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Inhibition of Transpiration from the Inflorescence Extends the Vase Life of Cut Hydrangea Flowers

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The relationship between transpiration from the inflorescence and the vase life of cut hydrangea 'Endless Summer' flowers was studied. In the defoliated cut flowers, the vase life increased with a decreasing number of decorative florets. Cut flowers having small inflorescences with 189 decorative florets exhibited a lower level of transpiration (7 g·day^{-1}) and longer vase life (15 days) than those having large inflorescences with 422 decorative florets. The stomatal conductivity of the decorative sepals was very low, ranging from 2.7 mmol·m^{-2}·s^{-1} to 3.3 mmol·m^{-2}·s^{-1}, and approximately 6% of the stomata were observed to be open microscopically. In addition, diurnal change of transpiration from a defoliated cut flower was not observed. These observations indicate that most of the transpiration from the sepals is through cuticular transpiration. The use of defoliated cut flowers that do not bear too many decorative florets and treatments that suppress transpiration from the surface of the decorative sepals would be effective for the vase life extension of cut hydrangea flowers.

Key Words: decorative sepal, hortensia, Japanese hydrangea market, stomatal conductance.

Introduction

Vase life is the major factor that determines the marketability of cut flowers, and is strongly affected by water balance, which is determined by a combination of transpiration and water absorption (Ichimura, 2010). When the transpiration rate exceeds the water absorption rate in a cut flower, turgor declines and wilting occurs. Treatments that suppress the stomatal or cuticular transpiration extend the vase life of many cut flowers through maintenance of turgor. For example, in roses, abscisic acid treatment, which induces the closing of the stomata on the leaves, or removal of the leaves results in vase life extension (Carpenter and Rasmussen, 1974; Halevy et al., 1974). Furthermore, the existence of a dark period, a treatment that closes the stomata, improves the water balance of cut flowers (Doi et al., 1999). In anthurium, waxy coating on the spathe extends the vase life of cut flowers (Mujaffar and Sankat, 2003; Paull and Goo, 1985).

Hydrangeas (*Hydrangea* spp.) are popular ornamental plants cultivated in many countries. Hydrangea inflorescences have 2 types of floret. One is a non-decorative floret, bearing tiny sepals, and the other is a decorative floret, bearing large decorative sepals. The inflorescence of hydrangea is classified into two types: hortensia and lacecap. In hortensia, the decorative florets constitute most of the inflorescence, and in lacecap, decorative florets are situated around the periphery of the inflorescence (Uemachi and Nishio, 2005; Uemachi et al., 2006).

In Japan, consumption of cut hydrangea flowers has been increasing in recent years, and the market requires stable production of high-quality cut flowers. Cut hydrangea flowers often wither in a few days. Selection of cut flowers with small inflorescences and the removal of the leaves from cut flowers are known by farmers as effective techniques for extending the vase life of cut hydrangea flowers. Mega (1957) also reported that covering the stem with latex film or removal of the leaves extends the vase life of cut hydrangea flowers. However, the relationship between transpiration from the inflorescences and the vase life of cut flowers has not been studied in hydrangeas. Almost all of the hydrangea cultivars marketed as cut flowers have hortensia-type inflorescences. Transpiration from the decorative florets constituting most of the hortensia inflorescence

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can have a crucial influence on the vase life of cut hydrangea flowers. Although stomata exist on the abaxial side of decorative sepals, their functionalities have not been studied.

We hypothesized that suppression of transpiration from the inflorescences would be effective for extending the vase life of hydrangea cut flowers. In the present study, the effect of suppression of transpiration of hydrangea inflorescences on the vase life of cut flowers was studied. The stomatal conductance and the opening state of stomata were measured and the role of the stomata in the regulation of transpiration from the decorative sepals is discussed.

Materials and Methods

Plant materials

'Endless Summer', a cultivar bred as a garden shrub, was used for all experiments. 'Endless Summer' is not sold in the hydrangea cut flower market because of its short vase life. However, since we can easily verify the effect of treatment suppressing transpiration on the vase life of cut flowers, 'Endless Summer' was suitable for the present study. A mother plant for cuttings was purchased at a nursery in June 2010.

At Shinshu University experimental farm, all plants were grown in 32.5-cm-diameter pots filled with 8 L of medium composed of 75% Metro Mix 250 (SunGro Horticulture, Agawam, MA, USA) and 25% vermiculite (v/v) (Asahi Kogyo, Okayama, Japan) under full-sunlight conditions in summer and fall. The plants were grown from December 2010 to January 2011 in a greenhouse heated above 0°C. After February 2011, the plants were transferred to a greenhouse heated above 17°C. In 2012, plants were grown continuously in a greenhouse heated above 0°C. The coloration of the decorative sepals was visually assessed, and cut flowers with approximately 50 cm stems were harvested.

Vase life and transpiration from cut flowers

Leaving 40 cm stems, the end of the cut flower stem was recut under distilled water just after the harvest. The diameter of the recut stem end was measured. Leaves were trimmed to three pairs for the control cut flowers. All cut flowers were kept in conical flasks filled with 300 mL of distilled water. The flask was loosely sealed with parafilm (Pechiney Plastic Packaging Company, Chicago, IL, USA) to suppress evaporation from the vase water surface. Immediately following the preparation of the cut flower, the total mass of the cut flower, distilled water and flask was recorded. The mass was recorded in the same manner at the same time on the following day, and the daily transpiration rate from the cut flower was defined as the decrease in mass in this time period. Cut flowers were placed in an environmentally controlled room held at 25 ± 2°C and 50 ± 5% relative humidity (RH), and maintained under 12-h photoperiods at a light intensity of 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ provided by daylight fluorescent tubes (FL40SSN/37; Toshiba Lighting and Technology Co., Yokosuka, Japan). The vase life was terminated when withering and sepal browning became apparent. Decorative florets were counted at the end of vase life in all treatments except the inflorescence-covering treatment.

Measurement of the areas of decorative sepals and leaves

On 31 March, 2011, a cut flower was harvested. Images of 30 decorative florets, copied onto A4 paper, were clipped and weighed. The average area of a decorative floret was calculated using the ratio of the weight of a unit area of the A4 paper and that of the copied paper of a decorative floret. The total sepal area of the whole inflorescence was calculated by multiplying the number of decorative florets by the average area of a decorative floret. Leaf area was calculated in the same manner.

Suppression of transpiration from cut flowers

Four cut flowers for each treatment were harvested on 31 March, 2011. Cut flowers with 3 pairs of leaves were prepared as intact cut flowers. Transpiration from cut flowers was suppressed by 3 treatments: leaf removal, reduction of the number of decorative florets, and covering of the inflorescence. Leaf removal was performed immediately after harvest. Using defoliated cut flowers, the number of florets was reduced as follows: 2 or 4 secondary inflorescences on the first and second nodes of the primary inflorescence axis were removed. Inflorescences of cut flowers from which leaves had been removed were covered with polyvinyl chloride film (Asahikasei, Tokyo, Japan).

Transpiration and the vase life of small and large inflorescences

Five cut flowers bearing small inflorescences and 4 cut flowers bearing large inflorescences were harvested on 31 March, 2011. All leaves were removed from cut flowers just after harvest. Transpiration and the vase life of cut flowers were investigated as described above.

Stomatal conductance and the state of stomata of the decorative sepals

Three cut flowers were harvested from 11 to 18 June, 2012, all leaves were removed and the stem bases were placed in deionized water to be hydrated. Stomatal conductance of the decorative sepal was measured with a leaf porometer (SC-1; Decagon Devices, Pullman, WA, USA) on the day after harvest. One decorative sepal was chosen randomly from 5 secondary inflorescences each on the first and second nodes of the primary inflorescence axis. Stomatal conductance was recorded 2 min after the sensor head had been clipped to the sepal. The average of 5 readings from 5 sepals was cal-

culated as the stomatal conductance of the cut flower.

Under the light conditions on the day after harvest, one decorative floret was chosen randomly from each inflorescence and a mold of the abaxial epidermis of the sepal was made with dental paste (EXAFINE; GC Corporation, Tokyo, Japan). A replica of the abaxial epidermis of the sepal was prepared by pouring epoxy resin (Konishi Bond; Konishi, Osaka, Japan) into the mold. Under a light microscope (BH2; Olympus, Tokyo, Japan), more than 400 stomata were observed for each replica, and the percentage of open stomata was recorded. To estimate whether the stomata on the decorative sepal are functional or not, diurnal change of transpiration from a cut flower was studied. The total mass of the cut flower, distilled water and flask was recorded hourly using a digital camera. The decrease in mass per hour was calculated as the transpiration rate per hour from the cut flower. Measurements were continued for 48 h.

Results

Suppression of transpiration extends the vase life of cut hydrangea flowers

In all cut flowers, except for those with the inflorescence covered, rapid progress of wilting of the decorative sepals marked the end of the vase life of cut flowers. The vase life of inflorescence-covered cut flowers was terminated when browning of the decorative sepals was observed. The cut flower characteristics measured immediately after the end of the vase life are shown in Table 1. Numbers of decorative florets on intact cut flowers, defoliated cut flowers, and defoliated cut flowers with 2 or 4 of the secondary inflorescences removed were approximately 366, 422, 222, and 117, respectively. The stem diameters of the cut flowers used in all transpiration suppression treatments were almost identical. Transpiration levels in intact cut flowers, defoliated cut flowers, defoliated cut flowers with 2 or 4 of the secondary inflorescence removed, and with the intact inflorescences covered with polyvinyl chloride film were approximately 28, 20, 14, 7, and 5 $g \cdot day^{-1}$, respectively (Fig. 1). Vase lives of those cut flowers were approximately 2, 6, 10, 14, and 15 days, respectively.

Inflorescence size determines the vase life of cut flowers

Large and small inflorescences had approximately 422 and 189 decorative florets, respectively (Table 2). The diameters of the stems of the cut flowers bearing large and small inflorescence were approximately 5.4 and 4.3 mm, respectively (Table 2). The transpiration levels of those cut flowers were approximately 19 $g \cdot day^{-1}$ and 7 $g \cdot day^{-1}$, respectively (Table 2). The vase lives were approximately 5 and 15 days, respectively (Table 2).

Table 1. Number of decorative florets, leaf area and total sepal area of cut flower in each transpiration suppression treatment.

Treatment	Number of decorative florets	Leaf area (cm^2)	Total sepal area (cm^2)	Diameter of stem end (mm)
Intact	366±88[z]	303±48	2215±536	5.4±0.3
Defoliated	422±76	—	2554±461	5.4±0.3
Removal of 2 secondary inflorescences and leaves	222±62	—	1343±379	5.2±0.2
Removal of 4 secondary inflorescences and leaves	117±19	—	712±120	5.1±0.6

[z] The values are the mean±SE (n=4).

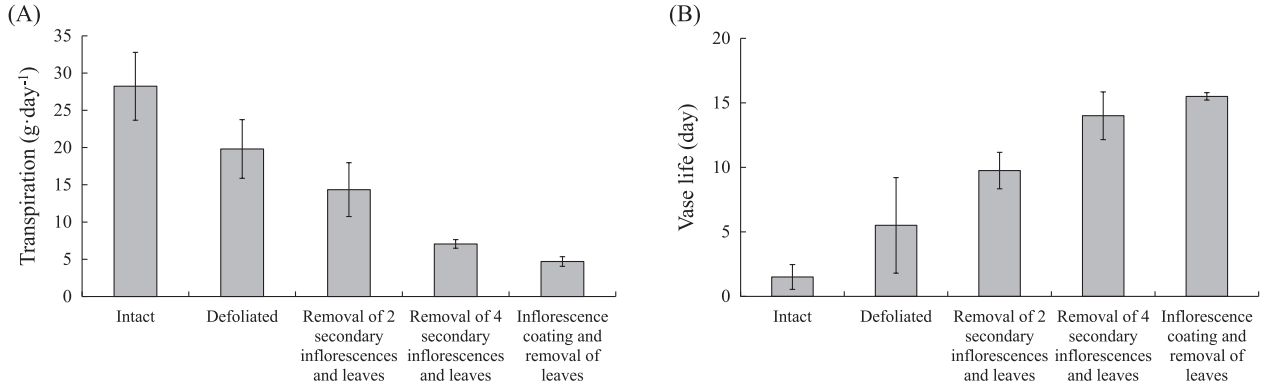

Fig. 1. Transpiration and vase lives of transpiration-suppressed cut hydrangea flowers. (A) Transpiration from transpiration-suppressed cut flowers. (B) Vase life of transpiration-suppressed cut flowers. Immediately following the preparation of the cut flower, the total mass of the cut flower, distilled water and flask was recorded. The mass was recorded in the same manner at the same time on the following day, and the daily transpiration rate from the cut flower was defined as the decrease in mass in this time period. The values are the mean ± SE (n = 4).

Table 2. Vase life, number of decorative florets and transpiration in cut flowers with large or small inflorescence.

Inflorescence type[z]	Vase life (day)	Number of decorative florets	Transpiration from cut flower ($g \cdot day^{-1}$)	Diameter of stem end (mm)
Large inflorescence	5±1[y]	422±76	19±3	5.4±0.3
Small inflorescence	15±2	189±38	7±1	4.3±0.4

[z] Leaves were removed from all cut flowers.
[y] The values are the mean±SE (n=4).

Table 3. Stomatal conductance of the sepal and transpiration in cut flowers.

Sample[z]	Stomatal conductance ($mmol \cdot m^{-2} \cdot s^{-1}$)	Transpiration from cut flower ($g \cdot day^{-1}$)	Number of decorative florets	Transpiration per decorative floret ($mg \cdot day^{-1}$)[y]
a	3.3±0.5[x]	18	439	40
b	2.8±0.4	14	428	33
c	2.7±0.4	12	303	41

[z] Three cut flowers were randomly sampled and used for the investigation.
[y] Transpiration rate from a decorative floret was derived from the transpiration rate of the cut flower and the number of decorative florets. The transpiration rate of the cut flower was divided by the number of decorative florets.
[x] Measurements were conducted on the day after harvest. The values are the mean±SE (n=5).

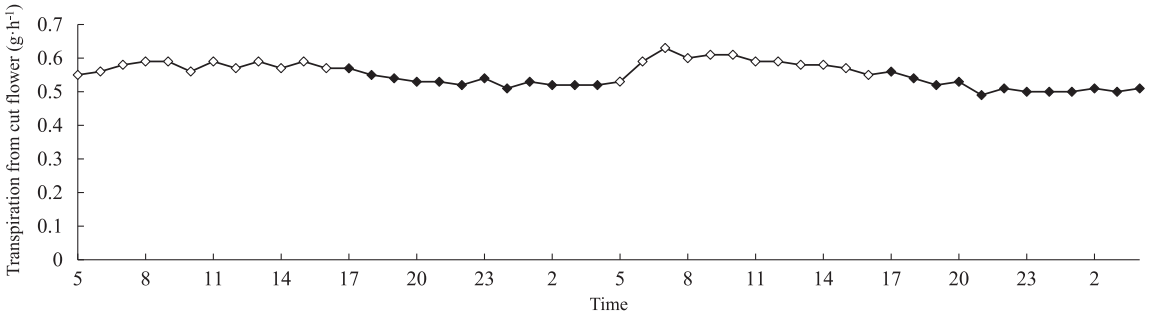

Fig. 2. Diurnal change of transpiration from a defoliated cut flower. ◇, Light period; ◆, Dark period. Investigation was continued for 48 h.

Table 4. Percentage of open stomata observed on the abaxial sides of the decorative sepal.

Number of observed stomata	Open (%)
491±40[z]	6±2

[z] A replica of the abaxial epidermis of the sepal was prepared the day after harvest.
The values are the mean±SE (n=3).

Closed stomata dominated the abaxial side of the decorative sepals

The average stomatal conductance of the abaxial side of the decorative sepals ranged from 2.7 to 3.3 $mmol \cdot m^{-2} \cdot s^{-1}$ (Table 3). There was no correlation between transpiration per decorative floret and stomatal conductance. Closed stomata dominated the abaxial sides of the decorative sepals. The percentage of open stomata was 6.3 (Table 4). Obvious diurnal change of transpiration was not observed in the defoliated cut flowers (Fig. 2).

Discussion

Suppression of transpiration from inflorescences, using a variety of treatments, elongated the vase life of the cut hydrangea flowers. This result is considered to have been due to the reduction of the water required for maintenance of the water balance of the cut flowers. In addition, extra time prior to wilting would have been generated.

In a wide variety of cut flowers, occlusion of vessels results in disruption of the water balance and progress of the wilting of these flowers (Loubaud and van Doorn, 2004; van Doorn and Cruz, 2000; van Doorn et al., 1989). The stems of the cut flowers used in all experiments were of almost identical diameter, except for the cut flowers bearing small inflorescences. The hydraulic conductivity of the stems of the cut flowers bearing a small inflorescence would be less than those of others; however, in the present study, a negative effect on vase life in cut flowers with thin stems was not apparent.

In cut rose flowers, browning of petals, caused by infection of *Botrytis cinerea*, is facilitated under high-humidity conditions (Williamson et al., 1995). In the present study, browning of decorative sepals, which was frequently observed in covered inflorescences, may

have been due to the high humidity in the cover and pathogens in the environment. In a previous study on anthurium, an artificial wax coating on the spathe suppressed the transpiration from cut flowers and resulted in extension of the vase life (Mujaffar and Sankat, 2003). Anti-transpirants, materials that coat the surfaces of cut flowers without raising the humidity around them, would be useful for extending the vase life of cut hydrangea flowers.

Although the effect of a difference in hydraulic conductivity of the stem remains to be elucidated, the number of decorative florets can be used as an indicator of the longevity of cut hydrangea flowers. Cut flowers with large inflorescences, carrying approximately 420 decorative florets, had a vase life of 5 days. On the other hand, the vase life of small inflorescences, carrying approximately 190 decorative florets, was approximately 15 days. The Japanese market prefers cut hydrangea flowers with rather small inflorescences (personal communication with market staff), so smallness of the inflorescences would not spoil the marketability of cut hydrangea flowers in Japan.

Some studies of stomata formed on the decorative organ have been reported. Hew et al. (1980) reported that stomata on tepals of orchids do not react to abscisic acid treatment, and concluded that those stomata contribute little to transpiration control of the flower. Azad et al. (2007) studied stomata on the tepals of tulips, and suggested the involvement of stomatal transpiration regulation in flower opening and closing. In anthurium, almost all of the stomata on the spathe are closed, and no correlation was identified between the stomatal density and the vase life of cut flowers (Elibox and Umaharan, 2010). In the present study, most of the stomata formed on the decorative sepals were closed even under light conditions (Table 4), and low stomatal conductance was detected on the abaxial epidermis of the sepal (Table 3). In the defoliated cut flowers, diurnal change in transpiration was not observed (Fig. 2). Thus, most stomata formed on the decorative sepal would not be functional. Transpiration from the decorative sepals would thus be determined by cuticular transpiration.

In the present study, it was suggested that the suppression of transpiration from the inflorescences was effective for extending the vase life of hydrangea cut flowers. We conclude that using cut flowers that do not bear too many decorative florets and treatments that suppress transpiration from the surface of the decorative sepals would be effective for extending the vase life of cut hydrangea flowers.

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