Plant Growth and Physiological Responses to Various Surfactants Injected in Irrigation Water: Tween 20 as a Method for Reducing Water Use in Plant Production

by

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Abstract

Nursery and greenhouse growers who heavily rely on groundwater and/or are located in drought prone regions should be hard-pressed to minimize water waste for the sake of their plants, their budgets, and the environment. Members of the green industry are recognizing the dangers of depleting water from the heavily relied upon aquifers and are seeking to be more efficient with natural water resources even in times when water is plentiful. The research presented in this thesis was directed at contributing to the goal of keeping water waste to a minimum and minimizing the overall water requirement of plants during production. In research previously conducted at Auburn University, the surfactant Tween 20 applied at 100 mg/L reduced the crop water demand by up to 40% for *Impatiens hawkerii* 'Celebrate Salmon' grown in Fafard 3B substrate, and reduced transpiration in *Spathiphyllum floribundum* 'Viscount' by 64% and in *Impatiens hawkerii* 'Celebrate Salmon' by 101% when grown hydroponically. Soil surfactants are generally understood to mitigate soil and substrate hydrophobicity and help soils and substrates retain more water. However, very specific data on actual quantities of water that could be saved by utilizing a soil surfactant in container production are difficult to find. To further test the validity and usefulness of Tween 20 as a means of reducing water waste, Tween 20 was compared with similar available soil surfactant products and tested on a woody plant species to determine if similar results would be observed in woody plants as was observed in prior experiments on herbaceous plants.

In prior research conducted at Auburn University, the surfactant Tween 20 has shown potential for decreasing transpiration and increasing plant water use efficiency (WUE). Water use efficiency does not intrinsically equate to drought tolerance therefore the objective of this study was to determine if applying Tween 20 to herbaceous landscape plants would affect

drought tolerance as well as WUE. A secondary objective was to determine how Tween 20 would affect plant growth and drought tolerance in relation to two other commercially available products (AquaGro L with PsiMatric Technology and Hydretain ES Plus). The first treatment factor consisted of four solution treatments: 100 ppm Tween 20, 100 ppm AquaGro L with PsiMatric Technology, 320 ppm Hydretain ES Plus, and plain water (Control). A second treatment factor consisting of the treatments "first-time", "every-time", and "last-time" were also included. The three treatments mentioned above refer to when the product solutions were injected into the irrigation stream and applied to plants. Shoot dry weight, size index, SPAD readings, drought ratings, and ET did not vary amongst individual treatments. Most data did not suggest that Tween 20 or the other two products effected the growth or drought tolerance of *Solenostemon scutellarioides* 'Wasabi'.

In a second experiment, three-gallon *Ligustrum japonicum* 'Recurvifolium' were irrigated with treatments of 0, 50, 100, and 200 ppm Tween 20 solutions. Whole plant evapotranspiration (ET) and transpiration (T) were measured gravimetrically on select days throughout the experiment. Leaf-level T, photosynthesis (Pn), and water use efficiency (WUE) were measured once while plants were well hydrated and once again, two days later, when plants were experiencing substantial drought stress. Plant water potential was measured on a weekly basis. Leaf level Pn, T, and WUE did not vary by treatment when plants were well hydrated, however, WUE of plants treated with 50 and 100 ppm Tween 20 decreased compared with control plants.

The objective of the third study was to determine if Tween 20, when mixed with water, alters the evaporation rate of the solution. If Tween 20 were traveling through the plant and made it all the way to the leaf surface, evaporation from the leaf surface could potentially be

altered by the interfacial properties of surfactants. Concentrations of 0, 25, 50, 75, 100. 125, 150, 175, and 200 ppm Tween 20 solution were placed in 12-ounce capacity open bowls on a bench in a greenhouse at Auburn University, AL. Evaporation rate was measured every morning by weighing each bowl. Results indicate that solutions containing Tween 20 have a slightly increased rate of evaporation compared with water with no Tween 20.

For the fourth study, *Spathiphyllum* 'Emerald Star' liners transplanted into 6.5-inch azalea pots were treated with a single 800 mL drench of the surfactants Tween 20 (100 mg/L), AquaGro L with PsiMatric Technology (1.2 mL/L) and the humectant Hydretain ES Plus (2 oz/gal). A Control treatment (plain water) was also maintained. A second treatment factor consisting of the treatments "covered" and "non-covered" was also included. Covered-treated plants had a 4-gallon white plastic bag enclosing the container, wrapped over the substrate surface, and snugged against the base of the plant by pinning the bag into the substrate with an unfolded paperclip. Non-covered-treated plants did not have a bag around the container and substrate. The purpose for the non-covered/covered treatment was to allow (non-covered) or inhibit (covered) evaporation from the substrate surface. Data were collected on evapotranspiration, transpiration, substrate water retention, and leaching. Results indicate that AquaGro L with PsiMatric Technology and Hydretain ES Plus have certain merits regarding beneficial substrate-water relations. Tween 20 had few notable beneficial effects on water savings. More notably, covered-control-treated plants, which had no substrate evaporative loss, had an extended growing period of 21.5 days and produced 107% more dry weight than non-covered-control counterparts.

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Ψ_p	Water Potential
AE	Application Efficiency
CMC	Critical Micelle Concentration
DW	Dry Weight
E	Evaporation
T	Transpiration
ET	Evapotranspiration
IE	Irrigation Efficiency
WUE	Water Use Efficiency
SDW	Shoot Dry Weight
SFW	Shoot Fresh Weight
SWP	Stem Water Potential
SWR	Soil Water Repellency
RDW	Root Dry Weight

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Food consumption and hydration are two basic requirements of human life and both are contingent on water availability, which places water in the highest demand for all humans in every region of the globe. Roughly seventy-one percent of the earth's surface is comprised of 1.4 billion km^3 of water (Gleick, 1996), however, 97% of that water is saline, unsuitable for both agriculture and human consumption. Of the 3% freshwater that remains, 2% is tied up in glaciers that is also unavailable for human use. About 1% of the total global water supply, theoretically, can be withdrawn for various anthropogenic uses (Oelkers et al., 2011). Water is not created or destroyed, rather it constantly travels through different phases of the hydrologic cycle, therefore water will never "run out" when it is used, in the sense that fossil fuel will run out when it is used. An estimated net 45,500 km^3/year of rainwater is considered to be the amount of global renewable fresh water available for human use (Oki and Kanae, 2006). Based on the world population in 2010, available renewable fresh water resources were considered to be over 10 times greater than global demand (Oelkers et al., 2011) which is an estimated 3800 km^3/year, only 10% of the total available renewable fresh water resources (Oki and Kanae, 2006). Although global renewable fresh water resources are much greater than the demand, imbalances of water distribution in space and time limit the amount of water that is actually available where it is needed. This imbalanced supply of usable water is the cause for an estimated 2.4 billion people globally living in moderate to highly water stressed conditions as of 2006 (Utsumi, 2006). The greatest predicted pressure on global water resources in the upcoming years is the increasing world population as well as the rise in economic standards around the world (Vorosmarty et al., 2000). An unchanging amount of water will be necessary to support the dietary and domestic needs of roughly 2.0 billion additional people by the year 2050 (United Nations, 2013). The current and predicted continual water crisis is becoming the "oil crisis" of the 21st century (Solomon, 2010). In the future, every sector that withdraws freshwater will be evaluated and leveraged to get the most productivity out of every drop, all the while seeking to eliminate water waste. This thesis research project serves to contribute to the task of increasing efficient water use for the irrigated agriculture sector, specifically focusing on the irrigation of horticultural greenhouse and nursery plants, although the technique proposed need not be limited to this particular application of irrigation.

Outlook on Water Supply

Water supply in the United States. In 2010 a total of 306,000 million gallons per day of fresh water were withdrawn within the United States (Maupin et al., 2014). Of the withdrawals, surface water supplied 75% (230,000 Mgal/d) and groundwater supplied the remaining 25% (76,000 Mgal/d). Roughly 90% of all freshwater withdrawals were used for thermoelectric power, irrigation, and public supply. Irrigation accounted for 38% (115,000 Mgal/d) of the total daily fresh water withdrawals. Surface water accounted for 57% of irrigation water withdrawals while the remaining 43% was from groundwater withdrawals. To gain some perspective, total fresh water irrigation withdrawals peaked in the United States in 1980 at 150,000 Mgal/d, which is 23% higher than the withdrawal rate in 2010. The current irrigation fresh water withdrawal rate is at its lowest since 1960 when it was 110,000 Mgal/d. The drastic reduction in withdrawal rate has occurred in spite of a 43% (133.7 million) increase in population since 1960. Total groundwater withdrawals have declined 4% since 2005 and 10% since the peak in 2000. A 14% increase in micro irrigated acreage between 2005 and 2010 has been a contributing factor to the overall decrease in irrigation withdrawals, yet super-efficient micro irrigation only accounts for

1% of irrigation systems globally (Postel, 1993). Total freshwater withdrawal has hit the lowest point since 1965, attesting to the adaptation of farmers and growers produced by demand (drought). Additionally, the conterminous US receives an average of 4800 Bgal/d of rainwater daily, of which, two-thirds rapidly evaporates, leaving one-third to recharge surface and ground water (Frederick, 1995). The one-third (1,400 Bgal/d) that contributes to average daily recharge is 78% greater than total daily fresh water withdrawal rates (Frederick, 1995).

Even with the ample rainfall and decreases in withdrawals from previous years there is still reason to be concerned about water supply in light of population growth and predicted long term droughts. From a broader perspective, water management is a balancing act between available water, many forms of consumption, and the environment, making water allocation decisions quite difficult, especially in times of drought. In periods of drought, groundwater is often heavily relied upon, and unsurprisingly, is not as quickly recharged in the absence of rainfall. Groundwater depletion can and does occur even when there is rainfall but is highly exacerbated under drought conditions, a consistent reality for much of the Western United States in recent years and throughout the future. Deleterious effects of groundwater depletion can be very serious and include increased pumping costs, need to drill deeper wells, reduced base flow to springs, streams, and other surface water bodies, loss of wetlands, irreversible land subsidence, salinity encroachment and contamination, and ultimately complete water depletion (Alley et al., 1999; Bartolino and Cunningham, 2003; Konikow and Kendy, 2005; Konikow, 2015). Geological surveys indicate that 800 km^3 have been depleted from aquifers in the United States during the 20th century. From 2000 to 2008 alone, 200 km^3 were depleted, a 25% increase in the total depletion over just an 8-year period (Konikow, 2015). The potential damage to the environment along with water rights conflicts, government regulations, and increasing water costs create an imperative for progressing towards a better approach to sustainable water management. Given both the human and ecosystem needs for water, Gleick et al. (1995) have provided an excellent definition of sustainable water use: "the use of water that supports the ability of human society to endure and flourish into the indefinite future without undermining the integrity of the hydrologic cycle or the ecological systems that depend on it."

Irrigated agriculture. Globally, an estimated 270 million ha (667 million acres) of land are irrigated for agriculture, comprising 70% of the total fresh water withdrawals, which is nearly 4 billion m^3 yr^{-1} (Perry, 2007). For perspective, the second highest global withdrawal is 23% for industrial use (Horrigan et al., 2002). Horticultural irrigation typically gets lumped into the total agriculture irrigation statistics, yet when separated from agricultural crop production, horticulture water use is only a small drop in the large bucket of agriculture. In the United States in 2013, an estimated 55.2 million acres of farmland were irrigated with 88.4 million acre-feet of water (U.S Department of Agriculture, 2014). Compare that with 556.5 thousand acres of greenhouse and nursery production withdrawing 683.4 thousand acre-feet of water in 2013. Horticulture production only comprises 1% of total irrigated land and 0.7% of total irrigation water withdrawn for agricultural use in the United States. In a two-year survey of Alabama nurseries, Fare et al. (1994) learned that the average nursery applied 0.3 – 1.3 acre inches per day (9000 – 35,100 gallons) based on a traditional one-hour period of overhead irrigation. In the public eye, agricultural irrigation can be seen as wasteful and inefficient, which is not altogether untrue. In the past few decades, and especially now, the agriculture and horticulture industry, the scientific research community, and government agencies are vigorously working to optimize water use in irrigation practices. In a report on global water management, the International Water

Management Institute concluded that increased irrigation effectiveness could reduce the need for developing global water supplies by 50% (Seckler et al., 1998).

Plants and Water Use

Many factors are fundamental for plant growth such as mineral nutrients, radiation, temperature, humidity, and water. When all factors are at the optimal level with the exception of one, the exception is considered the limiting factor, controlling plant growth whether it be for the best or for the worst. Water is the primary driving factor in plant growth and is the single greatest reason for yield losses worldwide when not sufficiently supplied (Boyer, 1982). The present discussion will focus on water as the limiting factor of plant growth, and ultimately the maturity of a saleable product, as contingent upon satisfactory supply of quality freshwater. For decades, the nursery industry has been supplying water to plants by way of overhead irrigation (Lu and Sibley, 2006), churning out beautiful saleable plants for the public to enjoy, while wasting a lot of water in the process. Although once acceptable, water waste is increasingly being discouraged by the horticulture industry and academia due to current and perceived future water shortage crises.

Managing water – the big picture. Attempting to eliminate all water waste during the plant production period implies that the ultimate desire is to ensure that all water withdrawn from the environment and applied for plant production purposes is only used for intended and beneficial purposes and at minimal rates necessary. In container-grown plant production, water is considered to be beneficially used if up to 15% of it leaches from the bottom of the container to flush out excessive salts, or if the water ultimately enters the plant through the roots (Southern Nursery Association, 2013). Some researchers have attempted to develop nutrient and irrigation regimes that would altogether eliminate the need for a leaching fraction (Sammons and Struve, 2008; Warren and Bilderback, 2005). Although evaporation (E) from substrate is minimally beneficial, it is often viewed to be an insignificant fraction of total ET and therefore not a viable means to decrease water waste (Fereres et al., 2003). Therefore, traditional non-beneficial water use can be defined in two categories: (1) water that does not reach the root zone of the plant (evaporated in the air, intercepted by plant canopy, or simply misses the root zone due to poor application accuracy); and (2) water that does not stay in the root zone of the plant (leaching in excess of 15% or flooding over container edges). A secondary, yet non-traditional method for decreasing the amount of water required to grow a horticultural crop is to decrease the amount of water transpired per unit of carbon dioxide fixed by the plant. If more carbon can be fixed per unit of water transpired or if less water can be transpired per unit of carbon fixed, overall water use will decline. Transpiration benefits the plant by helping draw water (mass flow) and nutrients from the soil towards plant roots where they become accessible to the plant (Barber et al., 1963). Additionally, transpiration can help cool leaf surfaces and expedite nutrient flow from root to shoot (Hopkins and Hüner, 2004). Yet, transpiration of large amounts of water is the leading cause of crop loss (Boyer, 1982). An estimated 97% of all water that enters a plant is lost to transpiration (Taiz et al., 2015). Although transpiration can provide certain beneficial effects to plants, many have questioned whether plant growth would be negatively affected if transpiration were eliminated or significantly reduced (Muenscher, 1922; Tanner and Beevers, 1991,2001). If possible, significantly reducing transpiration could greatly decrease the frequency of drought incidence and increase the efficiency of dry matter production per unit of water transpired. Muenscher (1922) provides an insightful review of research investigating whether or not transpiration rates are directly related to nutrient absorption rates. In his own research, Muenscher determined that transpiration rate in barley plants did not affect rates of nutrient absorption. Inspired by the work of Muenscher, Tanner and Beevers (1990,2001) conlcuded that transpiration was not necessary for uptake of nutrients in maize and sunflower and provocatively suggested, in contrast to popular belief, that transpiration may be entirely useless to plants and only occurs because it is an unavoidable side-effect of the Carbon fixation process. Smith (1991) promptly criticized the interpretation and conclusions of the 1991 study by Tanner and Beevers. A study of pear trees, germinating sunflowers, and growth of sunflowers showed that high humidity (reduced transpiration) had negative effects on plant growth (Winneberger, 1958). Although research is conflicting, there seems to be enough evidence to suggest that transpiration can be decreased in certain species with no deleterious effects on growth. Therefore, if it were possible to manipulate and decrease transpiration, large amounts of water savings could be obtainable. Kijne et al. (2003) coined the term "more crop per drop" to illustrate the desire to maximize the effectiveness of water in terms of directly translating water applications into yield. The "more crop per drop" concept is often quantified mathematically by a plethora of equations which are typically focused on the plant growth:water applied ratio and typically are relayed as an "efficiency" measurement.

Quantifying the plant growth: water relationship. The literature is saturated with "water efficiency" equations with several of these equations worthy of further discussion. Two broad categories of equations are used for water calculations. The first category of equations expresses the relationship between irrigation water applied to a plant and water applied that is actually beneficially used by the plant ("application efficiency"). Application efficiencies are designed to reveal and eliminate the application of wasted (unbeneficial) water so that it can be reallocated for use at a different time or place. The second category of equations focus on the relationship between plant biomass production and evapotranspiration (ET) or transpiration (T) ("use efficiency"), designed to quantify and maximize plant biomass production per unit of water evapotranspired. In the broadest sense, these are the two primary routes for reducing water use in irrigated crops.

Application efficiency (AE). Israelsen et al. (1944) is widely considered to be the first to formally propose irrigation efficiency (E_i) and water application efficiency (WAE) parameters to quantify irrigation performance. Israelsen's (1950) irrigation efficiency (IE) equation was $E_i = W_c/W_r$ where W_c is irrigation water consumed by the crop and W_r is water from a river or other source. Israelen's equation is not altogether without merit; however, it is somewhat simplistic in light of current understanding of water management. Since the inception of the IE equation, a diverse collection of meritable alternative equations have been proposed and vigorously critiqued (Burt et al., 1997; Jensen, 2007; Keller and Keller, 1995; Lankford, 2012; Passioura and Angus, 2010; Pereira et al., 2012; Perry, 2007; van Halsema and Vincent, 2012). The objective of many of these papers is to view IE from multiple perspectives such as societal, ecological, and economical, and propose an equation that incorporates water gains and losses from each of these perspectives. Unfortunately, it is impossible to create an equation that completely acquiesces the demands of each perspective due to the reality that water loss from a farmers perspective (economic), may be a gain to the environment from an ecological perspective or vice versa. Based on this premise, van Halsema and Vincent (2012) state, "we argue that IE's are defined from the proprietor's perspective – e.g. the allocated water belongs to (or is associated with) the irrigation system, and IEs provide a measure of how well the system handles/uses this water and is able to convey it without 'waste' (efficiency component) and convert it to productive use (efficacy component). The water leaving the system's management/engineering domain is subsequently regarded as a loss to the proprietor." Burt et al. (1997) have provided a comprehensive, well-illustrated guide for quantifying water uses to minimize wasted water in the irrigation process strictly from the irrigator's perspective. Although useful in many regards, the instruction guide is directed more towards farm and field irrigators and leaves out a few details specific to container plant production such as the calculation for a leaching fraction to expel excess salts from container substrate (Burt et al., 1997). The objective in nursery and greenhouse crop production is to sufficiently satisfy beneficial water demands (uses) and minimize non-beneficial water uses. Increasing "application efficiency," particularly IE and uniform application, is paramount to increasing "use efficiency" in greenhouse and nursery production.

Use efficiency. The term "water use efficiency" (WUE) is primarily used by plant physiologists, agronomists, and plant breeders when referring to the plant biomass produced to transpired water relationship (Sinclair et al., 1984). In some occasions, WUE has been carelessly used as an "application efficiency" term (Hsiao et al., 2007; Raviv and Lieth, 2008), serving to further confound its more widely accepted meaning. Within the limits of appropriate use of the term, there are several variations in meaning depending on the exact parameters being measured. For example, total crop biomass/ET is different from net CO^2 fixation/T. Both examples fall into the category of a "use efficiency" but they must be supplied with different names for the sake of clarity and cross communication. Sinclair et al. (1984) provides a detailed yet simplistic vocabulary for communicating the various types of use efficiencies. The nominator is either carbon dioxide assimilation (A), total crop biomass (B), or crop grain yield (G) while the denominator is either transpiration (T), evapotranspiration (ET), or total water input (I) to the system. Time scale is an important factor and is marked as either instantaneous (i), daily (d), or seasonal (s). A measurement of CO^2 assimilation/ transpiration would be designated as WUE (A,T,i). A measurement of leaf gas exchange [WUE(A,T,i)] is often thought to be reflective of whole plant [WUE (B,T,s) or WUE (G, T,s)] which is not necessarily the case (Martin and Thorstenson, 1988; Medrano et al., 2014), and great care should be taken to not unfoundedly correlate the two.

Evolution of irrigation. Overhead irrigation has been the primary method of water delivery to nursery crops in the United States since the 1960's (Lu and Sibley, 2006). Conventional overhead irrigation prescribes a daily (Karam et al. 1994) sixty minute application of water be made, which can result in water leaching from containers and running over pot edges. Furuta (1976) reported irrigation application efficiency (water retained in container/total water applied) for overhead irrigation was at best 80% and declined with increased pot spacing. The majority of research indicates that 10 to 40% of overhead irrigation water actually enters plant containers (Beeson and Knox, 1991; Witherspoon and Harrell, 1980). In response to extremely low overhead irrigation application efficiencies, the practice of cyclic irrigation, which involves irrigating multiple times per day for shorter periods, was introduced and has been reported to decrease the demand for water application by up to 34% by reducing run-off water (Fain et al., 1999; Fare et. al, 1994; Southern Nursery Association, 2013). Other recommendations include grouping plants with similar water demands together to decrease waste water (Burger et al., 1987). Many nurseries have retention ponds to capture, treat, and reuse irrigation run-off water which can help balance out the inefficiency of overhead irrigation (Fulcher et al., 2016). However, most nursery and greenhouse owners still utilize overhead irrigation because of its ease of installation, and lower up-front and maintenance costs in contrast to other more efficient, but costlier, methods of irrigation such as micro irrigation.

The art and science of irrigation technology advanced significantly in the early 1960's when S. Blass introduced the first commercially available drip irrigation system for agricultural crops in Israel (Fereres et al., 2003). Drip irrigation made it possible to apply water directly to the root zone with nearly 100% precision. Delivering water directly to the root-zone practically eliminates drift and atmospheric evaporation, reduces foliar disease incidence, and provided an easier method of fertilizer and chemical delivery. In addition to methods for precise water application, soils and substrates can be blended to increase water holding capacity and subsequently increase plant available water. Amending a peanut hull substrate with Canadian peat (1:1 by volume) resulted in significantly more available water in the substrate (Bilderback et al., 1982). Others have tried to reduce water loss due to evaporation from substrate by mulching but discovered overall water retention was negligible compared with the unmulched control (Amoroso et al., 2010). Although not a standard practice at the present, several projects have shown the potential for eliminating the need for a leaching fraction by using controlled release fertilizers instead of high concentration liquid fertilizers (Warren and Bilderback, 2005).

In recent years, much emphasis has focused on the development of techniques and autonomous systems for scheduling irrigation based on plant water needs (Fereres et al., 2003). Generally, irrigation timing is either static, plant based, or substrate based (Fulcher and Fernandez, 2013). Static scheduling is arbitrary and not accurately related to actual plant water requirements. Plant based scheduling utilizes indicators from the plant such as plant water potential (Zimmermann et al., 2008), stem and fruit diameter, leaf thickness, xylem cavitation, sap flow, and stomatal conductance (Jones, 2004). Because of variations in response among species, these measurements are hard to translate into usable scheduling indicators (Fereres et al., 2003). Substrate based irrigation scheduling is a more dependable method for providing water to plants. Substrate moisture is measured most commonly volumetrically, or gravimetrically. Having been extensively researched over the past 50 years, wetting agents are now commonly incorporated into potting substrates and applied extensively in sand-based golf greens to enhance soil moisture characteristics.

Surfactants in Horticulture

A wetting agent is a type of surfactant. The term “surfactant” is derived from the phrase “surface-active-agent” and refers to a group of chemicals that can alter liquid-gas, liquid-liquid, and liquid-solid interfacial properties to facilitate or accentuate the spreading, emulsifying, wetting, dispersing, or other surface altering properties of liquids (WSSA Herbicide Handbook, 1994), and is the most widely used type of adjuvant in agriculture (Miller and Westra, 1998). Based upon their chemical nature, adjuvants are grouped into four categories: surfactants, oils, inorganic salts, and non-traditional adjuvants (Guillén and Urrestarazu, 2012). In agriculture, the most common use for adjuvants is to enhance the efficacy of a chemical solution such as a fertilizer, pesticide, or herbicide by modifying its properties in one or more ways. Oftentimes the adjuvant will help emulsify, or mix the chemical and water together, as well as help spread the mixture more thoroughly on to the leaf surface and also help to solubilize leaf cuticles to enhance chemical penetration (Hazen, 2000). As mentioned in the prior section, another commonly used adjuvant in agriculture and horticulture is the surfactant that acts as a wetting agent allowing for better liquid penetration of soils and substrates that have developed soil water repellency (SWR). An estimated 230,000 tons of surfactants annually are incorporated into agrochemical products such as herbicides, pesticides, and growth regulators, with each formulated product typically containing 1 to 10% of a single or multiple surfactants (Edser, 2007).

Surfactants are categorized as either nonionic, cationic, anionic, or amphoteric based upon the ionization of the hydrophilic head (or lack of ionization, as is the case with nonionic surfactants) in aqueous solutions (Hazen, 2000). In solution, cationic surfactants will develop a positive charge (cation), anionic surfactants will develop a negative charge (anion), and amphoteric surfactants can develop a positive or negative charge depending on the pH of the solution. Cationic surfactants are not frequently used in agrochemicals with the exception of ethoxylated fatty amines, more frequently being used in disinfectants and antiseptics because of their bacteriostatic properties (Castro et al., 2013). Anionic surfactants are primarily used in liquid and powdery laundry detergents as well as household cleaners, however certain types are utilized in agrochemical formulations. Amphoteric surfactants have traditionally not been widely used in agrochemical (Hazen, 2000); however, they are currently gaining more traction (Castro et al., 2013). Nonionic surfactants comprise almost 40% of the total surfactant production worldwide (Bajpai and Tyagi, 2010) and are used in many applications such as immunocytochemistry (Sato and Myoraku, 2004), food emulsions, cosmetics, pharmacy (Bajpai and Tyagi, 2010), and agriculture (Castro et al. 2013). In general, nonionic surfactants have lower chemical activity, less phytotoxicity (Powell, 1986) and are less toxic to mammals (Young, 2003) which makes them well suited for agricultural uses. The focus of this research will be on the use of nonionic surfactants as a potential tool for increasing WUE in plants.

Properties of nonionic surfactants. Surfactants in general are comprised of a hydrophilic head and a lipophilic hydrocarbon chain (Hazen, 2000). Surfactants have two primary properties, the first being the ability to reduce surface tension of an aqueous solution. When introduced to a glass containing aqueous solution, surfactant molecules will orient themselves so that the hydrophilic head is in the solution and the lipophilic tail is oriented out of the solution and into the atmosphere. Surface energy is measured in dynes/cm and the surface tension of water is 73 dynes/cm (Penn state). When a surfactant is introduced to the water at the right concentration, the surface tension can be reduced to between 30 to 50 dynes/cm, which allows for more thorough coverage of a surface by the solution. In agriculture, this is exemplified in herbicide solutions. A liquid herbicide solution might naturally form beads of water on a waxy leaf surface and be relatively ineffective, but when the solution includes a surfactant, the herbicide will form fewer and flatter water beads that increase contact with the leaf, increasing its effectiveness. The second property of surfactants is the ability to form aggregates of surfactant molecules, called micelles, in a solution. Once all available interfaces have been occupied by a monolayer of surfactant molecules, the remaining surfactant molecules will remain in suspension and orient together to form micelles (Farn, 2008). In an aqueous solution, the lipophilic tails will clump together into micelles, whereas in an oil solution the hydrophilic heads would clump together. When this happens, the surfactant is said to have reached the critical micelle concentration (CMC) which will vary based on surfactant molecular properties.

Surfactant use in horticulture. Much research has gone into the testing of various surfactants and surfactant combinations for their effectiveness in emulsifying oil-based agrochemicals into water, their ability to solubilize cuticular waxes to allow for more thorough herbicide uptake, as well as the effectiveness of surfactants in spreading the agrochemical thoroughly over the surface of a leaf and keeping it stuck there (Hazen, 2000). The other well researched aspect of surfactants in horticultural use is that of how surfactants can help with plant-water relations.

Surfactants and Plant Water Use

In terms of maximizing the utility of water to plants, surfactants have played two primary roles. The first role is facilitating water infiltration into water repellant soils and substrates. The athletic turfgrass industry has been a heavy consumer of surfactants for increasing uniform water infiltration through the entire turf system, especially in areas of hydrophobicity (Cisar et al., 2000). Ethylene oxide/propylene oxide (EO/PO) block copolymer surfactants comprise the vast majority of surfactants used in the turfgrass industry for the remediation of SWR (Kostka and Bially, 2005). Surfactant efficacy for increasing water infiltration of hydrophobic soils has been convincingly shown (Baird and Calhoun, 1999; Cisar et al., 2000; Karnok and Tucker, 2001; Moore, 1975; Morgan et al., 1966; Ruemmele and Amador, 1994). Increased water infiltration results in reduced dry patches in turf systems and reduced water waste as a result of run-off. Kostka and Bially (2005) learned that by using an ethylene oxide-propylene oxide (EO/PO) block copolymer surfactant blended with an alkyl polyglycoside (APG) surfactant, infiltration time of hydrophobic soils can be significantly reduced in contrast with just using a single surfactant.

In addition to enhancing infiltration of water repellant soils, surfactants can also help to retain moisture in the soil (Blodgett et al., 1993; Ruemmele and Amador, 1998). The ability to "increase WUE" is oftentimes erroneously accredited to surfactants when growth increases or reduced water applications are reported. Surfactants have been positively associated with increased plant growth and reduced water applications, not typically as a function of increased water use efficiency but rather as a function of additional water moved to or stored in the root zone that is utilized in transpiration rather than wasted as run-off. In order for a surfactant to actually increase WUE in plants, it would have to either enhance intrinsic plant biomass production, decrease transpiration, or accomplish both simultaneously.

Corn plants irrigated at 80% of the evaporative demand with a nonionic surfactant have been shown to produce the same yield as untreated plants irrigated with 100% evaporative demand, demonstrating 20% water savings (Chaichi et al., 2015). When applied in saline soils and under severe water deficit conditions, a nonionic surfactant has been shown to significantly increase forage yield (kg ha^{-1}) of corn (Chaichi et al., 2016). In the same experiment, immediately after harvesting corn, wheat was grown in the same field where surfactant applications were made on corn. Under similar irrigation regimes with no new surfactant applications, wheat grown in the field with prior surfactant applications had a 58% increase in grain harvest (kg ha^{-1}) as compared with wheat grown in the field with no prior surfactant applications. The authors conclude that higher water retention rates and reduced evaporation were likely the cause for increased yield rather than a physiological alteration of biomass production or transpiration induced by surfactant. In a study with *Cotoneaster dammeri* 'Skogholm', Bilderback and Lorscheider (1997) showed that incorporation of a granular nonionic surfactant could increase shoot dry weight when irrigation supply was decreased below the evaporative demand of the plant. When full irrigation was supplied, surfactant use did not increase shoot dry weight. Yang (2008) showed that *Impatiens hawkerii* 'Celebrate Salmon' irrigated with 100 mg/L of Tween 20 at 60% of crop demand maintained similar growth as untreated, fully irrigated control plants. To our knowledge, Yang (2008) is the first to suggest that the increase in WUE could be attributed to direct physiological alterations on the plant caused by the surfactant rather than increased water retention in the root zone. To test this theory, Yang (2008) removed soil from the equation and grew peace lily and New Guinea impatiens in a hydroponic system with treatments of Tween 20 to see if similar results would be observed. Results showed up to a 40% decrease in transpiration, 46% increase in fresh weight, and a 62% increase in WUE for peace lily grown in a hydroponic system with 100 ppm Tween 20. New Guinea impatiens grown under the same conditions had a 50% decrease in total transpiration, a 34% increase in fresh weight, and a 69% increase in WUE. Physiological research has been conducted on how surfactants can enhance plant growth, however in applied research typical assumptions have been that any beneficial effects of surfactants are correlated with the soil-water-surfactant relationship. In physiological studies, extensive evidence exists indicating that Tween 20 (polyoxyethylene sorbitan monolaurate), can stimulate plant growth (Beal et al., 1954; Stowe, 1958, 1959, 1960, 1961; Stowe and Dotts, 1971).

With evidence mounting that nonionic surfactants have potential to increase agricultural crop yields (Chaichi et al., 2015; Chaichi et al., 2016) as well as increase WUE and total fresh weight and dry weight production in ornamental crops (Bilderback and Lorscheider, 1997; Yang, 2008), more research should be dedicated to this topic.

Research Objectives

The objective of this research is to further investigate the potential of utilizing the nonionic surfactant Tween20 as an irrigation additive for the purpose of increasing plant WUE by way of minimizing transpiration rates without altering rates of CO_2 fixation. The specific goals for this thesis research project are as follows:

1) Determine the effect of Tween 20 on WUE and drought tolerance of a woody plant species.
2) Compare the effects of Tween 20 and two other similar commercially available products, AquaGro L with PsiMatric Technology and Hydretain ES Plus on
3) Determine how plant water potential (Ψ_P) is effected by Tween 20 applied to the root zone of a woody plant.

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CHAPTER 2

EFFECTS OF APPLICATION TIME AND FREQUENCY OF TWO SURFACTANTS AND A HUMECTANT ON GROWTH AND DROUGHT TOLERANCE OF *SOLENOSTEMON SCUTELLARIOIDES* 'WASABI'

ABSTRACT

Regulations on water usage, primarily driven by water shortages in the western United States and by nutrient leachates contaminating surface and groundwater in various regions of the country, have forced many growers to adopt more efficient water practices. Soil/substrate surfactants are a tool that can be utilized to help reduce the overall water waste in container-grown plant production. Surfactants break the surface tension of water and help alleviate soil/substrate hydrophobicity by allowing water to uniformly infiltrate into the soils. Several studies indicate that by using a surfactant on agricultural crops, plant water use efficiency (WUE) is increased, which would suggest either a decrease in transpiration, an increase in plant biomass production, or both simultaneously occurring as a result of surfactant application. In prior research conducted at Auburn University, the surfactant Tween 20 has shown potential for decreasing transpiration and increasing plant water use efficiency (WUE). Water use efficiency does not intrinsically equate to drought tolerance therefore the objective of this study was to determine if applying Tween 20 to herbaceous landscape plants would affect drought tolerance as well as WUE. A secondary objective was to determine how Tween 20 would affect plant growth and drought tolerance in relation to two other commercially available products (AquaGro L with PsiMatric Technology and Hydretain ES Plus). The first treatment factor consisted of four solution treatments: 100 ppm Tween 20, 100 ppm AquaGro L with PsiMatric Technology, 320 ppm Hydretain ES Plus, and plain water (Control). A second treatment factor consisting of the

treatments “first-time”, “every-time”, and “last-time” were also included. The three treatments mentioned above refer to when the product solutions were injected into the irrigation stream and applied to plants. Shoot dry weight, size index, SPAD readings, drought ratings, and ET did not vary amongst individual treatments. Most data did not suggest that Tween 20 or the other two products effected the growth or drought tolerance of *Solenostemon scutellarioides* ‘Wasabi’.

INTRODUCTION

For the first time in California history, regulations on groundwater withdrawals were implemented in 2014 due to the severe drought conditions (Fulcher et al., 2016). Additionally, legislation affecting nurseries and greenhouses regarding water use and/or quality has also been enacted in Delaware, Florida, Maryland, Michigan, North Carolina, Oregon, and Texas (Fernandez et al., 2009). These regulations, coupled with pressures on regions that are under continual limited water conditions such as the Western United States as well as regions that experience shorter, but none the less, harmful droughts will likely be the driving force in getting growers to embrace more efficient irrigation practices. Many renowned and respected growers and irrigation researchers have predicted that efficient nursery irrigation practices “must and will be increased” in the upcoming years (Beeson et al., 2004).

Irrigation research emphasis has been placed primarily on developing strategies and mechanisms for efficiently and uniformly applying water precisely to the root zones of the desired plants without any losses. Methods concerned with designing an irrigation system to apply uniform amounts of water to many plants, while maximizing the amount of water captured by the container in relation to the total water applied is referred to as irrigation efficiency (IE). The IE equation is focused on evaluating the efficiency of water delivery techniques and systems and therefore can avert the attention of irrigators and researchers from other routes of significant water loss that can occur long after the irrigation process is completed such as evaporation from substrates and transpiration via stomata.

A secondary approach to reducing overall water use during plant production is to minimize the exchange rate of water lost through transpiration for the gain of atmospheric CO_2 through the stomata, which is referred to as water use efficiency (WUE). Because both water vapor and atmospheric CO_2 move in and out of a plant through the stomata, their relationship is linearly related and extremely difficult to alter one variable without equally altering the other. Manipulating WUE has been a primary objective in agronomic research where harvestable biomass is of utmost importance. However, in ornamental plant production, emphasis is instead placed on obtaining a "generally acceptable" saleable size as well as satisfactory aesthetic quality. For this reason, less research has focused on techniques to increase WUE efficiency that could only possibly lead to minute changes in overall water savings. Yet, conceptually, finding ways to decrease transpiration is not unmerited. Although transpiration can provide certain beneficial effects to plants, many have questioned whether plant growth would be negatively affected if transpiration were eliminated or significantly reduced (Muenscher, 1922; Tanner and Beevers, 1991, 2001). If possible, significantly reducing transpiration could greatly decrease the frequency of drought incidence and increase the efficiency of dry matter production per unit of water transpired. Muenscher (1922) provided an insightful review investigating the relation of transpiration rates are directly on nutrient absorption rates. Muenscher determined that transpiration rate in barley plants did not affect rates of nutrient absorption. Inspired by the work of Muenscher, Tanner and Beevers (1991, 2001) conlcuded that transpiration was not necessary for uptake of nutrients in maize and sunflower and provocatively suggested, in contrast to popular belief, that transpiration may be entirely useless to plants and only occurs because it is an unavoidable side-effect of the Carbon fixation process. Smith (1991) promptly criticized the interpretation and conclusions of the 1991 study by Tanner and Beevers. A study of pear trees, germinating sunflowers, and growth of sunflowers showed that high humidity (reduced transpiration) had negative effects on plant growth (Winneberger, 1958). Although research is conflicting, there seems to be enough evidence to suggest that transpiration can be decreased in certain species with no deleterious effects on growth. Therefore, if it were possible to manipulate and decrease transpiration, large amounts of water savings could be obtainable.

One way that water use efficiency (WUE) on the physiological level can be enhanced is by decreasing the transpiration rate while increasing or maintaining a steady state of carbon fixation on a leaf level basis. The surfactant Tween 20 applied at 100 mg/L reduced the crop water demand by up to 40% for *Impatiens hawkerii* 'Celebrate Salmon' grown in Fafard 3B substrate, and reduced transpiration in *Spathiphyllum floribundum* 'Viscount' by 64% and in *Impatiens hawkerii* 'Celebrate Salmon' by 101% when grown hydroponically (Yang, 2008). Water use efficiency does not intrinsically equate to drought tolerance. The objective of this study was to determine effects of Tween 20 on drought tolerance in response to altered transpiration rates. Coleus was selected as the study plant because it readily wilts under drought stress making it an ideal plant to observe. A second goal of this study is to determine if product application frequency will affect drought stress. A final goal was to compare the performance of Tween 20 with that of two similar commercially available soil conditioning products: AquaGro L with PsiMatric Technology and Hydretain ES Plus. Both of these products advertise that they can reduce the total water required during a production cycle, which is similar to what was discovered with Tween 20 by Yang (2008). If Tween 20 does not offer similar or superior benefits compared with AquaGro L with PsiMatric Technology and Hydretain ES Plus then it may not have much commercial potential.

MATERIALS AND METHODS

Location and experimental design. This experiment utilized a 2-way factorial treatment design. The first treatment factor consisted of four solution treatments: 100 ppm Tween 20 (Rocky Mountain Oils, Orem, UT), 100 ppm AquaGro L with PsiMatric Technology (Aquatrols Corp of America, Paulsboro, NJ), 320 ppm Hydretain ES Plus (Ecologel Solutions LLC, Ocala, FL), and plain water (Control). Mixture ratios for Hydretain ES Plus and AquaGro L with PsiMatric Technology were based on label recommended rates for weekly applications. The ratio for Tween 20 was selected based on prior work by Yang (2008). These solution treatments were applied with irrigation water by injecting solution concentrate into the irrigation stream with a Dosatron (Model D14MZ2VFII, Dosatron USA, Clearwater, FL). A second treatment factor consisting of the treatments "first-time", "every-time", and "last-time" were also included. The three treatments mentioned above refer to when the product solutions were injected into the irrigation stream and applied to plants. First-time refers to a single solution treatment application during the first irrigation (not counting the initial watering at time of planting) of the experiment, every-time refers to solution treatment applications being made during every irrigation of the experiment (not counting the initial watering at time of planting), and last-time refers to a single solution treatment application during the final irrigation before plants were allowed to dry down and experience drought stress. All three products were injected at the first-time, every-time, and last-time regimens, however, the control, which was irrigating with no product was not possible to split into the categories of first-time, every-time, and last-time. Therefore, 3 product solution treatments × 3 application time treatments = 9 treatments. Nine treatments plus a single control treatment equals a total of ten treatments consisting of 7 replicates per treatment equaling a total of 70 plants. Experimentation was conducted in a 2300 ft^2 fan and pad greenhouse at the Paterson Greenhouse Complex in Auburn University, Alabama. Replicates were arranged in a randomized complete block design on a single bench located in the center of the greenhouse.

Plant material and initial set-up. Solenostemon scutellarioides 'Wasabi' liners (Tagawa Greenhouses, Brighton, CO) were transplanted into 6.5-inch azalea pots filled with Fafard 3B substrate (a blend of peat, perlite, vermiculite, and pine bark, Conrad Fafard, Inc., Agawam, MA) in July 2016. Immediately after transplanting into azalea pots, all plants were initially hand-watered with a 200 ppm 20-10-10 liquid fertilizer until water leached from each pot. Treatment regimens were initiated during the next irrigation event.

Irrigation. Two days after the initial hand-watering, plants were irrigated 4 times (every other day) with a custom-built drip irrigation system (Figure 2.1). Each of the 10 treatments had its own individual mainline with spaghetti tubing attached to 5.0 GPH Non-Pressure Compensating spray stakes (Netafim, Fresno, CA) that were placed into the 6.5-inch azalea pots (1 stake per pot). Individual spaghetti tubes were attached to the mainline via a 3.2 GPH Woodpecker Pressure Compensating Emitter (Netafim, Fresno, CA). The irrigation system water pressure was maintained at 25 PSI with a 3/4 -inch, 25 PSI Drip Pressure Regulator (Ewing Irrigation and Landscape Supply, Montgomery, AL). Three, 5-gallon buckets were used as reservoirs for the concentrated Tween 20, AquaGro L with PsiMatric Technology, and Hydretain ES Plus solutions. Three Dosatron injectors (one for each product) were used to eliminate the possible cross-contamination of products that could occur if only a single Dosatron were used. A series of ball-valves was installed at various points along the irrigation system so that a single treatment could be isolated from the rest of the system and individually irrigated. A timing system was connected to a solenoid valve to irrigate plants for exactly 60 seconds which equated to an application of roughly 200 mL of irrigation water/treatment solution to each plant.

Soil moisture content and evapotranspiration (ET). All soil moisture measurements were made with a Delta T HH2 Meter (Delta T Devices, Burwell, Cambridge, UK) using the pre-set Organic Soil parameter on the device. Soil moisture readings were taken twice a day on the day plants were irrigated: once at 7am prior to irrigation and once again 2 hours later at 11am. Soil moisture data was not taken on the days in between irrigation events. ET for a given day was calculated by subtracting the 7am moisture reading of the current day from the 11am moisture reading of the previous day. Beginning on the final day of irrigation, soil moisture readings were collected at 7am daily for 12 days until experiment termination to compare substrate water retention among treatments as well as help interpret visual drought symptoms.

Drought ratings. Including the final day of irrigation, the drought phase of the experiment lasted ten days. On the tenth day plants were observed and given a drought rating based on visual symptoms of wilt. Plants were rated on a visual scale of 0 to 3 with zero indicating no signs of drought and three indicating heavy signs of drought (Figure 2.2).

Plant growth. After drought ratings were measured, above ground shoots were harvested to determine dry weight. Plant size index was measured the day after the final irrigation by averaging the plant height, width at widest point, and width perpendicular to widest point. Relative leaf greenness was measured with a SPAD-502 Chlorophyll Meter (Konica Minolta Inc., Tokyo, Japan).

Statistical analysis. Data were analyzed using ANOVA-type analyses using the GLIMMIX procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC). *P* values for multiple comparisons were adjusted using the Shaffer-Simulated method. Overall treatment effects were significant at P=0.05.

RESULTS AND DISCUSSION

Shoot dry weight, size index, SPAD readings, drought ratings, and ET did not vary amongst individual treatments (Table 2.1). Visual observation of the crop over the course of the experiment indicated that plants treated with Hydretain ES Plus and Tween 20 appeared larger than plants treated with AquaGro L with PsiMatric, although size index and dry weight indicate no differences in growth amongst all treatments. Also, based on visual observation, plants treated with AquaGro appeared to display fewer signs of drought stress leading up to the termination of the experiment.

Drought substrate moisture retention. There were significant differences in substrate moisture content amongst treatments on days 4, 10, and 12 (Table 2.2). On day 4, substrate treated with Hydretain every-time and Tween every-time had lower substrate moisture content than substrate treated with AquaGro the last-time. On day 12, substrate moisture content was highest in substrate treated with AquaGro the last-time and lowest in substrate treated with Tween every-time and Hydretain the first-time. A commonality between all three days where differences in substrate moisture content were observed is that Tween 20 applied every-time reduced overall substrate moisture content compared with other treatments. The reduction in soil moisture content could possibly be correlated to higher transpiration rates due to greater total leaf area in plants treated with Tween 20 every-time, although it would be necessary to actually measure leaf area in future studies in order to support this theory. In physiological studies, extensive evidence exists indicating that Tween 20 can stimulate plant growth (Beal et al., 1954; Stowe, 1958, 1959, 1960, 1961; Stowe and Dotts, 1971) which could have occurred in this study even if the growth increase were not statistically significant.

CONCLUSIONS

Based on data collected in this experiment, there is little evidence to suggest that any of the chemical products have water saving capabilities. Emphasis is placed on "based on data collected". It is very possible that all three products used may have water saving capabilities that simply went undetected because of insufficient data or unprecise data collection procedures. It was concluded that using soil moisture probes can helpful in scheduling irrigation events but they are not precise enough to make useful conclusions about plant and substrate water relations. In future studies ET, leaching, and substrate moisture retention should be measured gravimetrically and by collecting leachates.

In addition to the data collected in this experiment, there was also a plan to collect photosynthesis data with a LICOR 6400 photosynthesis machine (LI-COR Biosciences, Lincoln, NE) and chlorophyll fluorescence with a FMS 2 Fluorescence meter (Hansatech Instruments Ltd, Pentney, UK). While attempting to collect data with these machines it was discovered that both were malfunctioning and ultimately could not be used in the experiment. In future studies, the above-mentioned data collection devices would provide information that would be helpful in the discussion of drought tolerance and water savings.

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Table 2.1 Shoot dry weight (SDW), size index, SPAD readings from a SPAD-502 Chlorophyll Meter (Konica Minolta Inc., Tokyo, Japan), visual drought ratings, and evapotranspiration (ET) (measured as % water content by volume for first 9 days of experimentation) of *Solenostemon scutellarioides* 'Wasabi' treated with solutions of 100 ppm Tween 20, 100 ppm AquaGro L with PsiMatric Technology, 320 ppm Hydretain ES Plus, and plain water (Control). Each solution treatment was applied either at the first irrigation only (first-time), at every irrigation (every-time), or only at the final irrigation (last-time).

Treatment		Measurement parameter				
Product	Irrigation	SDW (g)	Size index (cm)[y]	SPAD[x]	Drought rating[w]	ET[v]
Control	Control	1.86^z	17.67	18.37	2.57	29.80
AquaGro	Every-time	1.77	17.52	16.47	2.43	33.91
AquaGro	First-time	1.76	17.10	17.07	2.43	33.94
AquaGro	Last-time	1.69	16.67	15.89	2.43	31.43
Hydretain	Every-time	1.96	18.90	20.03	3.00	24.77
Hydretain	First-time	1.91	18.00	18.63	2.86	26.14
Hydretain	Last-time	1.76	16.62	15.73	2.71	32.44
Tween	Every-time	2.10	19.67	20.59	2.86	26.10
Tween	First-time	1.70	17.81	18.27	3.00	31.80
Tween	Last-time	1.83	17.71	19.76	2.71	29.34

[z] When overall treatment effects were significant ($p \leq 0.05$), means followed by the same letter are not significantly different using the Shaffer-Simulated adjustment for multiple comparisons ($\alpha = 0.05$); otherwise, treatment means are presented without letter groupings.

[y] Size index was calculated as the average of plant height, width at widest point, and width perpendicular to width at widest point.

[x] SPAD units measure relative leaf greenness.

[w] A scale of 0 to 3 was used with 0 being no visual drought symptoms and 3 being severe visual drought symptoms.

[v] ET was measured only during the first 9 days of experimentation.

Table 2.2 Moisture content (% water by volume) of Fafard 3B substrate with transplanted *Solenostomen scutellarioides* 'Wasabi' measured daily beginning on the day of final irrigation (day 1) and ending on the day of experiment termination (day 12). Plants/substrate were treated with solutions of 100 ppm Tween 20, 100 ppm AquaGro L with PsiMatric Technology, 320 ppm Hydretain ES Plus, and plain water (Control). Each solution treatment was applied either at the first irrigation only (first-time), at every irrigation (every-time), or only at the final irrigation (last-time).

Treatment		Day											
Product	Irrigation	1	2	3	4	5	6	7	8	9	10	11	12
Control	Control	38.30^z	42.51	34.00	28.49ab	23.14	15.44	8.10	6.00	2.09	1.40ab	0.83	0.89ab
AquaGro	Every-time	38.50	40.20	33.83	27.81ab	21.59	16.34	7.70	5.96	2.06	1.50ab	1.40	0.86ab
AquaGro	First-time	41.41	44.64	34.30	29.16ab	22.70	16.90	8.61	5.17	1.74	1.50ab	1.10	0.90ab
AquaGro	Last-time	39.84	44.37	38.23	31.10a	23.90	18.40	9.30	6.23	3.17	1.83ab	1.47	1.54a
Hydretain	Every-time	37.94	39.99	33.31	25.81b	20.39	15.26	5.41	3.73	1.93	1.10ab	0.54	0.64ab
Hydretain	First-time	37.16	42.06	33.77	27.79ab	21.14	15.26	7.04	4.84	2.03	1.24ab	0.97	0.34b
Hydretain	Last-time	38.61	41.81	34.41	26.89ab	21.70	16.53	8.26	5.44	2.53	2.13a	1.56	0.64ab
Tween	Every-time	34.97	39.31	33.70	25.11b	19.57	14.41	6.39	3.20	1.14	0.54b	0.54	0.34b
Tween	First-time	37.84	42.81	35.37	26.66ab	22.64	16.56	7.97	5.11	1.96	1.64ab	1.16	0.97ab
Tween	Last-time	39.79	39.59	35.60	28.60ab	22.84	16.26	7.67	5.04	2.77	1.64ab	1.24	0.93ab

^{z}When overall treatment effects were significant ($p \leq 0.05$), means followed by the same letter are not significantly different using the Shaffer-Simulated adjustment for multiple comparisons ($\alpha = 0.05$); otherwise, treatment means are presented without letter groupings.

Figure 2.1 Custom built chemical injection system to inject Tween 20, AquaGro L with PsiMatric Technology, and Hydretain ES Plus into the irrigation stream to be delivered to Solonestemon scuttelarioides 'Wasabi' through drip tubes and spray stakes. The numerous ball valves were installed to direct water to individual Dosatrons so that only one treatment was ever irrigated at a single time. Irrigation was manually started by flipping the electrical control switch which would cut the water off after 60 seconds of run time.

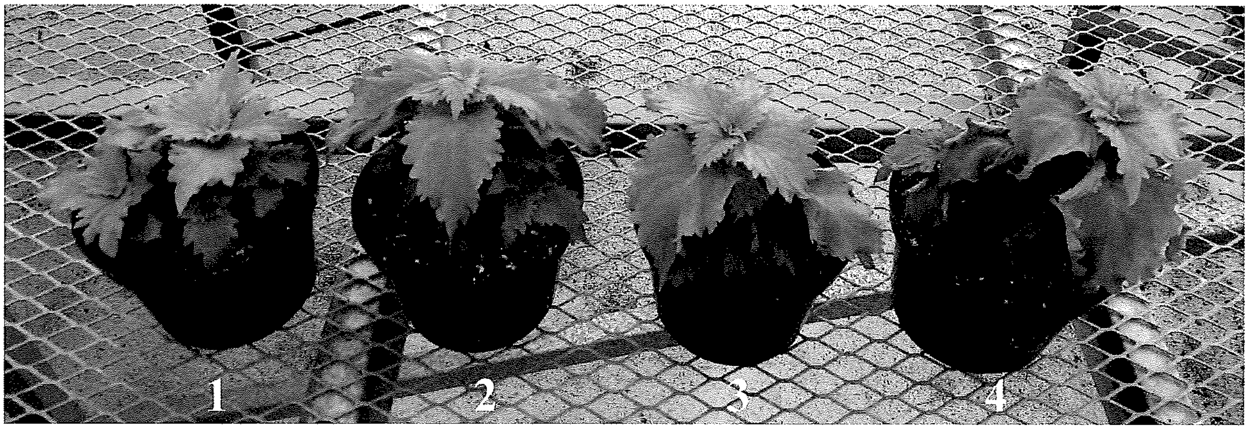

Figure 2.2 Visual drought rating of *Solenostemon scuttelarioides* 'Wasabi". 0 = no wilt; 1 = minor wilt (1-2 leaves); 2 = moderate wilt (3-5) leaves); 3 = heavy wilt (majority of leaves).

CHAPTER 3

EFFECTS OF MULTIPLE RATES OF A NONIONIC SURFACTANT ON WATER RELATIONS OF *LIGUSTRUM JAPONICUM* 'RECURVIFOLIUM'

ABSTRACT

Persistent and temporal droughts along with regulations limiting water use and allowable amounts of nutrients in irrigation leachates are forces driving the research and implementation of more efficient irrigation practices in ornamental plant production. Surfactants have long been used on sand-based golf greens and in potting substrates to overcome hydrophobicity and increase the "wettability" and water retention in soils and substrates. Several studies indicate that by using a surfactant on agricultural crops, plant water use efficiency (WUE) is increased, which would suggest either a decrease in transpiration, an increase in plant biomass production, or both simultaneously having occurred as a result of surfactant application. This study evaluated the effect of using the surfactant Tween 20 on WUE by reducing transpiration in a woody plant. Three-gallon *Ligustrum japonicum* 'Recurvifolium' were irrigated with treatments of 0, 50, 100, and 200 ppm Tween 20 solution. Whole plant evapotranspiration (ET) and transpiration (T) were measured gravimetrically on select days throughout the experiment. Leaf-level T, photosynthesis (PN), and WUE were measured once while plants were well hydrated and once again, two days later, when plants were experiencing substantial drought stress. Plant water potential was measured on a weekly basis. Leaf level photosynthesis, transpiration, and WUE did not vary by treatment when plants were well hydrated, however, WUE of plants treated with 50 and 100 ppm Tween 20 decreased compared with control plants.

INTRODUCTION

In a review of the economic impact of the green industry, Hall et al. (2006) compiled various data from national surveys, adjusting 2002 data for 2004 inflation rates, revealing a nursery and greenhouse industry comprised of 56,070 establishments producing a total of $26.1 billion in output, second only to the landscaping services sector ($53.0 billion) in contribution to the total green industry output ($147.8 billion). The nursery and greenhouse industry is unique in that it directly profits from the mass production and sale of live products. Although many inputs are required for creating an optimal growing environment for plant production, mineral nutrients and water are two of the primary inputs that are ultimately integrated into plant biomass. Of these two inputs, water is by far the most heavily applied, in terms of both volume and mass. Withhold nutrients, and plants will gradually decline in appearance and health and may eventually die, but withhold water, and in a matter of a few hours to a few days, plant death is imminent. This is especially true in container-grown plant production since a very limited amount of water can be held in container substrate and must be replenished frequently by irrigation (Majsztrik et al., 2011). Because the economic cost of applying water is typically low (Beeson et al., 2004) and the economic risk of plant death due to under watering is high, nursery growers traditionally water excessively, creating high leaching fractions and high run-off rates, having simply accepted the wasted water as a necessity to ensure plant livelihood (Majsztrik et al., 2011). Based on responses from an informal survey, Fulcher et al. (2016) concluded that "in general producers have not adopted more efficient and water conserving practices unless an economic incentive or legal imperative (return on investment or potential for regulation and/or fines) motivated the decision". For the first time in California history, regulations on groundwater withdrawals were implemented in 2014 due to the severe drought conditions (Fulcher et al., 2016). Additionally, legislation affecting nurseries and greenhouses regarding water use and/or quality has also been enacted in Delaware, Florida, Maryland, Michigan, North Carolina, Oregon, and Texas (Fernandez et al., 2009). These regulations, coupled with pressures on regions that are under continual limited water conditions such as the Western United States as well as regions that experience shorter, but none the less, harmful droughts will be the driving force in getting growers to embrace more efficient irrigation practices. Many renowned and respected growers and irrigation researchers are in agreement in predicting that efficient nursery irrigation practices "must and will be increased" in the upcoming years (Beeson et al., 2004).

Container-grown woody ornamental plant production nurseries rely on overhead irrigation as their primary system for water delivery (Besson and Knox, 1991). In a survey of 26 container nurseries in Georgia, Garber et al. (2002) discovered that nurseries essentially irrigated all 1, 3, and 5 gallon containers with overhead irrigation. Overhead irrigation is an attractive option to growers because it is relatively cheap, easy to install, and easy to maintain, however it typically has a very low interception efficiency (Majsztrik et al., 2011). As much as 74% to 87% of overhead irrigation water is never actually intercepted by plant containers (Witherspoon and Harrell, 1980). Based on standard industry spacing recommendations for 1 gallon containers (12 inches on-center) and 3 gallon containers (24 inches on-center), the maximum obtainable interception efficiency (water intercepted by container substrate/total water applied) for 1 and 3 gallon containers is 25% and 11% respectively (Garber et al., 2002). Seven, 15, and 25 gallon containers require much larger spacing due to plant canopy size leading to difficulties with water supply through overhead irrigation without creating mass amounts of run-off water. For this reason, some nurseries are adopting micro irrigation as an alternative for container sizes above 5 gallon. However, currently the majority of industry has not adopted micro irrigation practices for anything below a 7-gallon due to the high cost of installation, the labor required for system maintenance, and the fact that most 1,3, and 5 gallon potted plants are relocated once or twice during the growing season (Majsztrik et al., 2011). Both cyclic irrigation and the catchment and reuse of irrigation water provide avenues to increase the efficiency of overhead irrigation (Southern Nursery Association, 2013). In the case of irrigation run-off catchment and re-use, large amounts of water can be saved and reused.

Research emphasis has been placed primarily on developing strategies and mechanisms for efficiently and uniformly applying water precisely to the root zones of the desired plants without any losses. Methods concerned with designing an irrigation system to apply uniform amounts of water to many plants, while maximizing the amount of water captured by the container in relation to the total water applied is referred to as irrigation efficiency (IE). A secondary approach to reducing plant water demand is to minimize the exchange rate of water lost through transpiration for the gain of atmospheric CO_2 through the stomata, which is referred to as water use efficiency (WUE). Because both water vapor and atmospheric CO_2 move in and out of a plant through the stomata, their relationship is linearly related and extremely difficult to alter one variable without equally altering the other. Manipulating WUE has been a primary objective in agronomic research where harvestable biomass is of utmost importance. However, in ornamental plant production, emphasis is instead placed on obtaining a “generally acceptable” saleable size as well as satisfactory aesthetic quality. For this reason, less research has focused on techniques to increase WUE efficiency that could only possibly lead to minute changes in overall water savings. Yet, conceptually, finding ways to decrease transpiration is not unmerited. Of the total water that enters a plant, roughly 97% is lost to the atmosphere as transpiration, 2% supplies plant growth, and 1% is used in biochemical reactions such as photosynthesis (Taiz et al., 2015). Water movement through the transpiration stream has generally been considered vital for nutrient flow from roots to shoots. However, it is possible that transpiration could be reduced without negative consequence and ultimately save much water (Tanner and Beevers, 2001). Transpiration was decreased by 50% in new guinea impatiens without decreasing growth by irrigating with the surfactant Tween 20 (Yang, 2008). Reducing transpiration by applying surfactants to plant roots is a novel concept in need of further research. The objective of the current research project is to test for effects of Tween 20 on growth of a woody plant with particular focus on the effects on transpiration and drought tolerance.

MATERIALS AND METHODS

Initial set-up and experimental design. All research was conducted at the University of Georgia Trial Garden greenhouse in Athens, Georgia from 1 Oct. 2016 – 17 Nov. 2016. Twenty, 3-gallon *Ligustrum japonicum* 'Recurvifolium' obtained from a local wholesale nursery (RA Dudley Nurseries Inc, Thomson, GA) were placed onto two greenhouse benches in a completely randomized block design. Plants were treated with either 0, 50, 100, or 200 ppm Tween 20 by injecting a concentrated Tween 20 solution into the irrigation stream with a Dosatron (Model D14MZ2VFII, Dosatron USA, Clearwater, FL) and applying irrigation water directly to the substrate of plants with a watering wand until water visibly leached out of the bottom of the container. Three, 5-gallon buckets were used as reservoirs for the concentrated Tween 20 solutions. While irrigating, after switching the Dosatron uptake tube from one bucket to another, the irrigation valve was turned on and water from the wand was allowed to pour into the greenhouse drain for 2 minutes in order to "prime" the irrigation line with the new Tween concentration to avoid accidental cross contamination of Tween 20 concentrations. After the initial irrigation with Tween 20 solutions, plants were irrigated every other day at 7am and were irrigated with Tween 20 solution every other irrigation. In other words, plants were irrigated every 2 days but only treated every 4 days for the duration of the experiment (Table 3.1).

Data collection. Evapotranspiration (ET) was measured on days that irrigation occurred except for a few select days when Transpiration (T) was measured instead (Table 3.1). Both ET and T were calculated gravimetrically with an Ohaus scale (Model I10, Ohaus Corporation, Florham Park, NJ) by weighing plants 45 minutes after watering, to allow for gravitational water loss, and then again at 6:00 pm the same day. Evening weight was subtracted from morning weight to calculate gravimetric water loss from either ET or T for each given day. Plant water potential was measured once per week with a Plant Water Status Console, Model 3005 (Soil Moisture Equipment Corp, Santa Barbara, CA). Three 5-leaf cuttings were taken per plant and measured for plant water potential immediately after being removed from the plant. Plant water potential measurements were made at different times of the day depending on the day it was measured (Table 3.1). In the final week of experimentation, all plants were watered with treatment solutions one final time and then photosynthesis parameters were measured beginning at noon one day after the final irrigation with a Ciras II (PP Systems, Amesbury, MA). These same measurements were taken one more time 2 days after the initial measurement, again at noon, when plants were displaying significant signs of drought stress. The Ciras II cuvette was programed at 400 ppm CO_2, leaf measurement area was set at 2.5 cm^2, and LED light was set at 1000 PAR. These were the final measurements made before experiment termination.

Statistical analysis. Data was analyzed using an ANOVA-type analysis with linear mixed models using the GLIMMIX procedure of SAS. Block was included in the models as a random factor. Experimental unit was included in the models as a random factor when subsamples were used. *P* values for multiple mean comparisons were adjusted using the Shaffer-Simulated method.

RESULTS

ET/T. Upon visual inspection of Figures 3.1 and 3.2 it appears that plants treated with 100 and 200 ppm Tween 20 ride the lower end of the graph for most the measurements, however, at no point were there significant differences in transpiration rates, and only for two measurements were there significant differences in evapotranspiration (ET) rates. For ET measurements 4 and 5, plants treated with 100, and 200 ppm Tween 20 had significantly lower ET rates than plants treated with 0ppm Tween 20. Transpiration decreases of the magnitude which Yang (2008) observed in herbaceous plants grown in substrate and hydroponically were not observed in the woody plant *Ligustrum japonicum* 'Recurvifolium' in this experiment.

Photosynthesis. The parameters, transpiration, photosynthesis, conductance and water use efficiency did not vary by Tween 20 concentration on "pre" measurements that were made from 12:00 pm to 1:00 pm while all plants were well hydrated. Plants treated with 0 and 200 ppm Tween 20 had an elevated WUE on the second "post" measurement while under drought stress (Table 3.2). Although having increased WUE, both 0 and 200 ppm treated plants had much lower photosynthetic and transpiration rates during drought conditions than when fully irrigated. It is well known that during reduced water supply, non-drought tolerant plants typically experience an overall reduction in photosynthetic and have an increased WUE (Blum, 2009; Meyers et al., 1984; Peuke et al., 2006). The WUE rates of plants treated with 0 and 200 ppm did not differ from each other but rather were both higher than plants treated with 50 and 100 ppm Tween 20. It is somewhat confusing as to why WUE decreases with the middle two Tween 20 concentrations yet raises back to the same level as the control (0 ppm Tween 20). Although the difference is insignificant, it appears that plants treated with 0 ppm Tween 20 are operating at a lower, yet equally efficient rate as plants treated with 200 ppm Tween 20. It is likely that plants treated with 0 ppm Tween 20 are performing at a higher level of efficiency for the reason mentioned earlier, because they are experiencing a higher level of drought stress. Yang (2008) showed that Tween 20 can increase the moisture retention in a pine bark:peat:perlite substrate, which could be occurring in this experiment. If more moisture were being retained because of the increased moisture retention effects of Tween 20 it could explain the lower WUE measurements in 50 and 100 ppm. As for the anomaly, which is the similar WUE readings between 0 and 200 ppm, it is likely that there is variation in photosynthetic capabilities from leaf to leaf (Medrano et al., 2015). Individual readings among the plants treated with 0 ppm Tween 20, were fairly precise, however there was more variability in readings from plants treated with 200 ppm Tween 20. In either case, the photosynthesis data would have been bolstered with more experimental units and more subsample measurements per experimental unit. Additionally, it would have been useful to have made soil moisture content measurements to help determine if treatment differences amongst WUE measurements were correlated with a higher soil moisture status or if Tween 20 was altering plant physiology in some way. In retrospect, it also would have been useful to have made water potential measurements on the same day as photosynthesis measurements were made.

Water potential. Xylem water potential (Ψ_p) in *Ligustrum japonicum* 'Recurvifolium' fluctuated most noticeably based on the day it was measured rather than according to Tween 20 concentration. Considering the time of day at which Ψ_p was measured (Table 3.1) it became apparent that the likely cause for the day-to-day fluctuations was related to measurement time of day. Measurements made close to or after noon (midday) were consistently in in the range of -7 to -11 bar. At midday in fully watered almonds, xylem Ψ_p usually ranges from -6 to -10 bars, while in moderately stressed trees Ψ_p may range from -10 to -20 bars (Lampinen et al., n.d.). According to Taiz et al. (2015), typical mid-day (Ψ_p) in the xylem is in the range of -10 to -20 bar. On 8 Oct., plants treated with 200 ppm Tween 20 had a 16.9 % higher (less negative) Ψ_p than plants treated with 0 ppm Tween 20, indicating that 200 ppm treated plants were likely undergoing less transpiration (Figure 3.3). On the last day that Ψ_p was measured, plants treated with 50 ppm Tween 20 had a 15.7% higher Ψ_p than plants treated with 0 ppm Tween 20, again indicating that plants were likely undergoing less transpiration.

CONCLUSIONS

Some data indicates that Tween 20 concentration has an effect on plant water use efficiency. It is unclear exactly why WUE decreased in plants treated with 50 and 100 ppm Tween 20 but was similar in control plants and plants treated with 200 ppm Tween 20. From the trends in the data it seems worthwhile to perform a similar experiment but with 10-15 experimental units rather than 5 and by taking more subsample data when measuring photosynthesis parameters. Instead of utilizing nursery-grown plants, it would be better to purchase plant liners, substrate, and fertilizer and transplant plants in order to have more control over the variables that could skew data interpretation.

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Table 3.1 Daily temperatures and dates for irrigation applications, evapotranspiration (ET) measurements, transpiration (T) measurements, and water potential (WP) measurements for 3-gallon *Ligustrum japonicum* 'Recurvifolium' grown on benches in Trial Garden Greenhouse at the University of Georgia, Athens, GA in fall 2016.

Date	Daily temperatures (°F)[z]			Irrigation	Evapotranspiration (ET)	Transpiration (T)	Water potential (WP)
	min	avg	max				
9/25/16	64	79	94	w[y]			
9/27/16	66	78	90	t			
9/29/16	56	71	85	w			
10/1/16	49	66	82	t			12:30pm
10/3/16	60	74	87	w	Measurement 1		
10/5/16	60	72	83	t	Measurement 2		
10/8/16	63	78	92	w			1:30pm
10/9/16	50	68	85	t		Week 1	
10/11/16	47	61	75	w			
10/13/16	49	67	85	t	Measurement 3		
10/15/16	60	67	74	w		Week 2	11:45am
10/17/16	55	70	85	t	Measurement 4		
10/19/16	59	75	91	w	Measurement 5		
10/21/16	52	62	71	t	Measurement 6		
10/22/16	44	57	69	-			8am
10/23/16	44	61	77	w		Week 3	
10/25/16	48	63	77	t	Measurement 7		
10/27/16	53	68	82	w	Measurement 8		
10/29/16	51	69	86	t	Measurement 9		7am
10/31/16	54	71	88	w		Week 4	
11/2/16	58	70	81	t	Measurement 10		
11/4/16	53	67	80	w	Measurement 11		
11/5/16	45	59	72	-			9:30am
11/6/16	40	57	74	t		Week 5	
11/8/16	40	54	68	w			
11/10/16	36	53	70	t	Measurement 12		
11/12/16	46	55	63	w	Measurement 13		
11/14/16	33	51	69	t			

[z] Daily minimum (min), average (avg), and maximum (max) outside temperature recorded at the Athens Ben Epps Airport in Clarke County, GA, USA.

[z] A letter 'w' indicates irrigation with water only, a letter 't' indicates irrigation with treatments of 0, 50, 100, and 200 ppm Tween 20 injected into irrigation stream with a Dosatron (Model D14MZ2VFII, Dosatron USA, Clearwater, FL).

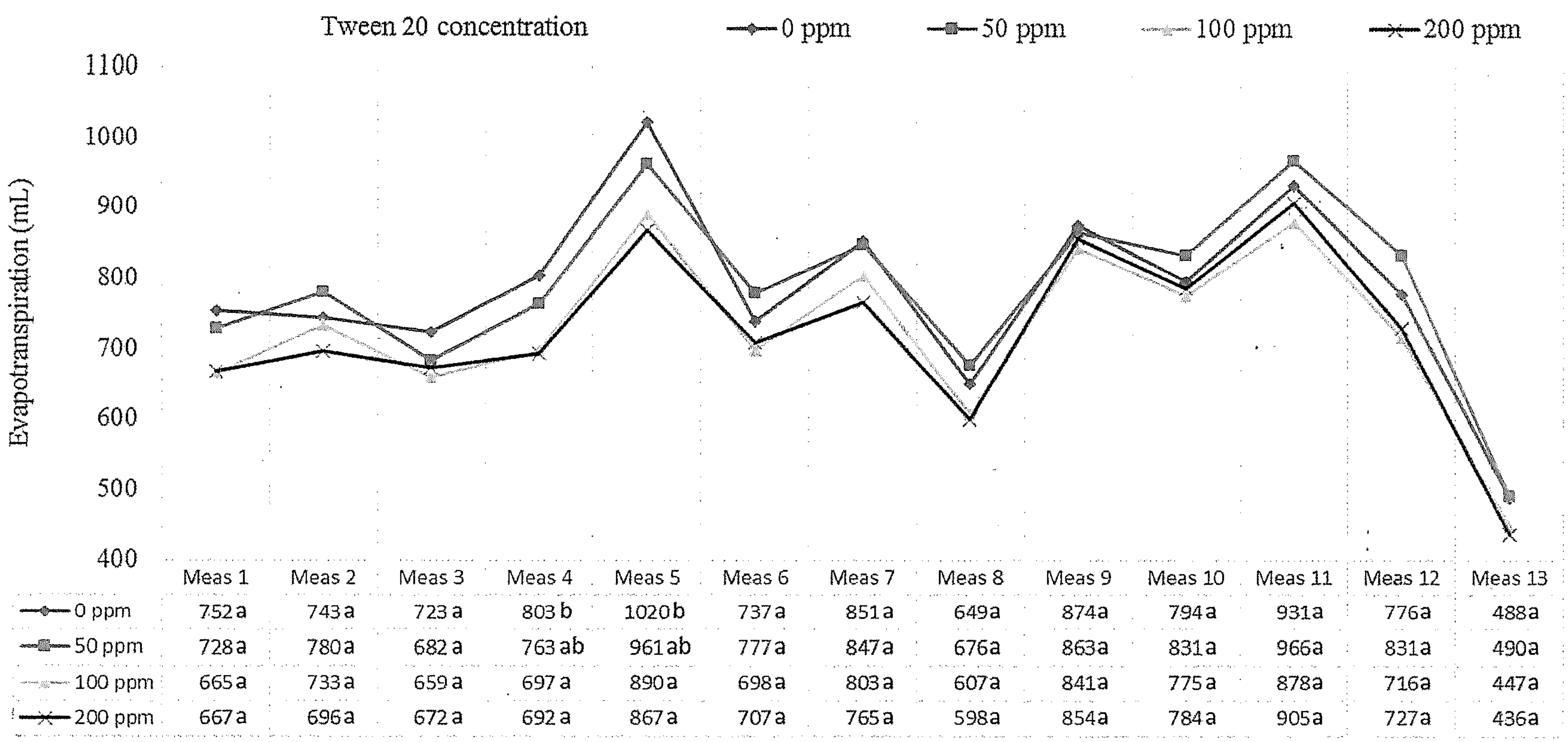
Table 3.2 Photosynthesis (µmol m^{-2} s^{-1}), transpiration (mmole m^{-2} s^{-1}), conductance (mmole m^{-2} s^{-1}) and intrinsic water use efficiency (µmol CO_2 mmole^{-1} H_2O) of 3-gallon *Ligustrum japonicum* 'Recurifolium' treated with 0, 50, 100, and 200 ppm Tween 20 grown on benches in Trial Garden Greenhouse at the University of Georgia, Athens, GA in fall 2016. Measurements were made once while plants were fully hydrated (Pre) and once more when plants were experiencing substantial drought stress (Post).

Parts per million (ppm) Tween	Photosynthesis[z]		Transpiration		Conductance		Intrinsic Water Use Efficiency[y]	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
0	9.6800a[x]	1.6200a	0.9880a	0.1220a	30.4a	2.6a	10.3920a	16.3220a
50	10.3800a	1.2400a	0.9960a	0.2360a	31.2a	5.8a	11.4160a	6.2740b
100	7.9000a	2.8800a	0.9020a	0.4260a	27.6a	10.4a	9.6120a	7.7900b
200	10.1800a	3.5400a	0.9820a	0.3760a	30.4a	9.4a	11.2360a	13.4120ab

[z] Photosynthesis, transpiration, and conductance measured with Ciras 2 (PP Systems, Amesbury, MA).

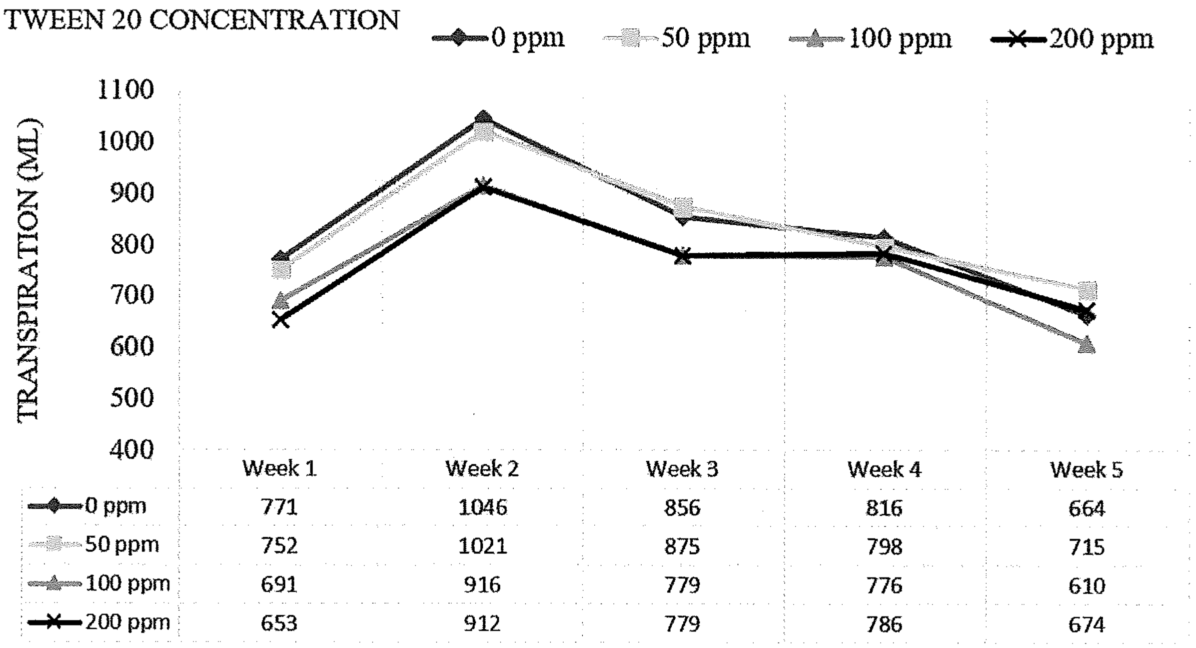
[y] Calculated by dividing photosynthesis by transpiration.

[x] Data analyzed using ANOVA with linear mixed models using the GLIMMIX procedure of SAS. Means with different letters are significant at $\alpha = 0.05$.



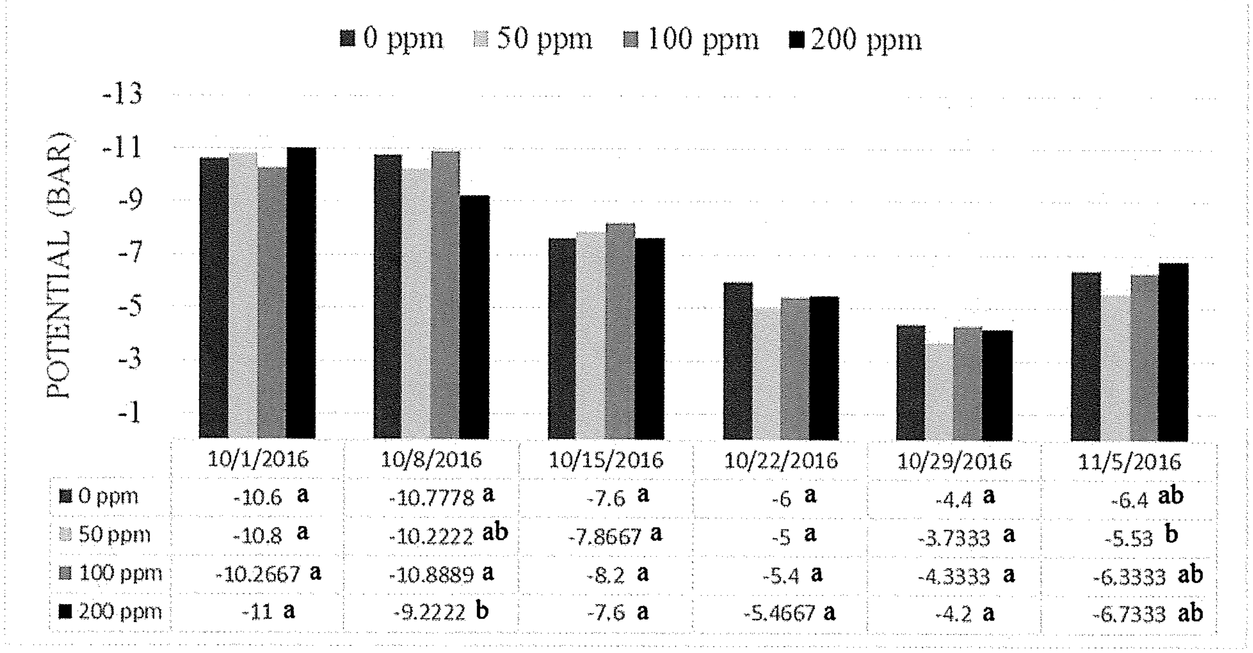
	Meas 1	Meas 2	Meas 3	Meas 4	Meas 5	Meas 6	Meas 7	Meas 8	Meas 9	Meas 10	Meas 11	Meas 12	Meas 13
0 ppm	752 a	743 a	723 a	803 b	1020 b	737 a	851 a	649 a	874 a	794 a	931 a	776 a	488 a
50 ppm	728 a	780 a	682 a	763 ab	961 ab	777 a	847 a	676 a	863 a	831 a	966 a	831 a	490 a
100 ppm	665 a	733 a	659 a	697 a	890 a	698 a	803 a	607 a	841 a	775 a	878 a	716 a	447 a
200 ppm	667 a	696 a	672 a	692 a	867 a	707 a	765 a	598 a	854 a	784 a	905 a	727 a	436 a

Figure 3.1 Milliliters of water loss from evapotranspiration of 3-gallon *Ligustrum japonicum* 'Recurvifolium' treated with 0,50,100, and 200 ppm Tween 20 grown on benches in Trial Garden Greenhouse at the University of Georgia, Athens, GA in fall 2016. Measurements 1-13 were taken at various times through the duration of the experiment (Table 3.1). Data analyzed using ANOVA with linear mixed models using the GLIMMIX procedure of SAS. Means with different letters are different at $\alpha = 0.05$.



	Week 1	Week 2	Week 3	Week 4	Week 5
0 ppm	771	1046	856	816	664
50 ppm	752	1021	875	798	715
100 ppm	691	916	779	776	610
200 ppm	653	912	779	786	674

Figure 3.2 Milliliters of water loss from transpiration of 3-gallon *Ligustrum japonicum* 'Recurvifolium' treated with 0,50,100, and 200 ppm Tween 20 grown on benches in Trial Garden Greenhouse at the University of Georgia, Athens, GA in fall 2016. Data analyzed using ANOVA with linear mixed models using the GLIMMIX procedure of SAS. No significant differences were observed.



	10/1/2016	10/8/2016	10/15/2016	10/22/2016	10/29/2016	11/5/2016
■ 0 ppm	-10.6 **a**	-10.7778 **a**	-7.6 **a**	-6 **a**	-4.4 **a**	-6.4 **ab**
▨ 50 ppm	-10.8 **a**	-10.2222 **ab**	-7.8667 **a**	-5 **a**	-3.7333 **a**	-5.53 **b**
▨ 100 ppm	-10.2667 **a**	-10.8889 **a**	-8.2 **a**	-5.4 **a**	-4.3333 **a**	-6.3333 **ab**
■ 200 ppm	-11 **a**	-9.2222 **b**	-7.6 **a**	-5.4667 **a**	-4.2 **a**	-6.7333 **ab**

Figure 3.3 Water potential measured on 5-leaf stem segments on 3-gallon *Ligustrum japonicum* 'Recurvifolium' treated with 0,50,100, and 200 ppm Tween 20 grown on benches in Trial Garden Greenhouse at the University of Georgia, Athens, GA in fall 2016. Three subsamples were measured on each plant. Data analyzed using ANOVA with linear mixed models using the GLIMMIX procedure of SAS. Means with different letters are significantly different at $\alpha = 0.05$.

CHAPTER 4

EVAPORATION RATES OF MULTIPLE SOLUTIONS CONTAINING VARIOUS CONCENTRATIONS OF A NONIONIC SURFACTANT

ABSTRACT

When a plant lacks the necessary water to fully perform normal functions that would otherwise occur in the presence of sufficient water, the plant is said to be experiencing water deficit stress. In container-grown plants almost entirely reliant on supplemental irrigation, water deficit stress usually occurs due to (1) poor irrigation system design, (2) faulty irrigation equipment, (3) or improper irrigation scheduling. The above-mentioned issues can also be a leading cause for water waste during container-grown plant production. As water frequently becomes scarce in the western US, it is important for the greenhouse and nursery industries to become more efficient with their irrigation practices. Surfactants help to mitigate soil and substrate hydrophobicity and can increase substrate moisture retention. The surfactant Tween 20 has shown potential for decreasing transpiration by up to 50% in New Guinea impatiens and Peace lily grown at Auburn University, AL. The objective of this study is to determine if Tween 20, when mixed with water, alters the evaporation rate of the solution. If Tween 20 were traveling through the plant and made it all the way to the leaf surface, evaporation from the leaf surface could potentially be altered by the interfacial properties of surfactants. Concentrations of 0, 25, 50, 75, 100. 125, 150, 175, and 200 ppm Tween 20 solution were placed in 12-ounce capacity open bowls on a bench in a greenhouse at Auburn University, AL. Evaporation rate was measured every morning by weighing each bowl. Results indicate that solutions containing Tween 20 have a slightly increased rate of evaporation compared with water with no Tween 20.

INTRODUCTION

Water is the primary driving factor in plant growth, and is the single greatest reason for yield losses worldwide when not sufficiently supplied (Boyer, 1982). When a plant lacks the necessary water to fully perform normal functions that would otherwise occur in the presence of sufficient water, the plant is said to be experiencing water deficit stress. In nature, water deficit stress occurs when soil moisture reaches a point where plants are at a permeant wilting point and there is insufficient rainfall to replenish soil water. In container-grown plants almost entirely reliant on supplemental irrigation, water deficit stress usually occurs due to (1) poor irrigation system design, (2) faulty irrigation equipment, (3) or improper irrigation scheduling. In addition to being the cause for water deficit stress, the three above issues are also the cause for much water being wasted during irrigation because water either never reaches the rootzone of plants or it doesn't stay in the rootzone due to leaching or flooding over container sidewalls. To address the issues of water deficit stress and water waste from suboptimal irrigation performance and practices, horticulture academia has performed countless research projects and made great strides towards more sustainable irrigation practices. In an effort to reduce the amount of water needed for irrigation, a secondary approach, not directly related to irrigation, can be taken. This approach is centered on either reducing plant transpiration rates, increasing CO_2 fixation rates, or accomplishing both at the same time. The relationship between CO_2 fixation and transpiration is typically referred to as water use efficiency (WUE) and is essentially a measurement of how efficiently a plant can trade water for CO_2 through stomata. In theory, both CO_2 fixation rates and transpiration rates can be altered, but both goals have thus far proven difficult to attain with much practical significance.

Transpiration benefits plants by helping draw water (mass flow) and nutrients from the soil towards plant roots where they become accessible to the plant (Barber et al., 1963). Additionally, transpiration can help cool leaf surfaces and expedite nutrient flow from root to shoot (Hopkins and Hüner, 2004). Yet, transpiration of large amounts of water is the leading cause of crop loss (Boyer, 1982). Roughly 95 to 97% of all water entering a plant during its lifetime is ultimately expelled, primarily through stomata, as transpiration (Kramer and Boyer, 1995; Taiz et al., 2015). Although transpiration can provide certain beneficial effects to plants, many have questioned whether plant growth would be negatively affected if transpiration were eliminated or significantly reduced (Muenscher, 1922; Tanner and Beevers, 1991, 2001). If possible, significantly reducing transpiration could greatly decrease the frequency of drought incidence and increase the efficiency of dry matter production per unit of water transpired. Muenscher (1922) provided an insightful review investigating the relation of transpiration rates are directly on nutrient absorption rates. Muenscher determined that transpiration rate in barley plants did not affect rates of nutrient absorption. Inspired by the work of Muenscher, Tanner and Beevers (1990,2001) conlcuded that transpiration was not necessary for uptake of nutrients in maize and sunflower and provocatively suggested, in contrast to popular belief, that transpiration may be entirely useless to plants and only occurs because it is an unavoidable side-effect of the Carbon fixation process. Smith (1991) promptly criticized the interpretation and conclusions of the 1991 study by Tanner and Beevers. A study of pear trees, germinating sunflowers, and growth of sunflowers showed that high humidity (reduced transpiration) had negative effects on plant growth (Winneberger, 1958). Although research is conflicting, there seems to be enough evidence to suggest that transpiration can be decreased in certain species with no deleterious effects on growth. Therefore, if it were possible to manipulate and decrease transpiration, large amounts of water savings could be obtainable.

The surfactant Tween 20 applied at 100 mg/L reduced the crop water demand by up to 40% for *Impatiens hawkerii* 'Celebrate Salmon' grown in Fafard 3B substrate, and reduced transpiration in *Spathiphyllum floribundum* 'Viscount' by 64% and in *Impatiens hawkerii* 'Celebrate Salmon' by 101% when grown hydroponically (Yang, 2008). The hydroponic study mentioned above is noteworthy because it demonstrated that a surfactant was inducing water savings by directly altering plant transpiration rather than by altering substrate moisture holding characteristics. The objective of this study is to determine if Tween 20, when mixed with water, alters the evaporation rate of the solution. If Tween 20 were traveling through the plant and made it all the way to the leaf surface, evaporation from the leaf surface could potentially be altered by the interfacial properties of surfactants.

MATERIALS AND METHODS

Materials and experimental design. Twelve-ounce capacity Styrofoam bowls (Great Value™, Walmart Stores Inc., Bentonville, AR) and nine, one-gallon jugs of distilled water (Great Value™, Walmart Stores Inc., Bentonville, AR) were obtained for use in this experiment. Recently purchased Tween 20 solution (Rocky Mountain Oils, Orem, UT) stored in a climate controlled room at 18°C was used to create 9 concentrations of Tween 20 solution. Experimentation took place in a 2300 ft^2 fan and pad greenhouse at the Paterson Greenhouse Complex in Auburn University, Alabama. Replicates were arranged in a completely randomized experimental design on a single bench located in the center of the greenhouse (Figure 4.1).

Initial experiment set-up. Nine, 3000 mL stock solutions were prepared in concentrations of 0, 25, 50, 75, 100, 125, 150, 175, and 200 ppm Tween 20 using the distilled water purchased from Walmart. From each of the 9 solutions, 10 Styrofoam bowls were filled with 200 mL of solution in each bowl, using a 100-mL graduated cylinder to measure and transfer stock solution to each bowl. A single rinse of the graduated cylinder with plain water was performed in between the fillings of each bowl in order to prevent the accumulation of foam from Tween 20 solution being transferred to subsequent bowls, and altering originally designed concentrations. In total, 9 solution treatments with 10 replicates per treatment were used in this experiment. Once all Styrofoam bowls were filled with 200 mL of Tween 20 solution, they were placed on a bench in the center of the greenhouse in a completely randomized design.

Data collection. Following randomization, each bowl was immediately weighed at 7am on 31 March 2017 to establish an initial weight before any solution had evaporated. Subsequent weight measurements were taken each of the following mornings at 7am until all water had evaporated by 7am on 9 April 2017. Daily evaporation rates were calculated by subtracting the weight of the following morning from the weight calculated on the morning of the desired day. In total, data for 9 days of evaporation were recorded. Solutions in bowls were considered to be completely evaporated when no visible residues remained. The entire experiment was considered terminated when contents of all bowls were completely evaporated.

Statistical analysis. Data were analyzed using ANOVA-type analyses using the GLIMMIX procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC). Means were compared at ($\alpha = 0.05$). *P* values for multiple comparisons were adjusted using the Shaffer-Simulated method. Additionally, a piecewise regression was conducted using the GLIMMIX procedure in SAS. To further analyze the fitness of the piecewise regression model, a lack-of-fitness test was also conducted.

RESULTS

On days 1 through 4 evaporation rate of the solution with no Tween 20 (0 ppm) was lower than all treatments containing Tween 20 and there were no differences in evaporation amongst treatments containing Tween 20 (25 ppm to 200 ppm) (Table 3.1). On day 7 evaporation rate did not vary amongst treatments. On days 8 and 9 the general trend established from day 1 to day 6 was reversed and the evaporation rate of 0 ppm Tween 20 solution was higher than all other treatments. On the morning of the ninth day several replicates from varying concentration solutions had completely evaporated, mostly from higher concentration solutions. Because a single daily measurement was taken at 7am, it was impossible to determine if the water had completely evaporated sometime between 7am and 7pm on the eighth day or at 7am on the ninth day. It is likely that in many cases the solution completely evaporated sometime in the afternoon of the eighth day which would mean that if more solution were in the bowl, the evaporation rate for that day would be higher than what was actually measured. Essentially, once the solution began to run out, it became a limiting factor to overall evaporation volume and rate that was able to be measured for a given day. It is believed that this is the cause for the general reversal of evaporation trends on days 8 and 9.

When looking at evaporation rate from a cumulative perspective (Figure 4.2 and Table 4.2) the piecewise regression analysis revealed that evaporation rate was positively related to increasing ppm of Tween 20 on every day except for the last day. On the last day increasing ppm Tween 20 was negatively related to evaporation rate. The reason for evaporation on the last day not following the same pattern as the previous days is likely the same as was mentioned in the previous paragraph. When looking at evaporation rates of individual days (Tables 4.3, 4.4 and Figures 4.3a-h) there was less consistency and there was not always a correlation between increasing Tween ppm and increasing evaporation rates. When considering days individually, only days 1, 2, 4, and 5 have significant parameters for all three parameters (intercept, tween, and ppm) (Table 4.3).

CONCLUSIONS

Data from this experiment indicates that incorporating Tween 20 into water increases the rate of evaporation compared with evaporation rate of water with no Tween 20. The biggest increase in evaporation rate occurs between 0 ppm and 25 ppm Tween 20. On several days, evaporation rate increased linearly with increasing Tween 20 concentration. If Tween 20 were traveling through the plant and made it all the way to the leaf surface, evaporation from the leaf surface would likely slightly increase based on results of this study. Future studies could pinpoint at what point between 0 ppm and 25 ppm Tween 20 does evaporation begin to increase.

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Table 4.1 Mean daily evaporation rates (% of total solution volume) of 0, 25, 50, 75, 100, 125, 150, 175, and 200 ppm Tween 20 solutions (200 mL per replicate) contained in 12-ounce Styrofoam bowls and placed in a completely randomized design on a single bench in a fan and pad greenhouse in Auburn, AL over an 8-day period in April 2017.

Treat[z]	Daily evaporation rates (% of total solution volume)							
ppm	1	2	3	4	5	6	7	8[x]
0	17.27b[y]	16.73b	15.83b	2.82b	12.18c	4.01b	12.61a	14.06a
25	18.13a	17.86a	17.00a	3.05a	12.52bc	4.01b	12.68a	13.32ab
50	18.28a	17.79a	16.86a	3.14a	12.74ab	4.19ab	12.84a	12.84ab
75	18.35a	17.73a	16.91a	3.16a	12.75ab	4.13ab	12.81a	12.93ab
100	18.38a	17.88a	16.89a	3.17a	12.69ab	4.15ab	12.83a	12.57ab
125	18.30a	17.79a	16.77a	3.14a	12.82ab	4.13ab	12.86a	12.70ab
150	18.40a	18.04a	17.06a	3.16a	13.02a	4.30a	12.99a	12.21ab
175	18.56a	18.23a	17.30a	3.19a	12.93ab	4.22a	12.76a	11.89b
200	18.74a	18.21a	17.19a	3.25a	12.95ab	4.23a	12.88a	11.77b

[z] Treatments consisted of 0, 25, 50, 75, 100, 125, 150, 175, 200 ppm Tween 20 solution.

[y] Means followed by the same letter are not significantly different using the Shaffer-Simulated method for multiple comparisons ($\alpha = 0.05$).

[x] On the morning of the ninth day several replicates from varying concentration solutions had completely evaporated, mostly from higher concentration solutions. Because a single daily measurement was taken at 7am, it was impossible to determine if the water had completely evaporated by 3pm on the eighth day or at 7am on the ninth day. It is likely that in many cases the solution completely evaporated sometime on the eighth day meaning that, if more solution were in the bowl, the evaporation rate for that day would be higher than actually measured. It is believed that this is the cause for the general reversal of evaporation trends on days 8.

Table 4.2 Coefficients for cumulative percentage evaporation used to create the piecewise regression model (Figure 4.2) for evaporation of 9 concentrations of Tween 20 solution over an 8-day period. The "intercept" coefficient represents the percentage of solution evaporated during that day from 0 ppm Tween 20 Solution (represented by dot in Figure 4.2). The "tween" coefficient is a variable that has a value of 1 if Tween 20 was in the solution (25 ppm or greater) or 0 for none (0 ppm). The "ppm' coefficient is the variable for the Tween 20 rate.

Day	Coefficients					
	Intercept		Tween[y]		Ppm[x]	
	Estimate	*P*[z]	Estimate	*P*	Estimate	*P*
1	17.27	<.0001	0.82	<.0001	0.0027	0.0038
2	33.99	<.0001	1.74	0.0001	0.0054	0.0090
3	49.82	<.0001	2.70	<.0001	0.0072	0.0171
4	52.64	<.0001	2.95	<.0001	0.0079	0.0130
5	64.82	<.0001	3.31	<.0001	0.0103	0.0061
6	68.83	<.0001	3.35	<.0001	0.0113	0.0035
7	81.45	<.0001	3.49	<.0001	0.0121	0.0035
8	95.50	<.0001	2.90	<.0001	0.0023	0.1104

[z] Estimates with $P \geq 0.05$ are not significant.

[y] The tween coefficient for day 1 (0.82) means that, if the regression line were extended all the way to the y-axis (which is 0 ppm), the line would cross the y-axis at 0.82% units greater than where the dot is located: 17.27% (intercept coefficient) + 0.82% = 18.086%. If the tween coefficient is not significant ($P \geq 0.05$) then the dot (prediction at 0 ppm) would likely fall on the regression line if it were extended to the left.

[x] The ppm coefficient for day 1 (0.0027) means that for every 1 ppm increase in Tween 20 concentration, the predicted percentage of solution evaporated will increase by 0.0027%. This value represents the slope of the regression line.

Table 4.3 Coefficients for piecewise regression models (Figures 4.3a-h) for day-by-day percentage evaporation of solutions containing 9 concentrations of Tween 20 over an 8-day period. The "intercept" coefficient represents the percentage of solution evaporated during that day from 0 ppm Tween 20 solution. The "tween" coefficient is multiplied by an indicator variable that has a value of 1 if Tween 20 was in the solution (25 ppm or greater) or 0 for none (0 ppm). The "ppm' coefficient is the slope (average change in percentage evaporation for each 1 ppm increase in Tween 20 rate).

Day	Coefficients					
	Intercept		Tween[y]		Ppm[x]	
	Estimate	*P*[z]	Estimate	*P*	Estimate	*P*
1	17.27	<.0001	0.82	<.0001	0.0027	0.0038
2	16.73	<.0001	0.92	0.0001	0.0026	0.0228
3	15.83	<.0001	0.96	<.0001	0.0018	0.0679
4	2.82	<.0001	0.25	<.0001	0.0008	0.0118
5	12.18	<.0001	0.36	0.0136	0.0024	0.0009
6	4.01	<.0001	0.05	0.4125	0.0010	0.0002
7	12.61	<.0001	0.14	0.2557	0.0007	0.2089
8	14.06	<.0001	-0.59	0.2699	-0.0084	0.0016

[z] Estimates with *P* values greater than 0.05 are not significant.

[y] The tween coefficient for day 1 (0.82) means that, if the regression line were extended all the way to the y-axis (which is 0 ppm), the line would cross the y-axis at 0.82% units greater than where the dot is located: 17.27% (intercept coefficient) + 0.82% = 18.086%. If the tween coefficient is not significant ($P \geq 0.05$) then the dot (prediction at 0 ppm) would likely fall on the regression line if it were extended to the left.

[x] The ppm coefficient for day 1 (0.00273) means that for every 1 ppm increase in Tween 20 concentration, the predicted percentage of solution evaporated will increase by 0.00273%. This value represents the slope of the regression line.

Table 4.4 R-squared values and results of the Lack-of-Fit (LOF) test for the piecewise regression model for evaporation rates (% of total solution volume) for 8 days of evaporation.

Day	Linear R-Square[z]	LOF test linear fit *P* values[y]
1	0.408	0.928
2	0.339	0.862
3	0.363	0.553
4	0.363	0.881
5	0.312	0.786
6	0.237	0.031
7	0.069	0.557
8	0.207	0.991

[z] R-squared represents the proportion of variability of the data that is explained by the regression model (equation). Values can run from 0 to 1. Higher values are preferred, but lower values do not necessarily indicated a poor fit of the regression line to the data.

[y] Small *p* values for the LOF test indicate a poor fit and larger values (e.g., ≥0.90) are evidence against a poor fit indicating that the slope is not zero.

Figure 4.1 Ninety, 12-ounce capacity bowls filled with 200 mL each of 0, 25, 50, 75, 100, 125, 150, 175, and 200 ppm Tween 20 solution and placed in a completely randomized design on a greenhouse bench in the Paterson Greenhouse Complex in Auburn University, AL.

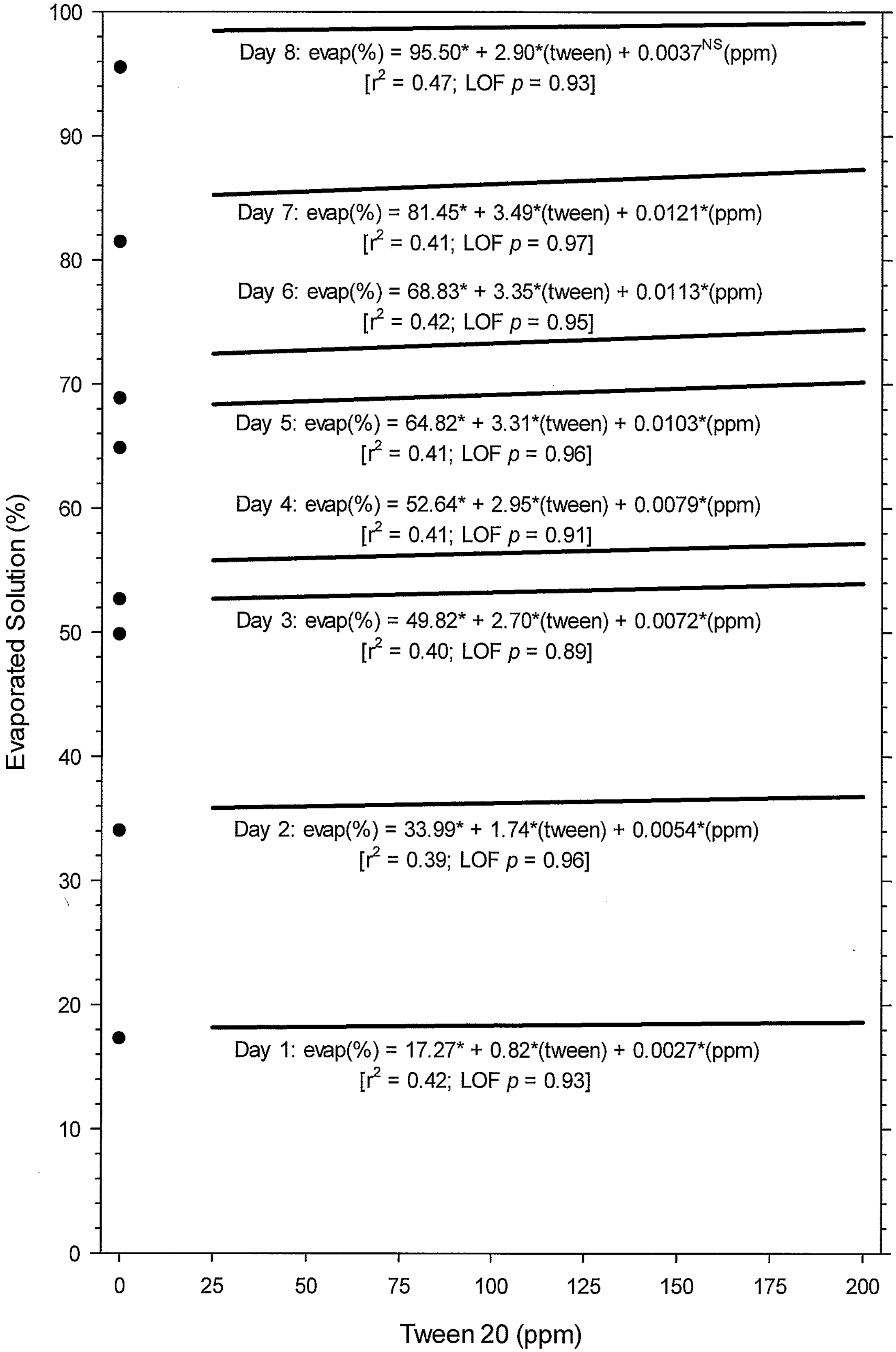
Evaporated Solution (%)
Tween 20 (ppm)
0
10
20
30
40
50
60
70
80
90
100
0
25
50
75
100
125
150
175
200
Day 8: evap(%) = 95.50* + 2.90*(tween) + 0.0037^{NS}(ppm)
[r^2 = 0.47; LOF p = 0.93]
Day 7: evap(%) = 81.45* + 3.49*(tween) + 0.0121*(ppm)
[r^2 = 0.41; LOF p = 0.97]
Day 6: evap(%) = 68.83* + 3.35*(tween) + 0.0113*(ppm)
[r^2 = 0.42; LOF p = 0.95]
Day 5: evap(%) = 64.82* + 3.31*(tween) + 0.0103*(ppm)
[r^2 = 0.41; LOF p = 0.96]
Day 4: evap(%) = 52.64* + 2.95*(tween) + 0.0079*(ppm)
[r^2 = 0.41; LOF p = 0.91]
Day 3: evap(%) = 49.82* + 2.70*(tween) + 0.0072*(ppm)
[r^2 = 0.40; LOF p = 0.89]
Day 2: evap(%) = 33.99* + 1.74*(tween) + 0.0054*(ppm)
[r^2 = 0.39; LOF p = 0.96]
Day 1: evap(%) = 17.27* + 0.82*(tween) + 0.0027*(ppm)
[r^2 = 0.42; LOF p = 0.93]

Figure 4.2 Piecewise regression models showing cumulative evaporation (%) of 9 concentrations of Tween 20 solutions over an 8-day period. Using Day 1 as an example, the first coefficient (17.27) represents the intercept for the first regression "line" for 0 ppm Tween 20 solution which is depicted with a black dot. The second coefficient (0.82) represents the change in the intercept for the second regression line in relation to the intercept for the first regression "line" (black dot); thus, if the line shown in the figure were extended to the left, the line will cross the y axis at y=17.27 + 0.82 = 18.09. The indicator variable "tween" takes a value of 1 when Tween 20 was in the solution or a value of 0 if Tween 20 was not in the solution. Since all values for the coefficient are positive and significant (asterisk next to the coefficient indicates significance), then adding Tween 20 increases evaporation in comparison to water alone. The third coefficient (0.0027) is the slope of the line on the figure and represents the average change in predicted percentage of evaporation for every unit (1 ppm) increase in rate of Tween 20. R-squared represents the proportion of variability of the data that is explained by the regression model (equation). Values can run from 0 to 1. LOF is a lack-of-fit test. Small p values for the LOF test indicate a poor fit. Larger values (e.g., ≥0.90) are strong evidence against a poor fit, indicating a good fit.

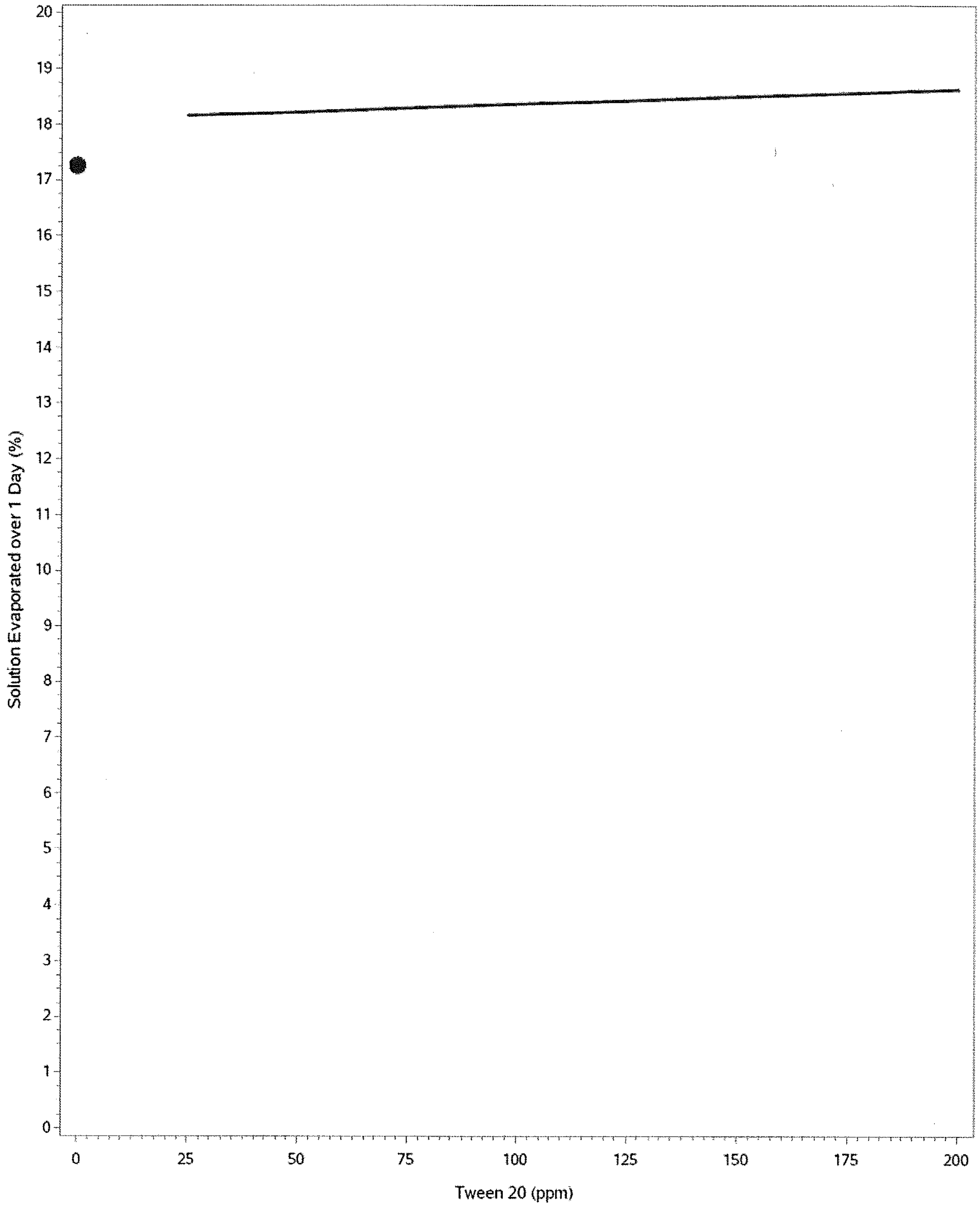

Figure 4.3a Day 1 piecewise regression model of evaporation rates (% of total) for 9 concentrations of Tween 20 solutions. Refer to Table 4.2 for coefficients used for plotting the model.

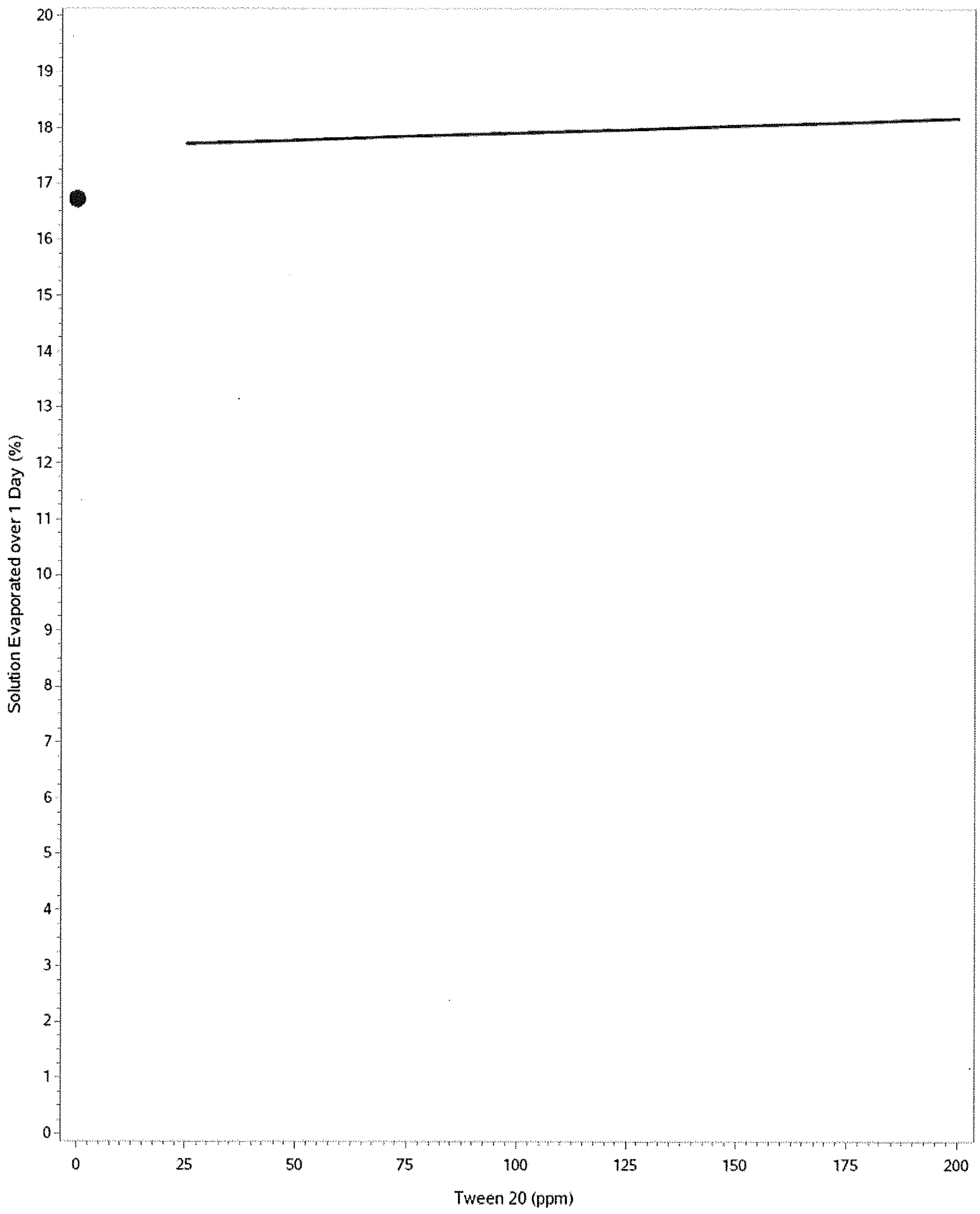

Figure 4.3b Day 2 piecewise regression model of evaporation rates (% of total) for 9 concentrations of Tween 20 solutions. Refer to Table 4.2 for coefficients used for plotting the model.

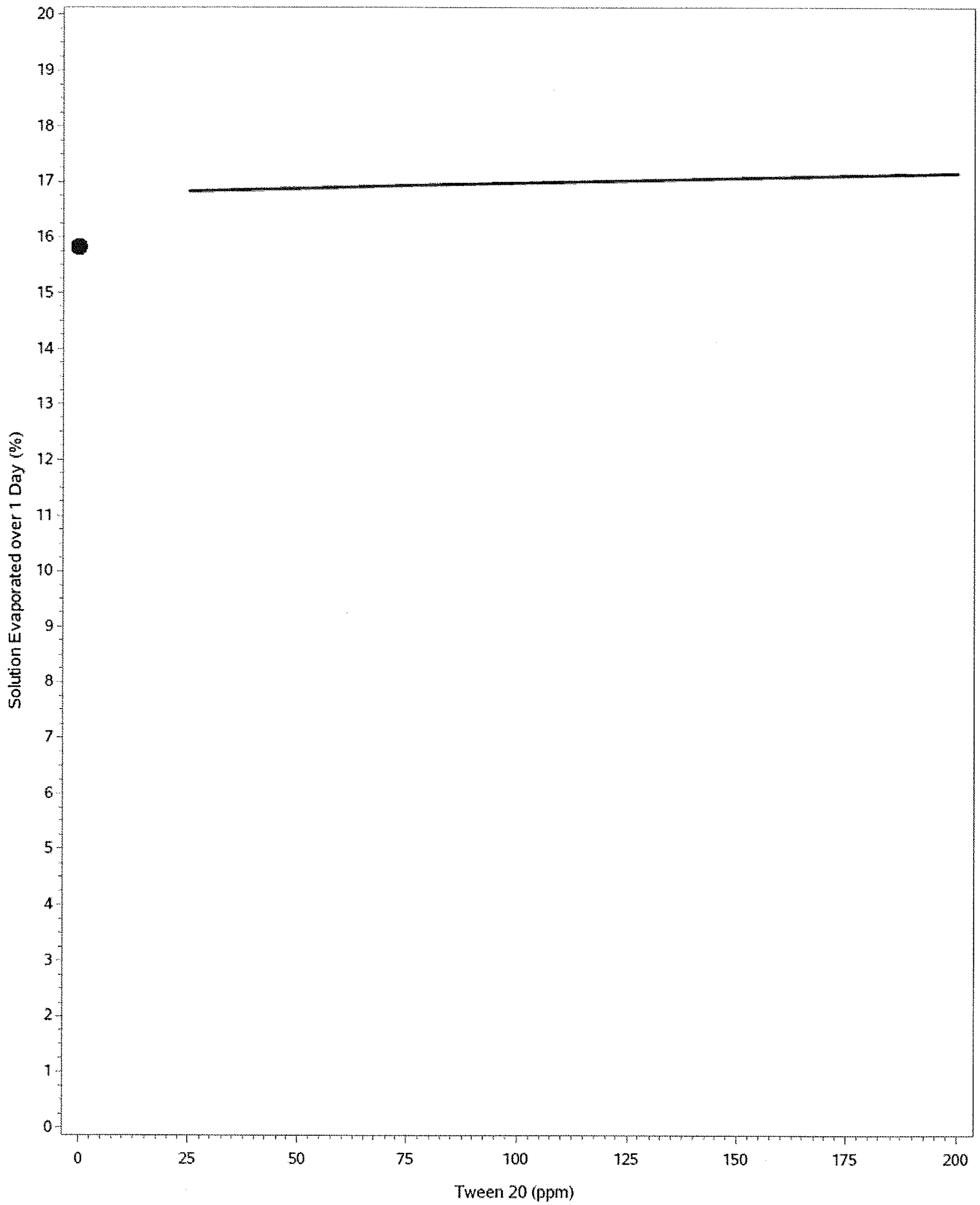

Figure 4.3c Day 3 piecewise regression model of evaporation rates (% of total) for 9 concentrations of Tween 20 solutions. Refer to Table 4.2 for coefficients used for plotting the model.

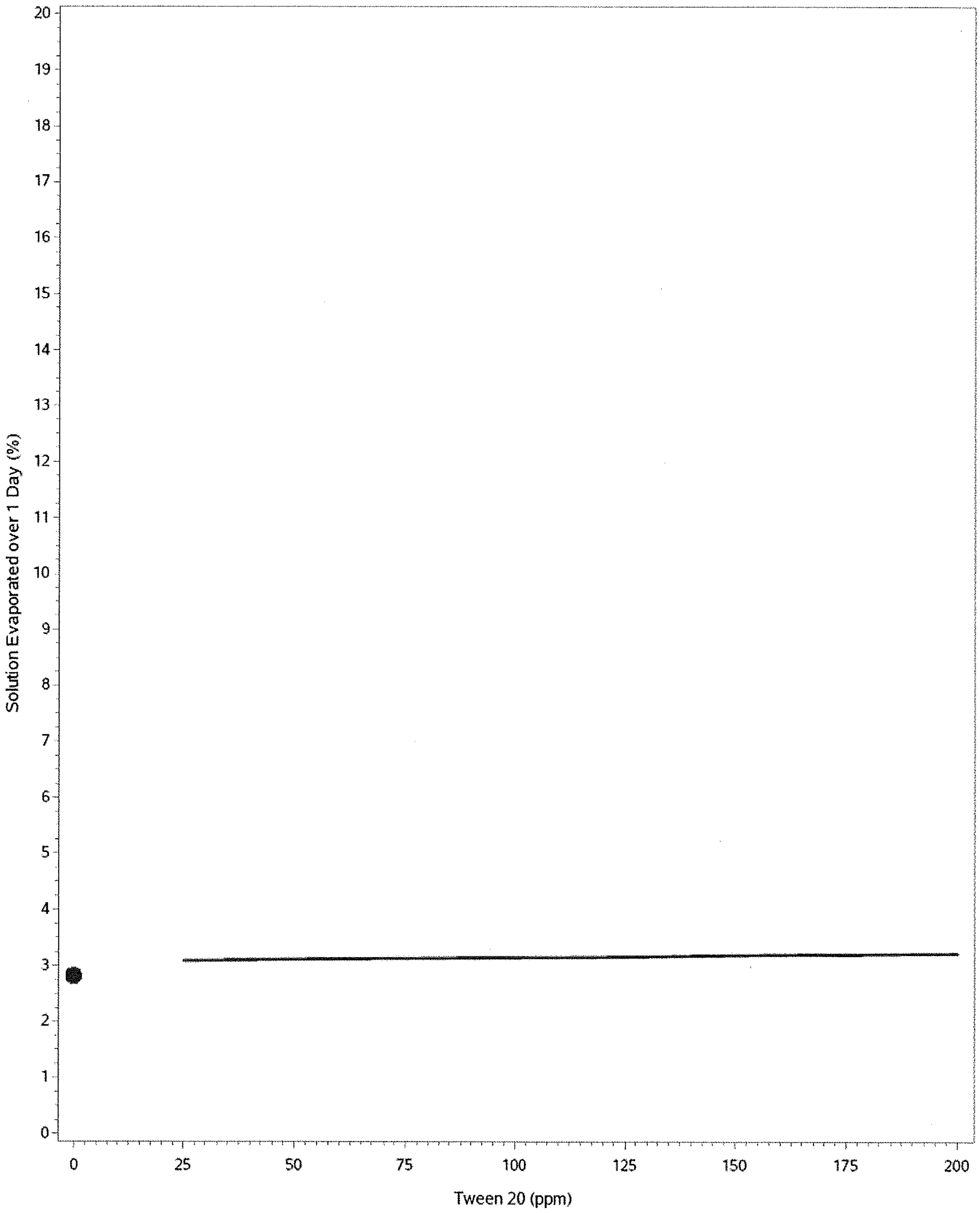

Figure 4.3d Day 4 piecewise regression model of evaporation rates (% of total) for 9 concentrations of Tween 20 solutions. Refer to Table 4.2 for coefficients used for plotting the model.

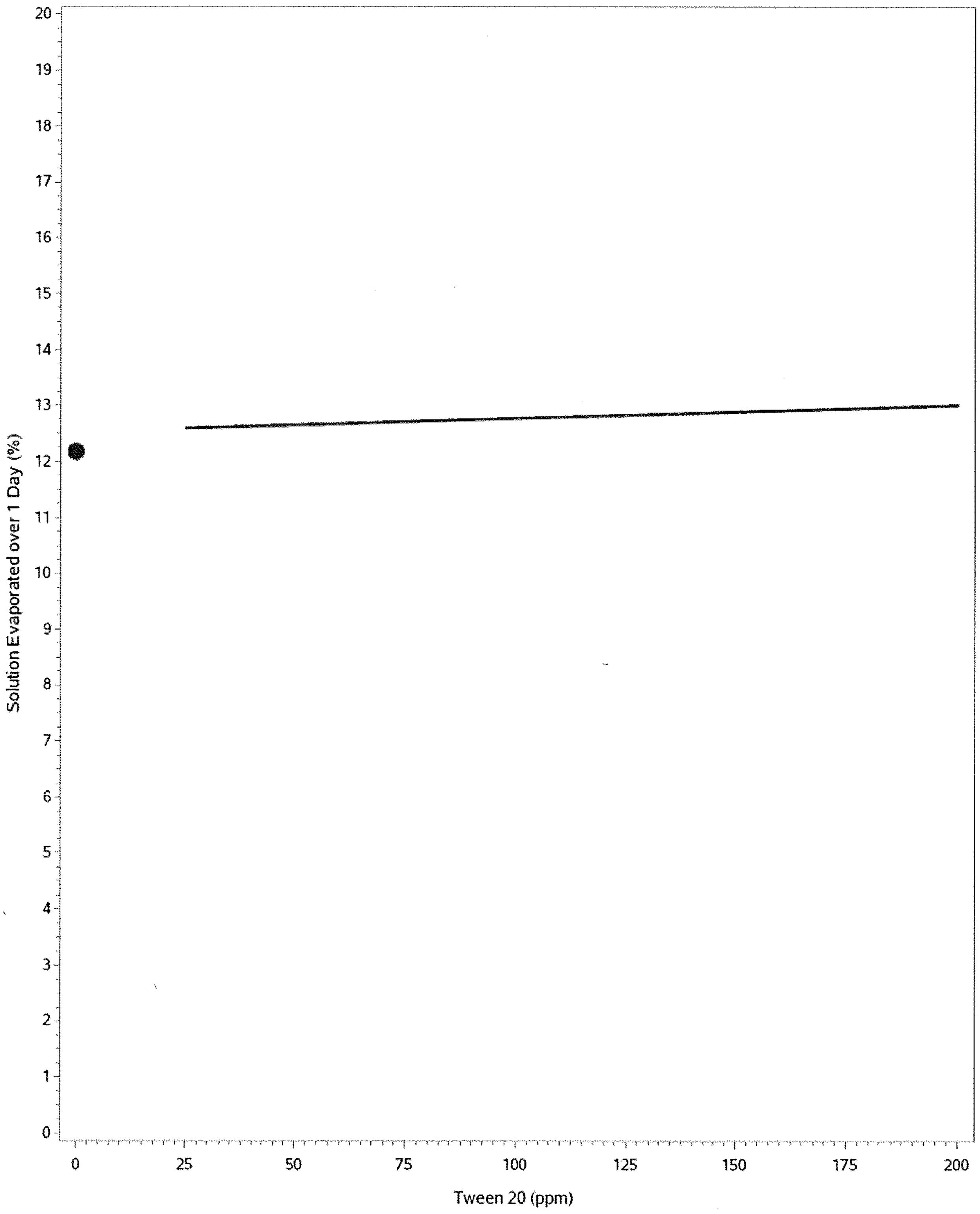

Figure 4.3e Day 5 piecewise regression model of evaporation rates (% of total) for 9 concentrations of Tween 20 solutions. Refer to Table 4.2 for coefficients used for plotting the model.

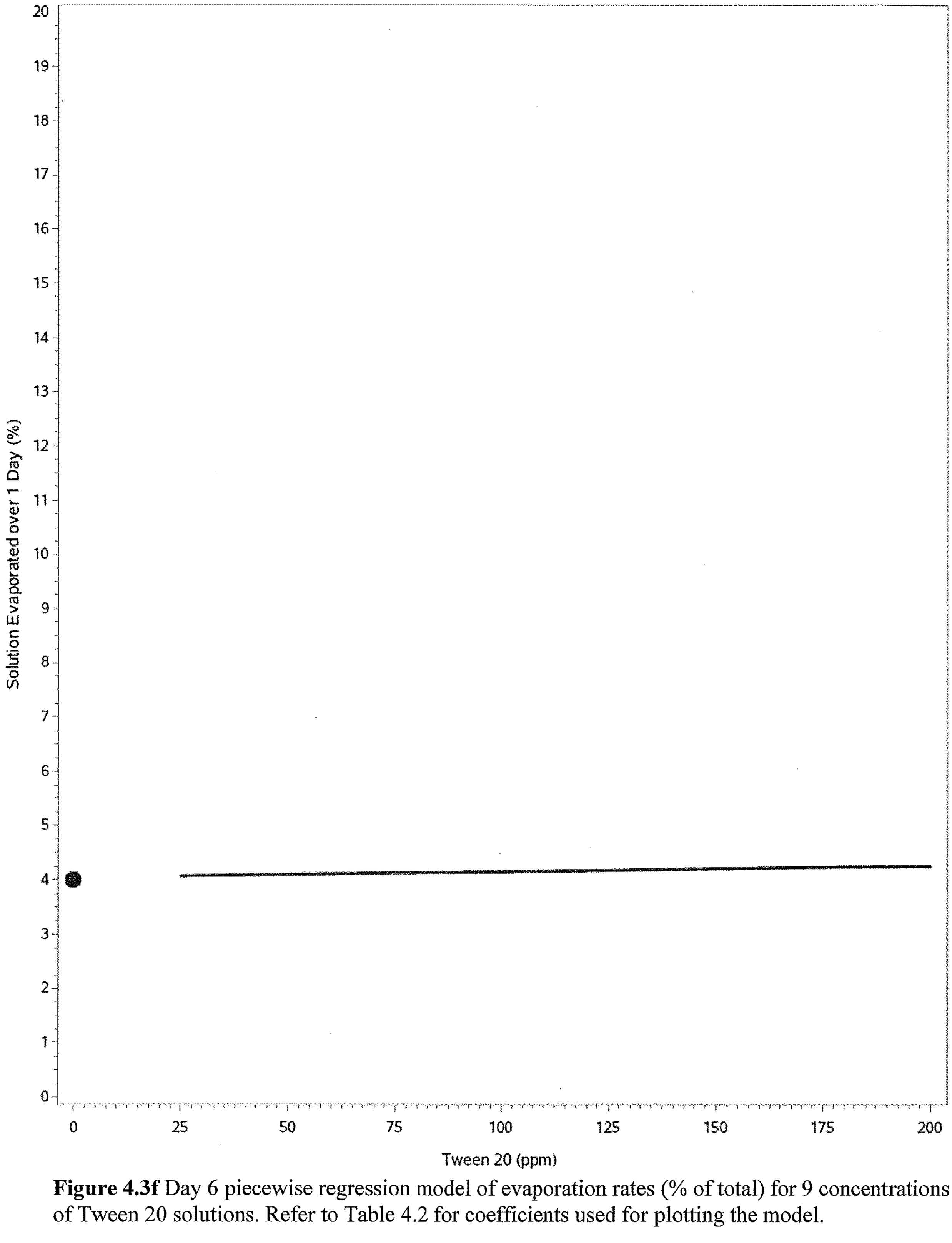

Figure 4.3f Day 6 piecewise regression model of evaporation rates (% of total) for 9 concentrations of Tween 20 solutions. Refer to Table 4.2 for coefficients used for plotting the model.

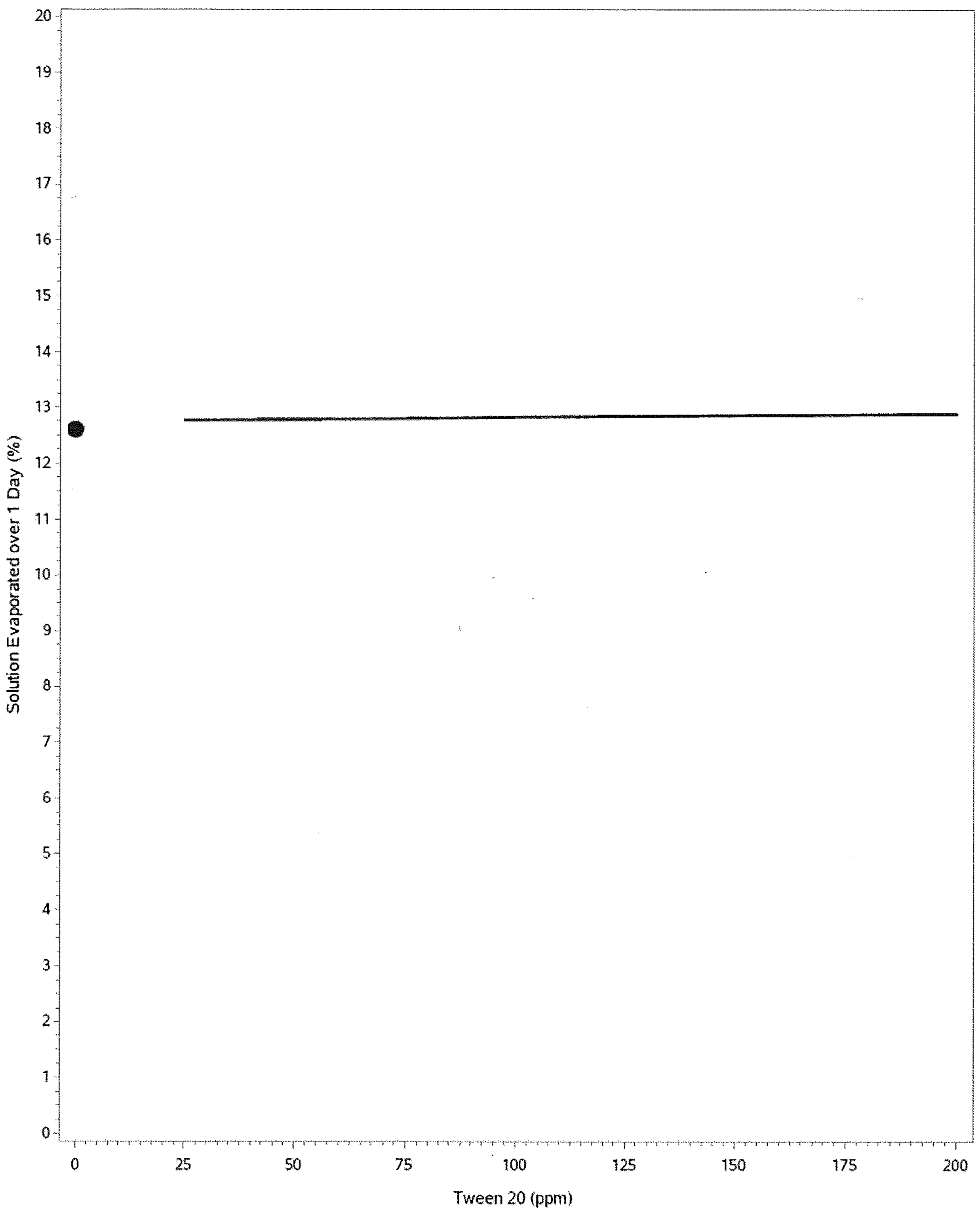

Figure 4.3g Day 7 piecewise regression model of evaporation rates (% of total) for 9 concentrations of Tween 20 solutions. Refer to Table 4.2 for coefficients used for plotting the model.

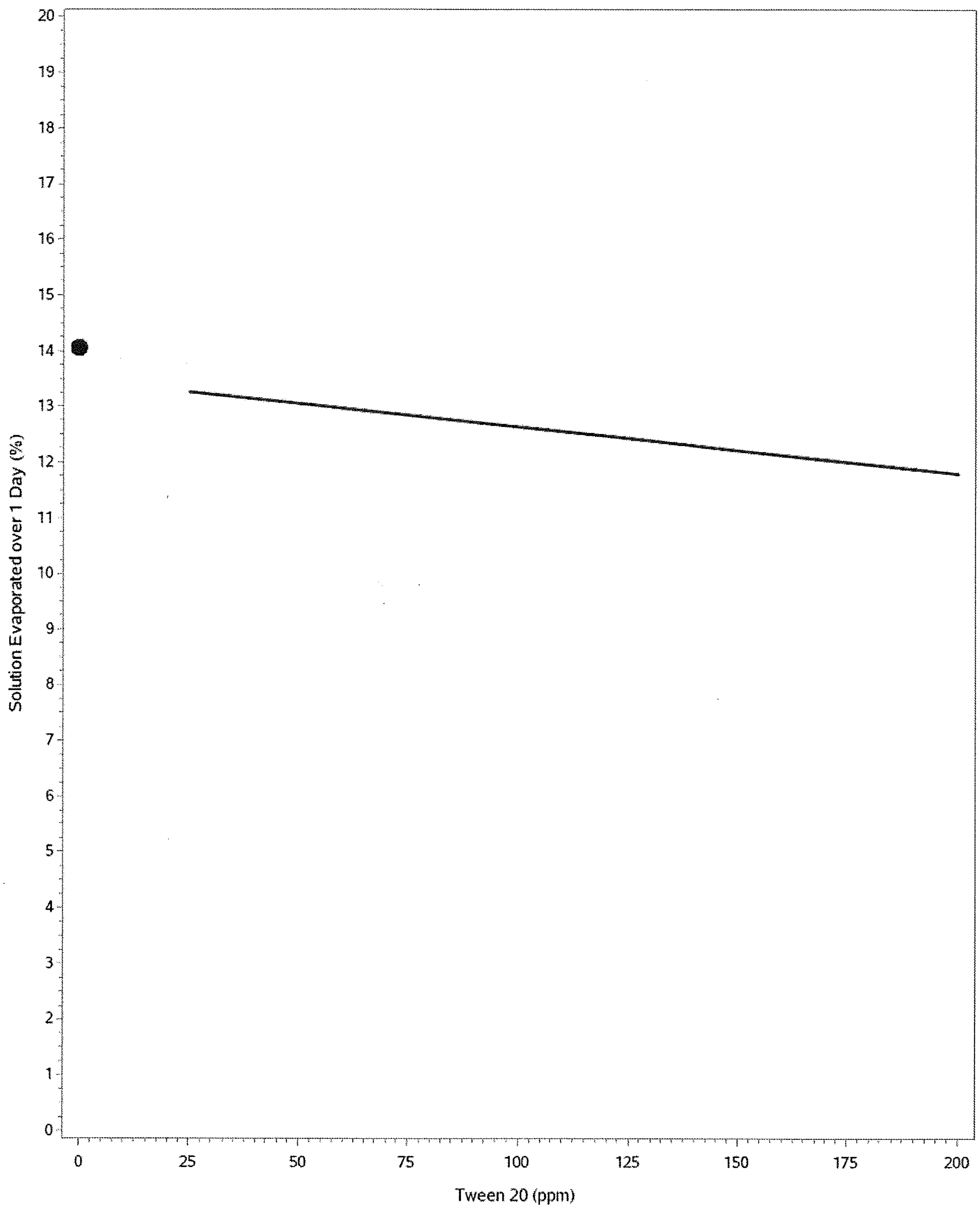

Figure 4.3h Day 8 piecewise regression model of evaporation rates (% of total) for 9 concentrations of Tween 20 solutions. Refer to Table 4.2 for coefficients used for plotting the model.

CHAPTER 5

WATER RELATIONS OF SUBSTRATE AND *SPATHIPHYLLUM* 'EMERALD STAR' TREATED WITH A SINGLE DRENCH OF TWO SURFACTANTS AND A HUMECTANT

ABSTRACT

Hydrophobic soils and substrates usually retain less water than similar but non-hydrophobic substrates. Surfactants are a class of synthetic and organic chemicals that are widely used to alleviate hydrophobicity and increase water retention in the sandy soils of golf greens and in organic potting substrates. However, there is little to no data available showing exactly how much water could be saved by using a surfactant while growing a container crop from plug stage to completion. The objective of this research project was to quantify exactly how much water is lost to leaching, transpiration, and evaporation from *Spathiphyllum* 'Emerald Star' liners transplanted into 6.5-inch azalea pots and treated with a single 800 mL drench of the surfactants Tween 20 (100 mg/L) and AquaGro L with PsiMatric Technology (1.2 mL/L), and the humectant Hydretain ES Plus (2 oz/gal), as well as a Control treatment (plain water). A second treatment factor consisting of the treatments "covered" and "non-covered" was also included. Covered-treated plants had a 4-gal white plastic bag enclosing the container, wrapped over the substrate surface, and snugged against the base of the plant by pinning the bag into the substrate with an unfolded paperclip. Non-covered-treated plants did not have a bag around the container and substrate. The purpose of the non-covered/covered treatment was to allow (non-covered) or inhibit (covered) evaporation from substrate surface. Results indicate that AquaGro L with PsiMatric Technology and Hydretain ES Plus have certain merits regarding beneficial substrate-water relations. More notably, covered-control plants, which had no evaporation loss, had an

extended growing period of 21.5 days and produced 107% more dry weight than non-covered-control counterparts.

INTRODUCTION

Historically, objectives for plant irrigation were quite simple: keep plants from wilting by providing enough water through irrigation. Oftentimes this meant overwatering plants to avoid risks of underwatering and inducing drought stress which could lead to diminished plant growth or death and economic loss. Overwatering causes harmful nutrient leaching from container substrate into surface and groundwater resources (Bilderback, 2002; Majsztrik et al., 2011) and is particularly wasteful in water limited regions such as the Western United States and any other region experiencing drought. Current irrigation objectives are often simplified in an equation called irrigation efficiency (IE). Irrigation efficiency can be defined as the amount of water beneficially used in relation to the total amount of water extracted (applied through irrigation) (Fulcher and Fernandez, 2013). This equation is designed to expose and eliminate water waste, also referred to as non-beneficial water use. Non-beneficial water use can be defined in two categories: (1) water that does not reach the root zone of the plant (evaporated, intercepted by plant canopy, or simply misses the root zone due to poor application accuracy); and (2) water that does not stay in the root %zone of the plant (leaching in excess of 15% or flooding over container edges). The IE equation is focused on evaluating the efficiency of water delivery techniques and therefore can avert the attention of irrigators and researchers from other routes of significant water loss that can occur well after the irrigation process is completed such as evaporation from substrates and transpiration via stomata.

Transpiration benefits the plant by helping draw water (mass flow) and nutrients from the soil towards plant roots where they become accessible to the plant (Barber et al., 1963).

Additionally, transpiration can help cool leaf surfaces and expedite nutrient flow from root to shoot (Hopkins and Hüner, 2004). Yet, transpiration of large amounts of water is the leading cause of crop loss (Boyer, 1982). Roughly 95 to 97% of all water entering a plant during its lifetime is ultimately expelled, primarily through stomata, as transpiration (Kramer and Boyer, 1995; Taiz et al., 2015). Although transpiration can provide certain beneficial effects to plants, many have questioned whether plant growth would be negatively affected if transpiration were eliminated or significantly reduced (Muenscher, 1922; Tanner and Beevers, 1991,2001). If possible, significantly reducing transpiration could greatly decrease the frequency of drought incidence and increase the efficiency of dry matter production per unit of water transpired. Muenscher (1922) provides an insightful review of research investigating whether or not transpiration rates are directly related to nutrient absorption rates. In his own research, Muenscher determined that transpiration rate in barley plants did not affect rates of nutrient absorption. Inspired by the work of Muenscher, Tanner and Beevers (1990,2001) conlcuded that transpiration was not necessary for uptake of nutrients in maize and sunflower and provocatively suggested, in contrast to popular belief, that transpiration may be entirely useless to plants and only occurs because it is an unavoidable side-effect of the Carbon fixation process. Smith (1991) promptly criticized the interpretation and conclusions of the 1991 study by Tanner and Beevers. A study of pear trees, germinating sunflowers, and growth of sunflowers showed that high humidity (reduced transpiration) had negative effects on plant growth (Winneberger, 1958). Although research is conflicting, there seems to be enough evidence to suggest that transpiration can be decreased in certain species with no deleterious effects on growth. Therefore, if it were possible to manipulate and decrease transpiration, large amounts of water savings could be obtainable. Transpiration is linearly related to biomass production because H_2O efflux and CO_2

influx occur simultaneously and inseparably through open stomata. Altering one parameter without the other is therefore very challenging. To decrease substantial water consumption by plants, decreasing transpiration rates, increasing CO_2 fixation rates, or both occurring simultaneously should be targeted. The term water use efficiency (WUE) describes the exchange rate of a unit of water lost through transpiration for the gain of a unit of atmospheric CO_2 diffusing into the plant. Increasing WUE is equivalent to increasing the relative amount of CO_2 fixed by the plant per unit of water lost to transpiration. Methods for increasing WUE are commonly accomplished through breeding (Condon et al., 2004) and have been almost entirely limited to agronomic row crops because relatively small increases in WUE can translate to relatively large increases in harvestable biomass. Although, to date, significant increases in plant WUE for horticultural plants have not occurred, if new methods for decreasing transpiration rates in plants were achieved, it could have massive implications for water savings.

Based upon their chemical nature, adjuvants are grouped into four categories: surfactants, oils, inorganic salts, and non-traditional adjuvants (Guillén and Urrestarazu, 2012). The term "surfactant" is derived from the phrase "surface-active-agent" and refers to a group of chemicals that can alter liquid-gas, liquid-liquid, and liquid-solid interfacial properties to facilitate or accentuate the spreading, emulsifying, wetting, dispersing, or other surface altering properties of liquids (WSSA Herbicide Handbook, 1994). Surfactants are commonly used to mitigate soil hydrophobicity in substrates by helping to prevent leaching (Baird and Calhoun, 1999; Cisar et al., 2000) while simultaneously increasing the water holding capacity of certain soils and substrates (Blodget et al., 1993; Ruemmele and Amador, 1998). Humectants are hygroscopic compounds which are a different type of adjuvant that have been reported to help decrease water use during plant production by reducing substrate evaporation (Ecologel Solutions, LLC, 2017).

Although surfactants are primarily utilized for their soil and substrate wetting properties, they can also directly affect plant growth and physiology (Parr and Norman, 1965). The surfactant Tween 20 was reported to decrease transpiration rates by up to 50% in New Guinea impatiens and peace lily without reducing overall plant growth (Yang, 2008). No other reports addressing how surfactants work to alter transpiration have been located. Research that quantifies exact amounts of water savings during the growth cycle of a horticultural crop attributed specifically to soil surfactants is also lacking. The objective of the current project is to evaluate the efficacy of the surfactants Tween 20 (polyoxyethylene sorbitan monolaurate, $C_{58}H_{114}O_{26}$) and AquaGro L with PsiMatric Technology (85% nonionic surfactant, 15% water), and a humectant Hydretain ES Plus (54% humectants, 10% nonionic surfactant, 36% inert ingredients) as a means to reduce overall water use and/or water waste in a practical growing environment by recording all water inputs (irrigation) and outputs (leaching, transpiration, evaporation) in the growing system.

MATERIALS AND METHODS

Location and experimental design. This experiment utilized a 2-way factorial treatment design. The first treatment factor consisted of four solution treatments: 100mg/L Tween 20 (Rocky Mountain Oils, Orem, UT), 1.2 mL/L AquaGro L with PsiMatric Technology (Aquatrols Corp of America, Paulsboro, NJ), 2 oz/gal Hydretain ES Plus (Ecologel Solutions LLC, Ocala, FL), and plain water (Control). Mixture ratios for Hydretain ES Plus and AquaGro L with PsiMatric Technology were based on label rates for a single application that would have efficacy for 4 to 6 weeks. The ratio for Tween 20 was selected based on prior work by Yang (2008). Eight hundred milliliters of treatment solutions were applied once as the initial irrigation. A second treatment factor consisting of the treatments “covered” and “non-covered” was also included. Covered-treated plants had a 4-gallon white plastic bag wrapped around the entire container, over the substrate surface, and snugged against the base of the plant by pinning the bag into the substrate with an unfolded paperclip (Figure 5.1). Non-covered-treated plants did not have a bag around the container and substrate. The purpose for the non-covered/covered treatment was to allow (non-covered) or inhibit (covered) evaporation from substrate surface. Bags from covered-treated plants were only removed during irrigation procedures and were promptly placed back on containers afterwards. Each of the 8 treatment groups consisted of 8 single plant replicates. For the duration of the experiment, gross transpiration (T) was measured for covered-treated plants and gross evapotranspiration (ET) was calculated for non-covered treated plants. Experimentation took place in a 2300 ft^2 fan and pad greenhouse at the Paterson Greenhouse Complex in Auburn University, Alabama. Replicates were arranged in a completely randomized experimental design on a single bench located in the center of the greenhouse.

Plant material and initial set-up. Spathiphyllum 'Emerald Star' liners from tissue culture (Oglesby Plants International, Apopka, FL) were transplanted into Fafard 3B potting mix (a blend of peat, perlite, vermiculite, and pine bark, Conrad Fafard, Inc., Agawam, Mass.) in 6.5-inch azalea pots in February 2017. Two bags of 2.8 cubic feet Fafard 3B were mixed together to minimize potential variations among the two bags. Before transplanting, individual pot weights were measured and recorded. While transplanting liners, each pot was placed on a scale, pot weight was tared, and 370g of substrate (48% water content) was gently placed into each pot. After pots were filled with substrate, liners were planted into the center of the pot. All planted pots were placed on top of PVC slices and placed in the center of aluminum leachate catchment pans. At this point, substrate was watered per treatment designation, with 800 mL of treatment solution being poured from a 1000 mL beaker evenly and slowly over the surface of the substrate. After 45 minutes allotted for gravitational water loss and catchment in the pans, leachates were poured into a 1000 mL graduated cylinder and volumetric measurements were recorded. After leachates were measured, one-half of the containers (8) in each solution treatment group were bagged with a 4-gallon white garbage bag (Great Value™, Walmart Stores Inc., Bentonville, AR). The bag was placed under the pot, wrapping over the substrate surface leaving only the plant exposed, and being pinned to the substrate with an unfolded paper clip. Following bagging, both covered-treated (bagged) and non-covered treated (non-bagged) containers were all weighed to establish the weight of plants at full water content so that ET/T could be measured gravimetrically from the next weight measurement. Aluminum catchment pans and PVC slices were removed and only used during subsequent irrigation events. Plants were watered a second time with 500 mL water, 9 days after the first irrigation and a third and final time with 500 mL water, 7 days after the second irrigation. Irrigation timing was determined by visually inspecting substrate for drying and picking up non-covered plants to estimate weight and water need. Once it was established that several non-covered plants were ready to be watered, all plants, both covered and non-covered, were watered at the same time.

Water measurements. Gross water use and ultimate destination of all water was accounted for throughout the duration of the experiment. At every irrigation, water retention was calculated by subtracting leachate volume from total water applied. ET was measured on non-covered treated plants by subtracting the weight of containers at the driest period immediately prior to an irrigation from the weight of containers 45 minutes after the last irrigation. T was calculated for covered-treated plants in the same manner as ET was measured for non-covered treated plants. During the first 24 days ET/T was measured once for each period between irrigations. Beginning on the eighth day after the final irrigation, ET/T was measured daily until experiment termination. Total water loss for each plant was calculated by adding together leachates from all 3 irrigations and total ET/T for each plant, total ET was measured by adding all weekly and daily ET measurements of non-covered treated plants, and total T was measured by adding the weekly and daily T measurements of covered-treated plants.

Evaporation rates (E) for non-covered-treated plants was not directly measured but rather derived by subtracting the directly measured T rates of covered-treated plants from the directly measured ET rates of non-covered-treated counterpart plants. The accuracy of the derived E value is contingent upon the assumption that plants from the non-covered treatment and plants from the covered-treatment having identical transpiration rates, which would be irresponsible and unscientific to assume. With the above in mind, E values should be viewed with caution as rough estimates. Water use efficiency (WUE) was calculated by dividing total dry weight (g) by total transpirational water loss (L) of plants. IE(ET), irrigation efficiency considering ET, was calculated by dividing water consumed in ET by total water applied (1800 mL). IE(T), irrigation efficiency considering T, was calculated by dividing water consumed in T by total water applied (1800 mL). Leaf chlorosis was observed on plants within the covered-treatment therefore leachates from all covered-treated plants were collected and measured for EC and pH in accordance with the Virginia Tech Extraction Method (VTEM) (Wright, 1986).

*Termination procedures***.** The experiment was terminated in segments by treatment groups. A treatment group was terminated when the average weight of plants in that group (container + substrate + plant) reached 360g or below when being routinely weighed every morning. At a weight of 360g, plants had typically displayed mild to moderate signs of drought such as leaf wilting and curling for 1 to 2 days and substrate was highly desiccated with most plant available water having been extracted. The first step of termination consisted of watering each plant with 800 mL of the appropriate treatment solution, catching all leachates in aluminum pans, waiting 45 minutes for gravitational water loss, measuring leachate volume in graduated cylinders, and pouring water from graduated cylinders back into the same plant. This process was repeated so that plants were watered a total of three times with the same water, each time measuring leachates to determine if additional water was retained in substrate after each subsequent watering. This procedure was designed to determine if the different treatments had differing effects on water retention in very dry substrate. Once plants had regained full turgor, usually after 3 to 4 hours, growth indices were measured on each plant. At the time of the first termination, growth indices of all plants were measured to establish the growth at that specific point in time. SPAD readings were made with a SPAD-502 Chlorophyll Meter (Konica Minolta Inc., Tokyo, Japan) on 3 leaves per plant on all plants when the first treatment group was terminated. After growth indices were recorded on plants of individual treatment groups, plants were harvested to measure root and shoot dry weight and fresh weight. Immediately after measuring shoot fresh weight and just before shoots were placed in the oven to dry, individual leaves were excised from petioles and measured for leaf area using a LI-3100C Area Meter (LI-COR Inc., Lincoln, Nebraska). Roots and shoots were placed into a Grieve SC-350 oven (The Grieve Corporation, Round Lake, Illinois) for 72h at 76.7°C and later removed and weighed to calculate dry weight.

Statistical analyses. The experiment was repeated two additional times (total of three times) during February, March, and April 2017. Data were analyzed using ANOVA-type analyses with linear models (to analyze data by experiment: 1, 2, and 3) and liner mixed models (with experiment as a random factor) using the GLIMMIX procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC). When the interaction term in the model was significant ($p \leq 0.10$), simple effects means (treatment means for all rates of one factor grouped within one rate of the second factor) were compared; otherwise, main effects means were compared ($\alpha = 0.05$). *P* values for multiple comparisons were adjusted using the Shaffer-Simulated method.

RESULTS

Water relations of *Spathiphyllum* 'Emerald Star' and substrate.

ET/T/E. Total transpiration volume from covered-treated plants was not different among solutions, however, total evapotranspiration volume from the non-covered-treated plants was decreased 2.5% by AquaGro and 3.5% by Hydretain compared with control plants (Table 5.1). Plants treated with Tween 20 had similar total ET volume as control plants and plants treated with AquaGro and Hydretain. Although total ET volume for plants treated with Tween 20 was not different from control plants, the ET volume of plants treated with Tween 20 during the first three weeks of growth was 5.3% lower than control plants (Table 5.2). As a main effect, total transpiration volume was not significant among solutions, however, it was 89% higher for covered-treated plants than for non-covered-treated plants. The drastic increase in transpiration volume is accredited to an average extended growing period of 21.5 days for covered-treated plants (Table 5.1). Eliminating the E component of ET (covered-treatment) extended the growing period of *Spathiphyllum* 'Emerald Star' by channeling "new" water to be used in transpiration rather than be wasted in evaporation. During the first week of the experiment, non-covered-Hydretain treated plants lost 15.3% less water from evaporation than control plants (Table 5.3). During the combined first three weeks of growth, non-covered-Hydretain treated plants lost 11% less water to evaporation than control plants. Roberts et al. (2012) noted that humectants, like Hydretain, have the potential to inhibit the evaporative loss of water as was observed in this study.

Leached/retained. In non-covered-treated plants, total leachate volume was increased 9.6% by AquaGro, 9.9% by Tween 20, and 10.3% by Hydretain as compared with control plants (Table 2.1). In contrast, Yang (2008) determined that a soil column (25.5 cm length × 10.5 cm i.d.) filled with 352 ± 0.05g Fafard 3B and treated with 100 mg/L Tween 20 retained 40.5% more water than control plants during the first irrigation. Fafard 3B, the substrate used in this experiment, has a proprietary surfactant pre-incorporated in the substrate, which initially helps to alleviate hydrophobicity. This is likely occurring here, especially with AquaGro which is advertised to increase moisture retention. Covered-treated plants and non-covered-treated plants were both irrigated at the same times despite the fact that substrate of covered-treated plants still retained large amounts of water at times of irrigation (saved by eliminating evaporation). This method of irrigation led to overall significantly higher leachate volumes in covered-treated plants as compared to non-covered-treated plants across the board (Table 5.1). Among non-covered-treated plants, control plants retained the highest amount of water, and Hydretain treated plants retained the least amount of water during the initial irrigation when plants were irrigated with 800 mL of treatment solution (Figure 5.3). For the two subsequent irrigation events when 500 mL was applied each time, there were no significant differences in retention among non-covered-treated plants. In covered-treated plants during the second irrigation, plants treated with Hydretain retained 38% (61.2 mL) more water than the control. The label for Hydretain instructs that for best results, "water thoroughly when rewatering Hydretain treated plants." The term "water thoroughly" is somewhat ambiguous yet when covered-treated plants were watered the second time (when the substrate was still quite moist), they would have been watered much more thoroughly relative to non-covered-treated plants which did not retain significantly more water than non-covered-Control plants. No other differences in retention were observed for the second irrigation. At termination, all plants were mildly to moderately wilting indicating that most plant available water was extracted. When treatment solutions were applied to the dried substrate for the first time, substrate treated with AquaGro retained the most water in both covered-treated and non-covered-treated plants (Figure 5.4). However, after a second application of water, control treated substrate retained more water than substrate treated with AquaGro in both covered-treated and non-covered treated substrates. After the third (final) watering, Control and Tween 20 treated substrate retained the most water in non-covered-treated plants while there were no significant differences in covered-treated plants.

Plant growth

Non-covered-treated plants treated with Tween 20 and water (control) had similar shoot fresh weight (SFW) and were both higher than SFW of non-covered plants treated with Hydretain and AquaGro. Root dry weight (RDW) was significantly lower in all plants treated with Hydretain (Table 5.4). Total dry weight (root +shoot) was not significantly different amongst solution types, however, total dry weight of covered-treated plants was 124% higher than in non-covered-treated plants (Table 5.1). RDW of non-covered-treated plants (0.91g) was 1.5 times lower than RDW of covered-treated plants (2.37g), which had an average of 21 more growing days than non-covered-treated plants. Six-and-a-half-inch azalea pots have a dimension of 16.5cm by 14.61 cm (6.5-inch Azalea-Green). When harvested, non-covered treated plants had a longest root length of 17.4 cm (Table 5.4). Only a few roots had actually reached this length while the majority of the roots were still concentrated around the original root mass established by the plug tray. In contrast, covered-treated plants, which were harvested 21 days later, had a longest root length of 25.1 cm, and visually, the root system was more extensively spread through the substrate. In both size index 1 (SI 1), measured on all plants at the single point in time of first treatment termination, and size index 2 (SI 2), the measurement of plant size of plants within each treatment group at the time of termination , plants treated with Tween 20 and water (control) were larger than plants treated with Hydretain and AquaGro (Table 5.4). Although larger according to SI 2, it was surprising that covered-treated plants were only 17.9% larger than non-covered plants considering that total dry weight for covered-treated plants was 124% higher than in non-covered-treated plants. Evidently, the increased shoot dry weight was due to an increase in smaller shoot and leaf growth as evidenced by significantly larger specific leaf area in covered-treated plants. In the final three weeks of experimentation it became evident that leaves of covered-Hydretain-treated plants were becoming chlorotic (Figure 5.5). Measuring relative leaf greenness with a SPAD meter revealed that covered-Hydretain leaves had the lowest SPAD readings. Furthermore, an EC test of leachates revealed that EC levels for covered-Hydretain treated plants was 3967 µS, 1069% higher than Control EC (Table 5.5). Leachates from all treatments were well outside the desirable range for EC in *Spathiphyllum* production which is 2,000-3,000 µS (Chen et al., 2015). Control, Tween 20, and AquaGro treated plants were well below the desirable range while Hydretain was well above. EC was lowest in Control plants (339 µS) and Tween 20 plants (355.8 µS) which was likely because only one application of 20-4.4-16.5 at 200 ppm liquid feed was applied during the experiment.

Efficiency

Water use efficiency (WUE) at the whole plant level, which is total g DW/ L H_2O transpired, was not significantly different in plants based on solution, however it was higher in covered-treated plants than in non-covered-treated plants. Irrigation efficiency, measured as water consumed by evapotranspiration + 15% allowable leachates /total water applied, is commonly accepted in the industry (Southern Nursery Association, 2013). For the sake of strictly evaluating the relationship between the amount water consumed in ET/T to the total amount of water applied, a leaching fraction has not been included in the following calculations. IE(ET), irrigation efficiency considering evapotranspiration water consumption, was 60% higher in non-covered treated plants than in covered-treated plants. However, IE(T), irrigation efficiency considering transpiration water consumption only, was 88.9% higher in covered-treated plants than in non-covered treated plants (Table 5.6).

CONCLUSIONS

The initial objective of this experiment was to determine how different soil conditioning products (surfactant and humectant) would affect transpiration from plants and evaporation and leaching from substrate. To measure transpiration, it was necessary to cover the substrate surface to eliminate the evaporative component. It became quickly apparent that the "covered" treatment had a much more drastic effect on evaporation and total water saving than did product type. Ultimately, covered-treated-control plants utilized an estimated additional 818.6 mL of water (44 % of total available water) to extend the growing period by an additional 21.5 days, producing 107% more dry weight than non-covered-control counterparts. The additional 818.6 mL of water available to covered-control plants was "created" by eliminating water lost from evaporation. Instead of watering every 6 to 8 days as was necessary with non-covered plants, eliminating evaporation could extend the period between necessary irrigation applications up to 21.5 days, which could significantly cut down on labor that would traditionally be required for frequent irrigations if hand watering were the method used. Ehlers and Goss (2016) state, "maximum exploitation of stored soil water for use in transpiration requires unproductive soil evaporation to be reduced to a minimum. For water, the soil needs to be managed so that it acts like a trap." This experiment provides a helpful initial assessment of the potential water saving, labor saving, and growth promoting benefits of redirecting E water into T water. Further experimentation on mass produced plants to determine if, or at what point, in the growth cycle evaporation no longer occurred would be extremely useful in modeling the water savings that could potentially be achieved by eliminating evaporation.

It should be noted that the sole purpose of bagging the container and substrate was to eliminate evaporation from the surface of the substrate, yet it is possible that additional growing parameters were altered as well. Gas exchange through drainage container drainage holes was prohibited and it is possible that temperatures in the canopy were altered by the lack of evaporation occurring as well as being altered by the white surface of the bag itself. Additionally, both covered-treated and non-covered-treated plants were all watered at the same time based on the demands of the non-covered-treated plants. Making irrigation decisions in this manner lead to an excessively high leaching fraction in covered-treated plants (varying slightly due to solution type) because substrate of covered-treated plants still retained a large portion of water (due to a lack of evaporation) at the time when it was necessary to irrigate non-covered-treated plants. The excessive leaching could have led to a larger proportion of the treatment solutions and nutrients being leached from covered-treated plants which could ultimately alter growth data. The higher moisture content that was maintained (although unintentionally) in covered-control plants would in and of itself likely alter plant growth. The above-mentioned issues should be accounted for and either measured or adjusted in future research focusing on the water saving potential of eliminating evaporation in container-grown plant production.

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Table 5.1 Total available water, total evapotranspiration (ET)/transpiration (T), total leachate volume, total number of growing days, and total dry weight for *Spathiphyllum* 'Emerald Star' transplanted into 6.5-inch azalea pots in Fafard 3B, treated with 800 mL of water (control) or solutions of the surfactants Tween 20 (100mg/L) and AquaGro L with PsiMatric Technology (1.2 mL/L) and the humectant Hydretain ES Plus (2 oz/gal). The second treatment factor was to cover substrate surface (to eliminate evaporation) or not cover substrate surface.

		Total applied water (mL)[z]	Total ET/T (mL)[y]	Total leached (mL)	Total water loss (mL)	Total growing days	Total dry weight (g)
			Significance of Treatment Factors[x]				
Solution		—	0.1134	<.0001	<.0001	0.0081	0.1815
Covered		—	<.0001	<.0001	0.0114	<.0001	<.0001
Solution*Covered		—	0.0192	0.0004	0.5086	0.9940	0.4226
			Treatment Least Square Means Grouped by Covered[w]				
Covered	Solution						
No	Control	1800	1237.1a	546.0b	1783.1	33.0	2.20
No	AquaGro	1800	1206.9b	598.3a	1805.3	34.3	1.89
No	Tween 20	1800	1215.9ab	599.8a	1815.7	33.3	2.22
No	Hydretain	1800	1195.8b	602.5a	1798.3	36.7	1.86
Yes	Control	1800	763.3	1010.6b	1774.0	54.0	4.55
Yes	AquaGro	1800	755.0	1051.3a	1803.8	55.8	4.56
Yes	Tween 20	1800	752.3	1044.0a	1796.3	54.3	4.66
Yes	Hydretain	1800	775.2	1006.9b	1782.1	59.0	4.56
			Least Square Means for Main Effects[v]				
Covered	Solution						
No		—	—	—	1800.6a	34.3b	2.04b
Yes		—	—	—	1789.0b	55.8a	4.57a
	Control	—	—	—	1778.5b	43.5b	3.37
	AquaGro	—	—	—	1804.5a	45.1ab	3.22
	Tween 20	—	—	—	1806.0a	43.8b	3.44
	Hydretain	—	—	—	1790.2b	47.8a	3.21

[z] 1800 mL water applied as initial treatment (800 mL) and two subsequent non-treatment irrigation applications (500 mL per application). In addition to the 1800 mL applied water, roughly 178 mL water was pre-incorporated into Fafard 3B.

[y] ET corresponds with non-covered-treated plants and T corresponds with covered-treated plants.

[x] *P* values.

[w] When the interaction term in the model is significant ($p \leq 0.10$), simple effects means (treatment means for all rates of one factor grouped within one rate of the second factor) followed by the same letter are not significantly different using the Shaffer-Simulated adjustment for multiple comparisons ($\alpha = 0.05$); otherwise, the treatment means are presented without letter groupings for informational purposes.

[v] When the interaction term in the model is not significant ($p > 0.10$), main effects means for rates within each treatment factor followed by the same letter are not significantly different using the Shaffer-Simulated method for multiple comparisons ($\alpha = 0.05$).

Table 5.2 Evapotranspiration (non-covered treatment) and transpiration (covered treatment) of *Spathiphyllum* 'Emerald Star' during the first three weeks of growth. Week 1 (9 days) was measured between irrigation 1 and 2, week 2 (7 days) was measured between irrigation 2 and 3, and week 3 (8 days) was measured immediately following the third (final) irrigation.

		Evapotranspiration (ET)/Transpiration(T) (mL)				
		Week 1	Week 2	Week 3	Week 1+2+3	Total
		Significance of Treatment Factors[y]				
Solution		<0.0001	0.0013	0.0002	<0.0001	0.1134
Covered		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Solution*Covered		<0.0001	0.1012	0.7391	0.0004	0.0192
		Treatment Least Square Means Grouped by Covered[w]				
Covered	Solution					
No	Control	361.5a	307.7	327.0	996.1a	1237.1a
No	AquaGro	339.2b	299.5	312.8	951.5b	1206.9b
No	Tween 20	349.1ab	298.3	317.0	964.3b	1215.9ab
No	Hydretain	306.3c	280.4	301.4	888.1c	1195.8b
Yes	Control	76.9	71.3	95.5	243.8a	763.3
Yes	Aquagro	66.2	64.6	83.3	214.1ab	752.6
Yes	Tween 20	69.9	72.0	88.9	230.8ab	752.3
Yes	Hydretain	65.4	63.9	79.9	209.1b	775.2
		Least Square Means for Main Effects[x]				
Covered	Solution					
No		—	296.5a	314.5a	—	—
Yes		—	67.9b	86.9b	—	—
	Control	—	189.5a	211.3a	—	—
	AquaGro	—	182.0ab	198.1bc	—	—
	Tween 20	—	185.1a	203.0ab	—	—
	Hydretain	—	172.2b	190.7c	—	—

[z] *P* values.

[y] When the interaction term in the model is significant ($p \leq 0.10$), simple effects means (treatment means for all rates of one factor grouped within one rate of the second factor) followed by the same letter are not significantly different using the Shaffer-Simulated adjustment for multiple comparisons ($\alpha = 0.05$); otherwise, the treatment means are presented without letter groupings for informational purposes.

[v] When the interaction term in the model is not significant ($p > 0.10$), main effects means for rates within each treatment factor followed by the same letter are not significantly different using the Shaffer-Simulated method for multiple comparisons ($\alpha = 0.05$).

Table 5.3 Evaporation (E) and evaporation as a percentage of total evapotranspiration (% of ET) from Fafard 3B substrate in 6.5-inch pots with transplanted *Spathiphyllum* 'Emerald Star' during the first three weeks of growth. Week 1 (9 days) was measured between irrigation 1 and 2, week 2 (7 days) was measured between irrigation 2 and 3, and week 3 (8 days) was measured immediately following the third (final) irrigation. E was derived for non-covered-treated plants by subtracting transpiration (T) of covered-treated plants from evapotranspiration (ET) of non-covered-treated plants. Because E values are derived rather than directly measured they should be viewed as rough estimates.

Treatment	Week 1		Week 2		Week 3		Week 1+2+3		Total[y]	
	E (mL)	% of ET	E(mL)	% of ET	E(mL)	% of ET	E(mL)	% of ET	E(mL)	% of ET
Control	284.6a[z]	78.8	236.3a	76.5	231.4	70.7	752.4a	75.5	818.6	66.1
Aquagro	273.0a	80.3	234.9a	78.4	229.5	73.4	737.4a	77.5	825.5	68.3
Tween 20	279.2a	79.7	226.3ab	76.1	228.1	71.6	733.6a	75.9	810.3	66.6
Hydretain	241.0b	78.5	216.5b	77.3	221.5	73.3	679.0b	76.4	790.4	66.2
				Significance of Treatment Factor						
Solution	<0.0001	0.3664	0.0133	0.3893	0.5288	0.3129	0.0003	0.5043	0.4959	0.6073

[z] When overall treatment effects were significant ($p \leq 0.05$), means followed by the same letter are not significantly different using the Shaffer-Simulated adjustment for multiple comparisons ($\alpha = 0.05$); otherwise, treatment means are presented without letter groupings.

[y] Total values were calculated by adding daily E measurements (began after week 3 measurements and lasted until termination) to E measurements from weeks 1,2, and 3.

Table 5.4 Shoot fresh weight (SFW), shoot dry weight (SDW), leaf area (LA), root dry weight (RDW), size indices (SI 1 & SI 2), and longest root length (LRL) of *Spathiphyllum* 'Emerald Star' treated with no product (control), AquaGro, Hydretain, or Tween 20 and with substrate that was either covered (to prohibit evaporation) or non-covered (to permit evapotranspiration).

		SFW (g)	SDW(g)	LA (cm^2)	RDW (g)	LRL (cm)	SI 1 (cm)[z]	SI 2 (cm)[y]
				Significance of Treatment Factors[x]				
Solution		0.0165	0.0746	0.1941	0.0204	0.0448	<.0001	<0.0001
Covered		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<.0001	<0.0001
Solution*Covered		0.0002	0.0132	0.0523	0.8224	0.5041	0.4178	0.1857
				Treatment Least Square Means Grouped by Covered[w]				
Covered	Solution							
No	Control	7.70a	1.26a	189.5a	0.94	17.4	17.6	17.6
No	Aquagro	6.16b	1.01b	162.5b	0.88	17.6	16.1	16.2
No	Tween 20	7.25a	1.19ab	184.3ab	1.03	17.8	17.2	17.2
No	Hydretain	5.88b	1.04b	161.9b	0.81	16.9	16.0	16.1
Yes	Control	13.52	2.19	313.4	2.36	25.8	19.2	20.1
Yes	Aquagro	13.19	2.13	309.5	2.43	25.5	17.9	19.3
Yes	Tween 20	14.07	2.17	310.3	2.49	25.1	19.7	20.2
Yes	Hydretain	13.24	2.33	321.3	2.22	24.1	18.4	19.7
				Least Square Means for Main Effects[v]				
Covered	Solution							
No		—	—	—	0.91b	17.4b	16.8b	16.8b
Yes		—	—	—	2.37a	25.1a	18.8a	19.8a
	Control	—	—	—	1.65ab	21.6	18.4a	18.8a
	AquaGro	—	—	—	1.65ab	21.5	17.0b	17.7b
	Tween 20	—	—	—	1.76a	21.5	18.5a	18.7a
	Hydretain	—	—	—	1.52b	20.5	17.2b	17.9b

[z] Size index 1 is the measurement of size indices of all treatments at the time of the termination of the first treatment. Size index was calculated as the average of plant height, width at widest point, and width perpendicular to width at widest point.

[y] Size index 2 is the measurement of size indices of all plants within a single treatment group measured at the time of termination for each treatment group. This measurement is a quantification of the maximum size of the plant before termination.

[x] *p* values.

[w] When the interaction term in the model is significant ($p \leq 0.10$), simple effects means (treatment means for all rates of one factor grouped within one rate of the second factor) followed by the same letter are not significantly different using the Shaffer-Simulated adjustment for multiple comparisons ($\alpha = 0.05$); otherwise, the treatment means are presented without letter groupings for informational purposes.

[v] When the interaction term in the model is not significant ($p > 0.10$), main effects means for rates within each treatment factor followed by the same letter are not significantly different using the Shaffer-Simulated method for multiple comparisons ($\alpha = 0.05$).

Table 5.5 Main effects of SPAD readings from a SPAD-502 Chlorophyll Meter (Konica Minolta Inc., Tokyo, Japan), and electrical conductivity (EC) and pH of leachates collected from substrate of plants at time of termination measured with a Pocket Pro™ + Multi 2 (Hach Company, Loveland, CO).

Treatment	SPAD[z]	EC (µS)	pH
Control	37.8ab[y]	339.5c	6.7a
Aquagro	36.0b	481.2b	6.4b
Tween 20	38.5a	355.8c	6.7a
Hydretain	32.3c	3967.6a	6.2b
	Significance of Treatment Factor[y]		
Solution	<0.0001	<0.0001	<0.0001

[z] SPAD units measure relative leaf greenness.

[y] When overall treatment effects were significant ($p \leq 0.05$), means followed by the same letter are not significantly different using the Shaffer-Simulated adjustment for multiple comparisons ($\alpha = 0.05$); otherwise, treatment means are presented without letter groupings.

Table 5.6 Gross transpiration, gross dry weight (root and shoot), leaf area (LA), water use efficiency (WUE), irrigation efficiency, evapotranspiration (IE(ET)), and irrigation efficiency, transpiration (IE(T)) of *Spathiphyllum* 'Emerald Star' transplanted into 6.5-inch azalea pots in Fafard 3B, treated with 800 mL of water (control) or solutions of the surfactants Tween 20 (100mg/L) and AquaGro L with PsiMatric Technology (1.2 mL/L) and the humectant Hydretain ES Plus (2 oz/gal). The second treatment factor was to cover substrate surface (to eliminate evaporation) or not cover substrate surface.

		Gross transpiration (mL)[z]	Gross dry weight (g)	LA (cm^2)	WUE (g DW L^{-1} H_2O)	IE(ET)[y] (mL^{-1} ET mL H^2O)	IE(T) (mL^{-1} T mL H^2O)
		Significance of Treatment Factors[x]					
Solution		0.2787	0.1815	0.1941	0.3050	0.1134	0.2787
Covered		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Solution*Covered		0.5871	0.4226	0.0523	0.9093	0.0192	0.5871
		Treatment Least Square Means Grouped by Covered[w]					
Covered	Solution						
No	Control	418.5	2.20	189.5a	5.3	68.7a	23.3
No	AquaGro	381.5	1.89	162.5b	5.2	67.1b	21.2
No	Tween 20	405.6	2.22	184.3ab	5.5	67.5ab	22.5
No	Hydretain	405.4	1.86	161.9b	4.9	66.4b	22.5
Yes	Control	763.3	4.55	313.4	5.9	42.4	42.4
Yes	Aquagro	755.0	4.56	309.5	6.0	41.8	41.9
Yes	Tween 20	752.3	4.66	310.3	6.2	41.8	41.8
Yes	Hydretain	775.2	4.56	321.3	5.8	43.1	43.1
		Least Square Means for Main Effects[x]					
Covered	Solution						
No		402.7b	2.04b	—	5.2b	—	22.4b
Yes		761.2a	4.57a	—	6.0a	—	42.3a
	Control	590.9	3.37	—	5.6	—	32.8
	AquaGro	567.7	3.22	—	5.6	—	31.5
	Tween 20	579.0	3.44	—	5.8	—	32.2
	Hydretain	590.3	3.21	—	5.3	—	32.8

[z] For non-covered plants, gross transpiration (T) is a derived rather than directly measured value and WUE and IE(T) are based on the derived value for T. This should be taken into consideration when interpreting data for T, WUE, and IE(T) for non-covered-treated plants.

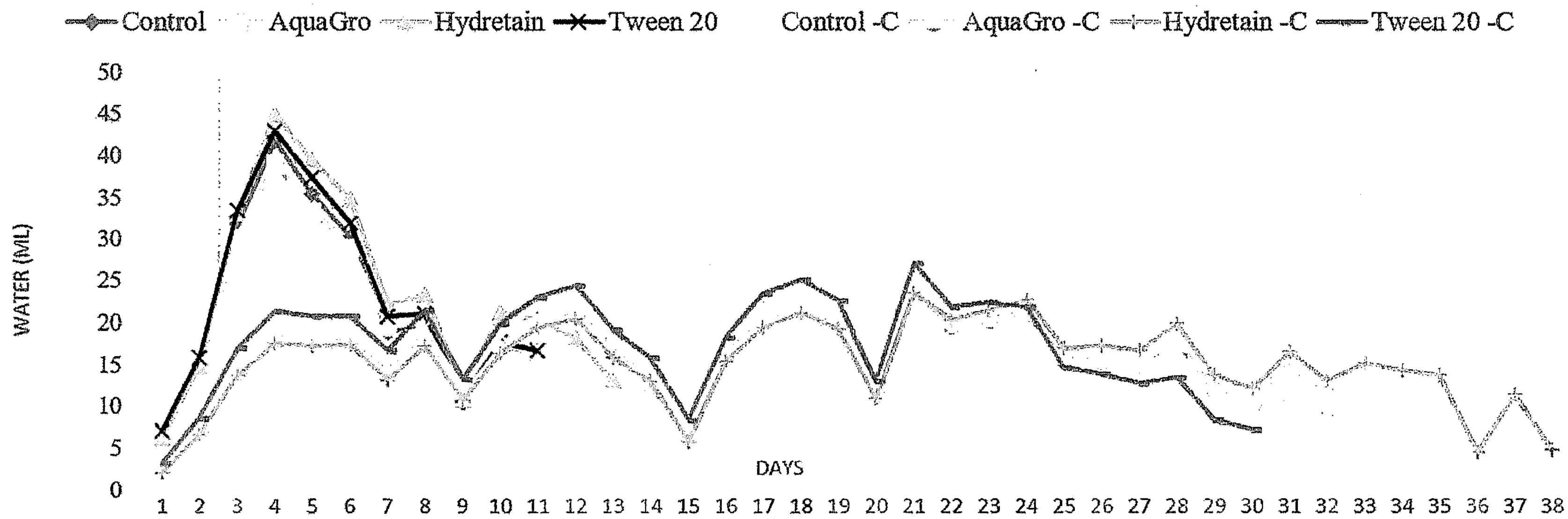
[y] IE(ET) = water consumed by evapotranspiration/water applied (1800 mL). IE(T) = water consumed by transpiration/water applied (1800 mL).

[x] *P* values.

[w] When the interaction term in the model is significant ($p \leq 0.10$), simple effects means (treatment means for all rates of one factor grouped within one rate of the second factor) followed by the same letter are not significantly different using the Shaffer-Simulated adjustment for multiple comparisons ($\alpha = 0.05$); otherwise, the treatment means are presented without letter groupings for informational purposes.

[v] When the interaction term in the model is not significant ($p > 0.10$), main effects means for rates within each treatment factor followed by the same letter are not significantly different using the Shaffer-Simulated method for multiple comparisons ($\alpha = 0.05$).

Figure 5.1 Six-and-a-half-inch azalea pots containing *Spathiphyllum* 'Emerald Star' illustrating the treatment factor "covered" with a 4-gallon white bag to eliminate water evaporation from substrate.



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
Control	ab	a	ab	ab	ab	ab	a	a	a	b	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
AquaGro	ab	a	b	b	b	b	a	a	a	b	b	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hydretain	b	a	a	a	a	a	a	a	a	a	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tween 20	a	a	a	ab	ab	ab	a	a	a	b	b	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Control -C	ab	ab	a	a	a	a	ab	a	a	a	a	a	a	ab	a	a	ab	ab	ab	ab	ab	a	a	a	a	a	a	ab	ab	ab	a	a	-	-	-	-	-	-
AquaGro -C	b	ab	a	a	a	a	ab	a	a	a	a	a	a	b	a	a	b	b	b	b	b	a	a	a	a	a	a	ab	ab	ab	a	a	-	-	-	-	-	-
Hydretain -C	c	b	a	a	a	a	a	a	a	a	a	a	a	ab	a	a	b	b	b	b	b	a	a	a	a	a	a	a	a	a	a	a	-	-	-	-	-	-
Tween 20 -C	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	b	b	b	-	-	-	-	-	-	-	-

Figure 5.2 Daily ET and T rates beginning after 24 days of growth and 9 days after the 3rd (final) irrigation. The final ET entry for each treatment represents termination of all experimental units within that treatment once wilting began to occur. When overall treatment effects were significant ($p \leq 0.05$), means followed by the same letter are not significantly different using the Shaffer-Simulated adjustment for multiple comparisons ($\alpha = 0.05$)

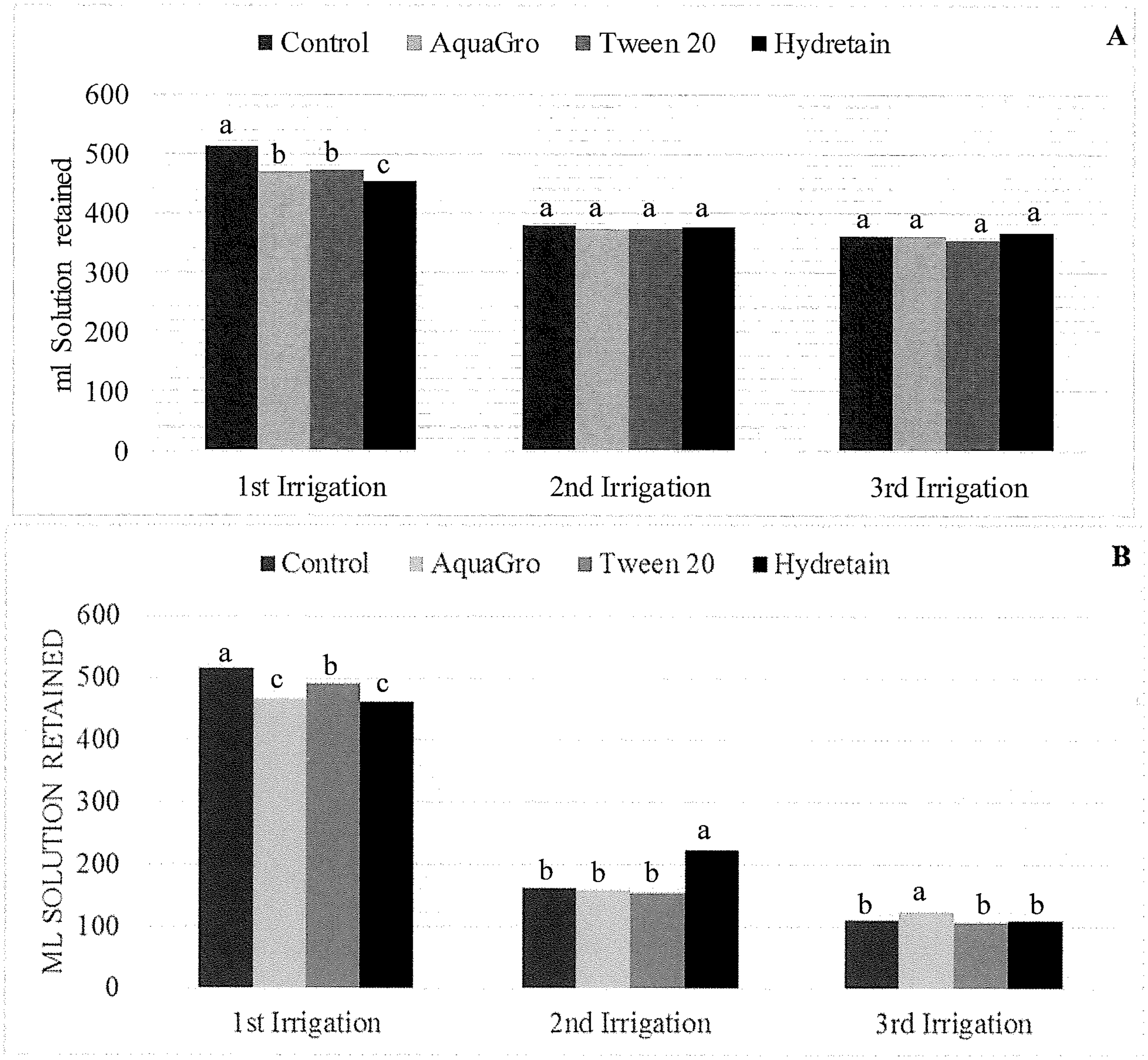


Figure 5.3 Irrigation solution retained in substrate, measured 45 minutes after applying solution by measuring leachate volume and subtracting that volume from total applied solution. Eight hundred milliliters of solution was applied at first irrigation and 500 mL was applied at following two irrigation events. (A) data from experimental units not treated with plastic bag (non-covered); and (B) data from experimental units treated with plastic bag (covered). When overall treatment effects were significant ($p \leq 0.05$), means followed by the same letter are not different using the Shaffer-Simulated adjustment for multiple comparisons ($\alpha = 0.05$)

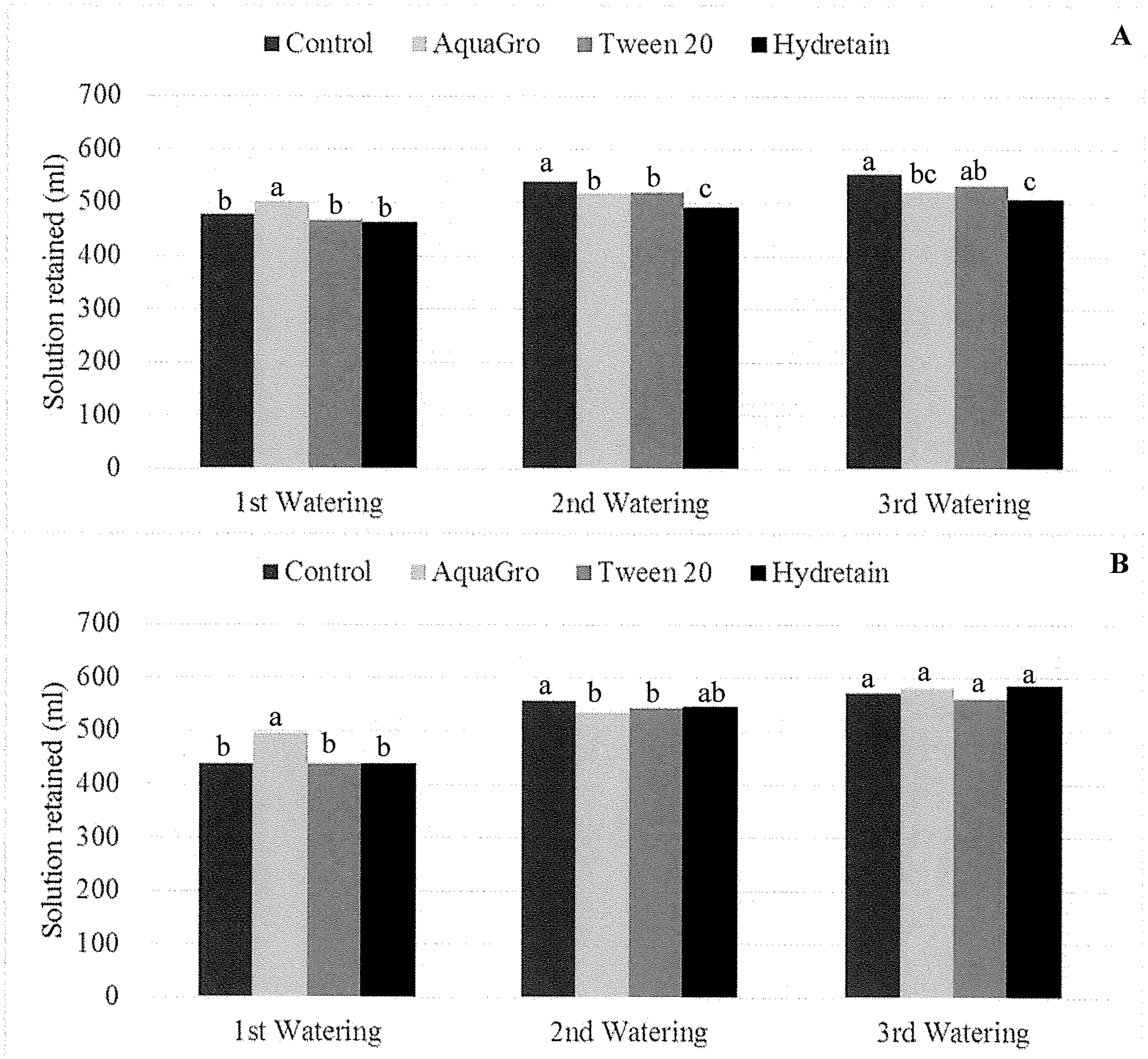


Figure 5.4 Data showing results of wetting hydrophobic substrates (all plant available water extracted) with plain water and solutions of AquaGro, Tween 20, and Hydretain at time of experiment termination. Quantities of 800 mL solution were applied to substrate surface from a beaker, leachates were collected and measured after 45 minutes, and then poured back into substrate a second and third time following the same procedure. (A) data from experimental units treated with plastic bag (to eliminate evaporation); and (B) data from experimental units not treated with plastic bag. When overall treatment effects were significant ($p \leq 0.05$), means followed by the same letter are not different using the Shaffer-Simulated adjustment for multiple comparisons ($\alpha = 0.05$)

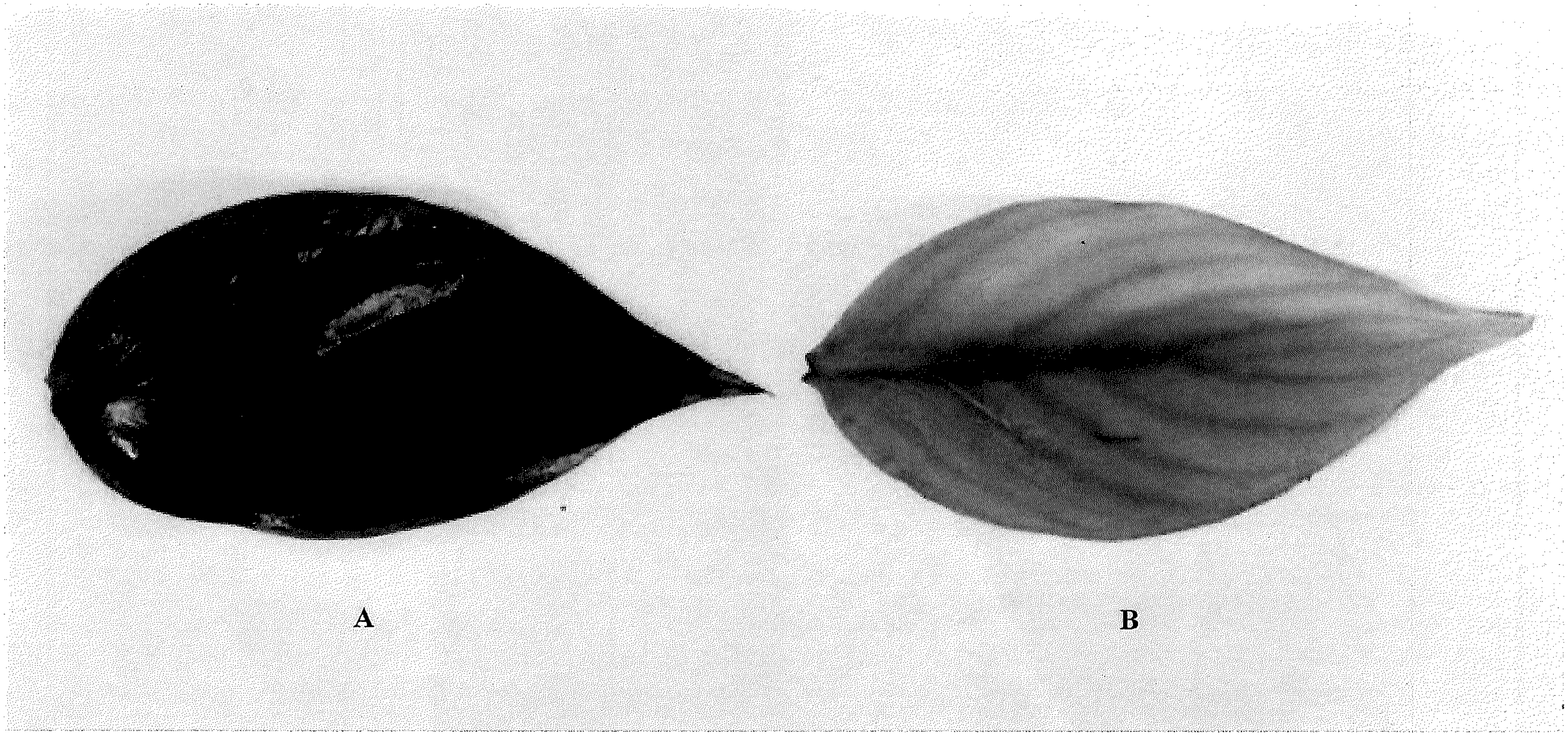


Figure 5.5 Leaves of *Spathiphyllum* 'Emerald Star' from (A) plant treated with AquaGro and with covered substrate (to eliminate evaporation) displaying healthy leaf coloration while leaf (B), displaying chlorosis, was from plant treated with Hydretain and with covered substrate.

CHAPTER 6

FINAL DISCUSSION

The experiments presented in this thesis came on the heels of previous research conducted at Auburn University focused on determining if the surfactant Tween 20 could be used as a novel method for reducing transpiration rates in plants. Yang (2008) hypothesized 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 will decrease". In two experiments Tween 20 significantly reduced transpiration of new guinea impatiens grown in Fafard 3B substrate and new guinea impatiens and peace lily grown in a hydroponic system (Yang, 2008). Tween 20 applied at 100 mg/L reduced the crop water demand by up to 40% for new guinea impatiens grown in Fafard 3B substrate, and reduced transpiration in *Spathiphyllum floribundum* 'Viscount' by 64% and in *Impatiens hawkerii* 'Celebrate Salmon' by 101% when grown hydroponically.

To expand upon the work of Yang (2008), three specific research objectives were developed for this work:

1) Determine how well Tween 20 affects the water budget and drought tolerance of plants in relation to two other similar commercially available products, AquaGro L with PsiMatric Technology and Hydretain ES Plus. Does Tween 20 have superior or alternate beneficial effects that would warrant its use commercially?
2) Determine if Tween 20 affects WUE and drought tolerance of a woody plant species.
3) Determine if Tween 20, when applied to the root zone, affects plant water potential (Ψ_p).

Conclusions

1) Determine how Tween 20 affects the total water budget and drought tolerance of plants in relation to two other similar commercially available products, AquaGro L with PsiMatric Technology and Hydretain ES Plus. Does Tween 20 have superior or alternate beneficial effects that would warrant its use commercially?

This objective was measured in the experiment with *Spathiphyllum* 'Emerald Star' transplanted into 6.5-inch azalea pots in Fafard 3B, treated with 800 mL of water (control) or solutions of the surfactants Tween 20 (100mg/L) and AquaGro L with PsiMatric Technology (1.2 mL/L) and the humectant Hydretain ES Plus. The second treatment factor was to cover substrate surface (to eliminate evaporation) or not cover substrate surface (to measure total evapotranspiration). For the sake of clarity, data for non-covered treated plants would be most relevant to traditional growing procedures. Hydretain treated non-covered plants had 3.5% less, and AquaGro treated non-covered plants had 2.5% less total evapotranspiration than non-covered Control plants. In the first week of experimentation, Tween 20 treated plants had 18% less ET than Control plants but in terms of total ET, plants treated with Tween 20 were similar to Control, Aquagro, and Hydretain treated plants. Tween 20, AquaGro, and Hydretain treated non-covered plants all had more total leachate volume than the Control. Tween 20 treated non-covered plants had 9.9% more leachates than Control plants. We speculate that because Fafard 3B already has a proprietary blend of surfactants pre-incorporated, the addition of surfactants could actually cause increased leaching. Growth parameters for non-covered Tween 20 treated plants were no greater than those of Control plants. In conclusion, data from this experiment does not support the hypothesis that a single drench application of 100 mg/L of Tween 20 provides advantages for water savings, drought tolerance, or growth compared with Control plants. However, it was unintentionally discovered that large quantities of water can be saved by covering the surface of container substrate to eliminate evaporation. Ultimately, covered-treated-control plants were able to utilize an additional 818.6 mL of water (44 % of total available water) to extend the growing period an additional 21.5 days producing 107% more dry weight than non-covered-control counterparts. The additional 818.6 mL of water available to covered-control plants was "created" by eliminating water typically lost from evaporation. It was necessary to water non-covered plants about once per week with 500 mL water. At this rate, 1500 mL (3 irrigations) are saved per plant for a 55-day growing period as compared to plants that are grown in substrate where evaporation occurs. If a grower were growing 10,000 *Spathiphyllum* plants for 55 days, he could potentially save 15,000 L (3,963 gallons) of water by covering the container substrate. According to Bailey et al. (1999) it takes 22,000 gallons of water per day to irrigate one acre of greenhouse, therefore the water saved by covering container substrate would supply 18% of the required water for 1 additional acre of greenhouse crop.

2) Determine if Tween 20 affects WUE and drought tolerance of a woody plant species.

Based on experimentation with 3-gallon *Ligustrum japonicum* 'Recurvifolium' treated with 0, 50, 100, and 200 ppm Tween 20, results show that rates of 50 and 100 ppm Tween 20 may reduce water use efficiency but only under drought conditions. This experiment was not as valuable as it could have been, had more experimental units been included and more subsample measurements made. Soil moisture was not measured at the same time as photosynthesis parameters, which make it difficult to determine if the alterations in water use efficiency were caused by Tween 20 altering soil moisture content or altering plant physiology.

3) Determine if Tween 20, when applied to the root zone, affects plant water potential Ψ.

Based on experimentation with 3-gallon *Ligustrum japonicum* 'Recurvifolium' treated with 0, 50, 100, and 200 ppm Tween 20, on the second measurement, plants treated with 200 ppm Tween 20 had a 16.9 % higher (less negative) Ψ_p than plants treated with 0 ppm Tween 20, indicating that 200 ppm treated plants were likely undergoing less transpiration (Figure 3.3). On the last day that Ψ_p was measured, plants treated with 50 ppm Tween 20 had a 15.7% higher Ψ_p than plants treated with 0 ppm Tween 20, again indicating that plants were likely undergoing less transpiration. Out of 6 measurements on 6 separate days, only two differences were found. The differences that would have been expected based on research done by Yang (2008) were not observed. It is possible that Tween 20 may not have the same effect on woody plants as observed on herbaceous plants.

Future Research

Based on the results of the present research and the research by Yang (2008), the following research projects are suggested:

1) A study very similar to the one conducted on *Spathiphyllum* 'Emerald Star' with a Control, Tween 20, Hydretain, and AquaGro is recommended utilizing a high-volume production annual plant such as petunia. Alterations to the experiment would include the use of a custom blended substrate with no pre-incorporated surfactants. Additionally, it is recommended that product applications be made on a weekly basis at recommended weekly rates rather than a single drench. Gravimetric water loss should be measured daily from the beginning of the experiment and irrigation decisions would be made for a single treatment group at a time based on gravimetric water measurements. A third treatment in addition to the treatments "covered" and "non-covered" should be added. This treatment would be called non-covered-continuous and would be exactly the same as the original

non-covered treatment up to the point of the termination of non-covered plants. At this point, non-covered-continuous plants would be irrigated again as necessary until the termination of covered-treated plants. The objective here is to see how non-covered plants would normally grow throughout the entire period that covered-treated plants are grown. Non-covered plants would be compared with covered plants on an irrigation quantity basis – after 3 irrigations and once plants begin to wilt, terminate. The second comparison would be between non-covered plants and covered-continuous plants to determine what can be accomplished by the two treatments in the exact same allotment of time. In this comparison, once a covered-treated plant treatment group is wilted it would be terminated alongside its non-covered continuous counterpart, regardless of whether or not it was wilting. Conducting the experiment in this fashion would also make it possible to determine when and if the evaporation component of ET naturally disappears due to plant canopy shading of the substrate and extensive root system and leaf canopy allowing for rapid rates of transpiration.

2) An experiment utilizing a potometer, which measures transpiration rate, should be conducted on stem cuttings of plants treated with a range of Tween 20 concentrations. This method is tedious but it would give a real-time look at transpiration rates, and it would allow for direct uptake of Tween 20.

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