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Postharvest Treatments and Shipping Conditions for Reducing Fruit Drop in Cut *Sarcandra glabra* Branches

Hiroko SHIMIZU-YUMOTO[1]*, Yumi UJIIE[2] and Takayuki OGAWA[2]

[1] Institute of Vegetable and Floriculture Science, National Agriculture and Food Research Organization, Tsukuba, Japan
[2] Ibaraki Agricultural Center, Kasama, Japan

Abstract
In Japan, cut *Sarcandra glabra* branches with fruit are used as a New Year's decoration, but frequent fruit drop reduces ornamental value. This study investigated the effects of the harvest season, postharvest treatments, and shipping conditions on the fruit drop of branches, in order to propose a suitable postharvest handling method for the shipment of cut *S. glabra* branches. Ethylene treatments accelerated fruit drop, especially in cut branches on which the pericarp turned red early in the growing season. Silver thiosulfate complex (STS), an ethylene action inhibitor, was effective in reducing fruit drop regardless of ethylene treatments. However, treatment with 1-methylcyclopropene (1-MCP), another ethylene action inhibitor, increased the cumulative percentage of dropped fruit after seven days with or without ethylene treatment. A synthetic auxin, 1-naphthaleneacetic acid (NAA), had an effect similar to that of STS, but NAA tended to accelerate leaf abscission. Treatments with 0.03 to 1.0 mM STS for three days at 10°C reduced fruit drop. Treatments with 0.03 to 0.1 mM STS for three days at 23°C also reduced fruit drop, but the cut ends of branches turned brown with more than 0.3 mM STS. For pretreatment with tap water, the cumulative percentage of dropped fruit after simulated shipment at 10°C increased earlier than at 2°C. Pretreatment with STS strongly suppressed fruit drop after simulated shipment at 2°C and 10°C. Sugar and antibacterial agents in the vase solution were also effective in reducing fruit drop. Finally, the cut branches of *S. glabra* were treated with 0.1 to 0.3 mM STS for three days in the grower's stockroom, and then dry-transported by reefer container at 2°C for 11 days. As a result, the cumulative percentage of dropped fruit from the branches was very low for ten days in a quality test room.

Discipline: Horticulture
Additional key words: ethylene, export, reefer container, silver thiosulfate complex, sugar

Introduction

Sarcandra glabra (Thunb.) Nakai is an evergreen shrub native to temperate and tropical Asia, with a broad distribution in the shady understory of forests in Japan, Korea, China, India, and Malaysia (Kunishige & Funakoshi 1994). Tiny flowers bloom from the end of June to July, followed by the formation of small drupe-type fruits that start to turn red by mid-November.

S. glabra has medicinal applications and is also used for decorations. In China, *S. glabra* has been used in traditional medicine as a treatment for bruises, bone fractures, and arthritis (Wu et al. 2012). In Japan, growers select and cultivate individual varieties that produce branches with fruits that will mature with optimal timing for use as a New Year's decoration for good luck. Branches with red-colored fruits are harvested from mid-November, and are allowed to absorb well water until shipment around mid-December. As red color is considered a lucky charm in China and other parts of Asia, Japanese growers intend to export *S. glabra* branches, but the postharvest characteristics and appropriate handling of *S. glabra* have yet to be examined in detail.

Ethylene is a plant growth regulator that promotes the wilting of petals, ripening of fruits, and abscission of leaves. In cut flowers of *Delphinium* hybrid and *Leptospermum scoparium*, ethylene accelerates sepal or petal abscission, and silver thiosulfate complex (STS), an ethylene action inhibitor, delays their abscission

*Corresponding author: hirokosh@affrc.go.jp
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(Ichimura et al. 2009, Zieslin & Gottesman 1983). Fruit drop reduces the ornamental value of cut *S. glabra* branches. Spray treatment with 2-chloroethyl phosphonic acid (CEPA), an ethylene generator, was found to accelerate fruit drop, and STS treatment for 24 h suppresses fruit drop in cut *S. glabra* twigs (Minosou & Tabuchi 2003). The effect of STS treatment on fruit drop in the cut branches of *S. glabra* has not been examined; consequently, Japanese growers do not use STS as a postharvest treatment for cut *S. glabra* branches. Another ethylene action inhibitor, 1-methylcyclopropene (1-MCP), is effective for extending vase life by delaying sepal drop in cut *Delphinium* flowers (Ichimura et al. 2002). Treatment with 1-naphthaleneacetic acid (NAA), a synthetic auxin, also delays bract drop in potted *Bougainvillea* (Chang & Chen 2001). These chemical treatments (called 'pulse treatments') are usually applied by growers for a short period before shipment. In addition, there are cultivar or line variations in the sensitivity to ethylene on flower senescence in carnations (*Dianthus caryophyllus* L.), *Petunia hybrida* L., and roses (*Rosa hybrida* L.) (Müller et al. 1998, Porat et al. 1993, Wu et al. 1991). Shipping conditions such as temperature and packaging method also affect the vase life of cut flowers (Doi et al. 1999, Halevy et al. 1978, Hu et al. 1998, Miyamae et al. 2007). For dry transportation, cut flowers are packed in a carton box without supplying water, and for wet transportation, cut flowers are placed in a bucket containing water. In this study, we investigated the effects of harvest time, postharvest treatments, and shipping conditions on fruit drop of branches in the vase, and propose a suitable postharvest handling method for the shipment of cut *S. glabra* branches.

Materials and methods

1. Plants

Ornamental branches of *S. glabra* were used for the experiments. The branches were harvested at Ibaraki Agricultural Institute, Kamisu, Ibaraki Prefecture or obtained from a grower in Kamisu, Ibaraki Prefecture in November or December.

2. Evaluation of fruit drop

Cut branches of *S. glabra* were evaluated for fruit drop in a quality test room under conditions of 23°C, 70% relative humidity, and a 12 h photoperiod of 10 μmol/m^2/s irradiance from cool-white fluorescent lamps. On the days of evaluation, branches were shaken gently three times, and then the number of dropped fruits was counted. On the final day of evaluation, the number of fruits remaining on the branches was counted. The cumulative percentage of dropped fruits was calculated as follows:

Cumulative percentage of dropped fruits (%) = (number of fruits dropped by shaking on current and previous drop test days / number of total fruits) × 100

3. Sensitivity to ethylene

On 14 and 28 November, and on 12 December 2016, ornamental branches of *S. glabra* were harvested at the Ibaraki Agricultural Institute to produce early, middle, and late color groups selected based on the season for pericarp to turn red. After harvest, branches were soaked in tap water for two days, and then dry-transported to the NARO Institute of Vegetable and Floriculture Science, Tsukuba, Ibaraki Prefecture, which took one day. The cut ends of the branches were recut (removed 5 cm) and soaked in tap water overnight at 5°C in the dark. The next day, the branches were recut to 55 cm. Branches were placed in glass vessels containing antibacterial agents (50 mg/L aluminum sulfate and 0.5 mL/L Kathon CG (Rohm and Haas Japan, Tokyo, Japan)) that contained 11.3 g/L 5-chloro-2-methyl-4-isothiazolin-3-one and 3.7 g/L 2-methyl-4-isothiazolin-3-one as active ingredients. The vases were placed in 70-L transparent acrylic boxes fitted with a septum through which ethylene was introduced to achieve a concentration of 2 or 10 μL/L. The control chamber was not treated with ethylene. The boxes were maintained for 24 h at 23°C and a 12 h photoperiod of 10 μmol/m^2/s irradiance from cool-white fluorescent lamps. After 24 h, the boxes were opened and the branches were held in the quality test room for ten days. From the next day, the number of dropped fruits was counted every day. On the final day of evaluation (day 10), the number of fruits remaining on the branches was counted. Replication entailed six branches per treatment.

4. Chemical treatments for pulsing

Ornamental branches of *S. glabra* were dry-transported from the grower in Kamisu, Ibaraki Prefecture to the NARO Institute of Vegetable and Floriculture Science on 11 January 2017. After arrival, the branches were recut to 55 cm and transferred to the quality test room. The branches were treated as follows: The cut ends of branches were immersed in 0.3 mM STS for 24 h; STS solution was prepared by mixing equal volumes of $AgNO_3$ and $Na_2S_2O_3$ at a molar ratio of 1 to 8. For the 1-MCP treatment, the cut ends of branches were immersed in distilled water and placed in an acrylic chamber (70 L), and then EthylBloc (Rohm and Haas Japan) was added to the distilled water to evolve 1-MCP at a concentration of 2 μL/L for 24 h. Fruits were fully wetted with a spray of 0.2 mM NAA solution, and the cut ends of branches were immersed in distilled water for 24 h. After chemical treatments, the cut ends of all branches were immersed in vase solution with antibacterial agents. The next day, half of the branches subjected to each chemical treatment were treated with 10 µL/L ethylene for 24 h. Other branches were left untreated. After ethylene treatment, the branches were held in the quality test room for ten days, and from the next day, the number of dropped fruits was counted every day. On the final day of evaluation (day 10), the number of fruits remaining on the branches was counted. Replication entailed six branches per treatment.

5. Temperature and STS concentration

Ornamental branches of *S. glabra* were harvested on November 16, 2018 at Ibaraki Agricultural Institute. After harvest, the branches were soaked in tap water for two days and then dry-transported to the NARO Institute of Vegetable and Floriculture Science, which took one day. The bottom of the branches was recut (removed 5 cm) and placed in tap water or in 0.03, 0.1, 0.3, or 1 mM STS solution; 7000, 2000, 700, or 200 times dilution of 'Chrysal K-20C' (Chrysal Japan, Osaka, Japan), respectively, for three days at 10°C or 23°C in the dark. After treatment, the branches were transferred to vase solution with antibacterial agents and held in the quality test room. The number of dropped fruits was counted on days 12, 17, and 20. Replication entailed four branches per treatment. The weight of the branches and vessel containing solution was measured before and after treatments to calculate solution absorption and estimate silver absorption.

6. Shipping conditions and post-shipment vase solution examination

Ornamental branches of *S. glabra* were dry-transported from the grower in Kamisu, Ibaraki Prefecture to the NARO Institute of Vegetable and Floriculture Science on 4 December 2017. The branches were recut (removed 5 cm) and placed in tap water or 0.3 mM STS solution; 600 times dilution of 'Chrysal K-20C' for 24 h at 23°C. After treatments, the shipping of branches was simulated using a dry or wet method lasting seven days at 2°C or 10°C in the dark. For dry shipment simulation, the branches were wrapped in newspaper and placed in a cardboard box. For wet shipment simulation, the branches were placed in a bucket containing solution with antibacterial agents.

After the shipment simulation, the branches were recut to a length of 55 cm. The branches were placed in glass vessels containing solution with antibacterial agents only or antibacterial agents and 1% (w/v) glucose and held in the quality test room for ten days. The number of dropped fruits was counted every day. On the final day of evaluation (day 10), the number of fruits remaining on the branches was counted. Replication entailed five or six branches per treatment.

7. Simulated shipment using a container

Branches of *S. glabra* were recut (removed 5 cm) and soaked in well water or STS solutions for three days in the stockroom of the grower in Kamisu, Ibaraki Prefecture on 30 November 2018. STS concentration at 0.1, 0.2 and 0.3 mM was prepared by diluting 'Chrysal K-20C' 2000, 1000, and 700 times, respectively. Twenty branches were placed in each solution. After three days, the 20 branches were wrapped in paper and bunches were packed two per carton. The carton boxes were transferred from the stockroom to a reefer container (20-foot container, 6,058 × 2,438 × 2,591 mm) placed at the port of Kashima in Ibaraki Prefecture, and then held at 2°C in the dark for 11 days. The carton boxes were then transferred to the NARO Institute of Vegetable and Floriculture Science. All branches were recut (removed 5 cm) and soaked overnight in antibacterial solution made by diluting 'Chrysal bucket' (Chrysal Japan, Osaka, Japan) 500 times at 10°C in the dark. The next day, 12 branches per treatment were recut to 55 cm and transferred to vase solution with antibacterial agents only or antibacterial agents and 1% (w/v) glucose, and then held in the quality test room for ten days. The number of dropped fruits was counted every day. On the final day of evaluation (day 10), the number of fruits remaining on the branches was counted. Replication entailed six branches per treatment.

Results and discussion

1. Effects of ethylene treatment on fruit drop

The cumulative percentage of dropped fruits was higher with 24 h treatment with 2 and 10 µL/L ethylene in the early, middle, and late colored groups of cut *S. glabra* branches (Fig. 1). This is consistent with the results of Minosou & Tabuchi (2003) showing that 500 ppm CEPA spray increases ethylene production from fruits and accelerates fruit drop in *S. glabra* twigs. Thus, fruit drop in *S. glabra* shows sensitivity to ethylene. In *S. glabra* twigs with red-colored fruits, cells forming an abscission zone between the fruit and the peduncle were observed by light microscope, and following the collapse of those cell walls, aperture occurred and finally, the fruit was abscised (Tabuchi & Misonou 2003). Electron micrographs showed that cell walls of the abscission zone become swollen, and cell separation commences within hours with ethylene treatment in *Coleus* explant (Reid 1985). In *S. glabra*, ethylene treatment likely accelerates cell wall degradation in the abscission zone.

In petunia, differential sensitivity to ethylene for petal wilting was shown to apparently determine flower longevity in two lines (Porat et al. 1993). In carnations, two cultivars with a long vase life were shown to have differential sensitivity to ethylene on petal wilting (Wu et al. 1991). In this study, the cumulative percentage of dropped fruits without ethylene was the highest in the middle colored group, while sensitivity to ethylene was moderate (Fig. 1). Therefore, fruit drop without ethylene may be irrelevant to the season of pericarp coloration in cut *S. glabra* branches. In the early colored group, the cumulative percentage of dropped fruits after ten days was 23.2% and 28.2% with 2 and 10 µL/L ethylene, respectively. In contrast, it was 3.6% and 7.1%, respectively, in the late colored group, and the middle colored group showed an intermediate response. These results suggest that the season of pericarp coloration may play an important role in the susceptibility to ethylene treatment.

2. Effects of chemical treatments for pulsing on fruit drop

In *S. glabra* twigs, ethylene production by fruits is low and fruit drop could be suppressed for 11 days by treatment with 0.3 mM STS for 24 h (Minosou & Tabuchi 2003). In this study, the cumulative percentage of dropped fruits of cut *S. glabra* branch was also very low in the STS treatment, irrespective of ethylene treatment (Fig. 2). Next, the effects of temperature and concentration of the STS treatment on solution absorption and fruit drop were examined as the cut ends of *S. glabra* branches are usually placed in water at cool temperatures for several days until shipment. The amount of solution absorption for three days ranged from 16.1 to 19.4 g/100 g fresh weight (FW) at 10°C and 45.9 to 50.3 g/100 g FW at 23°C (Table 1). The cut ends of branches turned brown with 0.3 and 1 mM STS treatments at 23°C, and whole branches wilted with 1 mM STS at 23°C. Compared with the non-STS treatments at 10°C and 23°C, there were very few dropped fruits in the STS treatments, except in the 1.0 mM at 23°C treatment where the branch wilted severely. These results indicate that 0.03 to 1.0 mM STS at 10°C or 23°C for three days effectively reduces fruit drop in cut *S. glabra* branches. The estimated silver absorption rates effective in reducing fruit drop were 0.5 to 17.3 µmol/100 g FW at 10°C, and 1.5 to 15.1 µmol/100 g FW at 23°C (Table 1). In cut flowers, silver absorption sufficient to delay petal wilting or sepal abscission was more than 2 µmol/100 g FW in carnations (Uda 1996), 4.5 to 13.2 µmol/100 g FW in *Eustoma grandiflorum* (Shimamura & Okabayashi 1997), and 2 or 3 µmol/100 g florets in *Delphinium* (Kuroshima et al. 2009). Compared to the ranges for flowers, the effective and non-harmful range of STS absorption is wide in *S. glabra* branches, which means that STS treatment may be easy for growers to implement.

The cumulative percentage of dropped fruits of cut *S. glabra* branches increased more than 10% after day 7 by 1-MCP treatment with and without ethylene treatment (Fig. 2). In *Pelargonium peltatum*, the effect of 1-MCP on the reduction of petal abscission by ethylene is transient, and re-treatment with 1-MCP is necessary to regain effectiveness (Cameron & Reid 2001). Moreover, STS delays petal senescence more than does 1-MCP in cut

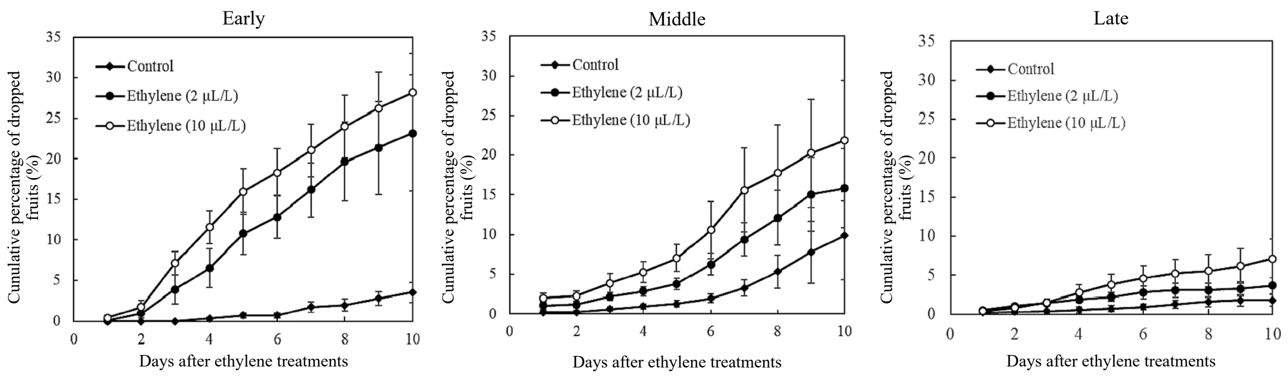

Fig. 1. Effect of ethylene treatment on cumulative percentage of dropped fruits in cut *S. glabra* branches
Branches were harvested on 14 November (early), 28 November (middle) and 12 December (late) based on the season of pericarp coloration. Cut branches were placed in vase solution containing antibacterial agents and treated with 2 or 10 µL/L ethylene for 24 h; the control branches were not treated. From the next day after ethylene treatment, the number of dropped fruits was counted every day. On the final day of evaluation (day 10), the number of fruits remaining on the branches was counted. Replication entailed six branches per treatment. Vertical bars represent S.E.

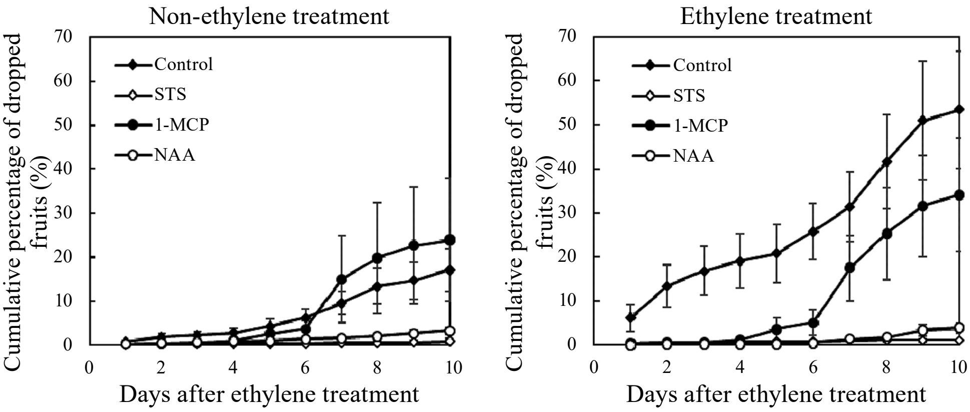

Fig. 2. Effect of chemical treatments for pulsing on cumulative percentage of dropped fruits in cut *S. glabra* branches

Cut branches were treated with distilled water (control) or 0.3 mM STS, 2 µL/L 1-MCP, and 0.2 mM NAA for 24 h, and cut ends were placed in vase solution containing antibacterial agents. The next day, half of the branches were treated with 10 µL/L ethylene for 24 h; the other half was not treated. From the next day after ethylene treatment, the number of dropped fruits was counted every day. On the final day of evaluation (day 10), the number of fruits remaining on the branches was counted. Replication entailed six branches per treatment. Vertical bars represent S.E.

Table 1. Effects of concentration of STS and temperature during treatments on solution absorption, estimated silver absorption, and number of dropped fruits in cut *Sarcandra glabra* branches

Treatment temperature	STS concentration (mM)	Solution absorption (g/100 g FW)	Estimated silver absorption[a] (µmol/100 g FW)	Number of dropped fruits[b]
10°C				
	0	18.4 ± 0.8	-	10.8 ± 4.7
	0.03	16.1 ± 0.7	0.5 ± 0.0	1.5 ± 0.6 *[c]
	0.1	19.4 ± 0.7	1.9 ± 0.1	1.3 ± 0.8 *
	0.3	17.0 ± 0.6	5.1 ± 0.2	0.8 ± 0.8 *
	1	17.3 ± 0.7	17.3 ± 0.7	2.0 ± 0.6 *
23°C				
	0	49.8 ± 1.6	-	14.8 ± 6.4
	0.03	50.3 ± 0.6	1.5 ± 0.0	0.0 ± 0.0 *
	0.1	48.5 ± 1.3	4.8 ± 0.1	0.8 ± 0.5 *
	0.3	50.3 ± 2.0	15.1 ± 0.6	1.3 ± 0.6 *
	1	45.9 ± 0.3	45.9 ± 0.3	-[d]

Cut branches were treated with 0, 0.03, 0.1, 0.3 and 1.0 mM STS for three days at 10°C or 23°C. After treatments, the cut ends were soaked in vase solution containing antibacterial agents, and the number of dropped fruits was counted on days 12, 17, and 20 of evaluation. Each value is the mean ± S.E (n = 4).

a) Estimated silver absorption was calculated from solution absorption and STS concentration.

b) The number of dropped fruits was the total number observed on three sampling days.

c) Values followed by * within each temperature treatment group were significantly different from 0 mM STS at that temperature at $P = 0.05$ by Dunnett's test.

d) The number of dropped fruits was not counted because the cut branches wilted severely.

carnation and *Delphinium* flowers, and in potted plants of *Campanula carpatica* and *Schlumbergera truncata* (Ichimura et al. 2002, Serek & Sisler 2001). In cut *S. glabra* branches, a single treatment with 1-MCP is insufficient to reduce fruit drop for ten days of vase life. Spraying with NAA reduced fruit drop with and without ethylene at the same level of STS treatment (Fig. 2). However, leaf abscission by ethylene was accelerated in cut *S. glabra* branches with NAA treatment (data not shown). In potted *Bougainvillea*, spraying with 50 ppm NAA is very effective in delaying bract abscission, but induces slight leaf abscission or chlorosis, depending on the cultivar (Chang & Chen 2001). In *Phaseolus vulgaris* L., 0.5 mM NAA treatment induces ethylene production in the root, stem, and leaf blade, and also accelerates leaf abscission of the petiole explants much more than in the control (Abeles & Rubinstein 1964). In cut *S. glabra* branches, the optimal concentration of NAA to delay organ abscission may differ between fruits and leaves.

3. Effects of handling method, temperature during shipment, and vase solution on fruit drop

A seven-day study of storage methods was conducted to simulate the shipment of cut *S. glabra* branches from Japan to other East Asia countries by ship. The cumulative percentage of dropped fruits of cut *S. glabra* branches quickly increased in tap water for pulsing and dry/wet transport methods at 10°C, whereas it increased after five or six days in tap water for pulsing and dry/wet transport at 2°C (Fig. 3). For tap water for pulsing, the cumulative percentage of dropped fruits was markedly increased by dry transport at 10°C as compared with dry transport at 2°C. The difference in the cumulative percentage of dropped fruits for wet storage in tap water for pulsing was small for wet storage at both 2°C and 10°C. In cut *Gypsophila paniculata* L. flowers, vase life was severely

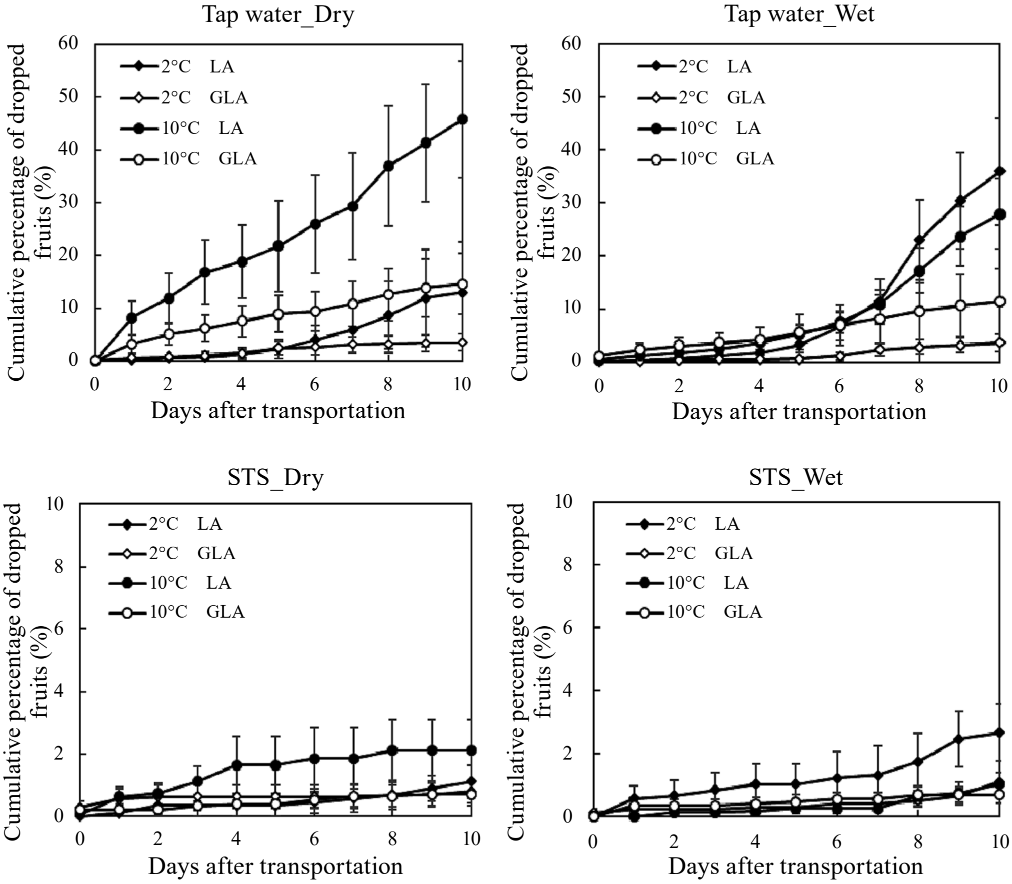

Fig. 3. Effect of temperature, simulated shipping method, and vase solution on cumulative percentage of dropped fruits in cut *S. glabra* branches

Cut branches were treated with tap water or 0.3 mM STS for 24 h at 23°C, and then packed in carton boxes (dry method) or in buckets containing antibacterial agents (wet method) for seven days at 2°C or 10°C to simulate shipping conditions. After seven days, the cut ends of the branches were soaked in vase solution containing antibacterial agents only (LA) or antibacterial agents and sugar (GLA) for ten days. The number of dropped fruits was counted every day. On the final day of evaluation (day 10), the number of fruits remaining on the branches was counted. Replication entailed five or six branches per treatment. Vertical bars represent S.E.

shortened by dry transport at high temperature (Doi et al. 1999, Miyamae et al. 2007), and the vase life of wet-transported flowers was not shortened more than that of dry-transported flowers, even when transported at 20°C (Miyamae et al. 2007). With the addition of sugar in tap water for pulsing as a vase solution, the cumulative percentage of dropped fruits for ten days was low for both transport methods and temperatures tested. In cut *Delphinium* flowers, sepal abscission delays and sensitivity to ethylene were reduced by sugar treatment (Ichimura et al. 2000). The cumulative percentage of dropped fruits was very low with STS for pulsing by either transport method, temperature, and vase solution (Fig. 3). These results suggest that STS pulse treatment is very effective in reducing fruit drop after putting branches in a vase for dry and wet transport at 2°C to 10°C for seven days.

4. Simulated shipment by reefer container

Based on our results and existing literature, a simulated transport by reefer container for cold storage was conducted. The appropriate treatment by growers and suppression of increases in temperature by shippers result in good quality cut flowers in the vase for carnation, chrysanthemum, and rose flowers (Halevy et al. 1978). The average temperature of the grower's stockroom where STS was used as a treatment was 13°C. The average temperature and relative humidity in the carton box during simulated transport was 1.5°C and 88%, respectively. With the use of well water for pulsing, the cumulative percentage of dropped fruits of cut *S. glabra* branches increased and reached 31% at day 10 after placing the branches in vase solution with antibacterial agents (Fig. 4). Fruit drop was reduced by using a vase solution with antibacterial agents and sugar, even without STS. All pulsing treatments with STS showed suppressed fruit drop, irrespective of the presence or absence of

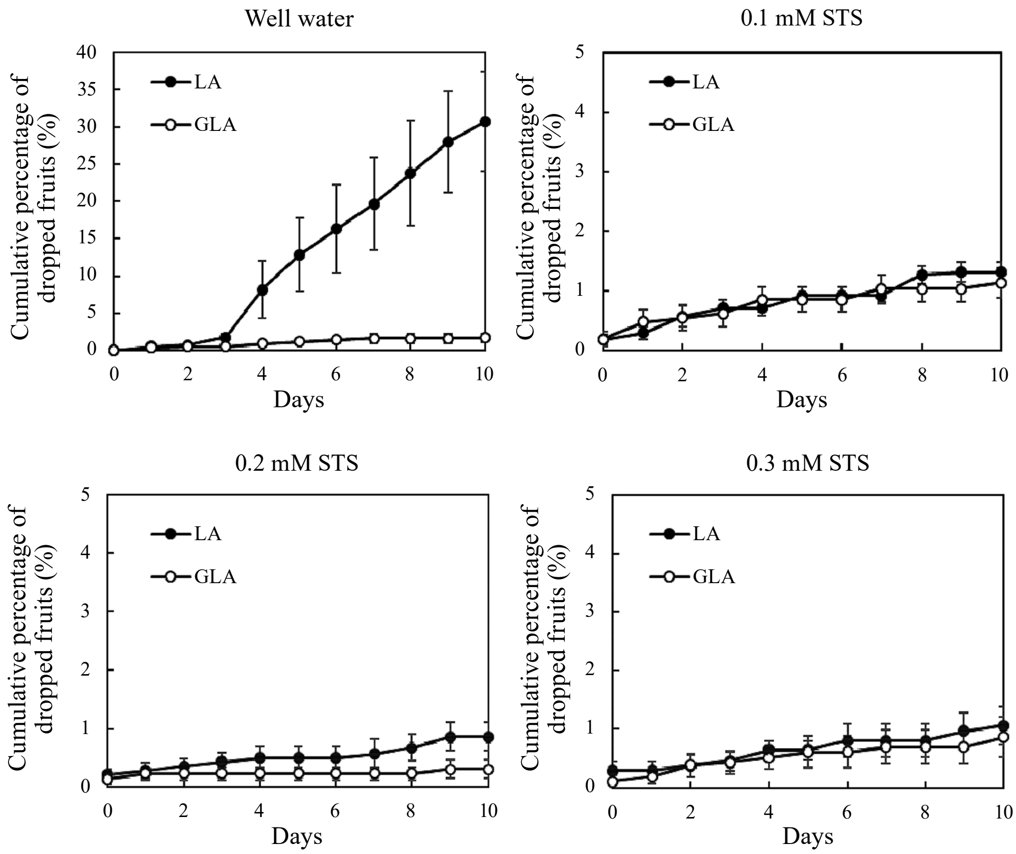

Fig. 4. Effects of STS treatments, simulated transportation by reefer container, and vase solution on cumulative percentage of dropped fruits in cut *S. glabra* branches

Cut branches were treated with well water or 0.1, 0.2, and 0.3 mM STS for three days in the grower's stockroom, and then packed in a carton box. The boxes were shipped in a reefer container for 11 days at 2°C, which simulated transportation. After 11 days, the cut ends of branches were soaked in vase solution containing antibacterial agents only (LA) or antibacterial agents and sugar (GLA) for ten days. The number of dropped fruits was counted every day. On the final day of evaluation (day 10), the number of fruits remaining on the branches was counted. Replication entailed six branches per treatment. Vertical bars represent S.E.

sugar in the vase. Taken together, the following method of suppressing fruit drop of cut *S. glabra* branches is proposed: after cutting, treat the cut ends with 0.1 to 0.3 mM STS at about 10°C for three days (initiated by growers), ship using the dry method at 2°C for up to 11 days, and then use a vase solution with at least antibacterial agents, but preferably with the addition of sugar.

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