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# Storage Temperature and Duration Affect Flower Bud Development, Shoot Emergence, and Flowering of *Leucocoryne coquimbensis* F. Phil.

**Kiyoshi Ohkawa, Hyeon-Hye Kim, Emiko Nitta, and Yukinori Fukazawa**
*Department of Biological Science, Faculty of Agriculture, Shizuoka University, Ohya 836, Shizuoka, 422-8529, Japan*

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**Abstract.** ***Leucocoryne*, a native to Chile, has violet, blue, or white flowers and is increasing in popularity as a cut flower. The effects of storage temperature and duration on flower bud development, shoot emergence, and anthesis were investigated. Bulbs stored at 20 to 30 °C for 22 weeks produced 3.4 flower stems per bulb between March and April. Bulbs stored at 20 °C flowered earliest, followed by those stored at 25 °C. Bulbs stored at 30 °C flowered last. After 16 weeks of storage at 20 °C, a further 2 weeks dry storage at 15 °C before planting resulted in 1 month earlier flowering with no reduction of the number of flowering stems. As dry storage at 20 °C increased to 11 months, the time to emergence and flowering decreased. After dry storage at 20 °C for 12 months, the primary flower stems aborted and secondary stems then developed. Secondary and tertiary flower stems tend to commence flower bud development after the flower bud on the primary flower stem has reached the gynoecium or anther and ovule stage of initiation.**

*Leucocoryne* (Liliaceae Family) is a genus of about fourteen species, including *L. ixioides* (Hook.) Lindl., *L. coquimbensis* F. Phil. and *L. purpurea* Gay., which flower normally in the spring (Crosa, 1988; Huxley, 1992; James, 1937; Uphof, 1945). All of the species of *Leucocoryne* are indigenous to Chile. However, individual species are widely distributed in mostly dry habitats from the coastal zones to the mountains. *L. coquimbensis* grows naturally around Coquimbo, from which the species name is derived, at latitudes 30 to 33°S (Hoffmann, 1989). This area is quite dry, with most rains in June (an average annual rainfall of 70 mm). From October to April, it seldom rains. Average temperatures are relatively constant, fluctuating from 12 to 17 °C. *L. coquimbensis* grows mainly near the coast where it is exposed to moist salt-laden winds from the sea (Zoellner, 1972).

*Leucocoryne* flowers are long-lasting, blue, violet or white, and have a pleasing fragrance. Furthermore, cultivation of this flower is relatively simple (Kroon, 1986, 1989; Van de Meer, 1993). For these reasons, *Leucocoryne* is increasingly popular as a cut flower.

In Japan, *Leucocoryne* bulbs, planted in the fall (September or October) and grown in greenhouses heated to 10 °C bloom from late March to April. In May, after the aerial parts senesce, bulbs are lifted and stored in dry conditions for reflowering the following spring.

These observations suggested that *Leucocoryne* would be good for commercial production although the specific temperature requirements to hasten or retard flowering are not known. In this paper, we investigated the effects of storage duration and temperature on flower development, emergence and flowering.

## Materials and Methods

**The effects of storage temperature on flower development, emergence, and flowering (Expt. 1).** Bulbs that bloomed in Mar. 1991 were lifted on 29 July and stored dry in nonperforated polyethylene film (20 µm) bag for 16 weeks at 0, 5, 10, 15, 20, 25 or 30 °C until 18 Nov. Five bulbs, each bulb weighing 3.0 ± 0.1 g, per treatment, replicated six times, were stored at each temperature. After storage, all bulbs were planted 3 cm deep in pots that were 15 cm diameter × 20 cm high. Pots were filled with a 4:1 (by volume) mixture of commercial compost and coco fiber, with five bulbs per pot. The compost (pH 6.4, EC 1.7 $mS \cdot cm^{-1}$) contained 0.4 $g \cdot kg^{-1}$ N, 1.5 $g \cdot kg^{-1}$ $P_2O_5$, 0.4 $g \cdot kg^{-1}$ $K_2O$, and 0.2 $g \cdot kg^{-1}$ MgO. Bulbs were grown under natural daylength (12.3 to 18.8 $h \cdot d^{-1}$) in a glasshouse (Shizuoka, Japan) ventilated when actual air temperature is 22 °C and heated to 10 °C from late November. No fertilizers were included when the growing medium was prepared and also during growth. Water was supplied by overhead irrigation regularly. The dates of emergence and flowering and the flower quality were recorded. In addition, the stage of flower bud development was observed during storage and growth by dissection of 10 bulbs per treatment, using the first florets of the scape that developed first. Experiment 1 was completed on 28 Apr.

**The effects of low temperature treatment after dry storage at 20 °C on flower bud development, emergence, and flowering (Expt. 2).** Bulbs that bloomed in Mar. 1992 were lifted on 11 June 1992 and stored dry at 20 °C for 16 weeks until 1 Oct. After this dry storage, five bulbs per treatment, replicated six times were stored dry at 0, 5, 10, 15, and 20 (control) °C, for a further 2 weeks. Each bulbs weighed 0.78 ± 0.14 g. Planting procedures, the growing medium, and the growing place were the same as Expt. 1. The dates of emergence and flowering, the quality and the number of flower stems per bulbs were recorded. Ten bulbs per treatment were dissected before planting to observe the stage of flower bud development, using the first florets of the first developed scape.

**The effects of dry storage duration at 20 °C on flower bud development, emergence, and flowering (Expt. 3).** Bulbs that bloomed in Mar. 1991 were lifted on 30 May and stored in dry conditions at 20 °C. From 30 Aug. 1991 (3 months storage) to 30 Sept. 1992 (16 months storage), five bulbs per treatment, replicated six times, were removed from storage and planted on the last day of every month. Each bulbs weighed 1.5 ± 0.1 g. For planting, the procedure was identical to Expt. 1. Ten bulbs per treatment were dissected to observe the stages of flower bud development as described above.

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Table 1. Effect of storage temperature on flower bud development of the first flower bud on the first flowering stem (Expt. 1). Storage duration was 16 weeks.

| Date of dissection | Storage temperature (°C) | No. of bulbs at each stage of flower bud development [z] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | F | G | H | I | J |
| Before storage | — | | 3 | 7 | | | | | | | |
| After 16 weeks storage | 0 | | 3 | 7 | | | | | | | |
| | 5 | | 3 | 7 | | | | | | | |
| | 10 | | | 10 | | | | | | | |
| | 15 | | | 3 | 7 | | | | | | |
| | 20 | | | | | | | 1 | 2 | 7 | |
| | 25 | | | | | 2 | 2 | 3 | 3 | | |
| | 30 | | | 3 | 7 | | | | | | |

z : A. No differentiation
B. Dome growing point
C. Trilocular growing point
D. Outer perianth initiation
E. Inner perianth initiation
F. Staminode (a rudimentary stamen) initiation
G. Androecium initiation
H. Gynoecium initiation
I. Anther and ovule formation
J. Flower stem elongation in the bulb

**FLOWER BUD DEVELOPMENT OF THE FIRST-, SECOND-, AND THIRD-ORDER FLOWER STEMS DURING STORAGE AND GROWING PERIODS (EXPT. 4).** Bulbs that bloomed in Mar. 1993 were lifted on 1 June and stored at 20 °C before planting on 1 Oct. 1993. Each bulbs weighed 2.5 ± 0.1 g. From lifting, flower bud development of the first-, second-, and third-order flower stems of 30 bulbs were observed on the first day of each month until May 1994.

Immediately after the harvest of cut flower in each experiment, stem lengths of all cut flowers were measured and data of stem mass were recorded on a fresh mass basis. Data were analyzed using the analysis of variance (ANOVA) procedure and means separation with least significant difference (LSD).

## Results

**THE EFFECTS OF STORAGE TEMPERATURE ON FLOWER DEVELOPMENT, EMERGENCE, AND FLOWERING (EXPT. 1).** At the time of lifting, most of the bulbs had growing points at the trilocular stage (Table 1). Development of floral organs was optimum at 20 °C, and was reduced at lower and higher temperature. After 16 weeks storage at 10 °C or lower, flower buds had not developed further. Flower bud development was very slow in bulbs stored at 15 °C. After 20 °C storage, stamens, anthers, and an ovule were detected. Flower bud development of bulbs stored at 25 °C was slightly retarded compared to those stored at 20 °C. The bulbs stored at 30 °C were at same stage as those stored at 15 °C (Table 1).

The emergence of bulbs stored at 15 °C or lower was considerably delayed. Since no plants flowered by 28 Apr. with these storage temperatures, data collection was discontinued. Bulbs stored at 20 °C emerged and flowered the quickest. Emergence and flowering were delayed with bulbs stored at successively higher temperatures. Number of stems per bulb was not affected by the storage temperature. Stem length, stem mass and number of florets were greater in bulbs stored at 25 °C than in those stored at 20 or 30 °C (Table 2).

**THE EFFECTS OF LOW TEMPERATURE TREATMENT AFTER DRY STORAGE AT 20 °C ON FLOWER BUD DEVELOPMENT, EMERGENCE, AND FLOWERING (EXPT. 2).** Bulbs stored at 5 to 15 °C for 2 weeks following the 16 weeks storage at 20 °C emerged earlier than those stored at 20 °C continuously (Table 3). Days to flowering decreased by ≈40 d in bulbs stored at 5 and 10 °C and ≈1 month in bulbs stored at 15 °C compared with those stored at 20 °C, although fewer flower stems developed in bulbs stored at 5 and 10 °C. The flower bud development stage following the 2-week storage treatment at 0 °C was the same as before the storage period. Storage at

Table 2. Effect of storage temperature on emergence, flowering and flower quality of *Leucocoryne coquimbensis* (Expt. 1).

| Storage temperature (°C) | Emergence | | | Flowering [z] | | | Stem length (cm) | Stem mass (g) | Number of florets | Number of stems (per bulb) |
|---|---|---|---|---|---|---|---|---|---|---|
| | % | Date | Days | % | Date | Days [y] | | | | |
| 0 | 50 | 15 Feb. | 89 d | - | - | - | - | - | - | - |
| 5 | 88 | 27 Mar. | 130 e | - | - | - | - | - | - | - |
| 10 | 100 | 5 May | 169 g | - | - | - | - | - | - | - |
| 15 | 100 | 10 Apr. | 144 f | - | - | - | - | - | - | - |
| 20 | 100 | 3 Dec. | 15 a | 100 | 2 Mar. | 91 a | 39.2 a | 3.2 a | 8.0 b | 3.4 a |
| 25 | 100 | 13 Dec. | 25 b | 100 | 29 Mar. | 107 b | 49.1 b | 4.1 b | 8.6 b | 3.5 a |
| 30 | 100 | 26 Dec. | 38 c | 100 | 13 Apr. | 109 b | 41.7 a | 3.1 a | 6.9 a | 3.3 a |

z : Flowering of the first flower stem floret.
y : Days from emergence to flowering.
x : Values in columns followed by the same letter are not significantly different at $P < 0.05$ (LSD).

Table 3. Effect of 2 weeks low temperature treatment after 16 weeks 20°C dry storage on emergence, flowering and flower quality (Expt. 2).

| Storage temperature (°C) | Emergence | | | Flowering [z] | | | Stem length (cm) | Stem mass (g) | Number of florets | Number of stems (per bulb) |
|---|---|---|---|---|---|---|---|---|---|---|
| | % | Date | Days | % | Date | Days [y] | | | | |
| 0 | 100 | 12 Dec. | 58 | 88 | 13 Mar. | 149 | 39.7 | 2.2 | 6.1 | 1.9 |
| 5 | 100 | 13 Nov. | 29 | 100 | 8 Feb. | 116 | 42.9 | 2.8 | 5.5 | 1.6 |
| 10 | 100 | 13 Nov. | 29 | 100 | 9 Feb. | 117 | 42.1 | 2.7 | 5.5 | 2.0 |
| 15 | 100 | 6 Nov. | 22 | 100 | 15 Feb. | 123 | 40.2 | 2.7 | 6.5 | 3.9 |
| 20 (control) | 100 | 5 Dec. | 51 | 100 | 18 Mar. | 154 | 38.6 | 2.9 | 5.9 | 3.2 |
| Significance | | | | | | | | | | |
| Linear | | | ** | | | ** | * | * | ** | ** |

z : Flowering of the first flower stem floret.
y : Days from planting to flowering.
NS, *, ** : Nonsignificant or significant at P ≤ 0.05 or 0.01, respectively.

Table 4. Effect of low temperature treatment after 20°C dry storage on development of the first flower on the first flowering scape (Expt. 2).

| Date of dissection | Low temperature treatment (°C) | No. of bulbs at each stage of flower bud development [z] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | F | G | H | I | J |
| After 20°C dry storage | — | | 3 | 5 | 2 | | | | | | |
| After 2 weeks low temperature treatment | 0 | | 2 | 3 | 5 | | | | | | |
| | 5 | | | 7 | 3 | | | | | | |
| | 10 | | | 6 | 4 | | | | | | |
| | 15 | | 1 | 6 | 3 | | | | | | |
| | 20 (control) | | | 3 | 2 | 5 | | | | | |

z : See Table 1.

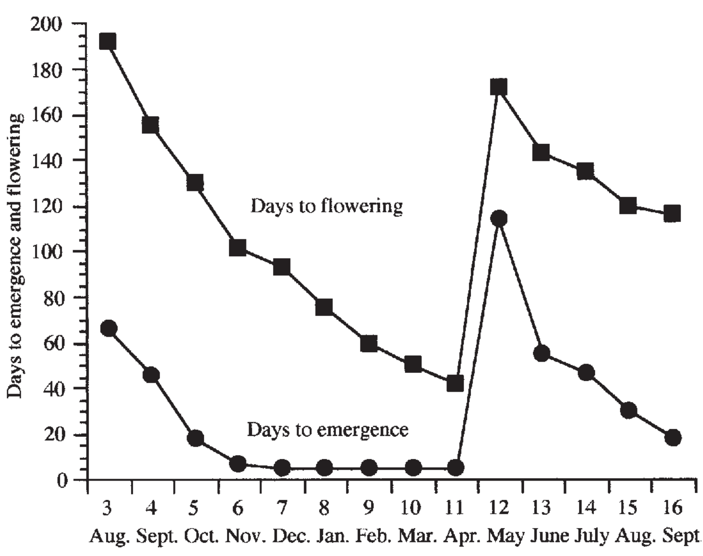


Figure. 1. Effect of dry storage duration at 20°C on days to emergence and flowering (Expt. 3).

5 to 15 °C advanced flower bud development slightly, but most rapid development was seen with the 2-week storage period at 20 °C (Table 4).

**The effects of dry storage duration at 20 °C on flower bud development, emergence, and flowering (Expt. 3).** Days to emergence and flowering decreased with increasing duration of storage, up to 11 months. However, after 11 months storage, the first stem aborted and the days to emergence and the days to flowering increased considerably. Thereafter it decreased gradually in storage for 12 or more months (Fig. 1). Rates of emergence and flowering were 100% in the bulbs planted from September to April (Table 5). Flower stem length ranged from 40.4 to 49.4 cm, the mass of flower stem ranged from 2.1 to 4.4 g, and the number of flowers per stem varied from 4.5 to 9.0. These three factors do not appear to be related to the duration of the storage period (data not presented). The number of flower stems per bulb was 1.0 to 3.5, and was larger in bulbs planted from August to April before the stem abortion that occurred in the 12th month of storage. In contrast, the number of flower stems per bulb was lower in bulbs planted from May to September after the stem abortion (Table 5).

Aspects of flower bud development are shown in Table 6. Following three to four months storage the growing points were at the dome to the outer perianth formation stage with the trilocular

Table 5. Effect of dry storage duration at 20°C on growth and flowering (Expt. 3).

| Planting month | Dry storage duration (months) | Emergence | | Flowering [z] | | Number of cut flower stems (per bulb) |
|---|---|---|---|---|---|---|
| | | % | Date | % | Date | |
| August | 3 | 80 | 5 Nov. | 70 | 10 Mar. | 3.0(0.8) [y] |
| September | 4 | 100 | 15 Nov. | 100 | 3 Mar. | 3.5(1.0) |
| October | 5 | 100 | 19 Nov. | 100 | 10 Mar. | 3.3(1.2) |
| November | 6 | 100 | 8 Dec. | 100 | 12 Mar. | 2.7(0.7) |
| December | 7 | 100 | 6 Jan. | 100 | 8 Apr. | 3.3(1.6) |
| January | 8 | 100 | 6 Feb. | 100 | 16 Apr. | 2.8(1.3) |
| February | 9 | 100 | 6 Mar. | 100 | 28 Apr. | 2.9(1.4) |
| March | 10 | 100 | 5 Apr. | 100 | 20 May | 2.6(1.3) |
| April | 11 | 100 | 6 May | 90 | 11 June | 2.6(0.5) |
| May | 12 | 88 | 22 Sept. | 63 | 19 Nov. | 1.8(0.8) |
| June | 13 | 75 | 24 Aug. | 75 | 19 Nov. | 2.5(0.8) |
| July | 14 | 88 | 16 Sept. | 75 | 13 Dec. | 2.5(1.4) |
| August | 15 | 75 | 30 Sept. | 75 | 29 Dec. | 1.8(0.8) |
| September | 16 | 88 | 19 Oct. | 38 | 25 Jan. | 1.0(0.0) |

z : Flowering of the first flower stem floret.

y : Standard deviation.

Table 6. Effect of dry storage duration at 20°C on development of the first flower on the first flowering scape (Expt. 3).

| Planting month | Storage duration (months) | No. of bulbs at each stage of flower bud development [z] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | F | G | H | I | J |
| August | 3 | | 3 | 6 | 1 | | | | | | |
| September | 4 | | 2 | 6 | 2 | | | | | | |
| October | 5 | | | | | | | 3 | 3 | 3 | 1 |
| November | 6 | | | | | | | 1 | 2 | 2 | 5 |
| December | 7 | | | | | | | | 1 | 1 | 8 |
| January | 8 | | | | | | | | | 1 | 9 |
| February | 9 | | | | | | | | | | 10 |
| March | 10 | | | | | | | | | | 10 |
| April | 11 | | (2)[y] | | | | | | | | 8 |
| May | 12 | | (2) | (2) | (2) | (2) | (2) | | | | |
| June | 13 | | (4) | (4) | (2) | | | | | | |
| July | 14 | | | (6) | (2) | (2) | | | | | |
| August | 15 | | | (4) | (6) | | | | | | |
| September | 16 | | | (4) | (2) | (2) | (2) | | | | |

z : See Table 1.

y : The flower bud of the second flower stem.

stage dominant. After 5 months storage, all flower buds had developed stamens. Following longer storage periods of 9 or 10 months, flower stem elongation had commenced in all bulbs. However after 11 months storage the first flower stem had aborted in 20% of bulbs and the growing point of the second flower stem was developing by that time. Furthermore, after 12 months storage, the first flower stem of all bulbs aborted and the second flower stem was at the dome to staminode formation stage.

**FLOWER BUD DEVELOPMENT OF THE FIRST-, SECOND-, AND THIRD-ORDER FLOWER STEMS DURING STORAGE AND GROWING PERIODS (EXPT. 4).** The first flower stem was initially at the dome to perianth initiation stages and some of the second stems were at the dome stage when lifted on June 1 (Table 7). In November, after planting, the first flower stem had formed a pistil, anther and ovule, and most of the second flower stems were at the trilocular stage (stage C). Two months after planting, when the first flower stem emerged from the bulb, the second flower stem had formed a pistil, anther and ovule. Until the aerial parts senesced from desiccation, the development of flower stems progressed regularly. After flower buds formed anthers and ovules (stage I), 1 month was required for flower stem emergence from the bulb (stage K). Flowering began in March and continued until late April. However, the flower buds which started to develop, or flower stems which started to elongate in the bulb, aborted after April (Table 7).

## Discussion

With temperatures of 20 °C or higher for a finite period, dormancy is broken. The most effective temperature for storage was 20 °C while 25 and 30 °C were less effective. The first flower stem in the bulb developed with 20 to 25 °C storage temperatures, but did not develop at 30 °C. Bulbs stored at this high temperature developed abnormally, probably because of the subsequent much lower planting temperatures. Bulbs stored at ≤15 °C appeared to be dormant as the emergence time was 89 to 169 d. The loss of dormancy in these bulbs probably

Table 7. Flower bud initiation and development of first, second and third flower stem florets during dry storage and growing period (Expt. 4). Bulbs were lifted on 1 June, stored at 20°C until 1 Oct. when they were planted, 10 bulbs were dissected at each sample date.

| Month | Flower stem | No. of bulbs at each stage of flower bud development [z] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | F | G | H | I | J | K | L |
| June | 1st | | 1 | 3 | 2 | 1 | 1 | 1 | 1 | | | | |
| (lifting) | 2nd | 6 | 3 | 1 | | | | | | | | | |
| | 3rd | 10 | | | | | | | | | | | |
| July | 1st | | 2 | 3 | 2 | 1 | 1 | 1 | | | | | |
| | 2nd | 6 | 3 | 1 | | | | | | | | | |
| | 3rd | 10 | | | | | | | | | | | |
| August | 1st | | 2 | 2 | 3 | 2 | 1 | | | | | | |
| | 2nd | 6 | 3 | 1 | | | | | | | | | |
| | 3rd | 10 | | | | | | | | | | | |
| September | 1st | | 1 | 7 | 1 | | | 1 | | | | | |
| | 2nd | 6 | 3 | 1 | | | | | | | | | |
| | 3rd | 10 | | | | | | | | | | | |
| October | 1st | | | | 3 | 1 | 3 | 2 | 1 | | | | |
| (planting) | 2nd | 4 | 3 | 2 | 1 | | | | | | | | |
| | 3rd | 10 | | | | | | | | | | | |
| November | 1st | | | | | | 2 | 1 | 5 | 2 | | | |
| | 2nd | 1 | 2 | 4 | 1 | | 1 | 1 | | | | | |
| | 3rd | 8 | 1 | 1 | | | | | | | | | |
| December | 1st | | | | | | | | | 2 | 2 | 6 | |
| | 2nd | | | | | 2 | 2 | 4 | 2 | | | | |
| | 3rd | 6 | 2 | 1 | 1 | | | | | | | | |
| January | 1st | | | | | | | | | | 2 | 8 | |
| | 2nd | | | | | | | | 1 | 3 | 6 | | |
| | 3rd | | 1 | 2 | 1 | 1 | 1 | 1 | 2 | | 1 | | |
| February | 1st | | | | | | | | | | | 10 | |
| | 2nd | | | | | | | | | 1 | 7 | 2 | |
| | 3rd | | | 1 | 2 | | 1 | 1 | | 4 | 1 | | |
| March | 1st | | | | | | | | | | | | 10 |
| | 2nd | | | | | | | | | | 4 | 6 | |
| | 3rd | | 1 | 2 | | | | | | 2 | 5 | | |
| April | 1st | | | | | | | | | | | | |
| | 2nd | | | | | | | | | | | | 10 |
| | 3rd | | | | | | | | | | 5 | 3 | |
| | wilt [y] | | | | | | | | | | | 2 | |
| May | 1st | | | | | | | | | | | | |
| | 2nd | | | | | | | | | | | | |
| | 3rd | | | | | | | | | | | | 6 |
| | wilt | | | | | | | | | | | | 4 |

z : FromA to J. See Table 1, K. Flower stem emergence from the bulb, L. Anthesis.
y : Aborted flower bud or stems which developed or elongated in the bulb (1st, 2nd or 3rd order).

occurred in the greenhouse while the greenhouse temperatures rose above 20 °C.

A similar result has been reported for *L. purpura* (Van Leeuwen, 1992). *L. purpurea* responded best to a temperature of 20 or 23 °C and storage temperatures below 17 °C resulted in nonflowering or dormant bulbs.

The growing period of *Leucocoryne* is short with flower development beginning at the start of the rainy season so that flowering is over as soon as possible after emergence. Furthermore, flower buds which are at the initiation stage when bulb dormancy begins can survive unfavorable conditions. Also, flower buds which have already formed stamens or a pistil when bulb dormancy begins tend to abort during storage.

In *Leucocoryne* a flower bud is formed after two scales are produced (K. Ohkawa, unpublished data). During the growing period, after the first flower buds have formed pistil, anther and ovule, the next flower bud or flower stem bud starts to develop.

In warm areas of Japan, the air temperature rises rapidly in May and the leaves of *Leucocoryne* wilt and die rapidly. At this point bulbs are dormant. After lifting, bulbs are stored at 20 °C and then planted in October or November when dormancy is broken. Flowering begins in mid March. However, when bulbs were stored for 2 weeks at 15 °C after 20 °C storage, flowering commenced after planting 1 month earlier than usual. Preplant storage temperatures of 5 to 10 °C for 2 weeks also advanced flowering, however, the number of flower stems per bulb was reduced. After planting, the development of the flower buds was rapid following this short cool storage period and the period until flower stem emergence from the bulb was reduced.

Because of climatic conditions in Japan, the earliest flowering after forcing is mid February. For flowering before mid February, bulbs need treatments which retard development. The first flower stems of bulbs stored at 20 °C abort when stored more than 11 months by which time the second flower bud development is initiated (Table 6). In its natural habitat, sometimes there is no rain from April to October. In that situation *L. coquimbensis* becomes dormant before the beginning of the dry season, the first flower stem aborts, and the second flower stem starts to develop. This development is halted when the flower buds are at the growing point division to outer perianth formation stage.

Experiment 3 shows that forcing is possible at 20 °C. When bulbs are lifted 1 year before planting in May or June, plants will bloom in November. When bulbs are planted in July or August, flowering will occur in December. However, the period from planting to emergence or flowering will be delayed and the number of the stems per bulb will be less. If abortion of the first flower stem can be delayed, it would be commercially feasible to plant bulbs in September or October and make them bloom from November to January.

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