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Final Report on Gus Poesch Grant
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Research project description

The ASHS conference provides a great opportunity for graduate students to present their research, gain feedback, and interact with other students and members of the scientific community. I started as a PhD student in the laboratory of Dr. Michelle Jones at The Ohio State University in Jan 2016. I plan to present some of my dissertation research investigating the use of beneficial bacteria in the greenhouse industry at the ASHS annual conference in Waikolah, Hawaii, in September 2017. With the increasing concern over the overuse of pesticides and other chemicals, there is a growing interest in biological products to promote plant growth and quality. These biological products often include beneficial bacteria. This is bacteria that can benefit the plant by reducing disease, stimulating growth, or overcoming an abiotic stress (Cepeda Miranda, 2012). The use of beneficial bacteria in the greenhouse industry is the focus of my PhD dissertation and the research that I will present at the ASHS conference.

The Microbial Bioproducts Scale-up and Applications (MBSA) team at the Ohio State University is studying the possibility of implementing plant-associated microbial products into production systems. Previous projects conducted by the MBSA team show promising results for the use of the microbes in the greenhouse industry. Past projects in the Jones lab at OSU have focused on several bacterial strains including *Pseudomonas* strains that can increase plant tolerance to drought stress, salt stress, low fertility stress, and Botrytis control in floriculture crops. A bacterial product that will work effectively and consistently is of interest to the floriculture industry to promote a more environmentally sustainable production system. This summer I will begin working on the AFE funded project "*Use of beneficial microbes to enhance plant growth, decrease disease severity and improve stress tolerance in ornamentals*". The goal of this project is to investigate the efficacy of selected *Pseudomonas* strains (identified by the OSU MBSA team) for increasing growth, drought stress tolerance and botrytis resistance in containerized ornamentals. A number of commercially available products will also be included in these trials to provide growers with additional and immediately applicable information about the use of these products in greenhouse production systems.

Impact of the project

The floriculture industry faces challenges of meeting demands, producing plants with quality and quantity, and taking into account the impact the production process has on the environment. Juggling the many aspects of growing beautiful floriculture crops is no easy task. Cutting back on chemical use is important when considering environmental impact, but to cut

back on chemical use, there has to be a viable alternative. When considering botrytis, this pathogen has a wide host range and can cause devastating losses. Botrytis is also resistant to several fungicides (Gould, et al. 1996). Beneficial microbes that could control botrytis would be an excellent alternative for growers to turn to as something to integrate into their systems when these issues arise. Salinity and drought issues can reduce crop growth and nutrient uptake (Siddikee et al. 2011). This will lead to a longer production time or unsalable plants. Utilizing plant-microbe relationships in the production system could lead to the plants ability to tolerate or recover from these abiotic stresses. For a bacterial product to be successful in a production system, the results of the product need to be consistent and easy to apply. There is a lot of information missing pertaining to the use of these microbes in greenhouse production systems, and the proposed research will lead to answers and additional questions. The industry also has a lot of questions related to bacterial products used as stimulants or biocontrol, and the aim of this project is to provide some answers and recommendations for growers.

Importance of attending the ASHS (American Society for Horticultural Science) conference

With the topic of biological products, there needs to be collaboration to learn new techniques, what not to do, and ways to build on others' research. A crucial aspect of research is communication with other researchers, departments, businesses, universities, and organizations. To achieve the goals of the floriculture industry, scientific meetings are needed to discuss and form ideas. People representing all of these aspects of the horticulture industry have different knowledge and backgrounds, which makes the calibration beneficial. Graduate students are an important part of these scientific meetings because of the people they meet and the network they form to use in their project and after they graduate. Not only that, but teaching the younger generation how the scientific meetings run and how ideas are shared is important in keeping this type of meeting continuing in the future. This meeting is beneficial to floriculture because this is an opportunity for floriculture researchers from across the United States to discuss new findings. It also gives an opportunity to learn from other aspects of horticulture and find new ideas to apply to the floriculture industry. The ASHS conference is the perfect place to combine these vital aspects of research. This week-long travel to present at the conference and visit greenhouse producers in Hawaii will bring about new ideas, new research relationships, and solutions to problems.

As a graduate student, I am learning about the floriculture industry and research as well as growing professionally. A scientific meeting gives the opportunity to learn from others through presentations, new technology, and one-on-one conversations. The ASHS conference would allow me to see other areas of research as well as research being done in the area that I am interested in. Learning sessions will give me an overview of current research areas of interest. It is important to me to be aware and interested in other topics outside of my specific area of study. I also would like to attend the ASHS meeting to grow professionally. Learning and practicing how to present and discuss research findings is also an important part of research. The project described previously would be presented at the 2017 ASHS conference. This would give me the opportunity to present my project, answer questions, and discuss possible new ideas. Not only would I gain experience

through presenting the research, but also learn from other presentation styles that are given throughout the conference. While a graduate student, I would like to become more involved in industry organization and events not only to learn but to give back to the floriculture industry. Participating in the ASHS conference would provide an opportunity to become more involved in the floriculture industry. Through attending the conference, I would have the opportunity to learn more of the industry, learn more related to my research topic, and gain tools necessary to complete my degree and become a contributing member of the horticulture scientific community.

FINAL REPORT: Attending the ASHS 2017 Annual Conference

I had the opportunity to attend the American Society for Horticultural Science 2017 Annual Conference in Waikoloa, Hawaii. This was a truly amazing conference, as I learned about many different research topics in horticulture, grew professionally, and experienced a part of the United States in which I had never traveled. Many research presentations and workshops were offered each day of the conference. I learned of research being conducted in different aspects of the floriculture industry across the U.S., and I learned about other topics such as grant writing, teaching methods, and climate change. I was also able to attend a few Professional Interest Group meetings, including the Floriculture meeting and the Graduate Student meeting. These meetings allowed me to see more of how the ASHS conference works and to meet new people.

Attending meetings such as this one is a great way to grow professionally. I presented a poster titled "Ice cube irrigation of potted *Phalaenopsis* orchids does not decrease display life" in the PhD Poster Competition. This poster contained data that has recently been published in the journal HortScience (see attached). The first year of my PhD, I worked on this project in collaboration with Green Circle Growers (Oberlin, OH) and the University of Georgia. I am currently working on research involving the effects of beneficial microbe applications on *Botrytis* tolerance in floriculture crops. Originally I had planned to also present the beneficial microbe work at the ASHS conference, but the results were too preliminary when abstracts were due. This work will be presented at future meetings. During the poster session, I gained feedback on the project with *Phalaenopsis* orchids and ice irrigation, learned from other horticulture graduate students, and also learned about other research being done with microbes in other horticulture crops. I made many new contacts, and got to reconnect with old contacts.

Another great learning experience during the conference was the professional tours. I was able to go on two of these tours. On the first tour, we traveled across the Big Island, visiting an orchid grower with many different cultivars of orchids, a macadamia nut producer and shop, and many volcano viewing spots. The second tour was on tropical fruits and propagation practices on the island. I got to see different kinds of fruit producers, ornamental plant producers, and research facilities. These tours gave me the opportunity to learn of Hawaii's history, traditions, and agriculture.

Overall, this meeting allowed me to travel and experience a place I never thought I would visit, present a research project, gain feedback on research methods, learn from fellow graduate students and researchers, network, and learn more about ASHS. Thank you for the opportunity to travel to the ASHS Annual Conference.

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ASHS abstract:

Ice cube irrigation of potted *Phalaenopsis* orchids does not decrease display life

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Historically, orchids have been associated with the wealthy and orchid enthusiasts, but today *Phalaenopsis* orchids are readily available to all consumers due to the advancements in production, breeding, and propagation. Proper irrigation is a challenge faced by consumers, but plant care instructions can alleviate the problems of over or under watering. Ice cube irrigation is a method promoted to give structured, straight forward instructions for irrigating orchids. Chilling or freezing damage caused by the ice is a concern because *Phalaenopsis* orchids are native to tropical regions. The objective of this study was to evaluate the health and display life of *Phalaenopsis* orchids irrigated with ice or room temperature water. The experiment was conducted at The Ohio State University (Wooster, OH) and University of Georgia (Athens, GA). At each location, 24 orchids (6 plants of 4 different cultivars) were irrigated with three ice cubes and 24 orchids were irrigated with the equivalent volume of room temperature water every week. The longevity of an individual flower and the overall display life of the whole plant were determined. Plants were maintained in an interior evaluation room for 4 to 6 months, until the last flower on the plant senesced. Leachate volumes were measured to determine how much water was used by the plant or held by the bark growing media. The chlorophyll content of the leaves, quantum yield of photosystem II of the leaves and roots, and final dry weights of the leaves and roots were used to monitor the effect of ice irrigation on the health of the plants. The temperature in the media was

also monitored during irrigation events. Flower longevity and display life were the same in ice and water irrigated plants of all cultivars. The efficiency of photosystem II and chlorophyll content also showed no treatment effect. The leachate volume after ice irrigation was equivalent or lower than the leachate volume after water irrigation. Leaf and root dry weights showed similar results between the two irrigation methods. The temperature of the bark after ice irrigation reached a low of 11°C. Ice irrigation did not cause early flower loss or damage the plant's photosynthetic health, while providing a sufficient amount of water to the orchid. A consumer's success with their potted plants is an important aspect of the industry, and irrigating with ice should be considered a viable irrigation option for *Phalaenopsis* orchids in bark media.

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Ice cube irrigation

For more information, see the article by South, et al. that begins on p. 1271

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Ice Cube Irrigation of Potted *Phalaenopsis* Orchids in Bark Media Does Not Decrease Display Life

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Abstract. ***Phalaenopsis*** **orchids are an increasingly popular potted house plant in the United States. New cultivars have a long display life in home environments, but these epiphytes are often overirrigated by consumers. Irrigating potted *Phalaenopsis* orchids weekly with ice cubes has been recommended as a simple solution to help consumers, but concern has been raised about whether the ice cubes will cause low temperature damage in these tropical plants. The effect of ice cube irrigation on the display life and quality of four cultivars of potted *Phalaenopsis* orchids was, therefore, evaluated. Irrigation treatments included weekly application of three ice cubes or the equivalent volume of room-temperature tap water. The longevity of individual flowers and the overall display life of the orchid plants were determined. Monthly measurements determined the volume of leachate in the outer decorative pots after irrigation. The quantum yield of photosystem II (Φ_{PSII}) in roots and leaves was evaluated monthly to determine if photosynthetic efficiency was affected by the ice irrigation. The temperature in the orchid bark growing media during irrigation events was recorded, and a programmable antifreeze bath was used to determine the temperature at which damage to PSII was observed in orchid roots. The flower longevity and display life were unaffected by irrigation treatment. In general, the leachate volume over time was the same or lower in ice irrigated orchids compared with those irrigated with the same volume of water. The lowest temperature in the bark media irrigated with ice cubes was ≈11 °C, while controlled freezing experiments showed that damage to photosystem II in orchid roots did not occur until bath temperatures were below −7 °C. The internal temperature of roots in direct contact with ice cubes decreased to around 4 °C. Ice cube irrigation had no detrimental effects on the quality or display life of potted *Phalaenopsis* orchids growing in bark, demonstrating that ice cubes are a viable method of irrigating these tropical house plants.**

Orchids are currently the top-selling potted flowering plant in the United States because of their long flower life and compact growth (Banks, 2005; Fitch, 2004; USDA, 2016). While the popularity of many potted plants is declining, the number of orchids that are sold each year is increasing. Most commercial orchid sales are *Phalaenopsis* sp., also called moth orchids (Griesbach, 2002). The USDA Floriculture Crops Summary reported the wholesale value of potted orchids (including *Phalaenopsis*) to be $288 million in 2015, an increase of $15 million over the 2014 value (USDA, 2016). The introduction of improved hybrids and the development of large-scale production protocols that reduce the time required to produce flowering plants have allowed potted orchids to be grown for mass-market consumers (Griesbach, 2002).

Phalaenopsis species, which originate from tropical and subtropical climates, have a long flower display life in the typical temperatures and low light levels of a home (Banks, 2005). These orchids are epiphytic in their native environments and grow on trees with their roots exposed to the air (Banks, 2005). The roots of *Phalaenopsis* orchids contain chlorophyll and are photosynthetic (Dycus and Knudson, 1957; Lopez and Runkle, 2005). Commercial production of *Phalaenopsis* is usually in a bark-based potting media that allows for good aeration and drainage (Griesbach, 2002). In the home environment, proper irrigation is the largest challenge to maintaining a healthy potted orchid. Underwatering the plant will result in wrinkled, flaccid leaves, whereas overwatering or letting the orchid sit in water can result in damaged roots (Cullina, 2004). Healthy roots of *Phalaenopsis* are vivid green when the plant is well-watered, and they have a silvery hue when dry. Unhealthy roots are tan or brown (Cullina, 2004).

The technique of irrigating *Phalaenopsis* orchids with ice cubes has been recommended to orchid owners to reduce the likelihood of over- or underwatering the plants (Onofrey, 2009). Ice cubes provide a convenient way to apply a set volume of water, which is released slowly as the ice melts. The idea is that the ice melt will move slowly through the porous bark media, allowing more water to be absorbed by the bark and roots and resulting in less water accumulation in the bottom of pots where it may cause root damage. Concerns about this technique include whether the melting ice will provide enough water for proper plant growth and whether the ice will cause low temperature damage or reduce the display life of the plants (Cullina, 2004).

Even brief exposure to low temperatures can cause chilling injury (CI) in tropical species (Wang, 2007). Mesophyll cell collapse is a physiological disorder that can occur in orchid leaves exposed to low air or water temperatures (Cating and Palmateer, 2009; Sheehan, 2002). Symptoms include the development of sunken, light-green to yellow areas on the upper surfaces of the leaves, which then turn brown and necrotic. The severity of the CI is dependent on the temperature, duration of exposure, and physiological age of the leaves (McConnell and Sheehan, 1978). Exposure to 2 °C for 8 h causes mesophyll cell collapse in *Phalaenopsis* leaves. Water soaked spots on the upper surface of the leaves can be observed 0.5 h after removal from the chilling temperature treatment and these progress to deeply sunken dark brown spots within 3 weeks (McConnell and Sheehan, 1978).

While CI can be induced in the leaves of some *Phalaenopsis* species at temperatures below 10 °C, there is no information about root damage or what temperatures the roots experience when plants are irrigated with ice cubes. The objectives of this research were to 1) evaluate the effect of ice cube irrigation on the flower longevity, display life, and quality of potted *Phalaenopsis* orchids growing in bark; 2) measure the temperature of the bark

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media after irrigating orchids with ice cubes or water; 3) determine if there were any visual or physiological symptoms of low-temperature damage in the roots or leaves of orchids irrigated with ice cubes; 4) identify the temperature at which the photosynthetic apparatus in orchid roots is damaged; and 5) determine the internal temperature of roots that are in direct contact with ice cubes.

Materials and Methods

Plant material. Potted orchids (*Phalaenopsis* sp.) were received on 21 Jan. 2016 at the Ohio Agricultural Research and Development Center in Wooster, OH, and on 22 Jan. 2016 at the University of Georgia, Athens, GA. The orchids were transported on a temperature-controlled truck from Green Circle Growers (Oberlin, OH). Plants were growing in a custom bark media (95% bark and 5% sphagnum peatmoss; Oldcastle Lawn and Garden, Columbus, OH) in round, 11.5-cm plastic pots (700 mL volume) inside decorative ceramic pots. The orchids were at a market-ready stage with two flower spikes per plant and three to five open flowers on each spike. Four popular commercial cultivars, 699, 322, 386, and 56-100975, were used in all experiments except where noted.

Expt. 1. Evaluating the effects of ice cube irrigation on the quality and display life of potted Phalaenopsis orchids

Experimental set up and environmental conditions. Experiments were conducted at two locations (Wooster, OH and Athens, GA) in an interior evaluation room set up to simulate an office or home environment. These locations are referred to as OH and GA, respectively. All windows in the rooms were blocked, and light was provided by fluorescent lights from 8:00 AM to 5:00 PM daily. The average photosynthetic photon flux (*PPF*) in the room at plant height in OH was 7.8 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and the range was from 3.0 to 12.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$ across the room (LI-190R Quantum; LI-COR BioSciences, Lincoln, NE). In GA, the *PPF* was 6.8 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and the range was 3.7 to 10.0 $\mu mol \cdot m^{-2} \cdot s^{-1}$ across the room (LI-190SA; LI-COR BioSciences). The plants were blocked to account for the varying *PPF*. The temperature set point in each room was 21 °C. In OH, a relative humidity around 40% was maintained using a humidifier (Space Saver Evaporative Humidifier Model 831000; Aircare, Little Rock, AZ). The mean relative humidity in GA was 49% ± 9%. The mean room temperature over the entire experiment was 21.2 °C with a range of 14.5 to 28.7 °C in OH (January to July), and a mean of 22.0 °C and a range of 14.0 to 26.9 °C in GA (January to May). Temperature and humidity were monitored using a Watchdog (Model 2475 Plant Growth Station; Spectrum Technologies Inc., Plainfield, IL) or a temperature/humidity data logger (Hobo U12; Onset Computers, Bourne, MA). Fans were used to increase air circulation, and in OH, air samples were collected monthly to measure ethylene levels in the evaluation room using a gas chromatograph (Varian 3800; Agilent, Foster City, CA). Ethylene was not detected during the experiment.

Treatments. Orchids were arranged on tables in a split-plot randomized complete block design that accounted for the gradient in *PPF*. The main-plot effects were irrigation treatment (ice vs. water) arranged in six complete blocks (replicates). The subplot effects were cultivar (699, 322, 386, and 56-100975) arranged randomly within each main-plot. The experiment comprised 48 experimental units (pots) at each location.

The two irrigation treatments included ice cubes or water. Identical ice cube trays filled with tap water were used at both locations to make ice cubes. A preliminary experiment using a weighing lysimeter was used to determine the daily evapotranspiration by each of the four orchid cultivars in the interior evaluation room in GA. All four cultivars showed similar patterns of water loss. The cumulative water loss from the orchids after 1 week was ≈80 mL (data not shown), which is equivalent to the volume of three ice cubes. Orchids, therefore, received either three ice cubes or the equivalent volume of room temperature tap water (80 mL) on the same day and time once a week. The three ice cubes were evenly distributed on the surface of the media. Ice cubes did contact aerial roots, but contact with leaves was avoided. Any leachate in the pots was poured out of the decorative pots 24 to 48 h after irrigation. Once a month, the leachate was collected from the decorative pots 24 h after irrigation to measure the volume.

Flower longevity and display life. A single flower per spike was tagged on the day of flower opening. Flowers at a similar location on the spike were chosen within a cultivar. There were six single plant replicates of the four cultivars ($n = 6$). The two tagged flowers per plant (one per spike) were treated as a subsample. Flower longevity was determined as the number of days from flower opening until the corolla of the flower wilted. *Phalaenopsis* flower senescence is first visualized when the corolla starts to wilt and then eventually the dried flower abscises. The display life was determined as the number of days from the start of the experiment (when plants were received) until the day the last flower on the plant wilted ($n = 6$).

Orchid bark media temperature after irrigation. In OH, the temperature of the growing media over time was recorded using soil moisture/EC/temperature sensors (GS3; Decagon Devices, Inc., Pullman, WA) and data loggers (Em50; Decagon Devices). Sensors were inserted from the top down into the first 5.5 cm of the media of five orchids irrigated with ice and five orchids irrigated with water, all from the cultivar 699. The data loggers recorded measurements every 10 min. In GA, type-T thermocouples, connected to a datalogger (CR23X; Campbell Scientific, Logan, UT), were placed in the growing media of six orchids irrigated with ice and six orchids irrigated with water. The thermocouple was placed in the bark ≈5 cm below an aerial root. Temperature data were collected every 5 min for 3 h after an irrigation event.

Quantum yield of photosystem II and chlorophyll content. At the beginning of the experiment, the newest-most fully expanded leaf on each orchid was identified and tagged ($n = 6$). This leaf was used for measurements of both quantum yield of photosystem II (OH) and chlorophyll content (OH and GA) throughout the experiment. Measurements were taken toward the middle of the tagged leaf each month. The quantum yield of photosystem II was measured for both the leaves and the roots using a chlorophyll fluorometer (FluorPen FP 100 MAX; Photon Systems Instruments, Czech Republic) monthly to determine if the ice or water treatments affected photosystem II. For leaf measurements, the chlorophyll fluorometer was held so that the light sensor was on the top surface of the leaf. A single aerial root that was in direct contact with the ice or water during irrigation was chosen, and the quantum yield of photosystem II was measured after all of the ice cubes had melted, ≈5 h after irrigation. The leaf chlorophyll content was also measured each month using a chlorophyll meter (SPAD-502; Konica Minolta Sensing, Inc., Japan or CCM-200 plus; Apogee Instruments, Logan, UT).

Final shoot and root biomass. Orchids were harvested for final dry weight (DW) determination at the end of the display life or when no flowers remained on any of the plants of a single cultivar ($n = 6$). The timing of this harvest varied by cultivar and by location. After the final monthly measurements were taken for that cultivar, the shoots were separated from the root systems with a pruner. The number of leaves per plant was counted and any yellow leaves or symptoms of cold damage were noted (OH). Leaf area per plant was determined with a leaf area meter (LI-3100; LI-COR BioSciences) (GA). Leaves were placed in paper bags, dried in a forced air oven (60 °C), and weighed to determine the total leaf DW per plant. Bark media was washed off the root systems, and roots were then separated into green (live) roots and brown (dead) roots. Live roots and dead roots for each plant were placed in separate paper bags, dried in a forced air oven, and weighed to determine DW.

Expt. 2. Identifying the temperature at which the photosynthetic apparatus in orchid roots is damaged

Controlled freezing tests. An aerial root section (≈5 cm long) was taken from four different plants of each cultivar. Initial dark-adapted quantum yield of photosystem II was measured with a fluorometer (FluorPen FP100; Photon Systems Instruments). Individual root sections, wrapped in moistened cheese cloth in test tubes, were placed in an antifreeze bath. Control root sections were left at room temperature (28 °C) to determine possible degradation of the photosynthetic apparatus of the roots after severing them

from the plants. The temperature of the antifreeze bath started at 5 °C and was programmed to decrease by 2 °C every hour. Actual bath temperatures and internal root temperatures (see next paragraph for details on root measurements) were monitored using regular and fine-wire thermocouples (Omega Engineering, Stamford, CT), respectively. Quantum yield of photosystem II gives a measurement of the efficiency with which photosystem II uses absorbed light for electron transport. Dark-adapted quantum yield of photosystem II was measured on all root sections every hour until the dark-adapted quantum yield declined, indicating damage to photosystem II in the roots ($n = 4$).

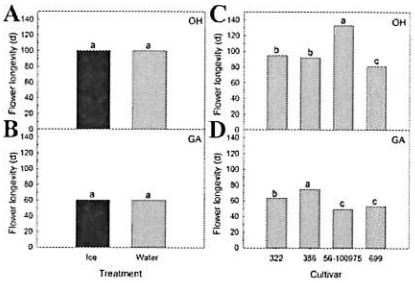

Fig. 1. Flower longevity when *Phalaenopsis* orchids were irrigated with ice cubes or water. Flower longevity was determined as the number of days from flower opening to flower wilting/senescence measured in OH (**A** and **C**) and GA (**B** and **D**). A single flower per spike was selected to monitor the longevity (total of two spikes per plant) on six plants from the ice treatment (black bars) and six plants from the water treatment (gray bars) from each of four cultivars (322, 386, 56-100975, and 699) ($n = 6$). Letters indicate significant differences among treatments based on least significant differences test ($P \leq 0.05$).

Expt. 3. Determining the internal temperature of orchid roots in contact with an ice cube

Internal root temperature. To determine the effect of ice cubes on the temperature of the stele of the aerial roots, 5-cm long root sections were cut from four different plants of cultivar 699. A fine needle was used to create a hole in the stele of the root. A fine-wire, type-T-thermocouple was then inserted into the stele to measure the internal root temperature. Both ends of the root were sealed with low melting temperature dental wax to prevent dehydration. Data were collected using a data logger (CR10; Campbell Scientific). An ice cube was placed on the root surface, and internal root temperatures were recorded every 5 min as the ice cube melted ($n = 4$). This experiment was repeated a total of four times. Results are presented for experiment one.

Data analysis. Data were analyzed with Proc GLIMMIX in SAS 9.4 (SAS Institute, Cary, NC). The analysis of Experiment 1 was conducted according to a split-plot design, with irrigation treatment tested using the irrigation treatment × block effect, and cultivar and the irrigation treatment × cultivar interaction tested using the residual error. Irrigation treatment, cultivar, and their interaction were considered fixed effects, and block and irrigation treatment × block were considered random effects. The *F*-test was considered significant at $\alpha = 0.05$. Mean comparisons were made using the least significant difference test ($P \leq 0.05$). The analysis of variance of data from Experiment 2 did not indicate any difference among cultivars, so data from all cultivars were combined and subsequently analyzed by fitting an exponential rise to a maximum curve to the data (SigmaPlot 11; Systat, San Jose, CA).

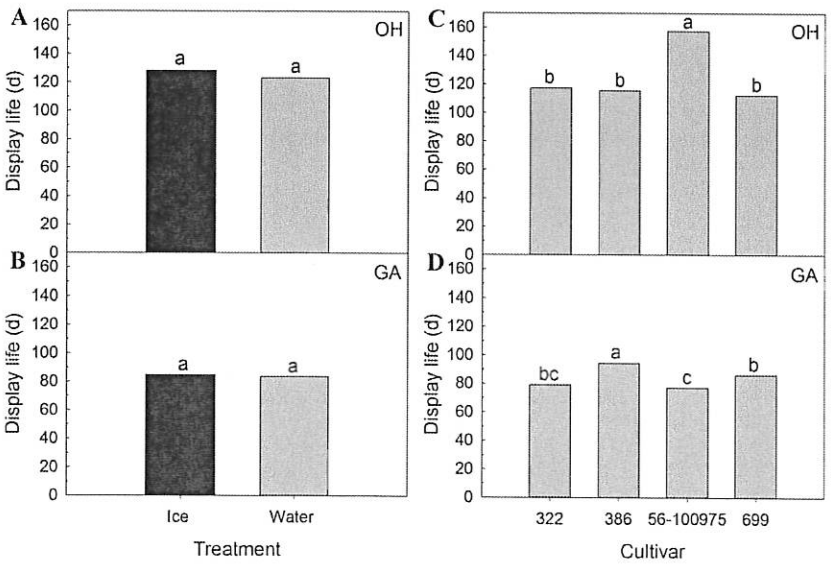

Fig. 2. Display life when *Phalaenopsis* orchids were irrigated with ice cubes or water. Display life of orchids in OH (**A** and **C**) and GA (**B** and **D**) was determined as the number of days from the date the orchids arrived to the date the final flower on each plant wilted. Ice (black bars) or water (gray bars) treatments were applied at the same time weekly to four different cultivars (322, 386, 56-100975, and 699) ($n = 6$). Letters indicate significant differences among treatments based on least significant differences test ($P \leq 0.05$).

Results

Expt. 1. Evaluating the effects of ice cube irrigation on the quality and display life of potted *Phalaenopsis* orchids

Flower longevity and display life. Orchid flower longevity was unaffected by irrigation treatment at either location (OH, $P = 0.999$ and GA, $P = 0.959$) (Fig. 1A and B). The mean flower longevity in OH was 100 d for both the ice- and water-irrigated orchids. In GA, the ice and water irrigation treatments had a mean flower longevity of 60 d. There were no significant irrigation treatment × cultivar interactions (OH, $P = 0.977$ and GA, $P = 0.110$). The display life of the plants, which was determined as the number of days from arrival until the last flower on the plant wilted, also showed no irrigation treatment effect (OH, $P = 0.304$ and GA, $P = 0.732$) (Fig. 2A and B) or irrigation treatment × cultivar interaction (OH, $P = 0.511$ and GA, $P = 0.542$). As expected, differences in flower longevity (OH, $P < 0.0001$ and GA, $P < 0.0001$) (Fig. 1C and D) and display life (OH, $P < 0.0001$ and GA, $P = 0.0005$) were observed among the cultivars (Fig. 2C and D).

Leachate volume and water use. Leachate was collected once a month and measured from each plant at 24 h after irrigation to determine the volume of water that was not taken up by the plant, but ran through the bark and accumulated in the bottom of the ceramic pot (Figs. 3 and 4). In OH, there was a significant interaction between irrigation treatment and cultivar in all six measurement months ($0.0045 \leq P \leq 0.13$). Irrigation treatments were, therefore, compared within a single cultivar. For cultivar 322, there was no difference in leachate volume between irrigation treatments, but the leachate volumes from cultivar 386 were significantly lower with ice cube irrigation compared with water irrigation in all six months. Cultivar 56–100975 had a significantly lower leachate volume for ice cube-irrigated orchids in the first 5 months. Orchids of cultivar 699 had a higher average leachate volume for ice cube-irrigated orchids only in the last measurement month (Fig. 3D). At the GA location, no irrigation treatment × cultivar interaction was found, so only the irrigation treatment main effects are reported. An irrigation treatment effect was observed in months one ($P = 0.0193$), three ($P = 0.0007$), and four ($P = 0.0038$), with ice cube-irrigation resulting in a lower leachate volume than water-irrigated orchids (Fig. 4).

Media temperatures. In OH, five probes per irrigation treatment were averaged for a single irrigation event in June to measure the media temperature changes after irrigation with water or ice cubes. The average media temperature remained between 20.2

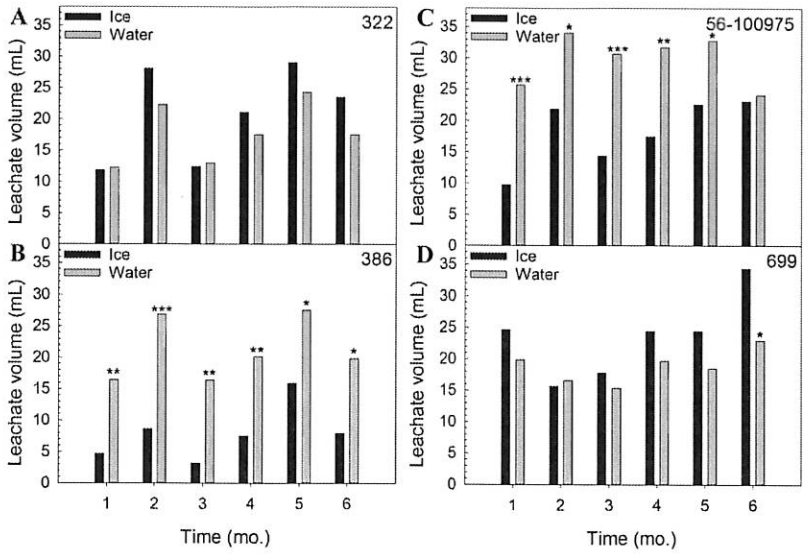

Fig. 3. Leachate volume measured 24 h after the irrigation of *Phalaenopsis* orchids in bark media with ice cubes or water in OH. The leachate was collected once a month for 6 months. Irrigation treatments were applied at the same time weekly to four different cultivars (322, 386, 56-100975, and 699) ($n = 6$). The average leachate volume (mL) for the irrigation method, ice cube (black bars) and water (gray bars), is compared within each cultivar for each month. The asterisks indicate significance between the irrigation treatments for a single month; *, **, *** significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

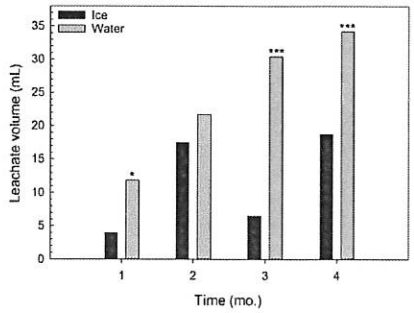

Fig. 4. Leachate volume measured 24 h after the irrigation of *Phalaenopsis* orchids in bark media with ice cubes or water in GA. The leachate was collected once a month for 4 months. Irrigation treatments were applied at the same time weekly. The average leachate volume (mL) (mean for four cultivars and six replication), for ice cube (black bars) and water (gray bars), is compared within each month. The asterisks indicate significance between the irrigation treatments for a single month; *, **, *** significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

and 21.3 °C after irrigation with room temperature water (Fig. 5A). Immediately following ice cube irrigation, temperatures in the bark decreased, with the lowest average temperature at 13.6 °C. The media temperature returned to the initial temperature of 21 °C 5 h after ice application. In GA, the average lowest temperature in the bark media with ice cube irrigation was 11.0 °C (Fig. 5B). Root zone temperatures during other recorded irrigation events showed similar trends.

Quantum yield of photosystem II. The quantum yield of photosystem II of the leaves and roots was monitored monthly over a 5-month period from the orchids at the OH location (Table 1). There were no irrigation treatment × cultivar effects on leaf ($P \geq 0.075$) or root ($P \geq 0.151$) quantum yield of photosystem II, so the cultivars were combined when comparing the two irrigation treatments. The orchid leaves ($P \geq 0.066$) and roots ($P \geq 0.076$) were unaffected by irrigation treatment over the 5 months.

Final harvest. Orchids were destructively harvested to determine the plant biomass at the end of the display life, after all the flowers within a cultivar had wilted. At the termination of the experiment in both locations, all leaves were dark green with no signs of discoloration or water soaking due to chilling or freezing damage. Leaf count, leaf area, and leaf DW were taken as measures of plant health. Leaf count in OH showed no significant irrigation treatment × cultivar interaction ($P = 0.606$), and no main effect of irrigation treatment ($P = 0.501$) was found. Leaf area in GA showed no significant irrigation treatment × cultivar interaction ($P = 0.887$), and the irrigation treatment had no effect ($P = 0.179$) on leaf area (data not shown). In OH, leaf DW showed an irrigation treatment × cultivar interaction ($P = 0.025$) (Table 2). Because of this, irrigation treatments were compared within each cultivar. Cultivar 322 receiving water irrigation had a 0.89 g higher leaf DW compared with ice irrigation ($P = 0.018$). No effect due to irrigation treatment was found within the other cultivars ($P > 0.097$). In GA, the leaf DW of orchids was unaffected by irrigation treatment ($P = 0.191$) (Table 3).

After leaf harvest, roots were separated into live, green roots and dead, and brown roots so that DW could be determined. In OH, a significant irrigation treatment × cultivar interaction was found for live root DW, so irrigation treatments were compared within each cultivar ($P = 0.018$). Live root DW of water-irrigated plants of cultivar 699 was 2.24 g higher compared with that of ice-irrigated plants ($P = 0.002$). No effect due to irrigation treatment was found within the other cultivars ($P \geq 0.188$) (Table 2). The GA live root DW ($P = 0.156$) and dead root DW at both locations (OH, $P = 0.922$ and GA, $P = 0.465$) showed no significant differences between the two irrigation methods (Table 3).

Expt. 2. Identifying the temperature at which the photosynthetic apparatus in orchid roots is damaged

Quantum yield of photosystem II as an indicator of low temperature damage in roots. While the temperature of the antifreeze bath was lowered, the internal root temperatures were monitored, and the dark-adapted quantum yield of photosystem II was measured as an indicator of damage. The internal temperature of the roots (cultivar 699) decreased from 7 to –8 °C as the bath temperature was reduced from 4.7 to –8.7 °C (Fig. 6A). Spikes in the root internal temperature were detected when the internal root temperatures reached –3.8 to –4.0 °C, indicating an exothermic energy release during the freezing of the water within the roots. There were no differences among the roots of the different cultivars (data not shown). The dark-adapted quantum yield of photosystem II of the orchid root segments declined rapidly when the bath temperatures dropped below –7 °C and the internal root temperature was about –4 °C (Fig. 6B), which is consistent with the bath temperatures at which the exothermic energy release was seen.

Expt. 3. Determining the internal temperature of orchid roots in contact with an ice cube

Internal root temperature. Thermocouples inserted into the stele of orchid root segments (cultivar 699) monitored the actual root temperature when roots were in direct contact with a melting ice cube (Fig. 7). The internal temperature of the roots dropped to 4.6 °C within 15 min and then gradually increased. Similar patterns were seen in two subsequent runs, but with minimum root temperatures reaching 3.1 and 3.3 °C (data not shown).

Discussion

Phalaenopsis orchids are marketed as interior potted plants, and their popularity is based on their long flower life and an overall floral display that can last from 3 to 6 months in the home environment (Blanchard and Runkle, 2006). Under our simulated interior environment, flower longevity and overall display life were similar between ice

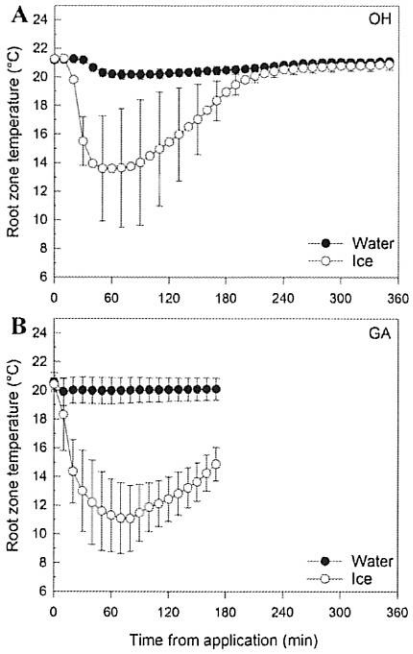

Fig. 5. Temperature of the bark media during irrigation of *Phalaenopsis* orchids with ice cubes or water. In OH, GS3 probes (Decagon Devices, Inc.) were inserted into the bark media to monitor the temperature in the top 5.5 cm of the root zone (**A**). Temperature was monitored in cultivar 699 from five plants treated with ice cubes and five plants treated with water ($n = 5$). The average temperature data are presented for an irrigation event on 27 June 2016 that was representative of other irrigation events monitored over the course of the experiment. In GA, type-T thermocouples, connected to a data logger (CR23X; Campbell Sci.) were used to monitor media temperature in six orchid plants (cultivar 699) irrigated with ice cubes and six orchids irrigated with water (**B**) ($n = 6$). Thermocouples were placed in the bark, 5 cm below the aerial roots. The average temperature data are presented for an irrigation event on 4 Mar. 2016. Plants were irrigated with three ice cubes (open circle) or the equivalent volume of room temperature water (solid circle) weekly. Vertical bars indicate standard deviation.

Table 1. Quantum yield of photosystem II of leaves and roots of *Phalaenopsis* orchids in bark media irrigated with ice cubes or water at the Ohio (OH) location. Measurements were taken once a month for 5 months.

Time (mo.)	Ice[z]	Water
	Leaf–Quantum yield of PSII	
1	0.80 a[y]	0.80 a
2	0.79 a	0.79 a
3	0.80 a	0.79 a
4	0.80 a	0.79 a
5	0.79 a	0.79 a
	Root–Quantum yield of PSII	
1	0.68 a	0.71 a
2	0.64 a	0.67 a
3	0.69 a	0.65 a
4	0.67 a	0.55 a
5	0.63 a	0.47 a

[z]Ice or water irrigation was applied weekly.
[y]Means are compared within a single row ($n = 6$) and means with different letters are significantly different at $P \leq 0.05$.

Table 2. Final dry weight (DW) measurements of leaves, live roots, and dead roots of *Phalaenopsis* orchids grown in bark media in OH irrigated with either ice cubes or the equivalent amount of room temperature water.

	Irrigation treatment			
Cultivar	Ice	Water	*P* value	Significance[y]
	Leaf dry wt (g)			
322	5.20[z]	6.09	0.018	*
386	4.69	5.16	0.194	
56-100975	4.96	4.41	0.134	
699	4.44	5.05	0.097	
	Live root DW (g)			
322	3.71	4.61	0.188	
386	5.44	5.33	0.867	
56-100975	4.94	4.13	0.235	
699	2.88	5.12	0.002	**
	Dead root DW (g)			
All cultivars	1.02	1.01	0.922	

[z]Means are compared within a single row ($n = 6$).
[y]Significance is indicated by *, **, or *** at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 3. Final dry weight (DW) measurements of leaves, live roots, and dead roots of *Phalaenopsis* orchids grown in bark media in GA irrigated with either ice cubes or the equivalent amount of room temperature water.

	Irrigation treatment			
Dry wt (g)	Ice	Water	*P* value	Significance[z]
Leaf	4.31[y]	4.67	0.191	
Live root	4.49	5.07	0.156	
Dead root	0.80	0.92	0.465	

[z]Significance is indicated by *, **, or *** at $P \leq 0.05$, 0.01, or 0.001, respectively.
[y]Means are compared within a single row ($n = 6$).

cube- and water-irrigated plants (Figs. 1 and 2). Flower loss from bud abscission and abnormal flower opening may be observed if flowers are exposed to extreme temperature stress (Cullina, 2004; Lopez and Runkle, 2005). Tracking the opening and wilting of individual flowers daily also allowed us to observe the overall quality of the flowers. No premature flower loss or abnormal flower development was observed in either the ice or water treatment. These experiments confirmed that ice irrigation did not have a negative impact on flower or plant quality.

Overall plant health and display life depends on proper irrigation. Irrigation instructions are not easy to give to beginning orchid owners, and the symptoms of overwatering can look similar to those of underwatering (Cullina, 2004). Consistent irrigation in the form of three ice cubes per week, rather than a measured volume of water, is a simpler, more convenient technique for consumers. The media used for the production of most commercial *Phalaenopsis* orchids consists of very coarse bark, and applied water drains very quickly (Griesbach, 2002). The volume of leachate was either the same, or in some irrigation events, it was greater in orchids irrigated with room temperature water compared with those irrigated with ice cubes. The slower release of water from ice cubes can allow for an increased uptake of water by the plant or greater retention within the media. The accumulation of leachate in the pots can lead to significant root damage (Cullina, 2004), and the extent of this damage is influenced by the sensitivity of the specific cultivar. In our interior evaluation room, the water use by the orchids and loss from media evaporation, as determined by a lysimeter experiment, was ≈80 mL per week. This is equivalent to the volume of three ice cubes. The water needs of potted orchids will vary based on seasonal temperature, light, and relative humidity changes in the home, and consumers may need to increase or decrease the number of ice cubes that are applied. Regular monitoring of media moisture will still be needed (Cullina, 2004).

The primary concern that has been raised about irrigating with ice cubes is that it may cause low temperature injury in these cold sensitive tropical orchids. Leaf mesophyll cell collapse is a physiological disorder in orchids that is caused by cold temperatures (Cating and Palmateer, 2009). The symptoms of CI in the leaves are visualized as sunken, yellow, water-soaked spots on the upper leaf surface that progress into brown necrotic spots (McConnell and Sheehan, 1978). Temperatures below 10 °C result in mesophyll cell collapse in *Phalaenopsis* leaves (McConnell and Sheehan, 1978). Growers have seen these symptoms at low production temperatures, and damage also occurs when orchid plants are exposed to low temperatures during shipping (Wang, 2007). In a preliminary experiment with *Phalaenopsis* orchids, ice cubes applied directly to the leaves resulted in the formation of water soaked spots (data not shown), but the recommendation for ice irrigation is to avoid placing the ice cubes directly on or against the leaves. In the current research, ice cubes were placed on the surface of the

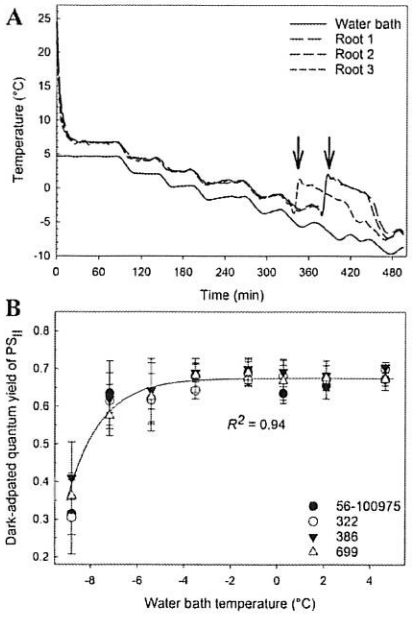

Fig. 6. Low temperature effects on internal root temperature and dark-adapted quantum yield of photosystem II in *Phalaenopsis* roots. Orchid root segments were placed in a programmable antifreeze bath, and the temperature was decreased by 2 °C every hour. Fine-wire thermocouples inserted into the stele of the root monitored the internal root temperature as the bath temperature decreased. Three individual root segments from cultivar 699 are represented by the three dashed lines and the bath temperature is represented by the solid line (**A**). The exothermic energy released during the freezing of the water within the roots is indicated by the arrows. The dark-adapted quantum yield of photosystem II of root segments was measured each hour. Symbols represent the four cultivars, and the vertical bars indicate standard deviation ($n = 4$) (**B**). The exponential rise to a maximum curve was fitted through the combined data that can be represented by the equation, Dark – adapted quantum yield of photosystem II = $0.647 + 0.000114 \times \left(1 - e^{(-0.904 \times \text{temp})}\right)$.

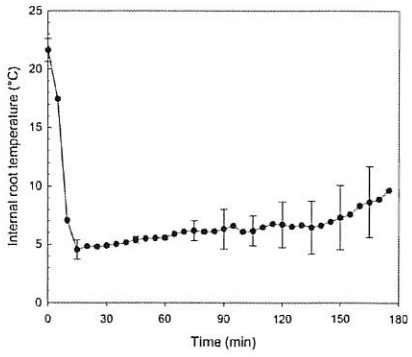

Fig. 7. Internal temperature when *Phalaenopsis* orchid roots were in direct contact with ice. Ice cubes were applied at time zero to root segments (cultivar 699) that had a fine-wire thermocouple inserted into the stele of the root. The line represents the average internal root temperature, and vertical bars indicate standard deviation ($n = 4$).

bark media. There was adequate space in all cultivars to avoid direct contact with the leaves. We did not observe symptoms of leaf mesophyll cell collapse in any of the cultivars at either location.

Chlorophyll fluorescence has been used as a physiological indicator of both chilling and freezing injury (Forney et al., 2000; Lurie et al., 1994; Maxwell and Johnson, 2000). Chlorophyll fluorescence measurements provide a nondestructive measure of quantum yield of photosystem II, which is sensitive to a range of environmental stresses, including cold (Maxwell and Johnson, 2000; Murchie and Lawson, 2013). These measurements showed no indication of chilling damage in the leaves of orchids that were irrigated with ice (Table 1). With the exception of a small increase in the average shoot DW of cultivar 322 orchids in OH irrigated with water, the final DWs, leaf counts, and leaf areas showed that the foliage of ice-irrigated orchids was as healthy as the foliage of water-irrigated orchids (Tables 2 and 3, and data not shown).

Phalaenopsis orchids have photosynthetic aerial roots covered with a spongy tissue comprising layers of dead cells called the velamen (Dycus and Knudson, 1957; Lopez and Runkle, 2005). During ice irrigation, aerial roots were often in direct contact with the ice cubes, and the roots in the bark media were in contact with the ice melt. Aerial roots that were in direct contact with ice showed no evidence of damage to the photosynthetic apparatus as determined by the monthly chlorophyll fluorescence measurements (Table 1). Thermocouples in the media were used to monitor the temperatures experienced by the roots within the top quarter of the media volume during many irrigation events throughout the 6 months of the experiment. Chilling temperatures between 0 and 15 °C can result in growth retardation, inhibition of photosynthesis, discoloration or lesions on the leaves, and leaf senescence or abscission in tropical species (Lyons, 1973). Less is known about CI in the roots. In this experiment, the bark temperature during ice irrigation decreased to a minimum temperature around 14 °C in OH and 11 °C in GA and returned to room temperature within 5 h (Fig. 5). In *Phalaenopsis aphrodite* plants, the growth rate of the white, nonvelamentous root tips decreased after 8 h of exposure to 4 °C, and they showed some withering by 12 h (Peng et al., 2014). The final harvest data in our experiment indicated that root health was not impacted by the irrigation treatment (Table 2). *Phalaenopsis* cultivars react differently to environmental and cultural practices. The differences in live root DW (OH only) are an example of such cultivar differences. Cultivar 699 water-irrigated orchids had higher live root DW than that of ice-irrigated orchids, while no differences were observed in the other cultivars. While the irrigation treatment may have had an effect on the growth of roots in cultivar 699, there was no difference in the DW of the dead roots between the ice and water treatments in this cultivar.

Experiments using an antifreeze bath did not show any effects on the quantum yield of photosystem II in orchid root segments after short-term exposure to chilling temperatures (above 0 °C), but inhibition of quantum yield of photosystem II was observed when bath temperatures dropped below –7 °C and root temperatures were about –4 °C (Fig. 6). This is well below the temperatures that could be caused by irrigating potted *Phalaenopsis* orchids with ice cubes because placing ice cubes directly on root sections did not cause internal root temperatures to drop below 3 °C (Fig. 7). While damage could be cumulative over the months of irrigation, our experiments also showed that at each irrigation event it only took 5 h for the root zone temperature to return to the initial temperature (Fig. 5).

Irrigating using ice cubes provides a viable solution to help orchid owners maintain healthy potted *Phalaenopsis* orchids and prevent over- or underwatering. Three ice cubes were sufficient in our low light, interior evaluation room, but this will vary based on changes in environmental conditions, and consumers should be encouraged to monitor the moisture levels in the bark and to watch for symptoms of underwatering, including wrinkled leaves and darker silver-colored roots (Cullina, 2004). Even with ice irrigation, which limits the volume of water that is applied, the remaining leachate in the bottom of any decorative pots should be poured out to prevent roots from sitting in water and allow them to get adequate oxygen. While ice cube irrigation causing low temperature damage in tropical plants is a valid concern, we found that irrigating *Phalaenopsis* orchids grown in 95% bark potting media with ice cubes or room temperature water resulted in high quality plants with excellent flower longevity and display life.

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