Substrate Water Content Influences the Flowering of Doritaenopsis Queen Beer ‘Mantefon’

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Abstract. Automated irrigation systems based on soil moisture sensor measurements can reduce water and fertilizer use while adequately meeting plant water requirements. In this study, the effects of substrate volumetric water content (0, v/v) on the flowering of 17-month-old Doritaenopsis Queen Bee ‘Mantefon’ (from the time of deflasking) were examined. The plants were transplanted in plastic pots (10.5 cm width × 9.5 cm height) filled with sphagnum moss and the 0 of sphagnum moss was maintained at 0.2, 0.3, 0.4, or 0.5 m·m⁻³ using an automated drip irrigation system. Plants grown at a 0 threshold of 0.2 m·m⁻³ had thinner leaves and lower SPAD value than those grown at higher 0 thresholds. The net CO₂ uptake of the uppermost fully expanded leaf increased with increasing 0 between 0.2 and 0.4 m·m⁻³, but there was no significant difference in the net CO₂ uptake between plants grown at 0.4 and 0.5 m·m⁻³ thresholds. The number of flower buds at the time of the first open flower was lower in plants grown at 0 thresholds of 0.2 and 0.3 m·m⁻³ as compared with that in the plants grown at 0.4 and 0.5 m·m⁻³ thresholds. Early flower abscission, flower bud dropping, and flower senescence during the 2 weeks after flowering occurred in 55% and 30% of the plants at 0.2 and 0.3 m·m⁻³ thresholds, respectively, whereas plants at 0 thresholds of 0.4 and 0.5 m·m⁻³ had negligible flower abscission. Although vegetative growth parameters were similar among 0 thresholds of 0.3 m·m⁻³ or higher, plants grown at a 0 threshold of 0.3 m·m⁻³ produced fewer flowers than those grown at 0.4 and 0.5 m·m⁻³ thresholds. During the 83-day experimental period, the system irrigated the plants =0.79, 1.93, 2.46, and 2.84 L/pot at 0 thresholds of 0.2, 0.3, 0.4, and 0.5 m·m⁻³, respectively. Overall, 0.4 m·m⁻³ was considered to be an optimal threshold 0 level for producing high-quality Doritaenopsis Queen Bee ‘Mantefon’ during the flowering period with most efficient water use.

Phalaenopsis has become the most important ornamental crop with high economic value in international flower markets (Gow et al., 2010). Phalaenopsis has been the best-selling pot plant in the Dutch flower-auctions since 2000, and 135 million pots were sold in 2016 (Vereniging van Bloemenveilingen in Nederland, 2017). In the United States, orchids are the most valuable flowering crop; 36.4 million pots worth $288 million were sold in 2015 (USDA, 2016). Among all the orchid genera sold in the United States, Phalaenopsis comprises most of the potted orchid sales owing to the ease of scheduling the flowering to meet specific market dates, high wholesale value, and long postharvest life, with the flowers often lasting for more than 3 months (Nash, 2003; Wang, 1997). Many studies have reported the effects of aboveground environmental factors such as temperature, light intensity, and CO₂ supply on the vegetative growth and flowering of Phalaenopsis hybrids (Guo and Lee, 2006; Lopez and Runkle, 2005; Ota et al., 1991; Wang, 1995). However, belowground factors, such as substrate moisture levels, have been rarely addressed, and orchids are often cultivated without knowledge of optimal water requirements. Irrigation control in Phalaenopsis production is commonly performed using timers or experience of the growers. Phalaenopsis is typically watered using overhead sprinklers or handheld watering at 6–21 d intervals, which varied depending on the season, medium, and plant and pot size (Hwang and Jeong, 2007; Wang and Lee, 1994). Human population growth, climate change, and increased agricultural and industrial water demands are likely to lead to a decrease in the availability of fresh water worldwide (IPCC, 2007). In the United States, laws and regulations currently limit the amount of water available for use in nurseries in across California, Florida, North Carolina, Texas, and Oregon, or in parts thereof. Moreover, such regulations are expected to become stricter (Fulcher et al., 2016). Nevertheless, water is commonly applied excessively in greenhouse production to assure complete wetness of the growing medium. Conventional irrigation with timers may result in unnecessary water application and runoff because a set irrigation volume is provided regardless of substrate moisture condition or plant water status (Alem et al., 2015). Models estimating daily water requirements from plant size and environmental factors such as daily light integral (DLI) and vapor pressure deficit (VPD) have been developed. However, they are not easy to adapt for ornamental plant production owing to varying environmental conditions across production areas (Kim et al., 2011). Capacitance soil moisture sensors have been successfully used to monitor moisture content in horticultural substrates and control irrigation to maintain a constant substrate moisture level with little or no leaching (Burnett and van Iersel, 2008; Nemali and van Iersel, 2006). By controlling irrigation using soil moisture sensors, experienced growers could save 22% to 83% of the water currently used in a commercial nursery setting (Pershey et al., 2015; van Iersel et al., 2009; Warsaw et al., 2009). Furthermore, soil moisture sensor systems allowing for real-time monitoring and thresholds for triggering irrigation can easily be adjusted to meet changing water demand or environmental conditions. The use of automated irrigation with soil moisture sensors to monitor and control substrate soil water content has been examined for several species, ranging from woody species, such as Rhododendron spp. (Lea-Cox et al., 2008) and Hydrangea (van Iersel et al., 2009), to herbaceous species, such as Petunia (Kim et al., 2011).

In the United States and Europe, bark is preferentially used as a substrate because it provides good aeration for roots. However, bark has a poor water-holding ability, which necessitates irrigation that is more frequent and results in water and fertilizer wastage (Wang and Gregg, 1994). Sphagnum moss holds water more effectively than bark and has been used as a substrate for epiphytic orchid cultivation mainly in Eastern Asia, including China, Japan, Korea, and Taiwan (Kim et al., 2016; Yen et al., 2011). The objective of the present study was to compare the flowering of Doritaenopsis Queen Beer ‘Mantefon’, potted with sphagnum moss and maintained at various substrate moisture levels, using soil moisture sensor-based automated irrigation.

Received for publication 12 May 2017. Accepted for publication 7 Nov. 2017.

This research was supported by Korea University Grant and the Korea Institute of Energy Technology Evaluation and Planning (KETEP), the Ministry of Trade, Industry and Energy of the Republic of Korea (No. 20142020103570).

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Materials and Methods

Plant material and growth conditions. Mature Doritaenopsis Queen Beer ‘Mantefon’ plantlets, growing for ≈17 months under ex vitro conditions planted on sphagnum moss in plastic pots (9.0 cm diameter, 8.5 cm height), were purchased from a commercial propagator (Goyang Farming Association Co., Gyeonggi, Korea) on 4 Mar. 2016. On arrival at the laboratory, these plants were transplanted in 475-mL transparent plastic pots (10.5 cm diameter, 9.5 cm height), filled with Chilean sphagnum moss (Sphagnum magellanicum; Lonquen Ltd., Pueto Montt, Chile) in the empty space of pots, and placed in a glasshouse located at Korea University, Seoul, South Korea, for 28 d before the experiment began. When filled with sphagnum moss, we used the same sphagnum moss as that used for the young plants, packed to a final bulk density of ≈0.3 g cm⁻³. Transplanted plants were watered manually for 4 weeks to acclimate them to the experimental glasshouse environment. We fully watered all the plants on 25 Mar. and did not irrigate until the automated irrigation began on 1 Apr. When treatments began on 1 Apr. 2016, the number of leaves, leaf span, and length and width of the uppermost fully expanded leaf were 8.0 ± 1.2 (mean ± SD, n = 4), 30.4 ± 2.3 cm, 18.4 ± 2.2 cm, and 7.2 ± 0.6 cm, respectively. Inflorescences had already initiated on all of these plants and were 9.2 ± 3.4 cm in length on 1 Apr. The experiment was initiated on all of these plants and were 9.2 ± 3.4 cm, 18.4 ± 2.2 cm, and 7.2 ± 0.6 cm, respectively.

Substrate hydraulic properties. The hydraulic properties of the Chilean sphagnum moss used in this experiment were determined using a modular instrument (Hyprop; UMS, München, Germany), following the method described by O’Meara et al. (2014).

Automated irrigation system. The experiment comprised four levels of substrate volumetric water content (θ, v/v): 0.2, 0.3, 0.4, and 0.5 m³ m⁻³. The 16 EC-5 capacitance soil moisture sensors (EC-5; Decagon Devices) were connected to a multiplexer (AM 16/32B; Campbell Scientific) which was connected to a data logger (CR1000; Campbell Scientific). The soil moisture sensors were powered by the data logger with 2.5 V excitation and calibrated to the specific substrate (sphagnum moss) used in this study (θ = 0.001415 x – 0.43315, r² = 0.9594). Each sensor (5.5 cm length and 1.0 cm width) was inserted diagonally from the top of the substrate in the center of a pot, where the roots were located, to a depth of ≈6.0 cm. The data logger was connected to one relay driver (SDM-CD16AC; Campbell Scientific) which controlled the power to 16 solenoid valves (S-390-2-R; Bermad, Kibbutz, Israel). Each experimental unit had five plants as subreplicates. The θ was measured every 10 s for one pot in the center for each experimental unit. Every 20 min, if the θ reading of the capacitance sensors in an experimental unit dropped below the assigned θ set point, the data logger opened the soil moisture valve for that experimental unit for 30 s. Plants were irrigated using a customized drip ring with a pressure-compensated drip emitter (Netafim 2 L h⁻¹; Netafim, Fresno, CA) placed around the plant at a rate of 16.7 mL per plant per irrigation. Each irrigation application was recorded by the data logger, and the daily and total water applied to the plants was calculated based on the last 83 d of the experiment. During the experiment, plants were fertigated with a 20N–8.7P–16.6K water-soluble fertilizer (Multifeed; Haifa Chemicals, Haifa Bay, Israel) solution at a rate of 500 mg L⁻¹. The pH and EC values of the fertilizer solution before application were about 6.54 and 0.7 dS·m⁻¹, respectively.

Vegetative growth and flowering. The date of observing the first open flower was recorded for each plant. We defined the date of the first open flower as the date on which one lateral petal had completely unfolded. On the date of the first open flower, the number of flower buds and inflorescence length were determined for each plant. Depending on the pots, there were one or two inflorescences in a plant. The total number of flowers was measured irrespective of the number of inflorescences, and the longest inflorescence in one pot was measured as inflorescence length. Because early flower abscission (flower bud dropping and flower senescence) was observed in response to the low θ treatment, flower abscission percentage within 2 weeks of flowering was calculated.
The flower bud dropping was based on the dropping of buds from the plant before flowering and flower senescence meant that a flower dropped within 2 weeks after opening. At the end of the experiment (on 23 June), the total number of leaves, leaf span, leaf thickness, length and width of the uppermost fully expanded leaf, total leaf area, SPAD value, inflorescence length, flower length, flower width, and fresh and dry weight of shoots and roots were determined. Flower size and inflorescence length were determined based on the largest one in each pot. SPAD value was measured using a chlorophyll meter (SPAD 502; Minolta, Osaka, Japan). Total leaf area was measured using a leaf area meter (LI-3100; LI-COR, Lincoln, NE). Dry weights were determined after drying samples in an oven at 80 °C for 4 d.

Gas exchange. Phalaenopsis absorbs CO₂ mainly during the night, demonstrating typical crassulacean acid metabolism (CAM) photosynthetic characteristics (Guo and Lee, 2006). To determine the best time for measuring the photosynthetic parameters of Doritaenopsis Queen Beer ‘Mantefon’, we measured diurnal variations in gas exchange using a portable photosynthesis system, CIRAS-3 (PP Systems, Amesbury, MA), with a PLC 3 universal leaf cuvette on 16 June 2016, which was a clear day. In the preliminary experiment, it was determined that the time from 0200 to 0400 HR to be the optimal period to measure CO₂ uptake in Phalaenopsis. The cuvette was placed in the middle of the leaf next to the vein on an uppermost fully expanded leaf. The rate of airflow through the cuvette was ≈200 mL-min⁻¹ with a CO₂ level of 400 μmol·mol⁻¹. After a steady state of gas exchange had been achieved, the net CO₂ uptake rate, stomatal conductance (gs), and transpiration rate were determined for 10–20 s until the values stabilized. For these measurements, the uppermost mature leaves on eight randomly selected plants per treatment were used. These leaves existed at the beginning of the treatment.

Stomatal frequency on leaf epidermis. The middle portion of an emerging new leaf was chosen to measure the stomatal frequency (number of stomata/total number of epidermal cells) in 1 mm² area, and leaves on four randomly selected plants per treatment were sampled. An abaxial leaf epidermis replica was prepared using clear commercial nail polish and the number of stomata was counted under a microscope (YS100; Nikon, Ibaraki, Japan) at ×100 magnification.

**Experimental design and analysis.** The experiment used a randomized complete block design with four treatments and four blocks. Each experimental unit had five plants and one was measured for one pot in the center of each unit. The effects of various θ thresholds on the inflorescence length, the number of flower buds at the time of the first open flower, and other growth parameters at harvest were analyzed using analysis of variance, followed by Tukey’s honestly significant difference (P = 0.05) procedure using SAS 9.4 (SAS Institute, Cary, NC).

**Results and Discussion**

**Substrate hydraulics.** The total porosity of the sphagnum moss was 0.57 m³·m⁻³ and the substrate matric potential decreased to

| Volumetric water content (θ, m³·m⁻³) | Days to first open flower | Inflorescence length (cm) | No. flower buds at first open flower |
|--------------------------------------|--------------------------|---------------------------|-------------------------------------|
| 0.2                                  | 50.2 a                    | 36.1 b                    | 13.4 b                              |
| 0.3                                  | 48.8 ab                   | 35.8 b                    | 12.7 b                              |
| 0.4                                  | 45.6 c                    | 40.1 a                    | 16.3 a                              |
| 0.5                                  | 47.4 bc                   | 39.4 ab                   | 15.4 a                              |

**Significance**

*Mean separation within columns by Tukey’s honestly significant difference test at P = 0.05.

*NS* Non-significant at P ≤ 0.001, respectively.

**Table 3. Effects of volumetric water content of substrate on the flowering and fresh and dry weight of Doritaenopsis Queen Beer ‘Mantefon’ grown for 54 d.**

| Volumetric water content (θ, m³·m⁻³) | No. flowers | Flower length (cm) | Flower width (cm) | Fresh wt (g) | Dry wt (g) |
|--------------------------------------|-------------|--------------------|-------------------|--------------|------------|
|                                     | Shoot       | Root               | Shoot             | Root         | Root       |
| 0.2                                  | 19.0 b      | 3.5 b              | 4.8 b             | 92.0 b       | 38.7 b     | 8.9 b     | 4.8 b     |
| 0.3                                  | 24.5 ab     | 3.8 a              | 5.3 a             | 125.8 a      | 54.8 a     | 10.4 a    | 5.3 a     |
| 0.4                                  | 28.1 a      | 4.0 a              | 5.3 a             | 129.0 a      | 56.3 a     | 10.5 a    | 5.1 a     |
| 0.5                                  | 25.6 a      | 3.8 a              | 5.3 a             | 127.7 a      | 59.2 a     | 10.2 a    | 5.1 a     |

**Significance**

*Mean separation within columns by Tukey’s honestly significant difference test at P = 0.05.

**NS** Non-significant at P ≤ 0.01 or 0.001, respectively.
–40 kPa at a substrate θ of ≈0.04 m³·m⁻³ (Fig. 1). When the substrate θ was 0.5, 0.4, 0.3, and 0.2 m³·m⁻³, the substrate matric potential was –0.4, –1.1, –2.6, and –13.6 kPa, respectively. Previously, Wang (2010) reported the substrate hydraulic properties in sphagnum moss for growing Phalaenopsis. In that study, moisture tension was maintained at 0 kPa until 33% of the total water being held by the sphagnum moss was lost. In our results, when a substrate θ was 0.4 m³·m⁻³, ≈30% of the total water was lost and the substrate matric potential dropped to –1.1 kPa (Fig. 1), which agrees with that reported by Wang (2010).

**Automated irrigation system.** The θ measurements in the moss decreased gradually until automated irrigation began on 1 Apr. (Fig. 2). Automated irrigation occurred when the substrate dried below the designated θ threshold and automation worked properly with very little variation (±0.004 m³·m⁻³ of θv) throughout the experiment. Irrigation started immediately on 1 Apr. in the 0.4 and 0.5 m³·m⁻³ θ threshold treatments, whereas it took 6 and 16 more days to start irrigation for the 0.3 and 0.2 m³·m⁻³ θ threshold treatments, respectively. Thereafter, the θ in each pot was maintained just above the allocated threshold consistently throughout the experiment. For petunia cultivation in a greenhouse, an automated irrigation system with capacitance soil moisture sensors could maintain the soil moisture status within a narrow range during cultivation (Kim et al., 2011).

**Vegetative growth and flowering.** Because all the plants had entered their reproductive stage with young inflorescences at the beginning of the treatment, and no new leaf was produced during the 89-d experimental period, the treatments had no effect on leaf growth. No significant differences were found in leaf span, length and width of the uppermost fully expanded leaf, and the total leaf area among the plants grown at the various θ thresholds (Table 1). The leaves of plants grown at a θ threshold of 0.2 m³·m⁻³ were 27%, 37%, and 38% thinner than those grown at θ thresholds of 0.3, 0.4, and 0.5 m³·m⁻³, respectively. SPAD value (greener leaves at higher values) was lower in plants grown at a θ threshold of 0.2 m³·m⁻³ (59.7) than in those grown at higher θ thresholds of 0.3, 0.4, and 0.5 m³·m⁻³ (65.1, 66.8, and 66.0, respectively). Similar effects have been reported in avocado and olive, where leaves were thinner and leaf total chlorophyll content was lower in water-stressed lower than in well-watered plants (Chartzoulakis et al., 2002; Guerfel et al., 2009). In general, although vegetative growth has been found to decrease with decreasing θ thresholds (Bayer et al., 2013; Kim et al., 2012; Nemali and van Iersel, 2006), there was no difference in vegetative growth due to θ thresholds in our study.

As the θ threshold decreased, the number of days to first open flower increased and inflorescence length and the number of flower buds at first open flower decreased (Table 2; Fig. 3), although there was no difference between the plants at 0.4 and 0.5 m³·m⁻³ thresholds. At harvest, plants grown at a θ threshold of 0.2 m³·m⁻³ had the fewest flowers and smaller flowers than those on the plants grown at higher θ thresholds (Table 3). Moreover, previous work has shown that plants grown at lower θ thresholds had fewer flowers than those at higher θ thresholds in petunia and impatiens (Blanusa et al., 2009), cut rose (Chimonidou-Pavlidou, 2004), and Big Bend bluebonnet (Niu et al., 2007). Fresh weight and dry weight accumulation showed a similar pattern, with lower fresh and dry weights at a θ threshold of 0.2 m³·m⁻³ than at higher θ thresholds (Table 3). There was no difference in these values among the plants grown at θ thresholds of 0.3 m³·m⁻³ and greater.

Under θ thresholds of 0.2 and 0.3 m³·m⁻³, early flower abscission occurred in 55% and 30% of the plants, respectively, occurring at ≈13 d from the first open flower (Fig. 4). Plants at the 0.4 m³·m⁻³ threshold showed reduced flower abscission, and no flower abscission was observed at a θ threshold of 0.5 m³·m⁻³. Fertigation at various θ thresholds might affect nutrient availability to plants and water availability. In previous studies, lower fertilizer concentrations produced smaller leaves in Phalaenopsis (Wang, 2010; Wang and Gregg, 1994) and showed symptoms of nutrient deficiency, such as yellowish leaves that eventually abscised (Wang, 2007). However, in our study there was no symptom of nutrient deficiency, such as yellowish leaves in any treatments. Early flower abscission under lower θ thresholds in our results was likely because of water stress and reduced turgor rather than because of lack of nutrition.

Early nutrient termination did not severely affect flowering longevity in Phalaenopsis compared with continuous application of fertilizer (Wang, 2000).
**Gas exchange and stomata.** The time from 0200 to 0400 hr was found to be the optimal time to measure the gas exchange of *Doritaenopsis* Queen Beer ‘Mantefon’ (Fig. 5). This pattern was similar to that observed in other *Phalaenopsis* spp. (Guo and Lee, 2006). Net CO$_2$ uptake rates from 0200 to 0400 hr at θ thresholds of 0.2, 0.3, 0.4, and 0.5 m$^3$·m$^{-2}$ were 1.23, 3.75, 4.48, and 4.40 μmol·m$^{-2}$·s$^{-1}$, respectively (Fig. 6). The photosynthetic rate increased with increasing θ threshold from 0.2 to 0.3 m$^3$·m$^{-2}$, with no additional increase as θ increased further. Similarly, $g_s$ and transpiration rate increased with θ threshold between 0.2 and 0.4 m$^3$·m$^{-2}$. Ota et al. (1991) reported that imposing water stress in *Phalaenopsis* by withholding water for 10 d induced lower CO$_2$ uptake from the leaves. It has been established that the relative water content of leaves controls the stomatal movement of CAM plants at night (Nobel, 1977), and that the stomata of CAM plants tend to close under water stress, thus reducing CO$_2$ uptake (Kluge and Ting, 2012).

In addition, our results indicated that *Phalaenopsis*, an epiphyte, suffers from drought stress at a θ level below 0.2 m$^3$·m$^{-2}$, which is about –15 kPa in a sphagnum moss substrate with the degree of compaction used in this study.

Stomatal density responds to environmental factors, such as drought and elevated CO$_2$ concentration (Xu and Zhou, 2008). In general, plants subjected to severe drought stress have low stomatal density as compared with plants grown in well-irrigated soils (Meng et al., 1999; Xu and Zhou, 2008). However, no significant differences were observed in stomatal density among the plants at four various θ thresholds in our experiment (data not shown). The reason for the absence of any difference in stomatal density among the treatments was likely because of the short experimental period for displaying any treatment effect on *Phalaenopsis*. However, our results showed that $g_s$ decreased with decreasing θ threshold between θ values of 0.4 and 0.2 m$^3$·m$^{-2}$ (Fig. 6). This is likely because of the direct closure of stomata. Drought stress is first sensed by the plant roots and activates the synthesis of the phytohormone ABA. The root-derived ABA is transported to leaves (Sauter et al., 2001) to induce stomatal closure and prevent stomatal opening through different pathways (Mishra et al., 2006).

**Water use.** The total fertigation volume decreased with the decrease in the θ threshold level (Fig. 7). In general, as compared with sprinkler systems and hand watering, drip irrigation reduces water and fertilizer losses due to leaching from substrates. In our experiment, no leaching was observed even at the highest θ threshold (0.5 m$^3$·m$^{-2}$). Moreover, the total volume of water applied at a θ threshold of 0.2 m$^3$·m$^{-2}$ was only 28% of that used by the plants grown at a θ threshold of 0.5 m$^3$·m$^{-2}$ (Fig. 7). Although treatment at a θ threshold of 0.2 m$^3$·m$^{-2}$ saved a considerable amount of water and nutrients, it produced poor quality *Phalaenopsis* with early flower abscission. Previous research has shown that sensor-controlled drip irrigation could be used to reduce irrigation volume without negative impacts on plant growth of bluebonnet (Niu et al., 2007), conifers (Pershey et al., 2015), geranium (Valdés et al., 2015), hibiscus (Bayer et al., 2013), and woody ornamentals (Warsaw et al., 2009) to a certain extent. Our results indicate that maintaining the sphagnum moss substrate at a θ of 0.4 or 0.5 m$^3$·m$^{-2}$ produced quality *Doritaenopsis* Queen Beer ‘Mantefon’ with comparatively little water and nutrients (2.84 L/pot for a θ threshold of 0.5 m$^3$·m$^{-2}$ for 83 d), without any leaching.

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