2010

MODELING WATER USE IN NURSERY CROPS

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ABSTRACT OF DISSERTATION

Amy Fulcher

The Graduate School
University of Kentucky
2010
MODELING WATER USE IN NURSERY CROPS

ABSTRACT OF DISSERTATION

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the College of Agriculture at the University of Kentucky

By
Amy Fulcher
Lexington, Kentucky

Director: Dr. Robert Geneve, Professor of Horticulture
Lexington, Kentucky
2010

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ABSTRACT OF DISSERTATION

MODELING WATER USE IN NURSERY CROPS

Water use is an important topic in the global agriculture community and is a critical input in nursery crop production. Several plants in the genus *Cornus* are important nursery crops. Not only are they economically relevant, they are found in grafted and seedling forms and parents and their hybrid are readily available in the trade, facilitating an assessment of water requirements. Anecdotal information suggests that *Cornus* taxa have differing stress tolerance and water use requirements. Research was conducted to characterize and model water use among *Cornus* taxa. Scanning electron microscopy and anatomy-based micromorphological studies as well as transpiration chamber-based studies revealed differences in the cuticle, epidermal thickness, stomatal density, total stomatal complex area, and gas exchange. A novel photosynthesis-based irrigation model was developed and evaluated, first on a model crop, *Hibiscus rosa-sinensis*, then with a range of *Cornus* taxa, including grafted specimens. The model allowed the identification of a setpoint or point at which irrigation is triggered. Producing plants under this model allowed a 27% reduction in water use while maintain growth when compared with controls.

KEYWORDS: *Cornus*, model, irrigation, anatomy, gas exchange

Amy Fulcher

May 7, 2010
MODELING WATER USE IN NURSERY CROPS

BY

AMY FULCHER

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Director of Dissertation

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Director of Graduate Studies

May 7, 2010
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DISSEPTION

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By

Amy Fulcher
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2010

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Chapter One

Overview

Nursery crop production is a substantial portion of the U.S. agricultural economy. Nursery production, especially container production, is dependent on irrigation for plant survival and optimal growth. Over-irrigation is not uncommon in part due to inefficient irrigation delivery techniques i.e. overhead irrigation, as well as poor scheduling of irrigation. Numerous technologies have been developed for estimating plant water use and refining irrigation scheduling. Unfortunately these techniques have not been adopted by nursery growers.

_Cornus_ (dogwood) is an important genus in the nursery industry. Flowering dogwood, _Cornus florida_ L. was selected as a target species for this research because it is one of the most popular landscape trees in the U.S. with $26,633,000 total sales and is in high demand in the Eastern U.S. Dogwood production has a very high income potential with gross returns of up to $60,000 per acre (Witte 1995). However, flowering dogwood, _Cornus florida_, is not suited to adverse rhizosphere or canopy conditions. Flowering dogwood is considered intolerant of drought, compacted soil, and flooded rhizosphere conditions, as well as high light intensity, while _C. kousa_ is considered generally more tolerant of a range of conditions.

_Cornus_ is a desirable genus on which to do irrigation research. Dogwood is a good research tool because there is at least limited research on the stress physiology of _Cornus_ species and there appear to be some difference among species. There are many species of dogwoods, in particular those in the big-bracted clade, which is popular in the trade. Dogwood has a wide geographic range across most continents. As noted, dogwood is a highly relevant nursery crop. Finally, both seedling and grafted _Cornus_ forms, as well as parents and their hybrids are found in the trade, facilitating a
comprehensive examination of water use and the potential transfer irrigation technology to related species.

This research had two main foci: 1) developing a novel irrigation model that could be easily adopted by the nursery industry, and 2) evaluating constraints to applying the model to *Cornus* species.

Research on developing a photosynthesis-based irrigation model for *Cornus* initially utilized a model crop, *Hibiscus rosa-sinensis* ‘Cashmere Wind’. *H. ‘Cashmere Wind’* met several criteria for the model crop outlined in Chapter Three. A model crop was utilized to accelerate development of the model since, unlike *Cornus, Hibiscus* can be grown throughout the year in a greenhouse. Model development, consisting of a sigmoidal moisture response curve, and a small-scale production scale experiment took place with *H. ‘Cashmere Wind’*. Experiments then focused on determining model parameters for *Cornus* seedlings under greenhouse conditions. Experiments were conducted to determine model parameters in outdoor conditions and utilized grafted plants. Under these conditions, the sigmoidal relationship persisted, however, the nature of the moisture response curve changed. Further experiments with self and reciprocal grafts were conducted to determine if the variation was caused by the outdoor environment, specifically high vapor pressure deficit, or by grafting.

Research was also conducted to elucidate various aspects of ecophysiological and micromorphological characteristics affecting water use among *Cornus* taxa. This line of research investigated drought response and recovery among *Cornus* taxa, the effect of grafting and canopy environment on water use. This research included the influence of foliar anatomy (stomatal aperture, size, stomatal density, epidermis and palisade thickness, etc.) on model parameters: water loss and photosynthesis.
Chapter Two

Literature Review

Water

Water defines life. As in all other living creatures, plants must have water to survive. Water is both provided to the plant by the environment and removed from the plant by the environment. Out of necessity, plants have achieved the delicate balance between water conservation and CO₂ fixation.

Water is used as a substrate in reactions, for temperature regulation, as a carrier for nutrients and plant hormones, and is the hydraulic force behind growth. Water is taken up by the roots and is lost to the environment either by exiting the plant through the leaf cuticle or through the stomates. A water deficit can affect biomass and yield (Taiz and Zeiger 2006).

Water Use and Plant Anatomy

Many morphological as well as physiological characteristics impact transpiration. Leaf size and shape, stomatal attributes, as well as epidermal cell, trichome, and cuticle characteristics can all influence water loss. Differences in morphology have been identified among related taxa native to varying ecosystems. Xerophytic species, those species adapted to arid environments, often have foliar morphology that limits water loss, such as smaller leaves than their mesic counterparts.

The connection between environment i.e. lobed leaves and drier environment, entire leaves and mesic environments, has long been established (Bailey and Sinnott 1916, Gentry 1969, Richards 1996, Wolf 1978). Deep versus a shallow sinus or no sinuses, can influence the formation of the boundary layer (Baker and Myhre 1969). Additionally, there is evidence that lobed leaves dissipate heat via convection better than entire leaves (Gottschlich and Smith 1982, Vogel 1968, Vogel 1970) such that
naturally occurring tears in banana leaves are associated with increased convective heat loss as well as reduced boundary layer resistance (Taylor and Sexton 1972). Some current forest ecosystem models that examine the role of climate change include morphological modifications in response to increased temperature (Shahba and Bauerle 2009). Finally, leaves of xeric plants tend to be smaller as are leaves that develop under water deficit (Galston et al. 1980).

Epidermal tissue, the outer cell layer of the leaf, includes the epidermis, trichomes, cuticle, guard cells, and stomates. Epidermal cells may contain protrusions, called papillae (Dickison 2000). The cuticle is a continuous layer of cutin and wax that forms on the surface of cellulose microfibrils (Holloway 1980) and may take several forms. All of these epidermal features affect water loss.

Thicker, lignified epidermal cells are common among plant native to dry areas, as are smaller leaves, for reduced transpirational area (Esau 1967, Oppenheimer 1960). Thicker epidermal cells have been associated with an enhanced ability to screen or filter UV-B light which would decrease the total radiation intercepted by the plants (Day 1993). Ristic and Cass (1991) found that Zea mays L. with greater drought resistance had thicker epidermal cells than drought sensitive Zea mays lines. Capparis spinosa L., a Mediterranean species, develops a very thick epidermal cell wall as leaves age, especially on the exposed cell surface (Rhizopoulou and Psaras 2003). Greenhouse versus outdoor-grown Prunus insititia L. ‘Pixy’ significantly reduced epidermal cell thickness, 19.7 and 26.2 µm respectively. Outer epidermal cell walls from Alchornea triplinervia Spreng. leaves from trees growing in a relatively dry microclimate in Brazil were 43% thicker, but not significantly so, than leaves from trees growing in a more humid microclimate (Rôças et al. 1997).

The cuticle is exposed to the biotic and abiotic factors that affect plant health and is a protective barrier. The cuticle is the final (non-stomatal) impediment to
moisture loss (Scott 1964, Scott 1966). However, plants may experience cuticular water loss in amounts up to 30% of the amount of stomatal water loss (Holmgren et al. 1965). Thus, loss of water vapor through the cuticle and during drought stress or other conditions that don’t stimulate stomatal opening can be relevant. Cuticle thickness has long been associated with the level of resistance to water vapor, however, research has shown that type and composition of cuticular waxes can be more important than the quantity of wax in preventing water loss (Lendzian 1982, Lendzian and Kerstiens 1991, Schönherr 1976, Schreiber and Riederer 1996, Tischler and Voigt 1990). The principle advantage of waxes over cutin is their highly ordered structure (Reynhard and Riederer 1991, Reynhard and Riederer 1994).

In research with Mexican redbud, *Cercis canadensis* var. *mexicana* Rose, a species adapted to an arid climate and characterized by a very glossy cuticle, and Eastern Redbud, *Cercis canadensis* L., a species adapted to a mesic environment with a less apparent cuticle, the cuticle was determined to be inconsequential in preventing water loss (Tipton and White 1995). In fact, for greenhouse-grown redbuds in this study, the rate of water loss for Mexican Redbud was 50% greater than that of Eastern Redbuds on a leaf area basis in spite of the fact that the cuticle was 35% thicker on the Mexican Redbud. In this case the overall makeup of the cuticle was considered to be more cutin than wax, thus explaining the seemingly disparate relationship between cuticle thickness and drought adaptability.

Trichomes are extensions of epidermal cells and, like the cuticle, serve to protect plants from biotic and abiotic agents. Trichomes are singular or multicellular structures and may be branched or unbranched. Some trichomes have secretion glands. Trichomes are often associated with xeric plants (Fitter and Hay 1993, Gibson 1996) and are known to increase the boundary layer depth (Donselman and Flint 1982) and reflectance (Ehleringer and Mooney 1978, Karabourniotis et al. 1994). For example, among several southwestern tree species, *Fraxinus veluntina* Torr. had the greatest
trichome density, 1836 trichomes/cm$^2$, and under drought stress and ensuing reduced leaf water potential was able to maintain high photosynthetic rates despite exposure to drought (Coye and St. Hilaire 2002). Among hybrids of the perennial *Piriqueta caroliniana* Urban and *P. viridis* Small, those plants with higher trichomes density were able to maintain higher water use efficiency under water deficit conditions (Picotte et al. 2007). While trichomes are associated with increase leaf reflectance, Smith and Hare (2004) found no difference among reflectance or absorptance of granular versus non-glandular trichomes in *Datura wrightii* (Regel).

Stomatal density and individual stomate size, as well as other characteristics, such as sunken stomates or stomates occluded with wax, trichomes, and/or papillae, can influence water use. In general, a greater stomatal density is considered a strategy for dealing with hot, arid climates. Increasing the stomatal density increases transpiration, which cools the plant (Maximov 1931). Among 22 of 32 trees species, stomatal density was greater under dryland production than when grown with irrigation (Gindel 1969). Stomatal density increased for container-grown *Pelargonium x hortorum* Bailey under drought cycles (Hassanein and Dorion 2006).

Increasing stomatal density on subsequent leaves and decreasing stomatal aperture are adaptive strategies for some plants under drought stress. Lines of drought resistant *Zea mays* had greater stomatal densities and smaller stomates than those of drought-sensitive lines (Ristic and Cass 1991). Abrams et al. (1992) found that with increased exposure to irradiance, *Prunus serotina* Ehrh., developed leaves with increased stomatal density but smaller stomates. Finally, Hilaire and Graves (1999) found that stomatal density increased and stomate size decreased for *Acer saccharum* Marsh. and *Acer saccharum* subspecies *nigrum* Desm. along a continuous latitudinal line (43°N) from a mesic to a more arid region of the United States.
Stomatal characteristics features of the surrounding anatomy can restrict moisture loss. Most guard cells have a ledge of cell wall that extends beyond the guard cell and shields the aperture (Esau 1967). Stomates may be sunken below the surface of the epidermis to reduce water loss (Esau 1967). Stomates may be partially or fully occluded by the cuticle to restrict transpirational water loss (Esau 1967). Berberis trifoliolat Moric. develops papillae as does, Becium burchellianum Benth. (Gibson 1996). SEM photomicrographs of numerous Cornus species reveal varying levels of cuticle occlusion of the stomates as well as papillae covering the stomates (Hardin and Murrell 1997). Cuticular ledges were apparent in all three Populus clones (two clones of Populus candidans Ait. x P. berolinensis Dipp. and one clone of Populus betulifolia Dipp. x P. trichocarpa Torr.) tested and the ledges were overlayed with cuticle (Pallardy and Kozlowski 1980). In addition, the cuticle was thicker and caused complete occlusion of the stomates in field-grown but not greenhouse-grown clones. Doritaenopsis (Doritis x Phalaenopsis) ‘New Candy’, a very light sensitive hybrid orchid, developed papillae during an acclimatization period under a range of low light treatments (Jeon et al. 2005). In addition, partial cuticle covering of stomates was visible in SEM photomicrographs.

Responses to and Consequences of Water Deficit

Water deficit during production can have serious consequences; water deficit can decrease yields, increase production time, and affect fruit quality (Jones and Tardieu 1998). Growth is considered more sensitive to water stress than photosynthesis (Hsiao and Xu 2000). This is because growth is driven by turgor pressure and photosynthesis is not. Leaf area is reduced in drought stressed plants because cell expansion is highly dependent on and sensitive to decreases in turgor pressure. However, some evidence exists for non-hydraulic signaling rather than response to reduced leaf water content being responsible for reduced leaf growth (Saab and Sharp 1989). In addition to slowed growth in response to drought, leaves often abscise in response to water deficit when the deficit occurs following growth. Leaf abscission adjusts the root to shoot ratio to be in accordance with the water supply.
As mentioned above, the root to shoot ratio adjusts in response to water deficit. The balance between above and below ground biomass is largely controlled by water supply and modulated by ABA. Roots grow until limited by photosynthate supplied by the shoots, and correspondingly, shoots will grow until limited by water supplied by the roots. Generally, roots are less sensitive to water deficit than shoots and roots may even continue to grow under water deficit conditions (Sharp and Davies 1989). Water deficit-induced growth can promote deeper roots systems in mineral soils, which can enhance not only water but also nutrient availability (Keller 2005). Chaves et al. (2002) found that *Lupinus albus* root growth was stimulated by water deficit. Non-irrigated *Vitis*, roots grew into deeper, more moist portions of the soil profile, while irrigated *Vitis* roots largely remained in the upper, irrigated portion of the soil profile (Bauerle 2007, Bauerle et al. 2008). ABA has been shown to inhibit ethylene production during water stress, stimulating root growth (Sharp and LeNoble 2002, Spollen et al. 2000) while also influencing shoot growth (Sharp 2002). Also, ABA is known to stimulate root growth in the *Vitis* hybrid ‘Kyoho’ (*Vitis labrusca* L. x *V. vinifera* L.) by increasing expansin production in root cells (but not stem or leaf cells) in response to a soil water deficit (Lovisolo et al. 2010).

Both stomatal and non-stomatal limitations can limit photosynthesis is response to water deficit. The loss of CO$_2$ as a substrate for photosynthesis due to stomatal closure is a stomatal limitation. Dehydration of mesophyll cells is a non-stomatal limitation.

A rapid water deficit elicits different responses than a gradual deficit. Water uptake causes the guard cells to be turgid and exposes the stomatal aperture to the atmosphere, permitting water vapor to exit and CO$_2$ to enter the leaf. Stomatal closure can occur due to water deficit by two different mechanisms. Stomatal closure can take place hydropassively when the relative humidity is so low that water evaporates from the guard cells and they become flaccid (Cowan, 1977, Farquhar, 1978, Grantz, 1990). This in turn closes the stomate and prevents transpirational water loss. This, however,
is an active field of research; Kaiser and Legner (2007) found that direct response to guard cell transpiration does not occur in *Sambucus nigra* L. and, rather, hydropassive stomate regulation involves sensing leaf water potential and perhaps signaling. Hydroactive stomatal closing is a stomatal response to whole leaf water relations rather than direct water loss through the guard cell cuticle (Taiz and Zeiger 2006). Guard cells become flaccid in response to water deficit in the whole leaf or root. Hydroactive stomatal closure relies upon metabolic processes within guard cells, not simply evaporative water loss.

ABA is involved in stomatal regulation in response to water deficit in two ways (Taiz and Zeigler 2006). ABA is synthesized in the mesophyll cells and accumulates in chloroplasts. Upon dehydration of mesophyll cells, some ABA is transported to the apoplast, allowing ABA to move with water via transpiration. Upon dehydration, ABA synthesis is up-regulated, and greater amounts of ABA accumulate in the apoplast. The newly synthesized ABA is thought to modulate sustained stomatal closure initiated by ABA previously synthesized and mobilized from the chloroplast. Overall, ABA levels can increase 50 fold in response to water deficit (Taiz and Zeiger 2006). When plants experience a soil moisture deficit, chemical signals from the roots appear to affect stomatal regulation, closing the stomates to limit water loss. ABA, pH, and inorganic ions are considered to be involved in root-to-shoot signaling.

Irrigation

Approximately 70% of the earth’s surface is water. Oceans, glaciers and the polar caps amount for 99.4% of surface water, while rivers, lakes, and ponds are just 0.6%. In the US, hydroelectric power and irrigation are the greatest consumers of water with irrigation responsible for 30% of water use (United States Department of the Interior 2009). In some developing countries, irrigation is responsible for 90% of water consumption (World Business Council for Sustainable Development 2009). On a worldwide basis, approximately 70% of freshwater is consumed by agriculture (World
Water deficit is considered the single greatest source of yield losses worldwide (Boyer 1982). While use of highly efficient irrigation delivery methods such as drip irrigation are increasing, they represent only 1% of irrigation delivery techniques worldwide (Postel 1993). Inefficient irrigation and competition for water could negatively impact the ability to produce enough food in the future. (World Business Council for Sustainable Development 2009).

Nursery production is a small percentage of agricultural acreage but is intensively managed and can consume a lot of water. Nursery irrigation delivery generally uses overhead emitters for #1-#5 containers and individual microemitters for #7 and larger-sized containers. Overhead irrigation can be very inefficient and great disparity often exists in water output across an individual zone (Yeager et al. 2007). Niemiera (1994) reported as 300% variability in water output within a single irrigation zone. Scientists and nursery producers predict a reduction in water availability for future nursery crop production (Beeson et al. 2004). Consequently, there is much interest in and research on irrigation efficiency for nursery crop production.

According to a survey of Alabama growers, nurseries tend to irrigate between 0.3-1.3 acre inches per day (9,000-35,100 gallons) (Fare et al. 1992). Beeson and Knox (1990) found that as linear spacing between plants increases, irrigation efficiency decreases exponentially with as little as 35% efficiency even at close spacing (a linear distance of half a container diameter between pots). Standard irrigation practices for container nursery production include 0.5-0.6” water per day during the summer (Harrison 1976; Yeager et al. 2007). Strategies to use water more efficiently in nurseries include grouping plants by relative water needs and using cyclic irrigation. Plants requiring little water can be located in the same irrigation zone. Grouping plants into irrigation zones by water needs along with proper spacing can reduce irrigation usage by 80% (Burger et al. 1987).
Another conservative irrigation strategy is cyclic irrigation. Cyclic irrigation takes the total daily volume of water and applies it as multiple, smaller volume irrigation events with a minimum of one hour between irrigation events (Yeager et al. 2007). Using cyclic irrigation can reduce runoff by 30% and nitrogen leaching by 41% compared with conventional continuous irrigation (Fare et al. 1994). Using amendments to increase the water holding capacity can also reduce water use. Owen et al. (2008) found that using 89% pine bark:11% calcined palygorskite-bentonite mineral aggregate decreased water use by 25% when compared with a 89% pine bark:11% coarse sand substrate.

Scheduling Irrigation

Scheduling irrigation has been the focus of much research in agronomic and horticultural crops. Scheduling can be relatively static and arbitrary (timer-driven), substrate moisture-based, environmental models, or plant-based (Jones 2004).

Arbitrary

Container irrigation has traditionally been initiated by automatic timers and scheduled for predawn to minimize evaporative loss. As much as 30% of overhead irrigation can be lost to evaporation on days with high temperature and irradiance (Ross 1994). Applications often occur during daylight hours for cyclic irrigation simply due to logistics of scheduling 2-3 times as many irrigation events. Other nurseries run irrigation all night to deliver water to every irrigation zone. While less efficient than predawn irrigation, research has shown that irrigating during the afternoon can increase growth (Warren and Bilderback 2002).

Substrate Moisture-based

Substrate moisture-based irrigation scheduling is either predicated on substrate water balance calculations or substrate moisture measurements (Jones 2004). Substrate moisture measurements consist of either substrate water potential or substrate water content and generally rely on moisture probes or gravimetric measurements. Tensiometers reflect actual water potential but are difficult to use in
coarse nursery substrates and require regular maintenance. Capacitance probes and gravimetric techniques measure substrate water content rather than water potential and, thus, are not a measurement of actual availability of water. A fundamental weakness in substrate moisture-based irrigation scheduling is that plant moisture status is based not only on the amount of water taken up by the plant, which is dependent on the substrate water potential, but also the rate at which it is moved through the plant. Substrate moisture-based irrigation does not take into account atmospheric influences, such as low vapor pressure deficit. It is possible under high vapor pressure deficit-conditions to have a well hydrated soil, but water-deficient plants because the rate of transpiration outpaces water uptake and transport (Grange and Hand 1987). A midday drop in photosynthesis has been described for some plants and may correspond to a temporary water deficit brought on by higher vapor pressure deficit (VPD) conditions (Iio et al. 2004, Kauhanen 1986, Portes et al. 2007). Determining the position for the substrate moisture probe is challenging and currently must be done empirically (van Iersel et al. 2009). Advantages of substrate moisture-based irrigation are that it can reflect root-to-shoot signaling in response to dry substrate conditions and can be automated. Substrate water balance calculations are used to determine substrate moisture status and are calculated as the difference between water applied to the plant (irrigation and precipitation) and water lost through evapotranspiration.

Environmental-based

Environmental-based models attempt to calculate evapotranspiration based largely on environmental conditions. The Penman-Monteith is the most widely recognized and comprehensive evapotranspiration model (Monteith 1965).

\[
LE = \frac{\Delta R_{\text{net}} + \rho Cp \Delta \text{VPD} \Delta \theta}{\Delta + \frac{\Delta \theta}{f}}
\]

where:

LE= latent heat of vaporization, expressed at latent heat flux, \((W \cdot m^{-2})\)

\(\Delta\)=slope of the saturated vapor pressure curve
\[ R_n = \text{net radiation at the canopy level} \ (W \cdot m^{-2}) \]
\[ G = \text{soil/substrate heat flux} \ (W \cdot m^{-2}) \]
\[ \rho_{\text{air}} = \text{density of air} \ (kg \cdot m^{-3}) \]
\[ c_{pa} = \text{specific heat of air at constant pressure} \ (J \cdot kg^{-1} \cdot ^\circ C^{-1}) \]
\[ \text{VPD}_{\text{air}} = \text{air vapor pressure deficit} \ (kPa) \]
\[ r_n = \text{resistance for sensible heat transfer by convection} \ (s \cdot m^{-1}) \]
\[ \gamma = \text{“psychometric constant”} \ (Pa \cdot ^\circ C^{-1}) \]
\[ r_s = \text{canopy surface resistance, i.e., resistance to evapotranspiration} \ (s \cdot m^{-1}) \]

Complications with using such models are that 1) prerequisite information may be necessary, e.g., for the Penman-Monteith model, crop coefficients, must be empirically derived for every species, perhaps even at the cultivar level, 2) it may be necessary to have a weather station on-site, and 3) the model may be complex, requiring that several variables be measured. In addition certain assumptions may be made. In the case of the Penman-Monteith model a uniform, closed canopy is assumed.

Plant-based

Plant-based systems are generally considered to be highly relevant and allow for environmental influence, but do not account for root to shoot signaling, are challenging to automate, and do not indicate the volume of water to apply (Jones 2004). Plant-based systems can respond to the physiological changes that occur directly due to changes in plant water status. However, this can be a disadvantage for conservative irrigation schedules in certain environments, as low plant water status induced by extreme midday conditions could trigger irrigation when the substrate moisture is not limiting. Leaf temperature (Prenger et al. 2005), plant water potential (Goldhamer and Fereres 2001, Zimmermann et al. 2008), stem diameter (Fereres and Goldhamer 2003), stem heat balance (Sakuratani 1981), and modeling based on empirically-derived plant characteristics (Baurle et al. 2002, Nui et al. 2006) are plant-based techniques that have been used to gauge water loss in horticultural crops.
In spite of the successes with plant-based measurements, they are not categorically superior. While the linkage between leaf temperature and transpiration has been established, thermal imaging is not well-suited for scheduling nursery crop irrigation. A closed canopy is ideal for use of infrared temperature sensors. However, conventional nursery plant spacing precludes development of a closed canopy. Additionally, the heterogeneity of stomatal closure and, thus, water relations and leaf temperature, necessitates precise placement if an individual leaf is used, which can be challenging even under controlled - environment production (Hashimoto et al. 1984). Other factors such as air temperature, humidity, and irradiance can affect plant temperature and infrared temperature sensors. Additionally, thermal imaging technology is expensive (Leinonen et al. 2006). A strong relationship between leaf water potential and transpiration has been observed in some trees (Nortes et al. 2005). However, there are both herbaceous and woody plants for which a relationship between leaf water potential and level of drought stress does not exist (Johnson et al. 2001; Jones 1985; Jones et al. 1983) and leaf water potential does not always reflect initial plant water deficit (Remorini and Massai 2003). For both *Malus domestica* Borkh. and *Zea mays*, it has been demonstrated that shoot growth can be restricted due to soil water deficit conditions before plant water potential decreases (Saab and Sharp 1989, Gowing 1990). Predawn leaf water potential requires either that measurements are made in the dark or after manually covering the leaves with a reflective surface for 2-3 hours, both of which are inconvenient. The petiole must be sufficiently long to fit the pressure bomb apparatus and sampling is destructive, which may limit biomass available for additional measurements or substantially change the total plant leaf area and thus change the rate of whole plant water loss for smaller plants. Additionally, leaf water potential can be variable depending on exposure to irradiance and few, if any, advances in automating irrigation to plant water potential have been made.

Stem diameter has been used to schedule irrigation applications in trees (Fereres and Goldhamer 2003, Goldhamer and Fereres 2004). Stem diameter is a reflection of xylem water potential and can be attributed to water movement to and from xylem
vessels and bark (Molt and Klepper 1973). Stem thickness can correlate well with evapotranspiration, but requires highly specialized sensors and can require a large number of replications due to high variability (Doltra et al. 2007). Sap flow technology requires detailed knowledge of anatomy of each species and sap flow measurements do not always keep pace with transpiration (Wronski et al. 1985). Additionally, some sap flow-based technology requires the use of invasive probes or, alternatively, heaters that wrap around the trunk and must be adjusted regularly to accommodate growth in diameter. Finally, highly parameterized models are complex and require extensive prerequisite information.

Nursery Crop Industry

The nursery crop industry is a high profit industry with cash receipts approaching $10,000 per acre for certain production types and plant sizes (Yeager et al. 2007). According to the 1997 Census of Agriculture, the nursery crop industry includes 26,297 acres and is valued at $6,617,038,558 total annual sales (USDA 2008). Collectively, nursery production in FL, GA, KY, NC, SC, and TN represent 1.5 billion dollars in farm gate values annually. Nurseries in these southeastern states employ hundreds of thousands of workers. Nursery crops are a high value segment of agriculture; liner trees cost as much as $7.00-$25.00 per plant at the onset of production and may require 2-5 years before harvest and sale. Plant sales are based almost solely on aesthetic quality.

Dogwood

Flowering dogwood, *Cornus florida* L. was selected as the target species for this research because it is one of the most popular landscape trees in the U.S. with $26,633,000 total sales and is in relatively high market demand in Kentucky and surrounding states (USDA 1998). Dogwoods are grown in many field nurseries in the Southeastern U.S., including Kentucky, and are widely planted in landscapes throughout the Eastern U.S. Kentucky ranks 19th in Flowering Dogwood production; by comparison, Tennessee, North Carolina, Alabama, and Virginia are ranked 1, 3, 7 and 8, respectively. Dogwood nurseries have a very high income potential with gross returns of up to
$60,000 per acre (Witte 1995). Kentucky appears to have the potential to produce more dogwoods than current production levels indicate.

Typically *C. florida* is the rootstock used for both *C. florida* and *C. kousa* Hance scions. Dogwood seeds are collected, cleaned and sowed in the fall (Halcomb 1993). The following August, cultivars and other selections are field budded onto seedling rootstock. Liners are root-pruned in late fall of the year they are budded. Liners are dug during the dormant season, sold bareroot, and replanted in the field at a spacing to allow growth to a salable size. Dogwoods are generally harvested as balled and burlapped trees after 2-4 years of field production. Dogwood has been a difficult plant to grow in containers (Witte and Tilt 1991), yet container-grown plants are increasingly preferred by retail customers over balled and burlapped plants (Basham et al. 2004).

Dogwood is widely represented in North America, South America, Europe, and Asia. Xiang et al. (2006) categorized *Cornus* into four major clades including the big-bracted dogwoods, the dwarf dogwoods, the blue- or white-fruited dogwoods, and the Cornelian cherries. The big-bracted clade includes: *C. florida*, *C. florida* subspecies *urbiniana* Rose, *C. nuttalli* Aud., *C. disciflora* Moc. et Sessé, *C. kousa*, *C. kousa* var. *chinensis*, *C. capitata* Wall., and several subspecies of *C. hongkongensis* Hemsl. In the US, dogwood trees are largely grown for showy bracts, often incorrectly referred to as flowers. Currently the dogwood trees which are grown in the trade for attractive bracts are members of the big-bracted clade, *C. kousa* and *C. florida*. *C. florida* is native to a wide range of the Eastern US, from north Florida to Michigan, New York, Massachusetts and as far west as eastern Texas and Oklahoma. *Cornus kousa* and its varieties are native to Japan, Korea, Taiwan, and China, where it grows in native in mixed woods, streamsides, and valleys at 400-2200 meters elevation (Hillier Nurseries et al. 2007). *C. florida* is an understory tree native to mesic forest areas. *C. florida* is native to the eastern half of the United States, from Texas and Ontario east to Florida and Maine. Currently *C. florida* is considered endangered by the Maine Department of Conservation.
Dogwood is also a good choice for this research because there is potentially a range of physiological responses to environmental stress in the big-bracted germplasm from North America and Asia. Anecdotal information suggests that *C. kousa* may be more drought tolerant than *C. florida* in the landscape (Dirr 2009). However, other information suggests that newly transplanted *C. kousa* may be drought intolerant, and perhaps more so than newly planted *C. florida*, as recently planted liners of *C. kousa* have been observed wilting before their *C. florida* counterparts (M. Moffett, personal communication). *C. florida* is reportedly more adapted to the lower South than *C. kousa* and *C. kousa x C. florida* hybrids (Hardin et al. 2002). Augé et al. (2002) found that many *C. kousa* seedlings experienced leaf curl and scorch under water deficit and high temperature conditions in the field. *C. florida* is known to respond to drying soil conditions with leaf drop and osmotic adjustment although it is not considered dehydration tolerant (Augé et al. 2002, Tschaplinski et al. 1998).

Stomatal conductance of both *C. kousa* and *C. florida* are lower than those of other deciduous trees and not correlated with plant water potential (Johnson et al. 2001). Well-watered *Acer rubrum* L., *Liriodendron tulipifera* L. and *Chionanthus virginicus* L. all had maximum stomatal conductance near 600 mmol m⁻²·s⁻¹ while *C. florida* had a maximum stomatal conductance of 200-300 mmol m⁻²·s⁻¹. Over the course of a two-year experiment, stomatal conductance for *C. kousa* seedlings never exceeded approximately 200 mmol m⁻²·s⁻¹ (Croker et al. 1998). Additionally, stomatal conductance was not related to irradiance for both *C. kousa* and *C. florida* (Augé et al. 2000, Augé et al. 2002). Williams et al. (1987) found that *C. florida* shoot length was reduced even under short intervals (five days) of withholding water, with stems length half of that of control regimes. *C. florida* is intolerant of flooded or compacted soils and is shallow rooted, and, thus, susceptible to drought (Day et al. 2000, McLemore 1990, Tschaplinski et al. 1998). No research directly comparing water use efficiencies or drought tolerance of *C. kousa* and *C. florida* has been conducted.
Research Objectives

The overall objectives of this research were to develop an irrigation model to more efficiently produce nursery crops and to understand water use in woody plants and thereby identify constraints to adapting the model to a range of nursery crops. Chapter Three addresses experiments designed to determine the utility of *Hibiscus rosa-sinensis* L. as a model crop for nursery research and development and evaluation of a photosynthesis-based irrigation model. In Chapter Four a number of experiments are described to determine if 1) *Cornus* species follow the same sigmoidal pattern between substrate moisture content and photosynthesis as *Hibiscus rosa-sinensis* ‘Cashmere Wind’, 2) a wide range of substrate moisture contents, from approximately 70 to 100% container capacity, support high photosynthetic rates, facilitating the determination of an irrigation setpoint that reduces the container capacity as much as possible while allowing photosynthesis to decrease by only 2% of maximum, and 3) grafting influences determination of the irrigation setpoint. Chapter Five explains the development of a novel transpiration chamber with the ability to independently control temperature and relative humidity in order to create various VPD_{air} treatments and experiments to determine the effect of vapor pressure deficit on transpiration as it relates to identification of environmental factors that may limit the use of the irrigation model developed in Chapter Three. Experiments in Chapter Six were designed to determine if net photosynthesis and gas exchange are related to leaf characteristics among several *Cornus* taxa, and if *Cornus* taxa differ for these morphological characteristics and plant water relations.

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Chapter Three

Development and Evaluation of a Photosynthesis-Based Irrigation System for Nursery Crop Production

Introduction

Worldwide, more water is consumed by agriculture than for any other use (Dobrowolski et al. 2008). In container nurseries in the southeastern U.S., historical use estimates are as high as 2820 mm of irrigation water applied annually, and as much as 33 mm applied daily (Beeson and Brooks 2008, Fare et al. 1992). Recent droughts in the U.S. have heightened awareness of the impact of water shortages on domestic and agricultural entities. From January 2006 to December 2008, approximately 50% of the contiguous U.S. was affected by drought (National Drought Mitigation Center 2009). In many areas the drought was at severe, extreme, or exceptional levels. The green industry has been severely impacted by this and other recent droughts (Ding et al. 2008) and by the ramifications of drought. Regulations have restricted container irrigation in some major nursery production areas. For example, irrigation restrictions are in place in Florida, where the cumulative irrigation application is limited to 1800 mm annually near metropolitan areas (Beeson and Brooks 2008). Additionally, scientists and nursery producers predict a reduction in water availability for future nursery crop production (Beeson el al. 2004).

Various methods have been used to estimate crop water use and refine irrigation schedules. Techniques can be broken down into plant-based, soil moisture-based, or based on environmental models of evapotranspiration. Plant-based systems are generally considered highly relevant and allow for environmental influence, but do not account for root-to-shoot signaling and are challenging to automate (Jones 2004). Plant-based systems can respond to the physiological changes that occur directly due to changes in plant water status. However, this can be a disadvantage for conservative
irrigation schedules, as low plant water status induced by extreme midday conditions could trigger irrigation when substrate moisture is not limiting. Alternatively, plant-based models may not be deployed until the plant is already experiencing water deficit conditions (Warren and Bilderback 2004). Leaf temperature (Prenger et al. 2005), plant water potential (Goldhamer and Fereres 2001, Zimmermann et al. 2008), stem diameter (Fereres and Goldhamer 2003), stem heat balance (Sakuratani 1981), and modeling based on empirically-derived plant characteristics (Bauerle et al. 2002, Nui et al. 2006) are plant-based techniques that have been used to gauge water loss in horticultural crops.

In spite of success with plant-based measurements, they are not categorically superior. While the linkage between leaf temperature and transpiration is established, thermal imaging is not well-suited for scheduling nursery crop irrigation. Conventional plant spacing precludes a closed canopy and the heterogeneity of stomatal closure and, thus, water relations and leaf temperature, necessitates precise placement if an individual leaf must be used (Hashimoto et al. 1984). This can be challenging even under controlled-environment production. Other factors such as air temperature, humidity, and irradiance can affect plant temperature and infrared temperature sensors. Additionally, thermal imaging technology is expensive (Leinonen et al. 2006). A strong relationship between leaf water potential and transpiration has been observed in some trees (Nortes et al. 2005). However, there are both herbaceous and woody plants for which a relationship between leaf water potential and level of drought stress does not exist (Jones 1985, Jones et al. 1983) and leaf water potential does not always reflect initial plant water deficit (Remorini and Massai 2003). Predawn leaf water potential requires either that measurements are made in the dark or after manually covering the leaves with a reflective surface for two to three hours, both of which are inconvenient. The petiole must be sufficiently long to fit in the pressure bomb apparatus and sampling is destructive, which may limit plant tissue available for additional measurements or substantially change the total plant leaf area altering the rate of whole plant water loss for smaller plants. Additionally, leaf water potential can be variable depending on
exposure to irradiance, and few, if any, advances in automating irrigation to plant water potential have been made.

Stem diameter has been used to schedule irrigation applications in trees (Fereres and Goldhamer 2003, Goldhamer and Fereres 2004). Stem diameter is a reflection of xylem water potential and can be attributed to water movement to and from xylem vessels and bark (Molz and Klepper 1973). Stem thickness can correlate well with evapotranspiration, but requires highly specialized sensors and can require a large number of replications due to high variability (Doltra et al. 2007). Sap flow technology requires detailed knowledge of the anatomy of each species and sap flow measurements do not always keep pace with transpiration (Wronski et al. 1985). Additionally, some sap flow-based technology requires the use of invasive probes or, alternatively, heaters that wrap around the trunk and must be adjusted regularly to accommodate growth in diameter. Finally, highly parameterized models are complex and require extensive prerequisite information.

Historically, some container producers have overwatered (Fare et al. 1992). Irrigation technology has not been adopted on a large scale by the nursery crop industry (Beeson et al. 2004, Warren and Bilderback 2004). This is partly due to the diversity of nursery crops and the need to develop individual crop coefficients or other requisite information. Developing an easily adopted, accurate method of modeling water use could improve water use efficiency. An irrigation model well-suited for the nursery industry would 1) be simple, 2) be easily configured to a large number of crops, 3) accurately estimate water use to prevent over and under irrigation and thus conserve water and minimize leaching, 4) not increase production time compared to current irrigation scheduling, and 5) have the ability to be automated.

A photosynthesis-based irrigation system is proposed in this paper. A photosynthesis-based irrigation system assumes that photosynthetic rate is a sensitive indicator of the water status of the plant, that growth would not be compromised due to a transient reduction in plant water potential, and osmotic adjustment, if it occurred,
would benefit plants grown under the model. Because photosynthesis is closely linked with stomatal conductance, and stomatal conductance is controlled by both root-to-shoot signaling and the environment, photosynthesis is logically a sensitive indicator of water status. Development of an irrigation system based on photosynthetic rates would require a minimum of data collection for model development and could easily be modified for use with other species. An irrigation system predicated on maximizing photosynthesis has been developed for container-grown apple (*Malus xdomestica* Borkh.) trees. This system utilizes a setpoint (trigger for irrigation application) based on the relationship between maximum photosynthesis and midday stem water potential (Steppe et al. 2008). To my knowledge, the irrigation system presented in this chapter is the first irrigation system based on the relationship between substrate water content and maximum photosynthesis.

Hibiscus (*Hibiscus rosa-sinensis* L.) is proposed as a model crop for nursery crop irrigation research. A model crop that could be grown year-round in either outdoor or controlled-environment conditions could accelerate development of an irrigation system. Additionally, the use of genetically identical plants growing on their own roots could reduce variation and also facilitate model development. Selection criteria for the model crop required that the plant 1) be a woody plant 2) be anatomically and physiologically representative of nursery crops, 3) be easily propagated, and 4) grow under a range of environmental conditions that are reasonably easy to create and maintain.

The objectives of this study were to 1) determine if *H. rosa-sinensis* is a good model crop for nursery research, 2) determine the relationship between substrate moisture content, photosynthesis, and biomass for *H. rosa-sinensis* ‘Cashmere Wind’, and 3) evaluate the hypothesis that plant biomass will be reduced only when substrate moisture levels cause a significant reduction in photosynthetic rate.
Materials and Methods

Stocks plants were established from rooted cuttings of *H. ‘Cashmere Wind’* which were purchased from a commercial greenhouse (Yoder Brothers, Inc., Barberton, OH). Cuttings were transplanted into trade one gallon (3.7 L) containers and fertigated with 100 ppm nitrogen at each irrigation. When roots reached the container sidewall, plants were transplanted to trade three gallon containers (10.8 L) and cut back regularly to encourage branching. Cuttings (three to four inches in length) were taken, subjected to a five-second 3000 ppm IBA quick dip, and placed in one-inch cubes of oasis, one cutting per cube. Flats of the oasis cubes were placed on bottom heat at 26 °C. Rooted cuttings of *H. ‘Cashmere Wind’* were potted into trade one gallon (3.7 L) containers (Nursery Supplies, McMinnville, OR) with a sphagnum peat moss and bark-based substrate (Metro Mix 280, Sun Gro Horticulture, Bellevue, WA) after 4-6 weeks of rooting time and one month prior to imposing treatments.

The relationship between light and CO$_2$ and photosynthesis was determined by conducting response curves with an infrared gas analyzer (LI-6400, LI-COR® Biosciences, Lincoln, NE). The second most recently matured, fully expanded leaf was used for gas exchange measurements. Photosynthetic response curves were conducted at both high and low irradiance. For high light response curves, the irradiance began at levels equivalent to ambient, approximately 800 μmol m$^{-2}$s$^{-1}$, and was set to 1200, 1800, 2000, 1800, 1500, 1200, 900, 600, 300, and 50 μmol m$^{-2}$s$^{-1}$ consecutively. For low light response curves, the irradiance was set to 300 μmol m$^{-2}$s$^{-1}$ and dropped in 50 μmoles m$^{-2}$s$^{-1}$ increments until reaching 50 μmol m$^{-2}$s$^{-1}$, when the irradiance was set to 25, 10 and 0 consecutively. For A-Ci curves, cuvette CO$_2$ concentrations were set to 400, 250, 100, 50, 0, 400, 600, and 800 ppm CO$_2$ consecutively at a constant irradiance of 800 μmol m$^{-2}$s$^{-1}$. SigmaPlot (SPSS, Chicago, IL) was used to fit the data from each light response curve. Data from high light curves were used to determine the maximum photosynthetic rate ($A_{\text{max}}$). Data from the linear portion of the low light curves (up to 150 μmol m$^{-2}$s$^{-1}$) were used to calculate apparent quantum efficiency ($Q_{\text{app}}$) and light
compensation point (LCP). Data from below 25 μmoles m$^{-2}$·s$^{-1}$ were not used to calculate $Q_{app}$; only the portion of the curve where light is limiting and respiration is constant was used in order to avoid overestimating the $Q_{app}$ due to the Kok effect region (Singsaas et al. 2001). $R_d$ was determined from the measured photosynthetic rate at 0 μmoles m$^{-2}$·s$^{-1}$. Leaf temperature and relative humidity in the cuvette ranged from 23-27 °C and 60–76%, respectively.

Substrate moisture levels were measured and controlled using ECH2O-5® dielectric probes (Decagon Devices Inc, Pullman, WA) connected to a CR1000 datalogger with a AM16/32 multiplexer and a SDM-CD16AC 16 channel relay controller to operate solenoid valves (Campbell Scientific Inc., Logan, UT). Probes were calibrated for the substrate to determine percent of container capacity and volumetric water content ($\Theta = 0.0015 \cdot$ millivolts - 0.3396, $r^2 = 0.999$). Calibration procedure is included in Appendix B. Probes were installed perpendicular to the substrate surface, halfway between the sidewall and the stem (5 cm) from the sidewall, with the sensor overmold just below the substrate surface. The photoperiod was set to 15 hours (light from 7 am and 10 pm). Supplemental lighting was used in the greenhouse when outside ambient light conditions were below 400 μmol of light per m$^{-2}$·s$^{-1}$. The daytime and nighttime thermostat setpoints were 24 and 20 °C, respectively.

For model development, plants were watered on the substrate surface by hand, and soaked for 60 minutes in one inch of irrigation water in order to fully and evenly saturate the substrate. Then containers were drained to container capacity. In order to determine the relationship between substrate water content and photosynthetic rate, photosynthesis was measured over a range of increasingly drier substrate moisture contents (100 to 45% of container capacity) by withholding irrigation. Single leaf gas exchange measurements were taken at 800, then 1500 μmoles of light per m$^{-2}$·s$^{-1}$ and at 400 ppm CO$_2$. Substrate water content and weight were recorded concurrent to photosynthetic measurements. The experimental design was a completely randomized
design with 12 single plant replications. SigmaPlot (SPSS, Chicago, IL) was used to graph the data and to determine a predictive curve that best fit the data.

For the system evaluation, just prior to initiating the experiment, plants were watered and soaked as described for model development. Four irrigation treatments were established based on the following setpoints: 89, 81, 69, and 61% of container capacity (corresponding to 49, 41, 30, and 22 m⁻³·m⁻³ volumetric water content). Irrigation valves were triggered when the average probe millivolt reading decreased below the setpoint. The irrigation valve remained open, delivering the volume of water necessary to reach container capacity, as determined by a preliminary experiment, in order to limit leaching. Preliminary data were used to determine that a 60 minute lag period was needed for those treatments which required the irrigation to run greater than 10 minutes, i.e., the two driest treatments. For these treatments, the irrigation came on for 10 minutes and then turned off and remained off for 60 minutes. Then the irrigation turned on for the remainder of the predetermined time that was necessary to return the container in each respective treatment back to container capacity. Soluble fertilizer (300 ppm N, 20-10-20, 200 ml per container) was manually applied one hour following the termination of an irrigation application. Fertilizer applications were scheduled such that each treatment received an equal number of treatments in a given time period.

Leaf water potential and gas exchange (at 800 μmol m⁻²·s⁻¹ and 400 ppm CO₂) were measured three times for each treatment when two criteria were met: plants were at the driest substrate water content permitted by their respective treatment, i.e. just prior to an irrigation event, and when the time was between 10 am and 3 pm. The data collection periods were as follows: Time 1: December 3–7, 2007; Time 2: December 18–24, 2007; and Time 3: January 7–14, 2008. A chilled mirror dew point potentiometer (model WP4-T, Decagon Devices, Pullman, WA) was used to determine water potential. For water potential and gas exchange measurements, one of the second most recently matured, fully expanded leaves was sampled.
One leaf per plant was selected for water potential measurements. One half of the leaf was prepared for water potential measurements by the following procedure: a droplet of purified water was placed on one half of the leaf blade. That half of the lamina was lightly and evenly sanded 8–10 strokes with 400 grit sandpaper (3M 400 TS4 TRI-MITE 413Q wet or dry, St. Paul, MN). The leaf was blotted dry with a lint-free cloth and detached from the plant. The leaf was cut along the midrib. The sanded half was immediately wrapped into Glad® Cling Wrap (The Glad Products Co., Oakland, CA), placed in a plastic bag with a lint-free cloth moistened with deionized water, sealed and placed in a cooler. Samples were taken by a core sampler made from sheet metal, fashioned to the circumference of the potentiometer sample cups. Each sample was processed for 20 minutes at 25.1 °C. The complete protocol development for leaf water potential is listed in Appendix C. Biomass was determined after ten weeks initially and after eight weeks when the system evaluation was repeated. The system evaluation experiment was arranged in a completely randomized design with four irrigation treatments and eight plants per treatment initially and ten replications when the system evaluation was repeated. The daily light integral was 8.7 and 8.1 mol m\(^{-2}\)d\(^{-1}\), respectively, for the first and second system evaluation experiments.

At the termination of each system evaluation experiment, height, width (plant width at the widest point and plant width transverse to the first width measurement) plant quality, leaf area, root and shoot dry weight, and branch number were measured. Plant quality was based on vegetative characteristics (1 = leggy and yellow-green leaf color, 2 = leggy or yellow-green leaf color, 3 = compact, green leaf color) and was assessed by three independent observers and averaged. A leaf area model developed for non-destructive individual leaf area measurements is included in Appendix D. Water use efficiency was calculated as the amount of water used per dry mass accumulation over the course of the experiment. In the initial run of the system evaluation, total number of flowers was also recorded each day. No floral buds matured in the shorter, second experiment so these data were not collected. When the system evaluation was repeated, branch length was also collected. Data were similar for the two runs of the
system evaluation experiments, so data are reported only for the first run of the experiment.

Results

The light response curves were used to calculate several gas exchange-based parameters. H. ‘Cashmere Wind’ had an \( A_{\text{max}} \) of 15.1 \( \mu \text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1} \), a \( Q_{\text{app}} \) of 0.049±0.002 \( \mu \text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1} \), a \( R_d \) rate of 1.91±0.010 \( \mu \text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1} \), and LCP of 32.4±2.7 \( \mu \text{mol light s}^{-1} \cdot \text{m}^{-2} \). Light and \( \text{CO}_2 \) response curves are included in Appendix E.

The photosynthetic rate during the model development experiment remained relatively constant between approximately 11 and 18 \( \mu \text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1} \) until the substrate dried to below 60\% of container capacity (Figure 3.1). A sigmoidal curve (\( r^2 = 0.62, P \text{ value} < 0.0001 \)) fit the data and was used to select irrigation setpoints. Four irrigation setpoints between 89 and 61\% container capacity were established to evaluate the hypothesis that plant growth would not be affected by reduced substrate moisture until photosynthesis also declined. The actual photosynthetic rates followed the predicted trends as indicated by percentage of maximum photosynthesis and mean prediction error (Table 3.1). However, the driest treatment showed a lower photosynthetic rate than was predicted. This was not surprising because this setpoint corresponds to a variable portion of the moisture response curve.

Leaf water potential, photosynthetic rate, and transpiration rate were generally not different for plants in the three wettest irrigation treatments, but were significantly reduced in the driest treatment (Tables 3.2–3.4). The same was true for stomatal conductance (data not shown). Plants grown under the wetter treatments used 1.4, 1.2, and 1.05 times more water during the course of the experiment than plants in the driest treatment. Water use efficiency was significantly greater for the three driest treatments compared to the wettest treatment (Table 3.1).

In general, growth was lowest for the wettest and driest treatments and greatest for the two intermediate treatments (Table 3.5). The wettest treatment, which
corresponded to 89% container capacity (49% volumetric water content), likely kept the substrate too wet. There was no effect of irrigation treatment on root dry weight or branch number. Plant quality was greatest for those plants in the three wettest treatments (data not shown).

Discussion

_Hibiscus rosa-sinensis_ ‘Cashmere Wind’ was used as a model crop to facilitate the development of the irrigation model. _H. rosa-sinensis_ is a fast-growing woody plant that can be grown throughout the year in a controlled environment in Kentucky. _H. rosa-sinensis_ cuttings root quickly and at a high percentage. Additionally, _H. rosa-sinensis_ has successfully been used in water deficit research (Egilla et al. 2005).

Metrics from light response curves for _H. rosa-sinensis_ ‘Cashmere Wind’ were consistent with those from other woody nursery crops. _H. ‘Cashmere Wind’_ had a comparable $A_{max}$ to many common nursery crops grown in container production (15.1±0.28 µmol CO$_2$ m$^{-2}$·s$^{-1}$ compared with 9.6, 12.9, 19.3, and approximately 16.2 µmol CO$_2$ m$^{-2}$·s$^{-1}$ respectively for _Acer rubrum_ L., _Cornus x Constellation®_, _Cercis canadensis_ L., and _Betula pendula_ Roth (Bauerle et al. 2003, Fulcher and Geneve 2009, Griffin et al. 2004, Ranney et al. 1991). Additionally, _H. rosa-sinensis_ ‘Cashmere Wind’ had a comparable $A_{max}$ to that of _H. rosa-sinensis_ ‘Leprechaun’, 15.1 vs. 18.0 µmoles CO$_2$ m$^{-2}$·s$^{-1}$, respectively (Egilla et al. 2005). _H. ‘Cashmere Wind’_ had a comparable $Q_{app}$ to _Acer saccharum_ Marsh., _Aesculus glabra_ Willd., _Fagus grandifolia_ Ehrh., _Liriodendron tulipifera_ L., and _Quercus rubra_ L., 0.049 vs. 0.066, 0.083, 0.042, 0.055, and 0.036, respectively (Singsaas et al. 2001). The $R_d$ rates and LCP were 1.91±0.010 µmol CO$_2$ m$^{-2}$·s$^{-1}$, and 32.4±2.7 µmol light s$^{-1}$·m$^{-2}$ respectively for _H. ‘Cashmere Wind’_, compared with 1.39 µmol CO$_2$ m$^{-2}$·s$^{-1}$, and 31.5 µmol light s$^{-1}$·m$^{-2}$ for _Acer rubrum_ and 1.24 µmol CO$_2$ m$^{-2}$·s$^{-1}$ and 25.0 µmol light s$^{-1}$·m$^{-2}$ for _Liriodendron tulipifera_ (Gronginger et al. 1996).
Unlike most woody species, *H. ‘Cashmere Wind’* has a viscous sap (image included in Appendix F). This limits the methods by which plant water relations can be measured. Appendix G contains results from experiments conducted to demonstrate the influence of the viscous sap on the consistency of plant water potential measurements. Accurate, consistent plant water potential measurements were possible with a chilled mirror dew point potentiometer. *H. ‘Cashmere Wind’* has a typical response to decreasing moisture (Figure 3.1); photosynthetic rates remain high as substrate moisture content declines until a critical point where stomates close (Boyer 1970). For these reasons, *H. rosa-sinensis* is a good model crop for nursery crop irrigation modeling.

Growth is considered more sensitive than photosynthesis to changes in water relations because it is a turgor-dependent process (McCree 1986, Taiz and Zeiger 2006). A decrease in water potential decreases turgor pressure and cell wall extensibility, while increasing the pressure that must be obtained to expand the cell wall (yield threshold) (Taiz and Zeiger 2006). Beeson (2006) suggested that irrigation must be maintained at container capacity to maximize growth and minimize production time for some woody container crops. However, other research with woody plants suggests that photosynthesis can be highly sensitive to water deficit (Kozlowski 1982). Photosynthesis requires water and CO₂ as substrates. Reduced stomatal conductance during water deficit restricts the loss of water vapor but also limits the availability of CO₂ and reduces photosynthesis (Jones 1998). Additionally, the degree of water stress may determine the relative influence water deficit has on various processes. Stomatal conductance of broom snakeweed (*Gutierrezia sarothrae* Pursh.) was reduced at low water deficits proportionately more so than biomass or photosynthesis. However, under moderate to severe water deficits, biomass was proportionately more affected than photosynthesis (Wan et al. 1993). Establishment of irrigation setpoints based on the relationship between photosynthesis and container water content, as described in this chapter, reduced water consumption by 17 and 27% for the two intermediate irrigation setpoints compared to the wettest treatment, without decreasing biomass.
The nature of water deficit, in addition to the extent of deficit, has an important influence on biomass. It is possible that a more severe reduction in dry mass did not occur for the driest treatment because plants were not subjected to a constant water deficit but rather maintained a substrate moisture content comparable with the other treatments for most of each irrigation cycle. These data show that conservative irrigation schedules for *H. ‘Cashmere Wind’* are possible without incurring a growth “penalty”.

Replacing daily water use (DWU), a measure of actual evapotranspiration from containers, is another strategy that can be used to conserve water in nurseries (Tyler et al. 1996). Replacing DWU is a water balance-based method whereas the photosynthesis-based irrigation model is plant-based. Irrigation based on DWU has been conducted on nursery crops using daily pre- and post-irrigation substrate moisture probe or gravimetric measurements to determine DWU (García-Navarro et al. 2004, Warsaw et al. 2009, Zahreddine et al. 2007). Water use has also been estimated by sap flow and by leachate fraction for both orchard and nursery crops (Bauerle et al. 2002, Fernandez et al. 2008, Roberts and Schnipke 1987). A comparison of 25 woody species showed that for all but two species, irrigation based on DWU was a more conservative irrigation regime than the industry standard control treatment (19 mm per application) (Warsaw et al. 2009). In the aforementioned experiment, DWU-based irrigation reduced water consumption by 6–75% of the control for most species. However, irrigation based on replacing DWU may use more water than a photosynthesis-based setpoint just above the point of stomatal closure, i.e., the 69% container capacity treatment in this study, without proportionately greater growth for some species. In a study that compared 100% of DWU with a repeated water deficit treatment, there was little difference in leaf area or water consumption for *Viburnum tinus* L., indicating that the 100% daily replacement-based treatment over-irrigated this drought-tolerant species (García-Navarro et al. 2004). A comparison with DWU would further evaluate the utility of a photosynthesis-based system and determine whether a photosynthesis-based system is more water conserving than a DWU-based system.
In this research, photosynthesis was an effective plant-based parameter for irrigation scheduling. It effectively estimated water use and prevented over and under-irrigation for the intermediate treatments. The species-specific information necessary to operate the system could be developed relatively easily by conducting a model development experiment (withholding water and measuring gas exchange) for each species of interest. In time, groups of similarly-responding plants could be identified and managed as a group. In addition, the system did not increase production time over current irrigation scheduling and was capable of being automated. However, further testing of this photosynthesis-based system is needed before its use on other taxa or at different substrate water contents or environmental conditions.

A photosynthesis-based irrigation system was developed and evaluated for container-grown H. ‘Cashmere Wind’. Substantial water savings without a decrease in growth was achieved by selecting irrigation regimes for efficient water use. This research demonstrates a novel irrigation model that could be adopted by the nursery industry with minimal development of species-specific prerequisite data and with the potential for considerable water savings.
Table 3.1 Actual and predicted photosynthesis and water use efficiency for *H. 'Cashmere Wind'* plants grown at four substrate moisture levels.

<table>
<thead>
<tr>
<th>Setpoint (% of container capacity)</th>
<th>Predicted photosynthetic rate (μmol CO₂ m⁻² s⁻¹)</th>
<th>Percentage of predicted maximum photosynthesis</th>
<th>Actual photosynthetic rate = MFE (μmol CO₂ m⁻² s⁻¹)</th>
<th>Percentage of actual maximum photosynthesis</th>
<th>Water use efficiency (dry matter [g]/ water applied [ml])</th>
</tr>
</thead>
<tbody>
<tr>
<td>89</td>
<td>14.7</td>
<td>100</td>
<td>13.8±0.66</td>
<td>100</td>
<td>1.62a</td>
</tr>
<tr>
<td>81</td>
<td>14.7</td>
<td>100</td>
<td>14.0±0.43</td>
<td>100</td>
<td>2.18b</td>
</tr>
<tr>
<td>69</td>
<td>14.4</td>
<td>98</td>
<td>13.6±0.52</td>
<td>98</td>
<td>2.32b</td>
</tr>
<tr>
<td>61</td>
<td>10.3</td>
<td>69</td>
<td>8.1±0.90</td>
<td>58</td>
<td>2.13b</td>
</tr>
</tbody>
</table>

*Means within a column followed by the same lowercase letter were not significantly different (Tukey's HSD α = 0.05)*

*Mean prediction error (MPE) is the square root of the summation of squared residuals divided by sample size.*

<table>
<thead>
<tr>
<th>Setpoint (% CC)</th>
<th>Time 1</th>
<th>Time 2</th>
<th>Time 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>89</td>
<td>-0.64a</td>
<td>-0.66b</td>
<td>-0.53a</td>
</tr>
<tr>
<td>81</td>
<td>-0.68a</td>
<td>-0.59ab</td>
<td>-0.53a</td>
</tr>
<tr>
<td>69</td>
<td>-0.59a</td>
<td>-0.53a</td>
<td>-0.51a</td>
</tr>
<tr>
<td>61</td>
<td>-1.01b</td>
<td>-0.92c</td>
<td>-0.73b</td>
</tr>
</tbody>
</table>

ANOVA *P* value | < 0.0001 | < 0.0001 | 0.0013

<table>
<thead>
<tr>
<th>Setpoint (% CC\textsuperscript{2})</th>
<th>Time 1</th>
<th>Time 2</th>
<th>Time 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>89</td>
<td>13.7a</td>
<td>15.1a</td>
<td>13.0a</td>
</tr>
<tr>
<td>81</td>
<td>14.4a</td>
<td>14.0ab</td>
<td>13.6a</td>
</tr>
<tr>
<td>69</td>
<td>13.9a</td>
<td>13.4b</td>
<td>13.5a</td>
</tr>
<tr>
<td>61</td>
<td>6.2b</td>
<td>7.5c</td>
<td>10.7b</td>
</tr>
</tbody>
</table>

ANOVA *P* value < 0.0001 < 0.0001 0.0002

<table>
<thead>
<tr>
<th>Setpoint (% CC₅)</th>
<th>Time 1</th>
<th>Time 2</th>
<th>Time 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>89</td>
<td>3.67a</td>
<td>4.84b</td>
<td>5.64a</td>
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<tr>
<td>81</td>
<td>3.97a</td>
<td>5.80a</td>
<td>1.71c</td>
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<tr>
<td>69</td>
<td>3.47a</td>
<td>4.03b</td>
<td>4.87b</td>
</tr>
<tr>
<td>61</td>
<td>0.99b</td>
<td>2.44c</td>
<td>0.72d</td>
</tr>
</tbody>
</table>

ANOVA P value < 0.0001 < 0.0001 < 0.0001
Table 3.5. Root and shoot growth for *H. ‘Cashmere Wind’* grown using four different irrigation regimes.

<table>
<thead>
<tr>
<th>Setpoint (% Container Capacity)</th>
<th>Total leaf area (cm²)</th>
<th>Increase in height (cm)</th>
<th>Stem dry weight (g)</th>
<th>Root dry weight (g)</th>
<th>Leaf dry weight (g)</th>
<th>Branch number</th>
</tr>
</thead>
<tbody>
<tr>
<td>89</td>
<td>1173b²</td>
<td>24.4b</td>
<td>6.4b</td>
<td>4.1</td>
<td>9.5ab</td>
<td>3.1</td>
</tr>
<tr>
<td>81</td>
<td>1407a</td>
<td>28.6a</td>
<td>8.1a</td>
<td>4.3</td>
<td>10.5a</td>
<td>3.8</td>
</tr>
<tr>
<td>69</td>
<td>1473a</td>
<td>28.9a</td>
<td>7.4ab</td>
<td>3.9</td>
<td>9.3ab</td>
<td>3.5</td>
</tr>
<tr>
<td>61</td>
<td>1224b</td>
<td>26.6ab</td>
<td>6.8b</td>
<td>3.5</td>
<td>8.2b</td>
<td>3.4</td>
</tr>
</tbody>
</table>

ANOVA P value | 0.0005 | 0.0056 | 0.0079 | 0.2350 | 0.0104 | 0.3800

*7 means followed by the same letter were not significantly different (Tukey’s HSD α = 0.05)*
Figure 3.1. Relationship between container moisture content and photosynthetic rate in container-grown *H. ‘Cashmere Wind’*. (Line is predicted from 136 photosynthetic measurements taken over a range of container water contents. Photosynthesis=14.6844/(1+exp(-(millivolts-361.9237)/15.4806)), $r^2 = 0.62$.)
Chapter Four

Comparison of the Relationship between Photosynthesis and Substrate Moisture Content for *Cornus* Taxa

Introduction

An irrigation model based on photosynthetic rates as an indicator of plant water status was developed using *Hibiscus rosa-sinensis* L. with the hypothesis that the model could be easily modified for use with other species. Specifically, the irrigation model was based on the relationship between substrate moisture content and photosynthetic rate, represented by the moisture response curve. The relationship was a sigmoidal curve with a wide range of substrate moisture contents supporting maximum or near maximum photosynthetic rates, $r^2=0.62$. An irrigation setpoint was established that reflected the substrate water content at which photosynthesis began to drop (photosynthetic rate was 98% of maximum), which corresponded with a reduction in stomatal conductance. By maintaining the substrate moisture content just above this setpoint, a crop could be produced using 27% less water than the control, and without adversely impacting quality, or production time. It is unknown how unique this sigmoidal relationship between substrate moisture content and photosynthetic rate is for woody plants, nor is the potential to exploit this relationship in order to identify water-conserving irrigation setpoints for nursery crops.

Members of the genus *Cornus* (dogwood) are ideal subjects for testing the extent to which an irrigation model based on the sigmoidal relationship between substrate moisture content and photosynthetic rate is applicable to woody plants. Flowering dogwood, *Cornus florida* L., is a valuable nursery crop (USDA, 1997). Numerous other *Cornus* species, as well as hybrids and their parents, are available in the trade. Use of related taxa facilitates a comparison of water use among similarly related
plants, which serves as an indication of the model’s applicability among nursery crops. Sensitivity to water stress differs among dogwood taxa and, thus, water requirements and irrigation setpoints may vary across taxa (Augé et al. 1998, Augé et al. 2002, Dirr 1998). Additionally, the Cornus genus is an ideal group of plants with which to test the effects of grafting on transpiration and, thus, identify any limitations for using the model on grafted plants. While seedling dogwoods are commonly sold, cultivars and other selections constitute a substantial portion of dogwood sales and are also readily available.

Grafting is a common propagation technique in horticulture. Fruit crops are commonly grafted, as are many ornamental plants. In the case of fruit trees, grafting is done to control size and as an asexual propagation technique. Ornamentals are grafted specifically as a means of asexual propagation. Recently, vegetable plants have been grafted to enhance stress tolerance (Liao and Lin 1996, Rouphael et al. 2008, Rivero et al. 2003).

The implications of grafting on the hydraulics of woody plants have been studied for some fruit crops, in particular the mechanism of dwarfing by rootstock and the effect of the graft union itself on plant growth and physiology. Initial anatomy-based experiments addressing the cause of dwarfing by rootstock indicated that the plant water status was reduced by the dwarfing rootstock (Beakbane 1956). Studies with apple, peach, and olive implicated low root hydraulic conductance in the dwarfing effect of certain rootstocks (Adkinson et al. 2003, Basile et al. 2003, Nardini et al. 2006). The graft union did not interfere with hydraulic conductance in olive and peach (Basile et al. 2003, Nandini et al., 2006); however, the graft union was implicated in increasing hydraulic resistance for apples (Adkinson et al. 2003, Cohen and Naor 2002) and cherries (Olmstead et al. 2006). Anatomical studies with avocado linked rootstock xylem anatomy with water use (Fassio et al. 2009). Studies with citrus indicate that competition between reproductive and somatic growth causes dwarfing by rootstock (Lliso et al. 2004).
Seedling rootstock is almost exclusively used in ornamental plant production. While rootstocks have been evaluated for drought tolerance in a number of fruit crops (Koundouras et al. 2008, Pérez-Pérez et al. 2008, Ranney et al. 1991) and, recently, for roses (Niu and Rodriguez 2009), there is a dearth of rootstock research for ornamental plants. The objectives of this research were to 1) determine if Cornus species follow the same sigmoidal pattern between substrate moisture content and photosynthesis as Hibiscus rosa-sinensis ‘Cashmere Wind’, 2) determine if a wide range of substrate moisture contents, from approximately 70 to 100% container capacity, supports high photosynthetic rates, facilitating the determination of an irrigation setpoint that minimizes container capacity while allowing photosynthesis to decrease by no more than 2% of the maximum photosynthetic rate, and 3) determine if grafting or substrate influences determination of the irrigation setpoint.

Materials and Methods
Seedlings: Bark-based Substrate

In February 2007, seeds of C. kousa (Korean provenance) and C. florida (KY, USA provenance) were stratified in damp peat moss at 5 °C. In May 2007 seeds were sown in a germination substrate in a greenhouse at the University of Kentucky in Lexington, KY. Seedlings were transplanted into 0.9L containers (Classic 100, Nursery Supplies, McMinnville, OR) with a bark-based substrate (Barky Beaver Professional Grow Mix, Barky Beaver Mulch and Soil Mix, Moss, TN) in August 2007. A model development experiment was conducted on October 3, 2007 as follows: a single ECH2O-5® probe (Decagon Devices, Pullman, WA) was placed in each container; probes were installed vertically, perpendicular with the substrate surface, halfway between the container sidewall and the trunk; plants were watered, allowed to drain to container capacity, and weighed; further irrigation was withheld. Gas exchange, substrate moisture content, and container weight were measured as the substrate dried, beginning at 100% container capacity, until photosynthesis was 25% or less of its maximum rate, approximately 2 μmol CO₂ m⁻² s⁻¹. Supplemental lighting (high pressure sodium) was
provided when ambient light conditions decreased to 400 μmol light per m²-s⁻¹. The experiment was a completely randomized design with eight replications. SigmaPlot (SSPS, Chicago, IL) was used to determine the relationship between photosynthetic rate and substrate moisture content for each species and to determine the curve that best fit the data.

Seedlings: Peat-based Substrate

Container-grown seedlings of *C. kousa* var. *chinensis* (identified internally as China accession 15) and *C. florida* (MO, USA provenance) (1/4” caliper) were purchased from a commercial nursery. On May 1, 2009 seedlings were transplanted into 3.8L containers (Classic 400 Nursery Supply, McMinnville, OR) with a peat and pine bark-based substrate (Metro Mix 280, Sun Gro Horticulture, Bellevue, WA). Wicks, one inch by eight inch strips of capillary mat material, were inserted vertically into the center drain hole such that four inches of wick extended into the center of the container and four inches were in contact with the capillary mat on the benchtop. Plants were grown in a greenhouse at the University of Kentucky on a controlled water table with 100-200 ppm N 20-10-20 (Peat-Lite Special®, The Scotts Co., Maryville, OH). A single ECH₂O-5® probe (Decagon Devices, Pullman, WA) was placed in each container. Probes were installed vertically, halfway between the container sidewall and the trunk. Plants were watered, drained to container capacity, and weighed. Further irrigation was withheld. Upon draining to container capacity, plants were placed in white plastic bags to minimize evaporation. Bags were sealed around the trunk and the ECH₂O-5® probe cable with wire ties and parafilm such that each container was enclosed in a bag. Gas exchange, leaf water potential, relative water content, substrate moisture content, and container weight were measured as the substrate dried, beginning at 100% container capacity. Gas exchange measurements were taken on the most recently matured, fully expanded leaves. Leaves were collected from half of the plants for relative water content and the other half for leaf water potential.
Leaf water potential was measured on the first day of the experiment with a chilled mirror dewpoint sensor (model WP4-T, Decagon Devices, Pullman, WA). A droplet of purified water was placed on one half of the leaf blade. That half of the lamina was lightly and evenly sanded 8–10 strokes with 400 grit sandpaper (3M 400 TS4 TRI-MITE 413Q wet or dry, St. Paul, MN). The leaf was blotted dry with a lint-free cloth and detached from the plant. The leaf was cut along the midrib. The sanded half was immediately wrapped in Glad® Cling Wrap (The Glad Products Co., Oakland, CA), placed in a plastic bag with a lint-free cloth moistened with deionized water, sealed and placed in a cooler. Samples were taken with a core sampler, made from sheet metal fashioned to the circumference of the potentiometer sample cups. Each sample was processed for 20 minutes at 25.1 °C. Leaves were collected throughout the experiment in order to determine the relative water content (RWC). Relative water content was determined as follows: Leaves of the same age as those for gas exchange were collected, immediately bagged in a sealable plastic bag with a moistened lint-free cloth and placed in a cooler. Leaves were transported to the lab and weighed immediately to record the fresh weight. Leaves were individually placed in a separate Petri® dish, and covered with deionized water. Leaves were stored in the water at 4°C for 24 hours. After 24 hours the leaf surface was dried with a lint-free cloth and weighed to determine the turgid weight. Leaves were then dried at 55 °C for 48 hours and weighed. Relative water content was determined according to the following equation:

\[
\frac{(\text{Fresh Weight} - \text{Dry Weight})}{(\text{Turgid Weight} - \text{Dry Weight})} \times 100
\]

Supplemental lighting (high pressure sodium) was provided when ambient light conditions decreased to 400 µmol light per m⁻².s⁻¹. The experiment was a completely
randomized design with 10 replications. SigmaPlot (SPSS, Chicago, IL) was used to determine the relationship between photosynthetic rate and substrate moisture content for each species and to determine the curve that best fit the data.

Self and Reciprocal Grafts

Seedlings of *C. florida* and *C. kousa* var. *chinensis* (internally identified as China #15) were purchased in late winter 2007, transplanted into 3.8 L containers with bark-based substrate without lime (custom Beaver Professional Grow Mix, Barky Beaver Mulch and Soil Mix, Moss, TN). Plants were grown under a lath structure at the University of Kentucky Horticulture Research Farm in Lexington, KY. Bud wood was collected from a field plot of *C. florida* ‘Cherokee Princess’ and *C. kousa* ‘National’. Bud wood was bagged and stored at 5 °C until grafts were made. Self-graft combinations (*C. florida* ‘Cherokee Princess’ scion on *C. florida* rootstock and *C. kousa* ‘National’ scion on *C. kousa* rootstock) as well as reciprocal grafts (*C. florida* ‘Cherokee Princess’ scion on *C. kousa* var. *chinensis* rootstock, *C. kousa* ‘National’ scion on *C. florida* rootstock) were made on September of 2007. *C. kousa* var. *chinensis* and *C. florida* rootstock with grafted buds that did not survive served as ungrafted controls. Following budding, plants were separated into two groups and either remained under a lath house at the University of Kentucky Horticulture Research Farm or were grown in a greenhouse at the University of Kentucky for three weeks before returning to the lath house.

In the spring, plants were brought into the greenhouse and fertigated at every irrigation with 100 ppm N of 20-10-20 (Peat-Lite Special®, The Scotts Co., Maryville, OH). ECH2O5® probes (Decagon Devices, Pullman, WA) were installed perpendicular to the substrate surface, 5 cm from the sidewall. Plants were watered, allowed to drain to container capacity, and weighed. Further irrigation was withheld. Supplemental lighting (high pressure sodium) was provided when ambient light conditions decreased to 400 µmol light m⁻²·s⁻¹.

Gas exchange and container weight were measured daily until photosynthesis declined substantially (60% of maximum photosynthetic rate), at which point the plant
was irrigated and removed from the experiment. Leaf water potential was measured on the first day of the experiment on two plants per treatment as previously described. Leaves were collected throughout the experiment in order to determine RWC as previously described. The experiment was a completely randomized design with five replications.

The experiment was repeated with an additional replication for a total of six replications. On day one of the experiment, a single leaf was harvested from each plant to determine leaf water potential as previously described. Leaves collected to test RWC were harvested throughout the experiment beginning on the first day according to the previously mentioned protocol.

Moisture response curves were graphed for each plant and regression was used to predict the relationship between substrate moisture content and photosynthetic rate and stomatal conductance. Predicted lines were used to determine photosynthetic and stomatal conductance rates at 90, 80, 70, 65, and 60 % container capacity. These predicted values were subjected to an analysis of variance and mean separation (Tukey’s HSD α= 0.05).

Related Cultivars

Bareroot liners (30–36 inches tall) of three related taxa, C. kousa ‘National’ (seed parent), C. florida ‘Cherokee Princess’ (pollen parent), and C. kousa x C. florida Constellation® were potted with a bark-based substrate (Barky Beaver Professional Grow Mix, Barky Beaver Mulch and Soil Mix, Moss, TN) into 23 L containers (Classic 2800, Nursery Supplies, McMinnville, OR) and grown in a pot-in-pot system with cyclic irrigation. Plants were fertilized with 90–100 g (90 in year one, 100 in year two) per plant of 19-4-8, 5–6 month release complete fertilizer (Harrell’s, Inc. Sylacauga, AL) each April. ECH2O-5® moisture probes (Decagon Devices, Pullman, WA) were installed vertically, perpendicular with the substrate surface, midway between the sidewall of the container and the trunk, so the overmold was five cm below the surface of the substrate. Plants were watered, drained to container capacity, and bagged and sealed.
around the trunk using the previously described technique. Substrate moisture content, stem water potential, and gas exchange were measured under initial (well-watered) conditions and once daily while water was withheld, except the second day of the experiment when data were collected twice. Irrigation was withheld from treated plants from August 21 – 25th. Cyclic irrigation (three times per day) was resumed for treated plants on August 26. Containers were weighed to determine the relationship between probe values for water content and actual substrate water content. On September 2, photosynthesis and stem water potential measurements were taken. The experiment was a completely randomized design with seven treated and five control replicates per taxa.

Results

The relationship between substrate moisture content and photosynthetic rate was examined for controlled environment-grown plants (seedlings and self and reciprocal grafts) and outdoor-grown grafted cultivars (a hybrid and its parents). Substrate moisture levels determined by dielectric probes ranged from 40 to 100 percent of container capacity and correlated well with gravimetric measurements, $r^2 = 0.85$ (data not shown). Average initial photosynthetic rates ranged from $5.6 \, \mu\text{mol m}^{-2}\cdot\text{s}^{-1}$ for *C. kousa* to $14.1 \, \mu\text{mol m}^{-2}\cdot\text{s}^{-1}$ for *C. x Constellation®* (Figures 4.1–4.3). A sigmoidal curve provided the best fit and maintained $r^2$ values above 0.62 for all seedlings, excluding non-grafted seedling controls in the reciprocal graft experiment (Figure 4.1). The fit for the reciprocal grafts ranged from $r^2 = 0.37$ to 0.69 (Figure 4.2) and was $r^2 = 0.70–0.78$ for the cultivars grown outdoors (Figure 4.3).

At 90, 80, 70, and 60 percent container capacity moisture levels, there was no difference in photosynthetic rate among *C. florida* scions and *C. florida* seedling control (Table 4.1). At 65% container capacity moisture levels, the *C. florida* control and the *C. florida* scion on *C. florida* rootstock had greater photosynthetic rates than *C. florida* on *C. kousa* rootstock. There was no difference in photosynthetic rate when comparing the *C. kousa* scion on *C. kousa* rootstock to the *C. kousa* control or the *C. kousa* scion on *C.
*Florida* rootstock at 90, 80, and 70% container capacity; however, at these same moisture levels there was a difference between the latter two plants. For *C. kousa* scions and the *C. kousa* seedling control there was no difference in photosynthetic rates at 65 or 60% container capacity.

There was no difference in photosynthetic rate at the 90, 80, and 70 percent container capacity for the *C. kousa* scions and the *C. kousa* seedling control. However, at 65 percent container capacity the photosynthetic rate decreased significantly from the 90 and 80 percent container capacity rates. Once the substrate moisture content dropped to 60 percent container capacity all photosynthetic rates were less than 0.7 µmol m$^{-2}$·s$^{-1}$.

At 90, 80, 70, and 60% container capacity moisture levels, there was no difference in stomatal conductance among the grafted selections and the ungrafted seedling controls for the *C. florida* scions and *C. florida* seedling control (Table 4.2). At the 65% container capacity moisture levels, the *C. florida* scion on *C. florida* rootstock had a greater stomatal conductance than *C. florida* on *C. kousa* rootstock. At the 90 and 80% container capacity, the *C. kousa* seedling had lower stomatal conductance rates than the other two combinations with *C. kousa* scions. At 70% container capacity *C. kousa* seedling had a lower stomatal conductance than *C. kousa* scion on *C. kousa* rootstock. At 65 and 60% container capacity, there was no difference in stomatal conductance with extremely low values in all plants combinations.

Stomatal conductance rates closely paralleled photosynthetic rates for individual plant types during the drying period (Table 4.1–4.2). This is not surprising because stomates are the openings by which CO$_2$ enters the leaf. Significant changes in stomatal conductance generally occurred between 70 and 60% container capacity, rather than at 65% as for photosynthesis.

From 65 to 100% container capacity, there was a sigmoidal relationship between leaf water potential and container capacity ($r^2 = 0.38$ and 0.73 for the peat-grown *C. kousa* and *C. florida*, respectively) (Figure 4.4). There was a linear relationship between
stem water potential and container capacity for *C. kousa* ‘National’, *C. florida* ‘Cherokee Princess’, and *C. kousa x C. florida* Constellation® (Figures 4.5-4.7). There was a linear relationship between stem water potential and photosynthetic rate ($r^2 = 0.67$, $r^2 = 0.75$, $r^2 = 0.64$, for *C. kousa* ‘National’, *C. florida* ‘Cherokee Princess’, and *C. kousa x C. florida* Constellation®, respectively) among the drought-imposed plants (data not shown).

As with hibiscus, calculations were made to determine the driest substrate moisture content that would support photosynthetic rates at 98% of the maximum. There was no difference in setpoints (percent container capacity to maintain photosynthesis at 98% or greater of the maximum rates) among the *Cornus* seedlings in either substrate, among *Cornus* taxa in reciprocal grafts including the seedling controls, or among the related *Cornus* selections within their respective experiments. The actual setpoints calculated to maintain photosynthesis at or greater than 98% of the maximum varied from 67–75% container capacity for experiments with seedlings and self and reciprocal grafts (Tables 4.3–4.4). For the hybrid and its parents, the calculated setpoints ranged from 93–95% of container capacity (Table 4.5).

**Discussion**

In previous research, a sigmoidal relationship with $r^2 = 0.62$ was an adequate basis for determining irrigation setpoints for controlled environment-grown *H. ‘Cashmere Wind’*, a cutting-propagated selection. For *Cornus* seedlings, the relationship between substrate moisture content and photosynthetic rate ($r^2 \geq 0.69$ for all but one seedling group) was comparable to that of *H. ‘Cashmere Wind’*.

For all seedlings and reciprocal grafts, a wide range of substrate moisture levels supported maximum or near maximum photosynthetic rates and there was a level (approximately 65–70% container capacity) at which stomates closed and photosynthesis declined substantially, regardless of substrate. It is common for photosynthetic rates to remain high in crop plants as the substrate moisture potential declines until a critical point where stomates close (Boyer 1970). However, prior to this research the moisture response curve was generally unknown for tree species.
While not different, the photosynthetic rate of *C. kousa* on *florida* rootstock was 12 times higher than that of *C. kousa* scion on *C. kousa* rootstock at the 65% container capacity. Also at 65% container capacity, *C. florida* plants grafted on *C. kousa* had a significantly lower photosynthetic rate and stomatal conductance than those self-grafted onto *C. florida* rootstock. More research is needed to examine a possible effect of *C. kousa* rootstock on stomatal conductance and photosynthesis and to determine if root-to-shoot signaling plays a role. *C. kousa* seedlings had low stomatal conductance, and as was expected, low photosynthetic rates at adequately moist substrate moisture levels. The *C. florida* scion on *C. kousa* rootstock and *C. kousa* scion on *C. kousa* rootstock seem to be compensating for the low stomatal conductance (and low photosynthetic rate) of *C. kousa* rootstock at adequately moist substrate moisture levels. While not different, photosynthetic and stomatal conductance rates for these plants are greater than for the *C. kousa* seedling control. Therefore, the scion appears to have some influence on gas exchange. There does not appear to be a hydraulic resistance at the graft union for any of these plants as the stomatal conductance is similar or greater for grafted combinations than for seedling controls.

The combined results of these experiments do not clearly indicate that one *Cornus* species is better suited to water deficit. It appears that both the scion and the rootstock are contributing to photosynthesis and/or stomatal conductance rates. In addition, there are some indications that *C. kousa* as a rootstock may contribute to earlier stomatal closing during a water deficit. In established landscape plants, *C. kousa* var. *chinensis* is considered more drought tolerant than *C. florida*. However, recently planted liners of *C. kousa* var. *chinensis* have been observed to wilt more quickly than their *C. florida* counterparts (M. Moffett, personal communication).

Rootstock can influence plant response to water deficit and gas exchange. In a study with *Betula* grown in a flooded rhizosphere, rootstock had a significant influence on photosynthesis and stomatal conductance and, at times, biomass (Ranney and Bir 1994). Grafted coffee selections (*C. arabica*) fared better during periods of water deficit.
than their ungrafted counterparts, which the authors theorized was due to a greater capacity of *C. canephora* root systems to supply water (Fahl et al. 2001). In a field situation where the root system is not restricted, the rootstock may play a more prominent role in plant water relations than seen in these experiments with container-grown plants. For example, citrus rootstocks have been selected for deep, expansive root systems (Castle and Krezdom 1977, Pérez-Pérez et al. 2008). Further experiments are necessary to determine how rootstocks of *Cornus* and other ornamentals plants influence plant water use and response to water deficit in field and container production settings.

Results from this research show that there is a relationship between plant water content and substrate moisture content. This relationship was sigmoidal for the plants in the greenhouse experiment (Figure 4.4) and linear for plants in the outdoor experiment (Figure 4.5–4.7). These divergent trends may reflect the disparity in vapor pressure deficit (VPD) in these two environments.

To examine how the model would perform in outdoor-grown grafted plants, an experiment was designed with a hybrid, *C. kousa x C. florida* Constellation® and its parents, *C. florida* ‘Cherokee Princess’ and *C. kousa* ‘National’. The sigmoidal relationship persisted, however, even as the nature of the moisture response curve changed. For the three cultivars in the outdoor experiment, there was not a wide range of container moisture levels that supported high photosynthetic rates and no clear point at which the stomates closed and photosynthetic rate decreased precipitously. Instead, there was a steady decrease in photosynthetic rate over the course of the imposed water deficit, indicating that these plants may need greater substrate water contents to maintain high photosynthetic rates. The irrigation setpoints calculated to maintain photosynthesis at or near maximum were unrealistically high, 93–95% container capacity, and would necessitate near constant irrigation.

Plants in the outdoor experiments were subjected to an extremely high VPD, (4.2–4.5 kPa) compared to the controlled environment experiments, which never
exceeded 2.7 kPa. VPD is known to govern plant water loss. For the outdoor experiment there was a strong linear relationship between photosynthesis and stomatal conductance, $r^2 = 0.89$ (data not shown). Mankin et al. (1998) found VPD to be a strong predictor of water use in New Guinea impatiens, *Impatiens hawkeri* W. Bull, and Clifton-Brown and Jones (1999) used VPD to alter transpiration in a monocot. Lorenzo-Minguez et al. (1985) found that increasing the VPD increased the transpiration rate of *Schefflera*. There may have been such extreme environmental pressure that water was lost faster than it was taken up by the roots and transported by the xylem, as has been documented in other tree species (Iio et al. 2004, Kauhanen 1986, Portes et al. 2007). VPD was very high (exceeded 4 kPa) within 24 h of the experiment’s initiation and then returned to more moderate levels (2.5–2.7 kPa), yet the photosynthetic rate dropped steadily throughout the experiment. Therefore, VPD alone may not cause the response.

The combination of decreasing soil moisture and high VPD was considered a factor in the overall plant response. Conditions that affect transpirational demand can also alter the point at which transpiration begins to decrease (Tardieu et al. 1992), such that the substrate moisture content at which water can no longer be absorbed by roots is not fixed, but instead fluctuates with changing environmental conditions. For example, during conditions of a high VPD, water exits the substrate or soil rapidly and the point at which water can no longer be taken up by the roots occurs at a greater substrate water content than under lower transpiration conditions (Ray et al. 2002, Tardieu et al. 1992). However, Ray et al. (2002) did not find a relationship between drying soil and VPD in *Zea mays* L., nor did Gollan et al. (1985) find a difference in the water content at which transpiration decreased for *Nerium oleander* L. under a range of VPDs. Further experiments are necessary to determine the effect of VPD on *Cornus* gas exchange and the impact of VPD on the proposed model.

Basing irrigation on photosynthesis is an unconventional approach. Photosynthetic rates are much less sensitive than growth to water deficit because, unlike growth, photosynthesis is not a turgor-dependent process. Development of a
photosynthesis-based irrigation model that minimizes water use without limiting plant growth and is applicable to a broad range of woody plants would provide a valuable tool for increasing water use efficiency in the nursery industry. *C. florida* and *C. kousa* var. *chinensis* seedlings and self and reciprocal grafts have a similar moisture response curve as *H. ‘Cashmere Wind’*. There was no difference in calculated setpoints for the taxa within each experiment. Production trials are necessary to evaluate the model on *Cornus*, determine the optimum irrigation setpoint, and quantify water savings from utilizing this irrigation model. Because of the drastic nature of the curve below 70% container capacity, plants irrigated at this threshold could suffer from a delay in irrigation or variability in substrate moisture content among containers of a single crop.

A sigmoidal curve best fit the data from cultivars in the outdoor experiment. However a clear point at which the stomates closed was undetectable. Future studies will address potential causes for the disparity in results between controlled environment and outdoor experiments, including VPD and the interaction of VPD and substrate moisture content. These studies are necessary to fully assess the utility of the model for nursery crops.
Table 4.1. Predicted photosynthetic rates for *C. florida* and *C. kousa* var. *chinensis* (access on 15) self and reciprocally grafted subjected to a range of substrate moisture contents. S = Scion, R = Rootstock.

<table>
<thead>
<tr>
<th>Graf Combination</th>
<th>90</th>
<th>80</th>
<th>70</th>
<th>65</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. florida</em> Scion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. florida</em> (non-grafted)</td>
<td>9.1±0.33a †</td>
<td>9.2±0.30a</td>
<td>7.4±0.78a</td>
<td>4.5±0.93A ‡</td>
<td>0.2±0.18c</td>
</tr>
<tr>
<td><em>C. florida</em> (S) / <em>C. florida</em> (R)</td>
<td>10.4±0.55a</td>
<td>10.7±0.35a</td>
<td>5.6±0.48a</td>
<td>6.1±1.03bA</td>
<td>1.4±0.99c</td>
</tr>
<tr>
<td><em>C. florida</em> (S) / <em>C. kousa</em> (R)</td>
<td>10.0±0.81a</td>
<td>9.5±0.76a</td>
<td>8.3±0.89a</td>
<td>0.0±0bB</td>
<td>0.0±0b</td>
</tr>
</tbody>
</table>

**ANOVA P Value**
- 0.219C
- 0.1756
- 0.1627
- 0.0013
- 0.1142

<table>
<thead>
<tr>
<th><em>C. kousa</em> Scion</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. kousa</em> (non-grafted)</td>
<td>7.0±0.56AaB</td>
<td>6.8±0.41AB</td>
<td>5.0±0.79abB</td>
<td>2.8±1.43bcA</td>
<td>0.7±0.51c</td>
</tr>
<tr>
<td><em>C. kousa</em> (S) / <em>C. kousa</em> (R)</td>
<td>8.6±0.99AaB</td>
<td>8.6±1.00AB</td>
<td>7.9±1.18AaB</td>
<td>0.2±0.81bA</td>
<td>0.0±0b</td>
</tr>
<tr>
<td><em>C. kousa</em> (S) / <em>C. florida</em> (R)</td>
<td>10.2±0.56Aa</td>
<td>10.1±0.63Aa</td>
<td>8.6±0.59aA</td>
<td>2.4±1.19bA</td>
<td>0.6±0c</td>
</tr>
</tbody>
</table>

**ANOVA P Value**
- 0.0261
- 0.0289
- 0.0421
- 0.3058
- 0.1029

†means within a row followed by the same lowercase letter were not significantly different (Tukey’s HSD α = 0.05).
‡means within a column followed by the same uppercase letter were not significantly different (Tukey’s HSD α = 0.05).
Standard error provided for within column comparisons.
Table 4.2. Predicted stomatal conductance for *C. florida* and *C. kousa* var. *chinensis* (accession 15) self and reciprocal grafts subjected to a range of substrate moisture contents, analysis by rootstock. S = Scion, R = Rootstock.

<table>
<thead>
<tr>
<th>Graft Combination</th>
<th>Substrate Moisture (% Container Capacity)</th>
<th>Stomatal Conductance (mol H₂O m⁻² s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>90</td>
<td>80</td>
</tr>
<tr>
<td><em>C. florida</em> Rootstock</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. florida</em> (non-grafted)</td>
<td>0.122±0.01a²</td>
<td>0.122±0.01a</td>
</tr>
<tr>
<td>*C. floridao S / C. floridb R)</td>
<td>0.163±0.02a</td>
<td>0.164±0.02a</td>
</tr>
<tr>
<td>*C. floridao S / C. kousa R)</td>
<td>0.176±0.03a</td>
<td>0.177±0.03a</td>
</tr>
<tr>
<td>ANOVA P Value</td>
<td>0.2590</td>
<td>0.2900</td>
</tr>
<tr>
<td><em>C. kousa</em> Rootstock</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. kousa</em> (non-grafted)</td>
<td>0.072±0.01aB</td>
<td>0.073±0.01aB</td>
</tr>
<tr>
<td>*C. kousa S / C. kousa R)</td>
<td>0.144±0.01aA</td>
<td>0.145±0.02aA</td>
</tr>
<tr>
<td>*C. kousa S / C. floridb R)</td>
<td>0.143±0.02aA</td>
<td>0.144±0.02aA</td>
</tr>
<tr>
<td>ANOVA P Value</td>
<td>0.0023</td>
<td>0.0033</td>
</tr>
</tbody>
</table>

²means within a row followed by the same lowercase letter were not significantly different (Tukey’s HSD α = 0.05).

³means within a column followed by the same uppercase letter were not significantly different (Tukey’s HSD α = 0.05).

Standard error provided for within column comparisons.
Table 4.3. Calculated irrigation setpoints (substrate moisture content) to maintain photosynthesis at 98% of maximum photosynthetic rates for *C. florida* and *C. kousa* seedlings in a peat-based substrate and a bark-based substrate.

<table>
<thead>
<tr>
<th>Substrate Accession/provenance</th>
<th>Setpoint to maintain photosynthesis at 98% of maximum (% Container Capacity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peat-based</td>
<td></td>
</tr>
<tr>
<td>C. <em>florida</em> MO, USA</td>
<td>69</td>
</tr>
<tr>
<td>C. <em>kousa</em> var. <em>chinensis</em></td>
<td>67</td>
</tr>
<tr>
<td>ANOVA P value</td>
<td>0.6757</td>
</tr>
<tr>
<td>Bark-based</td>
<td></td>
</tr>
<tr>
<td>C. <em>florida</em> KY, USA</td>
<td>66</td>
</tr>
<tr>
<td>C. <em>kousa</em> Korea</td>
<td>66</td>
</tr>
<tr>
<td>ANOVA P value</td>
<td>0.9687</td>
</tr>
</tbody>
</table>
Table 4.4. Calculated irrigation setpoints (substrate moisture content) to maintain photosynthetic rates at 98% of maximum for *C. florida* and *C. kousa* var. *chinensis* reciprocal and self grafts and seedling controls.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Setpoint to maintain photosynthesis at 98% of maximum (% Container Capacity)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. florida</em> (S) / <em>C. florida</em> (R)</td>
<td>74</td>
</tr>
<tr>
<td><em>C. florida</em> (S) / <em>C. kousa</em> (R)</td>
<td>72</td>
</tr>
<tr>
<td><em>C. kousa</em> (S) / <em>C. kousa</em> (R)</td>
<td>70</td>
</tr>
<tr>
<td><em>C. kousa</em> (S) / <em>C. florida</em> (R)</td>
<td>75</td>
</tr>
<tr>
<td><em>C. florida</em></td>
<td>74</td>
</tr>
<tr>
<td><em>C. kousa</em></td>
<td>74</td>
</tr>
<tr>
<td>ANOVA <em>P</em> value</td>
<td>0.6629</td>
</tr>
</tbody>
</table>
Table 4.5. Calculated irrigation setpoints (substrate moisture content) to maintain photosynthetic rates at 98% of maximum for *C. florida* ‘Cherokee Princess’, *C. kousa* ‘National’ and their hybrid Constellation®.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Setpoint to maintain photosynthesis at 98% of maximum (% Container Capacity)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. florida</em> ‘Cherokee Princess’</td>
<td>94</td>
</tr>
<tr>
<td><em>C. kousa</em> ‘National’</td>
<td>95</td>
</tr>
<tr>
<td><em>C. kousa</em> x <em>C. florida</em> Constellation®</td>
<td>93</td>
</tr>
<tr>
<td>ANOVA P value</td>
<td>0.8436</td>
</tr>
</tbody>
</table>
Figure 4.1. Relationship between substrate moisture content and photosynthesis for *Cornus* seedlings, *C. florida* (blue), *C. kousa* (red).

Graph A represents seedlings potted in a peat-based substrate, for which *C. florida* photosynthesis = \( \frac{8.7418}{1 + \exp(-(% \text{ container capacity} - 51.8019)/6.2852)} \), \( r^2 = 0.80 \), and *C. kousa* photosynthesis = \( \frac{8.4490}{1 + \exp(-(% \text{ container capacity} - 50.1342)/7.6951)} \), \( r^2 = 0.70 \).

Graph B represents seedlings potted in bark-based substrate, for which *C. florida* photosynthesis = \( \frac{7.5068}{1 + \exp(-(% \text{ container capacity} - 59.3595)/2.7943)} \), \( r^2 = 0.80 \), and *C. kousa* photosynthesis= \( \frac{7.0171}{1 + \exp(-(% \text{ container capacity} - 53.8189)/3.4671)} \), \( r^2 = 0.63 \). Circles represent irrigation setpoints calculated to maintain photosynthesis at or above 98% of maximum.
Figure 4.2. Relationship between substrate moisture content and photosynthesis for reciprocal grafts, self grafts, and ungrafted controls of *C. kousa* and *C. florida*, grown in bark-based substrate.

Plants are listed as “scion/rootstock”: f/f dark green, photosynthesis = 10.4565/(1+exp(- (% container capacity- 64.5042)/ 4.2819)), $r^2 = 0.52$; f/k light green, photosynthesis = 9.3001/(1+exp(-( % container capacity- 66.7340)/ 3.1750)), $r^2 = 0.37$; *C. florida* blue, photosynthesis = 8.9445/(1+exp(-( % container capacity- 65.1384)/ 2.8760)), $r^2 = 0.69$; k/f dark grey, photosynthesis = 10.1430/(1+exp(-( % container capacity- 64.3328)/ 3.8320)), $r^2 = 0.43$; k/k pink, photosynthesis = 8.3532/(1+exp(-( % container capacity- 68.2044)/ 1.5542)), $r^2 = 0.53$; *C. kousa* red, photosynthesis = 6.597/(1+exp(-( % container capacity- 63.484)/ 3.380)), $r^2 = 0.45$. Circles represent irrigation setpoints calculated to maintain photosynthesis at or above 98% of maximum.
Figure 4.3. Relationship between substrate moisture content and photosynthesis for three *Cornus* cultivars grown in a bark-based substrate.

*C. florida* ‘Cherokee Princess’, blue, photosynthesis \(= \frac{10.2301}{1+\exp\left(-\frac{\text{(% container capacity}-76.0913)}{9.8787}\right)}\), \(r^2 = 0.70\), *C. kousa* ‘National’, red, photosynthesis \(= \frac{13.6791}{1+\exp\left(-\frac{\text{(% container capacity}-83.4923)}{11.1622}\right)}\), \(r^2 = 0.78\), *C. x Constellation*®, purple, photosynthesis \(= \frac{13.8618}{1+\exp\left(-\frac{\text{(% container capacity}-80.1428)}{8.4879}\right)}\), \(r^2 = 0.76\). Circles represent irrigation setpoints calculated to maintain photosynthesis at or above 98% of maximum.
Figure 4.4. Relationship between leaf water potential and substrate moisture content for *C. florida* and *C. kousa* var. *chinensis*.

*C. florida* (MO, USA provenance) above, stem $\psi = -4.369/(1+\exp(-(\text{container capacity}-42.763)/-58.184))$. *C. kousa* var. *chinensis* (accession 15), below, stem $\psi = -1.463/(1+\exp(-(\text{container capacity}-114.947)/-14.939))$. 
Figure 4.5. Relationship between substrate moisture content and stem water potential for *C. kousa* ‘National’ grown outdoors.

\[ r^2 = 0.67, \psi = -3.861 + 0.027 \times \text{substrate moisture content}. \]
Figure 4.6. Relationship between substrate moisture content and stem water potential for *C. florida* ‘Cherokee Princess’ grown outdoors.

\[ r^2 = 0.75, \text{stem water potential} = -4.121 + 0.030 \times \text{substrate moisture content}. \]
Figure 4.7. Relationship between substrate moisture content and stem water potential for *C. kousa* x *C. florida* Constellation® grown outdoors.

\[ r^2 = 0.64, \text{ stem water potential} = -3.978 + 0.028 \times \text{substrate moisture content}. \]
Chapter Five

Development and Use of a Transpiration Chamber to Quantify Transpiration in Seedlings

Introduction

Water is critical to plant survival as a carrier for nutrients, a substrate in reactions, and the hydraulic force behind growth. Transpiration is the loss of water through the stomates or small openings in leaves. As CO₂ enters the leaf through stomates, oxygen and water vapor exit the leaves. Transpirational water loss in a crop is significant because it impacts a range of plant production issues, including growth, yield, and irrigation scheduling. By measuring and understanding water loss, models can be developed and irrigation practices refined. Development of transpiration chambers allows investigation of water loss under a controlled environment.

Plant water loss occurs through cuticular water loss and stomatal water loss (transpiration). One possible cause of differential water use among plants includes variation in micromorphological characteristics and morphological changes in response to an ongoing environmental condition, e.g. increasing foliar trichomes as a moist season progresses into prolonged drought. Stomatal regulation may be influenced by sensitivity to vapor pressure deficit (VPD), root system capacity, hydraulic conductivity of roots, shoots, and grafts, signaling, and osmotic adjustment. These are potential areas of control and variability in water use among plants.

Plant water loss is driven by environmental conditions and can be countered by the plant’s response to the environment. Both the rhizosphere environment and the canopy environment can influence water loss. Unless conditions are extreme, a hydraulic continuum exists between the soil, plant (roots, stem, and leaves) and air. Water is drawn from the leaf into the air because of the VPD, but water loss is mitigated by resistances between the soil and the air.
Vapor pressure deficit is the driving force in water vapor movement from plant tissue to the surrounding environment. VPD is the difference between the amount of water vapor in the air ($V_{air}$) and the amount of water vapor the air can hold at a given temperature ($V_{sat}$) (Prenger and Ling 2001). VPD is a function of the amount of water vapor in the air. When the VPD is high, air can hold more water, i.e. the humidity is low and the air is far from saturated with water vapor. A low VPD occurs when the air is at or near saturation with water vapor and is a high humidity situation; it does not stimulate much water vapor diffusion through the leaf. VPD is calculated as:

$$VPD = V_{sat} - V_{air}$$

To calculate the VPD$_{leaf}$, the $V_{sat}$ is assumed to be 100% saturated because the mesophyll space is at or near saturation (Taiz and Zeiger 2006). VPD$_{leaf}$ refers to VPD calculated with leaf temperature. The calculation of VPD$_{air}$ utilizes air temperature in the calculation rather than leaf temperature.

Vapor pressure deficit is superior to relative humidity because VPD gives an absolute rather than a relative indication of how much water is in the air, whereas relative humidity is essentially a ratio between the actual amount of water and the maximum amount of water that could be held in the air before condensation occurred (Anderson 1936). The calculation for VPD includes the affect of temperature changes on the ability of air to hold water vapor. In addition, VPD more clearly illustrates the challenge for a plant to maintain water status when faced with different temperatures (Anderson 1936).

Stomatal regulation of water loss occurs in response to the moisture level of the atmosphere and the root zone. Stomatal closure can occur when the relative humidity is so low that water evaporates from the guard cells and they become flaccid. This in turn closes the stomata and prevents transpirational water loss to the environment.
When plants experience a soil moisture deficit, chemical signals from the roots appear to affect stomatal regulation, closing the stomates to limit water loss. Evidence suggests that ABA, pH, and inorganic ions are involved in root-to-shoot signaling to stimulate stomatal closure (Bacon et al. 1998, Jia and Davies 2007, Tardieu et al. 2010, Thompson et al. 2007, Wilkinson et al. 2007, Zhang and Outlaw 2001). The extent to which environmental conditions, specifically, VPD, impact water consumption has not been well studied in woody ornamental plants.

Transpiration can be measured for an individual leaf, excised stem, or for the whole plant (Reigosa Roger 2001). Single leaf measurements use a steady-state porometer, and extrapolation to whole plant transpiration must be mathematically derived. Transpiration measurements taken from excised stems or whole plants measure water transfer from a solution or growing substrate through the plant/leaf into the atmosphere. This is either measured gravimetrically using container lysimeters or estimates of sap flow using stem heat balance. Whole plant measurements provide several advantages over single leaf methods including the ability to evaluate root and shoot communication as plants become increasingly drier or in graft combinations found in ornamental plants. Techniques may differ in accuracy. For example, Ramirez et al. (2006) showed that porometer and stem flow tended to overestimate transpiration compared to gravimetric measurements. Finally, while measurements can be made in ambient or greenhouse conditions, a controlled environment chamber allows for greater manipulation and uniformity of leaf temperature and VPD_{air}.

Several transpiration chambers have been constructed to report the impact of VPD_{air} on transpiration. However, most studies are designed to study air temperature effects on transpiration and VPD_{air} changes concurrent with the change in temperature. For example, in a chamber designed to study transpiration and water uptake in New Guinea impatiens, *Impatiens hawkeri* W. Bull ‘Equinox’, VPD_{air} increased from 0.58 to 1.54 kPa as the air temperature changed from 20 to 30 °C (Mankin et al. 1998). In order to study the relationship between relative humidity, transpiration and photosynthesis in
bamboo, Agata et al. (1985) used a thermo-electric module to control relative humidity by changing the inlet air temperature. Although relative humidity was controlled in this chamber, this system relied on air temperature to impact relative humidity. Few transpiration chambers have been reported with the ability to alter VPD and temperature independently. Clifton-Brown and Jones (1999) recognized that the failure to control both temperature and VPD_{air} may have impacted their ability to accurately study leaf expansion in a previous study. They constructed an elaborate transpiration chamber and used steam injection and desiccants to control VPD_{air}.

Research presented in Chapter Four indicated that the VPD may impact the ability to utilize a photosynthesis-based irrigation system in a variety of atmospheric environments. In Chapters Three and Four, the model development process demonstrated that greenhouse-grown *Hibiscus rosa sinensis* L. and *Cornus* taxa maintained high photosynthetic rates until the substrate moisture content dropped by approximately 25–30% container capacity. During these experiments the VPD_{leaf} never exceeded 2.9 kPa. However, in similar experiments on outdoor-grown *Cornus* taxa, while the relationship remained sigmoidal, there was a precipitous drop in photosynthesis as the substrate moisture decreased. During this experiment, the VPD_{leaf} reached a maximum of 4.5 kPa.

The objectives of this study were to 1) develop a transpiration chamber used to independently control temperature and relative humidity, creating various VPD_{air} treatments and 2) determine the effect of VPD on transpiration in order to identify environmental factors that may limit the irrigation model developed in Chapters Three and Four.

Materials and Methods

The basic transpiration chamber was a sealed plywood chamber with the ability to control temperature, light, and VPD_{air} (Figure 4.1). Vapor pressure deficit_{air} was controlled by introducing humidified and temperature-controlled air using a conditioning unit (Model J4S-5580A, Parameter Generation and Control, Inc., Black
The humidity control system moves a selected portion of the recirculating air through a spray chamber to meet a targeted dew point for a given air temperature. Air circulates out of the transpiration chamber through the conditioning system and returns through a floor plenum. Humidity and air temperature are measured as the air moves between the chamber and conditioner. In this chamber-conditioning system, there were approximately eight air exchanges per minute.

Floor and lighting racks were made of expanded sheet metal. Lighting was supplied by nine incandescent (100 watts) and two fluorescent bulbs (30 watts) mounted to a rack. Following the concept of Moreshet (1970), the light rack had a counterweight-based adjustable height and the lights were on two separate circuits allowing a range of light levels. Leaf temperature was measured using infrared temperature sensors (4000.4ZL, Everest Interscience, Inc., Tucson, AZ). Transpiration was measured gravimetrically with scales (ScoutPro, Ohaus Corporation, Pine Brook, NJ). The data acquisition systems consisted of two primary components: leaf temperature and weight. A Visual Basic computer program was written in order to record the gravimetric data (Measurement Computing, Middleboro, MA). Adapters (Keyspan Four Port USB Serial Adaptors, InnoSys Inc, Richmond, CA) were used to group the twelve scales into three units which connected to the computer by the USB port. Leaf temperature data were recorded with a datalogger (CR10, Campbell Scientific, Inc., Logan, UT) following calibration with a black body calibrator (BB701, Omega Engineering, Inc., Stamford, CT).

To test the ability of the transpiration chamber to maintain temperature at relative humidity at the setpoints, the setpoints were programmed into the conditioning unit and after approximately four hours the actual measurements were manually recorded one time from the digital output on the conditioning unit. Container-grown seedlings of *C. kousa* var. *chinensis* (internally identified as accession 15) and *C. florida* were purchased from a commercial nursery as ¼ caliper container-grown seedlings. Plants were maintained in the original bottomless container (2 7/8 inch x 5 ½ inch
Anderson Band, Anderson Die and Manufacturing, Portland, OR) in a University of Kentucky greenhouse in Lexington, KY on a controlled water table with 50 ppm nitrogen until the time of the experiments. Plants were grown on a controlled water table to ensure non-limiting water conditions. Before moving plants to the transpiration chamber, they were watered overhead, soaked for 20 minutes in one inch of water to thoroughly wet the substrate and reduce channeling of water. Containers were drained to container capacity and then bagged and sealed around the lower trunk with wire ties and parafilm to minimize evaporative water loss. Plants were individually placed on separate scales and weight was recorded every 15 minutes thereafter. The infrared temperature sensors were aimed at a single leaf at the top of the canopy. The sensors were focused on the blade of the leaf between the midrib and the margin, halfway between the petiole and blade tip. Lights were positioned eight inches above the top of the canopy. Initial steady state transpiration was recorded. Eventually, desiccation water loss occurred as water was depleted from the containers. Plants were tested under 0.5 (20 °C and 78.5%RH) and 1.5 kPa (23 °C and 47%RH) VPD\textsubscript{air} treatments. Two experiments were conducted, one at 0.5 (20 °C and 78.5%RH) and one at 1.5 kPa (23 °C and 47%RH) VPD\textsubscript{air}. There were six plants of each taxa in each experiment.

Results

A transpiration chamber was developed with the capability of measuring water loss (transpiration) gravimetrically while monitoring stomatal regulation with infrared temperature sensors under a range of VPD\textsubscript{air} conditions (Figure 5.1–5.2). The chamber air conditioning system was able to successfully control VPD\textsubscript{air} by adjusting the relative humidity, while maintaining a constant air temperature at both temperatures tested, 24 and 28 °C (Table 5.1). A calibration curve was established to determine light intensity at various heights of the rack light (data not shown). Because the chamber did not have to be opened and closed, the environmental conditions, including light intensity, temperature and VPD, were maintained constant.
Transpirational water loss was measured for two dogwood species at 0.5 and 1.5 kPa VPD_{air}. The transpiration chamber was found to effectively monitor and record the changes in substrate moisture (Figure 5.3). Rates of water loss were greater at the 1.5 kPa than the 0.5 kPa for *C. florida* and *C. kousa* seedlings (Table 5.2), indicating that water use and, thus, irrigation needs could change with varying environmental conditions for these two *Cornus* species. Leaf temperature was not significantly different between *C. kousa* and *C. florida* at the beginning of the experiment or at the end of the experiment (Table 5.3). Leaves were not significantly warmer at the end of the experiment than at the beginning of the experiment for either species.

**Discussion**

The transpiration chamber was able to deliver a range of VPD_{air} treatments at a constant air temperature, thereby avoiding a common confounding issue in similar research (Mankin et al. 1998, Agata et al. 1985) when air temperature is adjusted to manipulate the VPD_{air}. The system was also able to measure and record water loss gravimetrically. The observed wilting and the weight loss data suggest that this transpiration chamber system was able to accurately measure transpiration of these seedlings. Further work is needed to test the response of photosynthesis and transpiration to substrate moisture content under a wide range of VPDs and to determine the utility of infrared leaf temperature technology on woody plant water status and irrigation.

Unlike other systems, this chamber air conditioning system does not manipulate air temperature to adjust the VPD. Therefore, this chamber provides a novel system for creating VPD treatments that eliminate the confounding factor of changing air temperatures. Air temperature, and even changes in root zone temperature, such as those that often occur in potted plants, can influence transpiration rates (Apostol et al. 2007). One potential limitation of this system is the lack of a desiccant. Lowering the humidity of this system is dependent solely on increasing the volume of air that bypasses the mist station. During certain times of the year or in humid climates this
may impose a limitation on the range of this transpiration chamber. Future work will test the limits of this desiccant-free system and use the chamber to provide constant temperature over a wider range of VPD treatments while further examining plant water use in *Cornus*.
Table 5.1. Expected and actual values for the transpiration chamber.

<table>
<thead>
<tr>
<th>Air temperature (° C)</th>
<th>Relative humidity (%)</th>
<th>VPD_{air} (kPa)</th>
<th>Expected</th>
<th>Actual</th>
</tr>
</thead>
<tbody>
<tr>
<td>28</td>
<td>47.0</td>
<td></td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>72.0</td>
<td></td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>88.0</td>
<td></td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>24</td>
<td>33.0</td>
<td></td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>64.5</td>
<td></td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>83.0</td>
<td></td>
<td>0.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>
Table 5.2. Slope of container weight, as a percentage of initial weight, over time at 0.5 and 1.5 kPa VPDair for two Cornus species.

<table>
<thead>
<tr>
<th>VPDair (kPa)</th>
<th>C. florida (slope)</th>
<th>C. kousa (slope)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>-0.0010a(^2)</td>
<td>-0.0006a</td>
</tr>
<tr>
<td>1.5</td>
<td>-0.0019b</td>
<td>-0.0014b</td>
</tr>
</tbody>
</table>

ANOVA P Value

\( ^2 \)means within a column followed by the same letter were not significantly different (Tukey’s HSD \( \alpha = 0.05 \)).
Table 5.3. Leaf temperature for two *Cornus* species. Leaf temperature was recorded at well-watered conditions at the beginning of the experiment (12 hours after initiation) and at the termination of the experiment, after irrigation was withheld for five days.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Leaf temperature at 12 hours without water (°C)</th>
<th>Final leaf temperature (°C)</th>
<th>ANOVA P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. florida</em></td>
<td>25.7</td>
<td>26.1</td>
<td>0.2653</td>
</tr>
<tr>
<td><em>C. kousa</em></td>
<td>25.6</td>
<td>25.7</td>
<td>0.8463</td>
</tr>
<tr>
<td>ANOVA P Value</td>
<td>0.8412</td>
<td>0.6205</td>
<td></td>
</tr>
</tbody>
</table>
Figure 5.1. A transpiration chamber with adjustable irradiance, programmable air temperature and relative humidity for controlling $\text{VPD}_{\text{air}}$, replicated scales for detecting transpirational water loss gravimetrically, and infrared temperature sensors for leaf temperature.
Figure 5.2. A transpiration chamber with adjustable irradiance, programmable air temperature and relative humidity for controlling VPDair, and 12 replicated stations with one scale per station to measure transpirational water loss and one infrared temperature sensor per station to detect leaf temperature.
Figure 5.3. Transpirational water loss of *C. kousa* expressed as container weight loss over six days.
Chapter Six

Ecophysiology of Cornus: Big-Bracted Clade, Cornaceae

Introduction

Flowering dogwood, *Cornus florida* L., is a popular landscape plant. It is known for spring “blooms” characterized by white or pink bracts, exceptional fall color, and shiny, red drupes (Dirr 2009). Flowering dogwood is a valuable nursery crop for the southeastern U.S. Total sales exceed $11,000,000 for AL, KY, NC, TN, and VA combined (USDA 1998). Dogwood is traditionally produced in the field and sold balled and burlapped. However, there is an increasing demand for container-grown plants (Basham et al. 2004). Unfortunately, *C. florida* has been challenging to grow in above-ground containers, particularly small containers (Witte and Tilt 1991). The poor success of *C. florida* in containers has been attributed to insufficient root adaptation to supraoptimal temperatures and intolerance of shifts in substrate moisture levels, both common conditions in above ground production (Witte and Tilt 1991). Alleviating the cause(s) of this production dilemma would provide a substantial opportunity for nursery growers in the southeastern U.S.

Despite the popularity of flowering dogwood, it is susceptible to a number of pests (Dirr 2009). As aluded to above, *C. florida* is not well-adapted to many common landscape settings, including compacted, poorly drained soils (Day et al. 2000). *C. florida* also grows poorly in droughty sites, due at least in part to its shallow root system (Dirr 2009). Despite the marketing potential of *C. florida*, these detrimental qualities can limit survival and health of flowering dogwood in the landscape.

Kousa dogwood, *C. kousa* Hance, is an alternative to flowering dogwood. *C. kousa* has large, ornamental bracts that emerge after the leaves. It is relatively disease
and insect resistant, withstands full sun, and is considered more tolerant of drought (Dirr 2009). However, Augé et al. (2002) observed that leaves of average C. kousa seedlings became scorched and curled in full sun or afternoon sun exposures in Tennessee. As a newly transplanted liner, C. kousa has been observed to be less drought tolerant than C. florida in field production settings, scorching before any sign of drought stress on C. florida (Melvin Moffett, personal communication).

There is little scientific information on drought response of C. kousa and none on any of the C. kousa x C. florida hybrids. Much of the research on C. florida has been ecology-based research located in natural settings. In these works, the sample size is often low, hampering interpretation of results, and the potential for substantial site variation exists. Additionally, this information may not be transferrable to production, and in particular, container production settings.

Tschaplinski et al. (1998) and Gebre et al. (1998) found that C. florida is capable of osmotic adjustment, but nonetheless was not tolerant of drought and exhibited high mortality under drought stress. Both C. kousa and C. florida have low stomatal conductance rates (Abrams and Mostoller 1995, Augé et al. 2002, Croker et al. 1998, Williams et al. 1987). Research from Chapter Four showed that C. kousa plants were able to maintain slightly greater photosynthetic rates than C. florida seedlings as substrate moisture decreased (Figure 4.1). Unpublished data from experiments in Chapter Four demonstrated that substrate moisture content for C. kousa and C. florida decreased from 100 to 60% container capacity in 96 and 88 hours, respectively, implying that C. florida loses water more rapidly than C. kousa. However, the mechanism(s) by which C. florida loses water more rapidly than C. kousa is unknown.

Williams et al. (1987) found that container-grown C. florida shoot length was reduced under relatively short intervals (five days) of withholding water, with stem length half those of well watered plants. However, Augé et al. (1998) found that C. florida tolerated a lower leaf water potential than several other tree species. The lethal leaf water potential was -4.88 MPa for C. florida compared with -2.04, -2.38, -3.34, and
-3.98 MPa for *Nyssa sylvatica* Marsh., *Liriodendron tulipfera* L., *Quercus rubra* L., and *Oxydendrum arboreum* L., respectively.

Plant water loss occurs through cuticular and stomatal (transpiration) water loss and is governed by the vapor pressure deficit (VPD). Stomatal water loss is also affected by the boundary layer and morphological features which may affect vapor movement. Cuticular water loss is influenced by the epidermal cell thickness, cutin and waxes in the cuticle, and the boundary layer. Possible causes of differential water use among plants include variation in stomatal area per unit leaf area, thickness or absence of cuticular wax, thickness of epidermal cells, boundary layer thickness, stomatal regulation, which may be influenced in some plants by greater sensitivity to vapor pressure deficit, root system capacity, osmotic adjustment, and hydraulic conductivity of roots, shoots, and grafts.

*C. florida* and *C. kousa* are part of the big-bracted clade of Cornaceae (Xiang et al. 2006). *C. florida* is native to the Eastern U.S. from Texas to Georgia, north to Michigan and east to Maine. *C. florida* grows in mixed mesophytic forests as an understory tree from 300–1200 m (USDA Forest Service 1965). *C. kousa* is native to China, Taiwan, and Japan where it grows on both sparsely and densely wooded hillsides, along streams, in valleys, and along roadsides (Sargent 1917, Wu et al. 1995). *C. kousa* var. *chinensis* is considered a superior selection with more vigorous growth and larger flowers (Dirr 2009). *C. florida* subsp. *urbiniana* is a native of Central America, specifically, the Mexican states of Nuevo Leon and Veracruz (USDA GRIN). It grows in pine-oak forests in mountainous areas around 1400 m elevation (Flores et al. 1990).

The objectives of this research were to 1) determine if net photosynthesis and gas exchange are related to leaf characteristics among several *Cornus* taxa, and 2) determine if *Cornus* taxa differ in these morphological characteristics and plant water relations.
Materials and Methods

Plants were maintained prior to leaf collection as follows:

In February 2007, 30–36” bareroot liners of three related taxa, C. kousa ‘National’ (unnamed C. kousa seedling is seed parent), C. florida ‘Cherokee Princess’ (pollen parent), and C. kousa x C. florida Constellation® were potted with a bark-based substrate (Barky Beaver Professional Grow Mix, Barky Beaver Mulch and Soil Mix, Moss, TN) into 23 L containers (Classic 2800, Nursery Supplies, McMinnville, OR) and grown in a pot-in-pot system with cyclic irrigation. Plants were fertilized with 90–100 grams (90 in 2007, 100 in 2008) per plant of 19-4-8, 5–6 month release complete fertilizer (Harrell’s, Inc. Sylacauga, AL) each April.

Container-grown seedlings of C. kousa var. chinensis (accession 15) and C. florida (Missouri, USA provenance) (1/4” caliper) were purchased from a commercial nursery. On May 1, 2009 seedlings were transplanted into 3.8 L containers (Classic 400 Nursery Supply, McMinnville, OR) with a peat-based substrate (Metro Mix 280, Sun Gro Horticulture, Bellevue, WA). Wicks, one inch by eight inch strips of capillary mat material, were inserted vertically into the center drain hole such that four inches of wick extended into the center of the container and four inches of the wick extended out of the container and were in contact with the capillary mat on the benchtop. Plants were grown in a University of Kentucky greenhouse in Lexington, KY on a controlled water table with 100–200 ppm N 20-10-20 (Peat-Lite Special®, The Scotts Co., Maryville, OH) until the time of the experiment. Supplemental lighting (high pressure sodium lighting) was provided when ambient light conditions decreased below 400 μmoles of light per m$^2$·s$^{-1}$.

C. kousa from Korea, two accessions of C. kousa var. chinensis (accession 13 and # 14), and one accession of C. florida (Kentucky, USA) and C. nuttallii (California, USA) were started from seed. Between October 2007 and March 2008, seeds were purchased and stratified in damp peat at 5 °C. On November 28, 2007 C. florida subsp. urbiniana (Rose) Rickett (open pollinated plants) were obtained from USDA National
Arboretum and stratified. Upon germination, plants were transplanted into 3.7 L containers (Classic 400 Nursery Supply, McMinnville, OR) with a peat and pine bark-based substrate (Metro Mix 280, Sun Gro Horticulture, Bellevue, WA). Wicks, as described above, were used to provide contact with the capillary mat. Plants were grown in a University of Kentucky greenhouse in Lexington, KY on a controlled water table with 100–200 ppm N 20-10-20 (Peat-Lite Special®, The Scotts Co., Maryville, OH) until the time of gas exchange measurements and leaf collection for epidermal peels. Supplemental lighting was used as previously described.

Plants from a botanical garden (Yew Dell Gardens, Crestwood, KY) were used for the remaining stomate measurements. All plants except (C. kousa x C. nuttallii) x C. kousa Venus™ and C. kousa x C. florida Saturn™ were mature plants approximately 15 years old. Venus™ and Saturn™ were less than five years old. Plants were maintained within a turf-free zone approximately half the diameter of the dripline. A light layer (approximately one inch) of compost was applied annually to this turf-free area. Plants received no conventional fertilizer and no irrigation.

Container-grown seedlings of C. kousa var. chinensis (accession 15) and C. florida (MO provenance) (1/4” caliper) were purchased from a commercial nursery. Plants were maintained in the original bottomless container (27/8 x 51/2“Anderson Band, Anderson Die and Manufacturing, Portland, Oregon) in a University of Kentucky greenhouse in Lexington, KY on a controlled water table with 50 ppm nitrogen until the time of the transpiration chamber experiments.

**Foliar Micromorphology**

Epidermal peels were conducted as follows. The most recently matured fully expanded leaf was collected and stored in a sealed plastic bag with a lint-free cloth moistened with de-ionized water. Bags were kept in a cooler or a refrigerator set at 4 °C until peels were conducted (within 24 hours of collection). Two leaves were collected from each plant so the second leaf could be used as a reserve in case the initial leaf tore during removal from the slide. A line of glue (Duro® Super Glue, Henkel Corporation,
Avon, OH) 4 mm wide was spread across the slide (Clay Adams Gold Seal™ Rite-On™ microslides No 3051, 3”x1” Becton Dickinson Labware, Franklin Lakes, NJ). A section of the lamina between the midrib and margin, parallel with the midrib, was pressed against the slide. Pressure was continued for 45 seconds. The leaf was peeled off of the slide after 60 seconds. Each slide was placed under a light microscope (BX40, Olympus, Tokyo, Japan) with a 10x objective lens, connected by a camera (DP-25, Olympus Corp., Tokyo, Japan) to a computer. Photomicrographs were taken with DP2-BSW software (Olympus Corp., Tokyo, Japan).

Stomates were counted from digital images using Photoshop® (Adobe Photosystems, Inc.). Stomatal area was determined from the images using SigmaScan® (Systat Software Inc., San Jose, CA). Twenty stomates per image were traced around the outer edge. The area was calculated and the major and minor axes were recorded by SigmaScan® in pixels. A micrometer was used to calibrate pixels to mm². Leaf area was measured prior to conducting epidermal peels with a leaf area meter (LI-3100 LI-COR® Biosciences, Lincoln, NE).

Stomatal density was calculated as the number of stomates per mm² leaf area. Individual stomatal complex area (µm²) was calculated as the average area of a single stomata and surrounding guard cells. Total stomatal complex area (µm²/mm²) is an integrated value calculated by multiplying the individual stomatal complex area by the stomatal area (Wang et al. 2008). Stomate total (total number of stomates per leaf) was calculated as the stomatal density multiplied by the leaf area.

Leaf tissue to be used for sections and for epidermal peels was collected at the same time. Sections of leaf blade were fixed in FAA (formalin:acetic acid:alcohol), dehydrated using a tertiary butyl alcohol series, and embedded in paraffin. Microtome (RM 2135 Rotary Microtome, Leica Instruments, Wetzlar, Germany) sections (12 µm) were made and affixed to a microscope slide. Slides were stained with safranin-fast green (Johansen 1940). Sections were observed under a light microscope, and photomicrographs were taken as described above. Adaxial epidermis layer thickness,
palisade layer thickness, and total leaf thickness were determined using a light microscope (BX40, Olympus, Tokyo, Japan) under a 10x objective lens, connected by a camera (DP-25, Olympus Corp., Japan) to a computer. Linear measurements were taken using image acquisition and measurement software (DP2-BSW, Olympus Corp., Tokyo, Japan).

A single leaf from both *C. kousa* and *C. florida* were sputter coated with gold–palladium (Technics Hummer VI, Anatech Ltd., Alexandria, VA). The coated samples were scanned with a S-800 FE scanning electron microscope (Hitachi Ltd., Tokyo, Japan). Photomicrographs from scanning electron microscopy were used as a reference to compare with photomicrographs from epidermal peels.

All controlled environment experiments (except SEM) utilized a completely randomized design; a single plant (one leaf per plant) constituted one replication. There were between three and 10 replications. For all stomatal complex area measurements there were twenty subsamples (twenty stomates per leaf averaged). The outdoor experiment utilizing three related taxa in the pot-in-pot plot was a completely randomized design with between six and ten single plant (one leaf per plant) replications. The experiment utilizing plants from a public garden landscape was a completely randomized design with a single plant of each taxa. In this case, a single leaf was considered a replication and there were between five and 15 single leaf replications. For the leaf sections made with leaves from the cultivars in the pot-in-pot plot, there were five single plant replications per taxa; one leaf per tree was collected. For the leaf sections made from the greenhouse-grown seedlings, there were three single plant replications per species with one leaf per plant, except *C. florida* subsp. *urbiniana* of which there was a single plant. Epidermis thickness, palisade layer thickness, and total leaf thickness were an average of five subsamples, respectively, per replication.
Light and CO₂ Curves

The relationship between light and photosynthesis and CO₂ and photosynthesis was determined by conducting response curves with an infrared gas analyzer (LI-6400, LI-COR® Biosciences, Lincoln, NE). Photosynthetic response curves were conducted at both high and low irradiance. For high light response curves, the irradiance began at the equivalent of ambient, approximately 800 µmol m⁻²·s⁻¹, and was set to 1200, 1800, 2000, 1800, 1500, 1200, 900, 600, 300, and 50 µmol m⁻²·s⁻¹ consecutively. For low light response curves, the irradiance was set to 300 µmol m⁻²·s⁻¹ and dropped in 50 µmol m⁻²·s⁻¹ increments until reaching 50 µmol m⁻²·s⁻¹, when the irradiance was set to 25, 10 and 0 consecutively. For A-Ci curves, cuvette CO₂ concentrations were set to 400, 250, 100, 50, 0, 400, 600, and 800 ppm CO₂ consecutively for the outdoor container-grown plants and continued to 1000, 1100, 1200, 1500, and 1800 ppm CO₂ consecutively for the greenhouse experiments. CO₂ curves were conducted at a constant irradiance of 1000 µmol m⁻²·s⁻¹ in both experiments. SigmaPlot® (Systat Software Inc., San Jose, CA) was used to fit the data from each light response curve. Data from high light curves were used to determine the maximum photosynthetic rate (Aₘₐₓ). Data from the linear portion of the low light curves (≤ 100 µmol m⁻²·s⁻¹) were used to calculate apparent quantum efficiency (Qₐₚₚ) and light compensation point (LCP). Data from below 10 µmol m⁻²·s⁻¹ were not used to calculate Qₐₚₚ; only the portion of the curve where light is limiting and respiration is constant was used in order to avoid overestimating the Qₐₚₚ due to the Kok effect region (Singsaas et al. 2001). Leaf temperature and relative humidity in the cuvette ranged from 23–27 °C and 60–76%, respectively. Data from the linear portion (≤ 200 ppm Ci) of the A-Ci curves were used to determine the carboxylation efficiency (mesophyll conductance) and the CO₂ compensation point. The carboxylation efficiency was determined as the initial slope of the line. The CO₂ compensation point was calculated by setting the equation for the line equal to a photosynthetic rate of zero and solving for the internal CO₂ concentration.
Individual photosynthesis measurements were taken on several greenhouse-grown *Cornus* species. These species included *C. kousa* from Korea, three accessions of *C. kousa* var. *chinensis*, and two accessions of *C. florida*, from Kentucky and Missouri. A single gas exchange measurement was taken per plant at 1000 μmol light m⁻²·s⁻¹ and 400 ppm CO₂. Leaf temperature and relative humidity in the cuvette ranged from 23–27 °C and 60–76%, respectively. The experiment was a completely randomized design with between three and six single plant replications.

Transpiration Chamber

Plants were thoroughly watered by hand to prevent water channeling and drained to container capacity. Containers were bagged and sealed around the lower trunk to minimize evaporative water loss. Plants were individually placed one per scale (ScoutPro, Ohaus Corporation, Pine Brook, NJ) in the transpiration chamber described in Chapter Five, and plant weight was recorded every 15 minutes thereafter. Infrared temperature sensors (4000.4ZL, Everest Interscience, Inc., Tucson, AZ) were aimed at a leaf at the top of the canopy. The sensors were focused on the blade of the leaf between the midrib and the margin, halfway between the petiole and tip ends of the blade. Lights were positioned eight inches above the top of the canopy. Initial steady state transpiration was recorded. Eventually, desiccation water loss occurred as water was depleted from the containers. Two experiments were conducted, one at a VPD of 0.5 kPa and one at 1.5 kPa. Each experiment was arranged in a completely randomized design with six single plant replications of each taxa.

Plant-Substrate Moisture Relationship: Drought and Recovery

The drought and recovery experiment utilized the three related taxa growing in the pot-in-pot production system described above. Moisture probes (ECH2O® EC-5, Decagon Devices, Pullman, WA) were installed vertically, midway between the sidewall of the container and the trunk, and so that the overmold was five cm below the surface of the substrate. Plants were watered, drained to container capacity, bagged, and
sealed around the trunk utilizing a previously tested technique that allowed minimal evaporative water loss and excluded irrigation and rainwater. Substrate moisture content, stem water potential, and gas exchange were measured under well-watered conditions upon initiating the experiment, and daily, while water was withheld, except for the second day of the experiment when data were collected twice. Irrigation was withheld from treated plants from August 21 to Aug 24, 2008. Cyclic irrigation (three times per day) was resumed for treated plants on August 25, 2009. Containers were weighed to determine the relationship between probe values for water content and actual substrate water content. On Sept 2, 2008 photosynthesis and stem water potential measurements were taken. The experiment was a completely randomized design with seven treated and five control plants per taxa.

Plant-Substrate Moisture Relationship: Drought

*C. kousa* var. *chinensis* (accession 15) and *C. florida* (MO provenance) were maintained as described previously for leaf collection for leaf sections and epidermal peels. A single ECHO-5 probe (Decagon Devices, Pullman, WA) was placed in each container. Probes were installed vertically, halfway between the container sidewall and the trunk. Plants were watered, allowed to drain to container capacity, weighed, and further irrigation was withheld. Water potential and relative water content of the leaves, as well as container weight were measured as the substrate dried. The experiment was a completely randomized design with 10 replications. Half of the plants of each species were used for the relative water content treatments and half were used for the leaf water potential treatments; thus, for leaf water status measurements there were five replications.

For eight days, beginning on the last day that plants were irrigated, relative water content and leaf water potential were measured. The relative water content was determined as follows. Leaves were collected, immediately bagged in a sealable plastic bag with a moistened lint-free cloth and placed in a cooler. Leaves were transported to the lab and weighed immediately to record the fresh weight. Individual leaves were
placed in a Petri® dish, one leaf per dish and covered with de-ionized water. Leaves were stored in the water at 4 °C for 24 hours. After 24 hours, the leaf surface was dried with a lint-free cloth and weighed to determine the turgid weight. Leaves were then dried at 55 °C for 48 hours and weighed. The equation used for determining relative water content is:

Relative Water Content =

\[
\frac{(\text{Fresh Weight-Dry Weight})}{(\text{Turgid Weight-Dry Weight})} \times 100
\]

Results

Outdoor-Grown Taxa

There were no differences in adaxial epidermis thickness among the three outdoor, container-grown taxa (Table 6.1, Figure 6.1abc). Palisade layer thickness was greater for C. kousa ‘National’ than for C. florida ‘Cherokee Princess’ and C. kousa x C. florida Constellation® (110, 83, and 82 µm, respectively. The total leaf thickness was also greater for C. kousa ‘National’ than for C. florida ‘Cherokee Princess’ and C. kousa x C. florida Constellation®.

There was no clear trend in stomatal characteristics among the outdoor, container-grown plants (Table 6.2). Stomatal density was greater for C. kousa x C.
*florida* Constellation® (217 stomates/mm²) than for *C. kousa* ‘National’ and *C. florida* ‘Cherokee Princess’. *C. kousa* ‘National’ had a greater stomatal density than ‘Cherokee Princess’ (157 and 59 stomates/mm², respectively). The individual stomatal complex area was greater for *C. kousa* ‘National’ than for either of the other two taxa. The total number of stomates per leaf was greater for leaves of *C. kousa* x *C. florida* Constellation® than for *C. florida* ‘Cherokee Princess’ or *C. kousa* ‘National’. Total stomatal complex area was greater for *C. kousa* ‘National’ and *C. kousa* x *C. florida* Constellation® than for *C. florida* ‘Cherokee Princess’.

Selections of *C. kousa*, *C. florida*, and their hybrids grown in the landscape had great variation in stomatal density, with values ranging from 21 to 162 stomates/mm² (Table 6.3). *C. florida* cultivars had lower stomatal density than *C. kousa* or the hybrids. The two *C. florida* cultivars, ‘Hollmans’ and ‘Plena’ had the greatest individual stomatal complex size of all the selections. Total stomate number ranged from 10,985 for *C. florida* ‘Plena’ to 71,053 for *C. kousa* x *C. florida* Celest ial™. In general, *C. florida* cultivars had the lowest number of stomates/leaf. Total stomatal complex areas ranged from 25,760 to 154,083 µm²/mm² and were lowest for *C. florida* cultivars and greatest for the hybrids.

*C. kousa* x *C. florida* Constellation® plants had greater maximum photosynthetic rate, stomatal conductance, and transpiration rates than both *C. kousa* ‘National’ and *C. florida* ‘Cherokee Princess’ (Table 6.4). There was no difference in internal CO₂ levels for the three taxa. *C. kousa* ‘National’ and *C. kousa* x *C. florida* Constellation® had a greater quantum efficiency than *C. florida* ‘Cherokee Princess’ (Table 6.5). The light compensation point of *C. kousa* ‘National’ was greater than *C. kousa* x *C. florida* Constellation® but not greater than that of *C. florida* ‘Cherokee Princess’. There was no difference among the three taxa for CO₂ compensation point. *C. kousa* x *C. florida* Constellation® had greater carboxylation efficiency than *C. florida* ‘Cherokee Princess’ but not *C. kousa* ‘National’.
Container-grown parents and their hybrid were examined for photosynthetic and stem water potential recovery to drought. Plants were subjected to substrate moisture contents ranging from 100% to approximately 68% container capacity. For all three taxa, photosynthesis and stem water potential decreased significantly during the drought treatment (Table 6.6). During the drought, stem water potential and photosynthesis were similar among all taxa. The photosynthetic rate and stem water potential measured after the recovery period were not different from initial values for all taxa.

Upon initial irrigation and drainage to container capacity, the three container-grown *Cornus* taxa had the same stem water potential at -1.3 MPa (Tables 6.6 and 6.7). There was no difference in stem water potential between the three taxa at any of the substrate moisture levels (Table 6.7). During the course of the imposed drought, only three *C. florida* ‘Cherokee Princess’ plants had a stem water potential in the 81–90% substrate moisture content range, compared with seven *C. kousa* ‘National’ plants and 10 *C. kousa* x *C. florida* Constellation® plants.

**Greenhouse-Grown Taxa**

The *C. florida* accession had thinner adaxial epidermal tissue than all but one of the *C. kousa* accessions and *C. nuttallii* (Table 6.8). The *C. florida* accessions and *C. kousa* (accession 15) had significantly thinner palisade layer than the Korean *C. kousa* accession. The *C. florida* accessions had reduced total leaf thickness compared to all but the *C. kousa* var. *chinensis* accession 15 and *C. nuttallii*. *C. florida* subsp. *urbiniana* (no replication) characteristics appeared comparable with the two *C. florida* accessions and the *C. kousa* var. *chinensis* (accession 15).

Among the species grown in the greenhouse, there was no clear pattern with respect to *C. florida* and *C. kousa* stomatal density. Stomatal density among taxa (not including *C. florida* subspecies *urbiniana*) ranged from 70 to 154 stomates/mm² (Table 6.9, Figure 6.2ab). *C. florida* subspecies *urbiniana*, with just one replication, had 26
stomates/mm², approximately one third to one fifth the stomatal density of the other seedlings. *C. kousa* accessions tended to have larger stomatal complexes than *C. florida*. For all plants except *C. florida* subspecies *urbiniana*, the total number of stomates per leaf varied from 29,300 to 89,614. *C. florida* subspecies *urbiniana* had 10,587 stomates per leaf. The total stomatal complex area, an integrated value of individual stomatal complex area and stomatal density, was different only between two *C. kousa* var. *chinensis* accessions.

Like data from the epidermal peels, SEM images demonstrated that *C. kousa* (Korean provenance) had larger stomates than *C. florida* (KY provenance) and also illustrated that a portion of *C. florida* stomates appeared partially or fully occluded with wax. SEM images also revealed heavy cuticular wax on *C. florida* (Figure 6.2cdef) while the epidermal peel images (Figure 6.2ab) revealed papillae on *C. florida* but not on *C. kousa*. The abaxial surface of *C. kousa* leaves was covered in very light wax that emerged near the stomates and was generally limited to the area immediately around the stomate.

Photosynthesis and gas exchange varied considerably for the *Cornus* taxa (Table 6.10). Photosynthesis ranged from 10.2 to 6.1 μmol CO₂ m⁻² s⁻¹. *C. kousa* var. *chinensis* (accession 14) and the Korean accession had lower photosynthesis rates than *C. florida* (Missouri accession) and *C. kousa* var. *chinensis* (accession 15). Stomatal conductance was greater for the *C. florida* (Kentucky provenance) than for *C. kousa* (Korea accession) and *C. kousa* var. *chinensis* (accession 14). In general, trends in transpiration and internal CO₂ were similar and largely paralleled those of stomatal conductance.

Light and CO₂ based-parameters were calculated for *C. florida* (Missouri accession) and *C. kousa* var. *chinensis* (accession 15) because there were greater quantities of these plants than the others. There was no difference in quantum efficiency, light compensation point, CO₂ compensation point, or carboxylation efficiency (Table 6.11).
Transpiration was measured at two VPD levels for *C. florida* (MO accession) and *C. kousa* (accession 15). Water loss was expressed as the slope of the percentage of initial weight over time. Weight loss was significantly greater for *C. florida* seedlings than for *C. kousa* seedlings at 0.5 kPa (-0.0010 and -0.0006, respectively) and at 1.5 KPa (-0.0019 and -0.0014, respectively) (Table 6.12).

*C. florida* (MO, USA) and *C. kousa* var. *chinensis* (accession 15) were subjected to drought to determine if they responded to varying substrate moisture contents differently. *C. florida* plants transpired more than *C. kousa* plants at each 24 hour increment selected (Table 6.13). With respect to leaf water potential, there was no difference between the species except at the driest substrate moisture level (Table 6.14). At the driest moisture level, 61–70% container capacity, *C. florida* leaves had a significantly lower (more negative) leaf water potential than *C. kousa* var. *chinensis* (-1.8 and -1.4 MPa, respectively).

Discussion

Outdoor-Grown Taxa

Epidermis thickness varied by less than a micron for the three taxa (Table 6.1). The palisade layer thickness was 34% greater for *C. kousa* ‘National’ than *C. kousa* x *C. florida* Constellation® or *C. florida* ‘Cherokee Princess’. Leaves on *C. kousa* ‘National’ plants were 16% thicker than those of the two outdoor container-grown taxa. The palisade layer was 33% of total leaf thickness for *C. florida* ‘Cherokee Princess’ and *C. kousa* x *C. florida* Constellation® and nearly 40% for *C. kousa* ‘National’. While differences in palisade layer thickness were observed, they did not correspond with differences in photosynthetic rates. Leaf thickness for *C. kousa* ‘National’ was 13 and 16% greater than for *C. florida* ‘Cherokee Princess’ and *C. kousa* x *C. florida* Constellation®, respectively. Interestingly, double palisade layers were observed in all three outdoor-grown taxa and occasionally a triple cell layer was observed. The inner
palisade layer(s) appears much shorter for *C. florida* ‘Cherokee Princess’ than for *C. kousa* ‘National’ and *C. kousa* x *C. florida* Constellation® (Figure 6.1).

The stomatal density of outdoor-grown *C. florida* selections in this study was consistent with that of other outdoor-grown *C. florida* (44–56 stomates/mm²) (Abrams and Mostoller 1995). Values for both experiments involving outdoor-grown plants (both container-grown and landscape-established) were comparable (Tables 6.2 and 6.3). In both outdoor settings, *C. florida* had a lower stomatal density than either *C. kousa* or hybrids. Greater stomatal conductance rates for *C. kousa* x *C. florida* Constellation® compared to *C. florida* ‘Cherokee Princess’ or *C. kousa* ‘National’ may partially explain the greater maximum photosynthetic rate observed in this hybrid; stomata are the portals through which CO₂ enters into the leaf (Taiz and Zeiger 2006). Total stomatal complex area, the integrated value of stomate size and stomatal density, should reflect a plant’s physical capability for gas exchange better than any of the individual parameters. However, there was no relationship between maximum photosynthetic rate and total stomatal complex, individual stomatal complex, stomatal density, or total number of stomates (data not shown). There were some exceptions on an individual taxon basis. For *C. florida* (KY provenance) stomate size was linearly related to photosynthesis, and total stomatal number and photosynthesis were linearly related for three accessions: *C. florida* (MO) $r^2 = 0.66$, *C. florida* (KY) $r^2 = 0.85$, and *C. kousa* var. *chinensis* (accession 14) $r^2 = 0.99$. Despite the logic of total stomatal complex area, CO₂ uptake has long been linked to stomatal density; increasing stomatal density increases CO₂ uptake (Assman and Wang 2001). *C. kousa* x *C. florida* Constellation® had almost four times greater stomatal density than *C. florida* ‘Cherokee Princess’, 41% greater total stomatal complex area, and 30% greater photosynthetic rates.

*C. kousa* ‘National’ and *C. kousa* x *C. florida* Constellation® had a greater quantum yield than *C. florida* ‘Cherokee Princess’, indicating an enhanced ability to respond to increasing light at very low light levels ($< 100$ μmoles light m⁻²s⁻¹), i.e. for each unit increase in light, *C. kousa* ‘National’ and *C. kousa* x *C. florida* Constellation®
increased their photosynthetic rate more than *C. florida* ‘Cherokee Princess’ (Table 6.5). *C. kousa* ‘National’ had a greater light compensation point than *C. kousa x C. florida* Constellation® indicating that higher light levels were required for photosynthetic rates to exceed respiration rates for *C. kousa* ‘National’ and that it is less shade adapted than the *C. kousa x C. florida* Constellation®. *C. kousa x C. florida* Constellation® had a greater carboxylation efficiency than *C. florida* ‘Cherokee Princess’ but not *C. kousa* ‘National’. High carboxylation efficiency suggests that photosynthesis in *C. kousa x C. florida* Constellation® is more sensitive to changing levels of CO₂ at low CO₂ concentrations, indicating that Rubisco activity is enhanced in *C. kousa x C. florida* Constellation®. The higher carboxylation efficiency, stomatal density, and stomatal conductance may explain why *C. kousa x C. florida* Constellation® had a greater maximum photosynthetic rate than the other two outdoor container-grown taxa.

Transpiration rates were greater for *C. kousa x C. florida* Constellation® than for *C. florida* ‘Cherokee Princess’ or *C. kousa* ‘National’ (Table 6.4). Transpiration rate was not correlated to adaxial epidermal cell thickness, total leaf thickness, or stomatal density (data not shown). Stomatal regulation in response to atmospheric and rhizospheric conditions, not physical constraints, may dictate rates. Greater stomatal density suggests *C. kousa x C. florida* Constellation® and *C. kousa* ‘National’ may be more tolerant of water deficit than *C. florida* ‘Cherokee Princess’ because plants with greater stomatal density are considered better adapted to drought stress due to enhanced transpirational cooling (Gindel 1969, Maximov 1931); however, the instantaneous nature of this transpiration rate did not reflect this. Another measure of transpiration may better relate to stomatal density. Also, as noted, the volume limitations of the rhizosphere of a container-grown plant may play a role in the response to water deficit as may the graft union and the rootstock genetics.

Plants were subjected to a drought and recovery cycle with substrates drying to 68–70% container capacity. Based on previous work (Chapter Four), drying the substrate to 68% container capacity would cause photosynthetic rates to decrease
substantially, to 32, 27, and 22% of maximum for *C. florida* ‘Cherokee Princess’, *C. kousa* ‘National’ and *C. kousa x C. florida* Constellation®, respectively. The leaf water potential began at -1.3MPa. Bidwell (1974) defined the leaf water potential of a well hydrated tree to be -1.5 MPa; therefore, these plants appear to have started the experiment well-hydrated, as intended. Leaf water potential decreased to 62, 54, and 38% of maximum, respectively, for *C. florida* ‘Cherokee Princess’, *C. kousa* ‘National’, and *C. kousa x C. florida* Constellation®, thereby subjecting the plants to an apparently fairly extreme, albeit short, drought (Table 6.6). The lack of difference in stem water potential or photosynthesis following the drought indicates that these container-grown *Cornus* taxa tolerated fairly extreme drought conditions without incurring damage to the photosynthetic apparatus or xylem embolism. Despite the morphology of *C. kousa x C. florida* Constellation®, this plant seems to be no better suited to withstand a brief drought and recovery cycle when in a container.

There was no difference in stem water potential among taxa when grouped by substrate moisture levels that fell within an approximate range of 100% to 70% container capacity (Tables 6.7). However, with only three *C. florida* ‘Cherokee Princess’ plants with stem water potential measured at 81-90% substrate moisture content, compared with seven *C. kousa* ‘National’ plants and 10 *C. kousa x C. florida* Constellation® plants, *C. florida* ‘Cherokee Princess’ plants appeared to dry faster than the other two taxa. With two and zero plants, *C. kousa* ‘National’ and Constellation®, respectively, drying to the below 70% container capacity substrate moisture level these taxa appear to have maintained higher moisture contents than *C. florida* ‘Cherokee Princess’. Leaf area was not measured for these plants. It is possible that the rate of water loss could be influenced by leaf area. In Chapter Four, studies with *C. kousa* and *C. florida* that were of similar age and similarly managed, did not show a difference in leaf area. However, these plants were seedlings and much younger than those in the outdoor experiment.

*Greenhouse-Grown Taxa*
For epidermis, palisade, and total leaf thickness, the *C. florida* accessions were similar to one another and variation occurred in the *C. kousa* accessions (Table 6.8). Adaxial epidermis thickness ranged from 13 to 33 µm across taxa, a difference of 154%. Adaxial epidermis thickness varied within the *C. kousa* taxa, ranging from 17 to 33 microns, a difference of 94%. Palisade thickness was lower among *C. florida* accessions but variable within *C. kousa* taxa. Palisade layers ranged from 13-33 µm for *C. kousa* accessions. Total leaf thickness varied 61% among *C. kousa* selections. For the two *C. florida* accessions, the palisade layer averaged 26% of the total leaf thickness. The leaf thickness composition of *C. florida* subspecies *urbiniana* was 35% palisade layer. The palisade layer was 22-29% of total leaf thickness for the *C. kousa* accessions. *C. nuttallii* was intermediate with respect to epidermis, palisade layer, and total leaf thickness; palisade layer was 33% of total leaf thickness. The palisade layer thickness of *C. kousa* (Korean accession) was more than double that of the *C. florida*, Missouri accession.

There was no correlation between palisade thickness and photosynthetic rate for the greenhouse-grown species (data not shown). However, SEM imaging shows very tightly packed palisade cells in *C. kousa x C. florida* Constellation®, which would potentially compress more chloroplasts into a given area (Figure 6.1). Carboxylation efficiency data suggest that *C. kousa x C. florida* Constellation® may have an advantage due to Rubisco efficiency, however *C. kousa x C. florida* Constellation® may simply have more Rubisco.

*C. kousa* var. *chinensis* (accession 13) was fairly inconsistent with the other *C. kousa* accessions in characteristic such as stomatal density and individual stomate size. *C. kousa* accession 13 also appeared to be morphologically different from the other *C. kousa* accessions, having lanceolate leaves while the other accessions had ovate shaped leaves.

Overall, there was substantial variation in gas exchange within species (Table 6.10). *C. kousa* (Korean accession) and *C. kousa* var. *chinensis* (accession 14) had low stomatal conductance rates and correspondingly low photosynthetic rates. Preliminary
research in which CO₂ response curves were generated for other C. kousa seedlings from South Korea suggest that plants from this seed source are variable in response to CO₂ and that a low stomatal conductance isn’t the only factor limiting photosynthesis. In the trade, C. kousa var. chinensis is considered superior to C. kousa, exhibiting more vigor and larger bracts (Dirr 2009).

While great variation in gas exchange existed across the Cornus accessions, among the C. kousa var. chinensis and C. florida (MO, USA), there was little difference in gas exchange and no difference in gas exchange-based parameters (Table 6.10 and 6.11). These two accessions were also among the highest performing plants in terms of gas exchange. The seed collector of the C. florida (Missouri accession) stated that the mother plants are of a superior form, growth rate, and health (Judy Lovelace, personal communication). Perhaps both the C. kousa and the C. florida were both superior selections and that is why their performance is so similar.

The epidermal cell thickness of some C. kousa seedling accessions was more than double that of the C. florida (Missouri) accessions. The thicker epidermal cell may help reduce cuticular water loss (Esau 1969). While St. Hilaire and Graves (1999) did not find a relationship between epidermal cell thickness and drought tolerance in maples, Ristic and Cass (1991) found that Zea mays with greater drought resistance had thicker epidermal cells than drought sensitive Zea mays lines. Capparis spinosa L., a plant native to the arid Mediterranean region, develops a very thick epidermal cell wall as leaves age, especially on the exposed cell surface (Rhizopoulou and Psaras 2003).

SEM photomicrographs showed that abaxial leaf surfaces on C. florida were covered in coronate cutin and papillae very similar to that described by Hardin and Murrell (1997). Coronate wax patterns are characterized by tall papillae that have conspicuous bands that form webbing, are taller than papillose-striated or filigree patterns, and serve as supports around individual papilla (Hardin and Murrell 1997). Coronate wax characteristics are thought to further enhance light reflectance and enhance the boundary layer effect, reducing transpiration (Hardin and Murrell 1997).
Coronate wax may reduce leaf temperature and the concomitant need for transpiration. The heavy cutin and papillae (Hardin and Murrell 1997) hindered the micromorphology work, in particular when counting and measuring stomates. Papillae or papillose cell walls have been documented in a number of \textit{Cornus} species, including \textit{C. florida} (Hardin and Murrell 1997) and are thought to reflect light and decrease radiant heat load on leaves. \textit{C. kousa} had striated cutin which is also consistent with Hardin and Murrell (1997).

The cuticle is a barrier to moisture loss (Esau 1967). Cuticle thickness has long been associated with the level of resistance to water vapor. Research has shown that wax is more important than cutin in preventing cuticular water loss. The type and composition of cuticular wax can be more important than the amount of wax in preventing water loss (Lendzian 1982, Lendzian and Kerstiens 1991, Schönherr 1976, Schreiber and Riederer 1996, Tischler and Voigt 1990). The principle advantage of waxes over cutin is their highly ordered structure (Reynhard and Riederer 1991, Reynhard and Riederer 1994). The cuticle was determined to be inconsequential in preventing water loss in both Mexican redbud (\textit{Cercis canadensis} var. \textit{mexicana} Rose), a species adapted to an arid climate and characterized by a very glossy cuticle, and Eastern redbud (\textit{Cercis canadensis} L.), a species adapted to a mesic environment with a less apparent cuticle (Tipton and White 1995). In fact, for greenhouse-grown redbuds in this study, the rate of water loss on a leaf area basis for Mexican redbud was 50\% greater than that of eastern redbuds in spite of the fact that the cuticle was 35\% greater on the Mexican redbud. Because the wax component of these taxa was not analyzed it is impossible to ascertain the influence of the cuticle.

Both the wax and the papillae may mitigate environmental effects on water loss, but based on leaf water potential and transpiration data from the imposed drought, as well as data acquired from the transpiration chamber, \textit{C. florida} loses water faster than \textit{C. kousa} var. \textit{chinensis} in spite of the morphological advantages of \textit{C. florida}. Overall, there was no relationship for greenhouse-grown taxa between transpiration rate and
stomate size, number of stomates, stomatal density, or total aperture per unit leaf area. There were some exceptions on an individual taxon basis. *C. florida* (MO provenance) and *C. florida* (KY provenance) had a correlation between transpiration and stomate size, $r^2 = 0.74$, and 0.60, respectively (data not shown). For the *C. kousa* accession from Korea and *C. kousa* var. *chinensis* (accession 14), transpiration was linked to total stomatal complex area, $r^2 = 0.72$ and 0.87, respectively (data not shown). While some research suggests that water loss is most closely linked with aperture size, and secondarily with stomatal density (Lawson 1997), most studies support stomatal density as the main foliar characteristic affecting transpiration (Gindel 1969, Hassanein and Dorion 2006, Maximov 1931).

*C. florida* subsp. *urbiniana* did not appear to use epidermal cell thickness as an adaptive strategy as its thickness was not significantly greater than the species from mesic environments. *C. florida* subsp. *urbiniana* also did not have increased stomatal density as a foliar feature reflecting its adaptation to arid climates. It is possible that *C. florida* subspecies *urbiniana* has adapted to the arid regions of Mexico by reducing stomatal density as a mechanism for conserving moisture in an arid environment (Galston et al. 1980); although this is contradictory to much research. Plants in arid environments tend to have higher stomatal density in order to facilitate transpirational cooling (Maximov 1931). *C. florida* subspecies *urbiniana* may be more heat tolerant and require less transpirational cooling than *C. florida*.

Imposed droughts in the greenhouse and outdoors as well as under controlled VPD treatments showed that *C. florida* lost water faster than *C. kousa*. Additionally, *C. florida* became visibly wilted more quickly than *C. kousa*. The stomatal regulation of *C. florida* appears to allow significant water loss before stomatal closure occurs. Mesomorphic plants are known to increase transpiration under drought conditions but upon closing their stomata continue to lose large amounts of water due to less insulating leaf surfaces than xeromorphic plants (Maximov 1931). It is possible that
significant cuticular water loss took place in *C. florida*. This study did not separate cuticular transpiration from stomatal transpiration.

Morphology and gas exchange weren’t always consistent within a species. For example, photosynthetic rates for *C. kousa* accessions ranged from 6.1 to 10.0 µmol CO$_2$ m$^{-2}$·s$^{-1}$. Stomatal density ranged from 21 to 59 stomates/mm$^2$ among outdoor-grown *C. florida* taxa.

Gas exchange-based parameters from greenhouse-grown seedlings were generally consistent with those for the outdoor container-grown plants. An exception was the light compensation point, which for greenhouse-grown plants was approximately 50% of outdoor-grown taxa, although still within ranges comparable with other woody plants (Cordero 1999, Singsaas et al. 2001, Tenhunen et al. 1984). This may be because the greenhouse light conditions may have been at lower levels than the ambient light level during the outdoor experiment. Plants grown under lower light levels often have lower light compensation points than when grown under higher light levels (Björkman 1981).

Also the difference in greenhouse versus an outdoor environment appeared to play a role in micromorphology and gas exchange-based parameters. For example, stomatal density of *C. kousa* ‘National’ grown outdoors was more than double that of *C. florida* ‘Cherokee Princess’. However, in a greenhouse environment both *C. florida* accessions had greater stomatal density than three of four *C. kousa* accessions. Greenhouse-grown *C. florida* had a smaller individual stomatal complex than most outdoor-grown *C. florida* (Tables 6.2 and 6.3). In greenhouse-grown plants, the stomatal complex area for *C. kousa* tended to be greater than that of *C. florida*, but the opposite was true outdoors, with the average *C. kousa* stomatal complex being 37% smaller than the *C. florida* (Table 6.3). Stomatal complex size also varied within a species. It is possible that this could be a reflection of genetics within accessions.

Stomatal conductance, transpiration, and internal CO$_2$ varied somewhat across the two environments. Stomatal conductance averaged 40% greater in the controlled
environment-grown *C. florida* compared to the outdoor-grown *C. florida*, however, the difference in photosynthesis was just 6% (Table 6.4 and 6.10). Quantum efficiency and the CO$_2$ compensation point were conserved across environments. The light compensation point varied considerably between greenhouse and outdoor conditions, which likely reflected the lower light levels in the greenhouse compared with the outdoor environment. The carboxylation efficiency was 20% greater for *C. kousa* ‘National’ grown outdoors than for greenhouse-grown *C. kousa* var. *chinensis* and essentially identical to outdoor and greenhouse-grown *C. florida* taxa (Table 6.5 and 6.11). Again because *C. florida* ‘Cherokee Princess’, *C. kousa* ‘National’, and *C. kousa* x *C. florida* Constellation® weren’t grown in the greenhouse it is impossible to determine if this is genetically or environmentally controlled, or both.

Micromorphology was generally not related to photosynthesis or water use. For example, *C. florida* water loss was faster than that of *C. kousa* in spite of having coronate papillae and a waxy cuticle. However, total stomate number was correlated with photosynthesis in *C. florida* (MO), *C. florida* (KY), and *C. kousa* var. *chinensis* (accession 14), $r^2 = 0.66$, 0.85, and 0.99, respectively. For these data there were frequently just three single plant replications. For some taxa, such as *C. kousa* x *C. florida* Constellation®, the morphological characteristics were consistent with strategies to withstand water deficit such as greater stomatal density. If so, the other hybrids and *C. kousa* cultivars have characteristics of drought tolerance (greater stomatal density) and may in part be considered superior selections with regard to landscape adaptability because of this trait.

Plants have at their disposal numerous foliar features that can impact water use and they all are not used simultaneously, creating the appearance of contradictory morphological characteristics. For example, among several southwestern tree species, *Fraxinus veluntina* Torr. had the greatest trichome density, 1836 trichomes/cm$^2$, and under drought stress and ensuing reduced leaf water potential was able to maintain high photosynthetic rates (Coye and St. Hilaire 2002). However, it had a low stomatal
density suggestive of a more mesic species. In this study a great range of characteristics were observed that were not always consistent with other characteristics, water use, or photosynthetic data.

Stomatal density is a plastic micromorphological feature. Plants can respond to changing environmental conditions due to a signaling system that allows quick regulation of stomatal density in response to environmental stimuli (Lampard et al. 2008). Gindel (1969) found that among 22 of 32 trees species, stomatal density was greater under dryland production than when grown with irrigation. Stomatal density increased for container-grown Pelargonium x hortorum Bailey under drought cycles (Hassanein and Dorion 2006). In some cases very large stomates were offset by low stomatal density among the greenhouse-grown Cornus seedlings and the landscape-grown selections. In research with mutants, greater stomatal aperture was offset by lower stomatal density and vice versa preventing any impact of change in stomatal characteristics on stomatal conductance (Lawson 2009). Perhaps if subjected to water deficit conditions, the stomatal density would have increased for C. florida subspecies urbiniana and other accessions, compared with well watered controls.

C. kousa x C. florida Constellation® appears to have some hybrid vigor with greater stomatal density, photosynthesis, stomatal conductance, and transpiration, than C. florida ‘Cherokee Princess’ and C. kousa ‘National’ and greater carboxylation efficiency than C. florida ‘Cherokee Princess’. Stomatal conductance, stomatal density, and perhaps carboxylation efficiency and Rubisco quantity could explain the greater photosynthetic rate.

Conclusions

This research showed that Cornus taxa vary considerably in micromorphology and gas exchange parameters across and within species. In addition, Cornus taxa vary considerably in micromorphology and gas exchange parameters across environmental conditions. Individual micromorphological characteristics were not related to photosynthesis or water use except for some individual plants, i.e. C. kousa x C. florida
Constellation® had a high stomatal density and a high transpiration rate. *C. kousa* x *C. florida* Constellation® appears to have some hybrid vigor in micromorphological, gas exchange, and biochemical characteristics. *C. florida* and *C. florida* ‘Cherokee Princess’ appeared to use water at a faster rate under both well watered and deficit conditions in the rhizosphere as well as atmosphere when compared with *C. kousa*, *C. kousa* ‘National’, or *C. kousa* x *C. florida* Constellation®. Researchers and the horticulture industry may need to consider the genetics of plants and the environmental conditions for use in experiments and in landscapes as not all accessions or provenances are representative of the species and all won’t perform similarly in a range of environmental conditions.
Table 6.1. Adaxial epidermis, palisade, and total leaf thickness for three related outdoor-grown *Cornus* taxa.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Adaxial epidermis thickness (µm)</th>
<th>Palisade thickness (µm)</th>
<th>Total leaf thickness (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. florida</em> ‘Cherokee Princess’</td>
<td>22</td>
<td>83b</td>
<td>250b</td>
</tr>
<tr>
<td><em>C. kousa</em> ‘National’</td>
<td>23</td>
<td>110a</td>
<td>283a</td>
</tr>
<tr>
<td><em>C. kousa</em> x <em>C. florida</em> Constellation®</td>
<td>22</td>
<td>82b</td>
<td>245b</td>
</tr>
</tbody>
</table>

ANOVA P Value  | 0.7173  | 0.0007  | 0.0037  

*means followed by the same letter were not significantly different (Tukey’s HSD α=0.05).
Table 6.2. Stomatal characteristics for three related outdoor-grown *Cornus* taxa.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Leaf area (mm²)</th>
<th>Stomatal density (#/mm²)</th>
<th>Ind. stomatal complex area (µm²)</th>
<th>Total stomates (stomates/leaf)</th>
<th>Total stomatal complex area (µm²/mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. florida</em> ‘Cherokee Princess’</td>
<td>679a²</td>
<td>59c</td>
<td>766b</td>
<td>39,356b</td>
<td>45,298b</td>
</tr>
<tr>
<td><em>C. kousa</em> ‘National’</td>
<td>362c</td>
<td>157b</td>
<td>961a</td>
<td>56,557b</td>
<td>150,642a</td>
</tr>
<tr>
<td><em>C. kousa</em> x <em>C. florida</em> Constell.®</td>
<td>512b</td>
<td>217a</td>
<td>715b</td>
<td>110,741a</td>
<td>154,601a</td>
</tr>
<tr>
<td>ANOVA P Value</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.0002</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

*Means followed by the same letter were not significantly different (Tukey’s HSD α=0.05).*
Table 6.3. Stomatal characteristics for established *Cornus* selection (outdoors).

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Stomatal density (stomate/mm²)</th>
<th>Ind. stomatal complex area (µm²)</th>
<th>Stomate total (no. per leaf)</th>
<th>Total stomatal complex area (µm²/mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. florida</em> 'Hollmans'</td>
<td>22c</td>
<td>1,180b</td>
<td>13,301ef</td>
<td>25,760e</td>
</tr>
<tr>
<td><em>C. florida</em> 'Plena'</td>
<td>21c</td>
<td>1,362a</td>
<td>10,985f</td>
<td>28,188e</td>
</tr>
<tr>
<td><em>C. kousa</em> ‘Miss Satomi’</td>
<td>81b</td>
<td>857de</td>
<td>33,992cd</td>
<td>69,504d</td>
</tr>
<tr>
<td><em>C. kousa</em> ‘Wolf Eyes’</td>
<td>155a</td>
<td>997c</td>
<td>48,460bc</td>
<td>154,083a</td>
</tr>
<tr>
<td><em>C. x Celestial™</em></td>
<td>158a</td>
<td>944cde</td>
<td>71,053a</td>
<td>147,772a</td>
</tr>
<tr>
<td><em>C. x Ruth Ellen®</em></td>
<td>115b</td>
<td>712f</td>
<td>35,350cd</td>
<td>81,604cd</td>
</tr>
</tbody>
</table>
Table 6.3. Continued. Stomatal characteristics for established *Cornus* selection (outdoors).

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Stomatal density (stomate/mm²)</th>
<th>Ind. stomatal complex area (µm²)</th>
<th>Stomate total (no. per leaf)</th>
<th>Total stomatal complex area (µm²/mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. x Saturn™</em></td>
<td>114b</td>
<td>988cd</td>
<td>46,251bcd</td>
<td>112,458bc</td>
</tr>
<tr>
<td><em>C. x Star Dust®</em></td>
<td>106b</td>
<td>818ef</td>
<td>29,665de</td>
<td>86,240cd</td>
</tr>
<tr>
<td><em>C. x Stellar Pink®</em></td>
<td>162a</td>
<td>847ef</td>
<td>49,641bc</td>
<td>136,342ab</td>
</tr>
<tr>
<td><em>C. x Venus™</em></td>
<td>91b</td>
<td>859cde</td>
<td>54,412ab</td>
<td>81,584cd</td>
</tr>
<tr>
<td>ANOVA P Value</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
Table 6.4. Photosynthesis and gas exchange-based measurement for three related outdoor-grown Cornus taxa.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Maximum photosynthesis (µmol CO₂ m⁻²·s⁻¹)</th>
<th>Stomatal Conductance (mol H₂O m⁻²·s⁻¹)</th>
<th>Transpiration (mmol H₂O m⁻²·s⁻¹)</th>
<th>Internal CO₂ (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. florida</td>
<td>9.9b²</td>
<td>0.11b</td>
<td>2.6b</td>
<td>222</td>
</tr>
<tr>
<td>‘Cherokee Princess’</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. kousa</td>
<td>10.3b</td>
<td>0.11b</td>
<td>2.6b</td>
<td>214</td>
</tr>
<tr>
<td>‘National’</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. kousa x C. florida</td>
<td>12.9a</td>
<td>0.14a</td>
<td>3.4a</td>
<td>219</td>
</tr>
<tr>
<td>Constellation®</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ANOVA P</strong></td>
<td>&lt;0.0001</td>
<td>0.0007</td>
<td>0.0087</td>
<td>0.5545</td>
</tr>
<tr>
<td><strong>Value</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

²means followed by the same letter were not significantly different (Tukey’s HSD α=0.05).
Table 6.5. Gas exchange-based parameters for three related outdoor-grown *Cornus* taxa.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Quantum efficiency</th>
<th>Light compensation point (μmol light m²·s⁻¹)</th>
<th>CO₂ compensation point (ppm)</th>
<th>Carboxylation efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. florida</em> 'Cherokee Princess'</td>
<td>0.042b</td>
<td>50.2ab</td>
<td>70.4</td>
<td>0.05b</td>
</tr>
<tr>
<td><em>C. kousa</em> 'National'</td>
<td>0.047a</td>
<td>55.8a</td>
<td>66.9</td>
<td>0.06ab</td>
</tr>
<tr>
<td><em>C. kousa</em> × <em>C. florida</em> Constellation®</td>
<td>0.048a</td>
<td>43.9b</td>
<td>61.1</td>
<td>0.7a</td>
</tr>
</tbody>
</table>

ANOVA P Value 0.0067 0.0050 0.0935 0.0323

*Means followed by the same letter were not significantly different (Tukey’s HSD α=0.05).*
Table 6.6. Drought and recovery for three outdoor-grown **Cornus** taxa. Irrigation was withheld until there was a 30-35% reduction in initial photosynthetic rates.

<table>
<thead>
<tr>
<th></th>
<th><em>C. florid</em>a 'Cherokee Princess'</th>
<th><em>C. kousa</em> 'National'</th>
<th><em>C. kousa x C. florid</em>a Constellation*²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Photosyn. (µmol CO₂ m⁻² s⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stem Water Potential (MPa)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>9.7b¹A²</td>
<td>9.7bA</td>
<td>12.3aA</td>
</tr>
<tr>
<td>Drought</td>
<td>4.7aB</td>
<td>4.5aB</td>
<td>5.3aB</td>
</tr>
<tr>
<td>Final</td>
<td>9.7aA</td>
<td>8.7aB</td>
<td>13.0aA</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Photosyn. (µmol CO₂ m⁻² s⁻¹)</th>
<th>Stem Water Potential (MPa)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>9.7bA</td>
<td>12.3aA</td>
</tr>
<tr>
<td>Drought</td>
<td>4.7aB</td>
<td>5.3aB</td>
</tr>
<tr>
<td>Final</td>
<td>9.7aA</td>
<td>13.0aA</td>
</tr>
</tbody>
</table>

| ANOVA | P Value |                          |                |                |
|--------|---------|--------------------------|----------------|
|        |         |                          |                |                |
|        | 0.0004  | <0.0001                  | 0.0072         |
|        |         |                          | 0.0023         |
|        |         |                          | 0.0005         |
|        |         |                          | 0.0034         |

\[\text{P Value}\]

²ANOVA P Value provided for photosynthetic rate.

¹photosynthetic means for each parameter within a row followed by the same lowercase letter were not significantly different (α = 0.05).

*means within a column followed by the same uppercase letter were not significantly different (α = 0.05).
Table 6.7. Stem water potential for three outdoor-grown *Cornus* taxa. Plants were irrigated thoroughly by hand and then water was withheld.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Substrate moisture content (% of container capacity)</th>
<th>Stem water potential (MPa)</th>
<th>Plants in moisture content range (# of plants)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. florida</em> ‘Cherokee Princess’</td>
<td>91-100</td>
<td>-1.3</td>
<td>11</td>
</tr>
<tr>
<td><em>C. kousa</em> ‘National’</td>
<td>91-100</td>
<td>-1.3</td>
<td>10</td>
</tr>
<tr>
<td><em>C. kousa</em> x <em>C. florida</em> Constellation®</td>
<td>91-100</td>
<td>-1.3</td>
<td>11</td>
</tr>
<tr>
<td>ANOVA P Value</td>
<td></td>
<td>0.9798</td>
<td></td>
</tr>
<tr>
<td><em>C. florida</em> ‘Cherokee Princess’</td>
<td>81-90</td>
<td>-1.5</td>
<td>3</td>
</tr>
<tr>
<td><em>C. kousa</em> ‘National’</td>
<td>81-90</td>
<td>-1.1</td>
<td>7</td>
</tr>
<tr>
<td><em>C. kousa</em> x <em>C. florida</em> Constellation®</td>
<td>81-90</td>
<td>-1.4</td>
<td>10</td>
</tr>
<tr>
<td>ANOVA P Value</td>
<td></td>
<td>0.2159</td>
<td></td>
</tr>
<tr>
<td><em>C. florida</em> ‘Cherokee Princess’</td>
<td>71-80</td>
<td>-1.7</td>
<td>8</td>
</tr>
<tr>
<td><em>C. kousa</em> ‘National’</td>
<td>71-80</td>
<td>-1.8</td>
<td>8</td>
</tr>
<tr>
<td><em>C. kousa</em> x <em>C. florida</em> Constellation®</td>
<td>71-80</td>
<td>-1.9</td>
<td>7</td>
</tr>
<tr>
<td>ANOVA P Value</td>
<td></td>
<td>0.2597</td>
<td></td>
</tr>
<tr>
<td><em>C. florida</em> ‘Cherokee Princess’</td>
<td>≤70</td>
<td>-2.2</td>
<td>7</td>
</tr>
<tr>
<td><em>C. kousa</em> ‘National’</td>
<td>≤70</td>
<td>-1.9</td>
<td>2</td>
</tr>
<tr>
<td><em>C. kousa</em> x <em>C. florida</em> Constellation®</td>
<td>≤70</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>ANOVA P Value</td>
<td></td>
<td>0.2907</td>
<td></td>
</tr>
</tbody>
</table>
Table 6.8 Adaxial epidermis, palisade, and total leaf thickness for greenhouse-grown *Cornus* taxa.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Accession/Provenance</th>
<th>Adaxial epidermis thickness (µm)</th>
<th>Palisade thickness (µm)</th>
<th>Total leaf thickness (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. florida</em></td>
<td>MO, USA</td>
<td>13c&lt;sup&gt;1&lt;/sup&gt;</td>
<td>35b</td>
<td>145c</td>
</tr>
<tr>
<td><em>C. florida</em></td>
<td>KY, USA</td>
<td>14c</td>
<td>41b</td>
<td>151c</td>
</tr>
<tr>
<td><em>C. florida</em> subsp. urbiniana</td>
<td>Mexico</td>
<td>18&lt;sup&gt;y&lt;/sup&gt;</td>
<td>47</td>
<td>135</td>
</tr>
<tr>
<td><em>C. kousa</em></td>
<td>Korea</td>
<td>27ab</td>
<td>71a</td>
<td>243a</td>
</tr>
<tr>
<td><em>C. kousa</em></td>
<td>China 13</td>
<td>28ab</td>
<td>56ab</td>
<td>249a</td>
</tr>
<tr>
<td><em>C. kousa</em></td>
<td>China 14</td>
<td>33a</td>
<td>53ab</td>
<td>224ab</td>
</tr>
<tr>
<td><em>C. kousa</em></td>
<td>China 15</td>
<td>17bc</td>
<td>37b</td>
<td>155bc</td>
</tr>
<tr>
<td><em>C. nuttallii</em></td>
<td>CA, USA</td>
<td>18bc</td>
<td>60ab</td>
<td>183abc</td>
</tr>
</tbody>
</table>

ANOVA P Value | 0.0002 | 0.0007 | 0.0001

<sup>1</sup>Means within a column followed by the same letter were not significantly different (Tukey’s HSD α=0.05).

<sup>y</sup>*C. florida* subspecies *urbiniana* open pollinated plants from the USDA National Arboretum. Native to Mexico, no replication.
Table 6.9. Stomatal characteristics of several big-bracted *Cornus* seedlings grown in a controlled environment.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Accession/Provenance</th>
<th>Leaf area (mm²)</th>
<th>Stomatal density (stomate/mm²)</th>
<th>Individual stomatal complex area (µm²)</th>
<th>Stomate total (no./leaf)</th>
<th>Total stomatal complex area (µm²/mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. florida</em></td>
<td>MO, USA</td>
<td>678a²</td>
<td>130a</td>
<td>724c</td>
<td>89,614a</td>
<td>94,388ab</td>
</tr>
<tr>
<td><em>C. florida</em></td>
<td>KY, USA</td>
<td>314d</td>
<td>93b</td>
<td>826bc</td>
<td>29,300b</td>
<td>77,303ab</td>
</tr>
<tr>
<td><em>C. florida</em>²</td>
<td>Mexico</td>
<td>401</td>
<td>26</td>
<td>886</td>
<td>10,587</td>
<td>23,374</td>
</tr>
</tbody>
</table>

ANOVA P Value

“means within a column followed by the same letter were not significantly different (Tukey’s HSD α=0.05).

²*C. florida* subspecies *urbiniana* open pollinated plants from the USDA National Arboretum. Native to Mexico, no replication.
Table 6.9 Continued. Stomatal characteristics of several big-bracted *Cornus* seedlings grown in a controlled environment.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Accession/Provenance</th>
<th>Leaf area (mm²)</th>
<th>Stomatal density (stomate/mm²)</th>
<th>Individual stomatal complex area (μm²)</th>
<th>Stomate total (no./leaf)</th>
<th>Total stomatal complex area (μm²/mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. kousa</em></td>
<td>Korea</td>
<td>434bcd</td>
<td>79b</td>
<td>1,012a</td>
<td>34,524b</td>
<td>79,548ab</td>
</tr>
<tr>
<td><em>C. kousa</em></td>
<td>China 13</td>
<td>342cd</td>
<td>154a</td>
<td>701c</td>
<td>54,008b</td>
<td>106,851a</td>
</tr>
<tr>
<td><em>C. kousa</em></td>
<td>China 14</td>
<td>566abc</td>
<td>70b</td>
<td>1011a</td>
<td>39,931b</td>
<td>70,715b</td>
</tr>
<tr>
<td><em>C. kousa</em></td>
<td>China 15</td>
<td>609ab</td>
<td>84b</td>
<td>999ab</td>
<td>50,511b</td>
<td>84,816ab</td>
</tr>
<tr>
<td><em>C. nuttallii</em></td>
<td>CA, USA</td>
<td>379cd</td>
<td>81b</td>
<td>957ab</td>
<td>30,785b</td>
<td>77,143ab</td>
</tr>
</tbody>
</table>

ANOVA *P* Value

<0.0001 <0.0001 <0.0001 <0.0001 0.0120

1 means within a column followed by the same letter were not significantly different (Tukey’s HSD α=0.05).

2 *C. florïda* subspecies *urbiniana* open pollinated plants from the USDA National Arboretum native to Mexico, no replication.
Table 6.10. Photosynthesis and gas exchange measurements for greenhouse-grown *Cornus* taxa.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Accession/provenance</th>
<th>Maximum photosynthesis (μmol CO₂ m⁻²·s⁻¹)</th>
<th>Stomatal conductance (mol H₂O m⁻²·s⁻¹)</th>
<th>Transpiration (mmol H₂O m⁻²·s⁻¹)</th>
<th>Internal CO₂ (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. florida</em></td>
<td>MO, USA</td>
<td>10.2a²</td>
<td>0.135ab</td>
<td>1.81ab</td>
<td>261ab</td>
</tr>
<tr>
<td><em>C. florida</em></td>
<td>KY, USA</td>
<td>8.4ab</td>
<td>0.171a</td>
<td>2.24a</td>
<td>290a</td>
</tr>
<tr>
<td><em>C. kousa</em></td>
<td>Korea</td>
<td>6.1b</td>
<td>0.057c</td>
<td>0.84b</td>
<td>207c</td>
</tr>
<tr>
<td><em>C. kousa</em></td>
<td>China 13</td>
<td>7.4ab</td>
<td>0.113abc</td>
<td>1.45ab</td>
<td>269ab</td>
</tr>
<tr>
<td><em>C. kousa</em></td>
<td>China 14</td>
<td>7.0b</td>
<td>0.088bc</td>
<td>1.35ab</td>
<td>249abc</td>
</tr>
<tr>
<td><em>C. kousa</em></td>
<td>China 15</td>
<td>10.0a</td>
<td>0.109abc</td>
<td>1.47ab</td>
<td>234bc</td>
</tr>
<tr>
<td><em>C. nuttallii</em></td>
<td>CA, USA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ANOVA P Value</td>
<td></td>
<td>0.0004</td>
<td>0.0071</td>
<td>0.0129</td>
<td>0.0011</td>
</tr>
</tbody>
</table>

²means within a column followed by the same letter were not significantly different (Tukey’s HSD α=0.05).
Table 6.11. Gas exchange parameters for greenhouse-grown *C. florida* and *C. kousa* taxa.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Accession/provenance</th>
<th>Quantum efficiency</th>
<th>Light compensation point (μmol light m⁻²·s⁻¹)</th>
<th>CO₂ compensation point (ppm)</th>
<th>Carboxylation efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. florida</em></td>
<td>MO, USA</td>
<td>0.048</td>
<td>21.5</td>
<td>65.0</td>
<td>0.048</td>
</tr>
<tr>
<td><em>C. kousa</em> var. <em>chinensis</em></td>
<td>China #15</td>
<td>0.047</td>
<td>21.6</td>
<td>68.0</td>
<td>0.050</td>
</tr>
<tr>
<td>ANOVA <em>P</em> Value</td>
<td></td>
<td>0.7981</td>
<td>0.9850</td>
<td>0.7803</td>
<td>0.7569</td>
</tr>
</tbody>
</table>
Table 6.12. Slope of the relationship between container weight as a percentage of initial weight measured at 15 minute intervals for two *Cornus* species at two VPD\textsubscript{air} treatments.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Accession/Provenance</th>
<th>VPD\textsubscript{air} (kPa)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.5</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td><em>C. florida</em></td>
<td>MO, USA</td>
<td>-0.0010a\textsuperscript{2}</td>
<td>-0.0019a</td>
<td></td>
</tr>
<tr>
<td><em>C. kousa</em></td>
<td>China, #15</td>
<td>-0.0006b</td>
<td>-0.0014b</td>
<td></td>
</tr>
</tbody>
</table>

ANOVA P Value
<0.0001 0.0217

\textsuperscript{2}Means followed by the same letter were not significantly different (Tukey’s HSD \( \alpha \)=0.05).
Table 6.13. Percentage of initial weight for *C. florida* (MO, USA provenance) and *C. kousa var. chinensis* (accession 15). Plants were irrigated thoroughly by hand, containers were bagged and sealed around the container, and irrigation was withheld.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Time since last irrigation (hours)</th>
<th>Container Weight (% of initial)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. florida</em></td>
<td>24</td>
<td>88a²</td>
</tr>
<tr>
<td><em>C. kousa</em></td>
<td></td>
<td>92b</td>
</tr>
<tr>
<td>ANOVA P Value</td>
<td></td>
<td>0.0159</td>
</tr>
<tr>
<td><em>C. florida</em></td>
<td>48</td>
<td>76a</td>
</tr>
<tr>
<td><em>C. kousa</em></td>
<td></td>
<td>82b</td>
</tr>
<tr>
<td>ANOVA P Value</td>
<td></td>
<td>0.0062</td>
</tr>
<tr>
<td><em>C. florida</em></td>
<td>72</td>
<td>64a</td>
</tr>
<tr>
<td><em>C. kousa</em></td>
<td></td>
<td>73b</td>
</tr>
<tr>
<td>ANOVA P Value</td>
<td></td>
<td>0.0019</td>
</tr>
<tr>
<td><em>C. florida</em></td>
<td>96</td>
<td>57a</td>
</tr>
<tr>
<td><em>C. kousa</em></td>
<td></td>
<td>65b</td>
</tr>
<tr>
<td>ANOVA P Value</td>
<td></td>
<td>0.0056</td>
</tr>
<tr>
<td><em>C. florida</em></td>
<td>120</td>
<td>51a</td>
</tr>
<tr>
<td><em>C. kousa</em></td>
<td></td>
<td>58b</td>
</tr>
<tr>
<td>ANOVA P Value</td>
<td></td>
<td>0.0086</td>
</tr>
</tbody>
</table>

²means within a column section followed by the same better were not significantly different (Tukey’s HSD α=0.05).
Table 6.14. Leaf water potential for *C. florida* (MO, USA) and *C. kousa* var. *chinensis* (accession 15). Plants were irrigated thoroughly by hand and then water was withheld for eight days.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Substrate moisture content (% container capacity)</th>
<th>Leaf water potential (MPa)</th>
<th>Plants in moisture content range (# of plants)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. florida</em></td>
<td>96-100</td>
<td>-1.2a&lt;sup&gt;2&lt;/sup&gt;</td>
<td>4</td>
</tr>
<tr>
<td><em>C. kousa</em></td>
<td>-1.1a</td>
<td></td>
<td>5</td>
</tr>
</tbody>
</table>

ANOVA P Value | 0.3240

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Substrate moisture content (% container capacity)</th>
<th>Leaf water potential (MPa)</th>
<th>Plants in moisture content range (# of plants)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. florida</em></td>
<td>91-95</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td><em>C. kousa</em></td>
<td>-</td>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>

ANOVA P Value | -

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Substrate moisture content (% container capacity)</th>
<th>Leaf water potential (MPa)</th>
<th>Plants in moisture content range (# of plants)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. florida</em></td>
<td>86-90</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td><em>C. kousa</em></td>
<td>-</td>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>

ANOVA P Value | -

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Substrate moisture content (% container capacity)</th>
<th>Leaf water potential (MPa)</th>
<th>Plants in moisture content range (# of plants)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. florida</em></td>
<td>81-85</td>
<td>-1.34</td>
<td>3</td>
</tr>
<tr>
<td><em>C. kousa</em></td>
<td>-</td>
<td></td>
<td>3</td>
</tr>
</tbody>
</table>

ANOVA P Value | -
Table 6.14g Continued. Leaf water potential for *C. florida* (MO, USA) and *C. kousa* var. *chinensis* (accession 15). Plants were irrigated thoroughly by hand and then water was withheld for eight days.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Substrate moisture content (% container capacity)</th>
<th>Leaf water potential (MPa)</th>
<th>Plants in moisture content range (# of plants)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. florida</em></td>
<td>76-80</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td><em>C. kousa</em></td>
<td>-1.23</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

ANOVA P Value

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Substrate moisture content (% container capacity)</th>
<th>Leaf water potential (MPa)</th>
<th>Plants in moisture content range (# of plants)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. florida</em></td>
<td>71-75</td>
<td>-1.54a</td>
<td>3</td>
</tr>
<tr>
<td><em>C. kousa</em></td>
<td>-1.38a</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

ANOVA P Value

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Substrate moisture content (% container capacity)</th>
<th>Leaf water potential (MPa)</th>
<th>Plants in moisture content range (# of plants)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. florida</em></td>
<td>61-70</td>
<td>-1.8b</td>
<td>5</td>
</tr>
<tr>
<td><em>C. kousa</em></td>
<td>-1.4a</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

ANOVA P Value 0.0064

*Means followed by the same letter within the same column section were not significantly different (Tukey’s HSD α=0.05).*
Figure 6.1abc. Leaf sections of *C. florida* ‘Cherokee Princess’ (a), *C. kousa* ‘National’ (b), and *C. kousa* x *C. florida* Constellation® (c).
Figure 6.2. Epidermal peel (above) and electron microscopy (below) images of the abaxial leaf surface of controlled environment-grown *C. florida* (KY, USA provenance) (left) and *C. kousa* (Korean provenance) (right). Figure 6.2a-b depicts stomatal density. Red dots denote counted stomates, black dots are papillae. Figure 6.2c-d depicts surface wax and stomate size. Figure 6.2e-f depicts trichomes and surface wax patterns.
Appendix A
List of Symbols

LE = latent heat of vaporization, expressed at latent heat flux, (W·m⁻²)

Δ = slope of the saturated vapor pressure curve

Rₚₙ = net radiation at the canopy level (W·m⁻²)

G = soil/substrate heat flux (W·m⁻²)

ρₐᵢʳ = density of air (kg·m⁻³)

cₚₐ = specific heat of air at constant pressure (J·kg⁻¹·C⁻¹)

Aₘₐₓ = Maximum photosynthetic rate

Qₚₜₚ = Apparent quantum yield

LCP = Light compensation point

Rₜₜ = dark respiration

VP = vapor pressure

VPₐᵢʳ = water vapor in the air (kPa)

VPₚₙₜ = the maximum amount of water vapor the air can hold (kPa)

VPDₐᵢʳ = air vapor pressure deficit (kPa)

VPDₙₑₙₙₜ = leaf vapor pressure deficit (KPa)

rₗ = resistance for sensible heat transfer by convection (s·m⁻¹)

γ = psychometric constant (Pa·°C⁻¹)

rₛ = canopy surface resistance, i.e., resistance to evapotranspiration (s·m⁻¹)
Appendix B

Calibration of Soil Moisture Probes

The following process, modified from a procedure provided by the manufacturer of the ECHO-5 probe (Decagon Devices, Inc., Pullman, WA), was used to calibrate probes for model development and evaluation.

1. Weigh and label 5 identical containers. Enter container weight into spreadsheet cells E2–E7.

2. Weigh out 5 (or 6) identical batches of substrate and put each in a second set of containers.

3a. Put the first batch of substrate into container #1 without adding any water to the substrate. Press down with palm of hand firmly but gently on the surface of the substrate. Insert probe diagonally into substrate. Do not allow the end to touch the sides or bottom of the container. Press firmly but gently again on the surface of the substrate to ensure contact between probe and substrate.

3b. Record mV readings and the probe ID number.

Repeat for the other probes. Insert the next probe in the opening left where the previous probe was removed. Always firm the substrate after installing each probe. Conduct each step uniformly from probe to probe.

4a. Put the second batch of substrate into a bag. Add 50 ml water (any kind of water is fine).

4b. Mix extremely thoroughly. Put all of wetted substrate into the properly labeled container. Press down with palm of hand firmly but gently on the surface of the substrate. Insert probe diagonally into substrate. Do not allow the end to touch the sides or bottom of the container. Press firmly but gently again on the surface of the substrate to ensure contact between probe and substrate.

5. Record mV readings in the cell for that probe # and substrate sample #.

6. Repeat for the other probes as described above. Apply firm but gentle pressure when putting a new batch of substrate into the container. Insert the next probe in the opening left where the previous probe was removed. Always firm the substrate after installing each probe. Do each step uniformly from probe to probe, especially the pressure applied when firming the substrate. Seal the container with the lid.

7. Repeat with the third batch of substrate. Add 100 ml water and repeat steps listed in 4.b.

8. Repeat with 4th batch of substrate. Add 150 ml water and repeat steps listed in 4.b.

9. Repeat with 5th batch of substrate. Add 200 ml water and repeat steps listed in 4.b.

10. Repeat with 6th batch of substrate. Add 300 ml water and repeat steps listed in 4.b.
11. After recording the mV reading for the last probe, draw a line on the side of each container at the height of the surface of the substrate.
12. Weigh the container without the lid. Enter into spreadsheet cells C2 – C7.
13. Oven-dry each container at 55 °C.
15. Remove substrate. If container wasn’t weighed in the beginning, use wipe or paint brush to remove last bits of substrate and weigh empty container.
16. Pour water into each empty container to the line that marked the substrate surface level. Pour water into graduated cylinder, measure the volume of water, and record. Enter in cells J2-J7.
17. Graph the relationship between volumetric water content and mV output of probes.

ECHO-5 probes were calibrated and the data were regressed against volumetric water content (Θ) measured during the calibration (Figure B.1). The inverse of this equation was used to calculate Θ from the raw mV output of the sensors. Θ = 0.0015(mV) - 0.3396, r²=0.999.
Figure B.1. Relationship between mV output of ECHO-5 soil moisture probe and volumetric water content. $mV = 227.2167 + 690.7888 \times \text{VWC}$, $r^2 = 0.99$. 

![Graph showing the relationship between sensor output (mV) and volumetric water content (m^3 m^-3).](image)
Appendix C

Development of Leaf Water Potential Sampling and Measurement Protocol

*Measurement Period in Potentiometer*

An experiment was conducted to determine the minimum measurement time (time each leaf disk was in the potentiometer) needed before recording the leaf water potential. One leaf was collected predawn from each of five well-watered plants and prepared as follows: A droplet of purified water was dropped onto the leaf blade and 400 grit wet/dry sandpaper was gently stroked eight times across the leaf from the midvein to the margin. A lint-free cloth was used to dry the leaf blade. The leaf was then detached from the plant and cut along the midvein. The half that was sanded was wrapped in Glad® Cling Wrap (The Glad Products Co., Oakland, CA), sealed in a plastic bag with a lint-free cloth dampened with deionized water, placed on ice, and refrigerated until processed. Each sample was partially unwrapped, maintaining a seal on the sanded side of the leaf (adaxial). Sheet metal fit to the rim of a sample cup was used to cut a sample disk the proper size. The leaf disk was immediately placed in a clean sample cup, adaxial surface facing up, and placed in the potentiometer. Samples were measured in the potentiometer the same day at 25.1 °C. Harvested leaves were sampled in a random order. The experiment was a completely randomized design with five replications. A measurement period of 20 minutes was determined to be conservative yet efficient (Table C.1).
Table C.1. Relationship between sampling time and leaf water potential for well-watered *H. ‘Cashmere Wind’*.

<table>
<thead>
<tr>
<th>Minutes</th>
<th>MPa</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>-0.79c²</td>
</tr>
<tr>
<td>9</td>
<td>-0.70b</td>
</tr>
<tr>
<td>13</td>
<td>-0.65ab</td>
</tr>
<tr>
<td>17</td>
<td>-0.63ab</td>
</tr>
<tr>
<td>21</td>
<td>-0.59a</td>
</tr>
<tr>
<td>25</td>
<td>-0.61a</td>
</tr>
<tr>
<td>29</td>
<td>-0.61a</td>
</tr>
<tr>
<td>33</td>
<td>-0.58a</td>
</tr>
</tbody>
</table>

ANOVA P value < 0.0001

²means followed by the same letter were not significantly different (Bonferroni α = 0.05).
**Sanding**

An experiment was conducted to determine if sanding was an essential component to the leaf harvest and preparation procedure. Two leaves were collected predawn on each of five well-watered plants. One leaf was prepared for water potential measurements as described in “Final Protocol for Chilled Mirror Dew Point Potentiometer”.

Samples were measured in the potentiometer the same day using a minimum 20 minute runtime at 25.1 °C. The non-sanded leaves were treated identically with the exception of sanding. Harvested leaves from an individual pair were run consecutively and in a random order so that for some pairs the sanded leaf was sampled first and in other pairs the non-sanded leaf was sampled first. Sanding was a necessary component of the pre-sampling harvest/processing protocol in order to achieve leaf water potential measurements on well-watered plants that were consistent with the literature (Table C.2).
Table C.2. Relationship between sanding treatments and leaf water potential for *H. ‘Cashmere Wind’*.

<table>
<thead>
<tr>
<th>Sanding treatment</th>
<th>Leaf water potential (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sanded</td>
<td>-0.58&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Not Sanded</td>
<td>-1.89&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

ANOVA P value < 0.0001

<sup>«</sup>means followed by the same letter were not significantly different (Bonferroni α = 0.05).
Delay Prior to Measurement in Potentiometer

In an above mentioned experiment, a 20-minute sampling time was determined to be the shortest yet conservative measurement time acceptable. Use of a 20-minute measurement period required a total sample time of 30 minutes per sample, which restricted the number of samples that could be processed in the potentiometer each day. Therefore, an experiment was conducted to determine whether samples could be collected one day but not measured in the potentiometer until the following day, a delay of approximately 24 h. Two leaves from each of five plants were collected and prepared as described in “Final Protocol for Chilled Mirror Dew Point Potentiometer”. One leaf from each pair was measured the day of collection. The other leaf from each pair remained refrigerated until the following day when it was measured. A slight but significant reduction in leaf water potential occurred after a 24 delay (Table C.3). It was concluded that samples should only be measured on the day of collection.
Table C.3. The effect of delay after sampling on leaf water potential for *H. ‘Cashmere Wind’*.

<table>
<thead>
<tr>
<th>Delay (Hours)</th>
<th>Leaf water potential (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>-0.66a²</td>
</tr>
<tr>
<td>24</td>
<td>-0.75b</td>
</tr>
</tbody>
</table>

ANOVA P value < 0.0001

²means followed by the same letter were not significantly different (Bonferroni α = 0.05).
Time of Sampling for Leaf Water Potential

An experiment was conducted to determine if leaves for leaf water potential measurements could be collected predawn. A single leaf from each of eight plants subjected to one of four of irrigation setpoints was sampled. Leaves were prepared as described in “Final Protocol for Chilled Mirror Dew Point Potentiometer” except they were collected predawn. Leaves from plants in a single irrigation treatment were collected and measured on a given day. Leaves from all four irrigation setpoints were sampled and measured within a one-week period. The experiment used a completely randomized design with four treatments and eight replications. Predawn water potential measurements indicated plants were recovering or partially recovering from water deficits during the night; therefore, midday leaf collections were used in the model evaluation experiment (Table C.4).
Table C.4. Relationship between predawn leaf water potential and irrigation treatment for *H. ‘Cashmere Wind’*.

<table>
<thead>
<tr>
<th>Irrigation setpoint</th>
<th>Predawn leaf water potential (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>89/100</td>
<td>-0.51\textsuperscript{a}</td>
</tr>
<tr>
<td>81/100</td>
<td>-0.49\textsuperscript{a}</td>
</tr>
<tr>
<td>69/98</td>
<td>-0.68\textsuperscript{b}</td>
</tr>
<tr>
<td>61/69</td>
<td>-0.62\textsuperscript{ab}</td>
</tr>
</tbody>
</table>

ANOVA P value 0.0052

\textsuperscript{a} means followed by the same letter were not significantly different (Duncan’s \( \alpha = 0.05 \)).
Final Protocol for Chilled Mirror Dew Point Potentiometer

A chilled mirror dew point potentiometer (model WP4-T, Decagon Devices, Pullman, WA) was used to determine water potential during the model evaluation. One leaf per plant was selected for water potential measurements. One leaf was collected predawn from each of five well-watered plants and prepared as follows: A droplet of purified water was dropped onto the leaf blade and 400 grit wet/dry sandpaper was gently stroked eight times across the leaf from the midvein to the margin. A lint-free cloth was used to dry the leaf blade. The leaf was then detached from the plant and cut along the midvein. The half that was sanded was wrapped in Glad® Cling Wrap (The Glad Products Co., Oakland, CA), sealed in a plastic bag with a lint-free cloth damped with deionized water, placed on ice, and refrigerated until processed. Each sample was partially unwrapped, maintaining a seal on the sanded side of the leaf (adaxial). Sheet metal fit fashioned to the circumference of a sample cup was used to cut a sample disk the proper size. The leaf disk was immediately placed in a clean sample cup, adaxial surface facing up, and placed in the potentiometer. Samples were measured in the potentiometer after 20 minutes of equilibration time at 25.1 °C. Samples were processed the same day they were collected.
Appendix D

Development and Evaluation of a Leaf Area Model

A model was developed to determine if leaf area from a leaf biometric could be quickly and nondestructively measured. On October 30, 2007 forty leaves were collected from *H. ‘Cashmere Wind’* stock plants that had been maintained in a well-watered condition. Leaves were selected to range from small, immature leaves to fully expanded leaves (Figure D.1). Petioles were removed, leaves labeled, and length and width measured. Leaf area was measured with a standard conveyer leaf area meter (model LI-3100, LI-COR® Biosciences, Lincoln, NE). All possible combinations of length, width, length², width² were regressed against leaf area. Length, width, and width² were significantly related to leaf area. Width² was the factor most strongly associated with leaf area; the greatest portion of variation was attributable to width² (Table D.1). The relationship between leaf area and leaf width² was determined as a linear polynomial, 

\[
\text{width}^2 = -0.4499 + 1.1858 \times \text{conveyer leaf area}, \quad \text{with } R^2 = 0.99 \quad \text{(Figure D.2)}. 
\]

The inverse of this equation was used to calculate leaf area from width measurements, leaf area = 0.379+0.843*(width²). On November 20, 2008, 58 leaves were collected and subjected to the inverse equation to evaluate the leaf area model and the conveyer leaf area meter (Figure D.3). The slope for the predicted leaf area and the measured leaf area was 1.0614, showing a near 1:1 relationship and, thus, agreement between the two methods of obtaining leaf area.
Table D.1. Analysis of variance for a leaf area model for *H. ‘Cashmere Wind’* based on leaf width$^2$.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of squares</th>
<th>Mean square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>3</td>
<td>69080.75810</td>
<td>23026.9137</td>
<td>11119.8</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Error</td>
<td>37</td>
<td>76.61990</td>
<td>2.07081</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uncorrected</td>
<td>40</td>
<td>69157.37800</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Total**

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of squares</th>
<th>Mean square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-Square</td>
<td></td>
<td>69157.37800</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coeff Var</td>
<td></td>
<td>4.044663</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Root MSE</td>
<td></td>
<td>1.439030</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean leaf area</td>
<td></td>
<td>35.57850</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Source**

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Type I SS</th>
<th>Mean square</th>
<th>F Value</th>
<th>Pr&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length</td>
<td>1</td>
<td>64594.76283</td>
<td>64594.76283</td>
<td>31193.0</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Width</td>
<td>1</td>
<td>416.98910</td>
<td>416.98910</td>
<td>201.37</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Width$^2$</td>
<td>1</td>
<td>4069.00617</td>
<td>4069.00617</td>
<td>1964.94</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

**Source**

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Type III SS</th>
<th>Mean square</th>
<th>F Value</th>
<th>Pr&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length</td>
<td>1</td>
<td>161.699042</td>
<td>161.699042</td>
<td>78.08</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Width</td>
<td>1</td>
<td>129.420424</td>
<td>129.420424</td>
<td>62.50</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Width$^2$</td>
<td>1</td>
<td>4069.006169</td>
<td>4069.006169</td>
<td>1964.94</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>
Figure D.1. Range of leaf size collected for development of a leaf area model for *H. ‘Cashmere Wind’*. 
Figure D.2. Model development: relationship between non-destructive and harvested leaf area measurements for H. 'Cashmere Wind'

\[ \text{width}^2 = -0.4499 + 1.1858 \times \text{conveyer leaf area (cm)}, r^2=0.9872. \]
Figure D.3. Model evaluation: evaluation of leaf area model based on leaf width$^2$.

(Generated with predicted leaf area = width$^2$(0.8433) + 0.3794.)

This line is predicted leaf area = 1.0614 * (conveyer leaf area) - 1.8961, $r^2 = 0.9559$. 
Appendix E

Light and CO₂ Response Curves for *H. ‘Cashmere Wind’*

Figure E.1. Relationship between high light intensity and photosynthetic rate for *H. ‘Cashmere Wind’. Photosynthesis=17.8774*Light Intensity/(364.3904+Light Intensity), \( r^2 = 0.9986 \). The CO₂ provided to the cuvette was 400ppm.
Figure E.2. Relationship between low light intensity and photosynthetic rate for H. ‘Cashmere Wind’. Photosynthesis = -1.755 + 0.041* Light Intensity, $r^2=0.99$. The CO$_2$ provided to the cuvette was 400 ppm.
Figure E.3. Relationship between internal CO₂ concentration and photosynthetic rate for *H. ‘Cashmere Wind’*. Light intensity provided to the cuvette was 800 µmol m⁻² s⁻¹.
Appendix F

Viscous sap of *H. ‘Cashmere Wind’*

Figure F.1. Viscous sap exudates at severed end of petiole of *Hibiscus ‘Cashmere Wind’*. 
Appendix G

Experimentation to Determine an Effective Plant Water Potential Measurement Technique for *Hibiscus* ‘Cashmere Wind’

Leaf relative water content (RWC) was measured during the model development experiment. *H. ‘Cashmere Wind’* leaves were detached at 8 am and sealed in a plastic bag and weighed on an analytical scale. They were placed in a Petri® dish with deionized water for 24 hours at 40 °C in the dark. After 24 hours leaves were quickly patted dry, reweighed, and oven dried for 48 hours. One leaf per plant was collected daily. The experiment used a completely randomized design with four irrigation treatments and 12 replications. A viscous exudate drained from the cut end of the petiole after the 24 hour rehydration period (Figure F.1). There was no relationship between RWC and days withholding irrigation (Table G.1).
Table G.1. Relationship between days after last irrigation and leaf relative water content for *H. ‘Cashmere Wind’*.

<table>
<thead>
<tr>
<th>Day(s) after last irrigation</th>
<th>Relative water content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.923</td>
</tr>
<tr>
<td>3</td>
<td>0.898</td>
</tr>
<tr>
<td>4</td>
<td>0.922</td>
</tr>
<tr>
<td>6</td>
<td>0.908</td>
</tr>
<tr>
<td>7</td>
<td>0.898</td>
</tr>
<tr>
<td>8</td>
<td>0.913</td>
</tr>
</tbody>
</table>

ANOVA P value 0.1493

Superscripts followed by the same letter were not significantly different (Tukey’s HSD \( \alpha = 0.05 \)).
Preliminary results utilizing a pressure bomb for leaf water potential on *H. ‘Cashmere Wind’* indicated this technique was unreliable for this taxon. In order to determine the accuracy of pressure bomb-generated leaf water potential measurements for *H. ‘Cashmere Wind’*, uniform one gallon plants were placed on a controlled water table at 1, 4, 7, and 10 cm heights and allowed to equilibrate for four weeks. When the weight of the containers stabilized, the containers were considered equilibrated. One leaf was collected per plant, placed in a plastic bag, put on ice and immediately subjected to the pressure bomb. The experiment was a completely randomized design with eight replications. In a second experiment, pressure bomb measurements were taken on uniform, well watered *H. ‘Cashmere Wind’* plants. No differences in water potential were detected by the pressure bomb despite obvious differences in plant water content i.e. some plants fully turgid, others had very flacid leaves (Table G.2 and G.3). In addition, there was considerable variation in leaf water potential readings.
Table G.2. Pressure bomb measurements of stem water potential of *H. ‘Cashmere Wind’* subjected to four irrigation treatments (controlled water table heights).

<table>
<thead>
<tr>
<th>Controlled water table height (cm)</th>
<th>Stem water potential (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-1.31</td>
</tr>
<tr>
<td>4</td>
<td>-1.13</td>
</tr>
<tr>
<td>7</td>
<td>-1.07</td>
</tr>
<tr>
<td>10</td>
<td>-0.79</td>
</tr>
</tbody>
</table>

ANOVA P value 0.2624
Table G.3. Pressure bomb measurements of stem water potential of well-watered *H. ‘Cashmere Wind’.*

<table>
<thead>
<tr>
<th>Plant ID (number)</th>
<th>Stem water potential (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>-3.0</td>
</tr>
<tr>
<td>29</td>
<td>-0.5</td>
</tr>
<tr>
<td>12</td>
<td>-2.0</td>
</tr>
<tr>
<td>1</td>
<td>-13.0</td>
</tr>
<tr>
<td>38</td>
<td>-1.5</td>
</tr>
<tr>
<td>4</td>
<td>-1.5</td>
</tr>
<tr>
<td>42</td>
<td>-1.0</td>
</tr>
<tr>
<td>19</td>
<td>-2.5</td>
</tr>
<tr>
<td>30</td>
<td>-0.5</td>
</tr>
<tr>
<td>37</td>
<td>-0.5</td>
</tr>
<tr>
<td>11</td>
<td>-3.0</td>
</tr>
<tr>
<td>23</td>
<td>-1.0</td>
</tr>
</tbody>
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