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# Influence of Frozen Storage Duration and Forcing Temperature on Flowering of Oriental Hybrid Lilies

**Jong Suk Lee**
*Department of Horticulture, Chungnam National University, Daejeon 305-764, Korea*

**Mark S. Roh**
*U.S. Department of Agriculture–Agricultural Research Service, Floral and Nursery Plants Research Unit, Beltsville, MD 20705*

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***Abstract.*** **The effect of long-term storage of lily bulbs at –2 °C (frozen storage) and of high forcing temperatures on plant height and floral abnormalities was investigated with Oriental hybrid lilies from 1998 to 2000. 'Acapulco' and 'Simplon' bulbs were stored frozen at –2 °C for various lengths of time and were forced in fan- and pad-cooled greenhouses with temperatures ranging from 11 to 31 °C, depending on the season. The same cultivars were also forced in greenhouses and maintained year-round under refrigerated air conditioning with day/night temperatures of 16/15.5 °C or 18.5/18 °C. Floral development immediately after storage and at different intervals thereafter was observed by scanning electron microscopy (SEM). The prolonged frozen storage reduced the number of flowers. High greenhouse forcing temperatures during summer significantly accelerated flowering, resulted in short plants, and increased the number of abnormal flowers. Forcing at a low temperature (15.5 °C) after planting the frozen stored bulbs resulted in longer cut stems than those forced at 25 °C for 30 days after potting. Bulbs can be stored up to 9 months and still produce high-quality Oriental hybrid lilies.**

In order to produce Oriental hybrid lilies (*Lilium* hybrids) year-round as either potted plants or cut flowers, bulbs are typically stored at –2 °C (frozen storage) over an extended period. Short-term frozen storage of Oriental hybrid lilies may not affect quality (Boontjes, 1981; Franssen and Van der Hulst, 1985). However, long-term frozen storage of Asiatic hybrid lily (*L.* ×*elegans* Thunb.) bulbs resulted in increased flower bud abnormalities (Roh, 1990c). When frozen stored bulbs were planted during the summer, high temperatures (26 °C day/24 °C night) shortened the forcing period and reduced the number of flowers in 'Red Carpet', 'Sunray', and other cultivars of Asiatic hybrid lilies (Roh, 1990b, 1990c). High temperatures during floral development also resulted in a loss of flower buds in Asiatic hybrid lilies (Roh, 1990c; Zhang, 1991).

Oriental hybrid lily bulbs are generally harvested in November/December in the Netherlands and held at 5 °C during processing. They are shipped to the United States in December/January at 1 °C and are stored at –2 °C upon arrival ≈15 Jan. and are kept at that temperature until forcing (John Vandenberg, personal communication, 2000). Forcing Oriental hybrid lily bulbs during the summer requires the use of bulbs that were stored for an extended period of time, making it difficult to distinguish the growth and flowering of lilies influenced by two variables, storage duration and forcing temperature. Both high temperature and low light can cause a loss of flower buds (Durieux et al., 1982; Roh, 1990b). Without the use of an air-conditioned greenhouse or phytotron, it is impossible to distinguish between the effect of prolonged frozen storage duration and high forcing temperature.

This research was conducted to investigate the effect of the duration of frozen storage and forcing temperatures on the quality of cut Oriental hybrid lilies 'Acapulco' and 'Simplon'. The influence of the duration of frozen storage and forcing temperatures on shoot apex development was also observed by scanning electron microscopy.

## Materials and Methods

*Shoot apex development.* Bulbs stored at –2 °C since15 Jan. were shipped overnight from Vandenberg Bulb Co. (Chester, N.Y.) to the laboratory in Beltsville, Md. Upon receipt of 'Acapulco' bulbs (15 to 17.5 cm in circumference) on 23 Jan., 16 Apr., 29 May, and 10 July 1998, and of 'Simplon' bulbs (17.5 to 20 cm in circumference) on 4 Feb., 8 Apr., 5 Aug., 5 Oct., and 2 Dec. 1999, shoot apices from four or five bulbs were examined under a dissecting microscope to check the developmental stage.

In addition, some 'Simplon' bulbs received on 4 Feb. 1999 were packed with sphagnum peatmoss (50% moisture by weight) in a plastic bag. Five bulbs were sampled at 0, 5, and 10 d after storage in an incubator maintained at 16 °C in dark. Ten days after storage at 16 °C, the remaining bulbs were transferred to 28 °C and sampled 5 and 10 d after transfer. Shoot apices were fixed in 5% gluteraldehyde in a phosphate buffer at pH 7.0. Samples were dehydrated in ethanol, critical point-dried, coated with gold–paladium, and observed in a JEOL 6100 scanning electron microscope (JEOL USA, Peabody, Mass.).

*Forcing experiment (1998).* 'Acapulco' bulbs were potted singly in 15-cm pots filled with ProMix BM growing medium (Stamford, Conn.). After planting, 0.8 g of 14N–6.2P–11.6K controlled-release fertilizer was applied to the medium surface. Plants were grown in a fan- and pad-cooled greenhouse (FP–GH) or in two refrigerated air-conditioned greenhouses (AC–GH). Fifteen plants were grown in each greenhouse in a factorial experiment. A second group of frozen stored 'Acapulco' bulbs received on 29 May 1998 were potted and were then exposed to a continuous or interrupted 15.5 or 25 °C for 10, 20, or 30 d in growth chambers (0700 HR to 2000 HR light at 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$). After these treatments, all plants were grown in an AC–GH maintained at 16.5/16 °C. There were nine plants per treatment, which were completely randomized during greenhouse forcing.

Average monthly day/night temperatures of a FP–GH were: 16/12 °C (January) 19/15 °C (February), 23/18 °C (March), 25/19 °C (April), 26/22 °C (May), 29/24 °C (June), 31/27 °C (July), 31/28 °C (August), 29/24 °C (September), 24/19 °C (October), and 19/15 °C ( November) and for AC–GH at either 16.5/16 °C (Low AC–GH) or 18.5/18 °C (High AC–GH). Daily average temperatures in two AC–GH were maintained within ±0.2 °C from the set temperatures. Supplementary lighting from a high-intensity discharge–high-pressure sodium (HID–HPS) light source was given to maintain photosynthetic photon flux (*PPF*) at 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 16 h from 0600 to 2200 HR. Shadecloth was used to reduce *PPF* to between 350 and 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ throughout the year.

*Forcing experiment (1999–2000).* Frozen-stored 'Simplon' bulbs were potted on 4 Feb., 8 Apr., 5 Aug., 5 Oct., and 2 Dec. 1999. Bulbs were potted as described above and grown in a FP–GH or a Low AC–GH. There were 12 bulbs per treatment in a factorial experiment. The average monthly day/night temperatures of a FP–GH were: 18/15 °C (Feb. 1999), 23/18 °C (March), 24/19 °C (April), 27/23 °C (May), 29/25 °C (June), 32/27 °C (July), 31/28 °C (August), 23/18 °C (September), 18/16 °C (October), and 16/12 °C (November), 15/11 °C (Dec. and Jan. 2000), and 16/11 °C (Feb. 2000).

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*Data collection and statistical analysis.* The date of flowering was recorded when the first flower bud opened. The number of days to flower was counted from the date of planting. At flowering, the numbers of normal and abnormal (aborted and blasted) flower buds (Roh, 1990c) were counted. The number of dried leaves and green leaves were also counted separately. Stem lengths bearing dried leaves and green leaves were recorded separately. Ten leaves per plant were collected at the midpoint of the stem, and the length and the widths at the widest point of the leaves were recorded. Data were subjected to the analysis of variance and linear regression analysis, and average means were compared using the MSTA-C statistical analysis program (Michigan State Univ., 1989).

## Results and Discussion

*Development of shoot apex.* Shoot apices of 'Simplon' (Fig. 1a) and 'Acapulco' were vegetative for all dates when bulbs were received and examined microscopically (data not presented). After 5 d of storage at 16 °C, four out of five bulbs of 'Simplon' that were received on 4 Feb. 1999 had formed flower buds (Fig. 1b). After 10 d of storage, all bulbs became reproductive (Fig. 1c). When bulbs were transferred after 10 d at 16 °C and exposed to 28 °C for 5 d, flower buds were formed in all bulbs on 7.6-mm-long stems and had well-formed sepals and petals (Fig. 1d). Leaves were loosely embracing the flower buds and were easily removed, exposing the shoot apex. Stem roots were well developed. After 10 d at 28 °C, the stem length was 10.8 mm and flower buds were well developed (Fig. 1e).

*Forcing experiment.* When 'Acapulco' bulbs planted on 23 Jan. were forced in a FP–GH, shoots emerged and flowered in 14 and 104 d, respectively (Table 1). Flowering was accelerated by 35 to 48 d when bulbs were planted on 16 Apr. or at later dates. Flowering was earliest when bulbs were planted on 29 May, taking 56 d. Acceleration of flowering could be caused by high temperatures in a FP–GH. Long-day photoperiod could also influence the flowering responses, but this effect would be minimal because plants were grown under 16-h long-day photoperiod conditions provided with HID–HPS light sources throughout the year. Comparison of the plants grown in a FP–GH to those in an AC–GH demonstrated that FP–GH plants flowered 14 and 23 d later than those in Low AC–GH (16.5/16 °C) and High AC–GH (18.5/18 °C), respectively, due to lower greenhouse temperatures when bulbs were planted early in the season. When plants were forced in a Low AC–GH or High AC–GH, bulbs planted on 23 Jan. flowered in 90 and 81 d, respectively. Bulbs planted at later dates, after prolonged frozen storage, flowered in 71 to 75 d and in 69 to 75 d, when forced in Low AC–GH and High AC–GH, respectively. This acceleration in flowering time could be attributed to a difference in the total radiant energy level during the months between April and September as compared to the level during January and April.

The number of normal flowers that ranged from 3.5 to 5.0 was not significantly different when bulbs were planted on 23 Jan. and 16 Apr. and forced in a FP–GH and two AC–GH. Further, no abnormal flowers were formed. When forced in a FP–GH, there was an increase in the number of abnormal flowers, 3.2 and 3.6 in 'Acapulco' bulbs planted on 29 May and 10 July, respectively (Table 1, Fig. 2). This is attributed to high forcing temperatures, since the number of abnormal flowers was significantly lower when bulbs were forced in a Low AC–GH (Table 1). Stem length became significantly shorter when frozen bulbs were planted on 29 May and 10 July and forced

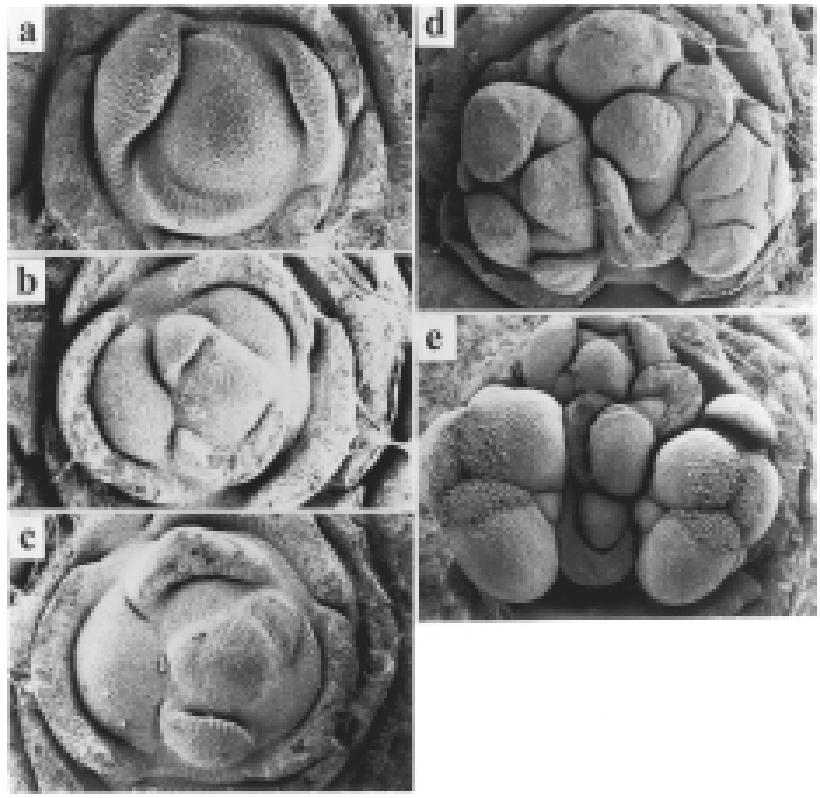

Fig. 1. Floral development of Oriental hybrid lily 'Simplon'. Bulbs were frozen and stored from 15 Jan. until 3 Feb. and shoot apex was observed upon (**a**) arrival, (**b**) 5 d, and (**c**) 10 d of storage at 16 °C, and (**d**) 5 d and (**e**) 10 d at 28 °C after transfer from storage at 16 °C.

Table 1. Effect of frozen storage duration and greenhouse forcing temperature on growth and flowering of Oriental hybrid lily 'Acapulco' (1998).

| Potting date[z] | Forcing | No. of days to: Shoot emergence | No. of days to: Flowering | No. of flowers: Normal | No. of flowers: Abnormal | No. of leaves: Dried | No. of leaves: Green | Stem length (cm) | Leaf area ($cm^2$) |
|---|---|---|---|---|---|---|---|---|---|
| 23 Jan. | FP–GH[y] | 14 | 104 | 3.5 | 0 | 5.3 | 21 | 60 | 23 |
| | Low AC–GH | 13 | 90 | 3.5 | 0 | 5.4 | 23 | 60 | 20 |
| | High AC–GH | 11 | 81 | 3.7 | 0 | 5.2 | 22 | 56 | 20 |
| 16 Apr. | FP–GH | 4 | 69 | 4.7 | 0 | 7.7 | 26 | 68 | 17 |
| | Low AC–GH | 4 | 79 | 4.9 | 0 | 8.5 | 28 | 61 | 15 |
| | High AC–GH | 4 | 75 | 5.0 | 0 | 9.0 | 28 | 62 | 15 |
| 29 May | FP–GH | 6 | 56 | 0.9 | 3.2 | 11.4 | 22 | 56 | 18 |
| | Low AC–GH | 6 | 71 | 3.9 | 0 | 10.7 | 25 | 71 | 17 |
| | High AC–GH | 6 | 69 | 4.2 | 0 | 10.0 | 26 | 69 | 17 |
| 10 July | FP–GH | 4 | 62 | 1.4 | 3.6 | 6.3 | 26 | 62 | 16 |
| | Low AC–GH | 5 | 75 | 4.1 | 0.3 | 8.7 | 25 | 75 | 16 |
| | High AC–GH | 4 | 69 | 4.4 | 0.2 | 8.3 | 24 | 69 | 18 |
| Level of significance | | | | | | | | | |
| Potting date (PD) (Lin) | | *** | *** | *** | *** | *** | *** | *** | *** |
| Forcing temp (FT) | | ** | *** | *** | *** | *** | NS | NS | *** |
| PD × FT | | * | *** | *** | *** | *** | * | *** | *** |
| HSD | | 1.5 | 4.9 | 1.4 | 0.8 | 3.9 | 2.4 | 5.3 | 2.6 |

[z]Bulbs were stored at –2 °C starting from 15 Jan.

[y]Fan- and pad-cooled greenhouse (FP–GH); see text for average monthly day/night temperatures; refrigerated air-conditioned greenhouse maintained at 16.5/16 °C (Low AC–GH) and at 18.5/18 °C (High AC–GH).

NS, *, **, ***Nonsignificant or significant at $P = 0.05$, 0.01, and 0.001, respectively. Lin = linear effect; honestly significant difference (HSD) by Tukey's ω-procedure at $P \leq 0.01$.

in a FP–GH as compared to plants forced in two AC–GH. Prolonged frozen storage reduced leaf area, but increased the number of dried leaves formed at the base of the stem (Table 1).

The effect of high forcing temperature on flower buds was demonstrated in a separate study in which the number of flowers was significantly reduced to 2.4 and plant height reduced to 43 cm when ‘Acapulco’ plants were forced continuously for 30 d at 25 °C (Table 2). Forcing at 25 °C in all three 10-d periods after potting significantly reduced the plant height as compared to forcing at 15.5 °C continuously for 30 d. However, the number of flowers was not reduced by high temperatures for 10 or 20 d during the first 30 d after potting. In *L.* ×*elegans*, 2 weeks at 26/24 °C given after potting reduced the number of flowers and plant height in ‘Red Carpet’ (Roh, 1990b, 1990c). When quality is judged by the number of normal flowers produced on a long stem, planting of ‘Acapulco’ bulbs stored for 6 months did not lower the quality of cut flowers, provided that forcing temperatures were maintained at or lower than 18.5/18 °C.

‘Simplon’ bulbs planted on 8 Apr. flowered at 70 d, due to high temperatures in a FP–GH between April and August. Bulbs forced in a Low AC–GH greenhouse flowered in 82 to 84 d, except when planted on 2 Dec. (Table 3), which could be attributed to a low total radiant energy during winter months. Planting of bulbs on 2 Dec. after a prolonged frozen storage since 15 Jan. significantly reduced the number of normal flowers to 2.4 and 2.6 when forced in a FP–GH and a Low AC–GH, respectively. This reduction was not the result of an increase in the number of abnormal flowers due to high forcing temperatures. Instead, we concluded that floral initiation is affected by an extended period of frozen storage.

Stem length of ‘Simplon’ was the shortest and the number of normal flowers was the lowest (2.3 flowers) when bulbs were planted on 5 Aug. and forced in a FP–GH (Table 3). The loss of flower buds due to abnormalities (Roh, 1990c) occurred generally on the upper portion of the inflorescence in both ‘Acapulco’ (data not shown) and ‘Simplon’ (Fig. 3). This may have resulted from the effect of the extended high temperature during floral initiation and development. In ‘Acapulco’, 30 d of 25 °C also reduced the number of normal flowers (Table 2).

The number of abnormal flowers increased and stem length decreased when frozen stored bulbs were forced in a FP–GH, starting in August when temperatures were high (Table 3, Fig. 4). Removal of flower buds in Easter lily ‘Ace’ (*Lilium longiflorum* Thunb.) reduced flower stem length, and the length of the uppermost internodes was directly related to the number of flowers (Gianfagna et al., 1986). Extended frozen storage reduced the length of stems with dried leaves, but not those with green leaves. Growing Oriental hybrid lily cultivars intended for cut flowers as potted plants during the summer months should not be attempted due to an increase in the number of abnormal flowers (Fig. 4).

Fig. 2. Oriental hybrid lily ‘Acapulco’ flowers showing abnormal development of buds ranging from flower bud blast to malformed flower petals.

Table 2. Effect of 25 °C treatment duration on growth and flowering of Oriental hybrid lily ‘Acapulco’.

| Temp (°C) treatment: Days after potting 0–10 | 10–20 | 20–30 | No. of days to: Shoot emergence | Flowering | No. of normal flowers | Plant height (cm) |
|---|---|---|---|---|---|---|
| 15.5 | 15.5 | 15.5 | 6 | 71 | 3.9 | 71 |
| 25 | 15.5 | 15.5 | 7 | 72 | 3.9 | 61 |
| 15.5 | 25 | 15.5 | 8 | 73 | 3.8 | 57 |
| 15.5 | 15.5 | 25 | 8 | 73 | 4.1 | 53 |
| 25 | 25 | 15.5 | 8 | 73 | 4.0 | 53 |
| 15.5 | 25 | 25 | 8 | 73 | 3.9 | 48 |
| 25 | 25 | 25 | 6 | 70 | 2.4 | 43 |
| Level of significance | | | NS | NS | ** | *** |
| HSD[z] | | | 3.1 | 4.8 | 0.98 | 7.9 |

[z]Honestly significant difference by Tukey’s ω-procedure at $P \leq 0.01$.
[NS, **, ***]Nonsignificant or significant at $P = 0.01$ and 0.001, respectively.

Table 3. Effect of frozen-storage duration and greenhouse forcing temperature on growth and flowering of Oriental hybrid lily ‘Simplon’ (1999–2000).

| Potting date[z] | Forcing | No. of days to: Shoot emergence | Flowering | No. of flowers: Normal | Abnormal | No. of leaves: Dried | Green | Stem length (cm): Dried | Green |
|---|---|---|---|---|---|---|---|---|---|
| 4 Feb. | FP–GH[y] | 19 | 90 | 3.4 | 0.45 | 12 | 27 | 23 | 52 |
| | Low AC–GH | 17 | 83 | 4.1 | 0.42 | 13 | 27 | 20 | 39 |
| 8 Apr. | FP–GH | 8 | 70 | 3.3 | 0.75 | 10 | 29 | 16 | 48 |
| | Low AC–GH | 8 | 82 | 3.7 | 0.08 | 10 | 28 | 17 | 48 |
| 5 Aug. | FP–GH | 8 | 81 | 2.3 | 1.92 | 11 | 21 | 10 | 23 |
| | Low AC–GH | 8 | 84 | 3.7 | 0.58 | 12 | 25 | 15 | 36 |
| 5 Oct. | FP–GH | 4 | 98 | 3.1 | 0.58 | 12 | 28 | 16 | 42 |
| | Low AC–GH | 5 | 84 | 3.1 | 0.33 | 11 | 29 | 16 | 40 |
| 2 Dec. | FP–GH | 10 | 101 | 2.6 | 0.08 | 11 | 33 | 12 | 39 |
| | Low AC–GH | 8 | 91 | 2.4 | 0.08 | 12 | 34 | 14 | 47 |
| Level of significance | | | | | | | | | |
| Potting date | Lin | * | * | ** | NS | NS | * | * | NS |
| | Quad | ** | ** | * | ** | NS | * | ** | ** |
| Potting date × forcing temp | | NS | * | * | ** | NS | NS | * | ** |
| HSD | | 2.8 | 4.2 | 1.2 | 0.31 | 3.7 | 2.6 | 4.9 | |

[z]Bulbs were stored at –2 °C starting from 15 Jan.
[y]Fan- and pad-cooled greenhouse (FP–GH); see text for average monthly day/night temperatures, refrigerated air-conditioned greenhouse maintained at 16.5/16 °C (Low AC–GH).
[NS, *, **]Nonsignificant or significant at $P = 0.05$ and 0.01, respectively. Lin = linear effect; Quad = quadratic effect; HSD = honestly significant difference by Tukey’s ω-procedure at $P \leq 0.01$.

Fig. 3. Two blasted buds (➔) observed in the upper inflorescence of Oriental hybrid lily ‘Simplon’.

Fig. 4. Oriental hybrid lily ‘Simplon’ plants forced in a (**left**) fan- and pad-cooled greenhouse (FP–GH) and (**right**) refrigerated air-conditioned greenhouse (AC–GH) maintained at 16.5/16 °C. Bulbs frozen-stored from 15 Jan. were received on 5 Aug. 1999. Blasted flower bud (➔) is observed in the plant grown in a FP–GH.

## Conclusion

The shoot apex of bulbs was vegetative when frozen stored bulbs were received. The shoot apex became reproductive within 5 d after transfer of ‘Simplon’ bulbs to 16 °C. An extended duration of the frozen storage reduced the height and the number of normal flowers. Forcing at 25 °C, 10 to 30 d after planting, reduced plant height and increased the number of abnormal flowers. ‘Acapulco’ and ‘Simplon’ bulbs can be stored frozen for 6 and 9 months, respectively, to produce high-quality cut flowers as judged by the number of normal flowers and stem length. This will make it possible to force these and other Oriental hybrid lilies year-round in places where summer temperatures are not excessively high.

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