  cm) of New Guinea impatiens did not significantly differ among the treatments at planting in August (one-way ANOVA for plant height:  $F = 1.39$ ,  $df = 18, 95$ ,  $P = 0.8766$ ; 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 independently. Post hoc tests revealed that, plants grown with 25, 50, 75, 100, or 125 mg·L<sup>-1</sup> Tween 20 had a significantly higher height, width, growth index, fresh and dry weight than those grown with 0 mg·L<sup>-1</sup> after three months growth, respectively (Table 1). As Tween 20 concentration increased from 0 to 100 mg·L<sup>-1</sup> 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 dramatically, especially fresh and dry weight, which decreased 51.52% and 52.79%, respectively.

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 H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>) 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% irrigation level combined with 25, 50, 75, 100, or 125 mg·L<sup>-1</sup> 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·L<sup>-1</sup> or in the treatment of the 40% irrigation level with 100 mg·L<sup>-1</sup> 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 ( $F = 6.20$ ,  $df = 2, 72$ ,  $P = 0.0033$ ). Post hoc tests on the irrigation factor showed that as the irrigation decreased from the 60% to 20% level, average stomatal conductance decreased from 165.71 to 97.83  $\text{mmol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ , average intercellular  $\text{CO}_2$  concentration decreased from 247.56 to 199.25  $\mu\text{mol CO}_2 \cdot \text{mol}^{-1}$  and stomatal limitation increased from 37.42 to 49.77 (Table 1). The intercellular  $\text{CO}_2$  concentration and stomatal conductance decreased with Tween 20 concentration increase from 0 to 125  $\text{mg} \cdot \text{L}^{-1}$ , but the decrease in the intercellular  $\text{CO}_2$  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  $\text{CO}_2$  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 net photosynthesis rate decrease accompanies an increase of intercellular  $\text{CO}_2$  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, the relationship between irrigation and the transpiration rate, the relationship

between irrigation and stomatal conductance and the relationship between irrigation 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, with Tween 20 concentration increased from 0 to  $100 \text{ mg}\cdot\text{L}^{-1}$ , 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  $5.5 \text{ mol}\cdot\text{m}^{-3}$  when Tween 20 was applied on the leaf surface of stawberry. The decrease of the transpiration rate could be probably explained by the following reasons. The most effective concentration of Tween 20 to reduce transpiration was near  $100 \text{ mg}\cdot\text{L}^{-1}$  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 transpiration rate partly confirmed our hypothesis, which was also supported by Ichimura et al. (2005) on studies of the cut rose flower, 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; Sánchez-Blanco et al., 2003), and the mechanism has been little understood.  $K^+$  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), but in repeated response to supplementation of the apoplast with KCl (Kasamo, 1979). 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  $100 \text{ mg}\cdot\text{L}^{-1}$  Tween 20 at the 20%, 40% or 60% irrigation level was 38%, 59% or 47% higher than those with fresh 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 photosynthesis was not significant, as discussed above (Fig. 2A).

Compared with the control, the transpiration rate of plants treated with 50 to  $125 \text{ mg}\cdot\text{L}^{-1}$  Tween 20 at the 60% irrigation level or plants treated with  $100 \text{ mg}\cdot\text{L}^{-1}$  at 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·L<sup>-1</sup> Tween 20 at 60% of full crop ET requirements or with 100 mg·L<sup>-1</sup> at 40% of full crop ET is reasonable.

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 benefit from the use of such surfactants in times of drought. Further trials are required 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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Table 1. Statistical summary showing that the results of two-way 3 × 6 ANOVAs and post hoc tests for the effects of irrigation, Tween 20 (mg·L<sup>-1</sup>), and irrigation × Tween 20 interaction on height (cm), width (cm), growth index (GI, cm), shoot fresh and dry weight (g), net photosynthesis rate (P<sub>n</sub>, μmol CO<sub>2</sub>·m<sup>-2</sup>·s<sup>-1</sup>), stomatal conductance (g<sub>s</sub> H<sub>2</sub>O, mmol CO<sub>2</sub>·m<sup>-2</sup>·s<sup>-1</sup>), intercellular CO<sub>2</sub> concentration (c<sub>i</sub>, μmol CO<sub>2</sub>·mol<sup>-1</sup>), stomatal limitation (L<sub>s</sub>), vapor pressure deficit at the leaf surface (VPDL, kPa), foliar transpiration rate (E, mmol·m<sup>-2</sup>·s<sup>-1</sup>), and water use efficiency (WUE, μmol CO<sub>2</sub>·mmol<sup>-1</sup> H<sub>2</sub>O) of *Impatiens hawkerii* 'Celebrate Salmon' after three-month growth in the greenhouse.

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Treatment	Ht	Width <sup>x</sup>	GI <sup>w</sup>	Fresh wt	Dry wt	P <sub>n</sub>	g <sub>s</sub> H <sub>2</sub> O	C <sub>i</sub>	L <sub>s</sub>	VPDL	E	WUE
<i>Irrigation<sup>z</sup></i>												
60%	28.00a <sup>y</sup>	35.63a	33.08a	171.40a	16.31a	12.39a	165.71a	247.56a	37.42b <sup>y</sup>	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
<i>Tween 20</i>												
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
<i>F</i>												
Main effect												
<i>Irrigation</i>	13.73	22.33	27.71	40.99	41.89	6.20	83.74	14.98	15.41	150.55	77.93	12.33
<i>Tween 20</i>	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
<i>P value</i>												
Main effect												
<i>Irrigation</i>	<.0001	<.0001	<.0001	<.0001	<.0001	0.0033	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
<i>Tween 20</i>	<.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

<sup>z</sup>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.

<sup>y</sup>Mean separation within columns by the post hoc Tukey's HSD test ( $P \leq 0.05$ ).

<sup>x</sup>Width was calculated as the average of the widest plant width and plant width perpendicular to the widest width.

<sup>w</sup>Growth index (GI) was calculated as the average of plant height, the widest plant width, and plant width perpendicular to the widest width.

<sup>v</sup>Stomatal limitation ( $L_s$ ) was calculated as  $(1 - C_i/C_a) \times 100$ , where  $C_a$  was ambient  $CO_2$  concentration.

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



<sup>z</sup>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<sup>-1</sup>. 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.

<sup>y</sup>Width was calculated as the average of the widest plant width and plant width perpendicular to the widest width.

<sup>x</sup>Growth index (GI) was calculated as the average of plant height, the widest plant width, and plant width perpendicular to the widest width.

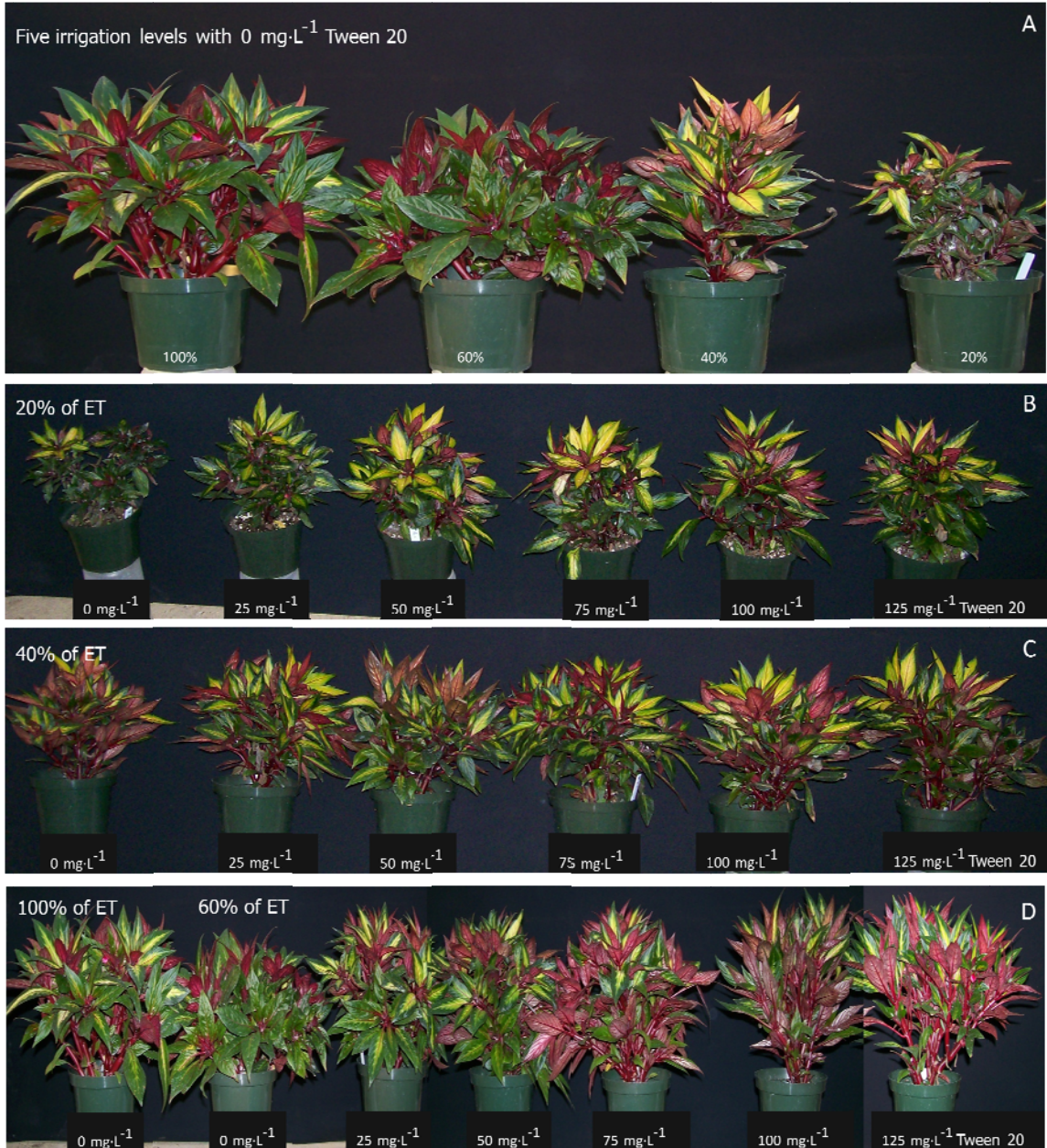
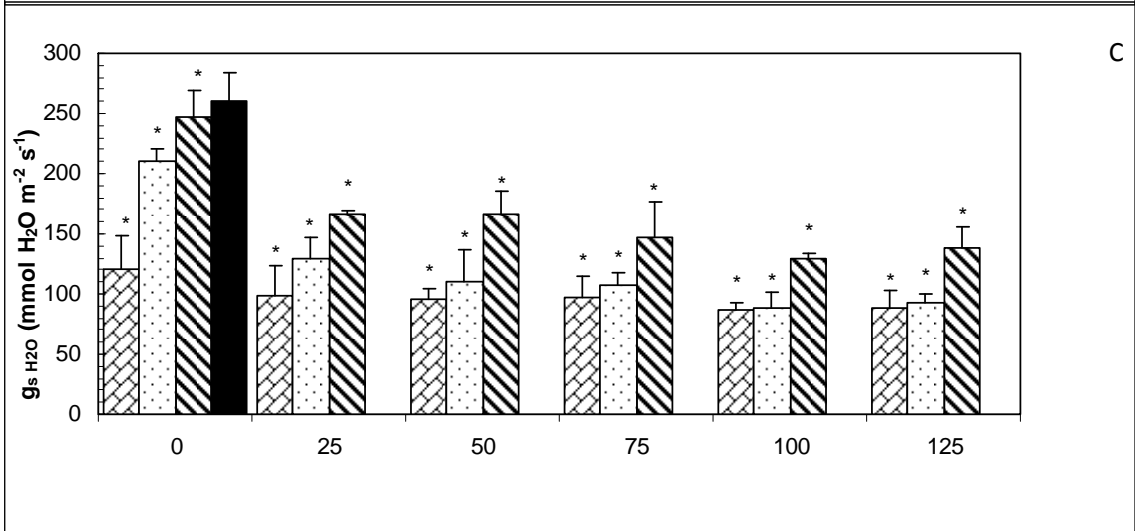
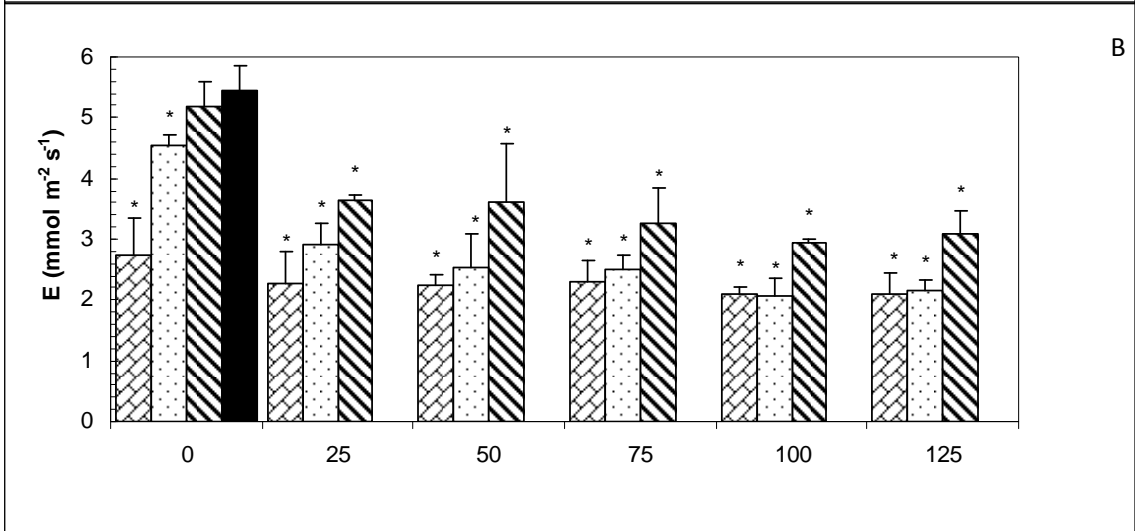
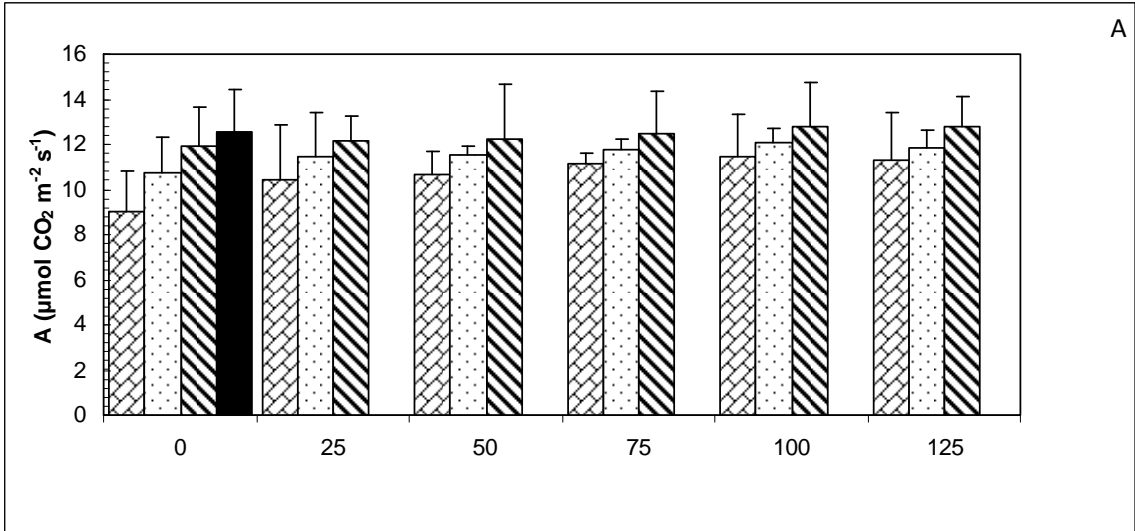


Fig. 1. Visual appearance of *Impatiens hawkerii* 'Celebrate Salmon' under 18 treatments and the control after three-month growth in the greenhouse. 18 treatments were combinations of irrigation levels of 20%, 40%, or 60% of the full crop evapotranspiration (ET) requirements with Tween 20 levels of either 0, 25, 50, 75, 100, or 125 mg·L<sup>-1</sup>. 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<sup>-1</sup> Tween 20 at 20% of full crop ET requirements. C) Plants were irrigated with 0, 25, 50, 75, 100, or 125 mg·L<sup>-1</sup> 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<sup>-1</sup> Tween 20 at 60% of full crop ET requirements.



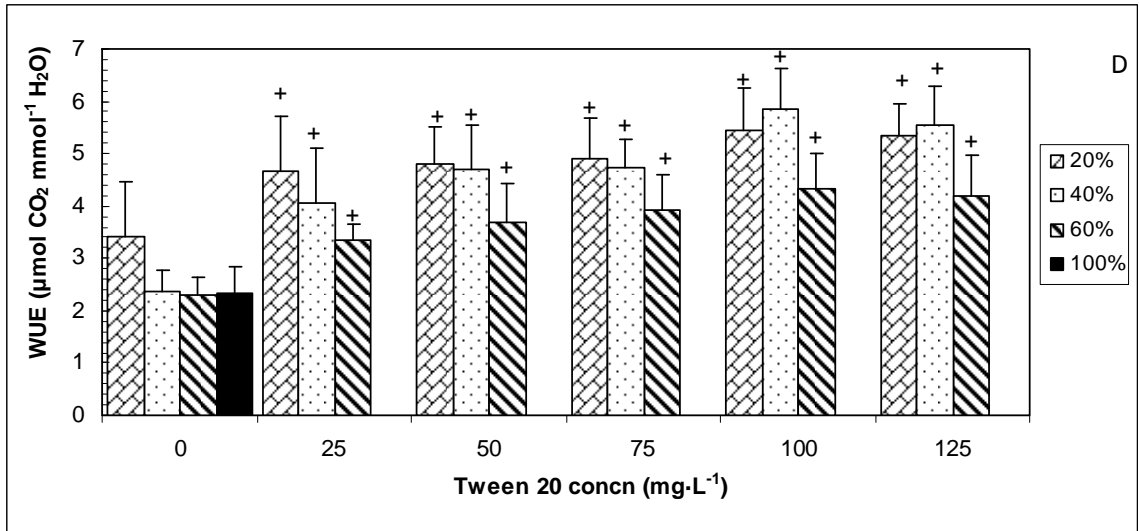
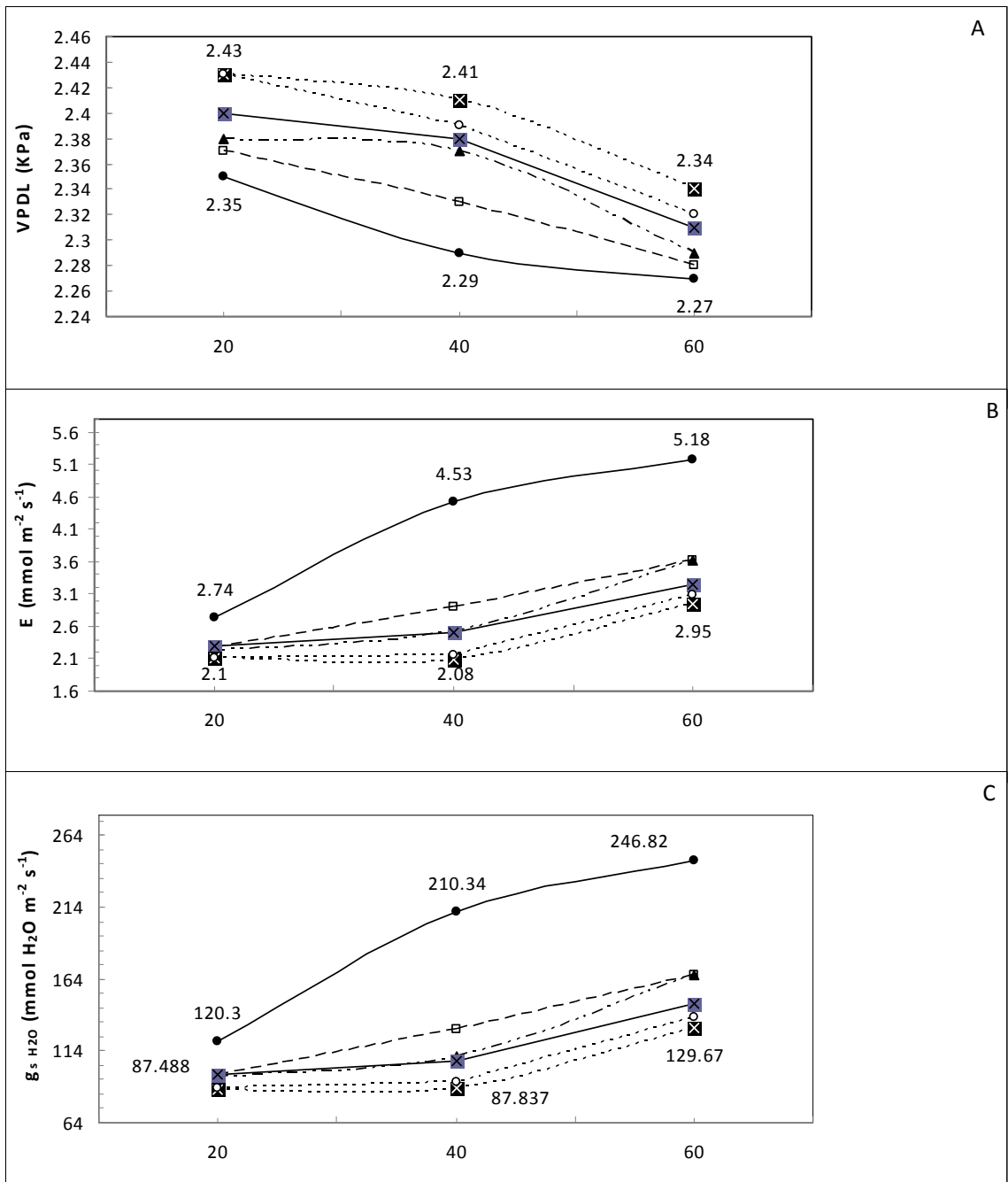


Fig. 2. Statistical summary showing that the results of Student's *t* tests between 18 treatments and the control on net photosynthesis rate ( $P_n$ ) (A), transpiration rate (E) (B), stomatal conductance ( $g_{s\ H_2O}$ ) (C), and water use efficiency (WUE) (D) of *Impatiens hawkerii* 'Celebrate Salmon' 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  $\text{mg}\cdot\text{L}^{-1}$ . 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  $\pm$  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. ( $P \leq 0.05$ ).



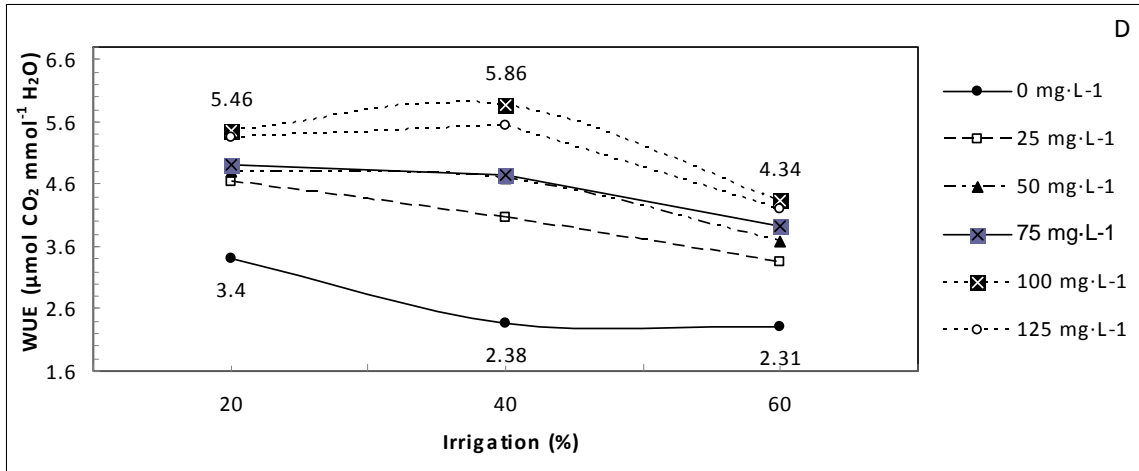


Fig. 3. Relationship between vapor pressure deficit (VPDL) and irrigation (A), transpiration rate (E) and irrigation (B), stomatal conductance ( $g_s$  H<sub>2</sub>O) and irrigation (C), and water use efficiency (WUE) and irrigation (D) at different Tween 20 concentrations for *Impatiens hawkerii* 'Celebrate 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<sup>-1</sup>. 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.

#### **IV. EFFECTS OF A SURFACTANT ON GROWTH AND WATER USE OF PEACE LILY AND NEW GUINEA IMPATIENS IN THE HYDROPONIC SYSTEM**

##### **Abstract**

Plant growth and water requirements were investigated in two species, peace lily (*Spathiphyllum floribundum* 'Viscount') and New Guinea impatiens (*Impatiens hawkerii* 'Celebrate Salmon'), grown in a controlled hydroponic systems containing either 0 (control), 50, 75, 100, or 125 ppm Tween 20 modified Hoagland solutions for 31 days. Fresh weight of peace lily grown in Hoagland solutions with 50, 75, 100, and 125 ppm Tween 20 increased 17%, 23%, 33% and 22%, while fresh weight of New Guinea impatiens increased 7%, 17%, 17% and 12% compared with control plants, respectively. Tween 20 increased total dry weight of peace lily and impatiens up to 33% and 18%. Relative growth rates of peace lily and impatiens in 100 ppm Tween 20 treatment were the fastest. Less water was transpired for both species treated with Tween 20. Guttation accounted for about one-third of the total water use of peace lily. Increased guttation was probably influenced by increased leaf weight from Tween 20. Water use efficiency of peace lily and New Guinea impatiens in 100 ppm Tween 20 solutions were increased 166% and 221%, compared to the control, respectively.



**Index words:** substrate; wetting agents, polysorbate, transpiration, ornamental, irrigation, adjuvant.

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

### **Significance to the Nursery Industry**

Moisture retention of container substrates is an important part of nursery irrigation management. Surfactants as soil amendments are popularly used to improve initial wetting of commercial peat or bark-based substrates. Limited information is available regarding effects of surfactants on plant growth and water use. Our hydroponic studies demonstrate that growth of peace lily and New Guinea impatiens increased while plant transpiration decreased when Tween 20 was included in the water supply, suggesting that Tween 20 has a potential to be a good irrigation additive in nursery crop production.

### **Introduction**

Water shortages and use conflicts have become more commonplace in many areas of the United States for irrigation of crops, for growing cities and communities, for energy production, and for the environment protected under law (30). Increasing water use efficiency is one way to reduce water crisis and sustain nursery development in horticulture. The physical condition of substrates is an important part of nursery water management. Moisture retention in proper range is critical to plant establishment and growth. One problem is that water intake is limited in most horticultural substrates at initial wetting although surfactants have been added in commercial pine bark, sphagnum peat moss and bark or peat –based substrates (2, 8). If substrates dry out during storage,

several extra steps must be taken before potting, such as watering substrates or irrigation with some commercial surfactants. However, if powdered fertilizers are incorporated to wet mixes, their distribution will be very uneven and this will result in large variations in plant growth (4).

Published data documenting the influence of surfactants on plant growth has been mostly “buried” inside of papers on other topics. Most surfactants are generally considered to be inert, non-phytotoxic, or phytotoxic with no effect on overall growth, or, as concentrations increase, growth is inhibited (3, 16). Tween 20 (Polyoxyethylene sorbitan monolaurate, C<sub>58</sub>H<sub>114</sub>O<sub>26</sub>)(19) is one of a few adjuvants that are recognized as promoting plant growth per se. Tween 20 stimulated growth of solution-cultured tobacco (14) and promoted hypocotyl growth of pea by 90% hydroponically (22). Méndez and his coworkers (15) demonstrated that 0.01% Tween 20 and IAA increased coleoptile elongation more than IAA alone. Low concentrations of Tween 20 have been shown to accentuate the activity of growth hormones (23, 24, 25, 26).

In a previous study, we tried to provide a new way to use surfactants as irrigation adjuvants to improve water use efficiency and decrease plant production periods in nurseries. Results showed that Tween 20 increased moisture retention of Fafard 3B at initial wetting, accompanying increased growth of New Guinea impatiens (*Impatiens hawkerii* 'Celebrate Salmon') under low irrigation levels in the greenhouse and the field, with decreased plant transpiration rate (data not shown). The increased moisture retention of substrate and decreased transpiration provided a good explanation of the increase of growth of impatiens under low irrigation levels. Two hydroponic studies were set up to

determine if the growth of two species New Guinea impatiens and peace lily (*Spathiphyllum floribundum* 'Viscount') was increased by Tween 20 and to investigate the influence of Tween 20 on plant water requirements in a hydroponic system.

## **Materials and Methods**

*Plant materials.* Peace lily plugs were purchased from a commercial tissue culture laboratory (Agri-Starts, Inc., Apopka, FL) on January 29, 2006. All plugs were planted into 15.24-cm (6-in) azalea pots filled with Fafard 3B mix (a blend of peat, perlite, vermiculite, and pine bark, Conrad Fafard, Inc., Agawam, MA) amended with 0.9 kg/m<sup>3</sup> (1.5 lbs/yd<sup>3</sup>) Micromax (The Scotts Co., Marysville, OH). Plants were placed in a double layer polyethylene-covered greenhouse with 60% black shade cloth under natural photoperiod conditions at the Paterson Greenhouse Complex, Auburn University, AL (32°36'N, 85°29'W, USDA Hardiness Zone 8a). Maximum photosynthetically active radiation in the greenhouse was 600 μmol/m<sup>2</sup>/s (3000 ft-c.) and daily maximum/minimum temperature in the greenhouse was 27 ± 6C (80 ± 10F)/18 ± 3C (65 ± 5F). All pots were watered by hand daily and fertilized at 100 ppm twice weekly using Pro-Sol 20-20-20 water-soluble fertilizer (Pro-Sol, Ozark, AL) for 30 days.

On June 1, 2006, stem cuttings of New Guinea impatiens were prepared as 2.5 cm (1 in) long, single-node terminal cuttings with 2 fully-expanded leaves and 3-4 immature leaves and 1.2 cm (0.47 in) of stem below the basal node. Cuttings were inserted into 36-cell trays filled with Fafard 3B and placed under a greenhouse mist system with municipal tap water for 6 s every 16 min during daylight hours for a rooting period of 14 days. After early root formation, plants were moved from the intermittent mist bed to a bench and

watered by hand daily and fertilized twice per week using Pro-Sol 20-20-20 water-soluble fertilizer applied as a drench at a rate of 100 ppm N for 45 days.

After establishment, 30 uniform plants of each species selected were shaken to dislodge substrate particles from root system on March 1 and August 2, 2006, respectively. The root system was rinsed gently under the tap until 99% of substrate particles were removed. Then the root system was submerged in the deionized water in a plastic container [57.15 cm (22.5 in) long × 37.47 cm (14.75 in) wide × 13.65 (5.375 in) cm high] and gently agitated by hand to further dislodge fine substrate particles.

*Hydroponic acclimation.* To acclimate to the hydroponic condition before the onset of hydroponic study, plants were transferred into 710-ml (24 fl oz) plastic containers (Ziploc<sup>®</sup> Brand Containers with Snap 'n Seal Lids, S.C. Johnson & Son, Inc., Racine, WI) (one plant per container) containing 600 ml (20 fl oz) modified Hoagland solution (9) in an environmental growth chamber (Model E15, Conviron Controlled Environments, Inc., Asheville, N.C.). Modified Hoagland solutions were mixed and allowed to stand for 24 h to allow for the nutrients to completely dissolve and the pH to stabilize. Solutions were then adjusted using 0.5 M NaOH to pH 6.5–6.7 measured by a Model 63 pH and conductivity meter (YSI Incorporated, Yellow Springs, Ohio). Two holes were drilled on each container lid, with one [5 cm (1.97 in) diameter] for the plant and the other [1-cm (0.39-in) diameter] for an airline tubing [0.7 cm (0.275 in) outside diameter]. Plants were sitting in containers and held upright in the lids and supported by polyester batting in the holes of container lids. Hoagland solutions were continuously aerated via 2.8-cm (1.10-in) long air stone (Aqua Culture<sup>®</sup> Aquarium Bubble Stone, Wal-Mart Stores, Inc.,

Bentonville, AR) by 120-V Aquarium air pumps (Aqua Culture<sup>®</sup> Aquarium Air Pump, Model MK-1504, Wal-Mart Stores, Inc., Bentonville, AR). Plastic containers and lids were totally covered with aluminum foil to produce healthy roots and discourage growth of algae. Hoagland solution was changed every two days to further prevent algae growth and reduce the risk of fungal contamination (20). The growth chamber was set with a 12-h photoperiod provided by fluorescent lamps at a light intensity of 400  $\mu\text{mol}/\text{m}^2/\text{s}$  (2000 ft-c.). All plants were maintained at day/night temperatures of 28/22C (82/72F) and under atmospheric CO<sub>2</sub> levels. A relative humidity was set at 40%. Plants were grown in this condition for two weeks.

For each species, 24 plants were further selected based on same height, leaf number and root length prior to the trial. Four of these plants were randomly selected and oven-dried at 70C (158F) for 3 days until a constant dry mass was obtained and whole plant dry weights were recorded as plant initial dry weight. Fresh weight of the other 20 plants was measured for the trial.

*Hydroponic studies.* Twenty peace lilies and New Guinea impatiens were grown hydroponically for 31 days in the same growth chamber from March 15 and August 17, 2006, respectively. Experiment designs were completely randomized designs. Five treatments were Hoagland solutions added with five Tween<sup>®</sup> 20 (Monomer-Polymer & Dajac Labs, Inc., Featerville, PA) concentrations at 0 (control), 50, 75, 100, and 125 ppm, respectively. There were 20 total containers. In each treatment, four containers were filled with 600 ml (20 fl oz) Tween 20-modified Hoagland solutions, and four randomly

selected plants were assigned into four containers with one plant each. Twenty containers were completely randomized in the growth chamber.

Guttation was observed on peace lily during night. Guttation fluid was collected from leaf tips carefully by means of a handheld pipette into a sealed bottle at 4-h intervals. Drops were combined to produce a single sample per plant and stored in the refrigerator at 4C until measurement. Prior to harvest, leaf greenness was estimated nondestructively using a portable chlorophyll meter (SPAD-520; Minolta Camera Co., Ltd., Tokyo, Japan) (32). Four readings per plant were taken on the separate leaves in the canopy with values expressed in SPAD units. Plant fresh weight was measured immediately after being wiped by paper towel until no water was visible on the plant surface. At the conclusion of the study, whole plant dry weight was measured after oven-drying at 70C for 3 days until a constant dry mass was obtained. Total water used by plants was determined by measuring solution volume left in the containers whenever solutions were changed. For New Guinea impatiens, the total water loss was the transpiration amount. For peace lily, the transpiration amount was the total water used minus the guttation volume. Water use efficiency (WUE, g/liter) was determined as described by Raven et al. (18):

$$\text{WUE} = (W2 - W1) / \text{total water used amount};$$

Relative growth rate (RGR, g/g/d) was calculated as:

$$\text{RGR} = (\ln W2 - \ln W1) / T$$

where W1 and W2 were whole-plant dry weights at the beginning and end of experiment, respectively (7, 10). T was time interval, 31 days.

*Statistics.* The hydroponic studies of peace lilies and New Guinea impatiens were repeated during May 1 ~ June 1 and Oct. 1 ~ Nov. 1, 2006, respectively. For each species, data of two runs was pooled, because there were no effects on runs and the interaction between treatments and runs. Data were tested for homogeneity of variance and normality of distribution (17). One-way factorial analysis of variance (ANOVA) was performed using GLM procedure in SAS for Windows v.9.1 (SAS Institute Inc., Cary, NC). Any statistical test with p-value  $\leq 0.05$  was considered as significant and reported as such. Means were separated by using Tukey's honestly significantly different (HSD) tests.

## **Results and Discussion**

Fresh weight of peace lily and New Guinea impatiens did not significantly differ among the treatments at the beginning of hydroponic study (one-way ANOVA for fresh weight of impatiens:  $F = 0.08$ ,  $df = 4, 35$ ,  $P = 0.9892$ ; fresh weight of peace lily:  $F = 1.36$ ,  $df = 4, 35$ ,  $P = 0.2662$ ). Both species had similar growth response to Tween 20 and Tween 20 notably enhanced plant growth after 31-day growth in the controlled hydroponics system. Fresh weight of peace lily grown in Hoagland solutions with 50, 75, 100, and 125 ppm Tween 20 increased 17%, 23%, 33% and 22% (Table 1), while fresh weight of New Guinea impatiens increased 7%, 17%, 17% and 12% compared to the control, respectively. 100 ppm Tween 20 treated Hoagland solution increased total dry weight of peace lily and impatiens up to 33% and 18%, respectively (Table 1 and 2). RGR of peace lily and impatiens in 100 ppm Tween 20 treatment were the fastest, 0.021 and 0.016 g/g/d, 75% and 45% faster than that of control, the smallest plants grown in the normal Hoagland solution, respectively. There were no differences in leaf greenness

(SPAD chlorophyll readings) among treatments for either species, respectively (Table 1 and 2).

The beneficial effect of Tween 20 on plant growth is the highest at 100 ppm, around its critical micelle concentration (29). The reason of promoting growth by Tween 20 alone could be attributed to increasing hormone activities as mentioned above. Another possible reason is that Tween 20 stimulated nutrient absorption through modifying the properties of the cell membrane (31), or loading nutrient molecules into cell as membrane micro-reactors (1).

Guttation, normally occurring at high water absorption, high root osmotic pressure, and low transpiration (21), is the phenomenon that roots actively absorb ions into the root xylem creating a high osmotic pressure that forces water up the stem and out pores at the tips of leaves. The pattern of localization of guttation drops on peace lily is similar to that of taro (*Colocasia antiquorum*), mustard (*Sinapis alba*), barley (*Hordeum vulgare*), or cucumber, in contrast to tobacco (*Nicotiana tabacum*), potato (*Solanum tuberosum*), lettuce or bean (*Phaesoleus vulgarus*), where guttation drops form principally on the entire surface of younger leaves (5, 6, 12, 27, 28, 33). Guttation was observed only from the tips of young leaves of peace lily. No guttation was observed on the oldest leaf of the guttating plant kept in the same moisture chamber. The significant amount of guttation peace lilies exhibited at night over the course of the study indicated that plants had sufficient water available and healthy root systems grown in the Tween 20 treated and non-treated Hoagland solutions (Table 1). Plants in 100 ppm Tween 20 solutions guttated 301.44 ml (10.19 fl oz), 30% higher than that of control, 233.58 ml (7.90 fl oz), over the



course of study, but there were no differences in guttation between all treatments if dry leaf weight was considered. The main factors influencing guttation fluid production was leaf weight instead of the surfactant. Guttation accounted for about one-third of daily water use by peace lily. Guttation fluid was collected at the average rate of 2.4 ml/g leaf dry weight/day (0.08 fl oz/g leaf dry weight/day; about 6 ml/plant/day (0.20 fl oz/g leaf dry weight/day). This number is comparable to published guttation rates of bean [6  $\mu\text{l}/\text{cm}^2/\text{day}$  (0.015 minum/in<sup>2</sup>/day)](33), tobacco [1-2 ml/g leaf dry weight/day (0.03-0.07 fl oz /g leaf dry weight/day) or 5  $\mu\text{l}/\text{cm}^2/\text{day}$  (0.013 minum/in<sup>2</sup>/day)](12), and taro [10 ml/plant/day (0.34 fl oz /plant/day)](5).

Tween 20 significantly decreased transpiration of both species. Total transpiration of peace lily and New Guinea impatiens in 100 ppm Tween 20 solutions decreased 40% and 50% compared to the control, respectively. Consequently, WUE of peace lily and New Guinea impatiens in 100 ppm Tween 20 solutions were markedly increased 166% and 221%, respectively. Our results are consistent with Kubik and Michalczyk (13) and Ichimura et al. (11), who reported that Tween 20 markedly decreased transpiration when Tween 20 was sprayed on the leaf surface of strawberry, or dissolved in the vase solution of cut rose flower.

In conclusion, these hydroponic studies demonstrated that Tween 20 alone increased the growth of peace lily and New Guinea impatiens. Guttation amount from peace lily were more related to plant weight than Tween 20. Tween 20 increased WUE and RGR, decreased plant transpiration rate and total water needed to produce crops. Our

results with peace lily and New Guinea impatiens indicated 100 ppm would be recommended for growers in irrigation solutions or hydroponics during production.

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Table 1. Effects of different Tween 20 concentrations on fresh and dry weight, SPAD reading (greenness), total water used amount, guttation, guttation /leaf dry weight/day, transpiration, fresh weight increase, water use efficiency (WUE), and relative growth rate (RGR) of *Spathiphyllum floribundum*

‘Viscount’ grown hydroponically after 31 days.

Tween 20 (ppm)	Fresh weight (g)	Dry weight <sup>z</sup> (g)	SPAD value <sup>y</sup>	Total water used amount <sup>x</sup> (ml)	Guttation <sup>w</sup> (ml)	Guttation/leaf dry weight/day (ml/g/d)	Transpiration <sup>v</sup> (ml)	Fresh weight increase (g)	WUE <sup>u</sup> (g/liter)	RGR <sup>t</sup> (g/g/d)
0 (control)	26.65d <sup>s</sup>	2.49d	52.95a	1481.4a	233.58d	2.42a	1247.82a	9.62d	0.50d	0.012d
50	31.07c	2.88c	54.10a	1367.4a	267.95c	2.40a	1099.45ab	14.58c	0.76cd	0.017c
75	32.89b	3.07b	55.50a	1278.9a	285.16b	2.39a	993.74ab	15.52b	0.94bc	0.019b
100	35.56a	3.31a	53.43a	1062.5a	301.44a	2.35a	761.06b	17.67a	1.33a	0.021a
125	32.47b	3.04b	53.93a	1143.2a	281.31b	2.38a	861.89ab	14.87bc	1.07ab	0.019b
F <sup>r</sup>	229.73	287.43	2.21	2.26	247.22	0.71	3.02	182.90	12.07	320.66
df <sup>q</sup>	4, 35	4, 35	4, 35	4, 35	4, 35	4, 35	4, 35	4, 35	4, 35	4, 35
p value	< 0.0001	< 0.0001	0.0881	0.0828	< 0.0001	0.59	0.0307	< 0.0001	< 0.0001	< 0.0001

<sup>z</sup>Whole plants were oven-dried at 70C until a constant dry mass was obtained.

<sup>y</sup>Leaf greenness of four recently matured leaves per plant using an SPAD 502 chlorophyll meter (Minolta Camera Co., Ramsey, NJ).

<sup>x</sup>The total water used amount was the sum of daily water used determined by measuring solution volume left in the containers when solutions were changed.

<sup>w</sup>Guttation was collected from leaf tips by a handheld pipette and transferred into a sealed bottle at 4-h intervals. Drops were combined to produce a single sample per plant.

<sup>v</sup>Transpiration amount was the total water used amount minus the guttation.

<sup>u</sup>WUE = (W2 – W1) / total water used amount, where W1 and W2 were whole-plant dry weights at the beginning and end of the study, respectively.

<sup>r</sup>RGR = (ln W2 – ln W1) / T, where T was time interval, 31 days.

<sup>s</sup>Mean separation within column by Tukey's studentized range (HSD) test ( $p \leq 0.05$ ;  $n = 8$ ).

<sup>t</sup>One-way factorial Analysis of variance was performed using GLM procedure in SAS.

<sup>q</sup>Degree of freedom.



Table 2. Effects of different Tween 20 concentrations on fresh and dry weight, SPAD reading (greenness), transpiration, fresh weight increase, water use efficiency (WUE), and relative growth rate (RGR) of *Impatiens hawkerii* 'Celebrate Salmon' grown hydroponically after 31 days.

Tween 20 (ppm)	Fresh weight (g)	Dry weight <sup>z</sup> (g)	SPAD value <sup>y</sup>	Transpiration <sup>x</sup> (ml)	Fresh weight increase (g)	WUE <sup>w</sup> (g/liter)	RGR <sup>v</sup> (g/g/d)
0 (control)	26.89b <sup>u</sup>	2.42b	46.11a	1228.40a	9.21d	0.58d	0.011b
50	29.01ab	2.60ab	44.86a	1013.40a	11.50c	0.88c	0.014ab
75	31.42a	2.83a	45.63a	853.40b	13.89ab	1.32b	0.016a
100	31.83a	2.84a	45.31a	609.65c	14.05a	1.86a	0.016a
125	30.23a	2.72a	45.34a	760.90b	12.53bc	1.34b	0.015a
F <sup>t</sup>	8.13	7.95	0.58	27.32	32.02	42.62	8.08
df <sup>s</sup>	4, 35	4, 35	4, 35	4, 35	4, 35	4, 35	4, 35
P value	< 0.0001	0.0001	0.6791	< 0.0001	< 0.0001	< 0.0001	0.0001

<sup>z</sup>Whole plants were oven-dried at 70C until a constant dry mass was obtained.

<sup>y</sup>Leaf greenness of four recently matured leaves per plant using an SPAD 502 chlorophyll meter (Minolta Camera Co., Ramsey, NJ).

<sup>x</sup>Transpiration was the sum of daily water used determined by measuring solution volume left in the containers when solutions were changed.

<sup>w</sup>WUE = (W2 – W1) / total water used amount, where W1 and W2 were whole-plant dry weights at the beginning and end of the study, respectively.

<sup>v</sup>RGR = (ln W2 – ln W1) / T, where T was time interval, 31 days.

<sup>u</sup>Mean separation within column by Tukey's studentized range (HSD) test (p ≤ 0.05; n = 8).

<sup>t</sup>One-way factorial Analysis of variance was performed using GLM procedure in SAS.

<sup>s</sup>Degree of freedom.

## V. EFFECTS OF AN IRRIGATION-APPLIED SURFACTANT ON GROWTH OF PEACE LILY AND THE ASSOCIATED COST ESTIMATION

### **Abstract**

Greenhouse and container nursery plant production requires large amount of high quality irrigation water. The availability of groundwater or public surface water by greenhouse and container nurseries are declining significantly in the new millennium, especially during unavoidable and unpredictable drought events. This study investigated the effects on growth and development of peace lily (*Spathiphyllum floribundum* ‘Viscount’) where the surfactant Tween 20 was continually injected into each irrigation event. Plants grew equally with as little as 60% irrigation compared to 100% irrigation when the reduced water regime included Tween 20 at  $75 \text{ mg}\cdot\text{L}^{-1}$  or higher concentrations. Cost estimation indicated the minimal increase in cost from Tween 20 was offset by decreased water costs based on current municipal water costs. Such decrease of water usage could be critical to maintain normal production capacity when water availability is severely reduced by government regulations or during extended drought.

**Index words:** irrigation management, chemigation, deficit irrigation, wetting agent, greenhouse production, economic analysis, Tween 20, adjuvant.

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

### **Significance to the Nursery Industry**

Irrigation management is an important issue in nursery crop production. Over the last 50 years, awareness of the importance of reducing water use in nurseries has increased. Luxurious irrigation with low irrigation efficiency are facing challenges, such as more frequent droughts, stricter regulations on nursery water use and nutrient release into public water system, and tougher competition for water resources with other sectors in large cities for some area. Nursery growers and researchers are searching for practical methods to reduce water use and maintain optimal plant growth and production at the same time. In this study we added a low concentration surfactant Tween 20 into the drip irrigation system to maintain growth of ‘Viscount’ peace lily while reducing water use by 40% when compared with recommended BMP irrigation amount to market size. Cost analysis indicated that at current city water prices, the increased cost of surfactant application was only a fraction of total operating cost. This study indicates a potential low cost, easily operated method to save a significant amount water when availability of water is dramatically reduced, such as during a drought.

### **Introduction**

Greenhouse and container nursery plant production requires large amount of high quality irrigation water. However, the availability and consumption of groundwater or public surface water by greenhouse and container nurseries will decline significantly in

the coming decade in many areas (Beeson et al., 2004; Beeson and Knox, 1991). Drought events, such as the unprecedented drought in the Southeast U.S. in 2007 (Fuchs, 2008), are likely to happen in an increased frequency and severity as a major impact of global change (IPCC, 2007). Severe drought often results in a significantly reduced amount of water allocated to nursery crops and dramatic decrease in sales (Muñoz-Carpena et al., 2003).

In order to increase water use efficiency in nursery production, various methods and technologies are used or proposed, such as grouping of plants according to water requirements and plant or container sizes, increasing the water holding capacity of substrates, increasing irrigation application uniformity, better irrigation system design, better scheduling of irrigation, and recycling or recirculation of water from collection structures (Beeson et al., 2004).

Due to limited volume of substrate and therefore rapid exposure to drought, crops grown in containers, whether in a greenhouse or on an outdoor container pad, are irrigated frequently and opulently to prevent possible water limitation to optimum crop growth. Regulated deficit irrigation (RDI) is an irrigation management strategy to improve water productivity and can be effectively applied in many agronomic crops and fruit trees (English and Raja, 1996; Intrigliolo and Castel, 2005; Playán and Mateos, 2006). However, largely due to above-mentioned limited substrate volume and high value of ornamental crops, RDI has only been tested in production of a limited number of ornamental crops (Cameron et al., 1999, 2006; Chimonidou-Pavlidou, 2004; Devitt et al., 1994; Fereres et al., 2003; Ku and Hershey, 1997). RDI was found to have no negative

effect on vegetative growth to significantly reduce growth, depending on degree and timing of drought imposed on crops (Cameron et al., 2006; Chimonidou-Pavlidou, 2004; Ku and Hershey, 1997). To a low degree of drought, RDI can be used to substitute pruning for some crops and improve crop quality (Cameron et al., 1999, 2006). With the prospect of severely reduced supply of irrigation water during drought, RDI will likely increase its application in ornamental crop production.

Surfactants have been widely used in potting substrates to improve water penetration and rewetting properties of hydrophobic substrate components and resulting in increased moisture retention (Bilderback and Lorscheider, 1997; Guillen, et al., 2005). While many studies reported the effectiveness of using surfactants to increase moisture retention in potting substrates, there have been few studies evaluating the effects of surfactants on plant water use efficiency and plant growth (Blodgett et al., 1993; Guillen et al., 2005). A few studies have reported decreased transpiration rate after spraying surfactants on leaves (Kubik and Michalczyk, 1993), or whole plants (Manthey and Dahleen, 1998), or after keeping cut flowers in a surfactant solution (Ichimura et al., 2005). Such a decrease in transpiration rate could be economically and scientifically significant if photosynthetic rate is not negatively affected.

We hypothesized that mass flow can transport certain types of small surfactant molecules from roots to other organs of the plant. Then such surfactant molecules can affect plant physiological processes, and one possible effect is decreased transpiration rate observed by Kubik and Michalczyk (1993) and Manthey and Dahleen (1998). A

novel method is to apply such surfactants through irrigation, which would require little or no change of current ornamental crop production system.

Based on surfactant properties and previous study results, a nonionic commercial surfactant Tween<sup>®</sup> 20 (polyoxyethylene (20) sorbitan monolaurate, C<sub>58</sub>H<sub>114</sub>O<sub>26</sub>, MW = 1227.54, Monomer-Polymer & Dajac Labs, Inc., Featerville, Pa.) was selected to examine its effects on growth of peace lily (*Spathiphyllum floribundum* ‘Viscount’) receiving different irrigation amounts. The production cost associated with application of Tween 20 through irrigation was estimated to find the economic feasibility of this new irrigation method.

## **Materials and Methods**

Two experiments were conducted using peace lily tissue culture liners from 72-cell plug trays obtained from Agri-Starts, Inc., Apopka, FL (Table 1). Upon receipt, liners were planted into 15.2 cm (6 in) azalea pots (one liner per pot). Pots were filled with Fafard 3B Mix (Conrad Fafard, Inc., Agawam, MA), incorporated with 6.6 kg/m<sup>3</sup> (11 lbs/yd<sup>3</sup>) controlled-release fertilizer 18N-2.6P-10K (Polyon 18-6-12, 8-9 mo., Pursell Technologies Inc. Sylacauga, AL) and 0.6 kg/m<sup>3</sup> (1 lb/yd<sup>3</sup>) Micromax (Scotts Co., Marysville, OH). Plants were grown in a double layer polyethylene-covered greenhouse at the Paterson Greenhouse Complex, Auburn University, AL (32° 36’N × 85° 29’W) under 60% shade cloth and hand-watered as needed until treatments started.

*Expt. 1.* Peace lily liners were planted on Nov. 18, 2005. On Dec 21, 2005, 360 plants were randomly assigned to treatments according to a split-plot design (Table 1). The split-plot design included two blocks; within each block, six Tween 20 concentrations of

irrigation water (factor A) were randomly assigned to whole plots (main plots) and five irrigation amounts (factor B) were randomly assigned to split plots (subplots) within each whole plot. Six Tween 20 concentrations were 0, 25, 50, 75, 100, and 125 mg·L<sup>-1</sup>. Five irrigation amounts were 100%, 90%, 80%, 70%, and 60% of pre-determined plant water requirements. The 100% irrigation amount was the irrigation amount that resulted a leaching fraction of 0.2 (leaching fraction, LF = irrigation water leached ÷ irrigation water applied) for treatment of 0 mg·L<sup>-1</sup> Tween 20 (control). LF was determined twice a week (Monday morning and Thursday or Friday morning) and an irrigation controller (Rain Bird Corp., Tucson, AZ) was set for the next three or four day's irrigation.

Each split plot included 6 pots of peace lily as replications. Thus, the design structure for factor B (irrigation amount) within each level of factor A (Tween 20 concentration) was one-way completely randomized design (CRD) with r = 6 replications.

Irrigation water was applied in the morning (0700HR) with a single pressure compensating drip emitter evenly distributed on irrigation pipes (flow rate coefficient of variation ≤ 3%; Toro AG Irrigation, El Cajon, CA). The irrigation pipes had pressure ~ 50 psi (recommended pressure range of emitters: 8 – 60 psi) and irrigation volumes were controlled by a combination of flow rates and irrigation times. Tween 20 was delivered to irrigation pipes by Dosatron liquid dispensers (Dosatron International Inc., Clearwater, FL) to deliver different concentrations of Tween 20 stock solutions. For control (0 mg·L<sup>-1</sup> Tween 20), tap water was used to substitute stock solution.

Plants were under treatments for a total of 11 weeks. Every three weeks, plants were drenched with tap water for two days to avoid salts and Tween 20 accumulation.

Plant growth was measured by taking plant height, width at widest point, and width perpendicular to width at widest point. Growth index (GI) was calculated as (height + width at widest point + width perpendicular to width at widest point)/3. Growth measurement was taken on 0 (date of treatments started), 7, and 11 weeks after treatments started. Leaf greenness (chlorophyll content) was nondestructively measured on the youngest, fully developed leaves with a portable chlorophyll meter (SPAD-502) (Minolta Camera Co., Japan). Three replicate measurements from leaves in the canopy of each plant were averaged and expressed in SPAD units.

*Expt. 2.* This experiment was a repetition of *Expt. 1* with several modifications. Three irrigation amounts were used: 100%, 80%, and 60%. Four Tween 20 concentrations were used: 0, 50, 100, and 125 mg·L<sup>-1</sup>. Three blocks were used instead of two. Peace lily liners were transplanted in pots on Jan. 16, 2007 and under normal culture conditions for a total of 6 weeks. On Feb. 27, 2007, Peace lilies with uniform growth were subjected to Tween 20 and irrigation amount treatments as described in *Expt. 1*.

Plants were under treatments for a total of 14 weeks. Plant growth (height and GI) was measured on 0 and 14 weeks after treatments started. Leaf greenness was measured in the 14<sup>th</sup> week after treatments started.

*Cost Estimation.* A partial budget analysis was used to evaluate the economic trade-offs between conventional irrigation and irrigation with Tween 20 surfactant in the water. Partial budgeting is a standard technique to evaluate the economics of a change in a farm enterprise and is frequently used to estimate the impact of a variety of alternative



production techniques when the change involves only part of the production system (Schuch et al., 2008; Sydorovych et al., 2006).

Cost was estimated using a hypothetical middle-scale wholesale greenhouse grower with gutter-connected greenhouses located in middle Alabama. The model grower had a crop mix that allocated greenhouse space to grow 1,000 ‘Viscount’ peace lily as we experimented in this study. Fixed costs for the conventional irrigation production of peace lily included depreciation (greenhouse, equipment, and machinery), indirect labor, advertising, insurance, tax, interest, repairs, supplies, travel and utilities, etc. Variable costs included water cost, cost of direct material other than water (peace lily liner, pot, substrate, fertilizer, pesticide, and plant growth regulator), direct labor, and interest on variable cost.

We assumed that the crop mix was in full production and the 1,000 ‘Viscount’ peace lily were grown for a total production period of 20 weeks. The first 6 weeks were pot-to-pot arranged and each pot occupied a space of  $0.02323 \text{ m}^2$  ( $0.5 \times 0.5 \text{ feet}^2$ ); no surfactant was used and all plants received same culture practice. From 7<sup>th</sup> week, pots were spaced to  $0.09290 \text{ m}^2$  ( $1 \times 1 \text{ feet}^2$ ) and surfactant was applied through irrigation water until product finish for 14 weeks. The cost change of the alternative approach with surfactant treatment was cost of Tween 20 and reduced water cost.

The cost of production with Tween 20 applied through irrigation water included all above costs plus cost of Tween 20. Injectors for Tween 20 delivery were regarded as standard greenhouse equipment and regarded a part of fixed cost. Costs of materials were lowest market price as of 2007 and a discount of 5% to 10% was included for some of the

materials according to supplier's policy. However, we didn't factor any further discount which is possible for wholesale growers with purchasing in bulk. For water cost, we assumed three scenarios: \$1/m<sup>3</sup> (Scenario I), which represents the average city water price of U.S.A. in 2007, \$5/m<sup>3</sup> (Scenario II), which represents very high price and a possible price under certain conditions for some areas, and \$50/m<sup>3</sup> (Scenario III), which represents extreme high price and is not likely to be a real price in any place in foreseeable future. These three water price scenarios were used to describe how water costs can affect the total cost of greenhouse crop production.

*Statistics.* The data were analyzed using the mixed model procedures for split-plot experiments (Littell et al., 1996) using SAS for Windows v.9.1 (SAS Institute Inc., Cary, NC). Any statistical test with *p*-value < 0.05 was considered as significant and reported as such where appropriate.

## **Results and Discussion**

*Expt. 1.* Peace lily plants had uniform height ( $\bar{x} = 11.40$  cm) and growth index ( $\bar{x} = 14.65$  cm) when treatments started on Dec. 16, 2005. After 7 weeks, no interaction on plant height between Tween 20 concentration and irrigation levels was observed ( $P > 0.05$ ) and the height was not significantly affected by irrigation amounts ( $P > 0.05$ ). In contrast, the effect of Tween 20 treatment on plant height was significant after 7 weeks (Table 2), as the height was 12.51, 12.31 cm with treatment of 0, 25 mg·L<sup>-1</sup> Tween 20, respectively, and was significantly lower than height of 13.31, 13.55 cm with treatment of 50, 75 mg·L<sup>-1</sup> Tween 20, respectively. However, there was no statistical difference in height between plants receiving lowest concentrations of Tween 20 (0 and 25 mg·L<sup>-1</sup>) and the

highest Tween 20 concentration (125 mg·L<sup>-1</sup>). After 7 weeks, the interaction on plant growth index was significant ( $P < 0.05$ ). Growth index was significantly smaller in the treatment receiving 60% water than the other four irrigation levels (70% to 100%) under 0 mg·L<sup>-1</sup> Tween 20 (Table 3), while no irrigation effect was observed in the other 5 Tween 20 concentrations (25 to 125 mg·L<sup>-1</sup>). The effect of Tween 20 was significant only when receiving 60% water, as the plants with 50 to 125 mg·L<sup>-1</sup> Tween 20 had larger GI than plants without Tween 20 (Table 3). GI of plants (19.31 and 19.28 cm) with 50 and 75 mg·L<sup>-1</sup> Tween 20 was about 20% larger than GI of plants (15.44cm) without Tween 20.

At 11 weeks after treatments started, plant height was significantly affected by two main effects (Table 4). Plants receiving 60% water had significantly lower height (17.16 cm) than plants receiving more water (heights from 18.03 to 18.22 cm). However, such height difference at the lowest irrigation level (60%) disappeared once plants were treated with Tween 20 and significant height difference was only in the treatment without Tween 20 or plain water, as plant height with 60% water (12.41 cm) was 37% to 45% lower than that with 70% to 100% water (Table 4, column of 0 mg·L<sup>-1</sup> Tween 20). Plant height was similarly affected by Tween 20 treatment, as plants with 0 mg·L<sup>-1</sup> Tween 20 was lower than plants with Tween 20 from 25 to 125 mg·L<sup>-1</sup> and such Tween 20 effect was only significant when receiving 60% water (Table 4, row of 60% irrigation level).

At 11 weeks after treatments started, plant GI was similarly affected by irrigation and Tween 20 levels (Table 5). GI was the lowest at 60% irrigation level which is largely attributed to the GI difference in treatment of 0 mg·L<sup>-1</sup> Tween 20 (17.33 cm in 60%

irrigation level and 26.21 – 27.31 cm in 70% - 100% irrigation levels). Similarly to height, plant GI was only affected by Tween 20 treatment when receiving the lowest irrigation level 60%.

*Expt. 2.* When treatments started 6 weeks after peace lily liners transplanted into pots, all plants had uniform growth (height average: 11.13cm; GI average: 15.68 cm). After 14 weeks under treatment, no interactions on height or GI between irrigation levels and Tween 20 concentrations were observed and the main effects of irrigation and Tween 20 on height and GI are presented in Table 6. Plants with 100% and 80% water had larger height than plants with 60%. With increasing irrigation levels, the GI increased accordingly from 34.6 cm to 38.0 cm. Tween 20 had similar effect on height and GI: plants with 100 mg·L<sup>-1</sup> Tween 20 had greater height and GI than plants with 0 and 50 mg·L<sup>-1</sup> Tween 20 (Table 6).

With 100% irrigation amount pots had LF of 0.2, the LF was 0.1, 0, -0.1 (theoretical), -0.2 (theoretical) for 90%, 80%, 70%, and 60% irrigation amount, respectively. LF of 0.2 is a recommended BMP water application amount which will ensure enough water and at the same time prevent salt accumulation. However, in *Expt. 1*, height of peace lilies was not affected by irrigation amounts 7 weeks after treatments started and regardless of Tween 20 treatment, both height and GI was the same for plants receiving as low as 70% (LF: -0.1) as that of 100% irrigation amount 11 weeks after treatments started. At 60% irrigation amount, GI was significantly lower when receiving no Tween 20 and /or low Tween 20 concentration (Table 3 – 5). When plants were under treatments longer, negative effects of less irrigation on GI were present in plants with 80%

irrigation amount (Table 6). In the meantime, plants had better growth when Tween 20 concentration was greater than  $50 \text{ mg}\cdot\text{L}^{-1}$ . Above results indicated that the negative effect of 0 or slight negative LF (-0.1 or 70% in *Expt. 1* and 2) may only be observed for a certain period after treatments started. We also observed that substrates with Tween 20 in irrigation water was obviously wetter than substrates with no Tween 20 at the same irrigation amount. Such higher wetness was attributed to two factors: 1) the wetting properties of surfactant, and 2) reduced transpiration rate. The second notion was supported by our measurements of leaf physiology (data not shown).

*Cost estimation.* Based on results of *Expt. 1* and 2, we chose  $100 \text{ mg}\cdot\text{L}^{-1}$  Tween 20 as the concentrate to estimate production cost.

The hypothetical grower was estimated to have a fixed cost of  $\$1.61/\text{m}^2$  per week ( $\$0.15/\text{feet}^2$  per week). The total fixed cost to grow 1,000 peace lily for 6 weeks from liner transplanting into pots was \$225 for all three water price scenarios (Table 7). Water cost for this period was increased from \$3 in Scenario I to \$16 in Scenario II and \$80 in Scenario III. However, the total cost under Scenario II was only increased 0.8%; but under Scenario III the total cost was increased by 5.1%.

For the second stage, the fixed cost for growth of 1,000 peace lily for 14 weeks was \$2,100. The conventional production method without surfactant in irrigation water had a variable cost of \$2,153 in Scenario I, which was increased to \$2,194 in Scenario II and \$2,666 in Scenario III. The total cost of conventional production for second stage was increased from \$3,737 in Scenario I to \$3,802 in Scenario II (1.5% increase) and to \$4,341 (15.9% increase) for Scenario III.

With surfactant Tween 20 applied in the irrigation water and using 60% of irrigation volume of the conventional method, the total variable cost was increased from \$2,153 to \$2,166 in Scenario I and the total cost was increased from \$3,747 to \$3,760 (0.3% increase). However, the total cost was decreased slightly from \$3,802 to \$3,799 (< 0.1% decrease) and from \$4,341 to \$ 4,149 (4.4% decrease) under Scenario I and II, respectively.

Our results indicate that water cost was a small fraction compared with other costs even when growers are paying city water cost. In an unlikely Scenario III, when water fee increased 50 fold of normal level, the total cost was increased 15.9% at most with conventional irrigation methods, a cost increase that could be absorbed by the market under such extraordinary condition.

In fact, a majority of nursery growers (all 3 types of greenhouse, container and field nurseries) use wells instead of city water (Muñoz-Carpena et al., 2003) with growers generally only paying for pumping equipment and electricity of pumping. And yet, most growers suffer dramatic loss in sales during drought as showcased by a survey of Miami-Dade county, FL (Muñoz-Carpena et al., 2003). An important conclusion is that when facing drought, the availability of water and water use efficiency will be the key to the success of nursery business and a water cost hike is probably an effective marketing leverage to distribution limited water resources. In the meantime, proper use of surfactant like Tween 20 into irrigation has the potential to maintain same production capacity when significantly reduced water amounts are available to use.

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Table 1. Experiment setup of peace lily (*Spathiphyllum floribundum* ‘Viscount’) growth in a split-plot design with Tween 20 concentrations as whole plots and irrigation amounts as split plots.

	<i>Expt. 1</i>	<i>Expt. 2</i>
Whole plot levels	6: 0, 25, 50, 75, 100, 125 mg·L <sup>-1</sup>	4: 0, 50, 100, 125 mg·L <sup>-1</sup>
Split plot levels	5: 100%, 90%, 80%, 70%, 60% <sup>z</sup>	3: 100%, 80%, 60%
Blocks used	2	3
Repetition used	6	6
Liner receipt date	Nov. 18, 2005	Jan. 16, 2007
Treatment starting date	Dec. 16, 2005	Feb. 27, 2007
Data collection date	0, 7, 11 WAT <sup>y</sup>	0, 14 WAT

<sup>z</sup> 100% irrigation amount was the irrigation amount required to have 15% leaching fraction (leaching fraction = irrigation water leached ÷ irrigation water applied) for the treatment with 0 mg·L<sup>-1</sup> Tween 20 (control).

<sup>y</sup> WAT: week(s) after treatment started.

Table 2. Height (cm) of peace lily (*Spathiphyllum floribundum* ‘Viscount’) seven weeks after treatment started due to Tween 20 effect of *Expt. 1*.

Tween 20 (mg·L <sup>-1</sup> )	Height <sup>z</sup>
0	12.51 c
25	12.31 c
50	13.31 ab
75	13.55 a
100	12.70 bc
125	12.61 c

<sup>z</sup> LS Means of height with different letters in the same row are statistically different at  $\alpha = 0.05$ .

Table 3. Growth Index (cm) of peace lily (*Spathiphyllum floribundum* ‘Viscount’) seven weeks after treatments started due to interaction between Tween 20 and irrigation of *Expt. 1*.

Tween 20 (mg·L <sup>-1</sup> )		0	25	50	75	100	125
Irrigation	100%	18.58 a <sup>z</sup>	18.63	18.84	19.33	19.01	18.29
	90%	19.00 a	18.79	19.67	18.59	18.74	18.37
	80%	19.07 a	18.31	18.12	19.93	18.81	18.95
	70%	19.93 a	18.55	19.36	18.43	18.35	19.05
	60%	15.44 bC <sup>y</sup>	17.61 B	19.31 A	19.28 A	18.98 AB	18.74 AB

<sup>z</sup> Least squares means (LS means) with different lowercase letters within a column of the same Tween 20 concentration were significantly different at  $\alpha = 0.05$ .

<sup>y</sup> LS means with different uppercase letters within a row of the same irrigation amount are statistically different at  $\alpha = 0.05$ .

Table 4. Height (cm) of peace lily (*Spathiphyllum floribundum* ‘Viscount’) 11 weeks after treatments started due to Tween 20, irrigation and interaction between Tween 20 and irrigation of *Expt. 1*.

Tween 20 (mg·L <sup>-1</sup> )	0	25	50	75	100	125	Irrigation main effect <sup>x</sup>	
Irrigation	100%	17.96 a <sup>z</sup>	18.04	18.22	18.67	17.92	17.79	18.10 a
	90%	17.45 a	17.97	18.21	19.13	18.00	17.45	18.03 a
	80%	17.06 a	18.48	18.83	18.98	180.2	17.97	18.22 a
	70%	17.07 a	18.08	19.09	19.21	18.07	18.00	18.22 a
	60%	12.41 aB <sup>y</sup>	17.75 A	18.57 A	18.67 A	18.12 A	17.46 A	17.16 b
Tween 20 main effect <sup>w</sup>		16.39 C	18.06 AB	18.58 AB	18.93 A	18.03 AB	17.73 B	

<sup>z</sup> Least squares means (LS means) with different lowercase letters within a column of the same Tween 20 concentration were significantly different at  $\alpha = 0.05$ .

<sup>y</sup> LS means with different uppercase letters within a row of the same irrigation amount are statistically different at  $\alpha = 0.05$ .

<sup>x</sup> This column is the LS means of the main effect irrigation amount; LS means with different lowercase letters are statistically different at  $\alpha = 0.05$ .

<sup>w</sup> This row is the LS means of the main effect Tween 20 concentration; LS means with different uppercase letters are statistically different at  $\alpha = 0.05$ .

Table 5. Growth Index (cm) of peace lily (*Spathiphyllum floribundum* ‘Viscount’) 11 weeks after treatment started due to Water and interaction between Tween 20 and Water of *Expt.1* .

	Tween 20 (mg·L <sup>-1</sup> )	0	25	50	75	100	125	Irrigation main effect <sup>x</sup>
Irrigation	100%	26.26 a <sup>z</sup>	27.40	26.94	26.89	26.56	27.26	26.87 <sup>w</sup> a
	90%	26.50 a	27.04	28.64	27.12	27.14	26.91	27.27 a
	80%	26.21 a	26.76	27.73	27.52	27.28	27.35	27.14 a
	70%	27.31 a	27.4	27.68	27.69	27.63	27.32	27.51 a
	60%	17.33 bB <sup>y</sup>	25.81 <sup>y</sup> A	27.52 A	27.08 A	27.28 A	26.12 A	25.19 b

<sup>z</sup> Least squares means (LS means) with different lowercase letters within a column of the same Tween 20 concentration were significantly different at  $\alpha = 0.05$ .

<sup>y</sup> LS means with different uppercase letters within a row of the same irrigation amount are statistically different at  $\alpha = 0.05$ .

<sup>x</sup> This column is the LS means of the main effect of irrigation amount; LS means with different lowercase letters are statistically different at  $\alpha = 0.05$ .

Table 6. Height and growth index (GI) of peace lily (*Spathiphyllum floribundum* ‘Viscount’) 14 weeks after treatments started of *Expt. 2*.

Treatment	Height (cm) <sup>z</sup>	GI (cm)
<i>Irrigation</i> <sup>z</sup>		
100%	26.76 a	37.99 a
80%	25.26 a	35.71 b
60%	23.31 b	34.57 c
<i>Tween 20 (mg·L<sup>-1</sup>)</i>		
0	22.69 c	33.68 c
50	24.82 bc	35.33 bc
100	27.41 a	38.10 a
125	25.53 ab	37.25 ab
<i>p-value</i>		
Main effect		
<i>Irrigation</i>	0.0067	<.0001
<i>Tween 20</i>	0.0154	0.0064
<i>Interaction</i>	0.9331	0.9919

<sup>z</sup> Least squares means of height or GI with different letters within a column are statistically different at  $\alpha = 0.05$ , as indicated also by the *p*-value of the statistical test.

Table 7. Estimated cost (\$) of growing 1,000 peace lily (*Spathiphyllum floribundum* ‘Viscount’) for six weeks from liners (plugs) transplanting into 15.24 cm (6-in) azalea pots in a typical wholesale greenhouse grower with gutter-connected, double-polyethylene greenhouses in middle Alabama.

Cost	Six week cost		
	Scenario I <sup>z</sup>	Scenario II <sup>z</sup>	Scenario III <sup>z</sup>
<b>Fixed cost<sup>y</sup></b>	225	225	225
Variable cost			
Plug	608	608	608
Substrate	220	220	220
Pot	100	100	100
Fertilizer	173	173	173
Water <sup>x</sup>	3	16	155
Direct labor <sup>w</sup>	200	200	200
Interest on variable cost <sup>v</sup>	65	66	73
<b>Subtotal variable cost</b>	1,369	1,383	1,529
<b>Total cost<sup>u</sup></b>	1,594	1,608	1,754
<b>Total cost per pot</b>	1.594	1.608	1.754

<sup>z</sup> Water price Scenario I: \$1/m<sup>3</sup> per week; water price Scenario II: \$5/m<sup>3</sup> per week; water price Scenario III: \$50/m<sup>3</sup> per week.

<sup>y</sup> Fixed cost = \$0.15/ft<sup>2</sup> per week × 0.25 ft<sup>2</sup>/pot × 1000 pot × 6 week (or using SI unit: \$1.614586/m<sup>2</sup> per week × 0.02323 m<sup>2</sup>/pot × 1000 pot × 6 week; note: in the text, the SI fixed cost was written as \$1.61/m<sup>2</sup> per week due to rounding). Fixed cost of \$1.61/m<sup>2</sup> per week (\$0.15/ft<sup>2</sup> per week) was the cost generated from a typical wholesale greenhouse grower with gutter-connected, double-polyethylene greenhouses in middle Alabama in year of 2007. The calculation process was not presented here due to space limit.

<sup>x</sup> Water used per pot: 3100 ml.

<sup>w</sup> Labor cost at \$10/hr.

<sup>v</sup> Interest rate was calculated at 10% annual percentage rate for 6 months.

<sup>u</sup> Total cost = fixed cost + subtotal variable cost.

Table 8. Estimated cost (\$) of growing 1,000 peace lily (*Spathiphyllum floribundum* ‘Viscount’) for 14 and 20 weeks from liners of 11.13cm height to market size in 15.24 cm (6 in) azalea pots under unlimited water supply scenario by a typical wholesale greenhouse grower with gutter-connected greenhouses in middle Alabama<sup>z</sup>.

Cost	Conventional irrigation			60% irrigation amount with surfactant <sup>y</sup>		
	Scenario I <sup>x</sup>	Scenario II <sup>x</sup>	Scenario III <sup>x</sup>	Scenario I	Scenario II	Scenario III
<b>Fixed cost<sup>w</sup></b>	2,100	2,100	2,100	2,100	2,100	2,100
Variable cost						
Chemicals	10	10	10	10	10	10
Water <sup>y</sup>	10	50	499	6	30	300
Tween 20	0	0	0	7	7	7
Direct labor <sup>u</sup>	30	30	30	40	40	40
Interest on variable cost <sup>t</sup>	3	5	27	3	4	18
<b>Subtotal variable cost</b>	53	95	566	66	91	374
<b>subtotal cost<sup>s</sup> (14 wks)</b>	2,153	2,194	2,666	2,166	2,191	2,474
<b>subtotal cost per pot (14 wks)</b>	2.153	2.194	2.666	2.166	2.191	2.474
<b>Total cost from liner to market size<sup>f</sup> (20 wks)</b>	3,747	3,802	4,420	3,760	3,799	4,228
<b>Total cost per pot from liner to market size (20 wks)</b>	3.747	3.802	4.420	3.760	3.799	4.228

<sup>z</sup> The cost of first six weeks was calculated on Table 6. The plant height of 11.13 cm was the average height of peace lily at the end of six weeks after liners transplanted into azalea pots as in *Expt. 2*.

<sup>y</sup> Surfactant was applied at 100 mg·L<sup>-1</sup>.

<sup>x</sup> Water price Scenario I: \$1/m<sup>3</sup> per week; water price Scenario II: \$5/m<sup>3</sup>; water price Scenario III: \$50/m<sup>3</sup>.

<sup>w</sup> Fixed cost = \$0.15/ft<sup>2</sup> per week × 1 ft<sup>2</sup>/pot × 1000 pot × 14 week (or using SI unit: 1.614586/m<sup>2</sup> per week × 0.09290m<sup>2</sup>/pot × 1000 pot × 14 week; note: in the text, the SI fixed cost was written as \$1.61/m<sup>2</sup> per week due to rounding). Fixed cost of \$1.61/m<sup>2</sup> per week (\$0.15/ft<sup>2</sup> per week) was the cost



generated from a typical wholesale greenhouse grower with gutter-connected, double-polyethylene greenhouses in middle Alabama in year of 2007. The calculation process was not presented here due to space limit.

<sup>v</sup> Water volume: 9986 ml/pot for conventional irrigation; 5992 ml/pot for 60% irrigation amount with surfactant application.

<sup>u</sup> Labor cost at \$10/hr.

<sup>t</sup> Interest rate was calculated at 10% annual percentage rate for 6 months.

<sup>s</sup> Subtotal cost = fixed cost + subtotal variable cost.

<sup>r</sup> Total cost from liner to market size = cost of six weeks from liner transplanting into azalea pots (from Table 7) + cost of 14 weeks from the week of surfactant application to market size (from this table).

## VI. FINAL DISCUSSION

Few studies have reported that surfactants reduce plant water use by plant itself. With water availability more of a concern for the horticultural industry, the objective of this research was to find an effective surfactant, or an irrigation additive to reduce plant water use. During the comparison and selection of surfactants, Tween 20 proved to be an excellent choice for nursery crops, in part because Tween 20 is cheap, safe, water-soluble, nonionic, and has a small molecule with low CMC. And a few studies reported Tween 20 promotes plant growth and reduce plant transpiration. While now in the market, surfactants, which routinely added to potting substrates to increase moisture retention, have no beneficial effects on plant growth and cause plant injury at high concentrations. Therefore, Tween 20 has a high potential to be an effective irrigation additive in the market.

In the soil column study of substrate hydrological characteristics, moisture retention of Fafard 3B treated with  $100 \text{ mg}\cdot\text{L}^{-1}$  Tween 20 solution increased 40.5% compared to that of the control (water) at initial wetting. It demonstrated that Tween 20 is efficient to reduce surface potential in the Fafard 3B at low concentration.

In the studies of plant water use in the greenhouse or in the outdoor containers, for New Guinea impatiens or peace lily, water use amount was significantly reduced and transpiration rate decreased with the application of Tween 20. Growth of impatiens in outdoor containers in the treatment combinations of 50, 75, 100, or  $125 \text{ mg}\cdot\text{L}^{-1}$  Tween 20

at the 20% or 40% irrigation levels were similar to those of the control over the course of the experiment. In the greenhouse study, New Guinea impatiens irrigated with Tween 20 from 25 to 125 mg·L<sup>-1</sup> at the 40% or 60% irrigation level had the same height and growth index as plants in the control after three months of growth. With Tween 20 concentration increased from 0 to 100 mg·L<sup>-1</sup> 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%.

Hydroponic study was carried out to determine how Tween 20 would affect photosynthesis and transpiration by Tween 20 per se, without interaction with substrate moisture. Tween 20 increased total dry weight of peace lily and impatiens up to 33% and 18%. Relative growth rates of peace lily and impatiens in 100 ppm Tween 20 treatment were the fastest. Less water was transpired for both species treated with Tween 20. Guttation accounted for about one-third of the total water use of peace lily. Compared with the control, water use efficiency of peace lily and New Guinea impatiens in 100 ppm Tween 20 solutions were markedly increased 166% and 221%, respectively.

Cost estimation indicated the minimal increase in cost from Tween 20 was offset by decreased water costs based on current municipal water costs. Such decrease of water usage could be critical to maintain normal production capacity when water availability is severely reduced by government regulations or during extended drought.

Each study demonstrated that Tween 20 is a unique irrigation additive with three-fold effects of increasing water retention of substrate and decreasing the amount of water required while simultaneously increasing plant growth when compared to the control.

Further research should investigate the use of Tween 20 to manage crop water stress in a broad line of species with different physiological characteristics. Plant physiological studies could be focused on how Tween 20 affects plant embolism, and how Tween 20 moves in the plant by radio-labeled Tween 20 or using HPLC, GLC to trace it. Additional studies are also needed to evaluate the efficacy of Tween 20 injection into irrigation of field crops for decreasing water requirements needed for plant growth and development. Studies should also address overhead versus direct irrigation application.

**APPENDIX. WATER ANALYSIS REPORT OF AUBURN CITY WATER.**

Table 1. Water analysis report of Auburn city water.

Minerals*	Ca	K	Mg	P	Al	As
Concn.( mg·L <sup>-1</sup> )	27.4	1.8	9.8	0.1	< 0.1	< 0.1
Minerals	B	Cd	Cr	Cu	Fe	Mn
Concn. (mg·L <sup>-1</sup> )	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Minerals	Na	Ni	Pb	Zn		
Concn. (mg·L <sup>-1</sup> )	2.3	< 0.1	< 0.1	< 0.1		
Alkalinity	HCO <sub>3</sub> <sup>-</sup>					
Concn. (mg·L <sup>-1</sup> )	80					

\*Sample was collected from Auburn city water from the Department of Horticulture of Auburn University on Feb. 12, 2008.